EVALUATION OF KNOWN VISUAL AND CHEMICAL CUES FOR SURVEILLANCE OF THE DENGUE AND CHIKUNGUNYA VECTOR, *Aedes aegypti,* IN BUSIA AND KILIFI COUNTIES, KENYA.

BY

OMONDI WYCKLIFF PETER, BSC (BOTANY/ZOOLOGY)

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Declaration

I declare that this thesis is my original work and has not been submitted elsewhere for examination or award of a degree. Where other people's work or not my own work has been used, this has been properly acknowledged and referenced (originality report attached)

Signature…..Date…………………………………………….

Wyckliff Peter Omondi

This thesis is submitted for examination with our approval as research supervisors

1. **Dr. Eunice Anyango Owino** **Signature………………………Date……………………...**

School of Biological Sciences, University of Nairobi, Kenya.

2. **Dr. David Odongo** **Signature………………………Date……………………...**

School of Biological Sciences, University of Nairobi, Kenya.

3. **Prof. Baldwyn Torto** **Signature………………………Date…………………………**

Behavioral and Chemical Ecology Unit, International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya.

4. **Dr. David P. Tchouassi** **Signature………………………Date……………………...**

Behavioral and Chemical Ecology Unit, International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya.

Dedication

I would like to honor the late mother Herina Awino for instilling sound educational principals in me. Special thanks to George K'oluoch and Teresa Odindo for guiding and shaping my educational life. Much appreciation to family members for the support they accorded me while studying.

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List of Abbreviations and Acronyms

Arboviruses	-Arthropod borne viruses
BGS	- Biogent Sentinel trap
CHIKV	- Chikungunya virus
CDC	- Center for Diseases Control and Prevention
CO_2	- Carbon dioxide
CYD-TDV	- Chimeric yellow fever dengue, tetravalent dengue vaccine
DENV	- Dengue virus
DDSR	- Division of Disease Surveillance and Response
DHF	-Dengue hemorrhagic fever
GLM	- Generalized linear model
GVCR	- Global Vector Control Response
HA	-Hexanoic acid
HLC	-Human landing catches
Icipe	- International Centre of Insect Physiology and Ecology
IFS	-International Foundation of sciences
KEMRI	-Kenya Medical Research Institute
KNBS	-Kenya National Bureau of Statistics
LEDs	-light emitting diodes
LO	-Linalool oxide
mg	-Milligram
MoH	- Ministry of Health
MSF	- Medicins san frontiers
ng	-Nano grams
NTDs	- Neglected Tropical Diseases
ORs	-Olfactory receptors
QGIS	-Quantum geographic information system
RVF	-Rift Valley fever

UoN - University of Nairobi

UV - Ultra Violet

WHO -World Health Organization of United Nations

Abstract

Dengue and chikungunya are arboviral diseases transmitted by *Aedes aegypti*. Currently there is an upsurge of these infections worldwide, Kenya included. Due to lack of effective human cure, vector surveillance is critical for control and prevention of these diseases. The purpose of this study was to improve sensitivity of existing surveillance tool using potentially identified light cues ultraviolet light and incandescent light as well as odor cues hexanoinc acid and linalool oxide in two study sites previously found to harbor *Aedes aegypti* the vector, in Busia and Kilifi counties. The identified cues were blended in biogent sentinel site trap (BGS-1) and effect tested following Latin square design. Trap catches for every treatment were entered in excel and performance in terms of counts compared in R using generalized linear model (GLM) relative to controls at 95% confidence level of significance. Generally, there were more *Ae. Aegypti* trapped in Kilifi than Busia. The results obtained show that BG traps baited with UV or incandescent light comparably increased catches of *Ae. aegypti* 40-57% in Kilifi and 24% in Busia although not significantly from the control BG trap. The findings of this study confirm the visual attractive effect of BG trap alone to *Ae. aegypti* with differential but marginal effect of UV or incandescent light on trap catches. On the other hand, results on odor cues showed decreased catches of *Ae. aegypti* among the blends constituted. In fact, the blends of the two odorants decreased captures about 2-4 fold when compared the individual compounds (LO or HA) to caches indicating that combining plant- and human-derived odors may elicit a masking effect in trapping this vector. Thus, the usefulness of combining plant and animal odorants in *Ae. aegypti* trapping needs further investigation. The results underscore the importance of detailed knowledge of interactive effect of combining plant and animal odorants for proper lure formulation and development.

CHAPTER 1 : INTRODUCTION

1.1 General introduction

Dengue and chikungunya are re-emerging arthropod-borne viral diseases (arboviruses) listed among twenty diseases that affect majorly the poor and these group of conditions are collectively termed, neglected tropical diseases (NTDs) (WHO, 2017). The causative agents of these disease conditions, dengue virus (DENV) together with chikungunya virus (CHIKV), are passed on chiefly through bite of infective female *Aedes aegypti* together with *Aedes albopictus* (WHO, 2017). Major distribution of dengue and chikungunya diseases, are ordinarily found in areas located within the subtropical and tropical countries of the earth (Kraemer *et al.,* 2015). Currently and in the last twenty years, the world has witnessed increased incidences and severity resulting from these infections, often leading to debilitating effects as well as fatalities among the affected individuals (Guzman *et al.,* 2010, Tsetsarkin *et al.,* 2011, WHO, 2014). Manifestation of these viral infections include headache, fever, pains on joints as well as muscles and rash in humans (Vasilakis *et al.,* 2011, Guzman *et al.,* 2010, WHO, 2009).

Overall, about one half of total global populace are in danger of infection by one or both of these arboviruses (WHO, 2012). In fact, new occurrences of dengue are approximated to affect about 50 to 100 million individuals yearly, while the number of incidences due to chikungunya infections are estimated to impact on 40 million individuals once a year (Bhatt *et al.,* 2013, WHO, 2012). It has been observed that no or little documentation of these viral infections take place before and after outbreaks. It is indeed noted that majority of the reported cases are documented during outbreaks and as such, the estimated figures may not accurately reflect the actual burden attributed to these diseases (WHO, 2016).

Despite of the noted global increase of dengue and chikungunya, continued unavailability of antiviral therapy as well as vaccine for treatment or prevention of human infections confounds control measures and strategies geared towards management of these infections (WHO, 2009). Notwithstanding of challenges in development of effective medicine and vaccine, currently advancement towards potential vaccine is on offing with WHO having approved a single dengue vaccine applicant with suitability among sero-positive patients in highly endemic countries for dengue virus (Capeding *et al.,* 2014, Villar, 2014, Lim *et al.,* 2013).

As a result of there being no effective human cure, control of vector or vectors that transmit these pathogens remain a key goal in ensuring reduced human vector contact (WHO, 2012). Reduced contact with infective vectors will cut on transmission cycle and in the long run yield few acquisition of infections among human and eventually lower number of deaths resulting from pathogens transmitted by these vectors (Esu *et al.*, 2010).

Unfortunately, not many dengue and chikungunya endemic countries have instituted proper vector control measures in readiness of these viral outbreaks. One major challenge fronted towards inadequate vector control is lack of adequate financial resources (Esu *et al.*, 2010). Consequent to these financial constraints, hurried emergency vector control operations have been mounted during disease increase in a local area (Esu *et al.,* 2010). Many a times, the mounted vector control mechanisms are usually conducted during or at the end of outbreaks and as such they mostly never achieve the desired results since majority of people in the endemic area will have been previously exposed to infective bites and probably infected (Esu *et al.,* 2010).

To this end, there is need of vector surveillance of these arboviral diseases to get information that are key for initiation of timely and appropriate interventions to mitigate potential infection risks before an outbreak (Esu *et al.,* 2010, Gu *et al.,* 2008).

Vector surveillance has been made possible through the collection of both immature and adult stages of mosquitoe vector (WHO, 2012). Collection of adult stages of disease transmitting mosquitoes provides not only population abundance trends but also reduces the contact of trapped vector with human. Collection can be achieved using established traps. It has been observed that, the efficiency of such trapping tools can be improved to enable them provide accurate trends in concentration and richness of vector in a specified endemic location (Ming *et al.,* 2018, Owino *et al.,* 2014, Tchouassi *et al.,* 2013). Trap improvements may be achieved by both olfactory and visual cues, mostly mosquitoes use these cues in locating host. Exploration of these cues have interestingly provided a window of opportunity to improve the efficacy of existing trapping tools by adding them to the already existing gold standard trap to maximize trap vector collection (Takken and Knols, 1991, Sutcliffe, 1987).

Vision is used for several purposes among insects, including mosquitoes. The main purpose of vision is not limited to, location of food sources, looking for mating partners, searching for ovipositional as well as resting areas but also involve searching of blood meal hosts (Allan *et al.*, 1987). Several properties such as color, light and movement enhance visual acuity (Allan *et al.*, 1987, Brown, 1954). Among these visual factors, black and white color property formed one of the basis on which the Biogent sentinel trap (here after referred to as BGS-1) was developed. The BGS-1 has been extensively acknowledged in its fitness for surveillance of the vector *Ae. aegypti* (Kroeckel *et al.,* 2006). *Ae. aegypti* operates and is largely full of life from day break in morning till dusk, with little activity at night (Estrada-Franco and Craig, 1995). In the course of the day, this mosquitoe species has been observed allured by white and black colors (Estrada-Franco and Craig, 1995, Gilbert and Gouck, 1957, Brown, 1958, Brett, 1938). Attraction to these colors is made possible due to the fact that this mosquitoe has ability to process and discern reflected colors using the ocelli (Allan *et al.,* 1987). In addition to the ability to discern reflected light colors, evidence equally point to the fact that *Ae. aegypti* similarly pay attention in the direction of transmitted light (Brett, 1938). However, attention to transmitted light, takes place within specific wavelengths of light (Brown, 1966). Achieving the production of light color that corresponds to these specific wavelengths is now possible through utilization of light emitting diodes (LEDs) technology (Burkett *et al.,* 2005, Burkett *et al.,* 1998). In previous field trials with a number of LEDs fitted on light traps originating from center for diseases control and prevention (CDC), increased catches of *Ae. aegypti* was observed in traps fitted with ultra violet (UV) and incandescent lights (Owino, *thesis*). Despite of this breakthrough in use of light traps in trapping of *Ae. aegypti*, no documented study exists in an effort to evaluate ability of LEDs to attract *Ae. aegypti* in tropical Africa.

Besides visual cues, there has been rising enthusiasm in identification of key attractive chemical components that mosquitoe vectors such as *Ae. aegypti* perceive for them to pinpoint hosts (Ghaninia *et al.,* 2008, Logan *et al.,* 2007). Among the numerous attractive odorants identified to increase *Ae. aegypti* catches are, carbon dioxide (CO_2), hexanoic acid (HA) and linalool oxide (LO) (Owino *et al.* ,2014, Nyasembe *et al.,* 2015, Rudolf, 1992). Carbon dioxide, a respiration product has been expansively used and well documented for its competence in increasing mosquitoe trap catches (Rudolf, 1992). It functions as an activator for flying among many insects including mosquitoes and its action has been noted even from long distances from the host (Gillies, 1980). Realization that vectors do not utilize only one compound to zero on the preferred host resulted to the discovery of additional odorants that work together. Combination of these odorants with CO_2 further enhances host location (Bernier *et al.,* 2002, Mukabana *et al.,* 2002). In fact, due to above, the synergy in exhaled CO_2 breath together with other odors released from the human body has made human landing catches (HLC) a successful method in trapping mosquitoes (Jones *et al.*, 2003, Canyon and Hii, 1997). However, safety of HLC method has raised ethical concerns making it disadvantageous in conducting surveillance more so those involving disease transmitting vectors (Jones *et al.*, 2003, Canyon and Hii, 1997). In order to avoid active use of human subjects to trap vectors, commercially produced odorants are currently in use in place. For instance, in the presence of commercially produced CO_2, synthetic hexanoic acid, comparable to human derived form, showed improved trap catches of *Ae. aegypti* in a field experiment done in Busia and Kilifi (Owino *et al.*, 2014). Similarly, CO_2 when jointly used with linalool oxide, showed extra value in trapping *Ae. aegypti* despite the fact that it was originally formulated for malaria vectors (Nyasembe *et al.*, 2015). Since the two specific host cues originate from various sources, they may connote diverse resource need (human and animal) and that their use has been independently evaluated, it is not known whether combining the two odorants would lead to higher trap catches thus, enhance surveillance of this vector of tremendous medical and veterinary importance (Jacob *et al.*, 2018).

The aim of this study was, first to investigate whether complementing BGS-1with incandescent or ultra violet light would improve attractiveness of *Ae. Aegypti* and secondly, evaluate whether combining Hexanoic acid (HA) and Linalool oxide (LO) would improve attractiveness of *Ae. aegypti*.

1.2 Problem Statement

Dengue and chikungunya are re-emerging arboviral diseases in the world, Kenya included. In Kenya, the Ministry of Health identified dengue and chikungunya among the NTDs of public health importance in the country (MoH NTD, 2016). Despite of the laid out strategy to reduce morbidities as well as mortalities that may arise due to these diseases by the year 2020, increased number of outbreaks continue to be witnessed more so in the last four years beginning 2016

Despite of the presence of vector and available disease pathogens, minimal control measures exist for the management and of infection in human as well as sustainable means to reduce vector population of *Ae. aegypti* that transmits these diseases. (MoH, 2019). This is compounded by lack of effective medicines and vaccine to manage human infections due to dengue and chikungunya viruses. As such, interventions directed toward the vector, *Ae. aegypti* that transmits these pathogens is a key option of reducing human infections. A number of challenges have been observed in controlling this diurnally active mosquitoe hence the need of providing effective tools to monitor adult vector populations. Monitoring of adult vector populations provide essential trends which can be used to initiate timely and appropriate infection risks of vector-human contact thus reducing transmission.

1.3 Justification

Currently, the preferred surveillance tool for dengue and chikungunya vector remains BGS-1 trap. Efficacy of this trap can be increased by adding more of the already identified potent attractants to or in the trap. Given that outbreaks are highly unpredictable, effective monitoring against adult *Ae. aegypti* populations is required. A number of visual and chemical attractants with ability to improve catches of the *Ae. aegypti* vector populations have exploited, although this area of research still remains poorly studied for populations of this species in Kenya. In light of this, supplementing previously identified visual cues, UV and incandescent lights on BGS-1 as well as blended chemical cues derived from HA and was evaluated.

1.4 Objectives

1.4.1 General objectives

To evaluate the efficacy of selected light emiting diodes (LEDs) and host related cues in enhancing collections of *Ae. aegypti,* in Busia and Kilifi Counties of Kenya.

1.4.2 Specific objectives

a) To determine the efficacy of incandescent and ultraviolet lights in trapping *Ae. aegypti* in Busia and Kilifi Counties

b) To evaluate the efficacy of combining hexanoic acid and linalool oxide in trapping *Ae. aegypti* in Busia and Kilifi Counties

1.5 Hypothesis

1.5.1 Null Hypotheses

a) There is no difference between UV and incandescent lights in trapping *Ae. aegypti* in the field

b) Combining hexanoic acid and linalool oxide is not effective in trapping *Ae. aegypti* in relation to the individual compound

CHAPTER 2 : LITERATURE REVIEW

2.1 Public health significance of *Aedian* mosquitoes species

Mosquitoes are key vectors of diseases globally and they are notoriously recognized to transmit not only parasites, helminthes and protozoans, but also other several viruses (Sallam *et al.* , 2017). *Aedes aegypti* remain one of the mosquitoes of both medical and veterinary importance globally (Kuno, 1997). It is better known to transmit yellow fever virus however, it additionally transmits chikungunya and dengue and viruses and is presently connected in spreading of zika virus to human (González *et al.*, 2019, Kuno, 1997).

2.2 Dengue and chikungunya diseases

Dengue and chikungunya diseases, are viral infections listed among the twenty neglected tropical diseases (NTDs) and the two infections are ear marked for control in respective endemic countries (WHO, 2017). These diseases are chiefly transmitted by *Ae. aegypti* as well as *Ae. albopictus* (Ibbara *et al.,* 2003, Kuno, 1997). Dengue and chikungunya are considered re-emerging (Brady *et al.*, 2012, WHO, 2012, Makenzie, 2004) as a result of their rapid spread rate, increased outbreaks, adoption of the viruses into new vectors and ability of some of the viral infections to easily cross zoonotic human barrier (Tsetsarkin *et al.,* 2011, Hanley and Weaver, 2008).

Dengue virus (DENV) is solely accountable for dengue fever. DENV is found in the family called *Flaviviridae*, and is classified finally in the genus *Flavivirus* (WHO, 2009). It is documented that four serotypes; DENV-1,2,3 and 4 are liable of human infections (WHO, 2009). On the other hand, chikungunya fever is attributed to chikungunya virus (CHIKV) belonging in *Togaviridae* family (Zeller *et al.,* 2016). The name of the disease, Chikungunya originates from Makonde community, in Tanzania in an attempt to describe the characteristic feature of affected individual assuming a bending posture (WHO, 2017).

2.3 Transmission of DENV and CHIKV

Main infection by either dengue virus or chikungunya virus happen when female *Ae. aegypti* or *Ae. albopictus* in search of blood meal uptake any of the viruses from an infected host while feeding (WHO, 2009, Gubler *et al*., 1986). The infected host may be either human or an animal (Hanley and Weaver, 2008). Once the virus is ingested by the mosquitoe and in the mid gut, the virus multiplies and systematically moves to other various parts of mosquitoe body and become infective after a period eight to ten days (WHO, 2009). Diseased female mosquitoes can spread the acquired virus to susceptible hosts during its life time. Transovarial transmission to offspring from infected females through the eggs has been observed (WHO, 2009). Infected human or animal hosts serve as virus source as well as the multipliers in the disease path way sequence (Durbin *et al.,* 2013). Inside infected human or animal host, the acquired virus circulates within the blood circulatory system for about two to seven days, a duration within which when fed upon by uninfected *Aedes* mosquitoe, infection is taken up (Durbin *et al.,* 2013).

Two cycles exist in vector transmission and these pathways are named based on the carrier host. Sylvatic cycle exists in the wild nature and involves population of wild primate animals (monkeys and baboons) for dengue and on the other hand, rodents and birds for CHIKV (Chevillon *et al.,* 2008, Hanley and Weaver, 2008). In sylvatic cycle, bite from infected vector to suitable host ensures the maintenance of the virus within the wild animal population (Chevillon *et al.,* 2008). Oppositely, the human cycle involves only human beings, and this cycle has been observed to have originated from the sylvatic arm. Infected *Ae. aegypti* together with *Ae. albopictus* perpetuate transmission of the infections between infected and un infected individuals (Hanley and Weaver, 2008).

2.4 Symptoms and signs of dengue and chikungunya

Infection due to DENV and CHIKV yields almost similar symptoms (WHO, 2009). Clinical manifestation resulting from these infections in humans include, increase in body temperature, headache that may tend to be severe, pains in the body joints and muscles, body rashes and general weakness which resolve with time (WHO, 2009). Incubation period of infections due to dengue virus may vary from seven to ten days, on the other hand infections from chikungunya virus may last for three to seven days (Breiman, and Powers, 2009, Guzmán and Kouri, 2002). Despite the fact that majority of these infections are self-limiting, a few of the DENV cases may deteriorate into life threatening situations, leading to hemorrhage as often seen in dengue hemorrhagic fever (DHF) especially in children under ten years (Vasilakis *et al.,* 2011, Guzman *et al.,* 2010, WHO, 2009). The utmost severe form DHF leads to dengue shock syndrome, which can quickly result to death. Persistent joint pains for several months or years may exist in individuals infected with CHIKV (Schilte *et al.*, 2013, WHO, 2008).

2.5 Diagnosis of dengue and chikungunya

Confirmation of infection is critical for clinical care and management. In most cases, diagnosis strive to observe, the virus itself, nucleic acid of the virus, and either viral antigens or antibodies produced against the virus infection (WHO, 2009). Combination of above methodologies are recommended for precise optimization of the test results (WHO, 2009). The virus may be detected in circulating blood cells, plasma or serum after onset of illness (Hunsperger, 2014)

Presence of virus can be observed in a number of ways. First, virus isolation can be conducted by culturing appropriate specimen in a suitable cell culture and later the cultures tested for viral antigens (WHO, 1997). Secondly, nucleic acid test which employs the usage of reverse transcriptase polymerase chain reaction technology provides specific as well as sensitive result about these infections (Shu *et al.,* 2004). Thirdly, enzyme linked immunosorbent assays can be conducted to detect antigens of the virus the peak phase of infection (Chanama *et al.*, 2004, Innis *et al.*, 1989). Additionally, antibody test measuring Immunoglobulin (Ig) M titer is useful within the first few days of infection whereas possibility exist of detecting IgG several months after acquiring infection (Chanama *et al.*, 2004, Innis *et al.*, 1989).

Last but not least, blood examination procedures for platelets and hematocrit values is useful in the peak phase of infection. During this period, observed drop in number of platelets count below 100,000 per micro liter, or an increase in hematocrit level above 20% may indicate viral infections of either dengue or chikungunya (WHO, 2009).

2.6 Burden of dengue and chikungunya

Increased incidences of dengue and chikungunya have been observed across the world in the previous forty years (Tsetsarkin *et al.*, 2011, Guzman *et al.,* 2010). The number of countries reporting dengue incidences increased from seven to over a hundred between 1970 and 2000. Currently, about one hundred and forty eight countries in the world are endemic for dengue; forty seven in Africa, forty-six in Americas, thirty in Asia, twenty-two in Pacific Ocean and three in Europe (CDC, 2019). As a result of this territorial increase, the burden of dengue infections doubled, from less than one million to over 2 million between 2000 and 2007 (WHO, 2012). To date, approximately 390 million infections occur among the at risk individuals residing in the endemic tropical and sub-tropical countries. Out of these newly acquired infections, about 96 million progress to severe stage of dengue (Bhatt *et al*., 2013). Figure 1 shows countries where dengue is either endemic or non-endemic in the planet earth (the map was produced with QGIS version 2.18 using relevant endemicity information).

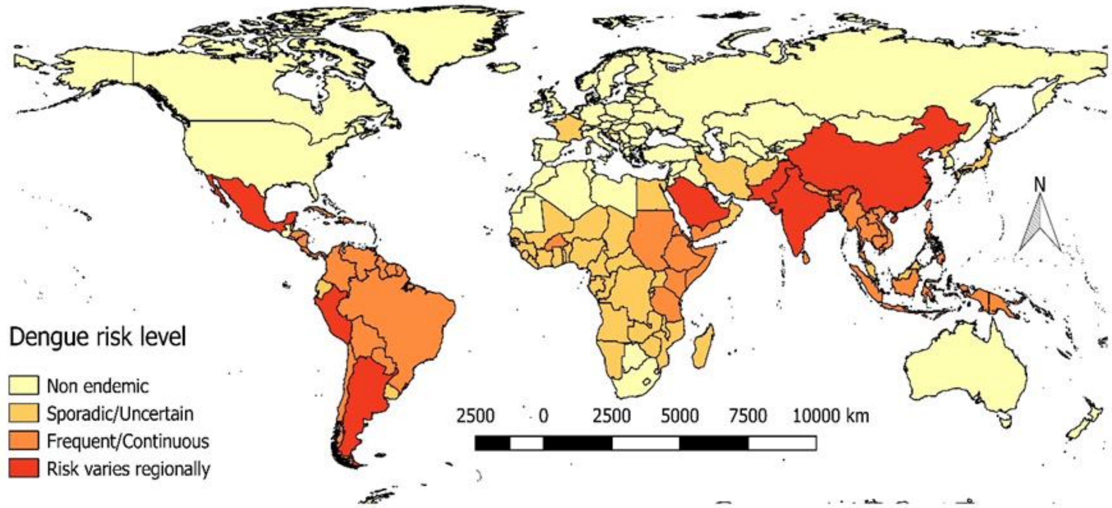

Figure 1: World endemicity of dengue

Equally, several reports have indicated increased spread of chikungunya in many countries in the world. About one hundred and ten countries reported cases of chikungunya; thirty in Africa, forty-seven in Americas, twenty in Asia, eleven in Pacific Region and two in Europe (WHO, 2008, Staples *et al.*, 2009). The current endemicity of chikungunya globally is indicated in Figure 2 (the map was produced with QGIs version 2.18 using relevant endemicity information).

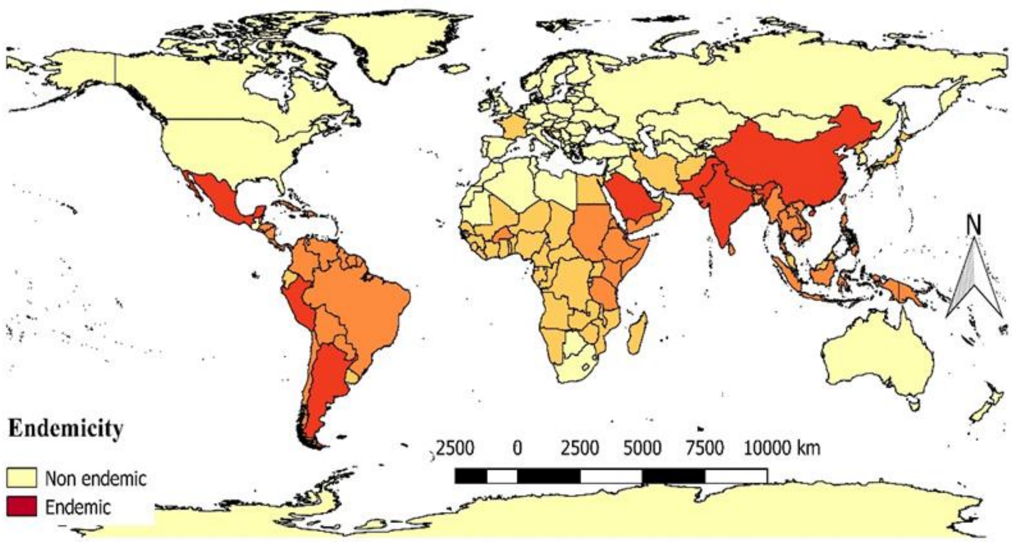

Figure 2: World endemicity of chikungunya

2.7 Status of dengue and chikungunya in Kenya

Kenya has witnessed increased dengue as well as chikungunya outbreaks recently. The first documented dengue was reported over thirty years ago and affected two coastal town Malindi town (currently in Kilifi County) and Mombasa town (currently in Mombasa County) in 1982 (Johnson *et al.,* 1982). As recent as March 2019, dengue outbreak was reported in Mombasa. Similar major outbreaks occurred in the month of May in two consecutive years in 2018 and 2017 after onset of long rains in county of Mombasa (MoH, 2018). In fact, in 2017, more than one hundred cases (190) were positively confirmed due to dengue (MoH, 2017) were confirmed. Prior to 2017 and 2018 outbreaks, the Division of Disease Surveillance and Response (DDSR), Ministry of Health documented dengue fever outbreaks between two counties of Mandera and Mombasa in 2016. In this year, more than five hundred people (500) were positively confirmed to have acquired dengue infection. In addition to these recent outbreak, in 2013, Mandera and Mombasa counties reported dengue infection in more than one thousand people (1900) with positively confirmed cases amounting to about two hundred people (190) (Lutomiah *et al.*, 2016, DDSR, 2013). It is also documented that in 2011, five thousand (5,000) people were infected in Mandera.

With improved diagnostics, the 2016 outbreak in Mandera positively confirmed more than five hundred people (500) infected with chikungunya. Earlier to this, two chikungunya outbreaks were documented in 2004 as well as 2005 in Mombasa and Lamu, coastal region during which more than one thousand individuals (1,300) were confirmed positive of chikungunya (WHO, 2017, MoH, 2016, Medicins Sans Frontiers (MSF), 2016, Daily Nation, 2016).

The witnessed current outbreaks are concerning due to their frequency and certainty. This worry is confounded with the fact *Ae. aegypti* is readily available in the country and that the circulation of both DENV and CHIKV has been confirmed in various regions of the country. Increase in circulating antibodies has been noted in studies conducted in various years. In 1983, prevalence due to DENV-2 increased by more than 50% reaching 57% in Malindi compared to an initial baseline of 7% observed in 1982. More recently, seroprevalence studies conducted in the year 2007 detected DENV antibodies in seven out of the eight (except Nairobi) former administrative provinces of Kenya, while that of CHIKV in about four of the former eight provinces (Ochieng *et al.*, 2015). Indication of circulating virus antibodies is and pointer of respective disease exposure hence presence of the virus. This denotes that only vector is required to enhance the transmission.

2.8 Factors related to the increase of dengue and chikungunya incidences

Increased incidences due to dengue and chikungunya have been attributed to several factors including uncontrolled urbanization, improved means of transportation, and inefficient vector control (Owino, 2018). Impact of people moving from rural set ups to urban settings has resulted to increased population density within these areas (Li *et al.*, 2014, Owino, 2018). Consequently, the population pressure created has resulted in low service provision of a number of infrastructures such as water and garbage collection enabled thriving conducive environment for *Ae. aegypti* vector to thrive (Li *et al.*, 2014). Additionally, improved international transport through air has enabled the wide spread of the both virus pathogens and vector importation to new areas (Huber *et al*., 2004). These importation, has resulted to expansion of regions initially occupied by vector and establishment in new areas together with spread of various virus strains to new foci (Tian *et al*., 2017).

2.9 Treatment of dengue and chikungunya

Although the world continues to witness rapid spread of dengue and chikungunya, human management of these infection continue to be a challenge due to lack of antiviral medicine or vaccine for cure as well as treatment of resulting viral ailments. However, advancement in vaccine development is ongoing (Carvalho *et al.*, 2014, Simmons *et al.*, 2012, WHO, 2009). In 2016, WHO approved the use of Dengvaxia (CYD-TDV) among sero-positive individuals residing in endemic areas with dengue infection prevalence above 70 percent, and none currently for chikungunya infection (WHO, 2017)

Management of symptomatic cases include medical advice for affected persons. It is also suggested that taking a rest and drinking plenty of fluids helps in relieving the associated conditional pain (WHO, 2009). Never the less, pain killers that are likely to increases risk of bleeding should be avoided and should be used only under medical guidance (WHO, 2009).

2.10 Measures on dengue and chikungunya prevention and control

The witnessed expansion of the two disease, dengue and chikungunya in the world may have come after the spread and establishment of the disease vectors (Charrel *et al.,* 2014). As observed with a number of NTDs, where drug and vaccine developments are not considered commercially viable, continued lack of effective cure for human infections may still be witnessed in future (WHO, 2017). As such, control measures directed towards minimizing vector transmission remains a major critical plan towards sustaining low mortality and morbidity of these pathogenic infections (WHO, 2012).

2.11 *Aedes aegypti* ecology and behavior

Aedes aegypti (Linnaeus) belongs to the subgenus *Stegomyia* (Theobald) within *Aedes* genus (Estrada-Franco and Craig, 1995, Edwards, 1932). Characteristically adult mosquitoes are marked with white and black colors. These colors are remarkably noticed on the abdomen, legs and thorax (Estrada-Franco and Craig, 1995). Morphologically, this mosquitoe species can be distinctively differentiated from other siblings according to white lyre-shaped pattern found on lower thoracic side together with white bands on the legs (Estrada-Franco and Craig, 1995).

Adult females lay a number of eggs that range of about 40-90 eggs in a batch, with an individual able of laying five times in its life time (Gubler, 1970). Single eggs are laid in a chosen breeding site, usually with damp substrates (Estrada-Franco and Craig, 1995). This mosquitoe species has adopted artificial breeding sites in addition to the naturally occurring sites. A number of house hold containers provide this mosquitoe with artificial breeding sites, with natural ones being; plant axil and tree holes (Estrada-Franco and Craig, 1995). The eggs that are produced by this mosquitoe species are capable of lasting for several months without water before hatching (Clements, 2000). In availability of water, the eggs hatch into larvae after a period of two to seven days (Clements, 2000). The hatched larvae feed on aquatic organisms for about four days then pupate (Trpis, 1977). The less active pupal stage takes about two to three days, a period after which an adult emerges (Kuno, 2014, Bacot, 1914). Adults are able to live for a period of about three weeks. Three to four days is the estimated period of egg production by female after every blood meal (Kuno, 2014, Bacot, 1914). However, autogenic production of mature eggs without blood-meals has been observed (Trpis, 1977).

2.12 Distribution of dengue and chikungunya vector, *Ae. aegypti*

Ae aegypti as well as *Ae. albopictus* are the two mosquitoe vectors responsible for dengue and chikungunya transmission and they are broadly distributed worldwide (Failloux *et al.,* 2002). Geographically, the mosquitoe vectors are limited in countries located inside the tropical region of the world, mostly corresponding to latitudes 35°N and 35°S as shown in figure 3. Historically, Africa is believed to be the origin of *Ae. aegypti* from where it had a world wide spread through intercontinental exchange of goods and services (Failloux *et al.,* 2002). This spread has resulted into distinction of African and non-African populations and at times beyond the tropical areas (Brown *et al*., 2011).

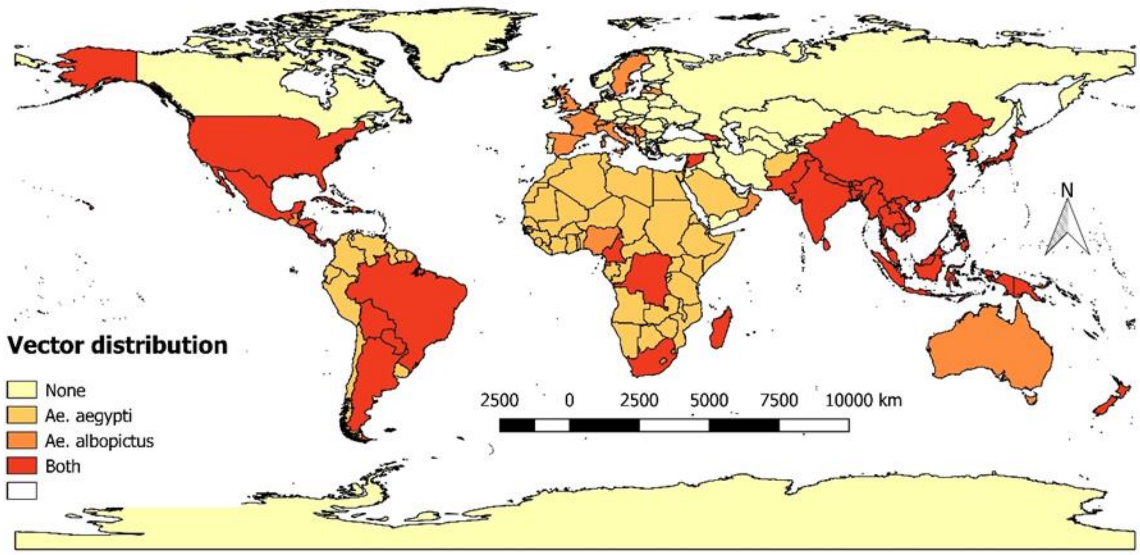

Figure 3 : Map showing the dispersal and co-endemicity of two major mosquitoe vectors causing dengue and chikungunya.

2.13 Feeding Behavior

Similar to other adult mosquitoes, both sexes of *Ae. aegypti* feed regularly on plant sugar (nectar) upon emergence for one to two days however, only females feed on animal hosts for blood meal (Haramis and Foster, 1990). In most cases, the female generally requires blood meal for the supply of vital proteins required for egg development (Briegel, 1985). To fulfill this need, it is necessary for the mosquitoe to locate host and when found, pathogen acquisition or transmission may be accomplished through this feeding mechanism.

This mosquitoe species operates largely in the day (Estrada-Franco and Craig, 1995). This diurnal activity mostly is seen in the morning hours as well as evening but the mosquitoes are capable of biting during the night in well-lit areas (Ho *et al.,* 1973). Behaviorally, these mosquitoes bite humans without being noticed, preferring ankles and elbows (Watson and Kay, 1999). Notwithstanding the fact that *Ae. aegypti* prefers biting people, they bite also domestic animals such as dogs together with other wild mammals (Ponlawat and Harrington, 2005). Only females bite animal hosts to obtain blood required for egg development (Kokoza *et al.*, 2000).

2.14 Host location by mosquitoes

Host location in mosquitoe is driven by physical as well as physiological conditions emanating from host in a step wise detailed process (Sutcliffe, 1987). The process entails appetitive search, activation, orientation, and attraction (Sutcliffe, 1987, Bidlingmayer, 1994). Appetitive search is premediated by hunger and the need to feed on a host (Sutcliffe, 1987). Appetitive search activity is marked by non-oriented flight directed by either visual and chemical cues or both (Sutcliffe, 1987, Bidlingmayer, 1994). When these cues are received by insect mosquitoe, oriented flight towards host is activated. In the final step of attraction, choice whether to feed or not is encountered (Sutcliffe, 1987). Other factors may influence the outcome of host choice. These factors include and are not limited heat, water vapor and visual attraction (Lehane, 1991).

2.15 Mosquitoe attractants

Adult mosquitoes use visual, chemical and physical indicators and signals to locate hosts (Allan *et al.,* 1987). Vision is used for several purposes among insects, including mosquitoes. The main use of vision includes location of food sources, looking for mating partners, oviposition as well as resting areas and additionally for blood meal hosts (Allan *et al.,* 1987). In order to achieve this, mosquitoes, they relate to several chemical attractants released by hosts (Allan *et al.,* 1987). Additionally, physical cues such as; moisture, surface structure, sound, and both radiant and convective heat are among other components that attracts mosquitoes (Laarman, 1955).

2.15.1 Visual attractants of mosquitoes

Adult mosquitoes, *Ae. aegypti* included possess two compound eyes and two ocelli (Brammer, 1970, Brammer and Clarin, 1976). The compound eyes are used majorly for navigation and sensing movement, patterns, contrast, and color (Allan *et al.,* 1987). However, it has been observed that the compound eyes have relatively poor resolution with respect to high light sensitivity (Muir *et al.,* 1992). The ocelli are believed to sense different light levels more so the polarized light (Allan *et al.,* 1987).

Visual attraction amongst insects, for instance mosquitoes and midges (Diptera) depend on specific colors and wavelengths (Headlee, 1932). Visual acuity is believed to be more strongly developed among the diurnally active insects than their nocturnal counterparts. It is indeed documented that response to host visual properties, such as color, brightness, pattern, and movement among others is better in the diurnally active mosquitoes than in the nocturnal ones (Allan *et al.*, 1987).

Ae. aegypti utilizes visual cues in many ways; mate location, oviposition sites, resting sites and host for food sources (animal or plant) (Allan *et al.,* 1987). This species, is able to discriminate ultraviolet (UV), blue, green of both naturally reflected light color's as well as artificially transmitted light color's (Burket *et al.,* 2005, Bret 1983, Gilbert and Gouck 1957, Brown, 1958) Moreover, they are able to contrast black and white color's (Burket *et al.,* 2005, Bret, 1983, 1957, Brown, 1958).

Response to transmitted light colors has been observed to occur in specific wavelengths (Brown 1966). Visualometer studies indicate that *Ae. aegypti* is highly attracted to ultra violet light color which corresponds to wavelengths range between 350-650 nanometers (Brown 1966). Production of light color corresponding to this wavelength is currently possible with the light emitting diodes (LEDs) technology (Burkett *et al.,* 2005, Burkett *et al*., 1998).

2.15.2 Chemical attractants for mosquitoes

There has been growing interest in identification of attractive cues that enable mosquitoe vectors such as *Ae aegypti* perceive in order to locate both animal and plant hosts. Cues mainly of human origin have been identified to attract this species of mosquitoe (Syed *et al*., 2015, Bernier *et al*., 2000), nevertheless, their behavioral impact assessment has largely been conducted in laboratory-based olfactometer assays. Some of the widely evaluated chemical attractants include carbon dioxide (CO_2) (Rudolfs, 1922), acetone (Bernier *et al.,* 2003), lactic acid (Kline *et al*., 1990), octenol (Kline *et al.,* 1991b, Takken and Kline 1989), phenols (Kline *et al.,* 1990), together with some amino acids, especially lysine and alanine (Roessler and Brown, 1964, Brown and Carmichael, 1961). Out of these CO_2, octenol, and lactic acid have been widely used in trapping mosquitoes (Qiu *et al*., 2004, Braks *et al.,* 2000). More chemical compounds of animals and plants origins continue to be explored. Promising results have been obtained with animal lure hexanoic acid (HA) and plant-based lure linalool oxide (LO) when used individually with CO_2 in collecting dengue and chikungunya vector, *Ae. aegypti* (Nyasembe *et al.,* 2015, Owino *et al.,* 2014). Linalool oxide (LO), an enantiomer obtained from some species of aromatic plants. This chemical compound was identified to be a potential mosquitoe attractant (Jhumur *et al.,* 2008). In evaluation of its field activity on malaria vectors, it showed increased catches in trapping *Aedes aegypti* (Nyasembe *et al.,* 2015).

On the other hand, hexanoic acid is a derivative of animal fats and oils. Studies using this volatile extracted from humans showed that it was attractive to *Ae. aegypti* (Owino *et al.,* 2014). Further field evaluation of this demonstrated that traps baited with this compound together with CO_2 significantly caught higher numbers of *Ae. aegypti* in comparison to other compounds tested (Owino *et al.,* 2015).

The above chemical odorants are released by hosts either animals or plants and they in turn evoke both behavioral and physiological responses on targeted mosquitoe species (Clements, 1999). One of the probably well understood host volatile is carbon dioxide (CO_2 (Gibson and Torr, 1999). It is a respiratory product universally known to attract mosquitoes (Gibson and Torr, 1999). In field set up, traps baited with CO_2 have been observed to capture eight to thirty times more mosquitoes than the ones without CO_2 (Shone *et al.*,2003, Kline and Mann, 1998). It is believed that CO_2 is an activator for flight and a long range attractant important for not only host orientation but also play synergistic role in presence of other host odors (Mboera and Takken, 1997, Gillies, 1980). To this end, relevance of combined CO_2 and other host related cues, has been demonstrated and successfully utilized in human landing catches (HLC) of *Ae. aegypti.* To avoid contracting diseases due to infected vector exposure, alternative potential and effective ways of collecting *Ae. aegypti*, that pose no danger to human are being explored in place of HLC (Schoeler *et al.*, 2004, Canyon and Hii 1997). In light of this artificially produced CO_2 (dry ice) have been synergistically used with other potent animal and plant odors which on their own are usually not strong attractants, (Schoeler *et al.*, 2004, Canyon and Hii 1997).

Mosquitoes perceive both single chemical cues as well as mixed odorants cues using olfactory receptors. It has been observed that when two or more chemicals cues are mixed, their perception is not as direct as that of that of the constituent component (Laing and Jinks, 2001). Detection of binary mixture occur in two ways. First each component in the mixture may separately remain identifiable or secondly, the resultant mixture may be detected as a unique entity different from its constituent components (Derby *et al*., 1996). Depending on how the odorant mixture is perceived, a weak or strong outcome is yielded by the mosquitoe. Since a number of chemically derived cues are needed to elicit vector attraction, mixed results have been yielded in an attempt to come up with synthetic mixtures to lure mosquitoes (Chan *et al*., 2018, Syed, 2015). For example, ammonia when used with lactic acid increases catches of *Ae. aegypti* (Geier and Boeckh, 1999). Additionally, increased attractiveness of *Ae. aegypti* have been observed in combinations of acetone plus carboxylic acids (Bernier *et al.,* 2003,Bosch *et al.,* 2000). Efforts to improve mosquitoe lures continue, with recent attempt of combining mammalian and plant tested on malaria vectors.(Jacob *et al.*, 2018). In this study, it was observed that combining odorants from both sources, decreased or increased trap catches according to the dose (Jacob *et al.*, 2018)

2.16 Mosquitoe control and preventive measures

To interrupt transmission of diseases linked to *Ae. aegypti*, control measures geared towards preventing humans from coming in contact with the vector is vital (WHO, 2012). This need for vector control has been echoed by the Global Vector Control Response (GVCR). The above call of action comes in even though both routine control measures as well as emergency measures having been recommended. With above recommendation, many endemic countries usually institute emergency measures during outbreaks which do not yield much of intended results because of lack of properly mainstreamed daily control operational activities to ensure low impact of vector densities (WHO, 2012). Control measures currently in use include, biological, chemical, physical, or an integrated approach, targeting mostly immature and adult stages of *Ae. aegypti*. (WHO, 2012).

2.16.1 Control directed towards immature stages of mosquitoe

Methods to control larval and pupal immature stages of *Aedes* with an intent to reduce emergence of adults exists (WHO, 2012) First, environmental management remains major source reduction method that can be operationalized in public and private places; both in natural and artificial places such as water storage containers and plant leaves that the *Aedes* larvae may develop in (WHO, 2012). Secondly, the aquatic stages can also be managed through larviciding (Yee *et al*., 2004, Gubler and Clark, 1996). Utilization of phosphate chemicals such as temephos, chlorpyrifos, pirimephos methyl, fenthion together with growth regulators are employed in killing of these immature stages (Ponlawat *et al*., 2005, Cheng *et al*., 2003, WHO, 2012). Thirdly, several biological methodologies continue to be explored to reduce both larval and pupal stages. One such biological techniques, employ bacterial toxin from *Bacillus thuringiensis* subspecies *israelensis* (Bti) against the immature mosquitoe stages. Additionally, predators like, *Gambusia sp* and copepod (Mesocyclops) can be employed to feed and eliminate the immature stages (Marten *et al*., 1994, Gerberg and Visser, 1978).

2.16.2 Avoidance and control of adult stages

Spraying of insecticides remain one of the most used mechanism for control of adult mosquitoes. For *Aedes*, this can be achieved through space spraying both indoors and outdoors (Baldacchino *et al*., 2015). Portable sprays are recommended for indoor spraying while other motorized equipment's and tools can be employed outdoors (Karunaratne *et al*., 2013, Gratz, 1993). In addition to use of insecticides, mass trapping of these active day time mosquitoes helps in reducing the encounter between the trapped mosquites and their animal host thus cutting on chances of disease transmission (Rapley *et al*., 2009). Lastly individual personal protection using topical mosquitoe repellant's applied directly on the skin to avoid bites from these mosquitoes (Schreck and McGovern, 1989). Moreover, insecticide treatment of clothes and other effects such as curtains and house screens goes a long way in enhancing personal protection (Morrison *et al*., 2008).

2.17 Surveillance of *Aedes aegypti*

A variety of traps have been utilized for surveillance of adult mosquitoes (Service, 1993). Of these traps, Center for Disease Control and prevention (CDC) trap remains widely used surveillance tool for several mosquitoe species. The CDC trap can be modified to accommodate visual as well as chemical cues. However, its inability to trap diurnally active mosquitoes has been noted (Service, 1993). To overcome this challenge, other traps efficient in trapping diurnally active mosquitoes have been developed. The BGS-1 trap is one of the robust traps with ability to collect day time mosquitoes (Meeraus *et al.*, 2008, Williams *et al.*, 2006).

BGS-1 trap is mostly used to sample adult phase of other dengue vectors such as *Aedes albopictus Aedes polynesiensis* (Schmaedick *et al.*, 2008). but remain preferred for *Ae. aegypti* (Owino *et al.*, 2015, Owino *et al.*, 2014, Meeraus *et al.*, 2008, Williams *et al.*, 2006) Both adult sexes of the mosqutoes in different physiological conditions are successfully caught by the trap (Owino *et al.*, 2015, Owino *et al.*, 2014, Maciel-de-Freitas *et al.*, 2006, Williams *et al.*, 2006b). BGS-1 trap generates convection currents from the power source. These currents imitate human conditions to attract *Ae. aegypti*. The trap is fitted with trap pockets to insert olfactory attractants to enhance catches. However, it has been reported that the BGS-1 with it commercially available lure is still inferior to natural human odors in attraction of *Ae. aegypti* in the field (Owino *et al.*, 2014). This is an active trap that needs a power source. It certainly has challenges for use in remote areas with no electricity and danger of the trapped mosquitoes lost when there is interruption in power source. Other improved version of the trap, BGS-2 is currently in the market. Compared to BGS-1, the new BGS-2 does not require support stands thus is self-supporting, and when power source is disconnected the trap entry closes to prevent the trapped mosquitoes from escaping (Unlu *et al*., 2018)

CHAPTER 3 : MATERIALS AND METHODS

3.1 Study area

The study was conducted in Busia and Kilifi counties, Kenya as displayed in Figure 4.

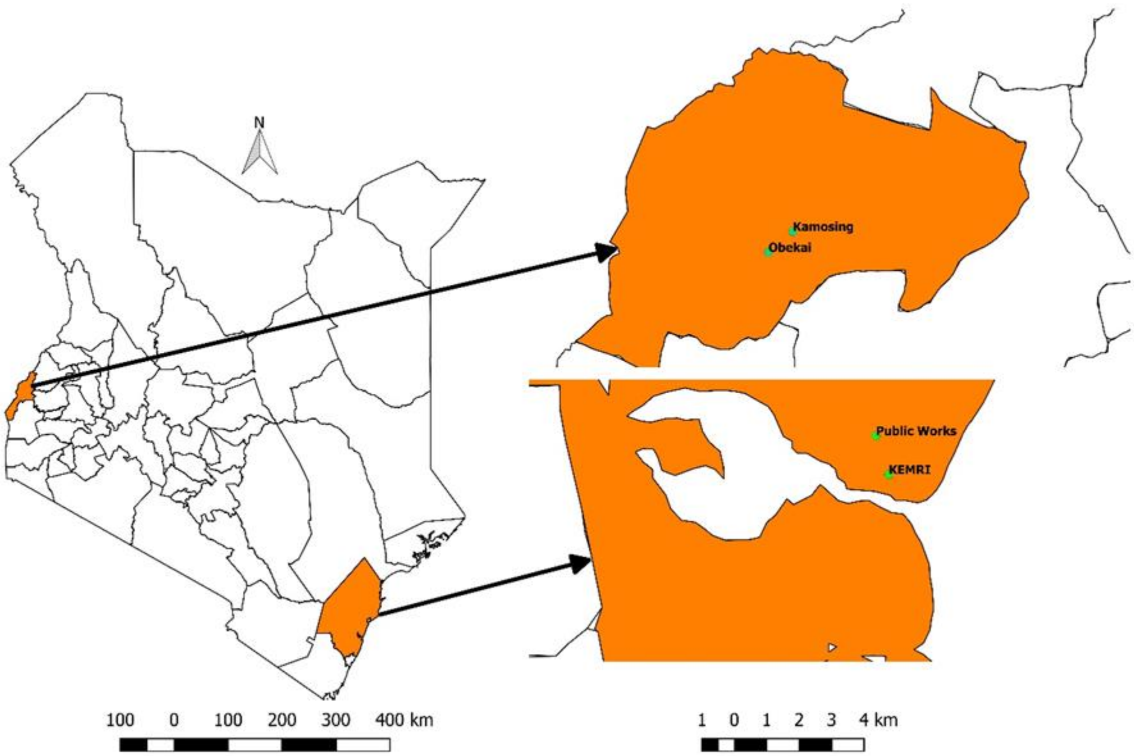

Figure 4 : Map showing study areas; Busia (top right) and Kilifi (bottom right) counties,Kenya

3.1.1 Busia County

Busia is a county in the former Western Province It is a gateway to the neighboring Uganda, with two border crossing points located at Busia and Malaba towns. The county is made of seven sub-counties; Teso North, Teso South, Nambale, Matayos, Butula, Funyula and Budalangi (Figure 4). It has an estimated population of 743,946 people (KNBS, 2009) dominated by the Luhya and Iteso communities among other Kenyan communities.

The study was conducted in Teso North in two villages of Obekai and Kamosing. Visual cues were evaluated at Obekai village (E 34.20556, S 0.51424) while chemical cues were conducted at Kamosing (E 34.21893, S 0.52562) both located in Teso North Sub county, in Busia County.

In the two selected sites, the main economic activities of the inhabitants include animal and crop husbandry as well as trading. Farming is on small scale basis with major produce consisting of maize, beans, groundnuts, cassava, sorghum, vegetables and fruits. The inhabitants of the area keep cows, sheep, goats and pigs. Whereas Busia town remain the main town center other small trading centers exists in Obekai and Kamosing areas in which mosquitoe collection was conducted.

The county has a tropical humid climate due to the presence of the lake. Annual temperatures range between 17°C and 30°C with average annual temperatures of between 24°C and 26°C with rainfall measuring between 900mm and 1,500mm during the year. The long rains are usually experienced between and March and June with short rains falling between September and October.

3.1.2 Kilifi County

Kilifi is one of the six counties in the coastal region. It borders, Mombasa, Kwale, Tana River and Taita Taveta. It consists of seven sub counties, Magarini, Malindi, Kilifi North, Kilifi South, Ganze, Kaloleni and Rabai. Kilifi County is home to 1,109,735 people (KNBS, 2009) from the following communities: Mijikenda, Swahili, Bajuni, Indians, Arabs and European settlers with other communities of Kenya including Kamba, Kikuyu, Luo, Kalenjin and Luhyia are found in the county.

In Kilifi, the study was conducted in Kilifi North Sub County. Visual cues were evaluated within Kenya Medical Research institute-KEMRI (E39.85672, N 3.63026) compound while chemical cue studies were conducted within the public works yard (E 39.85317, N 3.6195). The two sites are approximately 5 kilometers apart.

Economic activities in the study areas included, agriculture, tourism and trading. A number of crops are cultivated in Kilifi County. Main cash crops include, cashew nuts, coconut palms, mangoes, pineapples and sisal, other consumable agricultural produce include banana, cassava, cow peas, green grams and maize. Among the horticultural crops grown along the coastal plains include tomatoes, chillis, onions, brinjals and okra.

The county experiences cold period in June and July when temperatures usually is 21°C and hottest period in January and February. The temperature average of about 32°C is experienced in the county. Averagely the county is warm for most parts of the year. Rain falls mostly in two seasons, April-June (long rains and October-December (short rains). Yearly precipitation ranges from 900mm to 1000mm.

3.2 Materials

3.2.1 Center for Diseases Control and Prevention light trap

CDC light trap model manufactured by John W. Hock Company, in Gainesville, Florida was used. This model of trap is fitted with a 6 volt fan to suck mosquitoes together with incandescent light. The trap is powered with a 6 volt rechargeable battery as shown in Figure 5 (a) below;

Figure 5: Traps panel including modifications used in experimental evaluation of *Ae. aegypti* catches: a) incandescent CDC model light trap, b) Ultra violet CDC model light trap, c) BG-S 1 trap, d) BG-S 1 trap + UV light, e) BG-S 1 trap + CDC incandescent light

3.2.2 Center for Diseases Control and Prevention ultra violet light trap

The CDC trap model (John W. Hock Company, Gainesville, FL) was used. This trap model is fitted with a 6–volt fan to suck mosquitoes together with a 6.3 ultraviolet light. The trap is powered with a 6-volt rechargeable battery as shown in figure 5 (b) above.

3.2.3 Biogent Sentinel 1 Trap (BGS-1)

The BGS-1 (Biogents GmbH, Regensburg, Germany) was used. The trap is composed of white cylindrical container that is collapsible. It has an open top usually covered with white gauze. Triangular wire network that suspended a black cylinder on which the mosquitoe catch bag is attached where trapped mosquitoes are held. Beneath the catch bag lies a 12V fan. The fan creates suction pressure which leads the host seeking mosquitoes into a catch bag. The BGS-1is powered with a 12 V rechargeable battery. The trap is as shown in Figure 5 (c) above.

3.2.4 Biogent Sentinel 1 Trap (BGS-1) with UV light

The trap entry of the CDC UV light trap was sealed with masking tape. No catch bag was attached to the CDC light trap. The sealed trap was placed close to BG sentinel trap allowing light generated from CDC LED trap as the only additional cue attractant to direct mosquitoes into BG sentinel trap catch bag where captured mosquitoes was collected as shown in Figure 5 (d) above.

3.2.5 Biogent Sentinel 1 Trap (BGS-1) with CDC incandescent light

The trap entry of the CDC incandescent light trap was sealed with masking tape. No catch bag was attached to the CDC light trap. The sealed trap was placed close to BG sentinel trap allowing incandescent light generated from CDC trap as the only additional cue attractant to direct mosquitoes into BG sentinel trap catch bag where captured mosquitoes was collected as shown in Figure 5 (e) above.

3.2.6 Bio quip igloo

Plastic Bio Quip Ice Dispenser - capacity: 1/2 gallon, dimensions: 5.5 inches in diameter and 11.5 inches in height (14 x 29.3 cm), weight 0.45 kg) was used to dispense dry ice. The bio quip igloo is able to conserve dry ice consumption and be used for slow release of CO2 over a period of time. Figure 5 (c, d, and e) show blue suspended igloo above BGS-1 trap.

3.2.7 Rubber Septa

General purpose rubber septa ((Sigma-Aldrich) measuring 7.9 mm × 14 mm was used to dispense HA, LO and its constituent mixtures. The rubber septa were inserted in BGS-1 trap pocket.

3.2.8 Odor baits

Authentic commercial standards of HA and LO were used in field evaluations and laboratory electrophysiological assays. They included: (E-) linalool oxide (Aldrich, 99%) and Hexanoic acid (Sigma-Aldrich, ≥99%).

Additionally, sachet composed of three components that make BG-lure; ammonia, lactic acid and caproic acid was used. All treatments were baited with dry ice procured as pellets from Carb acid Limited in Nairobi.

3.3 Mosquitoe collection method

At each of the study sites, trapping location was selected near human dwelling. Traps were set after obtaining oral consent from house hold heads or institutional heads. Traps were set approximately fifty meters away from each other outside houses at about 9.00 am in the morning and left to run over night for twenty-four hours until the following day. To offset any positional bias, daily rotation following the Latin Square design was ensured.

Commercial dry ice was used to supply CO_2 in all treatments. Approximately one kilogram of dry ice was dispensed using Bio quip igloo. The igloos were suspended near each of the trap entrances.

On each day, trapped mosquitoes were collected from their respective trap catch bags, knocked down using trimethylamine and transferred into 50 milliliters plastic tube and preserved in liquid nitrogen while in the field. At the end of the field sampling period, the collected samples were taken to Behavioral and Chemical Ecology Unit, International Centre of Insect Physiology and Ecology (*icipe*), Dudu Ville Campus, Nairobi where captured mosquitoes were sorted and identified morphologically to species under a dissecting microscope with use of morphological keys (Huang YM 1981, Rueda LM 2004). Further categorization of *Ae. aegypti* into respective sexes was conducted. Daily number of identified mosquitoes per treatment and position were recorded and entered in an excel sheet. After scoring, the specimen was preserved in -80^0 C freezer.

3.3.1 Sampling procedure for selected platforms for visual cues

The effect of visual cue on BGS-1 platform to catch *Ae. aegypti* was evaluated in Busia between 29 April to 10 May 2017 and in Kilifi between 24 July to 4 August 2017. Visual acuity of mosquitoes was assessed using five treatments; CDC light trap fitted with incandescent light (Figure 5a), CDC trap fitted with ultra violet light (Figure 5b), BGS I (Figure 5c), BGS I baited with ultra violet light (Figure 5d) and BGS I baited with incandescent (Figure 5e). Each trap was approximately baited with 1kg of dry ice dispensed using thermos igloos. Daily rotation of the trap treatments following a 5X5 Latin square two times was adhered yielding a total of ten trapping days. Table 1 indicate how the rotations were positioned.

Table 1: Latin square arrangement for visual cue trap treatments

Day/Site	**Site_1**	**Site_2**	**Site_3**	**Site_4**	**Site_5**
Day_1	CDC	UV	BGUV	BGCDC	BG
Day_2	BG	CDC	UV	BGUV	BGCDC
Day_3	BGCDC	BG	CDC	UV	BGUV
Day_4	BGUV	BGCDC	BG	CDC	UV
Day_5	UV	BGUV	BGCDC	BG	CDC

***Key**: CDC (CDC trap with incandescent light cue), UV (UV light trap with ultraviolet light cue), BG (BG-sentinel 1 trap with CO2), BGCDC (BG sentinel trap 1 baited with incandescent light), BGUV (BG sentinel trap 1 baited with ultraviolet light)*

3.3.2 Sampling procedure for selected odor cues in trapping *Ae. aegypti*

The effect of combining the odor lures HA and LO on catches of *Ae. aegypti* was evaluated in Busia (29 April – 10 May 2017) and Kilifi (24 July – 4 August 2017). The compounds were each evaluated using established optimum doses- LO, 20 ng/µl (Nyasembe *et al.*, 2015) and HA at 0.5 mg/µl (Owino *et al.*, 2015) in BG-Sentinel 1 Trap. Six treatments were evaluated in a 6x6 Latin Square designed experiment as indicated in Table 2 and included i) LO dispensed using rubber septa +CO_2, ii) HA dispensed using rubber septa + CO_2, iii) blend of LO+HA dispensed together in one rubber septa + CO_2, iv) LO+HA dispensed separately in two rubber septa + CO_2, v) BG commercial lure + CO_2 and vi) CO_2 alone (positive controls.) Each trap was approximately baited with 1kg of dry ice dispensed using thermos igloos.

Table 2: Latin square arrangement for odor cue treatments

Day/Site	**Site_1**	**Site_2**	**Site_3**	**Site_4**	**Site_5**	**Site_6**
Day_1	BG	BGLOHA	BGHA	BGHALO	BGLO	BGL
Day_2	BGL	BG	BGLOHA	BGHA	BGHALO	BGLO
Day_3	BGLO	BGL	BG	BGLOHA	BGHA	BGHALO
Day_4	BGHALO	BGLO	BGL	BG	BGLOHA	BGHA
Day_5	BGHA	BGHALO	BGLO	BGL	BG	BGLOHA
Day_6	BGLOA	BGHA	BGHALO	BGLO	BGL	BG

***Key**: BGHALO (compound mixture of HA and LO dispensed in one septa in BG sentinel I trap), BGLO (Linalool oxide dispensed in one rubber septa in BG sentinel I trap), BGL (BG lure in BG sentinel I trap), BGHA (Hexanoic acid dispensed in one rubber septa in BG sentinel I trap), BGLOHA (Both HA and LO dispensed individually in separate two rubber septas in BG sentinel I trap), BGCO_2 (BG sentinel I trap with CO_2- control)*

3.3.3 Evaluating blend effect of HA and LO at different doses on catches of *Ae aegypti*

Since mosquitoes including *Ae. aegypti* respond to specified doses of odor attractants, varied doses of blend from HA and LO was limited to Kilifi County only between 9th and 22nd July 2018. Varied doses of LO and HA were formulated and tested on *Ae. aegypti* trap catches. Because the effect of a compound on trap catches is dose-dependent, two additional doses of each of the compounds *viz*: LO- (10-fold lower dose, 2ng/µl and higher dose (200 ng/µl); HA- 10-fold lower dose, 50ng/µl and higher dose (5 mg/µl). These, together with the established optimum dose of each compound were blended resulting in 9 different blend combinations (Table 3. Each of these blends was evaluated individually and compared to separate traps having CO_2 only, LO (at optimum dose) and HA (at optimum dose). Complete randomized design with days as replicates was used to evaluate the 12 trap treatments.

Table 3: Information on odor blends including the doses tested

Lure type	Treatment tested	Abbreviations		Dose
binary	(E)-linalool oxide+ hexanoic acid	LH1	LoLHAH	LO 2 ng/µl +HA 5 mg/µl
binary	(E)-linalool oxide+ hexanoic acid	LH2	LoLHAO	LO 2 ng/µl + HA 0.5 mg/µl
binary	(E)-linalool oxide+ hexanoic acid	LH3	LoLHAL	LO 2 ng/µl + HA 0.05 mg/µl
binary	(E)-linalool oxide+ hexanoic acid	LH4	LoHHAL	LO 200 ng/µl + HA 0.05 mg/µl
binary	(E)-linalool oxide+ hexanoic acid	LH5	LoHHAO	LO 200 ng/µl + HA 0.5mg/µl
binary	(E)-linalool oxide+ hexanoic acid	LH6	LoHHAH	LO 200 ng/µl + HA 5mg/µl
binary	(E)-linalool oxide+ hexanoic acid	LH7	LoOHAH	LO 20 ng/µl + HA 5mg/µl
binary	(E)-linalool oxide+ hexanoic acid	LH8	LoOHAO	LO 20 ng/µl + HA 0.5mg/µl
binary	(E)-linalool oxide+ hexanoic acid	LH9	LoOHAH	LO 20 ng/µl + HA 0.05 mg/µl
single	hexanoic acid	HA	HAO	HA 0.5 mg/µl
single	(E)-linalool oxide	LO	LOO	LO 20 ng/µl
	CO_2	CO_2		

***Key**: L(Low), O (Optimum) H (High)*

3.4 Data management and statistical analysis

Daily counts or abundance of *Ae. aegypti* and by sex in the different treatments served as the response variable in separate generalized linear models (GLM) with a negative binomial error structure. Treatment and site were the main predictor variables in the model for experiment I, while only treatment was included in the model for experiment II. All analyses were implemented in R version 3.6.1 (Team, 2013) at α=0.05 level of significance. Each treatment was compared to the control (CO2 alone) as the reference and incidence rate ratio (IRR) estimated, as a likelihood measure that mosquitoes chose other treatments other than the control. For the control, the IRR is 1 with values above this indicative of treatments with better performance and values below underperformance relative to the control (Tchouassi *et al*., 2012). Further, pair-wise comparison in *Ae. aegypti* catches between the treatments was performed by Tukey's HSD test

3.5 Ethical clearance

Sample collection was conducted in different study locations with probability of obtaining adult mosquitoes. The trapping sites were randomly selected near human dwelling after oral consents from home or institutional heads. Since the study was not involving human subject, ethical clearance was not sought from research boards.

CHAPTER 4 : RESULTS

4.1 Results for light cues

Overall, the visual cue experiment yielded 2,527 mosquitoes in total (Table 4). *Ae. aegypti* was the most abundant mosquitoe species accounting for 79% (n=1,985) of the total captures while other species were 21% (n=542). The other species encountered included anophelines (n= 12; mean = 0.12, range = 0-3), and other culicines (n= 530; mean = 5.3, range = 0-34) from both counties. Of the *Ae. aegypti* trapped, only 21% (n=416) was from Busia and 79% (n=1,569) from Kilifi with respective mean catch of 8.32 (range: 0-31) and 31.38 (range: 0-191).

The sex composition of trapped *Ae. aegypti* varied in the collections with more females (F) than males (M) captured (F=1,483; M=502). The sex composition by treatment were as follows: BGS-1=499 (M=140, F=359), BGS-1 +UV=681 (M=166, F=515), BGS-1+Incandescent= 715 (M=175, F=540), Incandescent=52 (M=11, F=41) and UV=38(M=10, F=28) (Table 4). Table 5 shows the respective total mean catches per treatment .

Table 4: Total number of mosquitoes captured using visual cues in Busia and Kilifi counties.

Treatment	**Busia**				**Kilifi**				**Overall**			
	Ae. aegypti		Total	Others	*Ae. aegypti*		Total	Others	*Ae. aegypti*			Others
	Male	Female			Male	Female			Male	Female	Total	
BG	37	81	118	8	103	278	381	83	140	359	499	91
BG&UV	34	113	147	9	132	402	534	115	166	515	681	124
BG&CDC	23	91	114	12	152	449	601	126	175	540	715	138
UV	9	14	23	20	1	14	15	52	10	28	38	72
CDC	1	13	14	41	10	28	38	76	11	41	52	117
Total	**104**	**312**	**416**	**90**	**398**	**1,171**	**1,569**	**452**	**502**	**1483**	**1985**	**542**

Others refers to collection of extra mosquitoes not Ae. aegypti (anopheline and culicine species caught in the trap)

Table 5: Mean catches *Aedes aegypti* recorded in the different treatments using light cues in Busia and Kilifi counties

Trap	**BG**			**CDC-Incandescent**			**UV**			**BG+UV**			**BG+Incandscent**		
Catches	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female
Mean	50	14	36	5	1	4	4	1	3	68	17	52	72	18	54
Standard deviation	41	7	35	5	1	4	4	1	3	36	10	28	58	15	46
Standard error	13	2	11	2	0	1	1	0	1	11	3	9	18	5	15

Treatment abbreviations are as indicated in table 1

For visual cues, significantly higher catches of *Ae. aegypti* was recorded in Kilifi than Busia [$\chi^2$1,94 =116.54, P<0.0001) and varied by treatments ($\chi^2$4,95=157.85, P= 0.002) (Appendix 1). This overall pattern was replicated on catches of females [site: $\chi^2$1, 94=116.13, P<0.0001; treatment: χ^2 4, 95=154.78, P=0.02) and males [site: $\chi^2$1, 94=100.15, P<0.0001; treatment: $\chi^2$4, 95=143.93, P<0.0001].

Higher *Ae. aegypti* catches were observed in the treatments BG&UV and BG&CDC incandescent traps when compare d with the control trap BG (IRR = 1.36, P = 0.334; IRR = 1.43, P = 0.263) respectively in the visual experiment, however, the differences were not significant. The individual light traps either having UV or CDC only recorded significantly decreased catches of *Ae. aegypti* compared to the control trap BG, (IRR = 0.08, P < 0.001, IRR = 0.10, P < 0.001) respectively. Similar results were observed in both Busia and Kilifi counties, as shown in Appendix 1. Figure 6 ,7 and 8 illustrates the mean *Ae. aegypti* catches in the using various treatments in Busia and Kilifi counties.

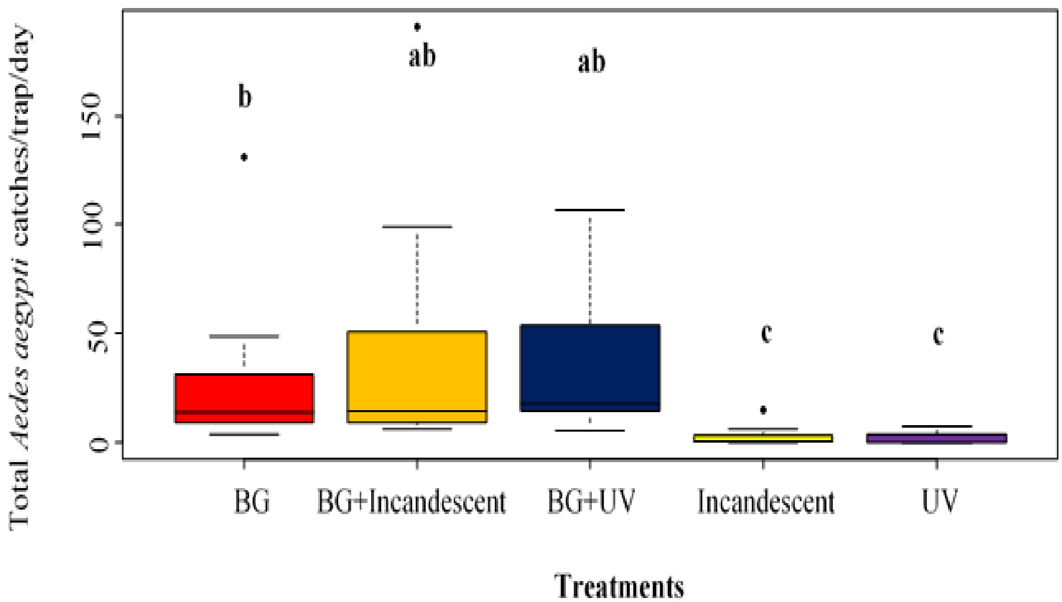

Figure 6: A dot-plot showing total number of *Ae. aegypti* collected using light cues in Busia and Kilifi counties. The whiskers represent lowest and highest counts recorded. Outliers are represented by dots outside the boundaries. Black line within the box represent the median number of catches.

Appendix 1 further provides comparison of the effect of the treatments on *Ae. aegypti* by sex. For the visual cues, both BG&UV&CO_2 and BG&CDC attracted higher catches of either sexes with more females compared to males, although with no significant difference. Significantly decreased captures of *Ae. aegypti* by sex was recorded in individual light traps with either incandescent CDC and UV traps, compared to BG the control: males (IRR =0.07, pP< 0.001; IRR =0.08 P < 0.001) respectively and females (IRR =0.08, P < 0.001; IRR =0.11, P < 0.001), respectively (Figures 7 and 8).

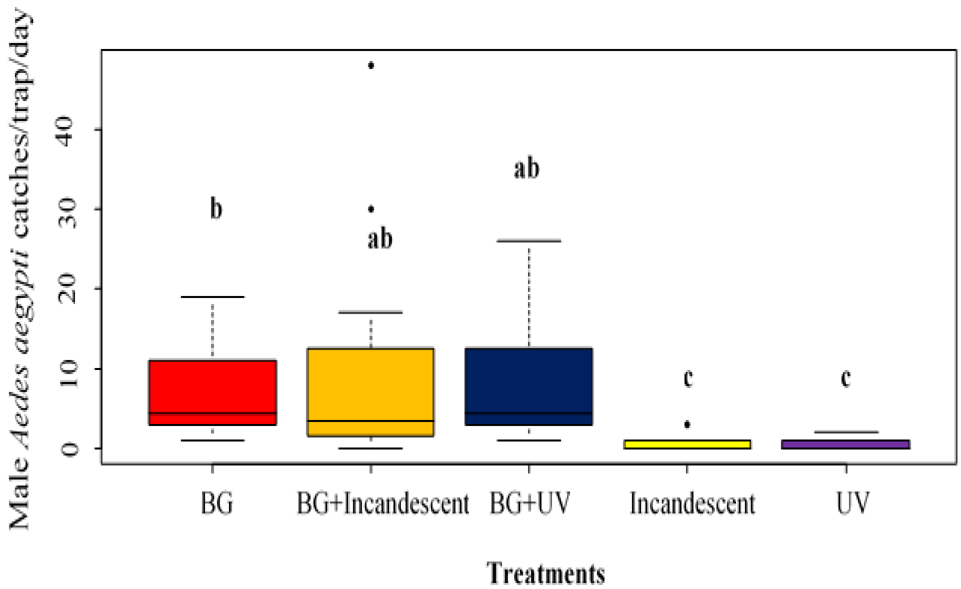

Figure 7: A dot-plot showing male number of *Ae. aegypti* collected using light cues in Busia and Kilifi counties. The whiskers represent lowest and highest counts recorded. Outliers are represented by dots outside the boundaries. Black line within the box plot represent the median number of catches.

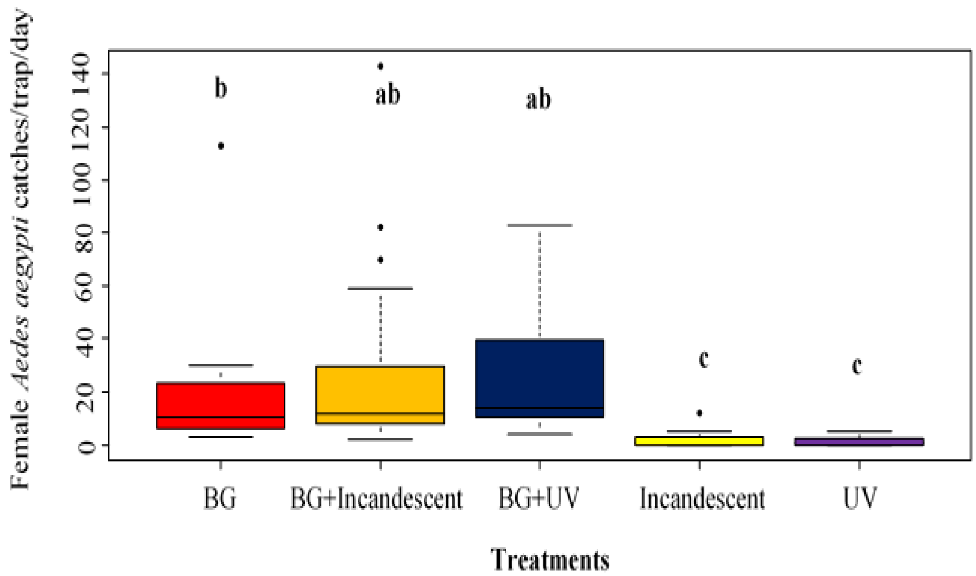

Figure 8: A dot-plot showing female number of *Ae. aegypti* collected using light cues in Busia and Kilifi counties. The whiskers represent lowest and highest counts recorded. Outliers are represented by dots outside the boundaries. Black line within the box plot represent the median number of catches.

4.2 Results for odor cues

A total of 10,987 mosquitoes were encountered in the experiment involving odor cues from the different treatments (Table 6). *Ae. aegypti* was the most abundant species (79%, n=8,728) with a minor representation of other species (21%, n=2,259). Of the *Ae. aegypti* trapped, 10.8% (n=945) was from Busia and 89.2% (n=7,783) from Kilifi with respective mean catches of 13.13 (range: 4-23) and 108.10 (range: 4-483). The other species comprised anophelines (n=1, mean = 0.01, range = 0-1), and other culicines (n=1,345, mean = 9.41, range = 0-102) from both counties.

The overall sex composition of trapped *Ae. aegypti* varied with more females (F) captures than males (M) (F=6,622 and M=2, 1062). The sex distribution by treatment was as follows: CO_2=762

(M=139, F=623), HA=2,278 (M=384, F=1,894), LO = 2,176 (M=788, F=1,388), LOHA=1.236 (M=313, F=923), HALO=1,083 (M=224, F=859) and BG Lure =1,193 (M=258, F=935) (Table 6). Table 7 show the mean catches of *Ae. aegypti* caught per treatment .

Table 6: Total number of mosquitoes captured using odor cues in Busia and Kilifi counties

Treatment	**Busia**				**Kilifi**				**Overall**			
	Ae. aegypti			Others	*Ae. aegypti*			Others	*Ae. aegypti*			Others
	Male	Female	Total		Male	Female	Total		Male	Female	Total	
BG	25	87	112	10	114	536	650	226	139	623	762	338
BG&HA	57	140	197	3	327	1754	2081	204	384	1894	2278	401
BG&LO	87	86	173	5	701	1302	2003	261	788	1388	2176	434
BG&LOHA	56	108	164	4	257	815	1072	213	313	923	1236	377
BG&HALO	41	115	156	2	183	744	927	206	224	859	1083	362
BG&LURE	37	106	143	8	221	829	1050	204	258	935	1193	347
Total	**303**	**642**	**945**	**32**	**1,803**	**5,980**	**7,783**	**1,314**	**2,106**	**6,622**	**8,728**	**2,259**

Others refers to collection of extra mosquitoes not Ae. aegypti (anopheline and culicine species caught in the trap)

Table 7: Mean catches *Aedes aegypti* recorded in the different treatments using odor cues in Busia and Kilifi counties

	BGL			BGHALO			BGLOHA			LO			HA			CO2		
Catches	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female
Mean	99	22	78	90	19	72	103	26	77	181	66	116	190	32	158	64	12	52
Standard Deviation	95	21	76	98	19	82	92	17	78	152	58	99	150	28	129	82	9	77
Standard error	27	6	22	28	6	24	26	5	22	44	17	29	43	8	37	24	3	22

Treatment abbreviations are as indicated in table

For odor cues *Ae. aegypti* catches were significantly higher in Kilifi than Busia (χ^2 1,142=170.63, P<0.0001) and varied by treatments (χ^2 5,137=151.19, P= 0.002) (Appendix 2). An analogous pattern was seen in catches of females [site: χ^2 1, 142=163.99, P<0.0001; treatment: χ ^{2}5, 137=150.64, P=0.02) and males [site: χ^2 1, 142=191.96, P<0.0001; treatment: χ^2 5, 137=145.30, P<0.0001] (Table 2).

Relative to the reference control (CO_2 alone), *Ae. aegypti* catches increased about two-fold in CO_2-traps baited with LO (IRR=2.2, 95%CI (1.42-3.56) or HA (IRR=2.4, 95%CI (1.53-3.83) (Appendix 2), with similar pattern in catches of females and males (Appendix 2). Between treatment effect in *Ae. aegypti* catches was evident between HA and BG-Lure (BGL) (IRR=1.7, 95%CI (1.07-2.65, P=0.024) and between LO and BGL (IRR=1.6, 95%CI (1.00-2.47, P=0.05). About a two-fold significant increase in catches of females was found in HA than BGL (IRR=1.7, 95%CI (1.06-2.79, P=0.028), and about 3-fold higher catches of males in LO than BGL (IRR=2.8, 95%CI (1.72-4.53), P<0.0001). After controlling for study site, we found an overall variation in *Ae. aegypti* catches by sex largely influenced by the effect of HA and LO. A significantly higher proportion of males was captured in traps with LO than HA in Kilifi (χ^2= 200.48, df = 1, P < 0.0001) and in Busia (87/173 vs 57/197, χ^2=16.783, df = 1, P<0.0001). Similar results were observed in both Busia and Kilifi counties, as shown in Appendix 2. Figure 7,8 and 10 illustrates the mean *Ae. aegypti* catches in the using various treatments in Busia and Kilifi counties

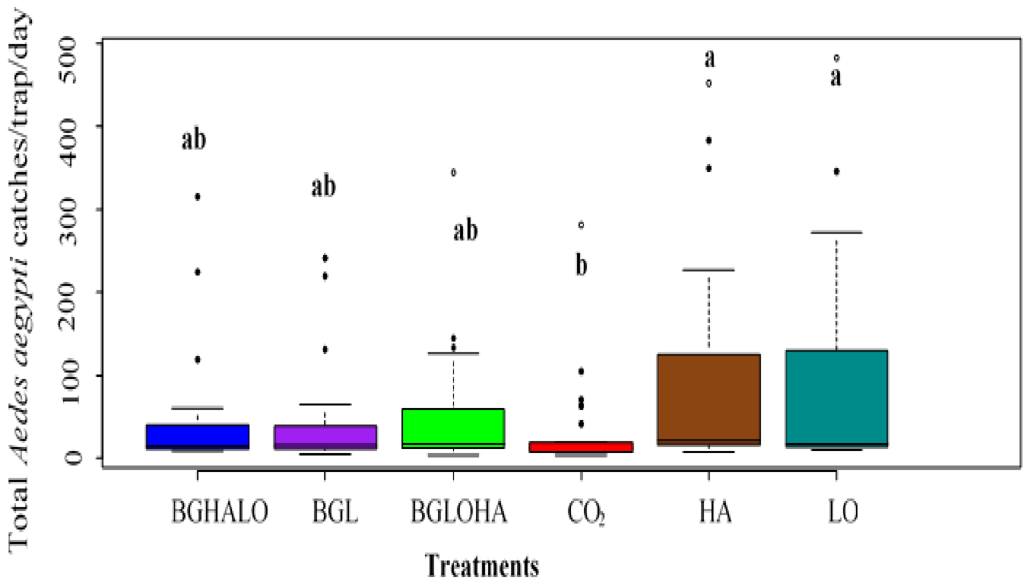

Figure 9: A dot-plot showing total number of *Ae. aegypti* collected using odor cues in Busia and Kilifi counties. The whiskers represent lowest and highest counts recorded. Outliers are represented by dots outside the boundaries. Black line within the box plot represent the median number of catches.

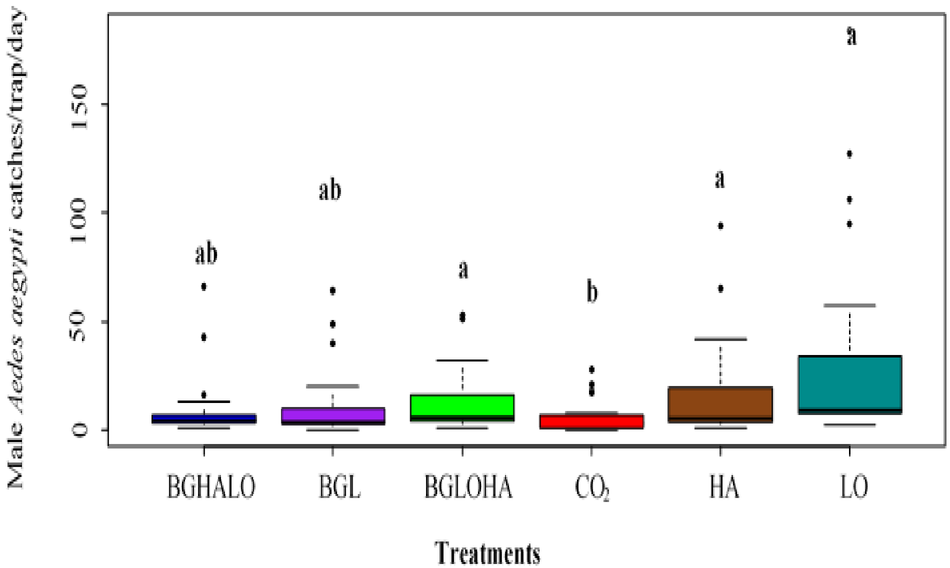

Figure 10: A dot-plot showing male number of *Ae. aegypti* collected using odor cues in Busia and Kilifi counties. The whiskers represent lowest and highest counts recorded. Outliers are represented by dots outside the boundaries. Black line within the box plot represent the median number of catches.

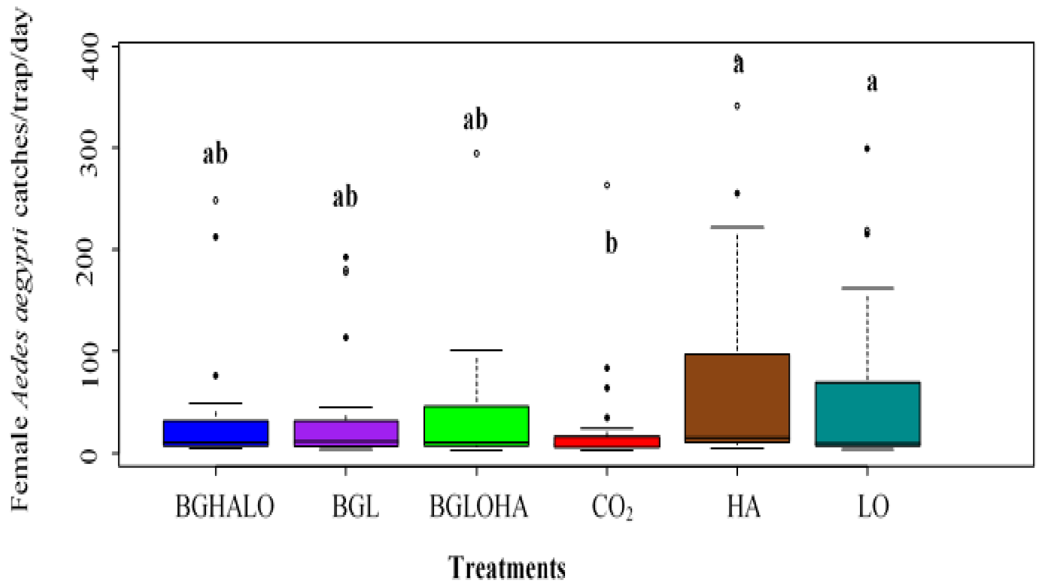

Figure 11: A dot-plot showing female number of *Ae. aegypti* collected using odor cues in Busia and Kilifi counties. The whiskers represent lowest and highest counts recorded. Outliers are represented by dots outside the boundaries. Black line within the box plot represent the median number of catches.

4.3 Results for dose-response in blends of HA and LO

This experiment was only conducted in Kilifi County. Evaluation of *Ae. aegypti* responses to varied doses of HA and LO yielded 3,667 mosquitoes (F=2,344 and M=1,323), with a mean of 25.47 and range of 0-180). The mean *Ae. aegypti* catches observed in each treatment are indicated in Table 8.

Table 8 : Total number and mean catches *Aedes aegypti* collected in odor dose response experiment in Kilifi County.

Dose Intervention		Total *Ae. aegypti* captured			Mean *Ae. aegypti*		
		Male	Female	Total	Male	Female	Total
LH1	LOLHAH	89	162	251	7	14	21
LH2	LOLHAO	61	123	184	5	10	15
LH3	LOLHAL	52	81	133	4	7	11
LH4	LOHHAL	78	115	193	7	10	16
LH5	LOHHAO	100	226	326	8	19	27
LH6	LOHHAH	87	139	226	7	12	19
LH7	LOOHAH	54	106	160	5	9	13
LH8	LOOHAO	82	231	313	7	19	26
LH9	LOOHAL	45	120	165	4	10	14
HA	HAO	233	492	725	19	41	60
LO	LOO	328	377	705	27	31	59
CO_2	CO_2	114	172	286	10	14	24
		1,323	2,344	3,667			

Treatment abbreviations are as indicated in table 3

Only the individual odorants LO or HA used as positive controls in this experiment significantly increased trap catches of *Ae. aegypti* (total and respective sexes) compared to the control (IRR = 2.53, P<0.005, IRR = 2.47, P= 0.006), respectively, for LO and HA) (Appendix 3). These traps baited with HA or LO significantly attracted over two times more *Ae. aegypti* than the control trap in Kilifi County. In contrast, increased catches of *Ae. aegypti* observed in the varied doses of LOHHAO and LOOHAO (IRR=1.14, P=0.692) and (IRR=1.09, P=0.785), respectively, however, the increases were not significant. The other blends, LOHHAH (IRR=0.79, P=0.478), LOHHAL (0.67, P=0.238), LOOHAH (IRR=0.56, P=0.083), LOOHAL (0.58, P=0.100), LOLHAH (IRR=0.88, P=0.694), LOLHAO (IRR=0.64, P=0.186), LOLHAL (IRR=0.47, P=0.023) attracted less number of *Ae. aegypti* compared to the control. Appendix 6 gives the comparison of the effect of varied dose of the treatment blends on total catches of *Ae. aegypti* and by sex, and illustrated in Figure 12. Almost all the blends of LO and HA reduced catches of *Ae. aegypti* relative to the control. In fact, most of the blends significantly decreased *Ae. aegypti* catches 2-4 fold compared LO or HA in the presence of CO_2.

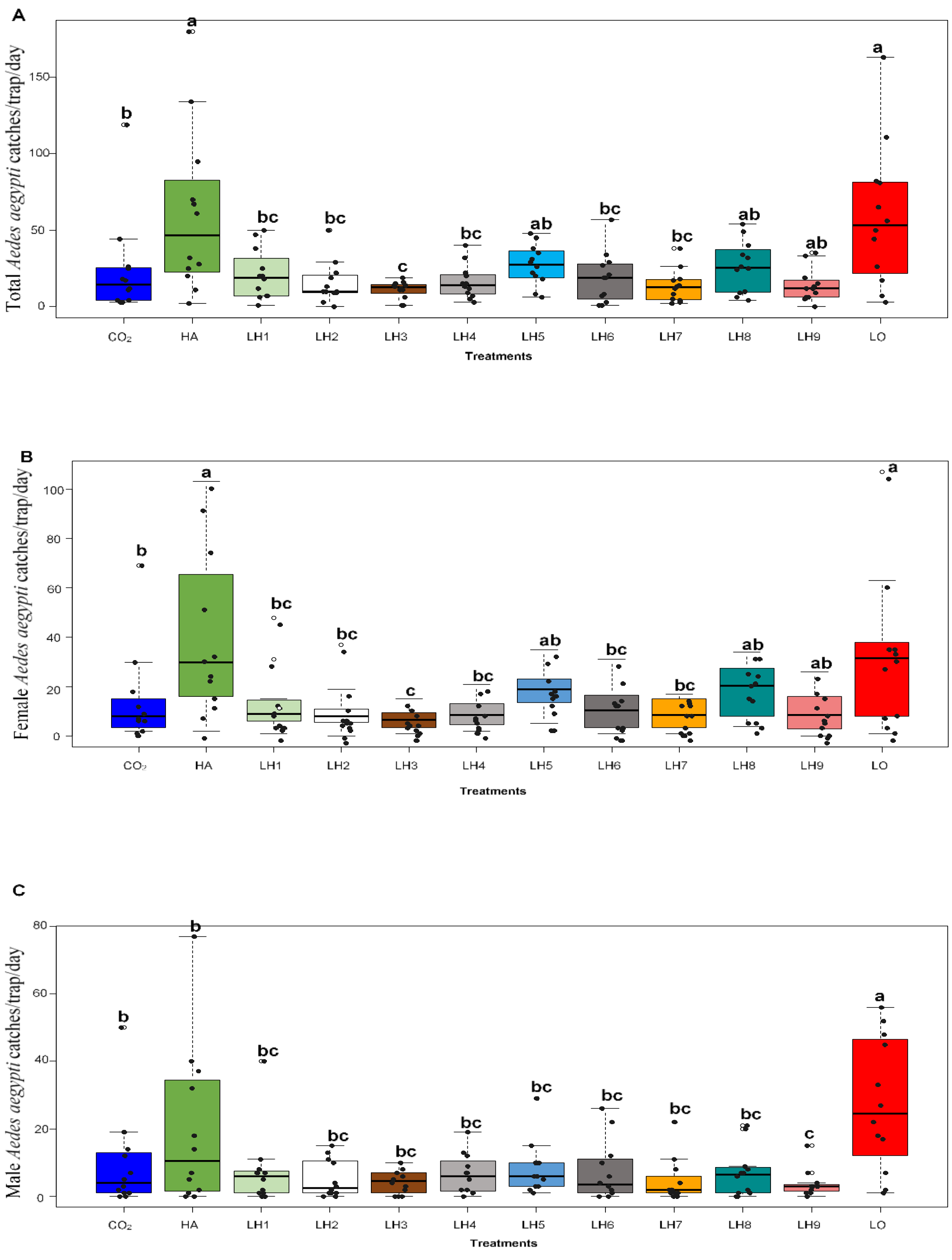

Figure 12 : A dot-plot showing a) total *Ae. aegypti*, b) female *Ae. aegypti*, c) male *Ae. aegypti*. collected using light cues in Busia and Kilifi counties. Treatment abbreviation are as indicated in

Table 3. The whiskers represent lowest and highest counts recorded. Outliers are represented by dots outside the boundaries. Black line within the box plot represent the median number of catches.

CHAPTER 5 : DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

In this study, effectivity of visual and odor cues to attract *Aedes aegypti* were assessed in Busia and Kilifi counties, Kenya.

In both of the visual and odor cues evaluation, the results generally showed that additional *Ae. aegypti* were trapped in Kilifi than Busia. The disparity in richness of *Ae. aegypti* in these two counties may have been influenced both by abiotic and biotic factors, known to impact its ecology, of these factors, Dada *et al.*, 2018 noted that, rainfall and humidity are key fundamental environmental variables that affect the spatial abundance of mosquitoes.

Availability of water is key to mosquitoe regeneration for the adult females to lay eggs and subsequent larval development. *Aedes* eggs are recognized to be laid in both artificial and natural surfaces (Medronho *et al*., 2009, Schaeffer and Touzeau, 2008). In light of this, the dominant rural nature of Busia County more so Obekai and Kamosing where the experiments were conducted provided only natural habitat for the species to breed and propagate. This is in contrast to urban Kilifi County sites; KEMRI and public of works yards where mosquitoes reproduced in natural as well as other man made artificial sites such as; abandoned old tires, uncovered water drainage system, dump site, enabling additional breeding areas (Agha *et al*., 2017) The increased number of breeding sites in Kilifi, may have resulted to the observed higher abundance of adult *Ae. aegypti* in Kilifi compared to Busia. In fact, the rapid expansion of this mosquitoe to newer territories has been attributed to it successful utilization of artificial habitats such as containers for breeding (Owino, 2018, Huber *et al*., 2004).

The annual precipitation in the two counties also vary. Busia receives approximately 1500 mm of rainfall per year compared to only about 950mm in Kilifi. (Owino *et al*., 2015). Rainfall and water quantity play critical role in abundance of mosquitoes. While adequate rainfall or water is required in both natural and artificial habitats for larval and pupal development, it has been observed that standing water is preferred to complete growth cycle (Barrera and Clark, 2006). Excess of this, may lead to washing away of the immature stages, more so eggs (Barrera and Clark, 2006). Occurrence of this may result to diminished number of adults that emerge from aquatic dependent stages. This phenomenon of aquatic stages being destabilized from breeding sites is likely to occur in Busia where high amount of rain is received than Kilifi.

Secondly, it has been observed that despite of complex interactions for the available resource and sibling competition, the growth of the larval instars is heavily dependent on temperature (Couret and Benedict, 2014). Low temperatures (around 15°C) inhibit *Ae. aegypti* larvae development and the larvae may remain in a particular instar for several months in the presence of sufficient water supply (Bar and Andrew, 2013a, Brady *et al*., 2013). Busia temperature varies between 14^0C-30^0C while in Kilifi, the temperature range is between 21^0C-32^0C. The conducive temperature in Kilifi therefore may have resulted in faster development of immature stages to adult observed than in Busia.

Unlike surveillance of nocturnal biting mosquitoes, light traps are not typically well represented in surveillance efforts towards day biting mosquitoes such as *Ae. aegypti*. As a result of increased public health importance of day biting mosquitoes, development of effective traps targeting adult stage of diurnal mosquitoe species is on the rise. Currently, BG sentinel trap serves as the gold standard surveillance tool for adult *Ae. aegypti*. The trap is designed in such a way that it utilizes both chemical and visual cues as attractant to the target mosquitoe species. Exploitation of visual and chemical cues has enhanced its collection of not only *Ae. aegypti* but also other diurnal mosquitoe species (Balestrino *et al*.,2016).

In the first experiment, effectiveness of two visual cues UV and CDC incandescent previously found to be attractive to *Ae. aegypti*, was evaluated. Individual UV and CDC light traps and the same lights cues supplemented on BG trap was evaluated. Individual light traps (UV and CDC incandescent) caught significantly less *Ae. aegypti* compared to the reference BGS trap. The results are in conformity with other studies that compared effectiveness of BGS to other commonly used surveillance traps for the day biting mosquitoes. For instance, field trials conducted by Horward *et al.,* 2015 using four different traps showed that, *Ae. aegypti* preferred BGS2>BGM>Zumba>CDC. Similar order of observation in which light trap tested yielded low number of diurnal mosquitoe species; BGS collected more *Ae. aegypti* than CDC was noted by Li *et al.*, 2016.

Low amount of *Ae. aegypti* caught with individual light traps reflects variation in the tested trap design, compared to BGS trap. Whereas, photo taxis is the main attraction in light traps, visual cue are used in BGS. Additionally, the operational mechanism of BGS mimics animal host conditions contrary to the light traps used. The simulated host environment in BGS therefore could be attributed to the higher catches. Overall, the low quantities of *Ae. aegypti* collected with individual light traps suggest that may not be a suitable surveillance tool for *Ae. aegypti.*

Contrary to low numbers captured with individual light traps, when these lights, UV or CDC incandescent were supplemented on BGS trap, higher numbers of *Ae aegypti* were realized. However, the increased catches were not significant in relation to the control BGS trap. Lack of significance could have been attributed to short trapping period, the outcome remains unknown if the trapping period could have been conducted over longer period. Interestingly, the field results from individual light traps, showed a reversed pattern in entire amount of *Ae. aegypti* caught in Busia and Kilifi counties. Even though this trend was similarly observed in previous field trials

(Owino, *thesis*), the same pattern was not replicated when the light platforms were supplemented on BGS. This pattern observed on light traps suggest a possible difference in populations in the counties.

The results show that combining LO and HA reduces trap catches of *Ae. aegypti* and even in both sexes. This implies that when combined each compound masks the attractive effect of the other. Previously, such interactive effect has been reported in the malaria vectors for blends of compounds albeit in a dose-dependent manner (Jacob *et al*., 2018). Here, irrespective of the doses tested, there was overall decreased *Ae. aegypti* catches in blends of HA and LO compared to the individual compounds in the presence of CO_2. Dispenser types can affect effectiveness of vector catches to semiochemicals (Torr *et al*., 1997, Mweresa *et al*., 2014), thus, further research employing different dispensers are needed to confirm our findings. Nonetheless, the results underscore the importance of a detailed knowledge of interactive effect of odorants for improved lure formulation. On the other hand, knowledge of compounds with masking effects (otherwise known as attractant-inhibitors (Bernier *et al*., 2006), can be exploited in disease control. For example, to mask attractiveness of humans, thereby limit mosquitoe bites and hence, pathogen transmission risk. For instance, ethyl pyruvate was found as an antagonist that masks attraction of *Ae. aegypti* to CO_2 and human skin odor (Tauxe *et al*., 2013).

Based on the trap catches, LO used as one of the positive controls lured both male and female *Ae. aegypti*. Generally, adult male mosquitoes depend exclusively on plants for exogenous sources of energy, whereas females derive energy both from vertebrate blood and plant sugars (Foster, 1995, Nyasembe and Torto, 2014). *Ae. aegypti* females have recently been found to feed on plant tissue in nature (Nyasembe, 2018). Thus, it is not unexpected that both sexes were attracted to LO which serves as a cue for sugar sources as previously reported (Nyasembe *et al*., 2015). More intriguing was the finding that males were generally more attracted to traps with host odors, especially to the human-derived odorant HA another positive control in our study. The reasons for this occurrence however, is unclear although it could be related to their bio ecology. Male *Ae. aegypti* commonly seek females for mating around a human host (Hartberg, 1971), a behavior which possibly involves olfactory cues (Grant, 1969). Perhaps males may detect and respond to human host-derived cues to locate and mate with females and this could explain their response to the host odors. Thus, studies that constitute evaluating male responses to host odors (plant and animal sources) may be explored in the development of improved lures that that can maximize their catches in traps. This could be rewarding for the sterile insect technique where improved monitoring of male populations is crucial to assess success. Notably, trap catches with HA were higher for females than males. This could be attributed to HA signifying host attraction for a blood meal that only females indulge in.

Odor-dispensing is very important in improving odor-baited trapping systems (Torr *et al*., 1997, Mweresa *et al*., 2014).Comparable *Ae. aegypti* catches were found when the blends of HA and LO were dispensed either separately or as a mixture (Appendix 2 and 3). Possibly both dispending modules did not affect the adsorption and slow-release of the odorant compounds. Further research is however, required to measure the release rates of each component in the different blends to confirm this suggestion.

The decreased *Ae. aegypti* catches as a result of combining LO and HA was very striking. Whether the observed decrease is associated with the mosquitoe’s peripheral coding of these odorants needs further study. Olfactory receptors (ORs) are involved in reception of odorants and mediate decoding of specific behavioral responses in insects such as mosquitoes for instance how they find food be it blood or sugars (Ray, 2015, Bohbot and Pitts, 2015). The overall few ORs repertoires in most insects has led to the hypothesis that a majority of ORs are broadly tuned to a range of odorants (Bohbot *et al*., 2007). In the *Drosophila* fruit fly, peripheral coding of odorants for odors give the impression that they are to a large extend functionally conserved (Linz *et al*., 2013,Goldman-Huertas *et al*., 2015). Narrow range tuning has been suggested for chemical cues involved in food selection, mate selection and reproduction, and (Bohbot and Pitts, 2015). A possible explanation for the reduced catches to the blends include peripheral coding of both odorants by functionally conserved receptors to facilitate broad detectability of plants or animal sources. It is worth noting however, that ORs differ in their specificity, and can be from narrowly to very broadly tuned (Bohbot and Pitts, 2015). Future research should focus on the molecular mechanisms underlying how HA and LO attracts *Ae. aegypti* and to identify which receptors determine behavior toward them.

Previously, studies have documented that mosquitoe response to odorants is affected by the feeding status. For instance, *Ae. aegypti*, expresses avoid seeking for host following a blood meal while waiting for oviposition (Klowden and Lea, 1979, Davis, 1984). Similar behavior has been noted in the malaria vector *An. gambiae*, showing reduced responses to hexanoic acid, after blood feeding (Takken *et al*., 2001). In this study, the feeding status of the mosquitoes caught in the different treatments was not ascertained, hence could not establish whether the mosquitoes caught in each odor-baited trap reflects their resource need. Wild mosquitoes caught in traps generally comprise of a varied population of unfed, pre-gravid and gravid cohorts (Gillies, 1954,Lyimo and Takken, 1993). Nonetheless, it may be helpful in future studies to correlate trap catches and physiological status by examining the proportion of blood fed, gravid or half-gravid, plant-fed in the different trap treatments.

5.2 Conclusion

In general, higher catches of *Ae. aegypti* were observed in Kilifi than Busia County for both visual and odor cues. On specific treatment, the data show that BG traps baited with UV or incandescent light comparably increased catches of *Ae. aegypti* 40-57% in Kilifi although not significantly different from the control BG trap. Only UV light increased captures of *Ae. aegypti* by 24% in Busia although not significantly different from BG trap control trap or when baited with incandescent light. On the other hand, blends of the individually attractive odorants HA and LO, regardless of the dose tested reduced trap catches of *Ae. aegypti* in the presence of CO_2, for example both odorants are competitive attractants. The compounds signify different resource needs to the mosquitoe LO for sugar and HA for blood, and could be peripherally encoded by functionally conserved receptors to facilitate broad detectability of plants or animal sources.

5.3 Recommendation

Even though there was no significant trap catches yielded from light cues from the control, longer evaluation period is recommended to assess seasonal trends. On the other hand, additional studies should be conducted to investigate different dispensers on the trapping efficiency of these odorants. Also, the molecular mechanisms underlying how HA and LO odorants are detected should be pursued to identify which receptors determine behavior toward these odorants.

Appendix 1: Statistical output of visual treatments on adult *Ae. aegypti* abundance

Factor		Total catches/captures		Female		Male	
		IRR(95%CI)	P-value	IRR(95%CI)	P-value	IRR(95%CI)	P-value
Site (1,985)	Kilifi (1,569)	2.98 (2.15-4.12)	<0.0001	3.1 (2.17-4.35)	<0.001	3.43 (2.38-4.95)	<0.001
	Busia (416)	1(reference)					
Treatment (n=10)	BG + CDC (715)	1.30 (0.81-2.05)ab	0.27	1.39 (0.86-2.27)ab	0.18	1.08 (0.68-1.72)ab	0.75
	BG+ UV (681)	1.33(0.84-2.10)ab	0.22	1.42 (0.87-2.31)ab	0.16	1.11 (0.70-1.78)ab	0.65
	UV (38)	0.09 (0.05-0.17)c	<0.0001	0.09 (0.05-0.17)c	<0.0001	0.08 (0.03-0.16)c	<0.001
	CDC (52)	0.10(0.6-0.18)c	<0.0001	0.12(0.07-0.22)c	<0.0001	0.07 (0.03-0.15)c	<0.001
	CO_2 (499)	1(reference)b		1b		1b	

Number of replicate trials: number of replicates (n=10) All traps were baited with CO_2; CO_2 only traps served as control. Treatment rows followed be different letters indicate significant difference between the treatments at α=0.05. Blend abbreviations are defined in table 1.

Appendix 2: Comparison of the effect of odor treatments on adult mosquitoe abundance

		Total catches/captures		Female		Male	
Factor		IRR(95%CI)	P-value	IRR(95%CI)	P-value	IRR(95%CI)	P-value
Site (8728)	Kilifi (7783)	7.7(5.89-10.01)	<0.0001	8.7(6.58-11.62)	<0.0001	5.5(4.12-7.35)	<0.0001
	Busia (945)	1(reference)		1(reference)		1(reference)	
Treatment	BGHALO (1,083)	1.4(0.89-2.24)ab	0.14	1.36(0.83-2.23)ab	0.22	1.6(0.97-2.75)ab	0.06
	BGL (1,193)	1.4(0.90-2.28)b	0.12	1.37(0.84-2.25)ab	0.21	1.7(1.03-2.92)b	0.04
	BGLOHA (1,236)	1.6(0.98-2.47)ab	0.06	1.37(0.84-2.25)ab	0.21	2.3(1.36-3.80)ab	0.002
	HA (2,278)	2.4(1.53-3.83)a	0.0001	2.4(1.44-3.84)a	0.001	2.6(1.57-4.35)ab	0.0002
	LO (2,176)	2.2(1.42-3.56)a	0.0005	1.6(1.0-2.68)a	0.048	4.9(2.94-8.02)a	<0.0001
	CO_2 (762)	1(reference) b		1b		1b	

Number of replicate trials: number of replicates (n=12) All traps were baited with CO_2; CO_2 only traps served as control. Treatment rows followed be different letters indicate significant difference between the treatments at α=0.05. Blend abbreviations are defined in table 2.

Appendix 3: Comparison of the effects of treatment blends of HA and LO on adult *Ae. aegypti* abundance in Kilifi County

	Ae. aegypti		Female *Ae. aegypti*		Male *Ae. aegypti*	
Treatment (n)	IRR (95%CI)	P-value	IRR (95%CI)	P-value	IRR (95%CI)	P-value
HA (725)	2.5 (1.33-4.84)a	0.005	2.9 (1.52-5.38)a	0.001	2.0 (0.84-4.99)b	0.11
LH1 (251)	0.9 (0.46-1.69)bc	0.69	0.9 (0.49-1.79)bc	0.85	0.8 (0.31-1.93)bc	0.59
LH2 (184)	0.6 (0.33-1.24bc)	0.19	0.7 (0.37-1.37)bc	0.31	0.5 (0.21-1.34)bc	0.18
LH3 (133)	0.6 (0.24-0.90)c	0.02	0.5 (0.24-0.91)c	0.02	0.5 (0.18-1.15)bc	0.09
LH4 (193)	0.7 (0.35-1.30)bc	0.24	0.7 (0.35-1.28)bc	0.22	0.7 (0.28-1.70)bc	0.41
LH5 (326)	1.1 (0.59-2.19)ab	0.69	1.3 (0.69-2.49)ab	0.4	0.9 (0.35-2.17)bc	0.77
LH6 (226)	0.8 (0.41-1.52)bc	0.48	0.9 (0.42-1.54)bc	0.52	0.8 (0.31-1.89)bc	0.55
LH7 (160)	0.6 (0.29-1.08)bc	0.08	0.6 (0.32-1.18)bc	0.14	0.5 (0.19-1.19)bc	0.11
LH8 (313)	1.1 (0.57-2.10)ab	0.78	1.3 (0.71-2.54)ab	0.36	0.7 (0.29-1.79)bc	0.47
LH9 (165)	0.6 (0.30-1.12)ab	0.1	0.7 (0.36-1.34)ab	0.27	0.4 (0.16-0.99)c	0.047
LO (705)	2.5 (1.29-4.71)a	0.006	2.2 (1.16-4.13)a	0.01	2.9 (1.18-7.01)a	0.02
CO_2 (286)	1 (reference)b		1 b		1 b	

Number of replicate trials: number of replicates (n=12) All traps were baited with CO_2; CO_2 only traps served as control. Treatment rows followed be different letters indicate significant difference between the treatments at α=0.05. Blend abbreviations are defined in table 3.

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