EVALUATION OF 2-BUTANONE AS A SUBSTITUTE FOR CARBON DIOXIDE IN MALARIA MOSQUITO ATTRACTANTS

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DECLARATION

I, Monicah Mirai Mburu, declare that this is my original work and it has not been presented for a degree in any other university.

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DEDICATION

To the family of Mr. and Mrs. Gabriel Mburu as well as Miss Dorothy Wambui.

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ABSTRACT

Odor-baited traps (OBTs) are increasingly being considered for use in sampling, surveillance and control of malaria mosquitoes. Most odor baits contain carbon dioxide, which apparently enhances trap catches given its role as a mosquito activator. Unfortunately, organic CO_2, which is the most locally amenable source of the gas, must be replaced after each experimental night. This precludes the application of carbon dioxide-based odor baits for prolonged area-wide use. This study explored the possibility of replacing organically-produced CO_2 with 2-butanone in odor blends targeting malaria mosquitoes. Experiments were conducted under field and semi-field conditions in western Kenya. A fresh preparation of organic carbon dioxide was made on each experimental night. The ketone 2-butanone was impregnated on nylon strips and used repeatedly for entire durations of the experiments. In preliminary investigations it was observed that the numbers of laboratory mosquitoes attracted to a reference blend (MB5) were significantly more than those attracted to MB5 without its CO_2 component ($P < 0.001$), CO_2 only ($P < 0.001$) or an unbaited trap ($P < 0.001$). Whereas the unbaited trap caught significantly fewer mosquitoes than all the baited traps ($P < 0.001$), the trap containing CO_2 only caught more *Anopheles gambiae* sensu stricto (hereafter referred to as *An. gambiae*) mosquitoes than the one containing MB5 minus CO_2 ($P < 0.001$). In all cases the reference blend attracted a significantly higher number of mosquitoes than its variants containing the different dilutions of 2-butanone used to replace CO_2 ($P = 0.001$). The highest catches were associated with the 99.5% and 1.0% concentrations of 2-butanone. The reference blend formed the best barrier for reducing house entry of *An. gambiae*. Although more wild female *An. gambiae* sensu lato, *An. funestus* and *Culex* spp. were attracted to a variant of MB5 containing pure 2-butanone, the catches did not differ significantly from those due to the intact reference blend ($P = 0.450$, $P = 0.090$, $P = 0.075$, respectively). When compared to existing sampling methods, female *An. gambiae* s.l. mosquitoes were highly attracted to a CDC light trap than to a human subject ($P = 0. 001$), MB5 ($P = 0.001$), or MB5 with its CO_2 component replaced with pure 2-butanone ($P = 0.001$). This study, which demonstrates that 2-butanone can serve as a good replacement for CO_2 in synthetic mosquito attractants, further underscores the possibility of using OBTs for monitoring and surveillance of malaria and other mosquito vectors.

ACRONYMS

CDC	Centre for Disease Control and Prevention
CO_2	Carbon dioxide
EIR	Entomological Inoculation Rate
icipe	International Centre of Insect Physiology and Ecology
IRS	Indoor Residual Spraying
ITNs	Insecticide treated nets
IVM	Integrated Vector Management
LLINs	Long lasting insecticidal nets
MM-X	Mosquito magnetic-X trap
OBTs	Odor baited traps

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Malaria is a life threatening disease (WHO, 2012). In sub-Saharan Africa the disease is mainly transmitted by female mosquitoes in the *Anopheles gambiae* and *Anopheles funestus* complexes (Gilles and Coetzee, 1987). Malaria vectors require a blood meal in order to develop their eggs (Clements, 1999). These mosquitoes are guided to their blood meal hosts primarily by odors released from the skin and/or breath of the hosts (Lehane, 1991; Takken, 1991, Takken and Knols, 1999; Smallegange and Takken, 2010). Carbon dioxide is a major and important component of human odor (Gillies, 1980). The gas is reported to activate mosquitoes by eliciting take-off and sustaining them in flight (Gillies, 1980; Dekker *et al*., 2001), guiding them towards blood meal hosts (Takken and Knols, 1999) and enhancing entry into odor baited traps (OBTs) (Costantini *et al*., 1996). It is not surprising therefore that carbon dioxide is used as a key ingredient in synthetic attractants targeting malaria mosquitoes.

Unlike other chemical constituents which can be used over long periods of time without replacement (Mukabana *et al.,* 2012a), the carbon dioxide component of mosquito attractant blends needs to be replaced daily. This makes the use of carbon dioxide to be logistically demanding in terms of cost of ingredients used, time and labour. This undermines the potential application of synthetic mosquito attractants for surveillance, monitoring and control of vectors under field conditions.

Through electrophysiological studies 2-butanone (C_4H_8O) was identified as a dose-dependent activator of the cleavage product A (cpA) neuron of *An. gambiae, Aedes aegypti*, and *Culex quinquefasciatus* (Turner *et al*., 2011), so simulating the activity of carbon dioxide. It has also been found that a combination of butanone with carbon dioxide as baits, may reduce or increase the attraction of mosquitoes to traps (Kline *et al*., 1990., Kline and Mann 1998). Since both electrophysiological and behavioral studies can provide valuable information on a chemical signal that the malaria vector can perceive as well as the optimal concentration at which the compound is effective (Davis and Bowen, 1994), the potential of using 2-butanone as a replacement of carbon dioxide in mosquito attractants was evaluated in this study.

1.2 LITERATURE REVIEW

Host-seeking mosquitoes are mainly guided by chemical cues released by their blood meal hosts (Sutcliffe 1987, Takken 1991, Takken and Knols 1999). Some of these cues which are released from the human skin have already been identified for the malaria transmitting mosquito *Anopheles gambiae* and include ammonia, lactic acid and carboxylic acids (Knols *et al*., 1997, Okumu *et al*. 2010a). A synthetic blend comprising of lactic acid, ammonia, tetradecanoic acid, 3-methyl-1-butanol and carbon dioxide produced from molasses have served as baits in malaria mosquito traps (Okumu *et al*., 2010a, Mukabana *et al*., 2012b).

1.2.1 Transmission of malaria

The dominant vector species of human malaria in Africa are *Anopheles gambiae* and *Anopheles funestus* (Gilles and Coetzee, 1987). From literature, *Anopheles arabiensis* is also a malaria vector (White, 1974). These dominant vector species are responsible for transmitting the malaria parasite *Plasmodium*. The five *Plasmodium* species that are known to cause malaria of humans include *Plasmodium vivax, P. ovale, P. malariae, P. falciparum* and *P. knowlesi*. The *Plasmodium* parasite requires two hosts namely a vertebrate host (human being) and an insect vector (female *Anopheles* mosquito) to complete its life cycle.

Various factors such us feeding habits, rainfall patterns, ecological location of a region can enhance malaria transmission. *Anopheles gambiae* and *Anopheles funestus* have unique characteristics in that they feed and rest indoors and have preference for feeding on humans.

These characteristics portray a strong mosquito-host interaction that can enhance malaria transmission (Beier, 1996; Takken and Knols, 1999; Hay *et al*., 2010). Given that swampy areas as well as human activities such as irrigation schemes are potential larval breeding sites for *Anopheles* mosquitoes (Fillinger *et al*., 2004), malaria transmission can be intense due to the abundance of the vectors. *Anopheles arabiensis* and *An. gambiae* are known to thrive in sunlit water bodies (Holstein, 1954; Gilles and DeMeillon, 1968). Studies have also revealed that the structure of a house can affect mosquito entry and therefore to eliminate indoor malaria transmission, house designs should be improved (Lwetoijera *et al*., 2013). Other important malaria vectors such as *An. pharoensis* have been identified as the main malaria vector in certain part of West Africa (Carrara *et al*., 1990). *Anopheles Merus* has also been documented to play a role in malaria transmission (Cuamba and Mendis, 2009).

There are mosquito species that do not transmit malaria but are known to cause diseases of medical importance. Most of these female species are nuisance biters and are known to harbor arboviruses. Examples include *Culex pipiens* which has been found to harbor West Nile Virus (Al- Ali *et al*., 2008), *Culex quinquefasciatus* was documented to be highly competent for West Nile Virus transmission (Jansen *et al*.,2008), *Aedes albopictus* as well as *Aedes japonicus* are known to transmit West Nile virus (Turell *et al*.,2001), *Aedes aegypti* has also been found susceptible to Chikungunya virus (Reiskind *et al*., 2008) as well as a vector for transmitting yellow and dengue fever viruses (WHO 2009).

1.2.2 Life cycle of malaria parasites

Female *Anopheles* mosquitoes undergo host seeking behavior to acquire a blood meal, which they use to mature their eggs (Clements, 1999). This host seeking behavior triggers the vector to transmit the malaria parasite in form of sporozoites via a bite on the human skin. The parasite invades hepatocyctes (Shortt and Garnham 1948, Shortt *et al*., 1948, Shortt *et al*., 1949, Garnham *et al*., 1954) and undergoes asexual reproduction to form a progeny though for *P. vivax* and *P. ovale* asexual reproduction does not occur as the parasite undergoes a dormant phase (Krotoski *et al*., 1982) referred as hyponozoite. Upon reactivation of a hyponozoite a relapse can occur.

Upon the rupture of hepatocytes, merozoites are released. The merozoites exit the hepatocytes and invade the erythrocytes. Within the erythrocytes, merozoites begin to enlarge as uninucleate forms (referred as trophozoites) whose nuclei divide asexually to produce a schizont. Schizonts divide and produce mono-nucleated merozoites. Some merozoites in the erythrocytes differentiate into gametocytes. Upon rupture of erythrocytes, toxins are released resulting in a cycle of fever and chills in the human host. Gametogenesis is induced when gametocytes from an infected individual are ingested by a mosquito. Sporogony occurs in the mosquito where there is fusion of male and female gametes to form a zygote within the lumen of the mosquito's gut. The zygote then develops into an ookinete (MacCallum, 1897). Ookinetes transverse the mosquito's midgut epithelium and after reaching the space between epithelial cells and basal lamina, they develop into an oocyst which undergoes sporogony to form sporozoites. Sporozoites migrate through the haemocoel and invade salivary glands of the mosquito ready for re-invasion.

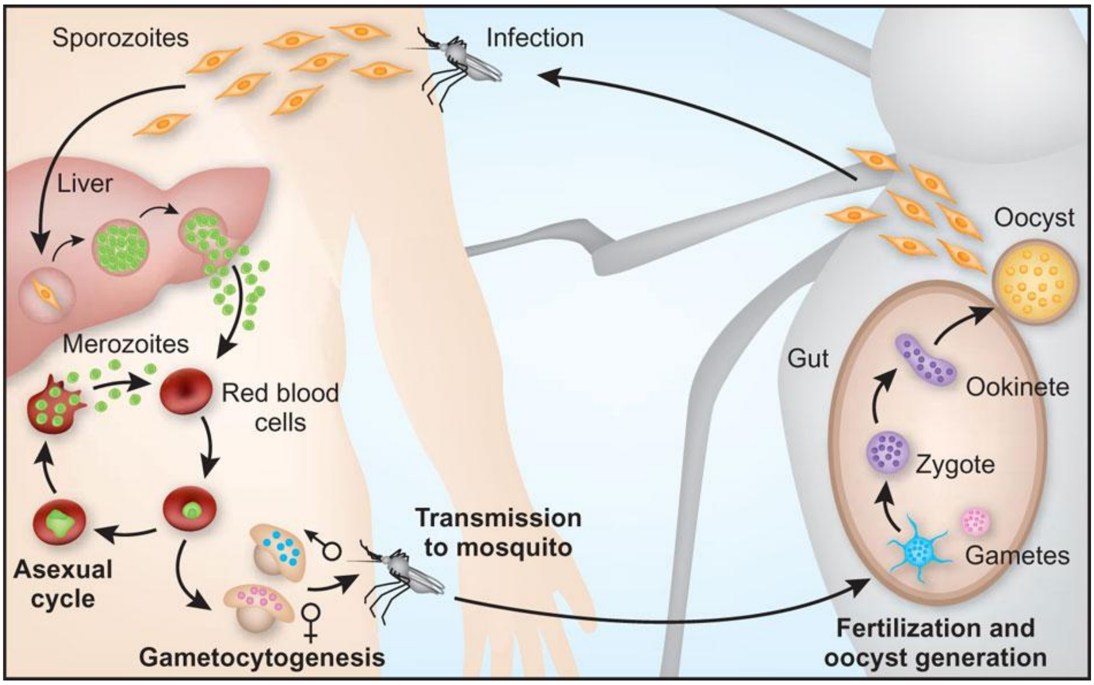

Figure 1: A generalized malaria parasite life cycle (Source: Pasvol, 2010.)

1.2.3 Mosquito life history behaviors

For mosquito survival and species maintenance, various behavior sequences are essential. These include the reproductive and foraging behaviors. Reproductive behavior includes oviposition and mating behavior while foraging behavior includes feeding and host seeking behavior.

1.2.3.1 Reproductive behavior

For mosquito reproductive success, oviposition and mating are of great significance. In the life history of all mosquito species, oviposition is vital and can be described as location and selection by mosquitoes of a suitable site to lay eggs. This involves visual, olfactory and tactile responses. The sensory chemical signals are usually detected by sensory receptors on the mosquito antennae (Davis and Bowen, 1994). The oviposition pattern may be related to the location and availability of suitable larval habitats (Leunita *et al*., 2004) for example for *An. gambiae*, its categorical in its oviposition (Muirhead, 1945) and its preferred larval habitats are fresh water pools that are devoid of vegetation but that are sunlit. Abiotic factors such as temperature, wind speed, relative humidity and rainfall (Michael and John, 1989) as well as blood feeding and distance from daytime resting places (McCrae, 1983) can influence oviposition patterns.

Although mating does not play a role in mosquito egg development and maturation, it has been shown that eggs can only be deposited when insemination has occurred (Clements, 1999). Swarming behavior is a mechanism of mate recognition (Manoukis *et al*., 2009) where males locate females by acoustic signals. *Anopheles gambiae* in many locations form daily mating swarms that last for about 30 minutes at dusk (Marchand, 1984, Charlwood *et al*., 2002a). Male mosquitoes can detect female flight tones as male auditory organ has been thought to act as an acoustic filter for female flight tones hence males can detect the flight of other males and hence differentiate between male and female mosquitoes (Gibson and Russell, 2006). Mosquito mating success can be determined by age and feeding behavior of males. If female mosquitoes mate with two day old males, the females are more likely to oviposit than when they mate with older males (Chambers and Klowden, 2001). Apparently size of male mosquitoes does not influence mating (Charlwood *et al.,* 2002b).

1.2.3.2 Foraging behavior

Sugar feeding is essential in the life history of mosquitoes. Various studies have shown that both male and female mosquitoes of all ages usually ingest sugar as floral and extra floral nectar (Foster, 1995). Thus sugar provides energy reserves for mosquitoes. Though female mosquitoes require a blood meal for the development of their eggs, they also require sugar to survive, fly and enhance reproduction. Female and male *Anopheles gambiae* mosquitoes have been shown to survive longer when provided with the vegetative parts of cassava (*Manihot esculenta*) rather than being fed on water or sucrose alone (Gary and Foster, 2004).

Male mosquitoes also require sugar to enhance mating success as a limited number of sugar deprived males can survive long enough to inseminate females (Gary *et al*., 2009)

Various studies have shown that host finding behavior is usually driven by olfactory cues given off by individual hosts (Lehane 1991; Smallegange and Takken 2010). These cues are complex olfactory signals from human skin and breath. An example is carbon dioxide emitted by human beings and is known to excite the chemo-receptors of mosquitoes. In a study where mosquito host seeking behavior was classified as plume finding and plume tracking, it was deduced that during plume finding (flight in search of odor plume), mosquitoes may choose to fly upwind, downwind or cross wind in search of carbon dioxide emitted by human beings. During plume tracking (flight within odor plume), mosquitoes encounter an odor plume such as carbon dioxide and use the odor plume and wind to guide their flight to locate the host (Cummins *et al*., 2012). Therefore carbon dioxide is an important plume that activates and helps maintain plume tracking (Gilles 1980, Bowen 1991, Gibson and Torr 1999). This can be related to the mosquito bites in that when human beings are in clusters and more tightly associated on smaller patches, odor plume is narrower and biting rate per host is decreased (Cummins *et al*., 2012). This can also be compared to reduction of groups at risk of malaria resulting from urbanization (Hay *et al*., 2005) as mosquitoes only require a fixed amount of blood and hence they will not attack additional individuals once they are engorged. Another study suggested that under windy conditions within the odor plume mosquitoes travel upwind to locate the source (Dekker *et al*., 2001).

1.2.4 Mosquito sampling

Human landing catches (HLC) for sampling malaria vectors remains the most direct method of monitoring human biting mosquito populations (WHO, 1975). Unfortunately this method subjects participants to great risks of acquiring malaria and some studies have documented that human subjects vary in their attractiveness to malaria mosquitoes (Lindsay *et al*., 1993, Knols *et al.,* 1995) and this variance is caused by host specific cues (Mukabana *et al*., 2002). Therefore, HLC may not serve as an effective method for sampling the malaria vectors as some human subjects may attract high number of malaria mosquitoes than others. Studies have also revealed that HLC show less productivity in collecting higher densities of mosquitoes than odor baited traps (Kweka and Mahande, 2009). Other sampling tools which have been documented include a resting box with a net for sampling malaria mosquitoes (Harbison *et al.,* 2006) that exhibit endophily, clay pots (Odiere *et al*., 2007) for sampling mosquitoes that exhibit exophagy, Centre for Disease Control and Prevention miniature light traps and Mosquito Magnetic -X traps. Sampling tools such as CDC light traps and Ifakara Tent Trap model C have been shown to capture more malaria mosquitoes like *An. funestus* (Sikaala *et al*., 2013).

Since haematophagus malaria vectors identify humans from more than 30m away by detecting and tracking the chemical cues that humans emit (Gilles and Wilkes 1972, Takken and Knols, 1999), traps such as MM-X traps can be impregnated with blends constituting of various compounds which serve as odor attractants. These compounds simulate human odors and their combination affects the host seeking behavior for example of the female malaria mosquito *An. gambiae* s.s. which has the preference for feeding on humans (Takken and Knols 1999). Mosquito Magnetic - X traps enhanced with odor baits have been used to sample malaria vectors (Njiru *et al.,* 2006). A synthetic blend which comprises of ammonia, lactic acid, tetradecanoic acid, 3-methyl-1-butanol and carbon dioxide (from yeast and molasses fermentation) have served as baits in MM-X traps for malaria mosquitoes (Okumu *et al*., 2010a; Mukabana *et al*., 2012b). The great challenge facing the carbon dioxide source is that the molasses preparation is on a daily basis thus the need to come up with a method which can be relied upon for longer periods of time. The attractants exclusive of carbon dioxide are usually delivered via nylon strips (Okumu *et al.,* 2010b). The baited traps under field conditions can be hung outside on eaves of houses next to the bedroom as eaves are the major route through which anopheline mosquitoes such as *An. gambiae* enter houses (Njie *et al*., 2009). Also for malaria mosquitoes such as *An. gambiae*, the key olfactory cues of human beings are derived from the foot region (De Jong and Knols, 1995) and it has been shown that cues are moderately produced by microbial flora present in or on the feet (Verhulst *et al.*, 2010). Therefore, the use of synthetic attractants as baits for malaria transmitting mosquitoes can help reduce malaria through mass trapping of mosquitoes.

1.2.5 Malaria vector control

Despite global and national efforts to control malaria, the disease burden remains high especially in tropical Africa. According to the World Health Organization, vector control remains the most effective measure to prevent malaria transmission (WHO, 2006). A variety of methods have been applied aimed at vector control to reduce malaria morbidity and mortality. Examples of these methods are: use of insect treated nets (ITNs), Long Lasting Insecticidal Nets (LLINs) and Indoor residual spraying (IRS). The nets act as physical barriers by blocking the malaria mosquitoes. Application of pyrethroid insecticides such as permethrin, deltamethrin adds a chemical barrier to the physical one, further reducing human-vector contact and increasing the protective efficacy of the mosquito nets (WHO, 2012). Resistance to pyrethroids has been reported to have developed (Chandre *et al*., 1999). Indoor residual spraying and insecticide treated nets only target mosquitoes that exhibit endophagy and endophily hence not effective for mosquitoes that exhibit exophagy. Other challenges that impede indoor residual spraying are vector resistance and replastering of sprayed surfaces.

Long Lasting Insectcidal Nets can also be used as a measure for control of malaria as the nets depend upon high proportions of human exposure occurring indoors to achieve maximum impact upon malaria transmission (Killeen and Moore, 2012) but for malaria mosquitoes that tend to feed and rest outdoors, the Long Lasting Insectcidal Nets are not effective. Biological control which entails use of larvivaporous fish such as *Gambusia affinis* (Baird and Girard 1853) as a predator has also successfully served as a means of control but this may not be effective due to lack of trained personnel on rearing of these fishes. Larviciding can also be employed to reduce burden of malaria (Fillinger *et al.*, 2006). Another method of vector control that compliments insect treated nets and indoor residual spraying include use of sampling tools such as traps, targets combined with techniques such as use of attractants. Such tools and techniques can be effective when mass trapping is applied. Mass trapping of mosquitoes would reduce the adult female malaria vectors (Kline, 2006).

Malaria control strategies can be complemented with integrated vector management (IVM) which can be regarded as a framework for malaria control (Beier *et al.,* 2008). Control strategies such as the use of bed nets and IRS complemented with IVM programmes have been shown to reduce entomological inoculation rates thereby reducing malaria transmission (Killeen *et al*., 2000). It is evident that community based IVM programmes can be implemented to help enhance malaria vector control (Mukabana *et al*., 2006). Such programmes incorporating other strategies such as larval source management have been in use (Fillinger *et al.*, 2008) and can help reduce the malaria burden.

PROBLEM STATEMENT

Carbon dioxide is a key ingredient of odor blends used for attracting malaria mosquitoes. The application of this gas from pressurized cylinders and or the use of dry ice present major challenges to using carbon dioxide based mosquito attractants under field conditions. Organic carbon dioxide derived from fermentation of refined cane sugar and sugar cane molasses offers a suitable solution but the sources must be replaced after each experimental night unlike other synthetic baits impregnated on nylon strips and this is very expensive and limits prolonged area wide use of carbon dioxide odor baits. There is need to develop a cheap, effective and reliable replacement for carbon dioxide for use in mosquito attractants. Two-butanone, through electrophysiology studies, has been suggested as a promising replacement for carbon dioxide in synthetic malaria attractants.

JUSTIFICATION AND SIGNIFICANCE OF THE RESEARCH

The use of human landing catches as a means of sampling mosquitoes has been rendered unethical in Kenya and this has necessitated the search for closely resembling mosquito sampling tools namely odor baited traps that are capable of providing more comparative epidemiologically useful information. The odor baited traps are limited by the lack of a suitable replacement for carbon dioxide whose current best source requires daily production. There is therefore need to develop a cheap, effective and reliable replacement for carbon dioxide for use in mosquito attractants. Two-butanone presents itself as a suitable candidate for this purpose. This study explored the possibility of replacing organically-produced carbon dioxide with 2-butanone in odor blends targeting malaria mosquitoes. Synthetic human odors can enhance surveillance control of malaria mosquitoes through mass trapping.

OBJECTIVES

1.3.1 General objective

The aim of this study was to determine whether 2-butanone was a suitable replacement for carbon dioxide in synthetic *Anopheles* mosquito odor baits.

1.3.2 Specific objectives

1. To determine the importance of carbon dioxide in malaria mosquito attractants.
2. To determine the optimal concentration at which 2-butanone can act as a substitute for carbon dioxide in synthetic attractants for malaria mosquitoes
3. To quantify the levels of attraction of mosquitoes to odor baits containing 2-butanone instead of carbon dioxide
4. To evaluate the effect of 2-butanone based odor baits on mosquito house entry behavior.
5. To quantify the levels of attraction of malaria transmitting mosquitoes to 2-butanone based odor baits versus other robustly proven mosquito sampling methods.

NULL HYPOTHESIS

The ketone referred to as 2-butanone is not a suitable replacement for carbon dioxide in synthetic *Anopheles* mosquito odor baits.

CHAPTER TWO: MATERIALS AND METHODS

This research was conducted under semi-field and field conditions. The semi-field studies were conducted at the Thomas Odhiambo Campus of the International Centre of Insect Physiology and Ecology *(icipe*-TOC) located near Mbita Point Township in western Kenya. The field studies were carried out near Ahero Township, also in western Kenya.

2.1. Mosquito rearing

All semi-field experiments utilized a laboratory colony of the Mbita strain of *An. gambiae* and *An. arabiensis*. The mosquitoes were reared under ambient environmental conditions at *icipe*-TOC. Mosquito eggs were placed in plastic trays containing filtered fresh water from Lake Victoria. Larval instars were fed on Go-cat Complete food (Purina, Nestle S.A)) supplied thrice a day. Pupae were collected in clean cups daily, transferred to the adult holding room and placed in mesh covered cages (30 × 30 × 30 cm) prior to adult emergence. The adults were fed on 6% glucose solution through wicks made from absorbent tissue paper. Semi-field experiments utilized 200 adult female *An. gambiae* or *An. arabiensis* mosquitoes aged 3-6 days. These mosquitoes were aspirated from the cages and kept in mosquito holding cups, starved for eight hours and did not receive any blood-meal before the experiments were started. They only had water from a wet cotton cloth material placed on top of the holding cups.

2.2. Synthetic attractants

A reference attractant blend named Mbita Blend 5 or MB5 (Mukabana *et al.,* 2012b) was used as a standard in all experiments. The chemical constituents of MB5 included ammonia (2.5%), lactic acid (85%), tetradecanoic acid (0.00025%), 3-methyl-1-butanol (0.000001%), 1-butylamine (0.001%) and carbon dioxide (approximately 63.23 ± 2.82ml/min). The chemical components in the synthetic attractant blend were purchased from Sigma Aldrich Chemie GmbH (Germany). Carbon dioxide was produced locally by mixing 250 g of molasses (Mumias Sugar Company Limited, Kenya), 17.5g dry yeast (Angel® Yeast Company Limited, China) and two litres of water (Smallegange *et al*., 2010, Mukabana *et al.*, 2012b). All the attractants with the exception of carbon dioxide were delivered via nylon strips (Okumu *et al*., 2010b) and dispensed using MM-X traps (Njiru *et al*., 2006). Carbon dioxide was dispensed into the plume tube of the trap through silicon tubing with a diameter of 7mm. The nylon strips (15 denier microfibers) contained 90% polyamide and 10% spandex (Bata Shoe Company, Kenya). The strips were cut into narrow pieces each measuring 26.5 cm × 1.0 cm.

2.3. Semi-field experimental setups

Most of the semi-field experiments were carried out inside screen-walled green houses at *icipe*-TOC. The floor of the screen-walled green house (11cm × 7cm) was covered with sand that was kept moist in order to regulate humidity as well as the temperature. Experiments employed 2 × 2 and 4 × 4 Latin Square experimental designs (Euler, 1782). The treatments were separately assigned to different MM-X traps which were placed diagonally and positioned 15cm above the ground level (Schimied *et al*., 2008; Jawara *et al*., 2009). In the 2 × 2 design, Mbita blend was used in one trap while the other trap utilized MB5 with its carbon dioxide ingredient replaced with different dilution of 2-butanone. Two different sets of experiments employed the 4 × 4 design whereby for set one, four MM-X traps were baited with MB5, MB5 without the carbon dioxide component, carbon dioxide alone and an empty trap (i.e. the negative control). For the second set of the experiment, the four MM-X traps were baited with MB5, MB5 + 2-butanone (99.5%; hereby referred as variant 1), MB5 + 2-butanone (1.0%; hereby referred as variant 2) and an empty MM-X trap (i.e. the negative control) . To rule out the positional effect, the traps were alternated after each experimental night.

2.4. Importance of carbon dioxide in malaria mosquito attractants

The primary aim of this part of the study was to establish the importance of carbon dioxide in malaria mosquito attractants. Thus, the effect on mosquito behavior of odors other than carbon dioxide and the potential synergism between carbon dioxide and the other odors on attraction of malaria mosquitoes were investigated. The experiments were conducted for a period of 16 nights under semi-field conditions. Two hundred laboratory-reared female *An. gambiae* mosquitoes aged 3-6 days were used per experimental night. Experimental mosquitoes were starved for eight hours prior to starting the experiments. Experiments were run from 20:00 to 06:30 hours (Verhulst *et al.,* 2011). A fully replicated 4 × 4 Latin Square experimental design was adopted. The four treatments included an un-baited MM-X trap, the trap baited with MB5 in its intact form, MB5 without carbon dioxide and carbon dioxide alone.

2.5. Optimizing the concentration of 2-butanone for use in malaria mosquito attractants

The underlying objective of the study was to determine the optimal concentration at which 2-butanone could act as a substitute for carbon dioxide in synthetic attractants for malaria mosquitoes. Binary assays were ran in a screen house at the Thomas Odhiambo Campus of the International Centre of Insect Physiology and Ecology *(icipe*), to determine the behavioral responses of malaria mosquitoes towards an attractant blend containing various concentrations of 2- butanone. This in turn helped to determine the optimal concentrations at which 2-butanone could act as a substitute for carbon dioxide in synthetic attractants for malaria mosquitoes. Two hundred laboratory reared *An. gambiae* mosquitoes aged 3-6 days were used per each experimental night. The mosquitoes had been starved for eight hours prior to starting of the experiments which run from 20:00hrs to 06:30hrs (Verhulst *et al*., 2011).

Below is a table showing the treatments used to evaluate the concentration of 2-butanone suitable for replacing carbon dioxide in malaria mosquito attractants.

Table 1: Treatments used to evaluate the concentration at which 2-butanone can be used as a replacement for carbon dioxide in malaria mosquito attractants.

Reference blend	Variant blend (showing concentrations of 2-butanone)	Replicates
MB5	MB5 (minus carbon dioxide) + 2-butanone (99.5%)	4
MB5	MB5 (minus carbon dioxide) + 2-butanone (10%)	4
MB5	MB5 (minus carbon dioxide) + 2-butanone (1.0%)	4
MB5	MB5 (minus carbon dioxide) + 2-butanone (0.1%)	4
MB5	MB5 (minus carbon dioxide) + 2-butanone (0.01%)	4
MB5	MB5 (minus carbon dioxide) + 2-butanone (0.004%)	4
MB5	MB5 (minus carbon dioxide) + 2-butanone (0.001%)	4
MB5	MB5 (minus carbon dioxide) + 2-butanone (0.0004%)	4

2.6. Mosquito attraction to odor blends containing 2-butanone instead of carbon dioxide

The central aim of this set of experiments was to determine how well 2-butanone-based blends competed with the reference blend (MB5) in attracting malaria mosquitoes. The assays were carried out through direct (semi-field) and indirect (field) comparisons. The evaluations were spread over a period of 16 days, assuming a 4 × 4 Latin Square experimental design in each case.

2.6.1 Direct comparisons on mosquito attraction to odor blends containing 2-butanone instead of carbon dioxide

To determine the best concentration at which 2-butanone could replace carbon dioxide in malaria mosquito odor baits, four treatments were used which included an un-baited MM-X trap, the trap baited with MB5 in its intact form (reference blend) and two variants of MB5 i.e. one where the carbon dioxide component was replaced with 2-butanone in its pure form (99.5%; variant 1) and a 1.0% dilution of 2-butanone (variant 2). The empty MM-X trap served as a negative control. Two hundred laboratory-reared female *An. gambiae* and *An. arabiensis* mosquitoes were released separately under semi-field conditions. These mosquitoes could freely access the four treatments, which were availed simultaneously in the screen house enclosure on each experimental night.

2.6.2. Indirect comparisons on mosquito attraction to odor blends containing 2-butanone instead of carbon dioxide.

To determine the best concentration at which 2-butanone could replace carbon dioxide in malaria mosquito odor baits under field conditions, four houses separated by a distance of greater or equal to 25 metres were selected and 4 × 4 Latin Square experimental design was employed. Four treatments were used which included an un-baited MM-X trap, the trap baited with MB5 in its intact form (reference blend) and two variants of MB5 i.e. one where the carbon dioxide component was replaced with 2-butanone in its pure form (99.5%; variant 1) and a 1.0% dilution of 2-butanone (variant 2). The empty MM-X trap served as a negative control. These treatments were allocated to each house on a rotational basis to rule out positional effects.

The studies were conducted at Kigoche village in Ahero (00° 08^{1}S, 034° 55^{1}E), Kisumu county, western Kenya. Kigoche/Ahero experiences long rain seasons through April – June while short rains occur through September – October. The village lies at an altitude of 1160m above the sea level with an average relative humidity of 65% and average annual rainfall of 1,000 - 1,800mm. Most of the residents engage in irrigated rice farming thereby creating breeding sites for malaria mosquitoes for enhanced malaria transmission. The houses are mainly mud walled with open eaves through which anopheline mosquitoes pass (Njie *et al.*,

2009) and with corrugated iron sheets for roofing. *Anopheles gambiae and An. funestus* are the chief vectors (Githeko *et al.,* 1996) at Kigoche/Ahero.

2.7. Effect of 2-butanone-based odor baits on reducing mosquito house entry behavior

The principal aim of the study was to evaluate the effect of 2-butanone based odor baits on mosquito house entry behavior. The study was conducted inside a modified semi-field set-up referred to as the *MalariaSphere* (Knols *et al*., 2002). This was achieved by testing the potential of 2- butanone based odor baits with an MM-X trap to reduce the house entry behavior of *An. gambiae* females (Snow *et al*., 1987, Njie *et al*., 2009). Three barriers were used namely 2-butanone based odor baits, carbon dioxide based odor baits and an empty MM-X trap, which served as a negative control. Five sets of experiments were conducted and each experiment had four replicates. For each set of the experiment, (a) two un-baited or baited MM-X traps (table 2) were hung 15 cm above the ground outside the hut, (b) Two unlit CDC traps were hung 150 cm from the ground inside the hut (with or without a human host) beside the bed net on the foot side end of the human being (figure 2). This is because the principal olfactory cues responsible for attracting *An. gambiae* mosquitoes originate from the human foot region (De Jong and Knols, 1995).

Table 2: Five sets of experiments conducted to determine the effect of 2-butanone based odor bait on reducing mosquito house entry

Experiments	**Replicates**	**Inside the hut**	**Outside the hut**
Set 1	4	No human host	Empty MM-X traps
Set 2	4	Human host	Empty MM-X traps
Set 3	4	Human host	MB5 (intact reference blend)
Set 4	4	Human host	MB5 + 2-butanone (99.5%)
Set 5	4	Human host	MB5 + 2-butanone (1.0%)

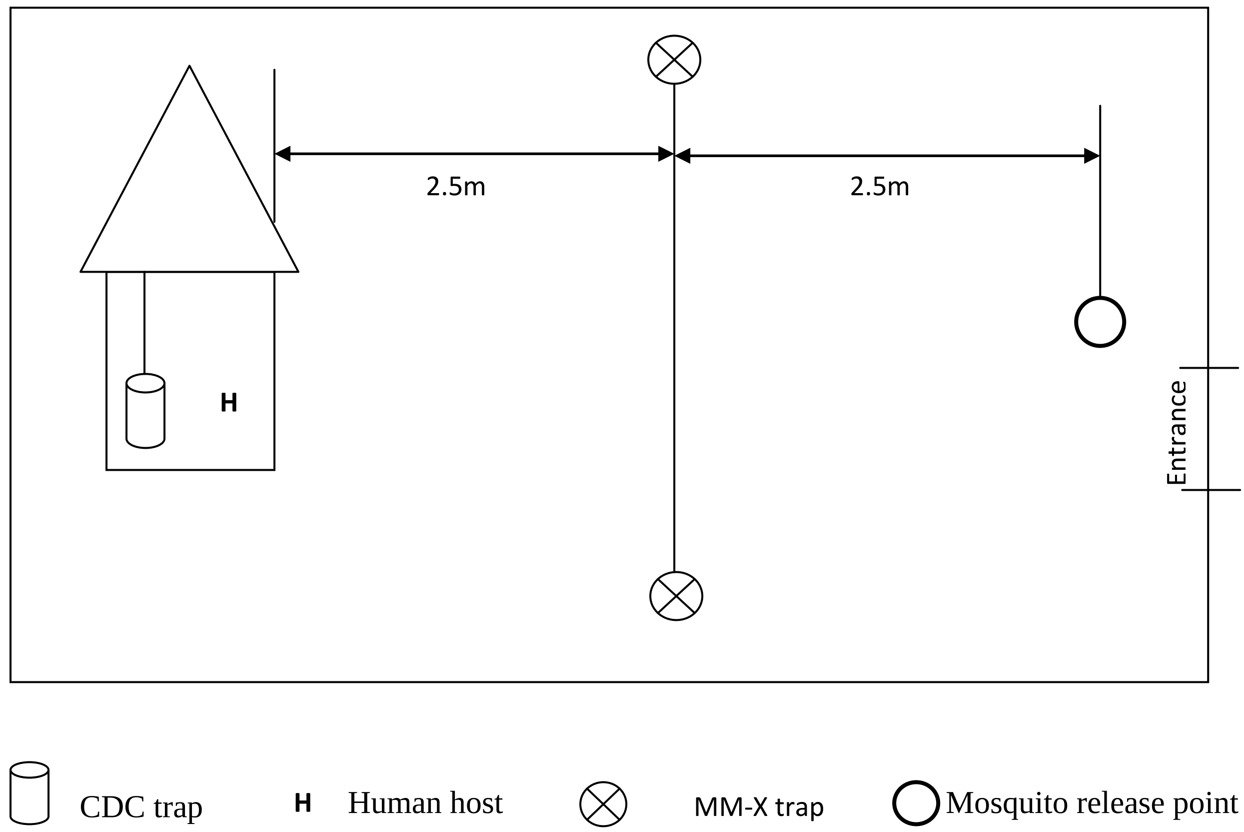

Figure 2: Experimental set up used to determine the effect of 2-butanone on the house entry bahavior of *An. gambiae* inside the *MalariaSphere*.

2.8. Effect of 2-butanone based odor baits versus other proven sampling methods

The key aim of this set of experiments was to quantify the levels of attraction of malaria mosquitoes to 2-butanone and carbon dioxide-based odor baits versus other mosquito sampling methods namely a human being and a CDC light trap. The parameters of interest were the species identity and abdominal status of trapped mosquitoes. Five houses in Kigoche village (see section 2.6.2.) were selected for these tests. Treatments were allocated to each house on a rotational basis to rule out possible site effects. The treatments included a reference odor blend namely MB5 in its intact form, MB5 with its carbon dioxide ingredient replaced with pure (99.5%) 2-butanone (variant 1), a human being, a fully functional CDC light trap and an un-baited MM-X trap. The un-baited MM-X trap served as a negative control. The odor baits (reference and variant 1) were dispensed using MM-X traps whose counter flow mechanism was disabled so that air could only be pushed out. The MM-X traps were hung 15cm above a bed under a net. Each house received only one treatment per night. In all cases the treatment was placed inside an untreated, well tacked in bed net. An unlit CDC light trap was hung outside the bed net 15 cm above the treatments (Jawara *et al*., 2009) in each case except where a human being posed as bait. The unlit CDC light trap was hung in the foot region of the bed (Mboera *et al*., 2000) in the case where a human being served as bait. The treatments served as mosquito bait while the unlit CDC traps served as the mosquito collection tools. A fully replicated 5 × 5 Latin Square experimental design was adopted. All the traps were powered with 6V (CDC traps) or 12V (MM-X traps) batteries. All the experiments ran from 20:00 to 06:30 hours. All traps were disconnected from power the following morning and the trapped wild mosquitoes were removed and taken to the laboratory where they were identified, counted and recorded according to species and abdominal status.

Table 3: Illustration of the 5 × 5 Latin Square design used to determine numbers of mosquitoes attracted to odor baits and other sampling tools

House	Day 1,6,11,16,21	Day 2,7,12,17,22	Day 3,8,13,18,23	Day 4,9,14,19,24	Day 5,10,15,20,25
1	MB5 (with no CO_2) + 2-Butanone (99.5%)	Empty trap	MB5 + CO_2	Lit CDC trap	Human
2	Human being	MB5 (with no CO_2) + 2-Butanone (99.5%)	Empty trap	MB5 + CO_2	Lit CDC trap
3	Lit CDC trap	Human	MB5 (with no CO_2) + 2-Butanone (99.5%)	Empty trap	MB5 + CO_2
4	MB5 + CO_2	CDC light trap	Human	MB5 (with no CO_2) + 2-Butanone (99.5%)	Empty trap
5	Empty trap	MB5 + CO_2	Lit CDC trap	Human	MB5 (with no CO_2) + 2-Butanone (99.5%)

2.9. Ethical considerations

This study was approved by the ethical review committee of the Kenya Medical Research Institute (KEMRI/RES/7/3/1). In all cases, the purpose and procedures of the study were explained to local leaders, household heads and resident volunteers before permission to carry out the study was sought.

2.10. Data analysis

For all semi-field experiments, Generalized linear models were used with Poisson distribution and data was transformed to assume linearity using a logarithmic linear function. Data collected during field experiments was analyzed by using a Generalized Linear Model (GLM) fitted with negative binomial distribution and a logarithm link function. The effects of treatments and house position on mosquito catches were tested as parameters in the model. All analyses were performed using IBM SPSS statistics, version 20.0.

CHAPTER THREE: RESULTS

The work reported in this thesis was carried out between August 2012 and May 2013. The semi-field experiments used a total of 20,000 mosquitoes. Of these 16,800 were *Anopheles gambiae* s.s and 3,200 were *Anopheles arabiensis*.

3.1. Importance of carbon dioxide in malaria mosquito attractants

Semi field data which sought to determine the role of carbon dioxide in malaria mosquito attractants was conducted for 16 nights in May 2013. Out of the 3,200 female *An. gambiae* mosquitoes released, only 1,743 (55%) were recaptured. The four treatments used were the intact reference blend (MB5), carbon dioxide only, MB5 with no carbon dioxide and an unbaited MM-X trap. Mosquito catches differed significantly among the four treatments (P = 0.001) with the reference blend catching the highest number (60%, n = 1053). On the other hand, carbon dioxide attracted more mosquitoes (22%, n=388) than the reference blend without the carbon dioxide component (15%, n = 264) (Figure 3).

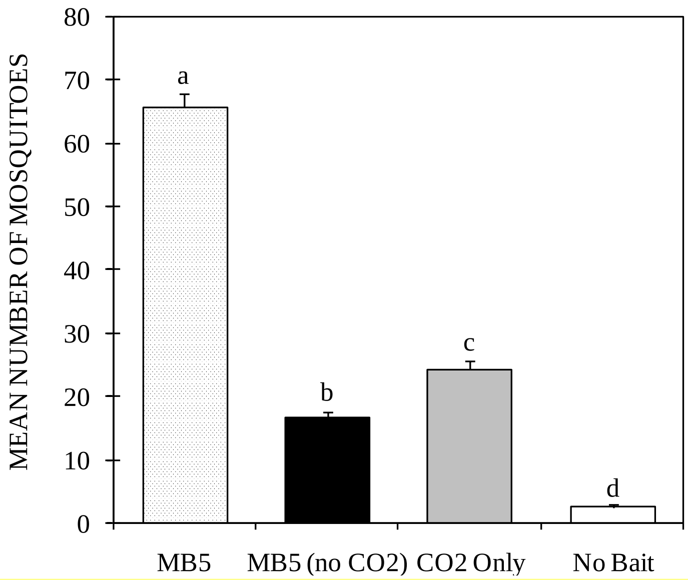

Figure 3. Mean numbers of *An. gambiae* mosquitoes caught in MM-X traps containing an intact reference attractant blend (hatched bar), the reference blend without its carbon dioxide component (black bar) or in ones baited with carbon dioxide only (gray bar) or no bait (blank bar). Error bars denote the standard error of the mean number of mosquitoes trapped. Bars with different letters denote significant differences in the number of mosquitoes trapped.

3.2. Optimizing the concentration of 2-butanone for use in malaria mosquito attractants

Semi-field data, which resulted from direct dual-choice competitions between experimental treatments over 32 nights, were collected from August 2012 to December 2012. Out of 6,400 individual mosquitoes that were released, a total of 4,271 (67%) female *An. gambiae* mosquitoes were collected in MM-X traps baited with the intact reference blend (MB5) or when its carbon dioxide component was replaced with a different dilution of 2-butanone (variant blend). The total percentage of mosquitoes that were trapped per experimental comparison ranged between 9.72% and 14.56%. In all cases the intact reference blend attracted a significantly higher number of mosquitoes than the variants (P = 0.001; table 3). The highest catches were associated with the 99.5% and 1.0% concentrations of 2-butanone. These two concentrations were selected for further testing.

Table 4: Mean number (±SE) of *An. gambiae* mosquitoes attracted to blend MB5 either in its intact form (reference blend) or with its carbon dioxide component replaced with a different dilution of 2-butanone (variant blend). The number of replicates N and the total number of responding mosquitoes n are shown. P values denote the level of statistical difference between the numbers of mosquitoes attracted to the two treatments.

Concentration of 2-butanone in variant blend	N	n	% average response	P-value	Mosquito trap catches (mean ±SE)	
					Reference blend	Variant blend
99.5%	4	622	14.56	0.001	122.75±5.54	32.75±2.86
10%	4	559	13.09	0.001	122.75±5.54	17.00±2.06
1.0%	4	588	13.77	0.001	118.75±5.45	28.25±2.66
0.1%	4	526	12.32	0.001	115.00±5.36	16.50±2.03
0.01%	4	415	09.72	0.001	89.75±4.74	14.00±1.87
0.004%	4	543	12.72	0.001	118.50±5.44	17.25±2.08
0.001%	4	535	12.53	0.001	118.00±5.43	15.75±1.98
0.0004%	4	483	11.31	0.001	106.50±5.16	14.25±1.89

3.3. Effect of 2-butanone-based odor baits on reducing mosquito house entry behavior

Experiments aimed at determining the effect of 2-butanone on reducing house entry behavior, were conducted under semi field conditions for 20 nights. The treatments which were used as candidate barriers against mosquito house entry were the intact reference blend (MB5) or the reference blend with the carbon dioxide constituent replaced with pure (99.5%; variant 1) or a 1.0% dilution of 2-butanone (variant 2). An unlit CDC trap was used to trap mosquitoes inside the hut in which a human subject slept overnight. Out of 4,000 female *An. gambiae* mosquitoes released, only 1,303 were recaptured. The intact reference blend attracted the highest number of mosquitoes thus served as the best barrier for reducing the house entry behavior of mosquitoes. Variant 1 and 2 of MB5 baited in traps which were placed outdoors attracted fewer mosquitoes while the human host in both cases attracted higher number of mosquitoes. The relative indoor attraction was greater than 0.5 an indication of high house entry of mosquitoes (2.24 and 2.77 respectively; figure 4). With MB5 baited in a trap which was placed outdoors as a barrier, fewer mosquitos were trapped inside the hut. The relative indoor attraction was 0.48 (figure 4) thus MB5 served as the best barrier.

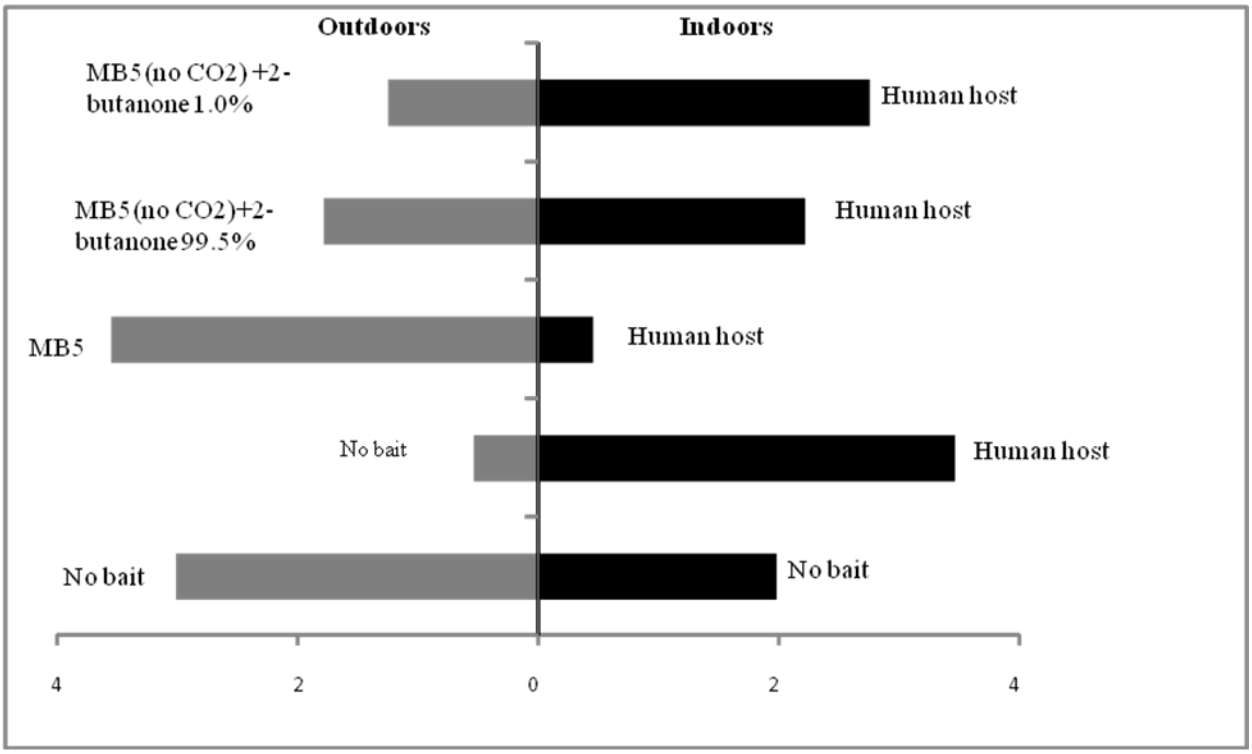

Figure 4: The house entry behavior of female *An. gambiae* s.s mosquitoes indicating the indoor and outdoor relative attractiveness. The reference blend (MB5) served as the best barrier as the indoor relative attractiveness was less than 0.5.

3.4. Mosquito responses to odor baits containing 2-butanone instead of carbon dioxide

The aim of these investigations was to compare the proportion of mosquitoes attracted to different odor baits under semi-field and field conditions. Under semi-field studies experiments were conducted using *An. arabiensis* and *An. gambiae* mosquitoes.

Semi-field testing with *An. arabiensis* was conducted for 16 nights in May 2013. Only 585 female *An. arabiensis* mosquitoes were recaptured out of the total of 3,200 released. The percentage of mosquitoes caught in the un-baited trap, the trap baited with the intact reference blend (MB5) or when the carbon dioxide component of MB5 was replaced with 2-butanone in its pure form (99.5%; variant 1) or a 1% dilution (variant 2) were 1.54%, 73.68%, 13.33% and 11.45% , respectively, (figure 5A). The reference blend attracted significantly higher numbers of mosquitoes than either variant 1 or 2 (P = 0.001). Variant 1 attracted higher number of mosquitoes than variant 2 but the catches were not significantly different (P = 0.361). The un-baited trap attracted the lowest number of *An.arabiensis* mosquitoes than either MB5, variant 1 or 2, (P = 0.001)

Semi-field testing with *An. gambiae* was conducted for 16 nights in December 2012. For this species 2,294 out of the 3,200 mosquitoes released were recaptured. The percentage of mosquitoes caught in the un-baited trap, the trap baited with the intact reference blend (MB5) or when the carbon dioxide component of MB5 was replaced with 2-butanone in its pure form (99.5%; variant 1) or a 1% dilution (variant 2) were 2.7% (n = 61), 80% (n = 1,835), 10.6% (n = 244) and 6.7% (n = 154), respectively, (figure 5B). The reference blend lured significantly higher numbers of mosquitoes than the un-baited trap (P = 0.001) or the two variant blends (P = 0.001). A significantly higher proportion of the mosquitoes was attracted to the variant containing pure 2-butanone than the one with a 1.0% dilution of 2-butanone (P = 0.001). The variant of MB5 containing pure 2-butanone also attracted a significantly higher proportion of the mosquitoes than the un-baited trap (P = 0.001).

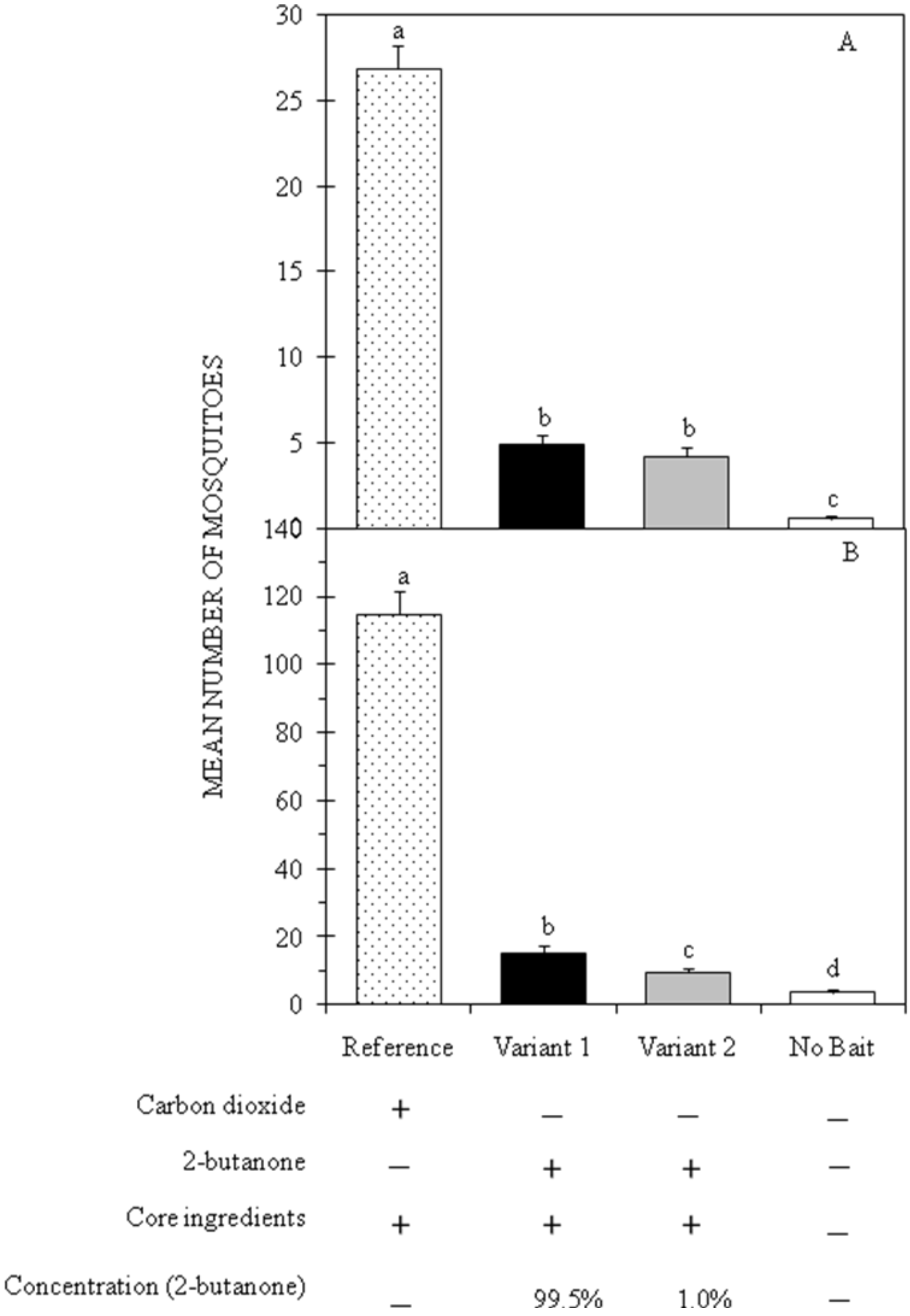

Figure 5. Mean numbers of *An. arabiensis* (panel A) *and An. gambiae* (panel B) mosquitoes caught in traps baited with a reference attractant blend (MB5) in its intact form (hatched bars) or with the carbon dioxide constituent replaced with the pure (99.5%; black bars; variant 1) or a 1% dilution of 2-butanone (gray bars; variant 2) or an un-baited MM-X trap (blank bar). Error bars denote the standard error of the mean number of mosquitoes trapped. Plus and minus signs indicate presence or absence of named ingredients of the attractants, respectively. Bars with different letters denote significant differences in the number of mosquitoes trapped. Core ingredients included lactic acid (85%), ammonia solution (2.5%), tetradecanoic acid (0.00025%), 3-methyl-1-butanol (0.000001%) and 1- butylamine (0.001%).

Field experiments, which sought to determine the proportions of mosquitoes attracted to the four treatments in-directly were conducted over a period of 16 nights in February 2013. The total numbers of adult female *An. gambiae* s.l., *An. funestus, Culex, Mansonia* and *Aedes* mosquito species that were trapped were 241, 115, 47, two and one, respectively (figure 6). Statistical analyses did not find a significant effect of hut and day on the numbers of *An. gambiae* (P = 0.763 for hut and 0.747 for day) and *An. funestus* mosquitoes collected (P = 0.497 for hut and P = 0.179 for day). The total numbers of unfed, blood-fed and gravid *An. gambiae s.l.* mosquitoes were 101, 37 and 103, respectively. Although there were no significant differences in the numbers of female *An. gambiae* s.l. mosquitoes (whether unfed or blood-fed) attracted to the reference blend or its two variants (P = 0.450), these catches were significantly more than those associated with the un-baited trap (P = 0.014). This was also the case for *Culex* mosquito species. Interestingly the numbers of gravid *An. gambiae* s.l. mosquitoes attracted to MB5 were not significantly different from those attracted to the un-baited trap and were lower, although not significantly so, to those attracted to the 2-butanone based blends.

Only unfed (n= 107) and gravid (n = 8) (but not blood-fed) *An. funestus* mosquitoes were trapped. Whereas the numbers of female *An. funestus* mosquitoes attracted to the intact blend and variant 1 (carbon dioxide replaced with 99.5% 2-butanone) were not significantly different (P = 0.090), those attracted to the intact blend and variant 2 (carbon dioxide replaced with 1.0% 2-butanone) differed significantly (P = 0.029). There were no significant statistical differences in the catches of *An. funestus* mosquitoes associated with variant 1 and variant 2 of the intact blend (P = 0.618). These findings were only true for the unfed mosquitoes. No attempt was made to verify these findings with gravid mosquitoes as the numbers collected were too few to merit statistical analyses. This was also the case with blood-fed (n = 2) and gravid (n = 3) *Culex* species, unfed (n = 1) and blood-fed (n = 1) *Mansonia* species and unfed (n = 1) *Aedes* mosquito species.

The total numbers for adult male *An. gambiae* s.l., *An. funestus* and *Culex* mosquito species that were trapped were 170, 22 and 21, respectively. No adult male mosquitoes belonging to the genera *Mansonia* and *Aedes* were trapped. Although there were no significant differences in the numbers of male *An. gambiae* s.l. mosquitoes attracted to the intact blend (MB5) or its two variants (P = 0.592), these catches were significantly more than those associated with the un-baited trap (P = 0.001). No attempt was made to verify these findings with male *An. funestus* and *Culex* mosquitoes as the numbers collected were too few to merit statistical analysis.

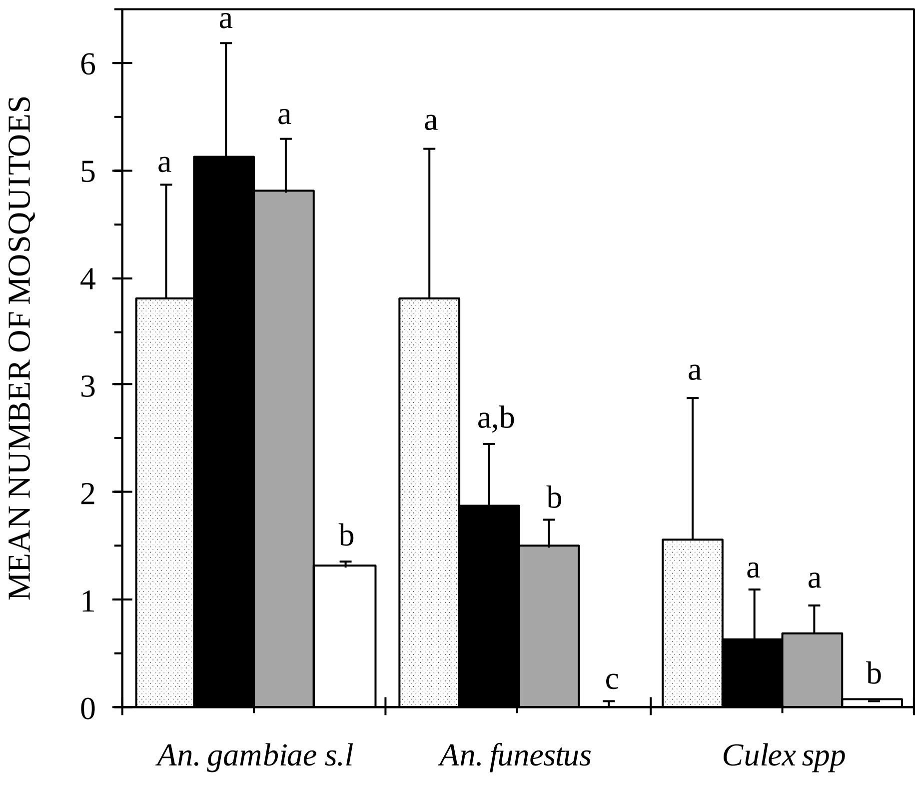

Figure 6. Mean numbers of wild mosquitoes caught in trap baited with a synthetic attractant blend (MB5) in its intact form (hatched bars) or with the carbon dioxide constituent replaced with pure (black bars) or a 1% dilution of 2-butanone (gray bars) or an empty MM-X trap (blank bar). Error bars denote the standard error of the mean number of mosquitoes trapped. Bars with different letters within a particular mosquito species denote significant differences in the number of mosquitoes trapped.

3.5. Attraction of mosquitoes to 2-butanone odor baits and other sampling methods

Data on relative attraction of mosquitoes to 2-butanone based baits against other robustly proven mosquito sampling tools was collected for 25 nights. The total numbers of female *An. gambiae*, *An. funestus*, *Culex* species, *Mansonia* species and other anopheline mosquito species trapped were 479, 658, 306, 12 and four, respectively (table 5). The numbers of unfed, blood-fed and gravid *An. gambiae* mosquitoes caught were 381, 27 and 71, respectively. Equivalent numbers of trapped *An. funestus* mosquitoes were 639, 3 and 16, respectively. Statistical analyses did not find significant differences in the numbers of female *An. gambiae, An. funestus* and *Culex* mosquitoes attracted to the reference blend or its variant (P = 0.840, P = 0.084, P = 0.313, respectively).

Table 5: Mean number (±SE) of wild female mosquitoes attracted to un-baited trap, CDC light trap, human host, MB5 either in its intact form or with its carbon dioxide component replaced with pure form (99.5%) of 2-butanone. The number of replicates is denoted by N. Means with different letter superscripts in the same column differ significantly.

Treatment	N	*An. gambiae* s.l	*An. funestus*	*Culex* spp.
CDC light trap	25	12.20 ± 2.54^{a}	7.40 ± 1.58^{a}	2.08 ± 0.51^{a}
Human	25	3.24 ± 0.74^{b}	12.40 ± 2.58^{b}	$5.12. \pm 1.12^{b}$
Reference odor bait (MB5)	25	$1.72 \pm. 0.43^{c}$	$4.08 \pm .0.91^{c}$	2.76 ± 0.64^{a}
Other odor bait (variant 1)	25	1.60 ± 0.41^{c}	2.32 ± 0.56^{c}	1.96 ± 0.48^{a}
Un-baited MM-X trap	25	0.40 ± 0.15^{d}	0.12 ± 0.07^{d}	0.32 ± 0.13^{c}

The numbers of female *An. funestus* mosquitoes attracted to a human host and the CDC light trap differed significantly (P = 0.001). The numbers of *Culex* mosquito species attracted to the human host and CDC light trap also differed significantly (P = 0.006). In general the CDC light trap caught significantly higher numbers of female *An. gambiae* and *An. funestus* mosquitoes (n = 490) as compared to catches with a human subject (n = 391), the reference blend (n = 145), the variant of MB5 (n = 98) and the un-baited MM-X trap (n = 13). The numbers of male *An. gambiae, An. funestus, Culex, Mansonia* and other anopheline mosquitoes caught were 254, 426, 93,one and one respectively.

With *An. gambiae* and *An. funestus* statistical analysis did not find any significant difference when comparing male mosquitoes attracted to the human volunteer and the reference blend (P = 0.619, 0.911), or with variant 1 (P= 1.000, 0.548; table 6) respectively. With *An funestus* there was a significant difference when comparing mosquitoes attracted to CDC light trap and reference blend (P=0.001) as well as comparing catches for *Culex* males attracted to the human volunteer or the reference blend (P=0.001).

Table 6: Mean number (±SE) of wild male mosquitoes attracted to an un-baited MM-X trap, a CDC light trap, a human host, an intact reference attractant blend (MB5) or the reference blend with its carbon dioxide component replaced with 99.5% 2-butanone. The number of replicates is denoted by N. Mean numbers with different letter superscripts in the same column differ significantly at P < 0.5

Treatment	N	*An. gambiae* s.l	*An. funestus*	*Culex* spp
CDC light trap	25	6.00 ± 1.30^{a}	13.92 ± 2.88^{a}	1.68 ± 0.42^{a}
Human	25	1.40 ± 0.37^{b}	0.84 ± 0.25^{b}	1.40 ± 0.37^{a}
Other odor bait (Variant 1)	25	1.16 ± 0.32^{b}	1.12 ± 0.31^{b}	0.36 ± 0.14^{b}
Reference odor bait	25	1.16 ± 0.32^{b}	0.88 ± 0.26^{b}	0.24 ± 0.11^{b}
Un-baited MM-X trap	25	0.44 ± 0.16^{c}	0.28 ± 0.12^{c}	0.04 ± 0.04^{b}

CHAPTER FOUR: DISCUSSION

In this study, I observed that the numbers of laboratory mosquitoes attracted to a reference blend (MB5) were significantly more than those attracted to MB5 without its CO_2 component, CO_2 only or an unbaited trap. In all semi-field investigations the reference blend attracted a significantly higher number of mosquitoes than its variants containing the different dilutions of 2-butanone used to replace CO_2. The highest catches were associated with the 99.5% and 1.0% concentrations of 2-butanone. The reference blend formed the best barrier for reducing house entry of *An. gambiae*. Although more wild female *An. gambiae* sensu lato, *An. funestus* and *Culex* species were attracted to a variant of MB5 containing pure 2-butanone, the catches did not differ significantly from those due to the intact reference blend. When compared to existing sampling methods, female *An. gambiae* s.l. mosquitoes were highly attracted to a CDC light trap than to a human subject, or MB5 with its CO_2 component replaced with pure 2-butanone.

With respect to the study which sought to determine the importance of carbon dioxide in malaria mosquito attractants, the mosquito catches were very low when an un-baited trap was used. The trap baited with carbon dioxide only attracted a higher number of mosquitoes than that containing MB5 without the carbon dioxide component but again the trap baited with the reference blend which constituted of MB5 and carbon dioxide attracted the highest number of mosquitoes among the other treatments (figure 3). This implies that carbon dioxide is a key component of baits as the gas activates mosquitoes by eliciting take-off and sustaining them in flight (Gillies, 1980; Dekker *et al*., 2001). It is possible that synergism exists between carbon dioxide and other odors on attraction of malaria mosquitoes. This potential synergistic effect has been shown in various studies such as the study which showed that carbon dioxide combined with foot odor increased mosquito trap catches (Njiru *et al*., 2006) and also studies that were conducted in Gambia which showed that carbon dioxide increased the mosquito catches in MM-X traps (Jawara *et al*., 2009). These findings correspond to the studies which demonstrated that compounds are more attractive when they are combined than when applied alone (Smallegange *et al*., 2005). The reference blend (MB5) without the carbon dioxide component was less attractive than when CO_2 was used alone thus CO_2 alone partially attracted many female *An. gambiae* s.s mosquitoes than MB5 without the CO_2 (figure 3) which correlates to a study which demonstrated that CO_2 alone attracted half as many *An. gambiae* s.l. mosquitoes compared to numbers trapped with human odor (Constantini *et al*., 1996). This also relates to the fact that the attractancy of candidate compounds to mosquitoes when used alone are less attractive than that of carbon dioxide alone (Kline *et al*., 1990).

In regard to the study that sought to determine the effect of 2-butanone based baits on reducing mosquito house entry behavior, it was evident that a trap baited with the intact MB5 blend placed outdoors served as the best barrier (figure 4) thereby reducing house entry of *An. gambiae* while the 2-butanone based blend did not fully prevent mosquito house entry**.** This finding was similar to the study that indicated that traps baited with human foot volatiles and carbon dioxide (yeast produced) reduced house entry of *An. gambiae* (Smallegange *et al*., 2010). Since the study was conducted within a *Malariasphere,* a human host who slept in the hut attracted higher numbers of *An. gambiae* than the trap baited with 2-butanone based baits or the unbaited trap placed outdoors. This indicates that *An. gambiae* mosquitoes which were released 5m away from the hut located the human host by following the cues released by the host (Smallegange and Takken, 2010) and passed through the eaves (Njie *et al*., 2009) of the hut to get into the hut. Since the 2-butanone based bait does not fully prevent mosquito house entry, this may mean that such blends may be used for mosquito surveillance but not for malaria control.

It was found that 2-butanone (pure form, 99.5%) has the potential to form a replacement for organic carbon dioxide in malaria mosquito attractants. Though this finding did not apply under semi-field conditions, it was evident that under field conditions there were no significant differences in terms of numbers of *An. gambiae* s.l and *An. funestus* mosquitoes attracted to the MB5 blend (intact form) or when the carbon dioxide ingredient was replaced with 2-butanone in its pure form (99.5%; figure 6). Therefore it is evident that 2-butanone mimics the activity of the carbon dioxide as it was shown through electrophysiological studies (Turner *et al.*, 2011). Failure of this finding to work under semi-field conditions may be associated with studies which showed that distance affects preferences of mosquitoes towards different stimuli (Okumu *et al*., 2010a). Both direct competitions and indirect competitions were carried out in semi-field and field conditions, respectively. Direct competitions were carried out in medium range phase of attraction while indirect competitions were done at long range. This therefore could suggest that stimuli in long range enhanced mosquito attraction towards 2-butanone based baits than to the carbon dioxide based baits.

The study that sought to quantify the levels of attraction of 2-butanone based baits versus other sampling tools showed that more *Anopheles gambiae* s.l were attracted to light rather than a human being (table 5). Human beings do not emit light yet we know that anthropophilic mosquitoes are highly attracted to human beings to obtain blood meals. The sibling of *An. gambiae* that is predominant in the field study area where the current research was carried out is known as *An. arabiensis* (Mukabana *et al*., 2012b). *Anopheles arabiensis* is known to be dominant in places with irrigated fields (Mwangi and Mukiama, 1992) which is also related to the study area where this research was conducted. This species is known to exhibit an opportunistic foraging behavior. It bites humans or animals indiscriminately depending on host availability. This non-specific behavior may partly explain why more *An. gambiae* s.l was attracted to light rather than man. The extended interpretation is that man ranks as a very low preference host for *An. arabiensis* and only becomes a preferred blood meal source for this species when there is no alternative host. It is not surprising, borrowing from this thinking that the very well known anthropophilic species namely *An. funestus* (Takken and Knols 1999) was more highly attracted to the human subject than to light (table 5). Also the finding that light attracted the highest number of wild female mosquitoes can be related to the study conducted in Tanzania whereby light traps caught a lot of malaria vectors that fed indoors (Lines *et al.,* 1991; Mboera *et al.,* 1998).

Contrary to two previous studies (Okumu *et al.*, 2010a; Mukabana *et al*., 2012b), the number of *An. gambiae* mosquitoes attracted to a human host was significantly less than those attracted to the synthetic odor blends. The only difference between the synthetic odor bait used in this study (intact reference blend namely MB5) and the one used by Mukabana *et al*., 2012b in the same study area is the inclusion of 1-butylamine in MB5. Thus, whereas 1-butylamine may have triggered significant mosquito behavioral responses in dose response experiments on its own, this compound probably has no synergistic effect with other chemical constituents of the MB5 blend. If this is true the inclusion of 1-butylamine in the reference blend may have compromised potential performance of 2-butanone as a possible replacement of carbon dioxide in malaria mosquito attractants as reported in this thesis. The issue needs further discussion and investigation.

It was found, under field studies, that the intact reference blend (MB5) or when the carbon dioxide ingredient was replaced with 2-butanone (99.5%) may be an effective strategy to control adult male mosquitoes as statistical analysis did not find significant differences in number of male mosquitoes lured into traps baited with the reference blend or variant 1 (table 6). This therefore may mean that the males were chasing after the females for mating thus the blend with 2-butanone can be employed as this would lead to reduction in the mating rate success. Again, if the mating rate is reduced, the number of gravid female mosquitoes will also decrease culminating into low prevalence of mosquito borne diseases. This corresponds to studies which proved that toxic attractant baits may be employed to control male mosquitoes (Xue and Barnand, 2003; Sandra, 2011)

Conclusion and Recommendations

This study demonstrated that 2-butanone has the potential to serve as a good substitute for carbon dioxide in synthetic mosquito attractants. The study further emphasizes on the possibility of using OBTs for monitoring, surveillance and control of malaria and other mosquito vectors. Since the field studies involved indoor experiments only, more research should be conducted to determine the effect of 2-butanone based baits outdoor. It is also important to evaluate the residual activity of 2-butanone. Under semi-field studies, none competitive experiments should be conducted to re-examine efficacy of replacing carbon dioxide with 2-butanone based baits in synthetic malaria mosquito attractants.

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