EVALUATION OF *BACILLUS THURINGIENSIS VAR ISRAELENSIS* AND *BACILLUS SPHAERICUS* BRUQUETS AS ANOPHELES MOSQUITO LARVICIDES IN VIHIGA COUNTY, WESTERN KENYA

BY

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SEPTEMBER, 2016
DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or any other award.

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DEDICATION

This work is dedicated to my wife, Dorice and children, Terrence and Tency for their endurance and moral support during my entire study period.
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### ABBREVIATIONS AND ACRONYMS

<table>
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<tr>
<td>ACTs</td>
<td>Artemisin Based Treatment Combination Therapies</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>Bs</td>
<td><em>Bacillus sphaericus</em></td>
</tr>
<tr>
<td>Bti</td>
<td><em>Bacillus thuringiensis var israelensis</em></td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control</td>
</tr>
<tr>
<td>EIR</td>
<td>Entomological Innoculation Rate</td>
</tr>
<tr>
<td>GPS</td>
<td>Global Positioning System</td>
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<tr>
<td>IGRs</td>
<td>Insect Growth Regulators</td>
</tr>
<tr>
<td>ITNs</td>
<td>Insecticide Treatment Bed Nets</td>
</tr>
<tr>
<td>KEMRI</td>
<td>Kenya Medical Research Institute</td>
</tr>
<tr>
<td>KM</td>
<td>Kilometre</td>
</tr>
<tr>
<td>MOH</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>LLINs</td>
<td>Long Lasting Insecticide Treated Nets</td>
</tr>
<tr>
<td>LSM</td>
<td>Larval Source Management</td>
</tr>
<tr>
<td>OBC</td>
<td>Oxitec British Company</td>
</tr>
<tr>
<td>RBM</td>
<td>Roll Back Malaria</td>
</tr>
<tr>
<td>SIT</td>
<td>Sterile Insect Technique</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>SSA</td>
<td>Sub – Saharan Africa</td>
</tr>
<tr>
<td>UNICEF</td>
<td>United Nation Children Educational Fund</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>VC</td>
<td>Vectorial Capacity</td>
</tr>
<tr>
<td>WDG</td>
<td>Water Dispersible Granular</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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ABSTRACT

Malaria is a key hindrance to the improvement of health in tropical and subtropical countries of the world with more than two billion people exposed to it. One of the approaches of controlling vectors of malaria is by targeting aquatic stages at source. This would lead to significant reduction in the adult vectors as well as malaria transmission. Entomopathogenic bacteria *Bacillus thuringiensis var israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) in the form of dispersable granular form has been one of the ways of managing malaria. This approach has had a number of drawbacks such as having short residual life span which increases treatment frequency leading to more labour cost. Moreover most are less effective since they have only one active ingredient of either *Bti* or *Bs*. Therefore, formulation of dispensing the entomopathogen in solid form would enhance slow release of insecticidal residue over a long period of time. This research set out to determine the impact of a new solid FourStar briquets formulation on aquatic Anopheline larval stages in Kenya. In addition, the study investigated the duration of briquets applied in the mosquito breeding larval sites. The effectiveness of FourStar briquet in identified selected three zones habitat was done within 180 days at Emakakha Location, Emuhaya sub-county in Vihiga County. Larvae and pupae after being sampled were picked from the tray by use of a pipette. The larvae were counted for ten dips at each of the ten identified mapped larval sites per the six habitat sectors. The post treatment was done after applying the 30 grams FourStar briquets as recommended by FourStar Microbial LLC company at a rate of one briquet of 30grams per nine square metres of water surface area on the identified zones sectors. A weekly post treatment data of mosquito larvae abundance was recorded in larval distribution survey forms in each larval site. The differences in the abundance between the control and intervention among the two mosquito species of *Anopheles gambiae* and *Anopheles funestus* were compared using student t-test. The correlation between mean numbers of *Anopheles gambiae* and *Anopheles funestus* collected in treated sites over time was calculated. All these analyses were carried out using the Statistical Package for Social Scientists (SPSS for windows version 20.0). In the three field treated larval habitat sites, larval productivity of *Anopheles gambiae* were greatly reduced by 100% for the first two months and 98-80% after third to sixth months. The results obtained indicated that there was significant difference among *Anopheles gambiae* larvae (P=0.021). This showed that *Anopheles gambiae* larvae are highly susceptible to the FourStar briquets. The briquets were effective and reduced *Anopheles funestus* larval productivity by 100% in the first two months and 99-87% after third to sixth months. Significant difference on early stages of *Anopheles funestus* was (P=0.032). Correlation between the mean numbers in treated larval sites over time *Anopheles gambiae* was significant (P=0.001) while *Anopheles funestus* was also significant at P=0.001. The larvicide reduced *Anopheles* larval abundance among the two Anopheline larvae instars significantly. The results of this study demonstrate the potential of using the solid briquette as a strategy to reduction of adult mosquito vectors as well as malaria transmission in Western Kenya Highlands.
1.1 Background information.

Malaria remains a major disease burden in many tropical and subtropical areas exerting an unacceptable toll on the health and economic welfare of the world's poorest communities (Breman et al., 2007). In Africa, the most vulnerable victims are children under five years and pregnant women because of lower or underdeveloped immunity (WHO, 2011).

In Kenya, malaria threatens the life of around 25 million out of the total population of over 41 million people (Pascaline et al., 2010). Rural and poor people suffer overwhelming burden of the disease due to extremely limited treatment. The infection rates are highest in the rural areas during rainy seasons because this is a time of intense agricultural activities that provide more breeding grounds for the Anopheles mosquitoes that transmit malaria (Yaw et al., 2004).

The epidemiology of malaria transmission and the severity of the disease vary greatly from region to region, village to village and even from person to person within villages (Oaks et al., 1991). Some of the differences are due to the particular parasite species. This being a life threatening disease, overwhelming fatality and infection rates, epidemiological analyses in recent times have shown that the medical impact of malaria may have been significantly underestimated (Breman et al., 2007). Inspite of all this, the enormous economic impact of malaria has never been adequately considered (Gallup et al., 2001). Malaria imposes a huge burden on human resources of a nation consequently, the Gross Domestic Product of the country gets reduced by as much as 1.3% (Gallup et al., 2001).
The World Health Organization (WHO) forecasted an annual growth of 16% in malarial cases (WHO, 2006). Considering these predictions as well as the current high transmission rates, it is paramount to develop research work on effective larval mosquito control strategy. FourStar briquet solid formulation with 1% *Bti* and 6% *Bs* concentration acts by releasing the insecticidal residue for many days in the water surface. It is recommended since it is easy to handle in the field, suitable for long distance transport and storage under tropical climatic conditions.

### 1.1.2 Ecology of malaria vectors

*Anopheles* mosquitoes, are responsible for transmission of malaria disease in specific geographical areas. The ability to discriminate between different breeding sites is more or less genetically determined by natural selection (Yih-shen *et al.*, 1980). Oviposition sites chosen by mosquito species may differ in size, appearance, in the presence or absence of vegetation, in types of aquatic vegetation, whether the water is stagnant or flowing, in salinity of the water, in degree and in presence or absence of shade and degree of turbidity (Chua *et al.*, 2004). For instance, gravid female *Anopheles gambiae* of the order diptera and family culicidae mosquitoes prefer to breed in sunlit pools, are usually temporarily persisting for about 4-5 weeks and are often associated with the presence of algae and absence of aquatic vegetation (Gimnig *et al.*, 2002).

Predation is also less prevalent in temporary habitats than in large, permanent ones (Sunahara *et al.*, 2002). These small sunlit habitats with higher water temperatures have shortened time of mosquito larval to pupal development (Munga *et al.*, 2006). The presence of less bacteria population in such habitats reduces the level of organic pollution
thus reducing mortalities associated with it. Larval mortality due to dessication or poor nutrient conditions may be reduced until water temperature rises above 30 degrees Celsius, consequently, enhanced larval habitat conditions may increase productivity of adult mosquitoes, which in turn increases the risk of malaria transmission (Munga et al., 2006).

In a study conducted by Ulrike Fillinger at Mbita area around lake Victoria in 2002, *Bti* and *Bs* water dispersible granules provided 88-100% mortality within 24 hours up to the fourth day after treatment. The present research work investigated a new solid briquet formulation with slow sustained release levels of *Bti/Bs* and extend the insecticidal activity for a longer period and low labour costs as compared with the water dispersible granules which requires frequent repeated application.

### 1.2 Statement of the problem

*Bti* and *Bs* are entomopathogenic bacteria which have been applied in aquatic habitats to control immature larval stages which cannot change their behavior to avoid control activities targeted at the larval habitat (Killeen et al., 2002). Larviding and source reduction have a major advantage in that they control mosquitoes before they disperse and transmit diseases (Killeen et al., 2002). It is being used mostly because it has less broad spectrum of activity hence cannot kill other insects that co-exist with mosquitoes in their natural breeding habitat. The dual active ingredients prevent resistance development over time and is environmentally compatible. Malaria control in endemic and epidemic regions remain too complex to be addressed by a single approach (Shiff, 2002). There is a
need to find alternative vector management strategies that are less resistant, low cost of insecticide and selective larval control measures that do not affect non-target organisms.

The new FourStar briquets formulation approach would be the most appropriate method in the reduction of larval population from instar to instar of the immature stages of mosquitoes. This formulation will be useful in promoting sustainable low cost method for vector management, reduce indiscriminate insecticide application among rural communities and control of mosquitoes before they disperse and transmit diseases (Killeen et al., 2002).

1.3 Justification of the study

The current impetus for disease control through reduced contact between mosquito vectors and humans (Insecticide Treated Nets and Long-Lasting Insecticide treated bednets), vector control (Indoor Residual Spray) and prompt treatment with artemisinin-based combination therapy (ACTs) have resulted in dramatic declines in malaria vectors and in the number of malaria cases (Mutuku et al., 2011). One major way of preventing malaria transmission is by controlling mosquito vectors from their breeding habitats. This has a major advantage in that it controls Anopheles mosquitoes before they disperse and contribute towards malaria transmission (Killeen et al., 2002). The use of Bti/Bs briquet can be mass produced, are toxicologically safe to human and wildlife, they are more or less specific in killing mosquito larvae and are easily applied (Service, 2004). When Bti/Bs is ingested mortality is caused by endotoxin acting as a stomach poison (Service, 2004). The research was carried out in Vihiga county which is one of the malaria endemic areas bordering the lake Victoria basin. In this study, microbial larvicide Bti/Bs
briquets was used which has a high efficacy, used during periods of heavy rains that dilute it and has longer insecticidal activity rate that last for up to six months. This is unlike granular formulation which has a residual activity span of a maximum of eleven days before reapplication (Fillinger et al., 2003).

1.4 Research questions

1) What is the effect of Bs and Bti FourStar briquet on the Anopheles mosquito larvae in Emakakha location Emuhaya sub-county, Vihiga County.

2) What is the duration of FourStar briquets of Bs and Bti activity in managing Anopheles mosquito larvae in Emakakha location Emuhaya sub-county, Vihiga County.

1.5 Hypotheses

1) Bacillus thuringiensis var israelensis and Bacillus sphaericus have no effect on Anopheles mosquito larvae.

2) The application of Bti and Bs briquets on larval habitats has no variation in duration.

1.6 Objectives of the study

1.6.1 General objective

To evaluate the effectiveness of Bacillus thuringiensis var israelensis and Bacillus sphaericus briquets as Anopheles mosquito larvicides, in Vihiga county in Western Kenya.
1.6.2 Specific objectives

1) To determine the effect of FourStar briquet of *Bacillus thuringiensis var israelensis* and *Bacillus sphaericus* on the abundance of *Anopheles gambiae* and *Anopheles funestus* mosquito larvae in Emuhaya sub-county.

2.) To establish duration of FourStar briquets in mosquito *Anopheles gambiae* and *Anopheles funestus* aquatic habitats in Emuhaya sub-county.

1.7 Significance and anticipated output

Control of mosquitoes at larval source is valuable in regions where the primary malaria vector is exophilic or bites before people go to bed making indoor residual spraying and impregnated bednets less effective (Shililu *et al.*, 2004). Long lasting *Bti/Bs* briquets were developed because malaria control is constrained by repeated applications of insecticides and development of chemical insecticide resistance. The use of briquets application has an advantage of taking a maximum of five months before declining in efficacy. Furthermore, release of the bio-insecticide in natural water bodies is gradual, can be sustained without frequent applications and it is environmentally compatible.

The study evaluated the possible duration and efficacy of *Bti* and *Bs* on *Anopheles* mosquitoes in selected breeding habitat in Emakakha location, Emuhaya sub-county in Vihiga County. *Bti* and *Bs* managed to cause an overall reduction in mosquito emergence in both *Anopheles gambiae* and *Anopheles funestus* and lasted for six months in all treated habitats. The study results information will be shared with the local community where the study was carried out in consultation with the county ministries of health, agriculture and other stake holders. The finding will also be disseminated through
publications. The findings of this study will be used to recommend *Bti/Bs* FourStar briquet formulation for application as larvicides in aquatic habitats to control mosquitoes
CHAPTER TWO: LITERATURE REVIEW

2.1 Malaria situation in the world

The World Health Organization (WHO, 2005) estimates that more than 85% of these episodes occur in the Sub-Saharan Africa, where approximately 3,000 people die every day especially children less than five years and pregnant mothers endure most of the morbidity and mortality rates (WHO, 2005). This inhibits fast achievements of millennium development goals on reduced child mortality, improved maternal health, achievement of universal education, fight against HIV/AIDS and other diseases, eradication of poverty and hunger thus impairing socio-economic development.

In the past decade, increased funding for malaria control has contributed to a reduction of malaria morbidity and mortality by using ITNs, IRS and effective anti-malarial therapies (WHO, 2012). The three strategies were estimated to reduce global incidence of malaria by 17% since 2000, and malaria-specific mortality rates by 26% (WHO, 2011). Whereas this is a major achievement, the reductions are lower than the internationally agreed targets of 50% by 2010.

Malaria continues to be a big challenge to mankind due to increased resistance to the anti malarial drugs, insecticides resistance, lack of effective vaccines, reduced immunity, loss of infrastructure for mounting systematic attack on the parasites and their transmission in countries with endemic infections (Shiff, 2002). Human malaria is a protozoan disease caused by five species of \textit{Plasmodium} namely: \textit{Plasmodium malariae}, \textit{Plasmodium vivax}, \textit{Plasmodium falciparum} and \textit{Plasmodium ovale} (Arora and Arora, 2002). The fifth species, \textit{Plasmodium knowlesi}, otherwise known to infect monkeys has also been shown to infect people in Malaysia and Indonesia (Anu \textit{et al.}, 2008). These \textit{Plasmodium
parasites have two hosts during their life cycle which involves female *Anopheles* mosquitoes as the definitive host and human as the intermediate hosts. However, *P. falciparum* is the most virulent species and predominates the sub-Saharan region causing up to 2,880 deaths in a day (WHO, 1999). In malaria endemic regions, individuals are often infected with more than one *Plasmodium* species (mixed species infections) (Mckenzie and Bossert, 1999).

Clinical manifestations and classical description of an individual with malaria progresses from shaking, anorexia, headache, vomiting, malaise, chills, through intense fevers to drenching sweats (Mbogo *et al*., 2003). Severe malaria due to infection with *P. falciparum* often leads to death in absence of prompt medical intervention. Malaria during pregnancy can cause miscarriages, fatal death, intrauterine growth retardation, low birth weight and premature delivery (Marsh, 2002). Malaria management strategies should therefore be governed by epidemiological factors prevailing in a particular region. The burden is largely attributed to potential threats from continued emergence and the spread of parasite resistance to antimalarial medicines and mosquito resistance to insecticides (WHO, 2012). Furthermore, *Plasmodium* parasites that cause human malaria are transmitted by many anopheline species with diverse breeding, resting, feeding habits and vectorial capacities coupled with lack of standardised reliable tools for monitoring vectors in different abdominal conditions (Alonso *et al*., 2011). The reported changes from indoor to outdoor biting behaviours and outdoor malaria transmission result in different disease patterns in various epidemiological settings (Bayoh *et al*., 2010). These challenges suggest that a single strategy cannot be effective in all malaria-prone regions. As a consequence, strategies used for malaria disruption and surveillance should also
target indoor and outdoor biting malaria vectors in various abnormal conditions (Alonso et al., 2011).

2.2 Factors affecting malaria distribution

Factors affecting distribution of malaria include the natural environment (climatic variability), vector population, interactions between vector and parasite, parasites determinants and some of its genetically controlled characteristics, host biological factors, behavioral, social and economic elements (McMichael et al., 2001). The slope of the land and the nature of the soil are some of the environmentally related factors affecting the type of surface water available and its persistence which subsequently increases the local malaria vector populations. The optimal range of temperature and relative humidity for most malaria vectors is 20-30 degrees Celsius and 70-80% respectively (Lindblade et al., 2006).

Irrigation schemes and hydroelectric projects are likely to increase the intensity of malaria transmission by maintaining a population of the vector anopheline mosquitoes throughout the year (Roberts et al., 1998). Human activities like agriculture and water management practices are important in the epidemiology of malaria because they provide breeding grounds for malaria vectors. The study of breeding habitats resulting from such practices can be vital in predicting the abundance, dynamics and fitness of the resultant adult mosquito populations (Paaijmans et al., 2008). Such type of information can be useful in understanding the vector competence of adult mosquitoes that cause malaria and timing of vector control programs with the aim of reducing malaria burden (Minakawa et al., 1999).
2.3 Diversity of malaria vectors in Africa

Principal mode of spread of malaria is by the bites of female \textit{Anopheles} mosquitoes. More than 480 species of \textit{Anopheles} mosquitoes have been described but only about 50 species transmit malaria and one or two species are known to be major drivers of disease transmission dynamics in a given area (CDC, 2007). Every continent has its own species of mosquitoes: \textit{An. gambiae} complex in Africa, \textit{An. freeborni} in North America, \textit{An. culicifacies}, \textit{An. fluviatilis}, \textit{An. minimus}, \textit{An. philippinensis}, \textit{An. stephensi}, and \textit{An. sundaicus} in the Indian subcontinent. \textit{Anopheles leucosphyrus}, \textit{An. latens}, \textit{An. cracens}, \textit{An. hacker} and \textit{An. dirus}, have been identified as the vectors for the transmission of \textit{P. knowlesi} (Cox-Singh et al., 2008).

The habits of most of the Anopheline mosquitoes have been characterised as anthropophilic which prefer human blood meal, endophagic, and nocturnal with peak biting at midnight, between 11 pm and 2 am. The blood meal from a vertebrate host is essential for the female mosquitoes to nourish their eggs. The mosquitoes find their host by seeking visual, thermal, and olfactory stimuli and of these, carbon dioxide, lactic acid, skin temperature, and moisture are more important mosquito attractants.

Malaria in Africa is mainly transmitted by three mosquito species namely \textit{Anopheles gambiae}, \textit{Anopheles arabiensis} and \textit{Anopheles funestus} (Minakawa et al., 2002b). The principal malaria vector in Sub-Saharan Africa (SSA) is \textit{Anopheles gambiae} complex (Ndenga et al., 2006). The seven siblings species members of the complex include \textit{An. arabiensis}, \textit{An. bwambae}, \textit{An. quadriannulatus}, \textit{An. commorensis}, \textit{An.gambiae} \textit{Giles sensu stricto}, the salt water \textit{An. melas} (Obbard et al., 2009) and type A species B which
have not been assigned scientific names but present in Kisii highlands. The *An. gambiae sensu strict* is the primary malaria vector species in Western Kenya highlands, constituting over 95% of the indoor-resting adult vector population and about 60-70% of the larval population (Besanky et al., 1994). *Anopheles funestus* occupies a wide range of ecological niches, is highly anthropophilic and is susceptible to human malaria parasites. Thus *Anopheles funestus* is considered a vector species that bridges malaria transmission during two wet seasons (Mbogo et al., 2003).

When a mosquito bites an infected individual, it sucks the gametocytes, the sexual forms of the parasite, along with blood. These gametocytes continue the sexual phase of the cycle within the mosquito gut and the sporozoites that develop then fill the salivary glands of the infested mosquito. When this female mosquito bites another person for a blood meal, the sporozoites are inoculated into the blood stream of the fresh victim, thus spreading the infection. *Anopheles funestus* and *An. gambiae s.s* are largely associated with humans and their habitations, preferring to feed on people and rest inside houses (Coetzee et al., 2000). *Anopheles arabiensis*, on the other hand will feed on humans or cattle, rest indoors or outdoors, and is slightly less efficient at transmitting malaria because of their low vectorial capacity and ability to feed on other animals when humans are not available (Gimnig et al., 2002).

### 2.4 The *Anopheles gambiae* complex

*Anopheles gambiae* complex comprises of the world’s most effective malaria vectors of human (Coetzee, 2000). For a long time this complex was considered to have six morphologically indistinguishable species, with five of them transmitting human malaria...
Anopheles gambiae s.s is the most efficient vector mainly because of its highly endophilic and anthropophilic characteristics (Githeko et al., 1996). It also has the highest sporozoite rate and predominant in rain saturated environments.

The vectorial capacity of An. arabiensis is slightly lower than that of An. gambiae. s.s because of its ability to feed on other animals when humans are not available (Coetzee et al., 2000). It is common in arid areas after a blood meal, majority of An. gambiae females rest indoors (endophily), prefers humid, higher altitude areas and peak season is shortly after onset of rainy season (Githeko et al., 1996). In general, the distribution of malaria vectors depends on the prevailing temperature, rainfall, humidity, vegetation, human population density, type and condition of the larval habitat and the distance from the breeding sites (Koenraadt et al., 2003).

2.4.1 Life cycle of Anophiline mosquitoes

Anopheles mosquitoes have four distinct stages of development in their life cycle: Egg, larva, pupa and adult (Figure 2.1). The first three stages are aquatic and last for 5-14 days, depending on the species and the ambient temperature. The adult is the active flying insect with the female Anopheles mosquito as the malaria vector. The adult females can live up to a month (or more in captivity) but most probably do not live for more than 1-2 weeks in nature.
Figure 2.1: Schematic illustration of the life cycle of *Anopheles* mosquitoes (Source: Google images generated from raywilson@googlemail.com).

**Eggs**: Adult females lay 50-200 brownish or blackish boat-shaped eggs per oviposition. Eggs are laid singly directly on water and are unique in having floats on either side and measure 1mm in length. Eggs are not resistant to drying and hatch within 2-3 days, although hatching may take up to 2-3 weeks in colder climates (Service, 1980).

**Larvae.** The first instar larva breaks the egg-shell with its “egg breaker” and becomes free in the water. Mosquito larvae have well-developed head with mouth brushes used for feeding, a large thorax, a segmented abdomen and have no legs. In contrast to culex
mosquitoes, *Anopheles* larvae lack a respiratory siphon and for this reason position themselves so that their body is parallel to the surface of the water (Service, 1980).

Larvae breathe through spiracles located on the 8th abdominal segment and therefore must come to the surface frequently (Crans and Mcnelly, 1977). The larvae spend most of their time feeding on algae, bacteria, and other microorganisms in the surface microlayer. They dive below the surface only when disturbed. Larvae swim either by jerky movements of the entire body or through propulsion with the mouth brushes. Larvae develop through four stages, or instars, after which they metamorphose into pupae. At the end of each instar, the larvae moult, shedding their exoskeleton, or skin, to allow for further growth (Mason, 1967).

**Pupae.** The pupa is comma-shaped when viewed from the side. The head and thorax are merged into a cephalothorax with the abdomen curving around underneath. As with the larvae, pupae must come to the surface frequently to breathe, which they do through a pair of respiratory trumpets on the cephalothorax. After a few days as a pupa, the dorsal surface of the cephalothorax splits and the adult mosquito emerges. The duration from egg to adult varies considerably among species and is strongly influenced by ambient temperatures. Mosquitoes can develop from egg to adult in as little as 5 days but usually take 10-14 days in tropical conditions (Gilles and Coetzee, 1987).

**Adults.** Like all mosquitoes, adult anophelines have slender bodies with three sections: head, thorax and abdomen. The head is specialized for perceiving and co-ordinating sensory information and for feeding. The head contains the eyes and a pair of long,
many-segmented antennae. The antennae are important for detecting host odours as well as odours of breeding sites where females lay eggs (Service, 1965).

The thorax is specialized for locomotion. Three pairs of legs and a pair of wings are attached to the thorax. The abdomen is specialized for food digestion and egg development. This segmented body part expands considerably when a female takes a blood meal. The blood is digested over time serving as a source of protein for the production of eggs, which gradually fill the abdomen. *Anopheles* mosquitoes can be distinguished from other mosquitoes by the palps, which are as long as the proboscis, and by the presence of discrete blocks of black and white scales on the wings. Adult *Anopheles* can also be identified by their typical resting position: males and females rest with their abdomens sticking up in the air rather than parallel to the surface on which they are resting.

### 2.4.2 Anopheline breeding sites

*Anopheles gambiae* and *Anopheles funestus* are common vectors of human malaria in Sub-Saharan Africa. Production of adults occur in small temporary sunlit, turbid pools of water (Fillinger and Lindsay, 2006). Habitats are often created by human or animal activity where larvae are found in small depressions such as hoof prints, edges of boreholes, burrow pits, roadside paddles formed by tyre tracks, irrigation ditches, ponds and drainage channels in valley bottoms.

In Western Kenya highlands majority of the Anopheline habitats are confined to valley bottoms where there is a continuous flow of water throughout the year. Homesteads are not far away from breeding habitats consequently humans provide a ready blood meal to adult *Anopheles* mosquitoes.
2.4.3 Mosquito larval source management

Larval source management involves use of a combination of techniques aimed at reducing mosquito larvae in the breeding habitats. This approach has been used to control the immature stages of mosquitoes (Walker and Lindsay, 2007). Larval source management is practiced by many countries in Africa where malaria is endemic (Fillinger et al., 2009). Field evaluations under various epidemiological conditions showed that larviciding reduced exposure to malaria transmission by 70-90% in sites where breeding habitats were well defined (Fillinger and Lindsay, 2006). Vector control programmes are being encouraged to develop integrated vector management strategies for the control of malarial (WHO, 2004).

2.4.4 Distribution of Anopheline mosquitoes in Western Kenya highlands

In the epidemiology of malaria, regions at and above 1500 meters above sea level in altitude are regarded as highlands (Cox et al., 1999). Indoor resting densities of anopheline mosquitoes decline with increasing elevation (Minakawa et al., 2002) as maximum and minimum temperatures correlate negatively with elevation. Therefore, climatic factors (particularly moisture index and temperature) strongly affect the distribution and abundance of malaria vectors. Anopheline mosquitoes inhabit houses around larval habitats (Minakawa et al., 2002) with more than 90% of Anopheles gambiae adults being found in houses within 300 meters from the nearest larval breeding habitat. These habitats are mostly created by human-made environmental changes that render aquatic habitats previously unsuitable for Anopheline mosquito breeding into suitable ones (Minakawa et al., 1999).
2.4.5 Productivity of larval habitat

The ability to discriminate between different breeding sites is more or less genetically determined by natural selection (Yih-Shen et al., 1980). Oviposition sites chosen by mosquito species may differ in size, appearance, in presence or absence of vegetation in types of aquatic vegetation, conductivity, whether the water is stagnant or flowing, in salinity of the water, degree of pollution and total dissolved solids (Edillo et al., 2006).

Mosquitoes do not necessarily lay eggs every day in each potential breeding site (Sattler et al., 2005), hence an aquatic habitat may not always contain larvae. However, Anopheles species larvae can breed in nearly every kind of water accumulation. The percentage of the eggs that result to adults is unknown, but there is usually heavy mortality especially among larvae due to predation, disease, drought and food (Service, 1980). However, productive larval habitats can be rendered less productive after the rains because of the flushing out of the larvae (Gimnig et al., 2002).

2.4.6 Survivorship of Anopheline larvae

Larval loss due to predation is one of the factors that reduce the numbers of larvae that develop into adults. Some of the predators of Anopheline larvae include: Mesocyclops (Copepoda: Cyclopoida) tadpoles of the genus Ptychadena (Service, 1965); mosquito-fish (Gambusia affinis), Coleoptera (Dytiscidae), Hemiptera (Notonectidae) and Odonata (Roberts et al., 1998). Interspecies competition (predation) between Anopheles gambiae and Anopheles arabiensis (Schneider et al., 2000). Other limiting factors include infections with viruses, bacteria, protozoans, nematodes and fungi (WHO, 1984).
The main endo and ecto-parasites of Anopheline mosquitoes in Africa and Asia are the water mold, *Coelomomyces indices*, and the *Vorticella sp.* (Whisler *et al.*, 1999). It has been observed that the ovipositing female mosquitoes would oviposit in habitats that appear to have low or no risk of predation (Star *et al.*, 1999).

### 2.5 Vector-parasite interactions

The ecology of malaria is associated with the availability of water which provides oviposition sites for malaria vectors. Proximity of human population to the breeding sites serves as an added advantage to the propagation of the vector population density and human contact (Koenraadt *et al.*, 2003).

The studies by Munga *et al.*, (2006) have shown that, larvae of *Anopheles gambiae sensu lato* (s. l), which is the primary malaria vector in Africa, occurs more frequently in sunlit temporal pools, or slow streaming waters in cultivated areas than in forested and natural swamps. Malaria parasite sporogonic development starts with susceptible female mosquito ingesting microgametocytes (male forms) and macrogametocytes (female forms) during blood feeding on an infected human. Once a female mosquito is infective, it remains so for life and is capable of transmitting sporozoites during each blood feeding episode, sometimes to multiple individuals during each feeding cycle (Boyd, 1947).

### 2.6 Malaria transmission

The number and species of Anopheline mosquitoes determine to a large extent the level of transmission in a given area. Malaria transmission is influenced by climate and geography and often coincides with the rainy season (Craig *et al.*, 1999). Other key components in malaria transmission are the entomological inoculation rate (EIR) and
vectorial capacity (VC). The EIR is the measure of the number of infective bites each person receives per night and is a direct measure of the risk of human exposure to the bites of infective mosquitoes. The EIR is the only direct measure of malaria transmission and the only useful index for predicting malaria epidemics (Grab and Onori, 1980).

Rainfall provides mosquito breeding sites and increases humidity thus enhancing mosquito survival (Craig et al., 1999). It is estimated that, an average of 80mm of rainfall per month for three to five months is reasonable amount for the availability of breeding sites to sustain malaria transmission.

Development projects in pursuit of economic development such as water schemes, construction of dams and bridges, oil drilling and mining activities, urban planning and logging activities lead to an increase in mosquito breeding sites, thereby increasing their numbers, human-mosquito contact and eventually transmission (Gubler, 1998).

Population movements of infected people from areas where malaria is endemic to areas where the disease has been eradicated has led to the resurgence of the disease (Gubler, 1998). Rural-urban migration, weak health systems, poor strategic development and inadequate funding of control programmes all play a role in transmission of malaria. The rural-urban migration in search of economic and educational opportunities has created densely populated communities, especially in peri-urban areas and weakened the health systems with the attendant increase and adverse effects on the environment creating breeding sites for malaria vectors (Bruce Chwatt, 1998).
2.6.1 Malaria transmission in Kenya

The level of endemicity of malaria in Kenya varies from region to region. Malaria endemicity ranges from hyperendemic to holoendemic areas at the coastal and lake regions of Kenya respectively (MOH, 2003). In the coastal and the lake regions of Kenya, malaria is transmitted all the year round and is described as stable malaria. The mortality rate is highest in children under five years of age (MOH, 2003). The rate of disease transmission is dependent on vector distribution, abundance and lifespan, degree of host-vector-pathogen contact, susceptibility of the vector to the pathogen and the effects of the pathogen on survivorship of both the vector and the host. Most common methods of malaria spread are mosquito bites, blood inoculation, needle stick injury, blood transfusion and mother to the growing foetus.

2.7 Malaria control measures

Mosquitoes play an important role in vectoring pathogenic organisms to humans and animals which cause major diseases in their hosts (Rodcharoen et al., 1991). Additionally, mosquitoes constitute a severe nuisance problem for humans and domestic animals in rural, sub-urban and urban areas of the world (Mulla et al., 1991).

2.7.1 Chemical control against vectors by use of long lasting bed nets

Insecticide treated bed nets (ITNs) come in different shapes and colours and provide a simple means of protection of humans against night biting mosquitoes including malaria vectors.

Long lasting insecticide treated nets have been impregnated with LLITN-Olyset to control malaria (Geissbuhler et al., 2009., Atieli et al ., 2010). This is evident in current
periodic mass distribution of long lasting bed nets campaigns (one net for every two people) to all targeted geographical areas, routine distribution in which pregnant women and children under one year are given free nets through antenatal care (Malaria Operational Plan Year 2014 report).

Pyrethroid treated bed nets and curtains seemed to be most promising as per the studies carried out in Senegal (Fontenille et al., 1997) which demonstrated the efficiency of ITNs for reducing infant mortality. These findings were confirmed in subsequent large scale multicenter studies across Africa including Ghana, Gambia and Kenya (Nevill et al., 1996). Randomized controlled experimental trials in sub-Saharan Africa have shown that insecticide treated bed nets have a profound impact on malaria transmission in areas where the main vector is Anopheline (Gimnig et al., 2002). Cases of pyrethroid resistance in the Anopheles gambiae in West Africa in cotton fields have been reported (Curtis et al., 1998). The alternative treatment is the use of organophosphates, carbonate and phenyl pyrazole insecticide as treatments for nets or curtains as in four villages near Kisumu as reported (Vulule et al., 1996).

2.7.2 Use of indoor residual spray.

The Indoor Residual Spray (IRS) is a standardized control method that involves spraying a dilute solution of insecticide on the interior walls and roofs of houses and domestic animal shelters so as to kill indoor resting and flying females or repel mosquito vectors from entering the houses (WHO, 2006 ). Indoor residual spraying has been used in much of the world including Asia, the Pacific, Latin America and Africa, where its use has been limited to areas where malaria is prevalent (WHO, 2006).
The objective of residual spraying is to spray houses at the right time, with the right insecticide, on the right surface, at the required dosage and at adequate intervals of time so that the lifespan of the vector species is shortened throughout the transmission season (Shiff, 2002). The Indoor Residual Spray (IRS) using lambda-cyhalothrin insecticide were carried out in targeted houses at Ighu village in Kakamega district of Western Kenya (Zhou et al., 2010). Insecticides used gave good results though with serious environmental setbacks and logistically demanding (Curtis, 1994). Irrespective of the particular ecosystem affected, the environmental, cost and public health impact of using insecticides for malaria control should be weighed against the potential benefit of improving and sustaining public health.

2.7.3 Personal protective measures against mosquito bites.

Mosquito repellent are substances that drive away mosquitoes and other biting insects. Currently used repellents are in the form of creams, lotions and aerosols (Service, 2004). Repellents like petroleum jellies are applied directly on the exposed skin or to clothing and other fabrics like bed nets and anti-mosquito screens to protect against mosquito biting (Curtis et al., 1998). Insecticide vaporizers like mosquito coils and vaporizing mats have a deterrent effect hence prevent mosquitoes from entering a room. They also have excito-repellent effect which irritates and disturbs mosquitoes after contact. Permethrin treated clothing prevent mosquito bites but the limitation is that protection lasts a few hours after which time reapplication is necessary. Commercial products may be too expensive for many communities so local plants and leaves are often burnt to produce smoke which repels mosquitoes (Seyoum et al., 2002). Other precaution observed is that
the mosquito repellent should never come into contact with eyes or mouth and should not be ingested under any circumstances (Miller et al., 1991).

2.7.4 Genetic control of mosquitoes

Most common method is through release of laboratory reared sterile male mosquitoes into the wild or production of sterile adults of both sexes. The creation of refractory mosquitoes as a vector that does not transmit human plasmodia or as a vector that feeds on animals instead of human is confounded with a lot of challenges based on ecological, epidemiological uncertainty, ethical and regulatory issues (Enserink, 2002). The goal of gene based mosquito control projects is either to kill the insect or make them benign.

Infecting mosquitoes with a transgenic fungus could drastically cut their ability to transmit malaria, existing efforts to develop fungal malaria control focus on slowly killing mosquitoes before they have the chance to pass on Plasmodium, the malaria parasite (Riehle et al., 2006). This approach relies on mosquitoes being inoculated with parasitic fungus soon after Plasmodium infection, which limits their use.

The researchers genetically modified (GM) the fungus Metarhizium anisopliae, which infects mosquitoes on contact, to express molecules which impede the entrance of sporozoites the cells that malaria parasites produce to infect new hosts to the salivary gland of the mosquitoes, reducing the number that can be passed to humans through a bite (Farenhorst et al., 2008).
The GM fungi reduced the number of sporozoites in mosquito salivary glands by up to 98 per cent compared to those infected with the non-GM fungi. Within just two days of infection 80 per cent of mosquitoes could not transmit malaria anymore compared to only 14 per cent of fungi-free mosquitoes and 32 per cent of those infected with non-GM fungi. The results confirmed that such transgenic fungi can block transmission of the disease even by mosquitoes with advanced malaria infections (Farenhorst et al., 2008).

**2.8 Environmental management control**

This is the planning, organization and evaluating of deliberate changes of environmental factors with the aim of preventing the propagation of vectors and reducing human vector pathogen contact (Shiff, 2002; Service, 2004). Specifications for environmental management vary with local ecosystem structure, and hence there is no uniform environmental management recipe that is appropriate in all settings (Singer et al., 2005). Environmental management practices can be very effective and was the primary tool used to eradicate malaria from US and Europe (CDC, 2004). Current environmental techniques used by researches include environmental modification, environmental manipulation and modification of human habitations/behaviours (Ault, 1994).

Environmental modification involves a physical change to potential mosquito breeding areas designed to prevent, eliminate or reduce vector habitat (Lindsay et al., 2004). Modification of larval habitats creates suitable conditions for different types of mosquito species that were previously either absent or uncommon (Service, 2004). Principal methods include drainage, land levelling, filling small ponds or water collecting depressions and planting of eucalyptus trees (Konradsen et al., 2004).
Environmental manipulation refers to activities that reduce larval breeding sites of the vector mosquito through temporary changes to the aquatic habitat in which larvae develop. Techniques include changing water levels in reservoirs, flushing streams or canals, providing intermittent irrigation to rice paddies, changing water salinity and management of vegetation in or around potential breeding sites (Rafatjah, 1988).

2.9 Biological control

Biological control involves the introduction or manipulation of predators, parasites and competitors to suppress vector populations (Scholte et al., 2003). Biological control tools have several advantages over chemical-insecticides. The most important ones include reduced risk of host resistance and minimal risk to the environment and living organisms (Service, 2004). The use of larval control is a very effective but under-utilized strategy for reducing malaria transmission intensity (Killeen et al., 2002). Currently, a number of novel tools based on biological interactions are undergoing development including fungal, bacterial, viral and protozoan pathogens. These methods aim at controlling mosquitoes before they disperse and acquire the potential to transmit diseases (Killeen et al., 2002).

2.9.1 Use of larvivorous fish

The most commonly used predator is the larvivorous fish *Gambusia affinis*. It is a warm water fish, small in size, relatively tolerant to polluted water, feeds at the water surface, a prolific life bearer thus making it highly adaptable to many different mosquito larval habitats. Effectiveness of *G. affinis* has been evaluated as a potential predator of *An. gambiae* at Ighu in Kakamega district (Kweka et al., 2011). The disadvantage is the
mass rearing and the restocking programmes required in the approach are very expensive. Besides, the fish may not survive in some temporary breeding sites.

The use of *Poecilia reticulate* fish to control *Culex quinquefasciatus* in rivers and lakes in Cuba have been effective (Lima et al., 2010). The fish species can be reared and released into mosquito breeding sites. In habitats that do not have year-round water, they have to be released annually. However, the efficacy is highly variable and significant negative impacts on native fauna has been reported (Floore, 2006).

### 2.9.2 Use of insect predators

There are diverse natural enemies of mosquitoes and each has played a significant role in regulating mosquito densities (Kumar and Jiang, 2005). Some mosquito predators that prey upon mosquito larvae are insects in the orders Odonata, Coleoptera, Diptera (primarily aquatic predators) and Hemiptera (primary surface predators) (Shallan and Canyon, 2009). The predators are able to reach mosquitoes in some habitats that may be difficult to be controlled with other biological control measures. Mosquito predators feed on many species (polyphagous), while some feed on only one species (monophagous). Majority of the documented mosquito predators are polyphagous (Kumar et al., 2005)). It has also been shown that, the predators are more active in old breeding places where *Anopheles* mosquitoes rarely occur and also solve the problem of resistant mosquitoes (Kweka et al., 2011).

### 2.9.3 Use of insect growth regulators

Larvicides continuous use have setbacks since most are harmful to arthropods that co-exist with mosquitoes in their natural breeding habitats (Hanafi- Bajd et al., 2006). They
include synthetic larvicide like insect growth regulator (IGRS) which has greater persistence, apparent lack of non-target effects and low probability of cross-resistance with other compounds used as larvicides. It acts by binding to Juvenile hormone receptors in the immature form of an insect (Rachid et al., 2009).

2.9.4 Use of fungi

The use of entomopathogenic fungi, as an alternative method for malaria vector control, seems promising. Fungal species belonging to the genera Coelomomyces, Culicinomyces, Beauveria, Metarhizium, Lagenidium, and Entomophthora were mostly considered when studying the role of fungus in vector disease control (Bukhari et al., 2010). Unlike other infectious agents, fungus does not require host ingestion; external contact with the insect’s cuticle is all that is needed to promote an infection.

The efficacy of *M. anisopliae* ICIPE-30 and *B. bassiana* I93-825 (IMI 391510) (2 × 10^{10} conidia m^{-2}) applied on mud panels (simulating walls of traditional Tanzanian houses), black cotton cloth and polyester netting was evaluated against adult *Anopheles gambiae sensu stricto*. Mosquitoes exposed to *M. anisopliae* conidia on mud panels had a greater daily risk of dying compared to those exposed to conidia on either netting or cotton cloth (P < 0.001) (Farenhorst et al., 2008). Mosquitoes exposed to *B. bassiana* conidia on mud panels or cotton cloth had similar daily risk of death (P = 0.14), and a higher risk than those exposed to treated polyester netting (P < 0.001). Residual activity of fungi declined over time; however, conidia remained pathogenic at 28 days post application, and were able to infect and kill 73 - 82% of mosquitoes within 14 days.
2.9.5 Zooprophylaxis

Zooprophylaxis is the control of vector-borne diseases by attracting vectors to domestic or wild animals to deviate the vector from humans to animals (Kawaguchi et al., 2004). The keeping of animals like cattle close to human habitation may reduce transmission of malaria by zoophilic and exophilic vectors like An. arabiensis.

However, introduction of domestic animals may increase mosquito density thereby enhancing, rather than reducing, malaria transmission (Sota and Mogi, 1989). It is now known that presence of livestock increases mosquito fitness by supplying more blood, but requires the basic reproductive ratio of malaria parasite since the livestock act as a dead end host of the parasite Plasmodium species has a closed transmission cycle between humans and mosquitoes (Kawaguchi et al., 2004).

Saul (2003) observed that zooprophylaxis may be inefficient with realistic values of host searching by mosquitoes and the associated vector mortality although use of animals as bait to attract mosquitoes is predicted to be a promising strategy. In an earlier study, (Muturi et al., 2007) observed that the zoophilic tendency of malaria vectors in irrigated areas of Mwea accounts partly for low malaria transmission rates despite the presence of higher vector density highlighting the potential of zooprophylaxis in malaria control. Therefore, the intervention of integrated vector management may be a better option.
2.9.6 Use of *Bacillus thuringiensis var israelensis* and *Bacillus sphaericus briquets*

FourStar larvicide is a unique microbial that terminates mosquito larval development using the naturally occurring bacteria, *B. sphaericus (Bs)* and *B. thuringiensis israelensis (Bti)*. These active ingredients are inherent to soil and contain protein crystals that, when ingested, rupture the gut wall of the larvae causing larval death. *Bacillus thuringiensis israelensis (Bti)* is a gram-positive, spore forming, aerobic bacterium that is found in a variety of habitats. During sporulation, *Bti* produces a spherical, parasporal inclusion that contains larvicidal proteins with activity against mosquitoes, blackflies, and fungus gnats. Some formulation advances have improved its activity, *Bti* has a relatively short life in heavily polluted waters, but it has a high level of safety for non-target organisms and humans (Lacey, 2007). *Bacillus thuringiensis israelensis (Bti)* provides broad spectrum activity, rapid control, and low potential for resistance; while *Bs* exhibits extended residual control, efficacy in polluted water, and high target specificity (Lacey, 2007).

Microbial larvicides have several advantages over other mosquito control agents due to high efficacy but also environmental safety (WHO, 1999), which makes them powerful vector control tools that are gaining more ground for disease control in Africa and other parts of the tropics. The efficacy and efficiency of some biological formulation of *Bti/Bs* of water dispersible granular (WDG) *Bti* (vector Bac.) and *Bs* (vecto/ex valent Bioscience Corp. Illinois USA) have been tested around Lake Victoria, Western Kenya for the control of larval *An. gambiae sensu lato* Giles mosquitoes (Fillinger *et al.*, 2007). The studies showed significant larval reductions up to eleven days post treatment at application doses of either 1 or 5 kg/ha.
The efficacy and persistence of two bacterial larvicides, Vectobac-DT (\textit{B. thuringiensis israelensis} (\textit{Bti}) and CulinexCombi (\textit{Bti} and \textit{B. sphaericus} (\textit{Bs})), were tested against \textit{An. gambiae} and \textit{C. quinquefasciatus} in temporarily unused swimming pools with rainwater in Malindi, Kenya (Kahindi \textit{et al}., 2008). Pre- and post-treatment larval densities were recorded by sampling with the standard WHO dipping technique for 8 consecutive days. The data showed that Vectobac-DT was highly effective against early instars of \textit{An. gambiae} with 89\% reduction within 24 h but not as effective against the early stages of \textit{C. quinquefasciatus} with reduction of only 46\%. CulinexCombi resulted in high mortalities to early instars of both species with over 97\% reduction within 24 h, but showed a drastic reduction 48 h after.
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study site

The study was carried out at Emakakha location (0.08° N to 0.13° N and 34.6° E to 34.65° E) in Emuhaya sub-county Vihiga County (Figure 3.1). The sub-county has an area of about 179.20km² with a population of over 190,580 people (Kenya National Census, 2009). The terrain consists of small hills with elevation ranging between 1,400-1,600m above sea level. A mosaic of land use types in the region, variation in climatic factors and the topography make it suitable and stable habitat occurrence of malaria vector larvae.

3.1.1 Climatic conditions of Emuhaya sub-county.

Emuhaya sub-county has a bimodal pattern of rainfall with long rains occurring from April to June and short rains between August to September with yearly inter-annual variations in precipitation. Dry season is from December to March with an annual average rainfall of about 1200mm and annual mean temperature between 18°C - 22°C.

3.1.2 Community living in Emuhaya sub-county.

Emakakha is inhabited by Abanyore sub tribe of the Luhya ethnic group. The community practice diversified subsistence farming mostly maize (*Zea mays*), beans (*Phaseolus spp*), pigweed (*Amaranthus spp*), finger millet (*Eleusine coracana*), bananas (*Musa paradisiaca*), cabbages and kales (*Brassica spp*) and sweet potatoes (*Ipomea batatas*), livestock is domesticated by use of tethering grazing methods in most homesteads and the settlement is densely populated. Most of the houses in the study area are mud walled, rusty roofs or grass thatched houses which allow mosquitoes to enter and leave. Permanent houses with large compounds due to extended families are a frequent phenomenon.
Figure 3.1: A map showing the study area, Emakakha location in Emuhaya sub-County.

3.2 Study design

The research was conducted within a period of eight months. Six design sites measuring 2km by 2km each were identified using satellite images on the land topography (Figure 3.2). Three sites were intervention sectors while the other three sectors were the control
sites. Each sector had 30 identified habitats (Figure 3.3). The criteria for site selection was based on the presence of relative permanence of aquatic habitats in the area.

**Figure 3.2:** Map of the study area larval aquatic sites. Zone 1 (site A control site, B intervention), zone 2 (site C intervention, site D control) and zone 3 (site E intervention, F control).
HABITAT FIELD STUDY: SAMPLE SIZE FLOW CHART

110 LARVAL HABITATS

Pre-application monitoring of habitat for two months

60 SELECTED HABITATS

Post application monitoring
Six months

30 INTERVENTIONS

30 CONTROLS

Figure 3.3: Sample size flow chart showing pre-application and post application monitoring of productive and suitable larval sites selection.
3.3 Larval habitat characteristics

Larval habitat mapping was done in six different sites, each site had ten larval productive sites making a total of sixty. Three sites were control while the other three intervention sites. A survey of the diverse larval habitats types present in each of the six sites was conducted and each identified habitat assigned a specific code and longitudinally followed for sampling. Larval habitat types were identified and sampled using a wooden dipper and a pipette separately for mosquito larvae and pupae since geographical positioning system coordinates had been recorded per habitat. Different larval habitats both man-made and natural were selected. They included larval habitats with different vegetative covered by couch grass growing along water banks (Plate 3.1 A) small water channels (Plate 3.1 B), manmade open water pool (Plate 3.1 C) and swamps (Plate 3.1 D). The habitats without any vegetation were grouped under 'no vegetation'. Vegetation was measured visually by estimating the percentage of the larval habitat covered. In case of mixed vegetation, the dominant vegetation (covering >5%) in the habitat was recorded.

The length, width and depth of each larval habitat were estimated visually and recorded in order to estimate the correct FourStar larvicide dosage. Other factors like water turbidity, presence or absence of other invertebrates were not assessed during the entire study period in all habitats.

Mean rainfall for the study site was obtained from the Kenya department of meteorology which assisted us during the study period from January to March 2013. Data collection on daily basis was done between 8.00am and 11.00am since Emuhaya sub-county receives convectional rainfall due to its proximity to Lake Victoria in Kisumu, rain was
experienced mostly in the afternoon. The month of June recorded the highest amount of precipitation, it reduced gradually the eighth month of the year. The larval habitats were grouped according to their stability and productivity into semi-permanent habitats and permanent habitats. Permanent habitats had water all the year round while semi-permanent had water for 3-6 months. In each site ten permanent and semi-permanent habitats were selected for mosquito larval sampling. To determine productivity, the habitats were sampled and monitored once per week for the presence of aquatic stages of Anopheline and Culicine mosquitoes.
Plate 3.1: Photographs showing larval habitats with different vegetative cover. (A) couch grass growing along water banks, (B) a small water channel, (C) manmade open water pool and (D) a swamp.
3.4 Field application of FourStar briquets microbial larvicides

Application of the FourStar briquets larvicide of *B. thuringiensis israelensis* (Bti) and *B. sphaericus* (Bs) was done by dropping briquets on targeted larval habitats. The habitats included abandoned ponds, water gardens, standing water in small channels, water holding receptacles but not intended for human consumption, manmade and natural habitats and small channels of stagnant water. Field application of briquets was done only to the intervention sites. One briquet was applied to aquatic habitat which was identified using Global Positioning System (GPS) measuring 3mx3m. Sites larger than 3mx3m one additional 30gm briquet was added to evaluate the extend of larvicidal activity for about 180 days.

The developmental stages of mosquitoes were recorded in a distribution table. Dipping was done in the morning hours between 8-10 am once per week in all the habitat sectors. Larval mortality sampling was ascertained within a period of twenty six weeks or 180 days.

3.5 Collection and identification of Anopheline larvae and pupae

Selection of mosquito larvae and pupae were done in both intervention and control sixty sites of aquatic habitat. The sampling and counting of mosquito larvae at different mosquito developmental stages were conducted daily at each larval site. Drainage ditches habitats were thirty, man hole fifteen habitats, small swamps ten and five ponds. This was done using a standard (350ml) blank white plastic larval sampling dipper (Clark R) with a wooden handle by dipping up to 10 times in each habitat. The dipper was immersed in the breeding pool at an angle of 45 degrees at water edge surfaces and close to aquatic plants (WHO, 2002). All individual *Anopheles* mosquito larvae in developmental stages
L1, L2, L3, L4 and pupae were transferred in a tray and picked using a pipette (Plate 3.2). The collection was done at intervals of 2-3 minutes between each dip.

Plate 3.2: Photograph of field assistants dipping in one of the larval aquatic habitat.

Mosquito larvae and pupae were identified in the field as according to their morphological characteristics as described by (Gillies and Coetzee, 1987), counted,
recorded and returned back to their aquatic habitat. Morphologically *An. gambiae* larvae are light in colour, big size and have whitish stripe on the neck while *An. funestus* are smaller in size, dark in colour with no stripe around the neck. The Anopheline larvae were further separated according to their developmental stages by morphological characteristics (Gillies Coetzee, 1987). The morphological features examined included the distance between the inner clypeal hairs, long mesopleural hairs, thoracic hairs, pulmate hairs, saddle hairs, tergal plates and accessory plates.

### 3.6 Differences between Anopheline and Culicine larvae

Anopheline mosquito larvae were differentiated from Culicine based on distinct morphological features that included the presence of abdominal palmate hairs and tergal plates. The pupae of Anopheline morphological features which were examined included the along breathing trumpet and abroad apical short peg like spine on abdominal segment 2-7 or 3-7.
Plate 3.3: Photograph showing sorting and counting larvae in different development stages.
3.7 Data management and analysis

Data was recorded in the larval distribution sheet (Appendix 1) which showed habitat number, habitat type vegetative cover percentage, Geographical Positioning System readings and the number of Anopheline individuals by species and developmental stages. The information was later transferred to the computer Ms excel spreadsheet. Monitoring of specific selected control and intervention sites were identified by use of Geographical Positioning Systems (GPS). Correlation between mean numbers of the Anopheline over time for two species in different treated and control larval habitat were tested.

The significant difference between the mean numbers of larvae obtained from treatments and control habitats during pre-treatment and post-treatment were analysed using student t-test. Significant effects tested were considered significant at P<0.05. These analyses were carried out using the Statistical Package for Social Scientists (SPSS for windows version 21.0).

3.8 Ethical clearance

Scientific and ethical approval to use FourStar briquets was granted by Kenya Medical Research Institute (KEMRI/ RES/7/3/1) before the onset of this research. Verbal consent to seek permission to access and sample mosquito larvae in aquatic habitats on private farms, was sought from households whose land were located on the study site in Emakakha location, Emuhaya sub-county.
CHAPTER FOUR: RESULTS

4.1 Larval habitat types of Anopheline larvae

The immature Anopheline developmental stages which were collected before treatment from both the control and treatments sites were 1,390. *Anopheles gambiae* was the most abundant species, accounting for 1010 (73%) of the total larvae collected during the pretreatment period, while *An. funestus* were 380 (27%) of the larvae collected (Table 1). *Anopheles gambiae* pupae were 50 (62%) while *An. funestus* were 31 (38%) before treatment. Of the 1060 *An. gambiae* sampled within the six months period were early larval instars 1 and 2 constituted 637 (60%), instars 3 and 4 had 373 (35.2%) and 50 (4.7%) pupae developmental stage. A total of 411 *An. funestus* were sampled with early larval instars 1 and 2 accounting for 235 (57.2%), late larval instars 3 and 4 were 145 (35.3%) and pupae 31 (7.5%).

Table 4.1: The number of Anopheline mosquito aquatic stages in different habitat types in Emakakha.

<table>
<thead>
<tr>
<th>Habitat type</th>
<th>Total number of Anopheline mosquito aquatic developmental stages.</th>
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<tr>
<td></td>
<td><em>Anopheles gambiae</em></td>
<td><em>Anopheles funestus</em></td>
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<tr>
<td></td>
<td>Early instars</td>
<td>Late instars</td>
<td>pupae</td>
<td>Early instars</td>
<td>Late instars</td>
<td>pupae</td>
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<tr>
<td>Drainage ditch (15)</td>
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<td>220</td>
<td>25</td>
<td>145</td>
<td>94</td>
<td>17</td>
</tr>
<tr>
<td>Rock pool and man hole (15)</td>
<td>123</td>
<td>93</td>
<td>13</td>
<td>60</td>
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<td>8</td>
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<tr>
<td>Small swamp (15)</td>
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<td>8</td>
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<td>10</td>
<td>5</td>
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<tr>
<td>Pond (15)</td>
<td>32</td>
<td>10</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total (60)</td>
<td>637</td>
<td>373</td>
<td>50</td>
<td>235</td>
<td>145</td>
<td>31</td>
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</tbody>
</table>
A total of sixty habitats were frequently colonized by *Anopheles* larvae during pretreatment monitoring. Habitats that had vegetation, as well as those without vegetation were present in different frequencies and percentages estimation among the sites. The most common vegetation was the short grass (*Cynodon dactylon*) and the long couch grass (*Cynodon nlemfuensis*) which grew in or along aquatic habitats.

The most frequently encountered larval habitats were drainage canals (50%), rock pools and manmade open pools (25%) while the small swamps recorded (17%) and ponds (8%). Drainage canals recorded a relatively higher larvae abundance of Anopheline immature stages 876 (60%) as compared to ponds which recorded the lowest numbers 58 (4%). Eighty three percent of all habitats monitored and recorded for the entire study period were either manmade or were associated with human activities namely drainage ditches, man-made holes, rock pools, and ponds (Table 4.1). Drainage canals, rock pool, manhole, small swamps, and ponds constituted most productive and stable mosquito breeding habitats.

### 4.2 Effectiveness of Bti/Bs on *Anopheles gambiae* mosquito larvae

All treatments were significantly different from the control ($P < 0.05$). *Anopheles gambiae* early (L1 and L2) instar stages significance was at ($P=0.021$) with over 80% reduction while the late (L3 and L4) *An. gambiae* significance was at ($P=0.018$) based on a two sample t-test (Figure 4.1). The first two months of larviding successfully reduced drastically the numbers of all larvae stages of *An. gambiae* by 100% in all the treatment sites while, the third to the sixth month 97-87% reduction was realized.
The highest mean densities of \textit{An. gambiae} larvae in the control sites were collected in the month of April while the lowest density was recorded in June. Comparing these to the intervention sites, the mean number of larvae which were collected were low with gradual increase in the third to sixth month.

\textbf{Figure 4.1}: Effects of FourStar briquets on \textit{Anopheles gambiae} larvae

\textbf{4.3 Effectiveness of Bti/Bs on Anopheles funestus mosquito larvae}

All the treated sites achieved almost 100\% larval mortality within the first two months after treatment (Figure 4.2). From the third to the sixth month, an overall 98-78\% reduction was achieved in all the intervention sites. When compared with the control, \textit{An. funestus} early (L1-L2) instar stages were significantly different (0.032). The \textit{An. funestus} late (L3-L4) instar stages were significantly lower at (0.03) using the student t-test.
Figure 4.2: Effects of FourStar briquets on *Anopheles funestus* larvae

4.4 *Anopheles gambiae* and *Anopheles funestus* mosquitoes larval densities in intervention and control habitat sites

*Anopheles gambiae* larvae responded to the Bti/Bs application and reached almost 100% mortality in the first two months (March and April) on all the four habitat types (Figure 4.3). Drainage canals recorded the highest mean larval densities in all the three treated sites at 38%, manmade open pools 32%, ponds 17% and the small swamps 13%. A strong correlation between the mean numbers of *Anopheles gambiae* in all the treated sites over time were significant ($P=0.001$).
The human action habitats of the manmade holes and drainage ditches comprised 70% mean densities of *An. gambiae* collected within the six months as opposed to local environmental conditions formed habitats of small swamps and ponds 30%. All the four habitats the mean larval increased progressively apart from the pond habitats where the mean larval density dropped in the month of June. Mean numbers of *Anopheles gambiae* larvae were highest in the month of August and lowest in the month of June in all the treatment larval habitats.

In the control larval habitats sites all the sites had no significance correlation over time with drainage ditches (*P*=0.240), manmade holes (*P*=0.644), ponds (*P*=0.053) and swamps (*P*=0.309). During all study period most larvae mean collected were from manmade holes (29%), drainage ditches (29%), ponds (22%) and swamps (20%) (Figure 4.3). *Anopheles gambiae* mean numbers were recorded the highest in the month of August in both drainage and manmade holes with mean numbers of (49.25) and (46.5) respectively. Control ponds and swamps larval habitat sites recorded highest means in March. Lowest mean numbers of larvae were recorded in June in three larval sites drainage (37.00), manmade holes (36.00) and small swamps (26.00). Ponds lowest mean numbers were recorded in the month of May (29.25) (Figure 4.3).

*Anopheles funestus* larvae were eliminated in all the treated habitat types within the first two months (March, April) reaching 100% mortality level (Figure 4.3). In the third month (May) the mean density of *An. funestus* started emerging in all the habitat types. All treated larval habitats showed a strong correlation significance (*P*=0.001) within months. Small swamps and ponds recorded the highest mean densities at 34% and 29% respectively within the six months period. The total mean densities of larvae within the
intervention period in the manmade holes and drainage ditches recorded 19% and 18% respectively.

In the control sites different mean densities were recorded in the six sampling months resulting to no significance correlation over months in three habitat types. Drainage ditches ($P=0.859$), manmade holes ($P=0.195$) and ponds ($P=0.442$). Swamps had a significance correlation over time at ($P=0.001$). More *Anopheles funestus* were recorded from small swamp habitats at 29%, ponds 25%, drainage ditches 24% and manmade holes 22%. Highest mean numbers per habitat type were drainage ditches (28.75) in the month of July, manmade holes (29.7) in March, ponds (39.5) March and small swamps (35.75) in August.
**Figure 4.3:** Mean numbers of *Anopheles gambiae* and *Anopheles funestus* larvae collected in (a) drainage ditches, (b) manmade holes, (C) ponds and (d) swamps treated with *Bti/Bs* and without *Bti/Bs* has control during the six months 2013.

### 4.5 *Bti/Bs* effectiveness on *Anopheles gambiae* pupal productivity

In the three treated larval habitat sites where FourStar briquette was applied pupal productivity of *An. gambiae* were greatly reduced by 100% for the first two months (March and April), 98-80% after the third to sixth months May, June, July and August (Figure 4.4). When *An. gambiae* productivity was compared within the treatment aquatic site B, C and E: P<0.05, the mortality for *An. gambiae* was lower at (P=0.049, P=0.031) and P=0.02 respectively.
**Figure 4.4**: Percentage reduction of *Anopheles gambiae* pupae using FourStar briquet of *Bti/Bs*.

4.6 *Bti/Bs Effectiveness on Anopheles funestus pupae productivity*

*Bti/Bs* briquets were effective and reduced *An. funestus* pupal productivity by 100% in the first two months (March and April), 99-87% after the third to sixth months May, June, July and August (Figure 4.5). *Anopheles funestus* as compared with the control sites, pupae were reduced by 93% (0.007). Treatment sites (B), (C) and (E) recorded different mortalities of *An. funestus*. Site E was higher at P=0.027, C was P=0.029 while site B had P=0.035
Figure 4.5: Effects of FourStar briquets on the percentage of the collected *Anopheles funestus* mosquito pupae.
CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

*Anopheles gambiae* was the abundant malaria vector and the most prevalent of the two species during the study period. The larval densities of mosquitoes reduced post treatment with microbial larvicide which was probably the result of a progressive reduction in ovipositional attraction of the breeding sites. Previous vector studies in Nandi indicated malaria vector composition of 98% *An. gambiae* complex and 2% *An. funestus* (Ernst *et al.*, 2006).

The use of microbial bio-larvicides long lasting briquets of *B. thuringiensis var israelensis* (*Bti*) and *B. sphaericus* (*Bs*) showed that in the three treated larval habitat sites larval productivity of *An. gambiae* were greatly reduced by 100% for the first two months, 97-87% after third to sixth months. This showed that *An. gambiae* larvae are highly susceptible to the FourStar briquets by overall 91% reduction in all intervention sites. These results agree also with the findings from Rydzanicz *et al.*, 2010 who found 100% mortality for three weeks and upto 60% in week five for *Bti/Bs* granules. Rydzanicz study outcome showed the importance of UV radiation from sunlight as a negative factor in the persistence of *Bti/Bs* in the environment hence reduction in microbial larvicide efficacy during degradation (Rydzanicz *et al.*, 2010). The two studies differed on larvicide formulations, application quantities, timing and larvicidal residual duration. These results of the present study confirm the observation of that formulation technology can enhance the activity of *Bti/Bs* larvicide products (Mulla *et al.*, 1999).
Equally the briquets were effective and reduced \textit{An. funestus} larval productivity by 100% in the first two months, 98-78\% after the third to sixth months. The results obtained agree with the findings from Majambere \textit{et al.}, 2007, where larviciding use of \textit{Bti} reduced Anopheline larval density by almost 90\%. It was noted that the early instars of both \textit{An. gambiae} and \textit{An. funestus} larvae were more susceptible to the \textit{Bti}/\textit{Bs} formulation at \textit{p}=0.0021 and \textit{p}=0.032 respectively. The late instars (L3 and L4) \textit{An. gambiae} \textit{p}=0.018 and \textit{An. funestus} \textit{p}=0.03 based on a two sample t-test. Larvae provided more reliable indicator for treatment efficacy hence FourStar larvicide had a significant reduction this is because they had more time to ingest lethal amounts of the toxins.

The choice for suitable places for female Anopheline to lay eggs is a key factor for the survival of immature stages, distribution pattern, density, development time, body size, survival and influence performance of the emerging adults (Mbogo \textit{et al.}, 2008). Temporary habitats held water for a short period of time approximately two weeks after the rainy season while permanent sites, on the other hand, held water for a longer period of time (approximately 2-3 months after the rains ended or fed by natural underground sources) and hence were more stable (Minakawa \textit{et al.}, 1999). The permanent habitats remained in the same location throughout the sampling period while temporary habitats changed depending on the availability of water. Man-made holes and drainage ditches comprised 70\% mean densities of \textit{An. gambiae} collected within the six months. These habitats are direct consequences of human actions, as opposed to small swamps and ponds 30\% which are mainly a result of the local environmental conditions.

The correlation relationship of the mean numbers of both \textit{Anopheles gambiae} and \textit{Anopheles funestus} over time on treated and untreated larval sites showed strong
significance and no significance results respectively. All treated sites were significant at (P=0.001) in drainage ditches, manmade holes, ponds and small swamps. All control with Anopheles gambiae were not significant over time, drainage ditches (p=0.240), manmade holes (p=0.644), ponds (p=0.053) and small swamps (p=0.309). Most of Anopheles gambiae larvae collected from treated sites were from drainage ditches sites (30%), manmade ditches (28%), ponds (22%) and small swamps (20%). Those Anopheles gambiae collected from control sites the highest percentage were obtained from both drainage ditches and manmade holes (29%) ponds (22%) and small swamps (20%). These results almost agrees with (Minakawa et al., 2002) findings on association between Anopheles gambiae larvae and habitat size conducted at Marani highland in Kisii county. He demonstrated that human action sites favours the proliferation of Anopheles gambiae larvae since they develop in freshwater habitats that are small size, temporal, clean and sun exposed. Further, larval density of small breeding sites might be increased due to a higher sampling intensity per unit area.

Anopheles funestus resurgence prevailed mostly in small swamps and ponds recording mean densities of 34% and 29% respectively within the six months period in intervention sites. Few larvae were found in manmade holes and drainage ditches recorded 18% and 19% respectively. All treated sites showed correlation significance over time at (p=0.001). Control untreated sites only three larval types recorded a no significance, drainage ditches (p=0.859), manmade holes (p=0.195) and ponds (p=0.442). Small swamps control sites recorded a significant correlation of (p=0.001). These may be attributed to higher percentages of vegetative cover and other aquatic forms in large habitats which served as other sources of food to mosquito larvae. Anopheles funestus
prefer permanent water bodies (>21 days) with aquatic vegetation with shade than sunny breeding sites (Gimnig et al., 2002). Manmade holes and drainage ditches had lower mean densities because they are relatively small, open sites exposed to sunlight for a long time increasing water temperature resulting to faster destruction of the protein toxin.

Nevertheless, efficacy of Bti/Bs was greater because of timely application, use of long lasting formulation and selection of productive and stable larval habitats. The briquet acted by releasing effective levels of Bs and Bti subspecies israelensis to the surface of water and extended the insecticidal activity for many days. The efficacy of the new briquets of 30 grams was 100% effective in all the two Anopheline species before gradually declining at the beginning of the third month, by the sixth month its residual activity was lesser effective. More importantly, pupation levels were very low in all the intervention sites which is considered the most important parameter for efficacy assessment of larval control measure (Mulla et al., 1999). Pupal population was drastically reduced and an overall reduction in mosquito emergence of 90% was achieved.

Effectiveness of the briquette was not realized 100% due to the onset of the long rains in western region as from April and possibly chemical contamination. Furthermore, the study was done under natural conditions and external factors such as water turbidity, nutrient content of the water, cannibalism, predation of immature stages, parasitism, pathogens, competition, water temperature and plant odours that could have either repelled or attracted female mosquitoes during oviposition were not controlled. The top five most observed predators that might have fed on Anopheline larvae and pupae were Gerrids, Veliids, Hydrometrids, Dytiscids and Notonectids. Predators are known to
increase as emergent vegetation becomes dense, considerably reducing the survival of An. gambiae and An. funestus larvae (Christie, 1958). Although predation among larvae was not determined in this study, some studies have shown that older larvae of Anopheline are able to prey on younger larvae as well as larvae of closely related species (Koenraadt and Takken, 2003). Though this kind of predation is also influenced by the abundance of food, cannibalism amongst larvae in this study was not observed. Other related studies have concluded that An. gambiae females tend to avoid oviposition sites containing older larvae instar (McCrae, 1984). Moreover, some larvae which survived may be due to feeding entirely on algae nutrition like spirogyra (Barbara et al., 2006) hence avoiding the protoxins crystal protein which released by Bti/Bs in water.

Nonetheless, occasional presence of heavy rains caused water out flow and the drifting of the briquets from sampling sites and therefore its insecticidal full dose could not have been achieved in all the instances hence compromising it’s duration of efficacy. In addition, when it rained heavily, flooding would occasionally occur especially in habitats along the river Etsaba and Jordan fringes.

Results obtained on the residual activity and larviciding duration efficacy of Bti/Bs disagree with the findings of (Fillinger and Lindsay, 2006), where larviciding reduced Anopheline larval density by 95%. The studies showed a shorter efficacy duration of eleven days since water dispersible granular were used.

This study is the first reported evaluation of FourStar briquet larvicides under operational conditions in rural western Kenya. The study demonstrates that malaria vector abundance
can be reduced by \textit{Bti}/\textit{Bs} briquets and sustained for extended time periods irrespective of high rainfall and large number of habitats available throughout the year.

The larval intervention strategies by use of microbial FourStar larvicide should therefore be appropriately focused at productive and suitable identified habitats for cost effectiveness and better results. The malaria vector distribution in the country is not uniform due to variation in climatic factors, particularly temperature and rainfall (President’s Malaria Initiative Kenya 2014).

The timing of briquets application is crucial since malaria is endemic with peaks of disease incidence occurring shortly after the rainy seasons. Increases in rainfall will directly result in an increase in the number and persistence of aquatic habitats, although the impact of rainfall will depend on parameters such as local evaporation rates, the soil percolation and the slope of terrain. If mitigation can be done just before the long rainy season in March-April and short rainy season in August-September then the number of mosquito vectors will be reduced drastically.

Mosquito larval control interventions can drastically reduce human exposure to malaria vectors, even in resource-poor rural African communities but needs to be further assessed in a variety of specific environments where they may find most utility. Rural Africa increasingly includes highly populated areas of endemic but low-to-moderate annual malaria transmission, where mosquito larval habitats are well defined, accessible and therefore manageable. In such environment, antilarval interventions can achieve significant reductions in mosquito numbers and may be highly appreciated and supported by local communities.
5.2 Conclusions

1. Overall, larviciding control reduced *Anopheles* larval density among the two anopheline larvae instars significantly when applied on productive and stable aquatic habitats. The results of this study suggest that under the circumstances in which it was conducted, the 30 grams *Bti/Bs* briquets was 100% effective in the first two months and gradually the efficacy started being less effective gradually in the subsequent months.

2. Intervention seasons timing resulted to three treated larval habitat sites larvae productivity of *An. gambiae* reduced by 100% for the first two months, 97-87% after third to sixth months. This showed that *An. gambiae* larvae are highly susceptible to FourStar briquets. The briquets were effective and reduced *An. funestus* larvae productivity by 100% in the first two months, 98-78% after third to sixth months.

5.3 Recommendations

1. Researches should integrate the use of Bti/Bs biolarvicide in vector control programs for reducing *Anopheles gambiae* and *Anopheles funestus* populations which lead to subsequent malaria transmission.

2. Timely re-application of *Bti/Bs* briquets for vector control and malaria prevention is recommended. The findings of this study suggest a two months re-application period for optimal results. Re-assessments of the effect of the *Bti/Bs* briquets treatments have to be conducted to observe persistence under specific environmental conditions.
REFERENCES


Appendix I

LARVAL DISTRIBUTION TABLE FORM AT EMAKAKHA, EMUHAYA SUB-COUNTY WESTERN KENYA.

ZONE.......... SITE............... DATE (day/month/year):-----/-------/-----

<table>
<thead>
<tr>
<th>Habitat No</th>
<th>Type</th>
<th>Veg. Cvr %</th>
<th>GPS Latitude</th>
<th>Reading Longitude</th>
<th>An. gambiae L1-L2</th>
<th>An. fune L1-2</th>
<th>Pupae L1-2</th>
<th>Pupae L1-2</th>
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</tbody>
</table>

Habitat Type (A, B, C AND D)

A. Drainage ditch         C. Swamp
B. Man made hole           D. Pond

Sample of GPS Reading:

Latitude N/S 0.12345        Longitude E/W 34.98765

Investigator

Name................................Signature................................