EVALUATION OF THE IMPACT OF *BACILLUS THURINGIENSIS ISRAELENSIS* AND *BACILLUS SPHAERICUS* ON POPULATION DYNAMICS OF *ANOPHELES* AND *CULEX* MOSQUITOES IN MWEA, KENYA

BY

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Evaluation of the impact of bacillus

DECLARATION

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

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We confirm that the candidate under our supervision carried out the work reported in this thesis and has been submitted for examination with our approval as supervisors.

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DEDICATION

To my daughters, Janet Koki and Monica Mumbua for their endurance in my absence during this study and my husband, Michael Kisini for his love and dedication.

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LIST OF ABBREVIATIONS AND ACRONYMS

BTI	*Bacillus thuringiensis israelensis*
BS	*Bacillus sphaericus*
DDT	Dichlorodiphenyltrichloroethane
DEET	Diethyl meta toluamide
DIMP	Di methyl phthalate
EIR	Entomological Inoculation Rate
IGR	Insect Growth Regulators
ICIPE	International Centre of Insect Physiology and Ecology
ITN	Insecticide Treated Bed nets
IRS	Insecticide Residual Spraying
IVM	Integrated Vector Management
MIAD	Mwea Irrigation and Agricultural Development Centre
MIS	Mwea Irrigation Scheme
PSC	Pyrethrum Spray Collection
WDG	Water Dispersible Granules
WHO	World Health Organization

ABSTRACT

Insecticide resistance of malaria vectors has caused great concern in malaria vector management and has accelerated the search for alternative methods of vector management. Integrated vector management (IVM) targeting both the adult and immature stages have been adopted with renewed interest in larval management with the aim of making it cost effective. The possibilities that targeting species succession processes in the mosquito larval habitats like irrigated rice agro-ecosystem calls for research to evaluate microbial larvicides as potential tool to be incorporated in IVM programmes. The population dynamics of the adult and larval mosquitoes in habitats within Mwea Irrigation Scheme and Mwea Irrigation and Agricultural Development centre (MIAD) was determined. A longitudinal study of 20 experimental plots was set up and treated with three different types of larvicides (*Bacillus thuringiensis israelensis* (Bti), *Bacillus sphaericus* (Bs) and a combination of Bti/Bs) and observed for a period of 16 weeks during rice growth. Three villages representing planned (Munyaka), and unplanned rice cultivation (Kiamachiri and Murinduko) were also studied. Eight different mosquito species that included culicine and *Anopheles* mosquito species were morphologically identified. The *Anopheles* species in decreasing order included *Anopheles gambiae* (72.5%), *Anopheles pharoensis* (24.84%), *Anopheles coustani* (1.25%), *Anopheles pretoriensis* (1.25%) *and Anopheles funestus* (0.16%). Among the culicines species, *Culex quinquefasciatus* (82.26%) were predominant followed by *Culex poicilipes* (10.33%) and *Culex annulioris* (7.41%). *Anopheles gambiae, Culex quinquefasciatus* and *An. pharoensis* occurred throughout the rice growth cycle, but their densities decreased each time after microbial larvicide application. The characteristics measured explained the vector density except for clear turbidity in the 3rd and 4th instars of *Anopheles* mosquitoes ($P<0.05$). Microbial larvicides reduced larval populations significantly ($F_{3,536}=7.56$ $p<0.0001$). Negative binomial regression showed *Anopheles* larval density to be significantly associated with habitat characteristics ($P<0.005$). Ditches had most *Anopheles* larvae with a mean of 0.82/dip (n=295) followed by pools 0.59/dip (n=99197) and seepage 0.44/dip (n=373). Indoor adult mosquito collection through Pyrethrum Spray Collection (PSC) showed that 97.01% (n=5780) of *Anopheles gambiae* and 2.83% (n=291) of *An. funestus* were the dominant species. These findings suggest that larvicidal application should target peridomestic habitats, paddies and associated canals between transplanting and tillering stages in order to achieve effective vector control.

CHAPTER ONE: INTRODUCTION

1.1 Background information

In the Sub-Saharan Africa, malaria infections are a major challenge to a large population and the crisis continues to worsen despite the ongoing efforts to fight the disease through integrated malaria management targeting both the vectors and the human reservoirs. The problem of malaria in Africa is largely aggravated by the presence of the highly efficient malaria vectors within the *Anopheles gambiae* Giles and *Anopheles funestus* Giles complexes that are widespread and difficult to control. Mosquito population densities tend to be high in aquatic habitats and rice fields in particular, and constitute an important source of vector mosquitoes (Lacey and Lacey, 1990). Mosquitoes are vectors of different diseases among them malaria, filariasis, rift valley fever, yellow fever, dengue, encephalitis as well as arboviruses. Vector-borne diseases are among the most important public health problems and obstacles to socio-economic development, particularly in the tropics.

Mosquito larval control by Integrated Vector Management (IVM) is essential as it helps to control the disease vectors before they disperse to transmit pathogens (Killeen *et al.,* 2002). Biological control where other living organisms are used as predators of mosquito larvae or their products as toxins against the larval mosquitoes has also been used. Other biological control agents are parasites, nematodes, worms and fungi that grow on the bodies of mosquitoes. Bacterial larvicides such as *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* are among the most widely used biological methods (De Berjac and Sutherland 1990). These interventions, however, ignore a fundamental biological phenomenon that species succession by avoidance

can substantially reduce effective coverage in terms of the proportion of the vector population that is covered and the overall impact on malaria transmission (Killeen *et al.*, 2002).

There have been no studies conducted in Mwea to show the effect of microbial larvicides on the succession pattern of mosquito species. Since *An. arabiensis* and *C. quinquefasciatus* outnumber the other species, it seems that at least in the rice fields during the growing season, the two species present the most significant public health problem in the area. *Anopheles arabiensis* is the main vector of malaria in the area and also a potential vector of Bancroftian filariasis and Onyong-nyong virus, whereas *C. quinquefasciatus* is a potential vector of Bancroftian filariasis and several arboviruses, (Mutero *et al.*, 2004a; Muturi *et al.*, 2006). The vector status of *An. pharoensis* in malaria transmission is considered secondary in the study area (Ijumba *et al.*, 1990). On the other hand, the status most of the other species collected, including *An. rufipes*, *An. coustani*, *An. maculipalpis*, *C. anulioris* and C. *poicilipes* remain uncertain. These findings demonstrate that rice fields have the potential to support a range of vector species capable of transmitting a myriad of mosquito borne diseases and could impact negatively on human health if not properly managed.

1.2 Statement of the problem

Mwea irrigation scheme is a rice growing area, which is grown in flooded conditions which are ideal breeding habitats for mosquitoes. The rapid increase in mosquito resistance to various insecticides and the growing public health concern has contributed to the search of other strategies of mosquito control. Insecticide resistance

has increased and is a significant barrier to the continued and dedicated use of organic chemicals such as organochlorines, organophosphates and synthetic pyrethroids.

1.3 Justification for the study

Due to toxicity and increasing ineffectiveness due to development of resistance, there is need to identify alternative vector control methods. There is no evidence of resistance to Bti and Bs that has been reported in the field situation despite over twenty years of use. This may be due to the multiple toxins present in the bacteria and unique mode of action. Microbial larvicides have several advantages over other mosquito control agents, among them high efficacy and environmental safety, for instance when applied in drinking water (WHO, 1999). This study investigated the effects of Bti and Bs on mosquito larvae in Mwea rice irrigated areas. The dynamics of the adult and larval populations and effects of the microbial agents on mosquito species successions pattern after microbial application in irrigated rice fields were established. The findings of this study will lead to implementing timely and species-specific larval control operations. Also the findings will provide a basis for developing guidelines for the long-term impact of individual mosquito control larvicides in aquatic habitats.

1.4 Research questions

i. How do microbial larvicides influence the colonization and succession of mosquito species in Mwea irrigated rice fields?

ii. What is the habitat diversity of mosquito species and their preferred breeding habitats in Mwea irrigation Scheme

iii. How does the population density of malaria vectors and species compositions in Mwea vary within months?

1.5 Null hypotheses

i. There is no significant impact of microbial larvicides on colonization and succession of mosquito species in Mwea irrigated rice fields.

ii. There is no significant variation in habitat diversity and preference of mosquitoes among different breeding habitats.

iii. There is no significant variation in population densities and species composition of mosquito vectors in Mwea.

1.6 Objectives of the study

1.6.1 General objective

To investigate the impacts of *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* on population dynamics of *Anopheles* and *Culex* mosquitoes in Mwea irrigation scheme.

1.6.2 Specific objectives

i. To determine the colonization and succession pattern of mosquito species in Mwea irrigation scheme after microbial larvicide application.

ii. To establish the habitat diversity of mosquito species and their preference for breeding in Mwea irrigation scheme.

iii. To determine the population densities of mosquito vectors and species composition in Mwea irrigation scheme.

CHAPTER TWO: LITERATURE REVIEW

2.1 Malaria vectors in Kenya

Female *Anopheles* mosquito species play an important role in the transmission of the most virulent malaria parasite *Plasmodium falciparum*. In Africa, malaria transmission is without doubt governed by the presence of the highly efficient anopheline vectors that are capable of maintaining transmission even at extremely low levels of vector abundance and causing severe malaria cases even during periods when little or no transmission is detectable thus highlighting the probability of severe malaria due to a single bite from infected mosquitoes (Mbogo *et al.,* 1995). Previous studies (Ijumba *et al.*, 1990; Mbogo *et al.*, 1993; 1995; Mutero *et al.,* 2004) in Kenya have shown that the overall vectorial situation in the country is dominated by *An. gambiae*, *An. arabiensis* and *An. funestus* but differing on importance with locality. For example, along the Coast and western Kenya, *An. gambiae s. s.* and *An. funestus* have been identified as the main vectors of malaria while in the irrigated areas like Mwea *An. funestus* and *An. arabiensis* are the main vectors of malaria (Ijumba *et al.*, 1990; Muturi *et al.*, 2007), the latter species being more predominant. These species occur throughout the year with peak population coinciding with seasonal rains.

2.2 Malaria parasites

There are four species of the genus *Plasmodium* that cause human malaria namely *Plasmodium falciparum* (Welch), *Plasmodium vivax* (Grassi and Feletti), *Plasmodium ovale* (Stephens), and *Plasmodium malariae* (Grassi and Feletti). *Plasmodium*

falciparum is probably the single most important parasite an African child encounters during his or her first few years of life. The transmission of *P. falciparum* is highly variable and is associated with severe disease and death for persons with little or no acquired immunity, such as infants, when the immunity gained through maternal antibodies during gestation has waned (McGregor, 1964; Greenwood, 1991). Seventy mosquito species of the genus *Anopheles* are responsible for the transmission of Plasmodia.

2.3 Medical importance of mosquitoes

Female *Anopheles* mosquitoes must feed on blood from animals and/ or humans to develop eggs as vertebrate blood contains essential proteins necessary for this process (Kogan, 1990). Malaria is transmitted through the bite of an infected female mosquito during feeding, which frequently takes place at night although daytime biting may occur. Different mosquito species have been shown to acquire their blood meal preferentially from either certain animals or from human hosts in different localities (Githeko *et al.*, 1993; Hadis *et al.*, 1997; Muriu *et al.*, 2008). Some species prefer to feed in forests, outside or indoors (WHO, 1997). Those that bite in the early evening are more difficult to avoid than species that feed at night.

Anopheles mosquitoes are well known as the major malaria vectors. *An. gambiae* is the major vector of malaria in sub-Saharan Africa and is probably the most important

vector of human pathogen in the world (Gwadz and Collins, 1996). The species is particularly infamous throughout tropical Africa as a prevalent killer and debilitator of human life by acting as a transmission agent of the human malaria parasite *Plasmodium* (Walker *et al.,* 1997). When the adult female *Anopheles* mosquito takes a blood meal from humans it is capable of transmitting the malaria parasites (Mbogo *et al.,* 1993).

Filariasis is a disease caused by infection with filarial nematode parasites of the family Filariadidae. The most common species are in genera *Wuchereria, Brugia, Mansoneilla* and *Dilofilaria. Wuchereria bancrofti* is the most common species in East Africa causing bancroftian (lymphatic) filariasis or Elephantiasis (Meillon, 1977). Though filarial diseases are rarely fatal the consequences of the infection can cause significant personal and social economic hardship for those who are infected (Nissen and Johann-Liang, 2001). Morbidity is usually due to host immune reaction to the microfilaria or developing adult in different areas of the body.

`Onyong` nyong virus an arboviral infection of humans causing a self-limiting febrile disease that is characterized by arthragia or arthritis in the knee, ankle and small joints of the extremities followed by a maculopapular rash in 60-79% of cases. The infection is usually found in Africa where Chandler and Highton, (1975) stated that it occurred in Ahero, Kenya. The virus is transmitted to humans through an infective bite from an infected *Anopheles* mosquito species especially *An. gambiae* and *An. funestus*. The incubation period is about eight days (Chandler and Highton, 1975).

Mosquitoes present greatest biting nuisance to humans and other animals apart from being vectors of pathogens. In their quest to get a blood meal the mosquito disturbs the serenity surrounding an individual. Hungry female mosquitoes especially the parous ones actively seek their host from early evening to late morning hours (Service, 1985). They become more troublesome when they are large in numbers especially after rainy period.

2.4 The life cycle of *Anopheles* mosquitoes

Under optimum conditions, the complete cycle from egg to adult takes 10-11days depending on the environmental conditions (Service, 1996). After mating and feeding on blood, the female *An. gambiae* lays some 50-200 brownish or blackish boat-shaped eggs. The eggs are laid singly on the water surface and measure 1mm in length. Viable eggs hatch into larvae within 2-3 days in the tropics, but in cooler temperate regions they may not hatch until after 4 to 7 days or longer (Service, 1996). The larvae are aquatic, metapneustic and pass through four larval stages. While on the water, they lie parallel to the surface to allow water intake and surface feeding. Mosquito larvae can be distinguished from all other aquatic insects by being legless and having a bulbous thorax that is wider than the head and abdomen. All mosquito larvae require water in which to develop. No mosquito has larvae that can withstand desiccation although they may be able to survive short period in wet mud (Service, 1996).

The pupae are unable to feed. Being less dense than water, they normally spend most of their time at the water surface breathing through the paired respiratory trumpets. The pupal coat splits dorsally and the adult emerges. Pupa duration is determined by temperature; in tropical countries, it is usually 2-3days (Service, 1996). The newly emerged adults inflate its wings, and separate grooming its head appendages before flying away (Kettle, 1992). When the progeny of any one egg batch emerge as adults, the males emerge first and become sexually competent within 24 hours after emergence. By the time the females emerge, the males are ready for mating. The females require a blood meal for ovarian development followed by the maturation and oviposition of a batch of eggs (Gillies, 1955).

The percentage of the eggs, larvae and pupae that survive to the adults is unknown. However, there is usually heavy mortality, especially among other factors (Service, 1996). Larval loss due to predation is one of the factors that reduce the numbers that develop into adults. The understanding of the life cycle is important as it can help one to know the crucial stage to target for control.

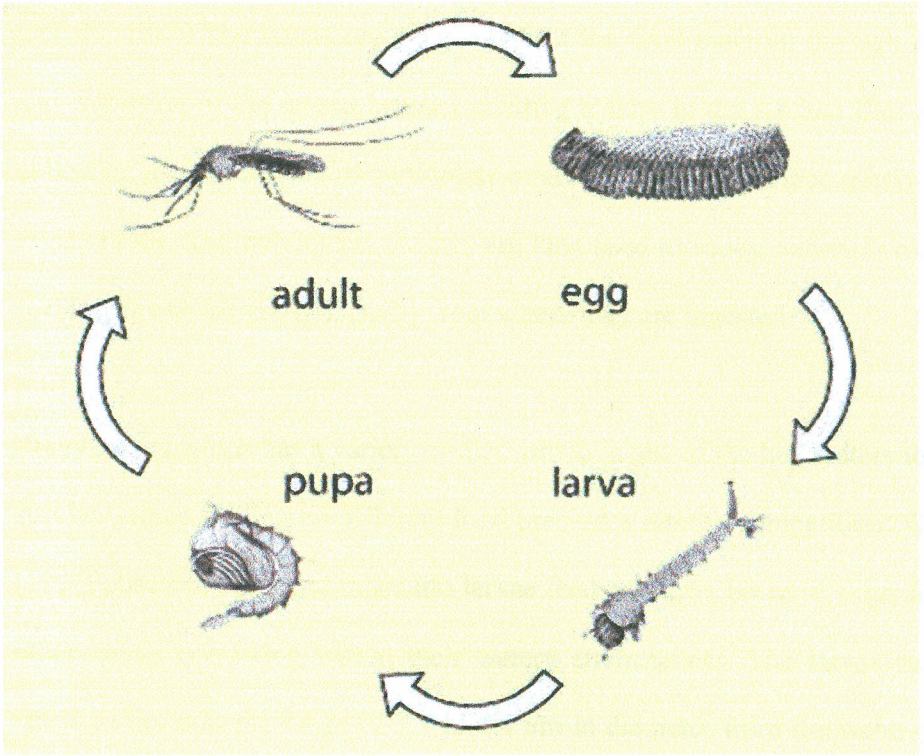

Figure 1: Schematic illustration of the life cycle of *Anopheles* vectors of malaria

2.5 Larval feeding behavior

There are different functional feeding behaviors that are recognized among mosquito larvae, for example; scavengers, bottom feeders, filter feeders and predatory species. Lane and Crosskey (1993) indicated that the filter feeders are in two groups; those that strain the phytoplanktons and zooplanktons from the water and those that feed from the water surface.

Anopheles gambiae is a surface filter feeder whose mouth and mouth-brushes are ventral and the larvae has to swivel the head through an 180°C in order to sweep the

under surface of water and waft very fine food particles towards the mouth (Mac Donald, 1986).The larvae are able to collect the food material through rapid flexion and extension of the lateral brushes creating a flow in the surface film allowing the collection and processing of particulate materials from the surface micro-layer (Merit *et al.*, 1990). The two lateral brushes are then used to scoop materials on or near the surface film to the pre-oral cavity from where they are ingested.

Anopheles gambiae has a variety of diet, which is one of the key factors to the species survival, since feeding on different food precludes intense competition. Walker *et al.*, (1997) observes that most mosquito larvae feed on algae, bacteria, organic debris and other larvae coexisting within their watery environment. The larvae mainly ingest small particles in the range of 1.5 to 4.5 um in diameter from the water-air interface (Qwartz and Collins, 1996; Rejminkova *et al.*, 2000). The abundance of the food allows rapid development of the larvae as the bacteria and the algae also secret exopolymers and other dissolved organic matter that promote rapid larval growth (Notton, 1996).

2.5.1 Larval habitats

Ovipositing female mosquitoes lay their eggs in habitats that would allow rapid development of immature stages into adulthood and where natural selection would favour them. Habitat selection in mosquitoes is influenced by many factors such as predators, conspecific immatures, vegetation, various chemicals, bacteria, size and

shape of the habitat (Blaustein, 1993). Larval habitats are important determinants of adult mosquito distribution and abundance.

The typical oviposition sites and eventual larval habitats are small temporary water bodies that are exposed to full sunlight (Gwadz and Collins, 1996). In essence *An. gambiae* larvae are associated with small turbid, temporary habitats with algae and little or no emergent aquatic vegetations. The larvae of *An. gambiae* generally colonize habitats such as hoofs and foot prints at the edges of streams and along trails, edges of small burrow pits used to build up road beds, forests pools, fallen leaves, empty snail shells and other small puddies (Lane and Crosskey, 1993). During the dry season the larvae may be found in temporary pools left by drying streams and pools associated with human activities (Qwartz and Collins, 1996). Ijumba *et al.* (1997) reported that *An. gambiae* are the pioneer species, which rapidly colonize recently flooded rice fields although they decline in abundance as the rice grows and begins to cover the water surface.

The larvae only survive under certain environmental conditions. They are unable to tolerate or survive desiccation although Qwartz and Collins (1996) observed that the larvae might remain quiescent in the unhatched eggs on dump soil for two weeks. They also noted that the larvae could crawl across dump soil and move to adjacent pools when theirs dries up. Temperature of the water is also essential in larval habitats. Moderate and extreme temperatures inimical to life are exceptional. The

larvae usually favour shallow unpolluted pools with some degree of oxygen, exposed to constant sunlight and protected from extreme heat (Walker *et al.*, 1997).

2.5.2 Larval mortalities

Most species colonizing temporary water collections such as *An. gambiae*, larval mortalities are caused by desiccation. Torrential rains may flush out some habitats and lead to high larval mortality. Density has also been shown to be a factor in larval mortality as indicated by Fisher and Carpenter, (1982) who observed that in *Aedes trieriatus* larval density increase resulted in decreased larval survivorship and population rates, clearly indicating density-dependent mortality. Gimnig *et al.*, (2000) showed that under conditions of extreme larval crowding or defined larval diet larval development is slowed, survivorship reduced and eventual adult body size decreased as larval density increases.

2.5.3 Larval predation

Among many causes of larval mortality, predation often seems to be the most important single factor determining population size (Gwadz and Collins, 1996). Predation is more intense on the immature stages of the mosquitoes than among the adults. Several studies have been carried on the contribution of predation on the larval mortality. Andis and Meek, (1982) showed that predation by *Psorophora columbiae* was the major source of larval mortality in rice fields of Lousiana. Sempaha (1982) also indicated that predators caused 83.2% - 91.0% of mortality recorded in *Aedes africana* larvae in Uganda. Predators also contributed to the larval death of *Culiseta*

longiareolata in artificial habitats. 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). In essence, predation influences the population dynamics of anophelines (Liard and Miles, 1985) and may be the most important single factor determining population size (Reisen *et al.*, 1989; Service, 1993).

Mosquito eggs seem to have few natural enemies probably due to the short period they take of the entire life cycle. Larvae and pupae have many predators such as adult flies of Dolichopiidae, Scathophagidae, Ephyridea, Anthomylidea and Muscidae, which prey upon larvae and pupae on the mosquito surface (Lane and Crosskey, 1993). Spiders, for example Lycosids, dash nimbly across water surface and seize emerging mosquitoes while cockroaches, mites, ants, and other scavengers devour stranded larvae and eggs. Walker *et al.*, (1997) found that dragon flies, tiger beetles, carnivorous plants for example, bladderwort (Urticilaria) prey on larvae, as is also the case with pond skaters, Gambusia and other surface feeding organisms. It has also been shown that, the predators are more active in old breeding places where *Anopheles* mosquitoes rarely occur. In his work on mortality of immature stages, Service (1995) noted that in pools and small ponds where *An. gambiae* colonize, there were fewer aquatic and non-aquatic species of predation.

2.6 Control of adult *Anopheles* mosquitoes

Current malaria control strategies emphasize domestic protection against adult mosquitoes with insecticides and improved access to medical services. However, malaria prevention by killing adult mosquitoes is generally favored because moderately reducing mosquito longevity can radically suppress community level transmission (Killeen *et al.,* 2002). House designs in tropical areas have ventilations that allow easy entry of mosquitoes. These ventilations are sometimes fitted with insecticide-treated screens that prevent entry while maintaining some ventilation (WHO, 1997). Since some malaria vectors enter houses to bite and rest, the use of insecticide treated nets (ITNs) for personal protection against A*nopheles* mosquitoes has become popular (Zaim *et al.*, 2000). Insecticide-impregnated bed nets have the advantage of acting as a physical barrier and also reduce mosquito densities by killing them.

2.6.1. House screening

House structures in the tropical countries are provided with ventilations. Openings such as windows and eaves allow easy entry of flying insects like mosquitoes. Thus screening of these openings prevents insects from entering while maintaining some ventilation (WHO, 1997). Treated screening or curtains provide a toxic barrier that prevent entry of mosquitoes into houses and may kill them at the same time. Mutinga *et al.* (1992) demonstrated that screens impregnated with permethrin can be effective against *An. gambiae s. s.* for about six months. However, the concept of house screening is not new. Towards the end of the 19th Century, it was demonstrated that screening of houses against mosquitoes can protect people from malaria (Lindsay *et*

al., 2002). Screening require less netting material compared to bed nets and hence cheaper, in addition to requiring little or no attention from members of household.

2.6.2 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 the malaria parasite since the livestock act as a dead-end host of the parasite because the human malaria 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 to insecticides is predicted to be a promising strategy. Furthermore, in Europe, changing of agricultural practices resulted in more effective zooprophylaxis and has been attributed to the disappearance of malaria (Bruce-Chwatt, 1985). However, zooprophylaxis alone may not be effective

intervention method against malaria (Bogh *et al.*, 2002). In an earlier study, Bogh *et al.* (2001) observed that a passive zooprophylaxis using cattle does not alter the individual exposure to parasites in Gambia. Muturi *et al.*, (2007) observed that the zoophilic tendency of malaria vectors in irrigated areas in 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.

2.6.3 Insecticide treated bed nets

Insecticide treated bed nets (ITNs) provide a simple means of protection of humans against night biting mosquitoes including malaria vectors. There is however little evidence that on a community basis untreated bed nets reduces malaria prevalence. During the 1980s it became fashionable to sleep under insecticides impregnated bed nets. The insecticides used have been pyrethroids, for example, permethrin, although in some countries such as China, the slightly more toxic deltamethrin was widely used. Such nets exert a repellent as well as an insecticidal effect on mosquitoes and moreover still reduce biting even when nets become torn.

The use of insecticide treated bed nets (ITNs) with pyrethroids seems to be the most promising available method of controlling malaria in endemic tropical countries. Several studies have shown that the use of ITNs is effective in reducing morbidity and mortality due to malaria (Lengeler, 2000). A trial of permethrin-impregnated bed nets in Gambia resulted in a 70% reduction in clinical cases of malaria in children who slept under nets (Alonso *et al.*, 1991). A series of impregnated bed net studies in

Kenya documented a reduction in incidence of infections in children under six years during both the high and low transmission seasons (Mbogo *et al.*, 1996).

Entomological inoculation rates (EIR) declined by 50% during the high season. Nevertheless acquisition of new infections still occurred at a high rate during the high transmission season, and it was estimated that 100% of the children would have been infected with *P. falciparum* within 13.6 weeks in the bed net villages and within 10.6 weeks for the controls (Beach *et al.*, 1993). According to Mbogo *et al.* (1996) permethrin impregnated bed nets exert a major impact upon abundance of the indoor resting principal vectors of *P. falciparum* in the coastal villages of Kenya. Densities of *An. gambiae s. l* and *An. funestus* were nine times lower in the houses where ITNs were in use as compared to households where no nets were used (Mbogo *et al.*, 1996). However, it has been observed that, there is a tendency of behavior change by vector species in the areas where bed nets are in use (Mbogo *et al.*, 1996). Studies in Kilifi showed that, a significant proportion of malaria vectors appeared to bite earlier in the evening in houses where ITNs were used, with greater tendency towards exophagy rather than the typical endophagy of most anthropophilic *An. gambiae s. l*(Mbogo *et al.*, 1996). This change in behavior renders the use of ITNs less effective as mosquitoes will often bite when bed nets are not in use. The insecticides for ITNs are not widely available or affordable to most communities in Kenya. Also non-compliance in the proper use of nets and failure to maintain the insecticide treatment rhythm has increased the malaria transmission rates.

2.6.4 Insect repellants

Repellents are substances that drive away mosquitoes and other biting insects. The best of this is the diethyl toluamide (DEET) that can sometimes remain effective for 6-13 hours. Di methyl phthalate (DIMP) is more effective against certain *Anopheles* species while cyclohexamethylene carbamide is effective against some mosquitoes for 10-16 hours. 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.,* 1999). Insecticide vaporizers like mosquito coils and vaporizing mats have a deterrent effect hence prevent mosquitoes from entering a room. They also have excitorepellent effect which irritates and disturbs mosquitoes after contact. Commercial products may be too expensive for many communities so local plants and leaves are often burned to produce smoke which repels mosquitoes (Seyoum *et al.,* 2002a). Placing branches or whole plants inside houses to repel mosquitoes is another method of application practiced by communities in western Kenya. Seyoum *et al.* (2002b) showed that live and intact potted plants can reduce exposure to malaria vector mosquitoes.

2.6.5 Indoor insecticide residual spraying

Adulticides are the products aimed at controlling the adult flying population of mosquitoes. The common insecticides used to control vectors are DDT, malathion and synthetic pyrethroids. Dichlorodiphenyltrichloroethane (DDT) is effective for residual

indoor spraying but was banned from use due to its non-biodegradable effects (Curtis, 1994; 1999). Malathion replaced DDT but has high refusal rate and shorter residual activity (White, 1999), hence could not be continued for spraying. Pyrethroids are quick acting and highly toxic to insects. They are also safer for humans and mammals at recommended dosages as well as being relatively safe for the environment because of their quick breakdown in the soil (WHO, 1997). However, most insects have developed resistance to most insecticides.

Indoor insecticide residual spraying (IRS) is the most effective and feasible method of chemical control of malaria vectors and is the principal method of killing adult mosquitoes that rest indoors. However, control programmes frequently lack well trained field staff to apply the insecticides and to maintain the application equipment (WHO, 2002a). Besides, residual insecticides pollute the environment and may have adverse effects on humans and other forms of animal life.

2.6.6 Outdoor protection (space spraying)

Space spraying is designed to provide a rapid knockdown and mortality of vectors with no or little residual effects as part of the integrated vector management. This method aims at rapid reduction of flying insects populations to reduce or interrupt the transmission cycle of insect-borne disease during emergency or epidemic situations (WHO, 2003). Nevertheless, space spraying is very costly and may not be economical in rural settings where homes are scattered.

2.7 *Anopheles* larval control

Vector control measures directed against the larvae may be the only effective approach especially when the mosquitoes bite outdoors. Larval control involves elimination of breeding places by drainage or filling, larviciding, biological and microbial methods. The anti-larval measures tend to be more successful when breeding places are not extensive and thus cheap (WHO, 1968). Planting of aquatic plants, for example *Azzola* species (Urticularia) around *An. gambiae* breeding sites help in their control as they become unattractive for habitation. The reduction in vector breeding areas involves a great deal of work without any immediate recognizable benefit to the community (WHO, 1984), with resultant lessening of community interest in such operations. Ecological changes, increase in weather variability and a warming trend appear to be playing a major role in the spread of malaria (WHO, 1997) and mosquito survivorship. This has necessitated search for new approaches to malaria control and adoption of an integrated approach, which include environmental management, chemical and biological control, health education and legislation.

2.7.1 Environmental management

This involves practices that create unfavorable habitats for larval survival. It may also involve the elimination of aquatic habitats. Environmental modification which is long term and this may be achieved through alteration of the breeding sites of the vectors by filling ponds and marshes on a permanent basis. Environmental manipulation which is short term and this can be done by repeatedly removing vegetation from ponds and canals and clearing premises (WHO, 1997). Deforestation can also

eliminate the malaria vectors by destroying adult mosquito resting habitats. Planting vegetation along streams and reservoirs make habitats inimical to sun loving *An. gambiae*. However, this approach has not achieved much because it is impossible, to fill in all the scattered, small and temporary collections of water (Service, 1996). Besides, the approach is labour intensive and costly thus untenable. There is therefore, need to focus on more practical larval control methods such as biological control and microbial larvicides.

2.7.2 Synthetic larvicides

Laviciding involves the application of insecticides to actual or potential larval habitats with an intention of killing the immature stages. This includes the use of chemical insecticides, biological agents/biotoxins or insect growth regulators (IGRs). The aim is to control larval populations or prevent adult emergence. Spraying breeding water surfaces with synthetic inorganic larvicides like temephos (Abate), methoprene or petroleum oils is also done to kill the immature stages of mosquito vectors although this may lead to pollution of the environment and mosquitoes develop resistance to the chemicals used.

The period of effectiveness of chemical larvicides depends greatly on the quality of the water treated and may vary from months in clean water to only a few days in more polluted water (WHO, 1984). The organochlorine such as DDT, are not recommended for larviciding because of their persistence in the environment. Likewise, insecticides with high mammalian toxicity are not recommended as larvicides. Although

considered ecologically unacceptable in some situations, oils such as malariol, flit MLO or fuel oil can be effective for limited periods when applied to breeding sites.

2.7.3 Biological control

Biological control is the use of natural enemies to control pests and disease vectors (WHO, 1999). Organisms used include predators, parasites and pathogens. These reduce the vector population to below an acceptable threshold level that impedes or lower the transmission of the pathogen in the vector or change the pathogen into a form that is not transmissible in the vector. Due to insecticide resistance and the adverse environmental impact of insecticide use, considerable resources have been devoted to the search for biological control agents. Several attempts have been made to control mosquito larvae by biological means.

2.7.3.1 Predators

To date, only larvivorous fish have been used successfully in malaria control projects, but these cases are few. The use of *Gambusia affinis* successfully reduced malaria incidences in Italy and Greece, where malaria transmission was unstable (Wickramasinghe and Costa, 1986). Prior to this, other fishes such as Armagosa pupfish (*Cyprinodea nevadensis armogosae*) and Guppies (*Poecillia reticulata*) were used (Moyle, 1976). These species reduced the number of mosquito larvae by almost half in most of the larval habitats during the entire study period (Moyle, 1976). The use of larvivorous fish, however, has its own disadvantages. The mass rearing and the restocking programmes required in the approach are very expensive. Besides, the fishes may not survive in some temporary breeding sites. Invertebrate predators such

as coleopterans, dipterans and hemipterans have also been considered as biological control agents but are difficult to rear *en mass*, feed non- specifically, and do not persist once vector target densities are reached (Rishikesh *et al.*, 1988).

2.7.3.2 Parasites

Rishikesh *et al.* (1988) have summarized efforts to identify useful pathogens and parasites including viruses, bacteria, fungi, nematodes and sporozoa. The main pathogens include the fungi *Coleomomyces* spp, *Culicinomyces clavosporus*, *Metarhizium anisopliae* and *Lagenidium gigantium* which have demonstrated little or no adverse effects on populations of invertebrate and vertebrate non-target organisms (Lawrence and Cynthia, 1990). Other control agents include the protozoan *Nosema algerae* and the mermithid nematode, *Romanomermis culicivorax* (Rishikesh *et al.*, 1988). None of these agents have shown any promise for wide scale larval control, having proven difficult to rear and store, as well as being unstable or inefficient under the field conditions.

2.7.3.3 Bacterial agents

The bacteria endospore toxins produced by various strains of *Bacillus* species such as *B. thuringiensis israelensis* H-14 and *B. sphaericus* have also been used as larvicidal agents (De Berjac and Sutherland, 1990; Davidson and Yousten, 1990). Their most attractive feature in vector control stems from the purported failure to induce mechanisms of resistance that confer cross-resistance to other classes of insecticides. They can also be produced on a local level with far less capital outlay that would be

required for traditional insecticides. Unfortunately the *Bacillus* toxins are still relatively expensive. Since they have no residual activity, they require frequent application or are only suitable for environments where a one-time control measure produces a valuable outcome.

Bacillus thuringiensis (Bt) (Bacillaceae) is a gram-positive rod shaped, spore forming bacterium capable of producing large crystal protein inclusions during sporulation, which are mainly responsible for the insecticidal properties of the species. The specificity and unique mode of action of Bt has historically been considered a safe option for pest control and often the preferred insect control method in IVM programs. Strains of *Bacillus sphaericus* with high toxicity to mosquito larvae have been used for the control of disease vectors. Larvicidal activity of *B. sphaericus* is caused by a parasporal crystal produced during sporulation. The crystal contains a binary toxin formed by the polypeptides of 42 and 51kDa, named bin A and bin B, respectively. The binary toxin complex act by binding specifically to midgut receptors of mosquito larvae, as has been demonstrated in some susceptible mosquito species (Charles and Nielsen Le Roux, 2000; Silva-Filha *et al.*, 1995).

2.7.4 Insect growth regulators

The insect growth regulators (IGRs) have also become more widely used over the past twelve years (WHO, 1996). IGRS can be divided into juvenile hormone analogues (juvenoids) such as pyriproxyfen, methoprene, fenoxycarb and hydroprene and chitin synthesis inhibitors such as triflumuron, cyromazine, diflubenzoron or chitin synthetase inhibitors (Laird and Miles, 1985). These compounds generally have no

toxicity to other non-target organisms. They are relatively specific to the insect and primarily active against the immature stages of mosquitoes, however they may kill beneficial insects. Currently, the most widely used IGR is Altosid® (Laird and Miles, 1985) that has no remarkable effects on non-target aquatic organisms. However, there is a great desire to obtain larvicides or IGR from in exhaustible natural sources such as plants that can be cultivated, extracted and biodegradable compounds obtained, to avoid environmental pollution (WHO, 1996).

2.7.5 Ethnobotanicals

It has been shown that some limonoids, quinones, alkaloids, flavonoids, terpenoids, polyacetylenes and butyl-amides extracted from plants show a high degree of larvicidal activity against mosquito larvae (Kubo *et al.,* 1984). For instance, piperine and wisanine are alkaloids that were isolated from *Piper guineense* and were found to be very active on *Aedes aegypti* larvae (Addae-Mensah and Achieng, 1986). The same extract has been shown to have larval activity against *An. gambiae* (Okinyo, 2002). Limonoids such as azadirachtin from *Azandiracta indica* and terpernoids such as 5-E - ocimenone from *Tagetes minuta* have been reported to possess larvicidal activity against mosquito larva (Maradufu *et al.*, 1978).

Larvicidal activity of long chain fatty amides such as N- isobuyl-2E, 4E, 8Z, 10Z-dodeca- 2, 4, 8, 10- tetraenamide isolated from *Spilanthes mauritiana* have been reported (Jondiko, 1989). The amides from *Zanthoxylum gilleti* (Fagara macrophylla) have also been reported as larvicides against *Culex* species (Kubo *et al.,* 1984). Their

efficiency against *Anopheles gambiae* has since been demonstrated (Okinyo, 2002). Other plants that have been successfully tested for larvicidal activity include:

Vernonia ammophila, Swartzia madagarensis, Pogestennon cablin, Sium suave, Datira candida, Achryrolcline satureoides, Petiverria alliacea and *Gardenia lutea* amongst others (Michael *et al.,* 1991). The efficacy of most of these plant extracts as potential larvicides have only been tested under laboratory conditions. However, their efficiency under natural field conditions against natural anopheline larval populations has not been investigated. As much as these investigations have not been done, their potential in mosquito control is thought to be high.

2.8 Factors affecting distribution of malaria

Several factors significantly affect the distribution of malaria in space and time, between persons, and the resulting morbidity and mortality. Some of these factors include; the natural environment through its vector populations, interaction between vector and parasite, parasite determinants and some of its genetically controlled characteristics, host-biological factors, behavioral, social and economic elements.

Factors pertaining to the natural environment for example, the availability of the larval habitats for malaria vectors influences the distribution of malaria in the area. The local rainfall produces rain pools favored by most malaria vector species for example *An. gambiae s. s* and *An. arabiensis*. The slope of the land and the nature of the soil are some of the other environmentally related factors, which affect the type of surface water available and its persistence and subsequently the increase of local

malaria vector populations. The optimal range of temperature and the relative humidity for most malaria vectors is 20-30°C and 70-80% respectively (Wernsdorfer and McGregor, 1986). Increasing the temperature increases the growth of vector population by shortening the interval from oviposition to adult emergence and vice versa. Biological factors such as immune response and genetics, as well as socio-economic status, living and working conditions, exposure to vectors and human behavior, all play a critical role in determining a persons risk of malaria infection and hence illness.

Greenwood, (1989) found that climatic and topographic features determine the ecology of both human and arthropod hosts as well as their contacts. Ponds and reservoir in an area were important in malaria transmission as they were the breeding sites for mosquitoes. Many other environmental factors have been found to influence the level of exposure to the mosquitoes of an individual resident in malaria endemic area. Greenwood, (1989) listed some of them as place and type of residence, the use of anti-mosquito measures and the position of the house.

Irrigation schemes and hydroelectric projects were likely to increase the intensity of malaria transmission and may change the seasonal transmission dependent on rainfall into perennial transmission by maintaining a population of the vector anophelines mosquitoes through out the year (Robert *et al.,* 1985). Large numbers of *An. gambiae s. l* found in the rice growing areas during the dry season at Ahero (Githeko *et al.*, 1993b) and at Mwea-Tebere irrigation scheme (Ijumba *et al.*, 1990) were maintained by irrigation water.

Schofied and white, (1984) demonstrated that the house design and situation was important in protecting its residents from mosquitoes. Better-designed houses with mosquito-proof screens at the windows and insectide treated curtains have fewer mosquitos compared to poor designed houses with large eaves and many openings at the walls.

The role of human behavior in relation to vector and the transmission of malaria were summarized by Greenwood, (1989). He asserted that human behavior which operates at several different levels depending on the number of others involved and the social structure within a community could greatly influence malaria transmission. He quantified human behavior in terms of the methods of avoidance of mosquito bites which included insecticide treated nets, house screening, mosquito coils, smoky fires, household insecticide and specialized house construction. In domestic situation zooprophylaxis was important in reducing the frequency of mosquito feeding on humans and hence malaria transmission (Hess and Hayes, 1970). Mosquitoes especially *An. arabiensis* would be defracted to feed on cattle and other vertebrates hosts if an attempt to feed on humans is thwarted. All these factors contributed to affecting the degree of man-vector contact.

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study area

This study was conducted in Mwea Division in Kirinyanga district, 100 km Northeast of Nairobi (Figure 3.1). Mwea Rice Scheme occupies the lower altitude zone of Kirinyanga district in an expansive low-lying area characterized by black cotton soil. The annual rainfall varies from a maximum of 1,626mm to a minimum of 356mm, with an average of 950mm per year – rainfall pattern is bimodal, long rains in March-June and short rains in October-December. The average temperatures are 21.3°C (range 16.0-25.6°C) and the relative humidity averages 59.5% (range 52-67%). According to the 1999 national census, Mwea division has an estimated 150,000 persons in 25,000 households (GOK, 2000). The irrigation scheme is located in the west central region of Mwea Division and covers an area of about 13,640 ha. Over 50% of the scheme is used for irrigation, while the remaining is used for subsistence farming, grazing and other community activities.

The current study was carried out in Mwea Irrigation and Agricultural Development Centre (MIAD) experimental plot measuring 1 acre (63m×63m) and three selected villages within and outside the irrigation scheme. The villages were selected from the scheme for larval and adult sampling. The selection plan was to assess villages within three categories as follows: 1) one village within the planned irrigation scheme known as Munyaka, 2) one village in the unplanned rice growing area known as Kiamachiri and, 3) one village falling in a non-irrigated area of Murinduko.

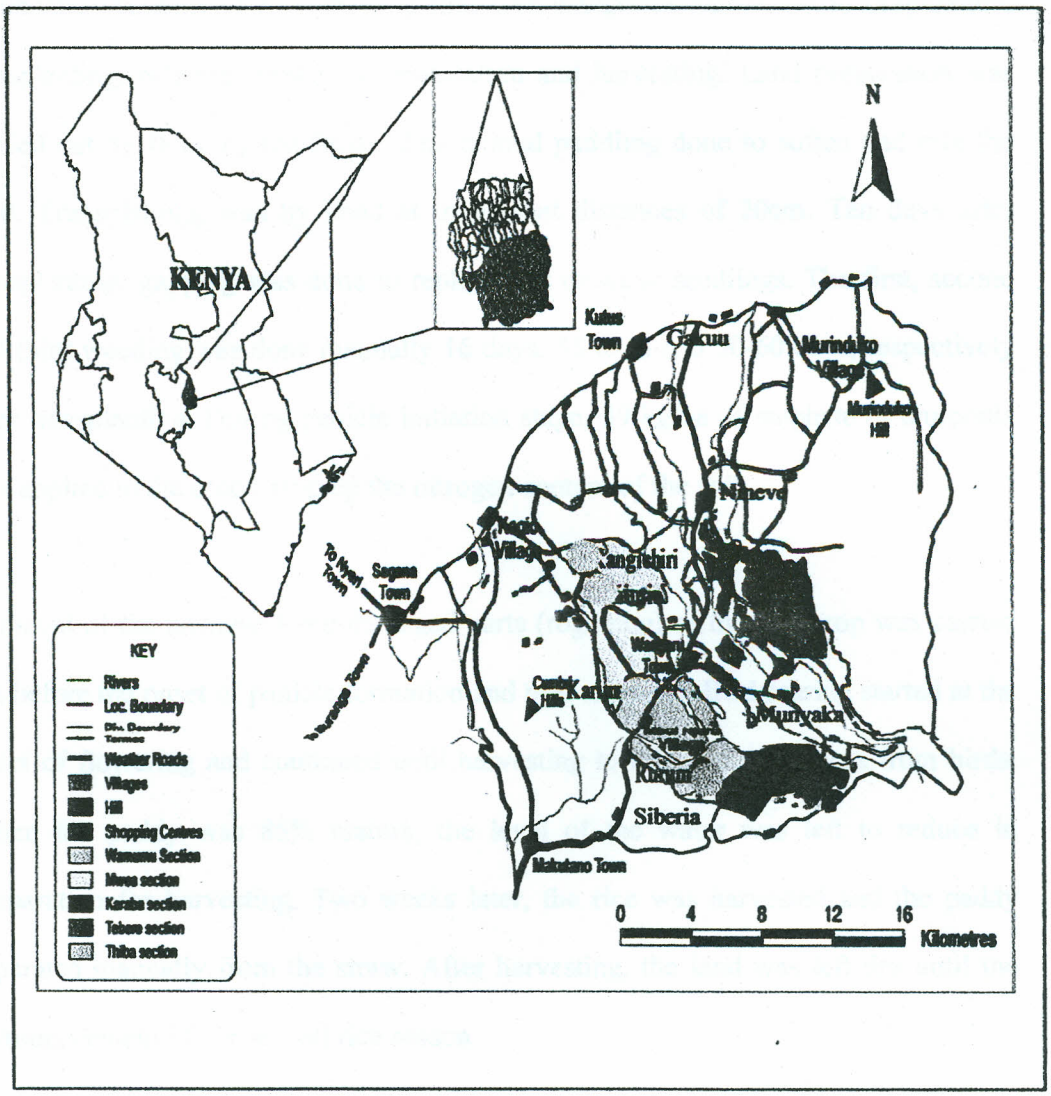

Figure 2: Mwea Rice Irrigation Scheme and study villages.

(Source: Mutero *et al.*, 2004)

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3.2 Rice growth cycle

Rice (aromatic variety: Basmati 217) was grown in a nursery according to normal practices of farmers in the scheme. Rice growth cycle was characterized using the agronomic standard of rice growing into five categories namely: land preparation, transplanting, tillering, flowering, maturation and harvesting. Land preparation was carried out by flooding the fields, then animal paddling done to soften and mix the mud. Transplanting was by hand at inter-plant distances of 20cm. Ten days after transplanting, gapping was done to replace dry or weak seedlings. The first, second and third weeding was done manually 16 days, 35 days and 50-60 days, respectively after transplanting. During panicle initiation stage, 39 kg/ha of sulphate of ammonia was applied to the crop to top up the nitrogen content of the soil.

Removal of the unwanted and damaged parts (rogueing) of the rice crop was carried out before the onset of panicle formation and near maturity. Bird scaring started at the onset of flowering and continued until harvesting to minimize crop loss from birds. When the paddy was 85% mature, the level of the water was left to reduce in preparation for harvesting. Two weeks later, the rice was harvested and the paddy separated manually from the straw. After harvesting, the land was left dry until the commencement of the second rice season.

3.3 Microbial larviciding

The experimental plot measuring 1 acre (63m×63m) was divided into nine blocks each measuring 50.4m×3.15m each with 10 sub-plots each measuring 6.3m×3.15m (Plate1). The 90 plots were hydrologically isolated using unidirectional inflow and

outflow canals to avoid water mixing between plots. Rice was planted in all the ninety subplots and 20 subplots were randomly selected, treated with microbial larvicides and monitored throughout the rice growing cycle.

Three treatments were used in the study- Bti (Vectobac VBC 60035), Bs (Vectolex ABG 6189) and the new improved combined formulation obtained from Valent Biosciences Corporation and the control. The three treatments and control each with five replicates were randomly assigned in the 20 subplots within the acre, and longitudinally monitored by sampling for mosquito species succession each alternate day for a period of three months from transplanting to rice maturity. The subplots were retreated with the microbial larvicides after every twenty days. During each sampling occasion 20 dips were sampled in each subplot using dippers assigned to each microbial larvicide. The 20 dips were pooled together to make a sample collection for the particular subplot during the sampling occasion and the immature stages sorted into respective stages. The numbers of the different mosquitoes in respective stages were counted in the field and recorded as well as the number of different predator species.

Plate 1: Land preparation in the experimental plots for rice growth in Mwea Irrigation and Agricultural Development centre (January 2008).

3.3.1 Mosquito species succession

A sub-sample of the early instar larvae (both culicine and anophelines) was reared in plastic containers within a screened house up to late instar stages (third and fourth stages) or adults for identification. The third and fourth larval instars and pupae collected were transferred to the laboratory for further identification and reared in pupal cages up to adult emergence for identification. The characteristics recorded for

each plot included rice height, water depth, water cover, rice stage, number of tillers and *Azolla* cover.

3.3.2 Description of larval habitats

The depth of water was measured from the same point every visit using a metal ruler. The height of the rice was measured weekly after transplanting. Water turbidity was examined against a white tray and scored in four classes namely: Clear, Low, Medium and High. Other factors which were assessed are emergent plant cover and presence or absence of other invertebrates. Rice growth cycle was categorized as: land preparation, transplanting, tillering, flowering, maturation and harvesting.

3.3.3 Experimental and mosquito sampling design

A standard mosquito dipper (350ml) was used in the treated and untreated habitats. Up to 20 dips were taken at 90 cm intervals along the edge of each larval habitat. Five replicates of each microbial larvicide were used as follows; five subplots for Bti, five for Bs, five for the new improved combined formulation and five for control. Rice was planted in the control subplots like the treated subplots but the control was not treated. A complete randomized design was used to compare untreated control plots to those treated with water dispersible granules of Bti and Bs. Utilization of the different subplots by mosquitoes was monitored through presence of mosquito egg rafts and larvae. Collection of egg rafts and first instar larvae from each subplot in the aquatic habitat was done each alternate day following microbial application.

3.3.4 Field application of the microbial larvicides

Bacillus thurgiensis israelensis and *Bacillus sphaericus* were applied on separate rice subplots two weeks after transplanting rice. Treatment of the rice subplots was with water dispersible granules of Bti Vectobac (VBC 60035) at a dosage of 22.4g per subplot, Bs Vectolex (ABG 6189) at a dosage of 44.8g per subplot and the new improved combined formulation at a dosage of 40.0g per subplot measuring 6.3m×3.15m. The WDG formulations were broadcasted on the water surface by hand as evenly as possible. Three treatment cycles with twenty-day intervals were carried out with the same application rate. The experiment was repeated during different rice growth stages (tillering and booting stage) to determine the effect of colonization and succession patterns of mosquito species.

3.4 Habitat diversity and preference by mosquito species

For larval habitat mapping an area of 0.5 - 1km around the perimeter of each village was divided into sections based on the number of potential larval habitats. A survey of the diverse larval habitats types present in each of the three selected villages was conducted and each identified habitat was assigned a specific code and longitudinally followed for sampling. Larval habitat types were identified and sampled separately for mosquito larvae. Representative larval habitats from each category were picked randomly and categorized as paddy, canal, ditch, marsh, pit, seepage, tyre track, stream, fish pond, pools, and water reservoirs. A maximum of 25 larval habitats from each village within and outside the village were selected. Up to 20 dips were taken at

intervals along the edge of each larval habitat using standard mosquito dipper (350ml). The larvae from each habitat were placed separately in plastic bags and transported to the laboratory where they were sorted counted, identified and recorded.

3.5 Indoor mosquito population

Adult mosquitoes were sampled fortnightly in ten randomly selected houses in each of the three villages. Most of the houses in the three villages were mud walled roofed with iron sheets. Few houses were built with stones. Almost all the houses had eaves. The adult indoor resting mosquitoes were sampled from the selected houses using the pyrethrum spray collection (PSC) method in the morning hours between 07:00-11:30h (WHO, 1975). White sheets were spread on the floor of each room of house after covering or removing foodstuffs and the house was sprayed with 0.3% pyrethrum in water. All the knocked-down mosquitoes were collected in labeled Petri dishes, and transported to the laboratory for identification and further processing.

3.6 Laboratory processing of mosquito samples

The mosquito larvae collected were sorted out and classified to the subfamily level and grouped according to the developmental stage. The late instars were preserved immediately in 70% ethanol and were later identified morphologically to species using taxonomic keys (Gillies and Coetzee, 1987; Hopkins, 1952). The morphological features which were examined included the distance between inner clypeal hairs, long

mesopleural hairs, which are simple, thoracic hairs, palmate hairs, saddle hair, main tergal plates and the accessory plates. The first and second instars were reared in plastic dishes under semi field conditions. Those instars that survived to third instars were preserved and identified morphologically. The pupae were kept in the insectary in cages to develop into adults. The emergent adult mosquitoes were used for species identification (Gillies and Coetzee, 1987; Hopkins, 1952).

3.7 Ethical considerations

An experimental permit for the use of microbial larvicides from Valent Biosciences Corporation was granted by the Kenyan Pest Control Products Board in Nairobi. Community consent for the implementation of operational mosquito larval control was sought through community meetings initiated by the local area chief. Individual consent was sought from every household where access was needed for adult collection and/or larval habitat monitoring and control. Clearance was also obtained from Kenyatta University.

3.8 Data management and analysis

The data collected was entered into Excel spreadsheets and analyzed using SAS (version 9.1) for windows and graphs were done in Microsoft Excel. The relative abundance of mosquito larvae was calculated as the number of mosquito larvae divided by the number of dips taken for each larval habitat. Log transformation (log1+n) was used for data that required normalization before particular statistical analysis. The student t-test was used to determine the density of mosquito species which were collected before and after application with microbial larvicides. Poisson

regression analysis was used to determine the relationship between mosquito density and habitat characteristics. Negative binomial regression was used to show the association between mosquito species counts and habitat characteristic

CHAPTER FOUR: RESULTS

4.1 Species composition and abundance after microbial application

A total of 2095 late instar larvae were collected from 20 experimental plots over the 16 week rice growing cycle. Mean *Anopheles* densities of 3.62 per dip for the late instar larvae were collected before microbial application and 0.69 larvae per dip after microbial application (Table 1). Morphological identification of 1483 late stage instars yielded five anopheline species which included *An. gambiae* (n = 484; 72.5%), *An. pharoensis* (n = 159; 24.84%), *An. pretoriensis* (n = 8; 1.25%), *An. coustani* (n = 8; 1.25%) and *An. funestus* (n = 1; 0.16%). *Culex* species composition included *Culex quinquefasciatus* (n = 677; 82.26%), *Culex poicilipes* (n = 85; 10.33%) and *Culex annulioris* (n = 61; 7.41%). Most of the larvae were collected between land preparation and transplanting stage. Between flowering and rice maturation, there was a decline of the number of mosquito larvae from the plots (Figure 2).

Table 1: Density of mosquito species (± SE) collected before and after Bti and Bs application in Mwea, Kenya (January-June 2008)

	Anopheles density				*Culex* density			
	Early Instars		Late Instars		Early Instars		Late Instars	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Mean +/- SE	12.78±1.96	3.23±0.47	3.62±0.68	0.69±0.08	31.94±4.88	7.22±1.58	3.98±0.78	1.50±0.27
t value	10.71		7.73		10.64		5.39	
p value	0		0		0		0	

4.1.1 Relationship between mosquito densities and rice growth cycle after Bti and Bs application

The mean number of *Anopheles* mosquitoes (L1-L4) differed significantly among the various rice stages after microbial larvicides application ($F=32.491$, $df = 4, 655$, $p<0.001$). Larval abundance fluctuated from a mean of 8.0 larvae per dip to 0.3 larvae per dip during land preparation. The *An. gambiae* larvae increased from 0.3 larvae per dip to 7.28/ larvae per dip immediately after transplanting and these were the highest numbers which were collected at the experimental plots. The highest larval production was recorded during transplanting. A day after the application of microbial larvicides, there was a significant reduction of *Anopheles* mosquito larvae. Thereafter the *Anopheles* larval densities were also high during the tillering stage (mean 0.49 larvae per dip). From the flowering of rice, the *Anopheles* larval densities declined from 5.0 larvae per dip to 3.0 larvae per dip in the maturation stage. Overall, larval populations in both the controls and treatments declined post treatment (Figure 3).

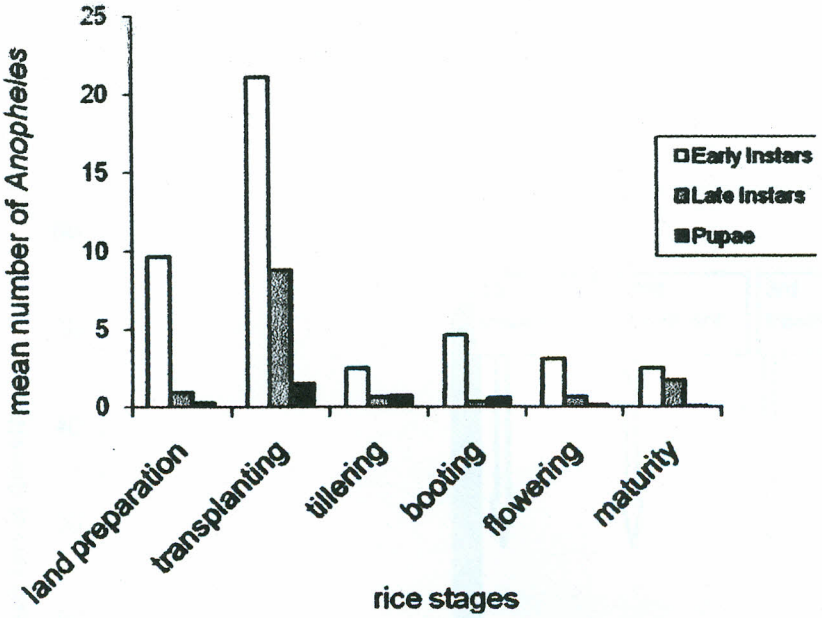
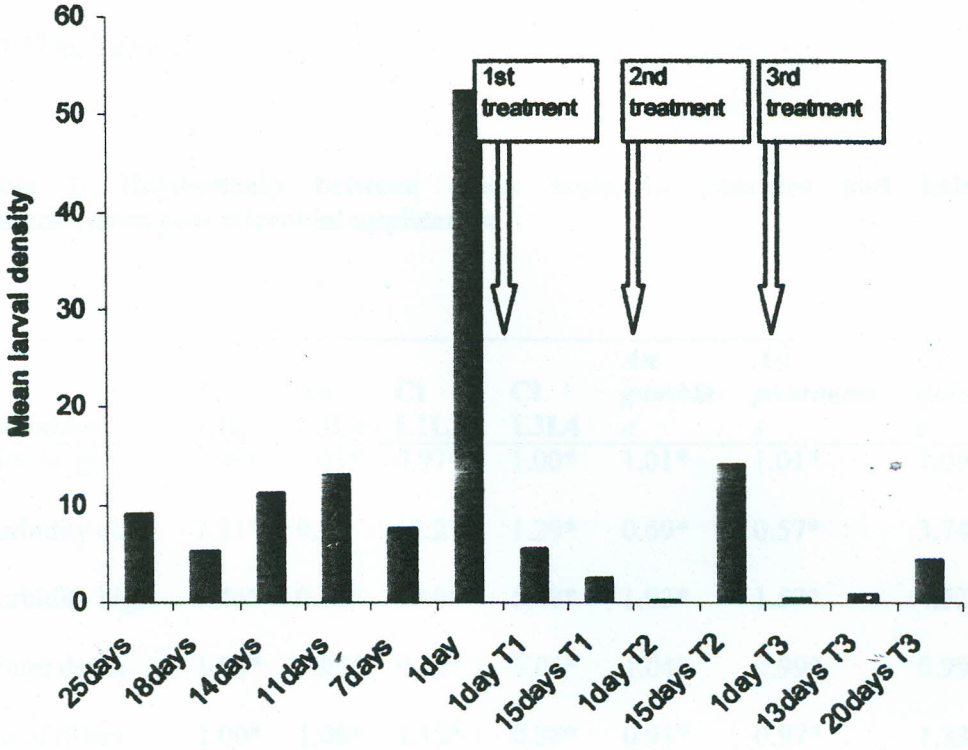


Figure 3: Mean number of pre-adult *Anopheles* mosquitoes during the rice growth stages in Mwea, Kenya (January-June 2008)

There was significant reduction (from 50.0 to 5.0/dip) of mosquito densities after the rice transplanting stage when the first set of microbial larvicides was applied. ($F_{(3,536)}$ 7.56 p=<0.0001). A day after the second set of microbial larvicides was applied, the mosquito densities declined from 2.0/dip to 0.01/dip. Fifteen days post the second microbial application, the population density of *Anopheles* mosquitoes was 13.0 larvae per dip while a day after the third set of microbial larvicides was applied, the density declined to 0.01 larvae per dip. However, larval samples taken in all treated subplots 6, 8, 10 days after treatment revealed the presence of early instar larvae,

showing that newly hatched larvae were not affected by ABG 6189, VBC 60035 and the new improved combined formulation (Figure 4).



Arrows indicate first, second and third treatment with microbial larvicides

25 days: 25 days pre-treatment
1 day: 1 day pre-treatment
1 day T1: 1 day after first treatment
1 day T2: 1 day after second treatment
1 day T3: 1 day after third treatment

Figure 4: Population dynamics of *Anopheles* larval instars before and after microbial application.

Poisson regression to assess the significance of the habitat characteristics to the vector density using procedure GENMOD in SAS reported in the incidence rate ratio (IRR) showed that rice height, water depth, number of tillers, vegetative cover were significant with the mosquito density. The characteristics measured were significantly explaining the vector density except for the clear turbidity which was not significant with the density of the 3rd and 4th instars of *Anopheles* mosquitoes ($F_{(7,182)} = 38.685$, p=0.7736, Table 2).

Table 2: Relationship between mean mosquito densities and habitat characteristics post microbial application

Variables	An L1L2	An L3L4	Cl L1L2	Cl L3L4	*An gambiae*	*An pharoensis*	*Cl quinquefasciatus*
Rice height	0.99*	1.01*	0.97*	1.00*	1.01*	1.01*	1.00*
Turbidity clear	2.21*	0.00	12.21*	1.29*	0.69*	0.57*	3.74*
Turbidity high	0.45*	0.33*	1.40*	5.48*	1.03*	1.57*	4.52*
Water depth	1.04*	1.02*	0.95*	1.06*	1.04*	0.99*	0.99*
No.of tillers	1.00*	1.00*	1.13*	0.88*	0.91*	0.97*	1.33*
Vegetation high	2.29*	3.98*	0.45*	3.00*	31.5*	4.68*	9.11*
Vegetation low	1.38*	2.75*	0.49*	1.78*	18.2*	6.06*	1.37*

* Significance measured at p value< 0.05

KEY
An.L1L2=*Anopheles* first and second instars
An. L3L4= *Anopheles* third and fourth instars
Cl. L1L2= *Culex* first and second instars
Cl.L3L4= *Culex* third and fourth instars

4.1.2 Succession pattern of the mosquito species after microbial application

Figures 5 and 6 show the changes in mosquito species composition in relation to the rice growing cycle after microbial application. *Anopheles gambiae* was common throughout the rice growing cycle but their densities were three folds higher during the early growth stages (transplanting) of rice crop than in the late stages of the rice development. A day after microbial application, the population density reduced from a mean of 50.0 larvae per dip to a mean of 0.5 larvae per dip. There was a peak of *An. gambiae* which was observed during transplanting. Other mosquito species included *Culex quinquefasciatus, An. pharoensis, An. coustani, An. pretoriensis, An. funestus, C. poicilipes* and *C. anulioris. Anopheles gambiae, C. quinquefasciatus and An. pharoensis* occurred throughout the rice cycle, and their density reduced each time after microbial application. *Anopheles funestus* and *C. poicilipes* colonized the rice paddies though in very small numbers with a peak at flowering stage. *Anopheles pretoriensis, An. coustani* and *C. annulioris* were recorded towards maturity of the rice, though the density was low (mean 0.01 larvae per dip).

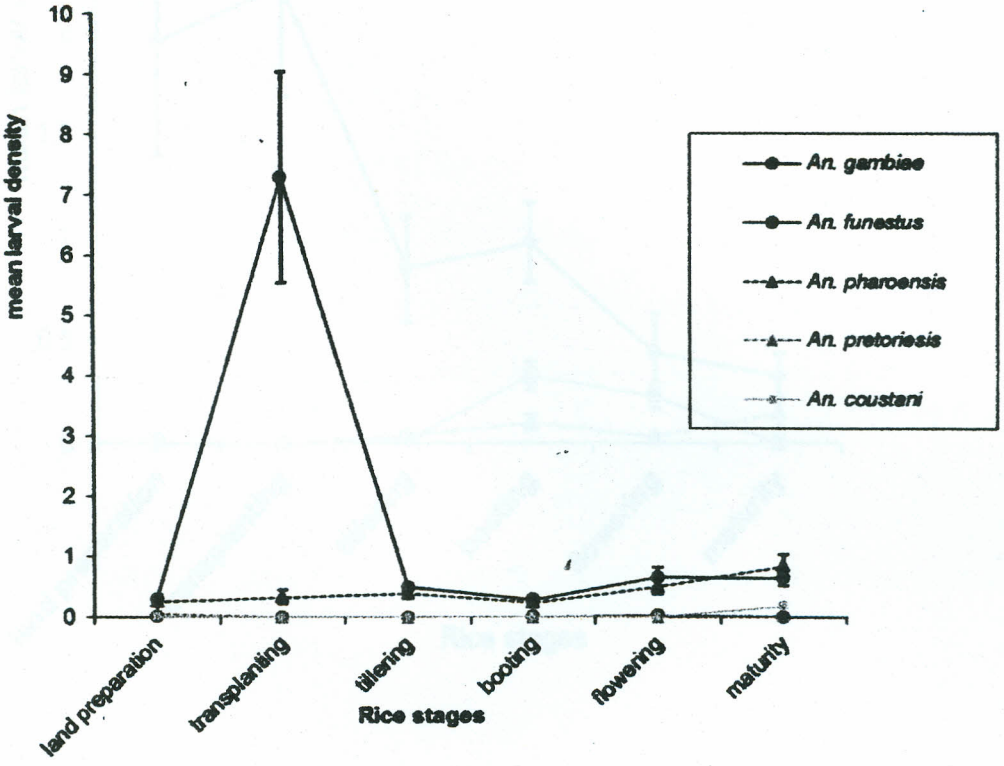


Figure 5: ***Anopheles*** **larval species succession from land preparation to maturation of rice**

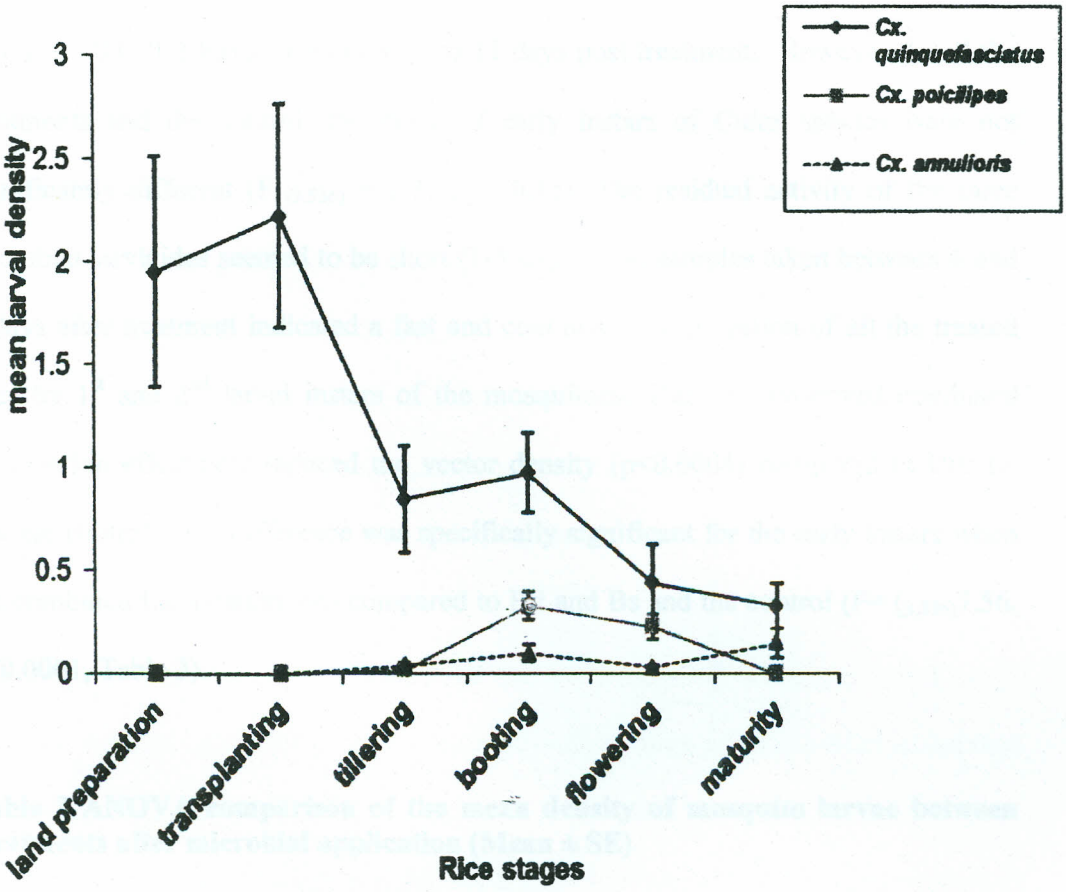


Figure 6: *Culex* larval species succession from land preparation to maturation of rice.

The application doses of the microbial larvicides were 22.4g Vectobac, 44.8g Vectolex and 40.0g of the new improved combined formulation per subplot measuring 6.3m×3.15m. Granular formulations, Vectobac VBC 60035, Vectolex ABG 6189 and the new improved combined formulation showed significant ($F_{(3,536)}$ = 7.56 p = <0.0001) larval reduction up to 11 days post treatment. However, in all the treatments and the control, the mean of early instars of *Culex* species were not significantly different ($F_{(3,536)}$ = 1.77 p = 0.15). The residual activity of the three microbial larvicides seemed to be short (1-5 days). Dip samples taken between 6 and 8 days after treatment indicated a fast and continual recolonization of all the treated sites by 1st and 2nd larval instars of the mosquitoes. The new improved combined formulation effectively reduced the vector density (p<0.0001) compared to Bti, Bs and the control. This difference was specifically significant for the early instars when the combined formulation was compared to Bti and Bs and the control ($F=_{(3,536)}7.56$, p<0.0001, Table 3).

Table 3 ANOVA comparison of the mean density of mosquito larvae between treatments after microbial application (Mean ± SE)

Treatment	AnL1L2	AnL3L4	CL1L2	CL1L2	F (df)	P-value
Control	4.78 ± 0.91^{a}	0.91 ± 0.16^{a}	0.91 ± 0.16^{a}	8.05 ± 2.70^{a}	7.56(3,536)	<0.0001
Bs	3.52 ± 1.24^{ab}	0.59 ± 0.13^{ab}	0.59 ± 0.13^{ab}	6.59 ± 1.92^{a}	2.81(3,536)	0.0391
Bti	3.18 ± 1.03^{a}	0.77 ± 0.18^{ab}	0.77 ± 0.18^{ab}	9.30 ± 4.52^{a}	1.77(3,536)	0.15
Combination	1.46 ± 0.32^{b}	0.53 ± 0.14^{b}	0.53 ± 0.14^{b}	4.94 ± 2.93^{a}	7.46(3,536)	<0.0001

Within columns same superscripted letter are not significantly different

KEY
An L1L2=*Anopheles* first and second instars
An L3L4= *Anopheles* third and fourth instars
Cl L1L2= *Culex* first and second instars
Cl L3L4= *Culex* third and fourth instars

4.2 Habitat diversity and preference

There were nine different larval habitats which were encountered in the study sites and these included water paddies, canals, marshes, pools, ditches, pits, fishpond and seepage. The nine habitat types supported larval development in Kiamachiri (unplanned rice agro ecosystem). The planned rice agro ecosystem (Munyaka) had five habitats types while the non-irrigated agro ecosystem (Murinduko) had six habitats types. Therefore, the number of habitat types in the different sites was variable, and the highest number of habitats sampled was recorded in Kiamachiri (n=9). Canals, paddies and marshes were common in the three habitat types. Ditches were only found in Kiamachiri and Munyaka while pit habitat was found in Kiamachiri and Murinduko. Pools formed near water tanks and also during the rainy season and they harbored fewer larvae in both Murinduko and Kiamachiri.

Paddies and irrigation canal habitat types had water most of the rice growing cycle and were the most productive habitat types throughout the sampling period in the rice growing villages. The peri-domestic habitats (including pools, tyre tracks and pits) were productive for the short periods when they had water making them important sources of *Anopheles gambiae* for extended periods of time in the study sites. Among the permanent or stable aquatic habitat categories (paddies, canals and marshes) paddies and the associated canals had high densities of anopheline larvae in both the planned irrigation scheme and unplanned rice growing areas. In general, canals were the most preferred habitat type for the early instars with a mean density of 0.23/dip (n=1303) followed by paddies with a mean density of 0.22/ dip (n=2695).

In the unstable or temporary aquatic habitats (pools, tanks, tyre tracks, pits and ditches), larval production was highly variable (mean range 0.82-0.05 larvae per dip). Ditches were the most productive with a mean density of 0.82 larvae per dip (n=295), followed by pools with a mean density of 0.59 larvae per dip (n=197) (Table 4).

Table 4: Mean number of pre-adult instars found in the different habitat types in Mwea, Kenya (June 2007-June 2008).

Habitat type	Early instars	Late instars	Pupae
Canal	0.23 (1303)	0.03 (140)	0.005 (27
Ditch	0.82 (295)	0.23 (85)	0.02 (8)
Fish pond	0.05 (20)	0	0
Marsh	0.142 (264)	0.01 (24)	0.03 (49)
Paddy	0.22 (2695)	0.04 (426)	0.01 (171)
Pit	0.27 (388)	0.04 (55)	0.02 (27
Pool	0.59 (197)	0.15 (47)	0.07 (27)
Seepage	0.44 (373)	0.05 (44)	0.06 (44)
Tank/ Pool	0.14 (109)	0.05 (57)	0.03 (36)

Number in parenthesis indicates sample size (n)

4.2.1 Factors associated with habitat preference

Negative binomial regression showed that habitat type, turbidity, water depth, other invertebrates, emergent and floating vegetation were the best predictors for *Anopheles* mosquito larval abundance in the habitats (Table 5 (a) and (b)). Habitat type, the presence of other invertebrates, water depth and floating vegetation had a significant effect on the early anopheline larval (1st and 2nd instars) densities in all the three villages ($p<0.0001$) while turbidity and emergent vegetation had no significant effect with the early larval abundance ($p>0.005$). Among the late stage larval instars (3rd and 4th larval stages), habitat type, turbidity and water depth were significant with the density of anopheline mosquitoes ($p<0.005$) while the presence of other invertebrates, emergent and floating vegetation were not significant ($p>0.005$) (Table 5 (a) and (b)

Table 5 (a) and (b) Factors associated with habitat preference of *Anopheles* mosquitoes in Mwea, Kenya (June 2007-June 2008)

Table 5 (a) Early instars

Factor	DF	Chi-square	p-value
Habitat type	10	577.73	<0.001
Emergent vegetation	1	0.15	0.6997
Floating vegetation	1	12.3	0.0005
Turbidity	2	0.79	0.6723
Water depth(cm)	1	34.3	0.0064

Table 5 (b) Late instars

Factor	DF	Chi-square	p-value
Habitat type	10	355.47	<0.001
Emergent vegetation	1	0.40	0.5256
Floating vegetation	1	0.70	0.4045
Turbidity	2	5.90	0.0525
Water depth(cm)	1	6.14	0.0465

4.3 Indoor mosquito collection

A total of 1237 female anophelines were collected between June 2007 and February 2008. *Anopheles gambiae* was the most dominant species collected by Pyrethrum Spray Collection in the three villages comprising 97.01%, followed by *Anopheles funestus* 2.83%, *Anopheles pharoensis* 0.08%, *Anopheles coustani* 0.08%. The planned agro-ecosystem of Munyaka had a high population density of female *Anopheles* mosquitoes (n=817), unplanned agro-ecosystem of Kiamachiri had 330 while the non-irrigated area of Murinduko had the least population density (n=90). The highest mean numbers of *Anopheles* mosquitoes were collected during the months of October and December mean 4.0, (Figure 7).

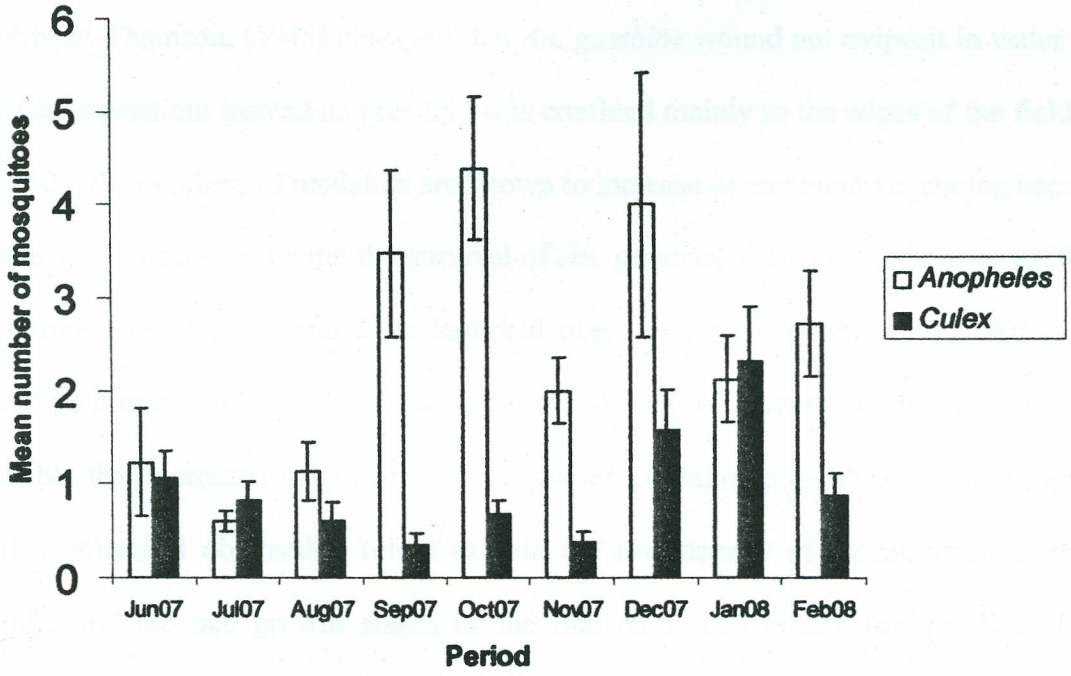


Figure 7: Mean number of mosquitoes collected by Pyrethrum Spray Collection in the three villages in Mwea.

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Anopheles gambiae s. l predominated most of the rice growing stages and their densities were at least three times higher during rice transplanting stage. Numerous shallow and sunlit pools and puddles, resulting from human footprints created extensive habitats conducive for intense breeding, particularly of *An. gambiae* species. This might have accounted for the overwhelming number of larvae collected in the period of transplantation. The larval densities of mosquitoes reduced post treatment with microbial larvicides which was probably the result of a progressive reduction in ovipositional attraction of the breeding sites. The increase in rice height during the middle and late growth stages of rice may have hindered oviposition by *An. gambiae*.

Muirhead-Thomson, (1945) observed that *An. gambiae* wound not oviposit in water with intense vegetation; instead its breeding was confined mainly to the edges of the fields. In addition, the numbers of predators are known to increase as emergent vegetation becomes dense, considerably reducing the survival of *An. gambiae s. l* larvae (Christie, 1958). In this study, the experimental plots harbored other aquatic invertebrates which included potential predators of mosquitoes during the middle growth stage of rice. It is therefore possible that increased height of the rice favored predators populations, which together with mechanical obstruction might explain the low density of mosquitoes during the middle and the late growth stages of the rice crop. The results further showed that although the density of mosquito larvae was low, *An. funestus* and *An. coustani* colonized the subplots at flowering stage. This signifies that these two *Anopheles* species preferred

to oviposit in the shaded habitats. *An. gambiae, An. pharoensis* and *Culex quinquefasciatus* colonized the experimental plots throughout the rice cycle and that there was a decline at the very late vegetative stages and also after microbial application.

In Africa, a number of studies have demonstrated a strong relationship between the rice cropping cycle and the mosquito species succession (Snow, 1983; Klinkenberg *et al.*, 2003; Muturi *et al.*, 2007). Studies in Gambia and Mwea (Snow, 1983; Muturi *et al.*, 2007) have shown that, in Gambia, *Anopheles gambiae s. l, Anopheles rufipes* and *Culex neavei* were predominant during the early stages of rice development. *Culex ethiopicus and Culex poicilipes* were present around the middle of the rice cycle while *Anopheles ziemanni* peaked as the rice matured. Studies by Muturi *et al.*, (2007) showed that *Anopheles arabiensis, Anopheles pharoensis,* and *Culex quinquefaciatus* were the predominant mosquito species throughout the growing cycle of rice in Mwea. Their abundance was greater one week before transplanting rice seedlings up to four weeks post transplanting after which their abundance declined with another peak occurring at week eight which was triggered by application of nitrogenous fertilizer.

Each mosquito species has its optimum abiotic and biotic characteristic that act as oviposition cues for gravid female mosquitoes and provide ideal environment for the development of the immature stages. These factors exhibit marked variation during a crop cycle and impact tremendously on the relative abundance (Sunish and Reuben, 2001) and succession of mosquito species in the course of the growing cycle. An increase in rice height and floating vegetation cover may inhibit oviposition by some species directly through physical obstruction or indirectly through reduced temperature

and microbial growth (Rao, 1984). The numerous shallow pools created by rice workers during rice transplanting create ideal sites for the breeding of *An. gambiae s. l.* (Chandler and Highton, 1976), whereas application of nitrogenous fertilizer increases its larval densities (Mutero *et al.*, 2004b). Rainfall also may dilute water in rice fields, altering their physicochemical properties, thus resulting in changes in larval densities and species succession.

Granular formulations of *B. sphaericus* (ABG 6189), *B. thuringiensis* (VBC 600035) and the new improved combined formulation enabled good penetration in the dense rice and this permitted even distribution of the bacterium in the fields. This caused a significant reduction of mosquito larvae in the rice subplots. Late instars (3rd and 4th stages) larvae provide more reliable indicator for treatment efficacy than early instars (1stand 2nd instars) larvae. Young larvae had little time to ingest lethal amounts of the toxins. The larval samples taken in all the treated sites 5, 6 and 7 days after treatment revealed the presence of early instar larvae, showing that newly hatched larvae were not affected by these microbial larvicides. These data is similar with those reported by other authors that describe short-term control of anopheline larvae due to rapid settling of spores out of the larval feeding zones (Davidson *et al.*, 1984, Nicolas *et al.*, 1987). The application of Bti, Bs and the new improved formulation can reduce the natural population of *An. gambiae* larvae in different habitats in the field, but the degree of reduction is limited due to different factors which intervene in the field.

Minimum effective dosages to achieve elimination of the larval population in a given habitat are extremely low and environmental impact is negligible (WHO, 1999).

Microbial products for larval control have therefore great potential within the integrated vector management programs and may augment control efforts against adult vector stages, such as the use of insecticide treated bed nets in many parts of Africa. Effective vector control relying on active monitoring and subsequent application of microbial larvicides throughout the year to targeted sites would substantially reduce malaria transmission. The main malaria vector in the study area is highly susceptible to these microbial control agents.

Rice growing contributed to high abundance of mosquito larvae but the importance was site-specific. In "planned" rice growing, the larval abundance and densities corresponded well with the rice growing system. Larval habitats in the villages with unplanned rice cropping tended to have higher larval densities than villages with the planned rice-cropping (organized) rice growing. Paddies and canals in the medium rice cropping are poorly drained which makes them more favorable for anopheline larval development, whereas paddies and irrigation canals in the organized/planned rice growing are well drained. The effect of the unplanned rice cropping which is unorganized rice growing and the subsequent unorganized water management meant that rice growing was undertaken throughout the year with rice paddies at different growth stages. This phenomenon not only increased the number of habitats but prolonged the period of active life of the larval habitat for production of *Anopheles* larvae.

In the low rice cropping (Murinduko), where <5% of the area is under rice, its cultivation has only recently been introduced along the river valleys. This has resulted in an exponential increase in breeding sites for mosquitoes. Initially, most of the habitats were

concentrated in stream edges and stream pools. The soil and topography of this village do not allow formation of rain-fed pools. Although even before the introduction of rice cultivation, the three villages were different in terms of hydrology and natural breeding sites, villages with unplanned and/or limited rice cultivation are expected to have diverse breeding sites than those within the scheme (Briet *et al.*, 2003).

Previous studies have demonstrated that *An. arabiensis* is associated with small, temporary habitats with algae such as foot prints, rain pools, puddles, tire tracks and garden wells (Robert *et al.,* 1998; Minakawa *et al.,* 1999, Gimnig *et al.*, 2001). The rest of the anopheline species were found in low proportions and were restricted to only a few aquatic habitats, with the streams being the main larval habitats, suggesting that conditions favorable for their development prevailed in this habitat type.

Although some variation in larval abundance could be explained by habitat factors such as water turbidity depth and vegetation cover, it was evident that interaction between these factors was not significant. This suggests that larval production is a function of complex interaction of several habitat characteristics, some of which were not measured in the current study although the occurrence and abundance of anopheline larvae is closely associated with physicochemical variables such as salinity and dissolved oxygen (Grillet, 2000; Mwangangi *et al.,* 2007).

Increase in turbidity resulted in a significant increase in the anopheline larval densities in the habitats. It is likely that increase in turbidity tended to affect the attractiveness of these breeding sites to ovipositing female *Anopheles* mosquitoes. McCrae (1984) found that *An. gambiae* preferred a dark to a light background as an oviposition substrate. In

this study clear and low turbid water had the most abundant *Anopheles* larvae. Mosquitoes view water bodies by the reflecting dark background of the soil substrate. The dark background increased the attractiveness of the breeding sites to ovipositing female *Anopheles* mosquitoes.

These findings are similar with previous observations by Muturi *et al.,* (2006) who reported that the most productive habitats per surface area are not necessarily the most important habitats for vector proliferation over space and time. In Venezuela, Grillet (2000) noted that a large number of low density, larvae but continuously productive habitats contributed more to the adult mosquito density than singly high density larval habitats. In areas of intense rice cultivation, the rice cropping cycle is considered to impact significantly on mosquito production. Conversely, in areas of little or no irrigation such as Murinduko, larval production is dependent upon temporary larval development habitats.

The findings of this study suggest that implementation of larval control activities should be targeted based on habitat productivity, which is governed by rainfall, rice cropping season and water management. However, the application interventions would have to consider habitat and site specific attributes of larval productivity. The fact that medium and low rice growing support more *Anopheles* larvae than high rice cropping system calls for better management of water in the rice cultivation and subsequent water distribution so as to reduce the active period when rice fields are flooded. This study further shows that critical surveillance and subsequent microbial application in the larval habitats are prerequisite for effective mosquito control.

5.2 Conclusions

1. Knowledge on the colonization and succession pattern may help in formulating larvicide application regimes that will reduce further increase in population density of *Anopheles* mosquitoes.
2. *Anopheles gambiae, An. pharoensis and Culex quinquefasciatus* colonized the experimental plots throughout the rice cycle. *An. funestus* and *Culex poicilipes* at flowering while *An. coustani, An. pretoriensis and Culex annulioris* from flowering to maturation of rice after microbial application but in small numbers.
3. The microbial larvicides (Bti, Bs) caused significant reduction of larval densities especially the new improved combined formulation of Bti and Bs.
4. Rice paddies and the associated canals were the most productive habitat types throughout the sampling period while the temporary habitats are productive only when they have water. For effective control of mosquito larvae, application of larvicides should be done between transplantation and the reproductive stage of the rice crop.

5.3 Recommendations

1. The field application of larvicides should be implemented between transplanting and tillering periods with 15-day intervals since the residual activity seemed to be very short.
2. Larval control programme should be implemented by individual farmers in Mwea Irrigation Scheme. This would ensure each farmer applies larvicides at the appropriate stage to maximize on the larvicidal effect.

3. In the treated subplots there was ovipositional attractancy since 1st and 2nd stage larval instars increased post treatment, but they died when they developed to late stages (3rd and 4th instars). This needs further investigation.
4. Temporary pools should be targeted for control as they were colonized by more *An. gambiae* mosquitoes. Those that are small should be back filled and the big ones applied with larvicides.

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