# EFFECT OF SUBLETHAL CONCENTRATIONS OF AGRICULTURAL CHEMICALS ON OVIPOSITION SITE SELECTION AND OFFSPRING HISTORY OF MOSQUITO VECTORS IN CENTRAL KENYA

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# Effect of Sublethal Concentrations of Agricultural Chemicals on Oviposition Site Selection and Offspring History of Mosquito Vectors in Central Kenya

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## DECLARATION

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

Signature …………………………… Date……………………………

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This thesis has been submitted for examination with our approval as university supervisors.

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## DEDICATION

I dedicate this thesis with much love to my husband Joseph, daughter Ivy and son Clive for their patience and encouragement during the time I was studying. I owe gratitude to God.

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## LIST OF ABBREVIATIONS AND ACRONYMS

**$(NH_4)_2HPO_4$** - Diammonium Phosphate

**$(NH_4)_2SO_4$** - Ammonium Sulfate

**An** - Anopheles

**ANOVA** - Analysis of Variance

**ARS** - Agricultural Research Service

**AS** - Ammonium Sulfate

**Cx** - Culex

**DAP** - Diammonium Phosphate

**Df** - Degrees of Freedom

**DT** - Development Time

**EIP** - Extrinsic Incubation Period

**ERC** - Ethical Review Committee

**ESACIPAC**-Eastern and Southern Africa Centre of International Parasite Control

**Ha** - Hectares

**JKUAT** - Jomo Kenyatta University of Agriculture and Technology

**KEMRI** - Kenya Medical Research Institute

**LF -** Lymphatic Filariasis

**LG -** Longevity

**MANOVA -** Multivariate Analysis of Variance

**Mg/l -** Milligrams per Liter

**MIAD -** Mwea Irrigation and Agricultural Development

**RH -** Relative Humidity

**SAS -** Statistical Analysis System

**SCC -** Standardized Canonical Coefficients

**S.L -** Sensu Lato

**SPSS -** Statistical Package for the Social Sciences

**S.S -** Sensu Stricto

**SSC-** Scientific Steering Committee

**USA -** United States of America

**USDA -** United States Department of Agriculture

**VC -** Vectorial Capacity

**WHO -** World Health Organization

**WL -** Wing Length

## ABSTRACT

Although many mosquito species develop within agricultural landscapes where they are potentially exposed to agricultural chemicals (fertilizers and pesticides), the effects of these chemicals on mosquito biology remain poorly understood. This study investigated the effects of sublethal concentrations of four agricultural chemicals on the oviposition site selection and on life history traits of *Anopheles arabiensis* and *Culex quinquefasciatus* mosquitoes. Field and laboratory experiments were conducted to examine how sublethal concentrations of four agricultural chemicals: an insecticide (cypermethrin), a herbicide (glyphosate), and two nitrogenous fertilizers (ammonium sulfate and diammonium phosphate) alter oviposition site selection, emergence rates, development time, adult body size, and longevity of *An. arabiensis* and *Cx. quinquefasciatus*. Both mosquito species had preference to oviposit in fertilizer treatments relative to pesticide treatments. Emergence rates for *An. arabiensis* were significantly higher in the control and ammonium sulfate treatments compared to cypermethrin treatment ($P = 0.009$), while emergence rates for *Cx. quinquefasciatus* were significantly higher in the diammonium phosphate treatment compared to glyphosate and cypermethrin treatments ($P = 0.007$). For both mosquito species, individuals from the ammonium sulfate and diammonium phosphate treatments took significantly longer time to develop compared to those from cypermethrin and glyphosate treatments ($P < 0.001$). Although not always significant, males and females of both mosquito species tended to be smaller in the ammonium sulfate and diammonium phosphate treatments compared to cypermethrin and glyphosate treatments. There was no significant effect of the agrochemical treatments on the longevity of either mosquito species *(An. arabiensis* ($P = 0.21$); *Cx. quinquefasciatus* ($P = 0.55$).These results demonstrate that the widespread use of agricultural chemicals to enhance crop production can have unexpected effects on the spatial distribution and abundance of mosquito vectors of malaria and lymphatic filariasis.

# CHAPTER ONE

# INTRODUCTION AND BACKGROUND

## 1.1 Introduction

Mosquitoes are blood sucking insects that not only cause biting nuisance but also transmit a variety of debilitating and life threatening parasites and pathogens to humans, domestic animals, and wildlife. These include malaria, lymphatic filariasis (LF), dengue, Rift Valley fever among others. Malaria is by far the most important vector-borne disease**,** affecting more than 300 million people with 627,000 fatalities annually (World Health Organization, 2015).

Further, despite ongoing control efforts, lymphatic filariasis (LF) continues to be a major public health challenge in Africa**,** where more than 50 million cases are reported each year, accounting for one-third of the global burden (Mbutolwe *et al*., 2013). Over the past few decades, mosquito-borne viruses that were thought to be unimportant or previously contained such as dengue, Chikungunya, West Nile and Zika viruses have emerged to become a global public health challenge.

Agricultural intensification by use of irrigation, synthetic fertilizers, and pesticides is considered a key factor in the changing dynamics of mosquito-borne diseases. These agricultural activities have been shown to modify the ecology of major mosquito vectors via diverse ecological pathways. Irrigation and use of fertilizers promote mosquito production by creating new and suitable breeding sites for mosquitoes (Mutero *et al*., 2004a, Mutero *et al*., 2004b, Muturi *et al*., 2007) while pesticide use in agriculture may not only select for insecticide resistance in the vectors (Muller *et al*., 2008) but also may enhance mosquito production by eliminating mosquito predators and competitors (Muturi *et al*., 2007). Alternatively, pesticide use in agriculture may influence disease transmission by altering the life history traits of vector mosquitoes.

Although many mosquito species including the primary vectors of malaria (*Anopheles gambiae* and *An. arabiensis*) and one of the major vectors of LF and arboviruses (*Culex quinquefasciatus*) are known to breed in artificial water bodies created by irrigation, and are frequently exposed to synthetic fertilizers and pesticides, we have a limited understanding of how these chemicals influence the life history traits of these vectors. To address this significant knowledge gap, a series of field and laboratory experiments were conducted to determine how *Anopheles arabiensis* and *Culex quinquefasciatus* mosquitoes respond to two commonly used pesticides (cypermethrin and glyphosate) and two commonly used fertilizers (ammonium sulfate and diammonium phosphate).

This was examined by quantifying several life history traits including oviposition site selection, development time, emergence rates, body size, and longevity. *Culex. quinquefasciatus* is a major worldwide vector of LF (Muturi *et al.*, 2006a, Muturi *et al*., 2006b; Mwandawiro *et al.*, 1997) while *An. arabiensis* is an important vector of both malaria and LF and has become the most dominant malaria vector in areas where insecticide-treated bed nets and indoor residual spraying have been implemented (Bayoh *et al.*, 2010; Mwangangi *et al.*, 2013). This study tested the hypotheses that: fertilizer and pesticide treatments alter oviposition site selection of the two mosquito species; and agrochemical treatments would differentially affect the development time, emergence rates, adult body size and longevity of the two mosquito species.

### 1.2 Statement of the problem

Given the widespread use of agricultural chemicals in irrigation agriculture, mosquito larvae are likely to be exposed to both lethal and sublethal concentrations of insecticide and inorganic fertilizers that may directly or indirectly affect aspects of mosquito biology relevant to transmission of pathogens.

Already, there are concerns that repeated exposure of mosquitoes to different kinds of insecticides is selecting for resistance among important disease vectors (Diabate *et al.*, 2002, Boyer *et al*., 2006) and considerable efforts have been directed at elucidating the molecular basis of resistance mechanisms (Harris *et al*., 2010, Ranson *et al*., 2011). There are also concerns in the short term, whether the effects of insecticides and inorganic fertilizers alter adult mosquito fitness and oviposition biology, but this area of research has rarely been the subject of investigation and very little is known about how these chemicals affect vector ecology.

## 1.3 Justification

Agricultural activities can influence mosquito ecology and the risk of mosquito-borne diseases by influencing the choice of mosquito oviposition sites by gravid mosquitoes as well as subsequent development of the aquatic stages of vector mosquitoes (Muturi *et al.,* 2007). In such situations, a clear understanding of how agrochemicals influence mosquito life history traits needs to be studied, because it may form a basis for designing integrated mosquito vector control management strategies through farm-based and possibly farmer-managed intervention strategies. For instance, it can be used in development of "attract – and – kill vector control strategy or be used to develop policies that enhance food production while alleviating negative impacts of agriculture on human health.

## 1.4 Null Hypothesis

Sublethal concentrations of agricultural chemicals (cypermethrin, glyphosate, diammonium phosphate and ammonium sulfate) have no effect on oviposition site selection and on offspring history of mosquito vectors in central Kenya.

## 1.5 Research questions

1. What is the effect of agricultural chemicals (cypermethrin, glyphosate, ammonium sulfate and diammonium phosphate) on oviposition site selection by *An. arabiensis* and *Cx. quinquefasciatus*?

2. What role is played by the insecticide cypermethrin, the herbicide, glyphosate and the inorganic fertilizers ammonium sulfate and diammonium phosphate on the emergence rate, development time, longevity and adult size of *An. arabiensis* and *Cx. quinquefasciatus*?

## 1.6 Objectives of the Study

### 1.6.1 General objective

To determine the effect of sublethal concentrations of agricultural chemicals (insecticide cypermethrin, herbicide glyphosate and inorganic fertilizers ammonium sulfate and diammonium phosphate) on the oviposition site selection and on offspring history of mosquito vectors (*Anopheles arabiensis* and *Culex quinquefasciatus)* in Mwea rice irrigation scheme, in central Kenya..

### 1.6.2 Specific objectives

1. To determine the effect of insecticide cypermethrin, herbicide, glyphosate and inorganic fertilizers ammonium sulfate and diammonium phosphate on oviposition site selection by *An. arabiensis* and *Cx. quinquefasciatus*.
2. To elucidate the role of insecticide cypermethrin, herbicide, glyphosate and inorganic fertilizers ammonium sulfate and diammonium phosphate on the emergence rate, development time, longevity and adult size of *An. arabiensis* and *Cx. quinquefasciatus*.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Introduction

Mosquitoes transmit some of the most devastating infectious diseases including malaria, lymphatic filariasis (LF), and dengue. Transmission of these diseases is largely influenced by mosquito distribution, abundance, and fitness, which are in turn dependent on the quality of aquatic habitats where egg hatch and larval development occurs (Alto *et al.*, 2005; Reiskind &Lounibos, 2009). Because mosquitoes do not provide parental care to their offspring, natural selection should favor the ability of gravid females to select aquatic habitats that maximize egg hatch and offspring fitness (Reiskind &Wilson, 2004). This process requires complex integration of biological, chemical, and physical cues by gravid females (Bentley &Day, 1989). Chemical contaminants can potentially disrupt this process by modifying the quality and attractiveness of the aquatic habitats and vector biologists are faced with the challenge of determining the impact of these chemicals on mosquito ecology, behavior, and ability to transmit pathogens.

Agrochemicals (fertilizers and pesticides) are one of the major classes of chemical contaminants that can potentially affect mosquito oviposition behavior and offspring fitness. Every year, an estimated 2.4 million tons of pesticides (Grube *et al.*, 2011) and 180.1 million tons of fertilizers (Food and Agricultural Organisation (FAO), 2012) are used worldwide to improve crop production and human health. The extensive use of these chemicals has led to their recurrent detection in many surface waters (Bogardi *et al.*, 1991; Gilliom *et al.*, 2007) increasing the potential for aquatic communities to be exposed to these chemicals. Immature stages (larvae and pupae) of many mosquito species including the vectors of malaria and LF develop in a variety of ephemeral and permanent water bodies situated within agricultural areas where they are potentially exposed to agrochemicals that are either applied to these farmlands or transported from nearby farmlands through spray drift, surface runoff, and/or leaching. However, despite the notable potential for mosquitoes to be exposed to agrochemicals, the implications of agrochemical use on the ecology and behavior of major vectors remains poorly understood.

## 2.2 Environmental impact of irrigated agriculture

### 2.2.1 Irrigation water

Irrigation has arguably contributed significantly to poverty alleviation, food security and improving the quality of life for rural populations (Smith, 2004) (see figure 2.1). However, in the past two decades the performance of irrigation projects in sub-Saharan Africa has greatly declined (Kikuchi *et al.,* 2005). Environmental factors and adverse human health impacts have been a prominent cause for the dismal performance of many schemes (Oomen *et al.,* 1990).

The environmental and human health aspects of irrigation schemes are closely interrelated and need to be considered in tandem. It is the changes in the environment together with the associated socio-economic change, which results in changes in health of the local populations. Irrigation schemes may affect the environment directly or indirectly by; modifying the river flow regimes, depleting the groundwater, sedimentation effects, soil salinization, waterlogging, water contamination and biological effects (Adams, 1992; Kay, 1999). Furthermore, water- related projects may aggravate the problem of mosquito-borne disease by increasing the number of larval habitats and extending the duration of transmission season (Oomen *et al.,* 1990) (see figure 2.1). However the potential environmental impacts and healthy consequences are rarely considered and the impacts of many thousands of water projects are unclear.

In Cameroon, the development of hundreds of small agro-pastoral dams led to a rapid spread of schistosomiasis (Ripert &Raccurt, 1987). Similarly, in Ethiopia, the construction of small dams in Trigray has led to malaria outbreaks, where previously there were none (Ghebreyesus *et al.,* 1999). Past literature strongly indicate that the development, management and operation of water projects have a history of modifying the frequency and transmission dynamics of malaria and other mosquito transmitted pathogens in sub-Saharan Africa (Mutero *et al*., 2004b). This therefore urgently calls for the need to take environmental and health considerations into account as part of ensuring sustainable development.

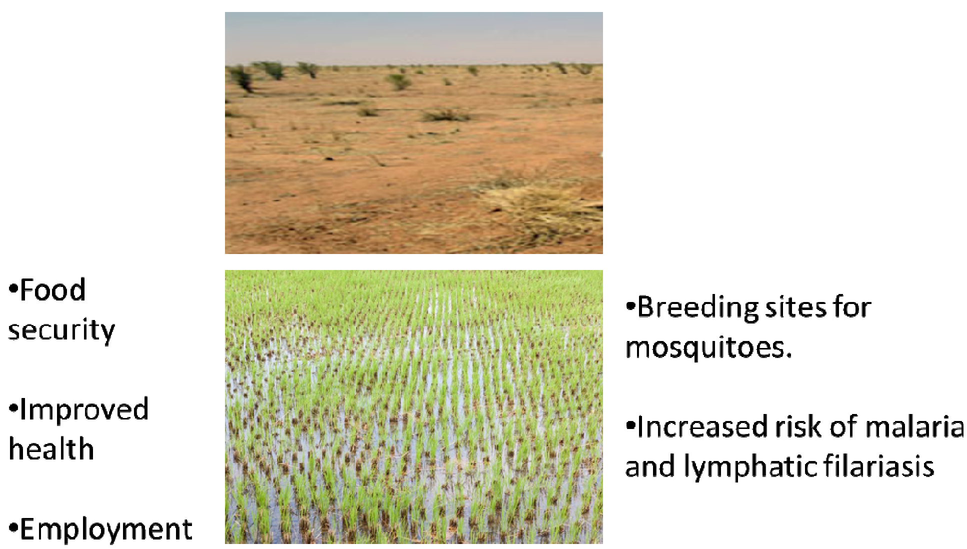


**Figure 2.1: Relationship between irrigated agriculture and mosquito-borne diseases**

### 2.2.2 Inorganic nitrogenous fertilizers

Agricultural application of nitrogenous fertilizers is a major source of nitrate contamination of aquatic systems. In many countries, nitrate concentration in surface and ground water ranges from 5 mg/L to > 100 mg/L, and due to its high solubility in water, nitrate has high mobility in the environment (Bogardi *et al.*, 1991). High levels of nitrates may promote mosquito production by enhancing proliferation of algal bloom and other microbial assemblages that serve as food for mosquito larvae (Sunish *et al.*, 1998; Victor &Reuben, 2000).

Fertilizer application may also alter the physical and chemical properties of the aquatic habitats making them attractive oviposition sites for mosquitoes (Mutero *et al.*, 2004a; Muturi *et al.*, 2007; Sunish *et al.*, 2003). This may explain why fertilizer application in rice fields is often associated with dramatic increase in mosquito larval populations (Mutero *et al.*, 2004a; Muturi *et al.*, 2007; Victor &Reuben, 2000). However, experimental studies to decipher the impact of nitrogenous fertilizers on epidemiologically relevant mosquito life history traits in the absence of other confounding factors are lacking.

### 2.2.3 Pesticides

Pesticides may also affect mosquito populations and communities both directly and indirectly. When initially applied, pesticides have lethal effects but can break down over time and switch from being lethal to sublethal and eventually to having no effects (Relyea & Hoverman, 2006). A shift in pesticide concentrations from lethal to sublethal is clearly demonstrated by the rapid reduction in mosquito larvae and their predators after pesticide application, followed by the resurgence of mosquito populations thereafter since they recover faster than their predators (Roger *et al.*, 1994; Muturi *et al.*, 2011a).

Lethal concentrations of pesticide may produce compensatory effects by killing a fraction of the population and releasing the survivors from larval competition (Juliano, 2007; Muturi *et al.*, 2010a). Conversely, insights from other aquatic insects and amphibians suggest that sublethal concentrations of insecticides can cause morphological, behavioral, and physiological impairments (Campero *et al.*, 2007; Pestana *et al.*, 2009a; Pestana *et al.*, 2009b; Relyea, 2012; Woodley *et al.*, 2015) that can become more deleterious when presented in a community context (i.e. in the presence of other environmental stressors)(Relyea, 2003; Relyea, 2004; Woodley *et al.*, 2015).

Similar effects have been reported in *Culex* and *Aedes* mosquitoes where larval exposure to certain pesticides alters their emergence rates, development time, longevity, body size, sex ratio, and vector competence (Bara *et al.*, 2014; Muturi & Alto, 2011; Muturi *et al.*, 2011a; Muturi *et al.*, 2010a; Muturi *et al.*, 2011b). In addition, C*ulex* mosquitoes preferred to oviposit in carbaryl-contaminated pools while *Anopheles* mosquitoes had no preference for either control or carbaryl-contaminated treatments (Vonesh & Buck, 2007). Despite this review of literature, a better understanding of how anthropogenic chemical contaminants influence mosquito ecology and biology is needed.

### 2.2.4 Environmental levels of agrochemicals (ammonium sulfate, diammonium phosphate, cypermethrin and glyphosate)

Ammonium sulfate [(NH4)2SO4] and diammonium phosphate [(NH4)2HPO4] are inorganic fertilizers that are commonly used to supplement the soil with three basic elements that are essential for plant growth nitrogen, sulfur and phosphorus. The annual world demand for nitrogen and phosphate fertilizers stands at 113.1 and 42.7 million tons, respectively and is expected to increase over the next two years (Food and Agricultural Organisation (FAO), 2015). These nutrients are important sources of ground and surface water pollution. Nitrate concentrations in water bodies near intensively cultivated and fertilized areas can be greater than 100 mg/l (George, 1987) while that of phosphate can be as high as 9.45 mg/l (Belagali, 2012).

Cypermethrin is a pyrethroid insecticide applied as an ultra-low volume spray to control insects in both large-scale commercial agriculture and small-scale agricultural settings. Pyrethroids are increasingly replacing organophosphates and carbamates in agriculture because of their effectiveness, lower application rates, and lower toxicity to mammals (Solomon *et al.,* 2001). Up to 3,114 µg/l of permethrin have been observed in water bodies (Norris *et al.,* 1983).

The herbicide glyphosate is of particular interest to understanding the consequences of pesticide use on pathogen transmission because of its widespread and abundant use (Kelly *et al.,* 2010). By volume, it is one of the most widely used herbicides and is commonly used for agriculture, horticulture, viticulture and silviculture purposes, as well as garden maintenance (Garthwaite *et al.,* 2010). Glyphosate is absorbed through foliage and minimally through roots and translocated throughout the plant (Franz *et al.,* 1997). Its primary action is blocking an enzyme that plants need to make aromatic amino acids and proteins thus killing the plants within days (Della-cioppa *et al.,* 1986).

Worldwide, around 650,000 tons of glyphosate products were used in 2011 (CCM, 2011). Glyphosate use has continued to increase largely due to the production of genetically modified crops and is expected to double by 2017 (Global Industry Analyst, 2011). The maximum concentration of glyphosate observed in water bodies is 5,200 µg/l (Edwards *et al.,* 1980).

As such, the use of chemicals in agriculture presents global alarm particularly for sub-Saharan Africa where irrigated agriculture is rampant, this is especially so because little is known about the effects of these chemicals on mosquito ecology and behavior.

## 2.3 Mosquito lifecycle

Mosquitoes (Diptera; Culicidae) are holometabolous insects which undergo a complete metamorphosis in their life history. They go through four separate and distinct stages of the life cycle: Egg, Larva, Pupa, and adult (Fig. 2.2). It normally takes between 7-14 days for an egg to develop into an adult, if weather conditions are suitable for mosquito development (Capinera, 2008). The first three stages occur in water, but the adult is an active flying insect. Eggs are laid singly (*Anopheles*) or attached together to form "rafts" (*Culex, Coquillettidina*) on water surface or on damp soil that will be flooded (Becker *et al.,* 2010).

Most eggs hatch within 48 hours. Larvae (wrigglers) lives in water and feeds on microorganisms and organic matter. Larvae molts four times; most species surface to breathe air (Smith *et al.,* 2013). During the fourth molt the larva changes to pupa. The pupal stage is a resting, non-feeding stage of development where the juvenile develops into an adult. Pupae are mobile responding to light changes and moving with a flip of their tails towards the bottom or protective areas. The pupal skin splits after about two days and the adult emerges (Smith *et al.,* 2013). The newly emerged adult rests on the surface of the water for a short time to allow its body parts to harden. The wings have to spread out and dry before it can fly to find food and mate. How long each stage lasts depends on both temperature and species characteristics.

Adult mosquitoes can feed and reproduce for several weeks before dying (Le Menach *et al.,* 2005). During this time female mosquitoes require one or more blood meals every 2-3 days in order to mature eggs after mating (Godfray, 2013; Minakawa *et al.,* 1999). Male mosquitoes feed exclusively on plant sugars (Smith *et al.,* 2013). The requirement for a blood meal makes the mosquitoes medically the most important group of insects (Okwa *et al.,* 2007; Thielman & Hunter, 2007), transmitting a number of diseases and causing great health problems (Araujo *et al.,* 2012). Since survival rate of the vector is one of the key factors for transmission dynamics, it is important to understand mosquito ecology and population dynamics (Smith *et al.,* 2013). Despite intensively ongoing research on mosquito ecology, relatively little is known about how agrochemicals influence mosquito life history traits.

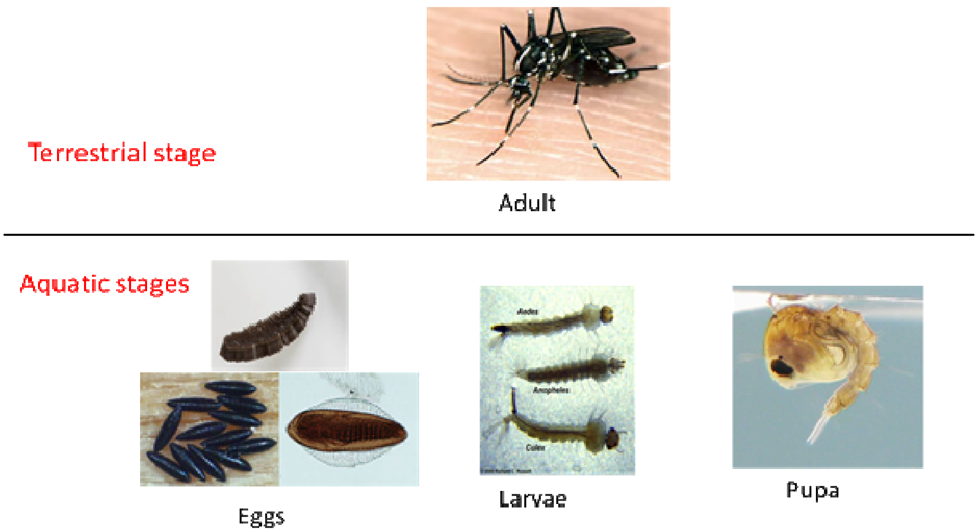


**Figure 2.2: Mosquito lifecycle. Source: (Muturi *et al*, 2010a)**

### 2.4 Mosquito-borne diseases

#### 2.4.1 Malaria

Malaria is still by far the world's most important vector-borne disease (Becker *et al.,* 2010), affecting more than 300 million people with 627,000 fatalities annually, more specifically in children under age of 5 years (WHO, 2015). Nearly 40% of the world's population lives in regions where malaria is endemic with the highest mortality at 85-90% occurring in the sub- Saharan Africa (Speight *et al.,* 2008, WHO, 2015).

Human malaria is caused by a parasitic protozoan from the genus plasmodium, (Bomblies, 2009) which are transmitted among humans by female mosquitoes of the genus *Anopheles* (Muriu *et al.,* 2013, Sinka *et al.,* 2013). Mosquito reproductive success is reflected by the fitness and amount of emerging adults, determining vector density, biting rate and life expectancy (Himeidan & Kweka, 2012). These influence vectorial capacity which determines the stability and intensity of disease transmission (Kiszewski *et al.,* 2004).

Substantial progress has been made globally to control and eliminate malaria, but it continues to be a significant public health problem with roughly 3.2 billion people worldwide at risk of the disease. To achieve sustainable control over malaria, healthcare professionals will need a combination of new approaches and tools, and research will play a critical role in development of those next-generation strategies.

### 2.4.2 Lymphatic filariasis

Lymphatic filariasis, commonly known as elephantiasis, is a neglected tropical disease. Over 947 million people in 54 countries worldwide remain threatened this disease, and require preventive chemotherapy to stop the spread of this parasitic infection (WHO, 2013). In 2012 over 67 million people were infected, with about 36 million disfigured and incapacitated by the disease (WHO, 2013). Globally, an estimated 19 million men suffer with genital disease (hydrocele) and over 15 million people , mostly women, are afflicted with lymphedema or elephantiasis of the leg (WHO, 2013)

Lymphatic filariasis caused by infection with thread-like worms called nematodes of the family Filarioidea: 90% of infections are caused by *Wuchereria bancrofti* and the remainder by *Brugia spp.*(Ottesen *et al.,* 1997). In 1995, WHO ranked LF as the second leading cause of disability worldwide after mental illness (WHO, 1995). The parasites are transmitted to humans through mosquitoes (*Culex, Anopheles* and *Aedes*) (Michael *et al.,* 1996). Infection is usually acquired in childhood causing hidden damage to the lymphatic system.

The most noted characteristic of this disease is the profuse enlargement of one or both limbs, one or both breasts in women, areas of the face, and genital area due to fluid accumulation and lodging of the parasites in the human lymphatic system (WHO, 2013). Despite ongoing control efforts, LF continues to be a major public health challenge in Africa, where more than 50 million cases are reported each year, accounting for one-third of the global burden (Mbutolwe et al, 2013). Eliminating lymphatic filariasis can prevent unnecessary suffering and contribute to the reduction of poverty.

### 2.5 Transmission dynamics of malaria In Sub-Saharan Africa

The observation that rice fields frequently generate large numbers of mosquitoes suggests that malaria transmission will increase in local communities. However, there is an increasing body of evidence that indicates that this is not the case, at least in areas of stable transmission, where malaria may be less of a problem than in surrounding communities outside the irrigated fields. A review of the literature shows that high vector density does not necessarily imply an increased risk of exposure to malaria parasites. For example, a field study to determine the association between irrigated rice cultivation and malaria transmission in Mwea, Kenya, found no difference in malaria transmission by *An. arabiensis* between irrigated and non-irrigated areas but in contrast, found less malaria transmission by *An. funestus* in irrigated than in non-irrigated areas (Muturi *et al.,* 2008).

Elsewhere, a study of two communities in the ValleÂe du Kou, Burkina Faso, revealed that malaria prevalence varied between 35-83% in the savannah and 16-36% in the rice-growing area (Boudin *et al.,* 1992). Introduction of a large-scale rice irrigation scheme in The Gambia was observed to result in less malaria near the rice field than in other rural communities (Lindsay *et al.,* 1991). Irrigated rice cultivation in Mali was associated with a reduction in the annual incidence of malaria and a 10-fold reduction in the sporozoite rate than was found in adjacent irrigated areas (Sissoko *et al.,* 2004). Githeko and others reported that the sporozoite rate of *Anopheles gambiae s.l* was five times lower in the Ahero rice scheme than in the neighboring sugarcane growing area (Githeko *et al.,* 1993).

In the Lower-Moshi rice irrigation scheme, Tanzania, the number of infective bites was 2.6 times lower in the irrigation scheme than in the control village and the malaria prevalence was four times lower in children living near irrigated rice cultivation compared with a nearby savannah village (Ijumba*,* 1997). Alternatively, in the rice-growing area of the Rusizi valley, Burundi, the vectorial capacity of *An. gambiae* s. l. was 150 times higher in the rice irrigation scheme than in an adjacent area growing cotton (Coosemans, 1985). Elsewhere, in the rice-growing areas of Bobo Dioulasso, Burkina Faso, the number of infective bites received in the local community was similar to that in the control area (Robert *et al.,* 1985). Introduction of irrigated rice cultivation in the Senegal River delta did not alter malaria transmission (Faye *et al.,* 1993).

Collectively these findings indicate that the relationship between irrigated agriculture and malaria transmission is complex i.e. irrigation can increase, decrease or have no effect on malaria transmission. Reason would probably be due to influence of environmental and socio economic factors (e.g. contaminants like agricultural chemicals) which may weigh heavily on arthropod vectors, in part because they determine vector abundance, fitness and competence for pathogens (Gimnig *et al*., 2002; Alto *et al*., 2005; Muturi *et al*., 2010a). There is need therefore to elucidate the role of agrochemicals on malaria transmission

**Table 2.1: Effect of irrigation agriculture on transmission dynamics of malaria**

| No difference | Less transmission | More transmission |
|---|---|---|
| Robert *et al.,* 1985 (Burkina Faso) | Dolo *et al.,* 2004 (Mali) | Baldet *et al.,* 2003 (Burkina Faso) |
| Audibert *et al.,* 1990 (Cameroon) | Sissoko *et al.,* 2004 (Mali) | Coosemans *et al.,* 1985 (Burundi) |
| Couprie *et al.,* 1985 (Cameroon) | Ijumba *et al.,* 2002a (Tanzania) | Robert *et al.,* 1992 (Cameroon) |
| Faye *et al.,* 1995 (Senegal) | Boudin *et al.,* 1992 (Burkina Faso) | Oomen *et al.,* 1988 (Sudan) |
| Faye *et al.,* 1993 (Senegal) | Thomson *et al.,* 1994 (Gambia) | Githeko *et al.,* 1993 (Kenya) |
| Muturi *et al.*, 2008 (Kenya) | Mutero *et al.,* 2004b (Kenya) | Yohannes *et al.,* 2005 (Ethiopia) |

## 2.6 "Paddies paradox"

An interesting phenomenon that is possible where higher vector densities in irrigated rice agro ecosystems are associated with lower risk of malaria transmission compared to adjacent non-irrigated agro ecosystems (Mutero *et al.,* 2004a; Mutero *et al.,* 2009; Ijumba *et al.,* 2002a; Ijumba & Lindsay, 2001). Ijumba and Lindsay (2001) coined the term "paddies paradox" to refer to this phenomenon.

Several theories have been advanced in an attempt to explain this paradox. For instance, the irrigation schemes generate short-lived mosquitoes due to density-dependent mechanisms at larval stage level. The basis for this theory would be that density-dependent competition during larval stages may lead to smaller-sized adult mosquitoes with lower longevity, thus compromising their vectorial capacity (Ameneshewa & service, 1996; Bruce-chwatt, 1993).

Another school of thought is that, *An. arabiensis* in irrigated villages tends to feed overwhelmingly on cattle (Mutero *et al.,* 1999). It has also been suggested that communities living within the schemes are economically empowered from rice farming proceeds as compared to those outside the schemes and as such can afford bed nets, better nutrition, adequate living standards and a better medical care (Ijumba & Lindsay, 2001). However, the underlying mechanisms still remains widely unknown and a proper understanding of the factors contributing to this paradox is therefore urgently needed.

### 2.7 Oviposition

In the nature, mosquito oviposition or egg-laying is intimately related to the search for aquatic habitats where immature stages (larvae and pupae) can develop to adulthood. The choice of an appropriate oviposition site has significant impact on the fitness of progeny, distribution of larvae, population dynamics and the overall maternal reproductive fitness and success (Gimnig *et al*., 2002; Alto *et al*., 2005; Muturi *et al*., 2010b). Different environmental cues may elicit oviposition behaviors beginning with the initial orientation towards a potential oviposition site and culminating in the deposition of eggs. The selection of oviposition sites by many mosquito species is greatly influenced by both physical and chemical stimuli.

Physical stimuli may include surface reflectance (Bentley and Day, 1989), water color (Beehler & DeFoliart 1990; Beehler *et al.,* 1993; Isoe *et al.,* 1995; Li *et al.,* 2009) and presence or absence of vegetation. Chemical factors mediating oviposition may result from the previous presence of conspecific pupae and larvae (Bentley Day, 1989), or from eggs which in several *Culex* species contain an apical droplet of pheromone (Laurence and Picket, 1985). Some mosquito species are known to select breeding sites that enable them to avoid competitors. For example, *Culex pipiens* oviposition is competitively inhibited in habitats where *Daphnia magna* is already established (Duquesne *et al*., 2011) while *Culiseta longiareolata* avoids to oviposit in aquatic habitats where insect predators such as dragonfly larvae and backswimmers are present (Stav *et al*., 1999; Blaustein *et al*., 2004; Arav & Blaustein, 2006).

Pesticides also influence oviposition site selection. For example, many insect repellents that were tested in laboratory trials effectively deterred oviposition by the mosquito *Aedes (Stegomyia) albopictus (*Xue *et al*., 2001, 2006). Extracts of *Solanum trilobatum* also prevented oviposition by *Anopheles stephensi (*Rajkumar &Jebanesan, 2005). However, unlike strong avoidance behavior imposed on ovipositing gray tree frogs (*Hyla chrysoscelis*), *Culex* mosquitoes were found to be attracted to and oviposit in pools treated with carbaryl (Vonesh &Buck, 2007). Elsewhere, *An. gambiae sensu stricto*, were two times likely to lay in lake water infused with the chemical cedrol than in lake water alone (Lindh *et al.,* 2015). In addition, a wide range of mosquito species, including *An. arabiensis* and *Culex spp*. (Mutero *et al.,* 2004b), *Cx. pipiens pipiens* (Olayemi *et al.,* 2012), *Cx. vishnui* and *An. vagus* (Mogi &Miyagi, 1990), *Cx. tritaeniorhynchus, Cx. pseudovishnui, Cx. vishnui, An. subpictus* and *An. vagus* (Victor &Reuben, 2000) are known to be attracted to inorganic nitrogenous fertilizers.

Overall, while major efforts have been directed towards the understanding of environmental cues that influence oviposition site selection by mosquitoes, little is known about how widely used agricultural chemicals affect oviposition site selection by the primary vector mosquitoes in Africa including *An. arabiensis* and *Cx. quinquefasciatus*.

## 2.8 Oviposition theories

### 2.8.1 Optimum oviposition theory

The key processes influencing successful development of mosquito larvae are considered to be site selection of gravid females and the survival of the larvae (Fillinger *et al.,* 2009). Both of them depend on the suitability and productivity of the habitat (Kenea *et al.,* 2011). Mosquitoes are thought to actively select suitable habitats rather than randomly colonize them (Minakawa *et al.,* 2004) in order to maximize offspring survival and fitness (Yoshioka *et al.,* 2012). Organisms that are actively selecting habitat must rely on environmental cues to help them identify high quality habitat (Bentley & Day, 1989). As such, they avoid ovipositing in habitats with potentially lethal chemicals as explained by optimal oviposition theory, which suggests that gravid insects lay their eggs in habitats that maximize the performance of their offspring (Scheirs & De Bruyn, 2002). If this hypothesis is correct, egg laying females are thus expected to select the most suitable sites for their offspring based on reliable cues of habitat quality (Craig *et al.,* 1989; Mayhew, 1997).

Previous examination of oviposition preference has shown that *A. albopictus* and *A. triseriatus* oviposit in water infused with oak leaves in preference to water without leaves (Trexeler *etal*., 1998). In addition, a wide range of mosquito species, are known to be attracted to inorganic nitrogenous fertilizers (Victor & Reuben, 2000; Mutero *et al.*, 2004b; Olayemi *et al*, 2012; Mogi, & Miyagi, 1990). Probably because the high levels of nitrates may promote mosquito production by enhancing proliferation of algal bloom and other microbial assemblages that serve as food for mosquito larvae (Sunish *et al.*, 1998; Victor & Reuben, 2000).

Gravid mosquitoes use chemosensory (olfactory, gustatory, or both) cues to select oviposition sites suitable for their offspring (Bentley & Day, 1989). In nature, these cues originate from plant infusions, microbes, mosquito immature stages and predators

(Lawrence & Picket, 1985; Stav *et al.,* 1999; Blaustein *et al.,* 2004). While attractants and stimulants are cues that could show the availability of food and suitable conditions, repellents and deterrents show the risk of predation, infection with pathogens, or strong competition.

Gravid females are thus expected to preferentially oviposit in containers with a superior performance agricultural chemical (higher survivorship, faster larval growth, greater adult size and longer lifespan), relative to containers with no agricultural chemical or with inferior performance agricultural chemical (low survivorship, slow larval growth, smaller adult size and shorter lifespan). This therefore, calls for a better understanding of agricultural practices that could influence habitat quality and habitat selection by major mosquito vectors.

### 2.8.2 Ecological trap theory

Understanding altered ecological and evolutionary dynamics in novel environments is vital for predicting species responses to rapid environmental change. One concept relevant to such dynamics is the ecological trap, which arises from rapid anthropogenic change. Ecological traps occur when formerly adaptive habitat preferences become maladaptive because the cues individuals preferentially use in selecting habitats lead to lower fitness than other alternatives (Crespi, 2000). Evidence for ecological traps has primarily been found in habitats modified by human activities (e.g. agricultural activities) (Battin, 2004). Thus although the female's prime goal may be to choose an oviposition site that maximizes offspring survival and performance, the outcome is not necessarily a perfect match between site selected and larval performance.

Many studies have demonstrated a good correspondence between preference and performance (e.g. Via, 1986; Craig *et al.,* 1989; Hanks *et al.,* 1993; Nylin & Janz, 1993; Barker & Maczka, 1996). However, an equally large number of studies found no correlation (e.g. Penz & Araujo, 1990; Valladares & Lawton, 1991; Underwood, 1994;

Larsson *et al.,* 1995). For instance, *Culex* mosquitoes were found to be attracted to and oviposit in pools treated with carbaryl (Vonesh & Buck, 2007). From the above examples it is clear that environmental cues, especially those that are anthropogenic in nature make ecological traps and those ecological traps can trigger extinction if all individuals of a population or a species are driven into them (Kokko & Sutherland, 2001).

Past literature has it that; ecological traps are 'attractive sinks' because they absorb immigrants but produce zero emigrants (Delebes *et al.,* 2001). This therefore, calls for a better understanding of role of agricultural chemicals in influencing habitat quality and habitat selection by major mosquito vectors. This understanding is needed to effectively model and map vector populations and develop suitable and cost-effective insect management strategies.

**2.9 Vectorial capacity**

Vectorial capacity of mosquitoes; is the daily rate at which future inoculations arise from a currently infective case (Smith & Mackenzie, 2004). It was originally devised by MacDonald in 1957 for malariologists, as a measure of the transmission potential of a vector borne pathogen within a susceptible population. (MacDonald, 1957; Smith & Mackenzie, 2004; Dye, 1992). It is directly related to man-biting rate, feeding habits and life expectancy of the mosquito. It is usually measured by collecting mosquitoes during an entire night using human bait. It represents several components and is given by the calculation below;

$$VC = \frac{ma^2p^Nb}{-\ln(p)}$$

Where **a** is the man biting rate and **m** is the mosquito density; these parameters are measures of contact between the vector and vertebrate hosts (Smith and Mackenzie, 2004). The probability of daily survival **p** is a measure of the mortality rate of the vector (Smith & Mackenzie, 2004). The extrinsic incubation period (**EIP**) **N** is the time, in days; it takes for a pathogen to infect the mosquito and disseminate to the salivary glands where it can be transmitted (Smith & Mackenzie, 2004). Also included is a transmission capability parameter, Vector competence **b**, which is the ability of an arthropod to transmit an infectious agent following exposure to that agent (Dye, 1986; Black *et al.*, 1996; Dye *et al.*, 1995; Hardy, 1988). Both vector competence and extrinsic incubation period have been used to evaluate differences in pathogen strains (Moudy *et al.,* 2007; Armstrong *et al.,* 2001, 2003). This formula expresses the capacity of a vector population to transmit malaria based on the potential number of secondary inoculations originating per day from an infective person (Smith &Mackenzie, 2004).

The prolonged exposure of mosquito immatures to agricultural chemicals may eventually modify these components. Insecticides have been shown to modify vector density by killing a fraction of the immature stages in the aquatic habitats, and this can in turn enhance survival of the remaining individuals resulting in more vectors than before exposure, especially when resources are limiting (Boone & semlitsch, 2001, Muturi *et al.*, 2010a). Recently, synthetic nitrogenous fertilizers were found to be responsible for a significant increase in anopheline and culicine larval populations in rice fields, which translates into a higher biting rate and probably increased pathogen transmission (Victor & Reuben, 2000; Mutero *et al*., 2004b).

Pesticides may also initiate a trophic cascade or a release from competition by decimating or impairing the sensitive species (e.g. kill predators that feed on larvae or change community structure and abundance that mosquitoes feed on. The resulting individuals may be bigger and more competent in pathogen transmission (Fleeger *et al.*, 2003; Juliano *et al.,* 2004). For instance, Service observed a resurgence of mosquito larvae after initial control with insecticides due to slow rebounding of predator populations (Service, 1993). In Korea, agricultural pesticide application reduced the density of the Japanese encephalitis vector *Culex tritaeniorhyncus* in rice-growing areas but had no effect on the main malaria vector *Anopheles sinensis* (Shim, *et al.*, 1995a, 1995b). It has been shown that individuals that survive exposure to low concentrations of pesticide benefits in terms of size due to release from competition or selection against weaker individuals (Antonio *et al*., 2008; Muturi *et al*., 2011a) .

Larger mosquitoes are associated with higher fecundity and longer adult life spans (Hawley 1985; Blackmore and Lord 2000; Briegel & Timmermann 2001) and may also be more or less competent for pathogens (Muturi *et al*., 2011a). Elsewhere, immunotoxicological studies have shown that pesticides can reduce fitness of an organism by causing structural and functional alterations to its immune system (e.g. elevating levels of cytochrome P450) that way compromising its vectorial capacity (Blankley *et al*., 1999a, 1999b). Pesticides have also been shown to change the physiology of mosquitoes (e.g. reduce food intake) which may interfere with the life history traits like adult size, longevity and fecundity, which impacts on vectorial capacity (Ribeiro *et al.*, 2001). Pesticides may also cause a diversion of energy to maintenance and/or modification of the level and activity of target biomolecules (e.g. inhibition of enzyme activity) (Campero *et al.*, 2007). Akogbeto and others suggested that insecticides inhibit hatching rate and development of larvae of *An. gambiae* (Akogbeto *et al.*, 2006).

These examples highlight the diversity of processes that should be considered when determining the overall consequences of cypermethrin, Glyphosate, Diammonium phosphate and ammonium sulfate treatment. This study will show that a better understanding of the ecological impacts of chemical disturbances is needed to facilitate development of effective strategies to control harmful species. Such knowledge will have direct benefits in designing mosquito control strategies.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study area

This study was conducted at the Mwea Irrigation and Agricultural Development (MIAD) experimental station and at Kangichiri and Kariua villages in the Mwea rice irrigation scheme, 100 km North East of Nairobi, in Mwea Division, Kenya. Mwea occupies the lower altitude zone of Kirinyaga County in an expansive low-lying area mainly characterized by black cotton soils. The mean annual rainfall is 950 mm with maximum amount falling in April/May (long rains) and October/November (short rains). The average maximum temperatures are in the range of 16–26.5 °C. Relative humidity varies from 52–67%. According to the 2009 national census, Mwea Division has approximately 150,000 persons in 25,000 households. The Mwea Rice Irrigation Scheme is located in the west central region of Mwea Division and covers an area of about 13,640 ha. More than 50% of the Scheme area is used for rice cultivation. The remaining area is used for subsistence farming, grazing and community activities.

## 3.2 Sampling sites

Two villages Kangichiri and Kariua were selected for the study. They both lie within the irrigation scheme and were selected based on the presence of large populations of *An. arabiensis* (Mwangangi *et al.,* 2013, Sinka *et al.,* 2016).and *Cx. quinquefasciatus* (Mutero *et al.,* 2000) and on their close proximity to MIAD experimental station.

The two villages are 500m apart and are located at the west central region of the scheme. They have 150 and 222 homesteads, respectively, with 650 residents in each village. Cows, goats, chickens, and donkeys are the primary domestic animals in the two villages. These animals are kept within 5m of most houses. More than 90% of the houses have mud walls with iron roofing. More than 75% of each village land is under rice cultivation, whereas people occupy the remaining area with 10% used for vegetables and bananas.

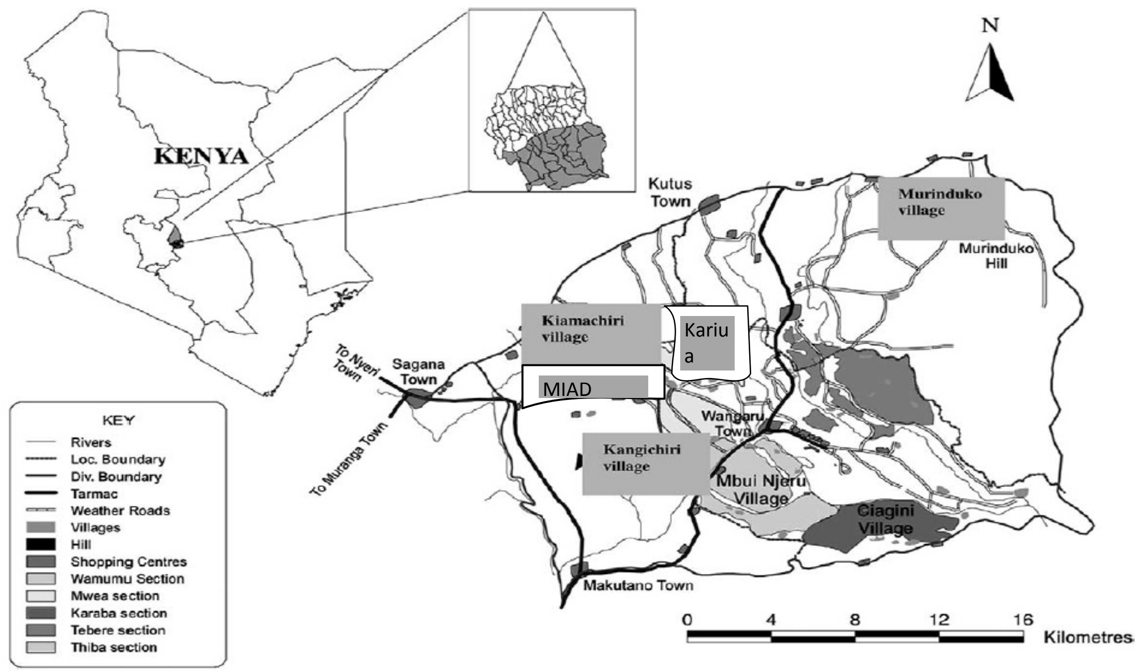


Fig. 1. A map of Kenya showing the sampling sites.

**Figure 3.1: A map of Kenya showing the sampling sites. (Source; Muturi *et al.*, 2008 )**

### 3.3 Study design

This was an experimental study where microcosms (laboratory-based experiments) and mesocosms (field-based experiments) were established and manipulated with the insecticides (alpha-cypermethrin), the herbicide (glyphosate) and the inorganic fertilizers (ammonium sulfate and diammonium phosphate) both under laboratory and field conditions. The study was conducted during the interphase of long rains and short rains (August and November) 2014.

### 3.4 Study Population

#### 3.4.1 Sample size

The study group population for laboratory oviposition experiment were gravid females of both *Anopheles arabiensis* and *Culex quinquefasciatus;* the sample size for the laboratory experiment was 9 (*An. arabiensis*) and 15 (*Cx. quinquefasciatus*) insect rearing cages (30 × 30 × 30 cm) each with 20 gravid females. The field experiment had 5 plastic buckets containing the oviposition substrates replicated 5 times; while the survivorship experiment had 6 replicates of the 5 treatments and control in tripour beakers each with 20 first instar larvae. A preliminary survey was conducted prior to embarking on the research work, and the information collected used to determine the choice of sampling size. For instance; mosquito abundance, environmental factors surrounding the houses (e.g. number of sleepers and presence of livestock), size of the village, consent by the leader of the home, large enough samples to draw a conclusion, availability of labour and economic implications.

#### 3.4.2 Sampling techniques

Blood-engorged females of *An. arabiensis* and *Cx. quinquefasciatus* were captured inside human dwellings (bedrooms and latrines) using manual aspirators in Kangichiri and Kariua villages in Mwea. The mosquitoes were collected from their resting and hiding places, under the beds, thatched roofs, upper regions of mud walls, dark and dump corners and ceilings, cracks and crevices. They were then transferred into cages, fed with 10% sucrose solution using pieces of cotton wool and transported to MIAD Centre.

### 3.4.3 Mosquito Identification

Gravid mosquitoes were collected inside human dwellings using manual aspirators, and taken to the laboratory where culicines and anophelines were separated, sorted into subfamily and subsequently identified morphologically to species (Edwards 1941, Gillies & Coetzee 1987). They were identified as gravid by examining the abdominal appearance.

## 3.5 Procedures of data collection

### 3.5.1 Laboratory studies on the effect of agricultural chemicals on oviposition site selection by *An. arabiensis* and *Cx. quinquefasciatus*

Blood-engorged females of *An. arabiensis* and *Cx. quinquefasciatus* were collected inside human dwellings in Kangichiri and Kariua villages using manual aspirators. At MIAD, 20 randomly


**Figure 3.2: The laboratory experiment set-up**

selected females of either species were transferred into one of 9 (*An. arabiensis*) and 15 (*Cx. quinquefasciatus*) insect rearing cages (30 × 30 × 30 cm) and provided continuous access to 10 % sucrose. For *Cx. quinquefasciatus* oviposition, each cage was provisioned with five Petri dishes containing either 50 ml of rain water (control) or 50 ml of agrochemical-spiked water generating four treatments with a final concentration of 0.1 mg/l α-cypermethrin, 0.5 mg/l glyphosate, 845 mg/l ammonium sulfate, or 845 mg/l diammonium phosphate. These pesticide concentrations are within the range that is commonly found in aquatic habitats (Norris *et al*., 1983; Edwards *et al*., 1980). In contrast, the fertilizer concentrations used in this study are much higher than observed in nature (George, 1987; Belagali, 2012). Because fertilizer application in rice fields within the study sites and other parts of Africa is done manually through broadcasting, it is expected that some parts of the rice fields receive high doses of fertilizers similar to those used in this study. Therefore this study sought to determine how potentially higher doses of fertilizers may affect mosquito ecology. A similar setup was used for *An. arabiensis* except that the Petri dishes were lined with filter papers moistened with respective agrochemical treatments. A single Petri dish was placed on each corner of a cage and the fifth one was placed in the middle of the cage. The treatments were rotated daily to eliminate positional bias. The number of eggs laid in each Petri dish were counted and recorded every day for 31 days and their sums computed. Agrochemical-treated filter papers were replaced each day.

### 3.5.2 Field studies on the effect of agricultural chemicals on oviposition site selection by *Cx. quinquefasciatus*

This experiment was conducted between 1$^{st}$ August, 2014 and 20$^{th}$ September, 2014 under field conditions since *Culex* egg rafts are easy to monitor compared to *Anopheles* eggs that are laid individually. The experiment was conducted in five randomly selected homesteads in Kangichiri village. In each homestead, five 20-litre plastic buckets containing either 3 liters of fermented grass infusion (control) or 3 liters of fermented grass infusion spiked with one of four agrochemicals for a final concentration of 1 mg/L α-cypermethrin, 2 mg/L glyphosate, 845 mg/L ammonium sulfate, or 845 mg/L diammonium phosphate served as the artificial oviposition sites. Each bucket had a top lid and large openings on their upper halves to facilitate access by mosquitoes. The infusion was prepared by mixing 1 kg of fresh grass with 100 litres of water and leaving it to ferment for 5 days. The homesteads were at least 60 meters apart and the buckets within a homestead were 2 meters apart. The buckets within each homestead were rotated daily to eliminate spatial effects. Every day, egg rafts were collected, counted, and transported to the laboratory in petri dishes lined with moist filter papers. The infusion was replaced every three days and 13 oviposition trials were conducted.


**Figure 3.3: Field experiment set-up**

### 3.5.3 Effect of agrochemicals on emergence rate, development time, adult wing length and longevity of *An. arabiensis* and *Cx. quinquefasciatus*

In this experiment, 20 first instar larvae (24 hours old) of either *An. arabiensis* or *Cx. quinquefasciatus* obtained by hatching eggs from control treatments of oviposition experiments were added into tripour beakers containing either 350 ml of rain water (control) or 350 ml of rain water spiked with one of four agrochemical treatments at the following final concentrations: 0.004 mg/L α-cypermethrin, 0.05 mg/L glyphosate, 845 mg/L ammonium sulfate, or 845 mg/L diammonium phosphate. Each treatment was replicated 6 times for a total 60 containers. The larvae were replenished with 0.05 g ground Tetramin® baby fish food once per week. Containers were examined daily until all individuals had either pupated or died. Pupae were removed daily and placed in plastic vials with a small volume of water until eclosion. The date and sex of newly eclosed adults in each replicate were recorded. The adults were held individually in 75 mm x 20 mm plastic cups covered by nylon net and provided continuous access to 10% sucrose solution. All cages were maintained at approximately 25-27 $^{0}$C, with 65-75% relative humidity (RH) and a 12:12 Light: Day photoperiod. Each individual adult mosquito was monitored daily until death. Dead adults (both males and females) were preserved in plastic vials and transported to the Eastern and Southern Africa Centre of International Parasite Control (ESACIPAC), Kenya Medical Research Institute (KEMRI) where their wings were removed and mounted on microscope slides. The wings were scanned and measured from the tip (excluding the fringe) to the distal end of the allula, using VHX KEYENCE digital microscope at the Department of Entomology, Nairobi National Museum.

### 3.6 Data management and analyses

Collected data was recorded in special designed data sheets and entered into computer using Microsoft-Excel software. Data was also recorded in the laboratory and field work books respectively and was backed up in Compact discs, flash discs and hard disc.

Data analyses was conducted using SPSS version 23 (IBM SPSS) and SAS 9.4 (SAS Institute) statistical packages. Data were checked for normality and homogeneity of variances using Kormogorov-Smirnov test. Oviposition data was log transformed (log + 1) to normalize the distribution. The means of each replicate of a treatment were compiled for each life-history trait and statistical analyses were based on these means. For each mosquito species, univariate analysis of variance (ANOVA) was used to determine the effect of agrochemical treatments on oviposition site selection, hatching rates, and emergence rates (males and females combined). In the field oviposition experiment, agrochemical treatment was used as a fixed factor while trial number was used as a random factor. Multivariate analysis of variance (MANOVA) was used to determine the effect of agrochemical treatments on both male and female development time, wing length, and longevity. Standardized canonical coefficients were used to describe the relative contribution of development time, wing length, and longevity to the significant treatment effects. When significant effects were obtained in both ANOVA and MANOVA tests, pair wise differences between treatment means were compared using a Tukey-Kramer adjustment test.

### 3.7 Study Approvals

The proposal was submitted for review and approval to KEMRI and JKUAT. Both scientific and ethical approvals were sought from these institutions.

### 3.8 Ethical Considerations

Free and informed written consent for participation were sought beforehand from the household heads in both villages, after meeting the criteria for participation in this study. The benefits and risks associated with participating in the study were explained, and privacy and confidentiality assured to participants in advance of seeking their consent. Ethical clearance was sought from the Ethical Review Committee (ERC), Kenya Medical Research Institute beforehand. The participants were given an opportunity to ask questions and seek clarifications on issues not well understood on the study, if they had additional questions or concerns about the study later, the participant were free to contact the researcher in charge of the study Tabitha W. Kibuthu, Cell no., 0725 978508 or through the email address tabwamboi@yahoo.com. If they had questions or concerns about their rights as a participant in the study, they were free to contact: The Secretary, KEMRI Ethics Review Committee, P.O. Box 54840-00200, Nairobi, Telephone numbers: 020-2722541, 0722205901, 0733400003; Email address : ERCadmin@kemri.org

# CHAPTER FOUR

# RESULTS

## 4.1 Effect of agrochemicals on oviposition site selection by *An. arabiensis* and *Cx. quinquefasciatus*

Agrochemical treatments had significant effects on oviposition site selection by *An. arabiensis* ($F = 24.02$, $df = 4, 40$, $P < 0.001$). The number of *An. arabiensis* eggs deposited were highest in DAP and ammonium sulfate treatments, intermediate in control treatment and lowest in cypermethrin and glyphosate.

Similarly, results of both laboratory ($F = 25.18$, $df = 4, 70$, $P < 0.001$) and field experiments ($F = 126.62$, $df = 4, 48.30$, $P < 0.001$) revealed that *Cx. quinquefasciatus* egg rafts were highest in DAP and ammonium sulfate treatments, intermediate in control treatment, and lowest in cypermethrin and glyphosate treatments (Fig 4.1B and 4.1C).

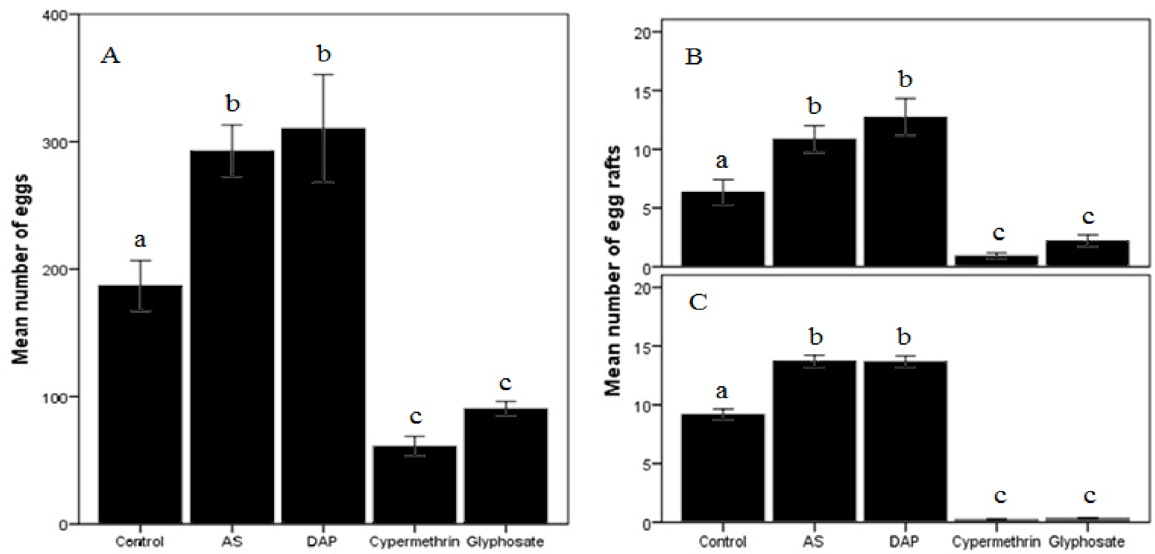


**Figure 4.1: Mean number (± SE) of A) An. arabiensis eggs and B,C) Cx. quinquefasciatus egg rafts laid in different agrochemical treatments. B and C are results for laboratory and field experiments, respectively. Different lower case letters indicate significant differences between treatments.**

## 4.2 Effect of agrochemicals on emergence rate, development time, wing length and longevity of *An. arabiensis* and *Cx. quinquefasciatus*

Agrochemical treatments had significant effects on emergence rates of *An. arabiensis* (F = 4.28, df = 4, 25, $P$ = 0.009), but not on those of *Cx. quinquefasciatus* (F = 4.54, df = 4, 25, $P$ = 0.07. Emergence rates for *An. arabiensis* in cypermethrin treatment were significantly lower compared to those of control and ammonium sulfate but not glyphosate and DAP (Fig 4.2A). Emergence rates for *Cx. quinquefasciatus* in cypermethrin and glyphosate treatments were significantly lower than those of DAP but not control and ammonium sulfate treatments (Fig 4.2B).

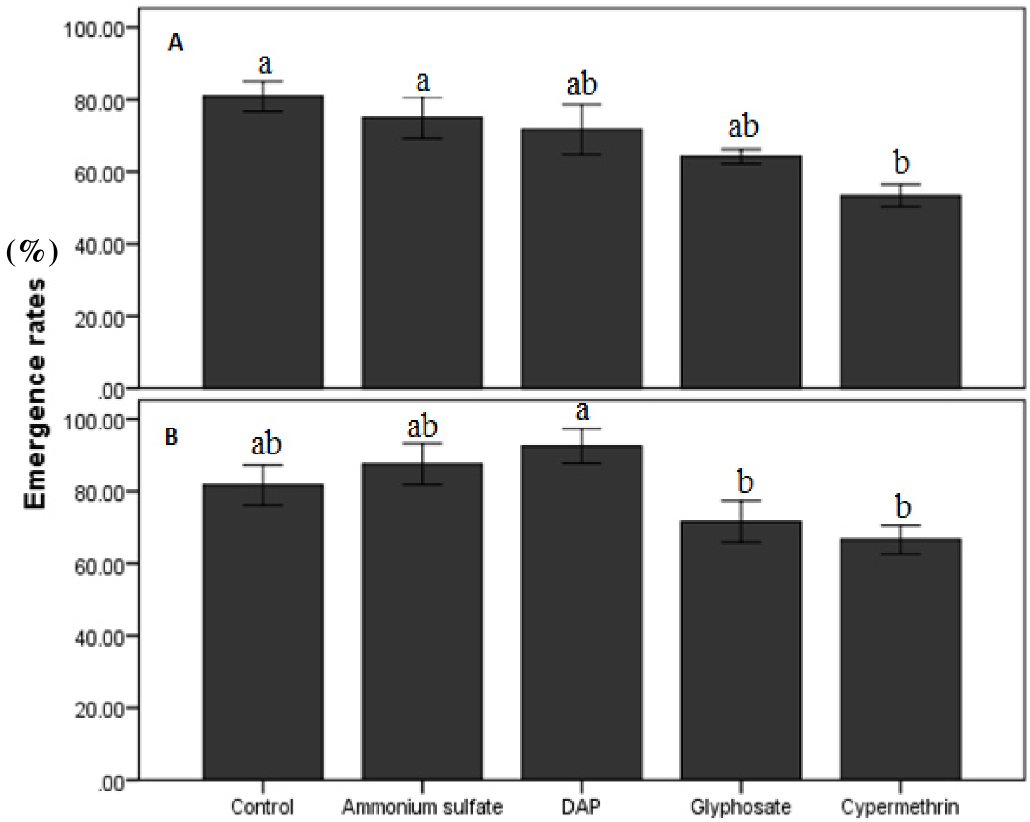


**Figure 4.2: Emergence rates (± SE) of A) An. arabiensis and B) Cx. quinquefasciatus mosquitoes in different agrochemical treatments. Different lower case letters indicate significant differences between treatments.**

For both mosquito species and sexes, multivariate analysis of variance revealed a significant effect of agrochemical treatment on development time to adulthood, wing length, and longevity with development time followed by wing length accounting for most variation (Table 4.1). *An. arabiensis* females from control, DAP, and ammonium sulfate treatments had significantly longer development times compared to those from glyphosate, and cypermethrin treatments (F = 17.71, df = 4, 25, $P < 0.001$; Fig. 4.3A). However, agrochemical treatment had no significant effect on *An. arabiensis* female wing length (F = 3.2, df = 4, 25, $P = 0.30$; Fig 4.3A) and longevity (F = 1.6, df = 4, 25, $P = 0.21$). *An. arabiensis* males from DAP and ammonium sulfate treatments had significantly longer development times compared to those from control, glyphosate, and cypermethrin treatments (F = 16.36, df = 4, 25, $P < 0.001$; Fig. 4.3B). *Anopheles arabiensis* males from ammonium sulfate treatment were significantly smaller compared to those from control, glyphosate, and cypermethrin treatments but not DAP treatment (F = 6.32, df = 4, 25, $P = 0.001$; Fig. 4.3B). In addition, males from DAP treatment were significantly smaller than those from cypermethrin treatments (Fig. 4.3B).

There were no significant effects of agrochemical treatment on *An. arabiensis* male longevity (F = 1.05, df = 4, 25, $P = 0.40$). *Culex quinquefasciatus* females from control, DAP, and ammonium sulfate treatments took longer to develop compared to those from glyphosate and cypermethrin treatments (F = 39.24, df = 4, 25, $P < 0.001$; Fig 4.4A). *Culex quinquefasciatus* females from DAP and ammonium sulfate treatments were significantly smaller compared to those from glyphosate and cypermethrin treatments but not control (F = 10.01, df = 4, 25, $P < 0.001$; Fig 4.4A). Females from control treatments were also significantly smaller than those from cypermethrin but not glyphosate treatment (Fig 4.4A). There were no significant effects of agrochemical treatment on *Cx. quinquefasciatus* female longevity (F = 0.77, df = 4, 25, $P = 0.55$). *Culex quinquefasciatus* male development time was longest in DAP and ammonium sulfate treatments, intermediate in control treatments, and shortest in glyphosate and cypermethrin treatments (F = 84.99, df = 4, 25, $P < 0.001$; Fig 4.4B). Males from DAP treatment were significantly smaller than those from glyphosate and cypermethrin treatments but not control and ammonium sulfate treatments (F = 7.99, df = 4, 25, $P < 0.001$; Fig 4.4B). In addition, males from control treatment were significantly smaller than those from cypermethrin treatment (Fig 4.4B).

**Table 4.1: MANOVA results on the effect of agrochemical treatments on development time to adulthood, wing length, and longevity of *An. arabiensis* and *Cx. quinquefasciatus* mosquitoes. Standardized canonical coefficients (SCC) describe the relative contribution of each response variable to significant treatment effects. Negative associations are denoted by (-).**

| | | | | | Standardized canonical coefficients | | |
|---|---|---|---|---|---|---|---|
| Mosquito species | Sex | Df | Pillai's trace | P | DT | WL | LG |
| *An. arabiensis* | Females | 12, 75 | 0.90 | 0.005 | 1.68 | -0.44 | -0.06 |
| | Males | 12, 75 | 1.07 | 0.0004 | -1.67 | 1.13 | 0.16 |
| *Cx. quinquefasciatus* | Females | 12, 75 | 0.96 | 0.002 | -2.55 | 0.63 | -0.38 |
| | Males | 12, 75 | 1.22 | <0.001 | -3.36 | 0.56 | 0.15 |

**DT** - Development time to adulthood

**WL** – Wing length of adult

**LG** - Longevity

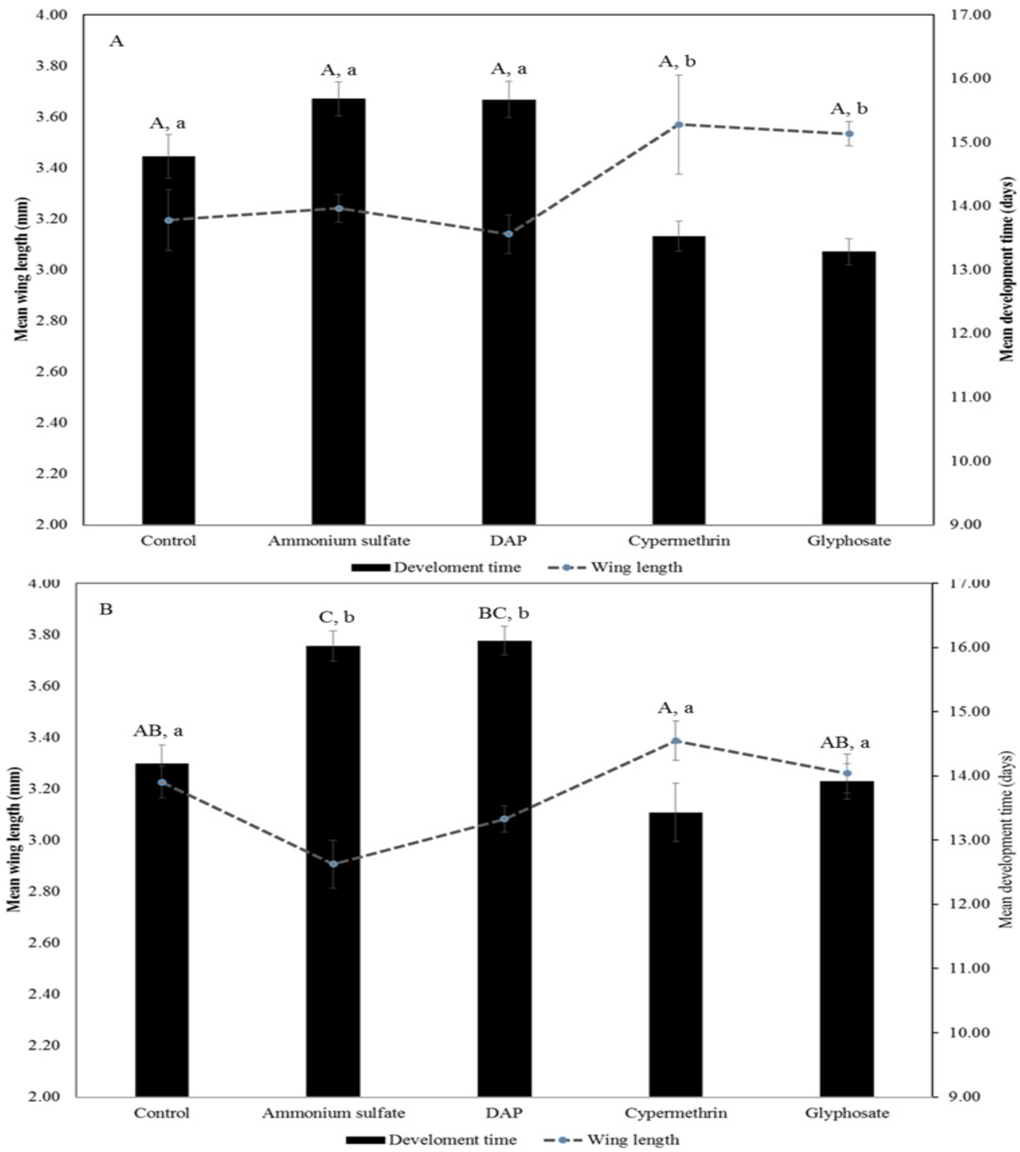


**Figure 4.3: Mean wing length (± SE) and development time (± SE) of An. arabiensis A) females and B) males in different agrochemical treatments. Lower case and upper case letters are used to compare treatment differences in development times and wing lengths, respectively. For each of the two life history traits, different letters indicate significant differences between treatments.**

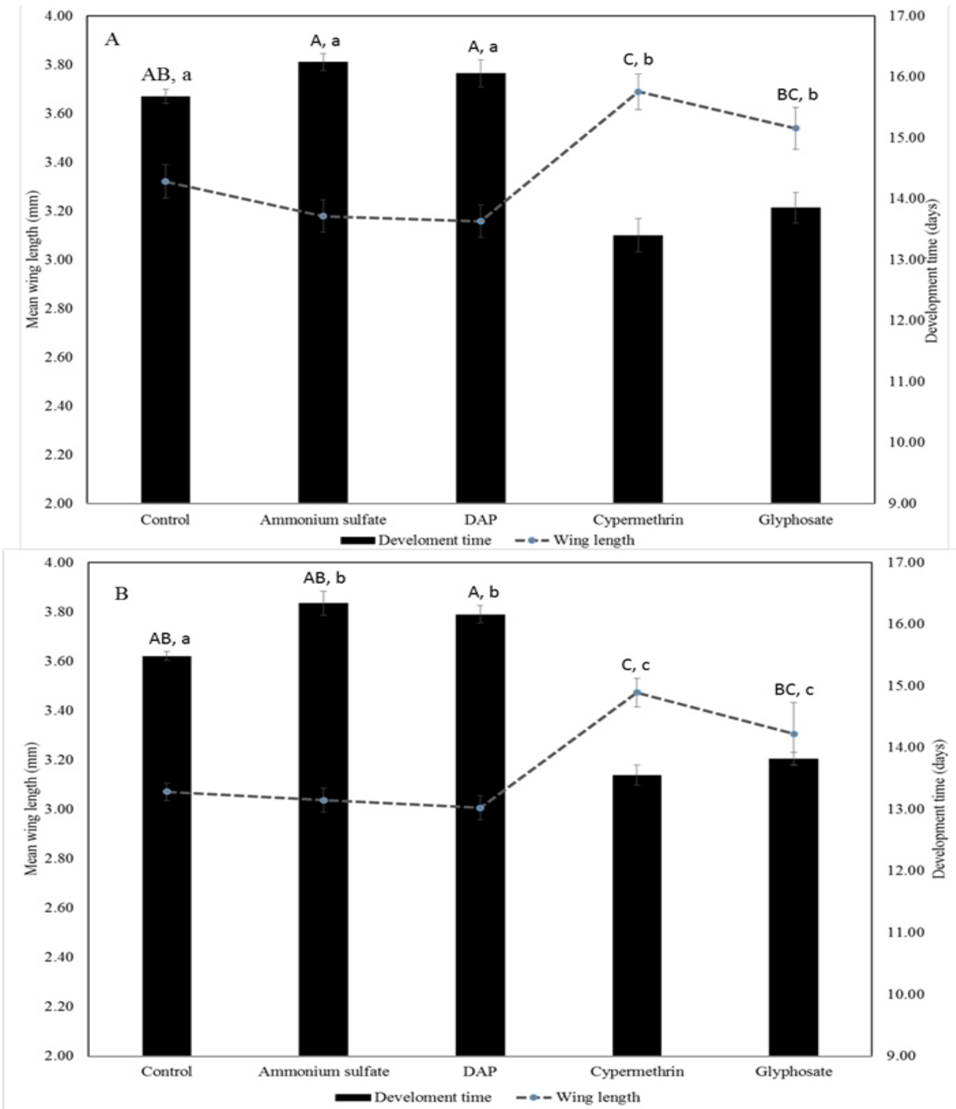


**Figure 4.4: Mean wing length (± SE) and development time (± SE) of Cx. quinquefasciatus A) females and B) males in different agrochemical treatments. Lower case and upper case letters are used to compare treatment differences in development times and wing lengths, respectively. For each of the two life history traits, different letters indicate significant differences between treatments.**

# CHAPTER FIVE

# DISCUSSION, CONCLUSION AND RECOMMENDATIONS

## 5.1 Discussion

Results for this study show that agrochemicals can alter the attractiveness and quality of *An. arabiensis* and *Cx. quinquefasciatus* larval habitats. When presented with a choice of fertilizer and pesticide-treated oviposition substrates, gravid females of both mosquito species preferentially oviposited in fertilizer-treated substrates. Fertilizer treatments were also associated with higher emergence rates, longer development times, and smaller adults relative to pesticide treatments. These findings are partially consistent with the optimal oviposition theory which predicts that egg laying females should select oviposition sites that maximize the probability for their offspring to reach adulthood and reproduce (Craig *et al.,* 1989, Blaustein & Kotler 1993). Looking at past literature, this is the first study to investigate how commonly used agrochemicals affect oviposition site selection and offspring survival of two of the most important mosquito vectors of human pathogens in sub-Saharan Africa.

The impact of fertilizers on mosquito larval populations is well documented. Application of nitrogenous fertilizers in rice fields is often associated with dramatic increase in larval populations of *Anopheles* and *Culex* mosquitoes (Sunish *et al.,* 1998, Mutero *et al.,* 2000, Victor &Reuben 2000, Mutero *et al.,* 2004a, Muturi *et al.,* 2007). Similarly, fertilizer-enriched mesocosms and wetlands had higher populations of mosquito larvae compared to control treatments (Duguma & Walton 2014, Young *et al.,* 2014). However, the mechanisms underlying fertilizer-mediated increase in mosquito larval populations are poorly understood. The results for this study suggest that fertilizer-mediated enhancement of habitat attractiveness and quality may be two of the major factors that account for dramatic increase in mosquito larval populations following application of nitrogenous fertilizers. Fertilizer application promotes microbial growth, which provide chemical cues that aid gravid females to locate suitable oviposition sites (Ponnusamy *et al.,* 2008, Ponnusamy *et al.,* 2010), stimulate egg hatch (Ponnusamy *et al.* 2011), and serves as food for mosquito larvae (Merritt *et al.,* 1992).

Avoidance of pesticide-treated oviposition substrates by the two mosquito species was expected as pesticides could be detrimental for egg and larval survival. However, previous studies have documented both positive and negative effects of pesticides on oviposition site selection by mosquitoes. *Aedes aegypti* avoided ovipositing in grass infusion treated with microbial larvicide *Bacillus thuringiensis* var. *israelensis* but did not discriminate between tap water or untreated grass infusion (Santos *et al.,* 2003). Gravid females of *Aedes aegypti* were attracted to spinosad-treated oviposition substrates but avoided temephos-treated substrates (Quiroz-Martinez *et al.,* 2012). Similarly, Eugenol, citronellal, thymol, pulegone, rosemary oil, and cymene acted as oviposition deterrents for *Ae. aegypti* while borneol, camphor and β-pinene acted as oviposition attractants for this mosquito species (Waliwitiya *et al.,* 2009). Carbaryl-treated pools were more attractive oviposition sites for C*ulex* mosquitoes but had no effect on oviposition behavior of *Anopheles* mosquitoes (Vonesh & Buck 2007). In addition, *An. gambiae s.s*, were two times likely to lay in lake water infused with the chemical cedrol than in lake water alone (Lindh *et al.,* 2015). Collectively, these findings suggest that pesticides can alter oviposition behavior of mosquitoes but the direction of the response is pesticide-specific. Further studies are needed to establish how a variety of mosquito species respond to different types of commonly used pesticides.

In general, mosquitoes from control and fertilizer treatments took longer to develop and were smaller compared to those from pesticide treatments. Given that pesticide treatments had lower emergence rates compared to control and fertilizer treatments, this could be due to random elimination of some larvae by pesticides which may have released the survivors from larval competition thereby promoting faster development and larger mosquitoes. It is also possible that pesticides may have selectively favored the survival of larger individuals with rapid growth and development. Both mechanisms have been used to explain why *Aedes* and *Culex* mosquitoes from experimental microcosms exposed to low concentrations of pesticides develop faster and are larger compared to those from control treatments (Muturi *et al.,* 2010a; Muturi *et al.*, 2011a;, Muturi, 2013). However, this study design could not allow one to determine which of the two mechanisms was responsible for the observation and further research is needed on this topic.

Mosquito body size and age are commonly used as a proxy for mosquito fitness and vector potential. Large mosquitoes consume bigger blood meals and lay more eggs compared to small mosquitoes (Briegel, 1990). Large mosquitoes also have longer life spans (Reiskind & Lounibos 2009), and are more likely to survive through the extrinsic incubation period of the pathogen compared to smaller mosquitoes (Bara *et al.,* 2015). On the other hand, younger mosquitoes have been associated with higher oviposition rates than older mosquitoes (Agyapong *et al.,* 2014). Thus, although fertilizer application may lead to production of large numbers of adult mosquitoes, this may not necessarily translate to increased risk of pathogen transmission since the majority of adults may be small and short-lived.

This may be one of the many factors explaining why rice cultivation in East Africa is often associated with large populations of malaria vectors but lower risk of malaria transmission compared to adjacent non-irrigated agroecosystems (Ijumba and Lindsay 2001; Ijumba *et al.*, 2002a; Ijumba *et al.,* 2002b; Mutero *et al.,* 2004b). However, nosignificant effect of agrochemical treatments on adult mosquito life span was observed. Moreover, both fertilizers and pesticides are used simultaneously in many agroecosystems and the large mosquitoes resulting from pesticide treatments may have higher fecundity (Muturi, 2013) and longevity both of which may increase the risk of pathogen transmission.

However, although exposure of mosquitoes to sublethal concentrations of pesticides is known to enhance arbovirus transmission (Muturi & Alto 2011, Muturi *et al.,* 2011b), their impact on malaria and LF transmission is poorly understood. Studies using insecticide-resistant and insecticide-susceptible strains of mosquitoes suggest that exposure to pesticides may reduce the ability of the vector to transmit malaria and LF (McCarroll & Hemingway 2002; Ferguson *et al.,* 2012; Alout *et al.,* 2014) but additional studies are needed to assess how short-term exposure of mosquitoes to sublethal pesticide concentrations affect vector susceptibility to malaria and LF parasites.

### 5.2 Conclusion

In conclusion these results demonstrate that the extensive and widespread use of agricultural chemicals to promote agricultural production can influence where mosquitoes lay eggs, how long they take to complete their development, how many adult mosquitoes are produced, and how big the resulting adults will be. In turn, these traits can influence the spatial and temporal distribution and abundance of mosquito populations and associated pathogens.

### 5.3 Recommendations

1. These findings may be used to develop policies that enhance food production while alleviating negative impacts of agriculture on human health. For instance;

- It can form a basis for designing integrated mosquito vector control management strategies through farm-based and possibly farmer-managed intervention strategies.
- The findings that some agricultural chemicals are favorable for mosquito production can be used in development of “attract – kill” vector control strategy.

2. Further studies are needed to examine how;

- Simultaneously applied agricultural chemicals affect the ecology of a variety of vector mosquitoes to illuminate its potential impact on human and wildlife health and inform the appropriate management options.
- A variety of mosquito species respond to different types of commonly used pesticides.
- Short-term exposure of mosquitoes to sub-lethal pesticide concentration affects vector susceptibility to malaria and LF parasites.

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# APPENDICES

## Appendix 1: Approval Letter by the SSC KEMRI

KEMRI

**KENYA MEDICAL RESEARCH INSTITUTE**

P.O. Box 54840-00200, NAIROBI, Kenya
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E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

**KEMRI/SSC/102820** **27 May, 2014**

Tabitha Wambui

Thro'
FOR: Director, ESACIPAC
**NAIROBI**

Forwarded 27/05/2014

**REF: SSC No. 2828 (Revised) – Effect of Sublethal Concentrations of Agricultural Chemicals on Selected Life History Traits of *Anopheles arabiensis* and *Culex quinquefasciatus* in Mwea Rice Irrigation Scheme, Central Kenya**

Thank you for your letter dated 23rd May, 2014 responding to the comments raised by the KEMRI SSC

I am pleased to inform you that your protocol now has formal scientific approval from SSC.

The SSC however, advises that work on the proposed study can only start after ERC approval.

**Sammy Njenga, PhD**
FOR **SECRETARY, SSC**

Encl(s)

In Search of Better Health

## Appendix 2: Approval Letter by the ERC KEMRI

# KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
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**KEMRI/RES/7/3/1** **August 1, 2014**

**TO:** **TABITHA WAMBUI**
**PRINCIPAL INVESTIGATOR**

**THROUGH:** **DR. SAMMY NJENGA,**
**DIRECTOR, ESACIPAC,**
**NAIROBI**

Forwarded 06/08/2014

Dear Madam,

**RE: SSC PROTOCOL NO. 2828 (*RESUBMISSION*): EFFECT OF SUBLETHAL CONCENTRATIONS OF AGRICULTURAL CHEMICALS ON SELECTED LIFE HISTORY TRAITS OF ANOPHELES ARABIENSIS AND CULEX QUINQUEFASCIATUS IN MWEA RICE IRRIGATION SCHEME, CENTRAL KENYA**

Reference is made to your letter dated 30$^{th}$ July, 2014. The ERC Secretariat acknowledges receipt of the revised study protocol on July 31, 2014.

This is to inform you that the Ethics Review Committee (ERC) reviewed the documents submitted and is satisfied that the issues raised at the 228$^{th}$ meeting of the KEMRI ERC on 24$^{th}$ June, 2014 have been adequately addressed.

The study is granted approval for implementation effective this **1$^{st}$ August, 2014**. Please note that authorization to conduct this study will automatically expire on **July 31, 2015.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **June 19, 2015**.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC. You are also required to submit any proposed changes to this protocol to the SSC and ERC prior to initiation and advise the ERC when the study is completed or discontinued.

You may embark on the study

Yours faithfully,

EAB

**PROF. ELIZABETH BUKUSI,**
**ACTING SECRETARY,**
**KEMRI/ETHICS REVIEW COMMITTEE**

In Search of Better Health

**Appendix 3: Published Manuscript in Parasite and Vectors Journal**

Kibuthu *et al. Parasites & Vectors* (2016) 9:500
DOI 10.1186/s13071-016-1788-7

Parasites & Vectors

RESEARCH Open Access

# Agricultural chemicals: life changer for mosquito vectors in agricultural landscapes?

Tabitha W. Kibuthu[1*], Sammy M. Njenga[2], Amos K. Mbugua[3] and Ephantus J. Muturi[4,5*]

## Abstract

**Background:** Although many mosquito species develop within agricultural landscapes where they are potentially exposed to agricultural chemicals (fertilizers and pesticides), the effects of these chemicals on mosquito biology remain poorly understood. This study investigated the effects of sublethal concentrations of four agricultural chemicals on the life history traits of *Anopheles arabiensis* and *Culex quinquefasciatus* mosquitoes.

**Methods:** Field and laboratory experiments were conducted to examine how sublethal concentrations of four agricultural chemicals: an insecticide (cypermethrin), a herbicide (glyphosate), and two nitrogenous fertilizers (ammonium sulfate and diammonium phosphate) alter oviposition site selection, emergence rates, development time, adult body size, and longevity of *An. arabiensis* and *Cx. quinquefasciatus*.

**Results:** Both mosquito species had preference to oviposit in fertilizer treatments relative to pesticide treatments. Emergence rates for *An. arabiensis* were significantly higher in the control and ammonium sulfate treatments compared to cypermethrin treatment, while emergence rates for *Cx. quinquefasciatus* were significantly higher in the diammonium phosphate treatment compared to glyphosate and cypermethrin treatments. For both mosquito species, individuals from the ammonium sulfate and diammonium phosphate treatments took significantly longer time to develop compared to those from cypermethrin and glyphosate treatments. Although not always significant, males and females of both mosquito species tended to be smaller in the ammonium sulfate and diammonium phosphate treatments compared to cypermethrin and glyphosate treatments. There was no significant effect of the agrochemical treatments on the longevity of either mosquito species.

**Conclusions:** These results demonstrate that the widespread use of agricultural chemicals to enhance crop production can have unexpected effects on the spatial distribution and abundance of mosquito vectors of malaria and lymphatic filariasis.

**Keywords:** Agricultural chemicals, Sublethal concentrations, Life history traits, *Anopheles arabiensis*, *Culex quinquefasciatus*

## Background

Mosquitoes transmit some of the most devastating infectious diseases including malaria, lymphatic filariasis (LF), and dengue. Transmission of these diseases is largely influenced by mosquito distribution, abundance and fitness, which are in turn dependent on the quality of aquatic habitats where egg hatch and larval development occurs [1, 2]. Because mosquitoes do not provide parental care to their offspring, natural selection should favor the ability of gravid females to select aquatic habitats that maximize egg hatch and offspring fitness [3]. This process requires complex integration of biological, chemical and physical cues by gravid females [4]. Chemical contaminants can potentially disrupt this process by modifying the quality and attractiveness of the aquatic habitats and vector biologists are faced with the challenge of determining

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