THE ROLE OF AGRICULTURAL CHEMICALS ON *ANOPHELES ARABIENSIS* VECTOR 'FITNESS' IN MALARIA TRANSMISSION IN A RICE AGRO-ECOSYSTEM IN KENYA

SOLOMON MURIU KAROKI

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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:

……….………………………

Solomon Muriu Karoki

This thesis has been submitted for examination with our approval as University Supervisors.

1. Signature: ……………………………..… Date:

……………………………….

Professor Zipporah Ng’an’ga

JKUAT, Kenya

2. Signature: …………………………..…… Date:

……………………………….

Dr. Evan Mathenge

KEMRI, Kenya

DEDICATION

I dedicate this work to my immediate and extended Karoki family. My late Dad Justin who left the work in progress and my late mum Lydia for their love of education, my children and the unwavering and encouraging wife Hannah.

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LIST OF ABBREVIATIONS AND ACRONYMS

A	Alpha- cypermethrin
ACT	Artemesinin Combination Therapy
AL	Artemeter Lumefantrine
DAP	Di-Ammonium Phosphate
DOMC	Division of Malaria Control
HMIS	Health Management Information System
IIM	International Irrigation Management Institute
IPM	Integrated Pest Management
ITM	Insecticide Treated Materials
ITNS	Insecticide Treated Nets
IRS	Indoor Residual Spraying
IVM	Integrated Vector Management
KARI	Kenya Agricultural Research Institute
KDHS	Kenya Demographic Health Survey
KMFS	Kenya Malaria Fact Sheet
NMS	National Malaria Strategy
LLINS	Long-Lasting Insecticide Treated Nets

LSP	Life Span (longevity) of a Mosquito from Eclusion to death in holding cup
MDG	Millennium Development Goals
MDSS	Material Data Safety Sheet
MOH	Ministry of Health
MOPHS	Ministry of Public Health and Sanitation
PECA	Programmatic Environmental Control Assessment
PMI	Presidential Malaria Initiative
RTI	Research Triangle International
SA	Sulphate of Ammonia
T	Denotes the exposure treatment to different agricultural chemical Combinations
T1	Di-Ammonium Phosphate, Sulphate of ammonia, Thiophanate methyl and Alpha-cypermethrin combined together
T2	Di-Ammonium Phosphate only
T3	Di-Ammonium Phosphate, Alphacypermethrin, and Thiophanate methyl combined
T4	Sulphate of ammonium, Thiophanate methyl and

	Alphacypermethlin combined
T5	Di-Ammonium Phosphate and Alphacypermethrin combined
T6	Di-Ammonium Phosphate and Thiophanemethyl combined
T7	Control – No chemicals
TM	Thiophanate Methyl
UNDP	United Nations Development Programme
UNICEF	United Nations International Children Educations Fund
USAID	United States Agency for International Development.
WHO	World Health Organization
WMR	World Malaria Report
WHS	World Health Statistics
WL	Wing length (size of a mosquito)

DEFINITION OF TERMS AS USED IN THIS STUDY

Agricultural chemicals:	Fertilizers and pesticides as inputs used in rice growing in Mwea irrigation scheme.
Anthropophilic:	Mosquitoes that prefer biting humans.
Aquatic habitats:	Limnologic or water bodies that malaria vectors breed in.
Density-dependent outcomes:	Behavioural outcomes in adult mosquitoes that may be resultant of larval exposure in different habitats.
Dispersal range:	The minimum and maximum distance a mosquito is capable of taking freight at a time
Ecological factors:	External factors which may produce different types of reaction in population having the same genetic characteristics.
Extrinsic incubation:	Sporogonic cycle of plasmodium parasites inside the mosquito vector.
Endophagic:	Mosquitoes that prefer feeding indoors.
Endophilic:	Mosquitoes largely found resting indoors.
Exophagic:	Mosquitoes that prefer to feed outdoors.
Exophilic:	Mosquitoes largely found resting outdoors
Malariogenic potential:	The capability of the main variables of the environment, parasite, vector and man, acting together to produce endemic or epidemic malaria in given locality.
Mosquito longevity:	The life span of a mosquito in days from emergence to death in holding cups
Mosquito resources:	Essential biotic and abiotic requirements for mosquito

	growth.
Mosquito size:	Wing-length as a proxy for mosquito size measured from the *alulla* notch to the distal end of the seventh venation
Survival rates:	The percentage of emergent mosquitoes that survive to attain reproductive age in a cohort.
Sporogony:	The period it takes a plasmodium parasite to develop in the female anopheline mosquito from time of ingestion to infective status.
Transgenic mosquitoes:	Mosquitoes that are genetically manipulated to curtail the parasite transmission.
Transmission index:	The percentage of new cases found among susceptible persons during given unit of time
Vector Bionomics:	Biology (autecology) which deals with the relationships of given species and its environment. This includes but not limited to development of immature stages, i.e., eggs, larvae, and pupae as well as the life of the adults under the influences exercised by the environmental conditions.
Mosquito Vector ‘fitness’:	Longevity in days and size of a mosquito as a function of its malaria transmission potential.
Wing length:	Signifies the size of a mosquito.

Zoophilic:	**Mosquitoes that prefer biting animals than Humans.**

ABSTRACT

Control of African malaria vectors is dependent on insecticides via residual sprays IRS or insecticide treated nets ITNs. Emergent vector resistance to pyrethroids in sub-Saharan Africa and development of alternative insecticides, treatments and vaccines against malaria is expensive and long-term. New tools for vector control to achieve interruption of malaria transmission needs priority considering the long-term challenge of malaria eradication. Increased knowledge on the ecology and behavior of malaria vectors in their habitats is necessary. The effects of agro-chemicals in rice cultivation on malaria vector ‘fitness’ was investigated in Mwea irrigation scheme Kenya, for its potential application as a novel vector control strategy. During the rice-growing period of September 2010 to January 2011, four agro-chemicals were evaluated for their effects on vector ‘fitness’ in paddy and simulated field experiments. Pupas were sampled in agro-chemical exposed paddies and mosquito eggs incubated in simulated conditions of different agrochemical combinations. Emergent female Anophelines were reared until death. Longevity in days and wing length in millimeters were used as parameters for mosquito ‘fitness’. Emergent adults had a mean lifespan of 6.5 days in paddies and 7.1 days in simulated trials. Experiment control mosquitoes had a mean longevity of 18 days in the paddies and 15 days in simulated trial. The mean wing length was 3.5 mm in the agrochemical paddy-exposed vectors and 3.7 mm for the simulated experiment compared to 3.1 mm and 3.2 mm in the field control and simulated experiment respectively. Longevity was significantly affected by agrochemicals in both paddy ($P = 0.003$) and simulated experiment ($P = 0.00$). Agrochemicals used in Mwea irrigation scheme decreased mosquito longevity with minimal size encumbrance. These findings suggest that systematic use of agrochemicals in rice irrigation contribute to low malaria transmission by shortened *Anopheles arabiensis* life span and proper agrochemical use is a potential malaria vector control strategy. The results of this study need to be experimented in other agro-ecosystems for use as a passive malaria transmission control tool.

CHAPTER ONE: INTRODUCTION

1.1 Background

Malaria is one of the most important diseases that affect humans. Worldwide there were 216 million cases of malaria in 2010, 81% of these occurred in the WHO African Region(WHO, 2011). An estimated 3.3 billion people were at risk of malaria in 2010 (WHO, 2011). An estimated 655 000 persons died of malaria in 2010, 86% of the victims were children under 5 years of age, and 91% of malaria deaths occurred in the WHO Africa Region (WMR, 2011).

Estimates indicate that there may be 300-500 million clinical cases each year, with countries in sub-Saharan Africa accounting for more than 90% of these cases (Greenwood and Mutabingwa 2002; WHO, 2010, WHO 2011). National control efforts have resulted in a reduction in the estimated number of deaths from almost 1 million in 2000 to 781 000 in 2009 (WMR, 2011). The estimated number of cases of malaria rose from 233 million in 2000 to 244 million in 2005 but decreased to 225 million in 2009 (WHO, 2011). An estimated 3.3 billion people were at risk of malaria in 2010, although of all geographical regions, populations living in sub-Saharan Africa have the highest risk of acquiring malaria (WHO, 2011).

In Kenya, malaria is still the leading cause of morbidity and mortality according to the Kenya Malaria Fact Sheet 2012. An estimated 25 million out of a population of 39 million Kenyans are at risk of malaria while the disease accounts for 30-50% of all outpatient attendance and 20% of all admissions to health facilities in the country `(MoPHS, 2011).

The Government of Kenya recognizes malaria as a health and socio-economic burden (MIS, 2010). Malaria control is a priority investment as articulated in the second National Health

Sector Strategic Plan (MoPHS II, 2005–2010, extended to 2012) and the Ministry of Public Health and Sanitation Strategic Plan, 2008–2012. The goal of the 2009–2017 National Malaria Strategy (NMS) is to reduce illness and death by two-thirds of the 2008 level. The Kenya Vision 2030 includes among its health sector objectives the intention to reduce the proportion of inpatient malaria fatality to 3 per cent. In 2010, clinically diagnosed malaria accounted for 34 per cent of outpatient hospital visits in Kenya (MIS 2010).

Early diagnosis and prompt treatment are two basic elements of malaria control. Early and effective treatment of malaria can shorten the duration of the infection and prevent further complications including majority of deaths **(**Global scenario**:** Malaria cases in India: 1971-2008). However, treatment of malaria cases alone may not be sustainable or a cost effective strategy to control malaria. Malaria prevention interventions play a pivotal role in the reduction of the disease burden among vulnerable populations. In spite of the current high level of research into the biology of the malaria parasite, control of malaria transmission in the foreseeable future will continue to depend on vector control (Clive *et al.,* 2002).

Vector control is the mainstay in malaria control as it reduces malaria parasite transmission intensity. The main objective of malaria vector control is to significantly reduce malaria transmission intensity in populations at risk. Vector control entails the reduction of the population and longevity of malaria vectors through larval source management by larviciding or environmental management; adult mosquito control and the reduction of human vector contact by using personal protection measures such as sleeping under insecticide treated nets (ITNs) or other repellants . In some malaria endemic regions, adult mosquito control and the use of insecticide treated nets (ITNs) is jeopardized by the occurrence of insecticide resistance in the major malaria vectors (Hargreaves *et al.,* 2000; Antonio-Nkondjio *et al.,* 2011). With reports from 45 countries around the world indicating insecticide resistance to at least one of the four classes of insecticides used for malaria vector control and 27 of these being in sub-Saharan Africa (WHO, 2011), the war against malaria is yet to be won. This scenario calls for the development and deployment of innovative and cost-effective malaria vector control strategies. One such method has been reduction of mosquito larvae habitats by environmental management and manipulation (Ault, 1994; Fillinger *et al.*, 2006).

Comprehensive environmental management of "mosquito larvae resources" is a promising approach of malaria control but has seen little application in Africa for more than half a century (Killeen *et al.,* 2002). In rice agro-ecosystems, resources in the form of aquatic habitats are always plenty and controlling them as a vector control intervention may not be feasible, since rice growing is viewed as an income generating activity aimed at poverty and hunger alleviation. Other methods should be explored in controlling these aquatic resources without jeopardizing the main stay economic activity of rice growing as it offers much needed income to the population. One way of restricting the availability of habitat resource to the aquatic stages of arthropods is to make them unsuitable for ovi-position, and therefore, reduce mosquito populations. Regardless of vector reduction being core to malaria control, it is not the number of mosquitoes that is critical in the transmission, but rather the longevity of mosquito survival that contributes to the efficient transmission of malaria (Gillies, 1972; Macdonald, 1951; Clive, 2002).

The use of agro-chemicals in rice agro-ecosystems could influence the choice of mosquito ovi-position sites by gravid mosquitoes as well as subsequent development of the aquatic stages of malaria vectors (Muturi *et al.,* 2007). A clear understanding of how agrochemicals influence mosquito ‘fitness’ needs to be studied. Adult mosquito ‘fitness’ in rice agro-ecosystems may form a basis for designing integrated malaria vector control management strategies (Diuk-Wasser *et al.*, 2004).

The current intra-domiciliary interventions, insecticide treated nets (ITNs) and indoor residual spray (IRS) remain the most effective means for malaria vector control (WHO, 2009). However, these interventions are not sufficient

to meet the goal of malaria elimination as their benefits are limited to indoor biting mosquitoes while neglecting outdoor biting populations (Pates *et al.*, 2005).

This study was designed to examine the role of agricultural chemicals on mosquito 'fitness', with focus on fertilizers (Sulphate of ammonia and Di-ammonium phosphate) in combination with pesticides (Thiophanate Methyl and Alpha-cypermethlin). These are the routinely used agrochemicals for rice production in the Mwea irrigation scheme (KARI, Mwea 2011).

1.2 Problem Statement

Malaria is still one of the most serious parasitic diseases with an enormous socioeconomic impact in tropical Africa. Recently, malaria treatment drugs faced parasite resistance and new treatment with artemesinin based drugs was introduced (Olliaro *et al.,* 2004). Malaria control programs have so far relied on controlling the mosquito vectors to reduce the disease burden. Current malaria vector control strategies rely on use of ITNs and IRS for disease prevention. Although these approaches have given promising outcomes, they may not provide a lasting solution to malaria control in the future due to vector and parasite resistance to available options. Drug and vaccine development may not be sufficient to halt transmission, while vector-based initiatives in other areas have proved effective (José *et al.,* 2009).

Vector control is likely to remain the main stay in malaria control. It is therefore important to continually develop new and innovative malaria vector control strategies based on sound understanding of vector biology and ecology. Not only do vector-based control strategies target an essential step in the transmission process, but they also address a step that precedes infection. In Africa, indoor spraying has eliminated the endophilic *A. gambiae* in some areas, only to have it replaced by the more exophilic *A. arabiensis* (Hargreaves *et al.,* 2000). An improved knowledge of mosquito life history could inform better vector control measures for integrated vector management (Gu *et al.,* 2011). Changes in the transmission pattern of malaria following irrigation development have been a perennial subject of debate (Ijumba *et al.,* 2001).

Home usage of ITNs and IRS remain the most effective means for malaria vector control (WHO, 2009). The benefits of these interventions are limited to indoor biting mosquitoes while neglecting outdoor biting populations (Pates *et al.,* 2005). There is a need to revisit and understand the life history of mosquitoes in rice agro-ecosystems to inform the requisite innovations in vector control. A detailed ecological field study on vector interactions with the environment is lacking. Studying the implication of agro-chemical use in rice agro-ecosystems is a valuable asset for multi-faceted approaches to effective malaria control.

1.3 Justification

In Africa, disease and food insecurity pose major obstacles to economic and social development. Malaria weakens the human labour force working in agriculture thus reducing agricultural productivity. Unpredictable climatic conditions have made rain fed agricultural practices untenable. The use of irrigation in agriculture is perceived as one way of enhancing food security and promoting economic development in Africa. However, irrigation, especially flooding of land in rice cultivation, has been associated with an increase in the number of disease vectors, and in certain cases, a corresponding increased health burden due to malaria.

To maximize the benefits of irrigation and minimize the risks of increasing the burden of vector and water-borne diseases, especially malaria, there is a need to understand, in details, the relationship between agricultural practices in irrigated cultivation and disease outcomes.

Irrigated agriculture, especially in rice agro- ecosystems, may result in creation of increased disease vector habitats. This occurs for mosquitoes of the genus *Anopheles arabiensis,* the commonest malaria vector in rice agro-ecosystems of sub-Saharan Africa whose population increases during rice planting and growing period (Muturi *et al.,* 2009).

Association of rice agronomy and mosquito vectors is not well understood**.** Fertilizer application in rice fields promotes mosquito productivity by stimulating microbial growth and probably by acting as an oviposition cue (Mwangangi *et al*., 2006). The influence of larval diet on the survival of emergent African malaria vectors remains to be elucidated in the field and understanding of the factors that determine the longevity of malaria vectors remains extremely limited (Oketch *et al.,* 2003).

No information is available on the effects of agricultural chemicals on vector immature and the effect that may transcend the emerging adults and the associated ‘fitness’ cost. Knowledge on agricultural chemical vector interaction needs to be generated for its potential application in evidence based malaria vector control. Such information will guide the malaria control programmes and the division of vector borne and neglected tropical diseases’ in the design of targeted and cost effective vector control interventions and ascertain on whether use of chemicals and pesticide to improve food security could be counter- productive.

This study attempted to evaluate and quantify the cumulative effects that agrochemical use in a rice agro eco-systems have on mosquito ‘fitness’ and its potential application in vector control.

1.4. Research questions.

1. Does the use of agricultural chemicals in rice growing fields induce effects in larval stage that affect emergent mosquito vector ‘fitness’ in longevity or size?
2. How do different combinations of agricultural fertilizer and pesticide application in a rice agro-ecosystem affect mosquito longevity?
3. How does fertilizer and pesticides application in a rice agro-ecosystem affect the resultant adult mosquito size?
4. What is the effect of different agro-chemical combination on mosquito egg hatchability in simulated conditions?
5. How do different agro-chemicals combination in simulated conditions affect emergent mosquito ‘fitness’ in size and longevity?

1.5 Objectives

1.5.1 Main objective

The main objective of this study was to determine the role of agricultural chemicals in the vector ‘fitness’ of *Anopheles arabiensis*.

1.5.2 Specific objectives

1. To determine the effect of agrochemicals on longevity of emergent *A.arabiensis* vectors in paddy fields in Mwea rice irrigation scheme.
2. To determine the effect of agrochemical application in rice fields on *A. arabiensis* size.
3. To determine *A arabiensis* egg hatchability rates in the presence of different combinations of agrochemicals in simulated experiments.
4. To determine the effects of agrochemicals on mosquito vector longevity and size in simulated experiments.

1.6 Null Hypothesis

Agricultural chemicals used in rice agro-ecosystems have no effect on vector 'fitness' of emergent *Anopheles arabiensis* malaria vectors.

1.7 Alternative Hypothesis

Agricultural chemicals used in rice agro-ecosystems have significant effects on 'fitness' of emergent *Anopheles arabiensis* malaria vectors.

CHAPTER 2: LITERATURE REVIEW

2.1 Current malaria situation

Out of the 216 million cases of global malaria in 2010; 81% occurred in the WHO African Region where Kenya belongs. (WHO, 2011).Most people at risk of malaria live in the world's poorest countries in Africa, Asia, and Latin America (WHO, 2011).

In Kenya, malaria is still the leading cause of morbidity and mortality according to the Kenya malaria fact sheet 2012. In 2007, there were 9.2 million reported clinically diagnosed malaria cases in the public health sector (HMIS, 2008). Up to 25 million out of a population of 39 million Kenyans are at risk of malaria. Malaria accounts for 30-50% of all outpatient attendance and 20% of all admissions to health facilities (KDHS, 2009; MIS, 2010; MOPHS-KMFS 2012; WHO, 2011). The most vulnerable group to malaria infections are pregnant women and children under 5 years of age (WHO, 2011).

Kenya has four distinct malaria epidemiological zones (MOPHS -NMS, 2009- 2017).

(i) Endemic zones - These are areas of stable malaria transmission with altitudes ranging from 0 to 1,300 meters. These zones include Lake Victoria in western Kenya and in the coastal regions. Rainfall, temperature and humidity are the determinants of the perennial transmission of malaria. The vector life cycle is usually short and survival rates are high because of the suitable climatic conditions. Transmission is intense throughout the year, with annual entomological inoculation rates of 30–100 (NMS, 2009-2017). (ii) Seasonal transmission - These zones include arid and semi-arid areas of northern and southeastern parts of the country, which experience short periods of intense malaria transmission during the rainy seasons. Temperatures are usually high and water pools created during the rainy season provide breeding sites for the malaria vectors. Extreme climatic conditions like the El Niño southern oscillation lead to flooding in these areas, resulting in epidemic outbreaks with high morbidity rates owing to the low immune status of the population (NMS, 2009 - 2017).

(iii) Epidemic zones - Malaria transmission in the western highlands of Kenya is seasonal, with considerable year-to-year variation. Epidemics are experienced when climatic conditions favor sustainability of minimum temperatures around 18^{o}C. This increase in minimum temperatures during the long rains favors and sustains vector breeding, resulting in increased intensity of malaria transmission. The whole population is vulnerable and case fatality rates during an epidemic can be up to ten times greater than those experienced in regions where malaria occurs regularly. This constitutes a third zone which is epidemic prone (NMS, 2009-2017). (iv) Low risk malaria areas. This zone covers the central highlands of Kenya including Nairobi. The temperatures are usually too low to allow completion of the sporogonic cycle of the malaria parasite in the vector. However, the increasing temperatures and changes in the hydrological cycle associated with climate change are likely to increase the areas suitable for malaria vector breeding with the introduction of malaria transmission in areas where it had not existed before (Patz *et al*., 2009).

Among Kenya's total population of thirty-nine million people, 70% live in endemic, epidemic or seasonal transmission areas where they are at risk of malaria (MoPHS-MIS 2010). The majority of those at-risk population lives in areas of low or unstable *Plasmodium. falciparum* transmission where the *P.falciparum* parasite prevalence is less than 5%. This includes several areas, most notably along the coast, that have transitioned recently toward low, stable, endemic conditions (MoPHS-MIS, 2010). However, an estimated 4 million people (10% of the population) live in areas of Kenya where the parasite prevalence is estimated to be > 40 % and malaria remains a serious risk (MoPHS-DMOC, 2011).

2.2 Malaria vector composition

In sub-Shaharan Africa malaria is transmitted principally by female anopheles mosquitoes of *Anopheles gambiae* complex (Sinka *et al.,* 2010). There are between 50 and 60 species of *Anopheles* mosquitoes that transmit malaria worldwide (William *et al.,* 1999).

The *Gambiae complex* is a combination of six morphologically indistinguishable siblings that have minimal morphological differences to classify them as different species but are reproductively isolated. The complex is composed of *Anopheles gambiae ss, An. arabiensis, An. quandrianalatus, An. bwambae, An. merus* and *An. melas. Anopheles funestus* is also an important vector of malaria in the sub-Saharan Africa (Giles *et al.,* 1987)

In Kenya, *An. gambiae* is represented by four sibling species namely *An. gambiae, An.arabiensis, An. merus*, and *An. quandriannulatus.* An. *arabiesis* is the most prevalent and efficient malaria vector in the Mwea irrigation scheme (Muturi *et aI.*, 2007).

2.3 Malaria transmission and epidemiology

Malaria transmission is a combination of three key components which are part of natural ecological system and which act as diverse factors to produce disease (Patz *et al.,* 2009). The main factors involved in the malaria transmission cycle are the parasite, the vector and the human host which interact with one another in their wider biological and physical environment for malaria transmission success (Ferreira *et al.,* 1986).

Physical environment includes temperature, humidity requirements and contact with human host while biological factors consist of reproductive and growth requirements, parasite species, and susceptibility to infection. Other biological factors may include but not limited to feeding and resting behavior, flight capabilities, seasonal distribution and longevity of the mosquito vectors (Michel *et al.*, 2008).

Malaria transmission is a combination of three key components which are part of natural ecological system and which act as diverse factors to produce disease. These are the plasmodium parasite, the mosquito and human's factors which act inter-dependently All living organisms have evolutionary pathways in which to defend their survival, ensure their preservation and continuity. The main factors involved in the malaria transmission eco-system are the parasite, the vector and the human host which react with one another in their wider biological and physical environment for malaria transmission success.(Ferreira, A.W. and Cochrane,1986.)

Physical environment

(a) Temperature (b) Humidity requirements (c) Contact with human host

Biological factors

(a)Reproductive and growth requirements (b) Parasite species (c) Susceptibility to infection

Other biological factors

(a) Feeding and resting behaviour (b) Flight capabilities

(c) Seasonal distribution (d) Longevity

Human host

These factors can be categorized as follows:-

(a) Social organization (urban/rural) – urban areas may benefit from lack of suitable breeding sites unlike rural settings

(b) Quality of housing, drainage, water supply, etc.

(c) Occupation – to some extent the occupation of certain human populations may be more venerable and exposed to mosquito bites than others.

(d) Agricultural pattern (irrigation, etc.) the agricultural practices of different human populations may influence the diversity of habitats. Irrigation schemes offer an all year round breeding habitat for vector populations. (Diuk-Wasser *et al*, 2004).

(e) Population movement (migration, nomadism)

(f) Immunity

(g) Intervention present (on parasite, mosquito environment).

2.4 Malaria in irrigated agro-ecosystems

In Africa, malaria is predominantly a rural disease where agriculture forms the backbone of the economy. Various agro-ecosystems and crop production systems have an impact on mosquito productivity, and hence malaria transmission intensity (Mboera *et al.,* 2007). The stagnant water in rice farms and the collection of water in ditches and burrow pits constructed during agricultural practices form conducive breeding habitats for malaria vectors (Jayawardene, 1995).

Consequently, in addition to nutritional and socio-economic benefits associated with irrigated rice cultivation (Dolo *et al.,* 2004; Ijumba *et al.*, 2001; Van *et al.*, 2003) there is creation of large and more permanent larval habitats that support higher densities of malaria vectors. Accordingly, proper understanding of the factors that promote mosquito population may provide useful information on how to mitigate the negative effects of irrigated rice cultivation on human health (Mwangangi *et al.,* 2010).

An entomological evaluation conducted in the Mwea area indicated a 30-300-fold increase in the number of the local malaria vector, *Anopheles arabiensis* in villages with rice irrigation compared to those without irrigation with malaria prevalence being significantly lower in these villages (Mutero *et al*., 2004).

Environmental conditions play an important role in the transmission of malaria and regulating these conditions can help to reduce disease burden (Randell *et al*., 2010). Agrochemical use changes larval habitat environment and may affect the emergent mosquito 'fitness' in transmission of malaria (Muturi *et al*., 2008). Addition of nitrogenous fertilizers acts as the attractant for oviposition by the gravid *An. arabiensis* mosquitoes (Mwangangi *et al*., 2006). Broadcasting nitrogenous fertilizers in rice fields enhances mosquito larval populations (Simpson *et al*., 1991; Victor *et al*., 2000).

Africa would have a more sustained approach to control of malaria vectors if the larval ecology of vector species resident in them is adequately understood (Albert *et al*., 2011). In Kenya, the assessment of agro-practices which promote mosquito breeding in rice-agro ecosystems has not been fully explored. Results from such study are useful for designing evidence-based malaria control interventions. Furthermore, understanding of anopheline larval ecology in rice agro-ecosystems is still insufficient and this affects the design and implementation of larval control (Mwangangi *et al.,* 2010).

2.5 Malaria Vector control

From the late nineteenth century to the early twentieth century, malaria vectors were managed through such methods as wetland drainage (water management) and improvements in housing and screening (Natacha *et al.,* 2009). During World War II, the chlorinated hydrocarbon pesticide DDT (Dichlorodiphenyltrichloroethane) was discovered to be extremely effective in controlling mosquitoes, and was used in malaria control as an indoor residual house spray **(**USAID, 2007). In the 1950s and early 1960s, WHO conducted mosquito eradication campaigns using DDT. These campaigns were highly effective. However, as mosquito resistance to DDT emerged, costs of the campaigns increased and efforts to expand campaigns to endemic tropical areas failed. The pursuit of worldwide malaria eradication was abandoned. Individual countries continued controlling malaria using IRS, with DDT and other chemicals.

In the years following the eradication campaigns, governments relied more heavily on curative services for malaria control. This strategy became problematic with the increasing spread of multi-drug resistant malaria, and consequently highlighted the importance of transmission reduction through vector control (PECA, 2007).

The distribution of bed nets treated with pyrethroid insecticides, or ITNs, was widely adopted as a malaria control strategy during the 1990 (WHO, 2006). Use of ITNs and ITMs has increased since 2000, but its success in reducing malaria has varied widely (WHO, 2006). Forty five countries around the world have identified resistance to at least one of the four classes of insecticides used for malaria vector control; 27(60%) of these are in sub-Saharan Africa (WHO, 2011).

The use of ITNs and LLNs in poor sub-Saharan countries might also not be economically sustainable method and must be taken as an opportunity to synergize the current control strategies. Larval control in their habitat is one of the unexploited and less developed areas in mosquito control (Natacha *et al.,* 2009).

Unless malaria control strategy adopts an integrated approach its success is far from being realized (Mboera, *et al.*, 2007). The approach involves management of land, water and farming practices in ways that discourage mosquitoes from breeding and to reduce human-mosquito contact. This is based on the fact that malaria is a disease that can be controlled by good management of the environment where the vector breeds (Oladimeji *et al.,* 2010).

Innovation and development require an intimate knowledge of three elements: 1) the molecular, physiological, and population biology of the mosquito; 2) the interactions between the parasite, vector, and host; and 3) potential delivery mechanisms. The synergy of these elements may be the key to eliminating a pest and eradicating a deadly disease (José *et al.*, 2009).

Currently in Kenya, vector control approaches intend to ensure use of insecticide treated nets by at risk communities, to significantly reduce rates of the disease and other methods through Integrated Vector Management (NMS, 2009-2017)**.**

2.6 Integrated vector management (IVM)

With diminished assurance that ITNs, LLNs and IRS can solve the malaria problem, IVM emerges as a widely supported malaria control strategy. IVM is intangible strategy, rather than a physical strategy. It is a decision-making process for the management of vector populations, to reduce or interrupt disease transmission (IIM, 1996).

Exhaustive studies on low malaria incidents in rice agro-ecosystems despite increased vector populations has not been done. In this study, agrochemicals role in vector ‘fitness’ in rice agro ecosystems is investigated in elucidating knowledge on factors that contribute to this phenomena.

2.7 Vector potential of mosquitoes

The vector potential of mosquitoes is inclined to their size and lifespan (longevity) (Ameneshewa and Service, 1996). Mosquito size affects the vector capacity by determining the chance of actively foraging for a host. Wing length is used as a proxy for determination of the size of a mosquito and is well correlated with weight in various studies (Ameneshewa and Service, 1996). A big sized mosquito may have advantage of covering a larger area when searching for a blood meal due to its advanced flight range. Small mosquitoes however might have increased transmission potential as they may have increased feeding cycles, which may increase their chance of infection with plasmodium parasites (Tanga *et al.,* 2010).

A recent study by Diuk-Wasser *et al,* (2004) demonstrated a positive relationship between adult survival and vectorial capacity at low mosquito densities and a negative relationship between the two variables at higher mosquito densities (Manoukis *et al.*, 2006). Bigger mosquitoes may suffer shortened lifespan and compromise their vectorial capacities (Foster *et al.,* 2004). Mosquitoes must live beyond 12 days, which is required for sporogony to complete to succeed as malaria vectors (Michel *et al.,* 2008).

2.8 Factors influencing the vector potential

Mosquitos' longevity, man-vector contact and mosquito density determine the transmission capacity of a vector population (Natacha *et al.,* 2009). A reduction in the lifespan of the mosquitoes will reduce the sporozoite rate and hence the proportion of infective bites. Secondly, a reduction in the human/vector contact will decrease the proportion of blood meals taken on human hosts (Mouchet *et al.,* 1998). Finally, a reduction of vector density by decreasing the number of adult or larvae will also reduce transmission intensity. Therefore, any factors that could have an impact on any of these components will influence malaria transmission.

According to the MacDonald model on malaria transmission, (MacDonald, 1957) factors influencing longevity will have more impact on transmission than factors affecting human-vector contact or density.

2.8 Malaria transmission in rice agro-ecosystems

Malaria parasite transmission depends on the availability of competent mosquito vectors (Omumbo *et al.,* 1998). Irrigated rice agro-ecosystems provide abundant malaria vector breeding habitats in the tropics. Malaria vectors can reach very high densities in villages near irrigated rice fields in Africa, leading to the expectation that malaria should be especially prevalent there. Surprisingly, this is not always the case (Manoukis *et al.,* 2006). The presence of high vector densities in African rice agro-ecosystems without corresponding increase in malaria transmission intensity has been referred to as the ‘paddies paradox’ (Ijumba *et al.,* 2001).

Malaria is a major tropical disease associated with irrigation schemes, and changes in the transmission pattern of this disease following irrigation development vary widely (Ijumba *et al.,* 2001). Paddies paradox is a phenomenon in which malaria transmission does not increase during rice planting seasons despite considerable increase in malaria vectors. Most studies have varied outcomes on the low malaria prevalence when vectors increase in irrigated communities than surrounding areas (Ijumba *et al*, 2001; Smith, 2003).

Malaria transmission intensity and knowledge in rice-agro ecosystems has differed widely in many studies. In some cases malaria transmission increased on introduction of rice irrigation while in others the transmission decreased showing some transmission control effects conferred by irrigated rice eco-systems. In Sri Lanka, introduction of the Mahaweli Irrigation Scheme resulted in a five-fold increase in malaria incidence among the local population (IIM, 1996). In contrast, irrigated rice cultivation in Mali was associated with a reduction in the annual incidence and a 10-fold reduction in the sporozoite rate than was found in adjacent non-irrigated areas. (Rogier, *et al.,* 2004; Robert *et al.,* 1985).

Ascertaining the means by which disease agents are transmitted is a major objective in epidemiology as it becomes possible to introduce measures to prevent transmission from taking place. This study aimed to contribute to existing knowledge on malaria vector ecology in irrigated agro-ecosystems.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

This study was carried out in Mwea rice irrigation scheme located about 120 km North East of Nairobi. Mwea occupies the lower altitude zone of Kirinyaga County in an expansive low-lying area. Geographically, Mwea lies between latitude 1 ˚ S of the equator, longitude 37.5 ˚ E and an altitude of 1900M above sea level. 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 - 29.5°C. Relative humidity varies from 52–67%. According to the 2009 national census, the Mwea irrigation scheme had approximately 250,000 persons in 35,000 households. The Mwea rice irrigation scheme is located in the west central region of Mwea District and covers an area of about 13,640 ha. More than 50% of the scheme area is under rice cultivation. The remaining area is used for subsistence farming, grazing and community's other activities. This is an area of stable, endemic malaria where *An. gambiae* Giles, *An. arabiensis* Patton, and *An. funestus* Giles all contribute to transmission (Ijumba *et al.,* 1990).

The longevity, survival and size experiments of *Anopheles arabiensis* were conducted during the months of October to December 2010, at the commencement of rice transplantation from nurseries to the paddies. On transplantation rice is top-dressed with fertilizers and pesticides fumigated to protect crop damage by pests and thus enhance yields. Mwea district hospital has a good vector borne disease laboratory, which was used for laboratory rearing of mosquitoes. The laboratory is under the Ministry of Public Health and Sanitation.

3.2 Study design

The study was a two-tier randomized experiment in which field sample and simulated field experiments were conducted. In order to get comparative data on lifespan and size of mosquitoes, two study designs were utilized. In the field studies, observational design was used. Pupa stage was sampled from paddies exposed to agrochemicals as a presumed agrochemical contaminated natural habitat. However, randomized experimental design was used in the simulated semi-field trials where the investigators exposed mosquito eggs to different agrochemical combinations and followed the emergent *Anopheline arabiensis* mosquitoes to establish their longevity and size.

3.4 Field experiments

In the field experiments, three 5-hectare plots were randomly selected in three villages with planned rice growing of Mbui-Njeru ,Kimbimbi and Unit 14 of Mwea irrigation scheme. The owners were identified from within the settlement areas by the investigator and informed consent obtained for use of their paddies in experiments. The study team obtained information on the transplanting, paddy fertilizing and pesticide spraying dates, which were of main interest.

Four days post transplanting, pupae were sampled in the 3 paddies on a weekly basis until the desired sample size of 192 female *Anopheles* mosquitoes was attained. These field experiments were based on the hypothesis that pupae sampled in the paddies had been exposed to agrochemicals used during rice growing (Mwangangi *et al.*, 2006). Control pupa samples were collected from areas without exposure to agrochemicals in the village settlement areas simultaneously. Sampled pupae were held in holding cups with paddy-water from the sampled plot and transported to the insectary. The cups were placed inside netted emergence wire cages until emergence.

Upon emergence, each mosquito was morphologically identified using taxonomic keys (Giles *et al.,* 1987) and female *Anopheles* mosquitoes transferred to individual netted holding cups using manual aspirators. All holding cups were appropriately labeled. Female *Anopheles arabiensis* in individual holding cups were placed in humified holding boxes and fed on 6% glucose soaked cotton wicks daily (Clements *et al.,* 1992; Foster, 1995) and observed until their death. The longevity in days was recorded as the time from emergence to time of death. Upon death, one wing was removed and measured to the nearest 0.01 mm using an ocular micrometer under a dissecting microscope. The wing length (size) and longevity in days were then recorded in designed laboratory data forms.

3.4.1 Field control samples

Mosquito pupae were sampled from animal hoof spools, bicycle and vehicle ruts, tree holes and discarded cans in settled areas of the three villages where rice paddies had been selected. The collection of pupae in these areas was based on the assumption that it was highly unlikely that the habitats had any agro-chemical exposure as these were found in the housing areas with no rice growing. Emerging female *Anopheles arabiensis* in individual holding cups were placed in humified holding boxes and fed on 6% glucose soaked cotton wicks daily (Clements *et al.,* 1992; Foster, 1995) and observed until their death.

3.5 Simulated field experiments

A tall grass vegetated ground was selected within the Kimbimbi District Hospital compound about 500 meters from the nearest paddies. Three replicate bowls (capacity 10 litres each) per agro-chemical combination were used to simulate field conditions at the selected site (Mboera *et al.,* 2000). Based on water loading rates of 100,000 liters of water per hectare and a 1:4 soil water ratio in rice paddies (KARI Mwea – Desk information unpublished, Muturi *et al.,* 2007) and agrochemical specific manufactures' instructions, bowl composition was arrived at as follows:-

Sulphate of ammonium– 50kgs/Hectare (0.845 g, /l)

Diammonium phosphate– 50kgs /hectare (0.845 g, /l)

Alpha-cypermethrin - 0.1mls /litre

Thiophanate methyl– 0.15mls/litre.

One litre of canal water was measured using a standard measuring jar and 8.45 g of each fertilizer dissolved. Nine more liters of water were added to make a final concentration of 0.845g/L, which is the standard concentration in paddies at 50kg/hectare. This concentration had been previously used in determining survival of *Culex quinquefasciatus* in inorganic fertilizers (Muturi *et al.*, 2007). 7.5 liters of the fertilizer and canal water solution were poured into the 10 liter bowl and soil used to fill up to imitate the 1:4 soil water ratio in paddies.

Anopheles arabiensis Kisumu strain eggs were collected from mouse fed mosquitoes in the Mwea DVBD laboratory. Standard rearing procedures and holding conditions for the experimental mosquitoes were used (Knols *et al.,* 2002; Mathenge *et al.*, 2002). Each bowl was inoculated with 50 eggs of *Anopheles arabiensis* Kisumu strain. Egg inoculation was done ten days after introduction of agrochemicals into the bowls this being the period observed for increased larvae densities after pesticide sprays in paddy fields (Mutero *et al.,* 2008). Netting material was used over the bowls to avoid undesired oviposition from natural wild populations. Observations were made daily and any pupa developing from the bowls was transferred to individual emergence cups. Emerging female *Anopheles arabiensis* in individual holding cups were placed in humified holding boxes and fed on 6% glucose soaked cotton glucose wicks daily (Clements *et al.,* 1992; Foster, 1995) and observed until their death like in the field collected samples. Replicate bowls without agrochemicals were incorporated in the experiments as controls.

3.5.1 Agrochemicals allocation and combinations in simulated treatment

Table 3.1: Agrochemicals combination in simulated experiments

TREATMENT	AGROCHEMICAL COMPONENT
TREATMENT 1 (DSTA)	Di-Ammonium Phosphate, Sulphate of ammonia, Thiophanate methyl and Alpha-cypermethrin
TREATMENT 2 (D)	Di-Ammonium Phosphate only
TREATMENT 3 (DAT)	Di-Ammonium Phosphate, Alphacypermethrin, and Thiophanate methyl
TREATMENT 4 (SAT)	Sulphate of ammonium, Thiophanate methyl and Alphacypermethrin
TREATMENT 5 (DA)	Di-Ammonium Phosphate and Alphacypermethrin
TREAMENT 6 (DT)	Di-ammonium phosphate and Thiophanate methyl
TREATMENT 7	Control – No chemicals

3.5.2 Determination of mosquito size

In determination of mosquito size, this study employed wing-length as proxy**.** Mosquito wing length is often used as a proxy for body size because it is a trait that is relatively easy to measure, and is positively correlated with body mass in most species of mosquitoes (Lyimo *et al*, 1992,; Nasci, 1991). Elsewhere, *Anopheline* mosquito wing length has been used as a significant predictor of traits such as fecundity and survival (Ameneshewa and Service, 1996; Hogg *et al.,* 1996; Kittayapong *et al.,* 1992; Lyimo *et al*, 1992).

3.5.3 Estimation of mosquito wing length

Upon mosquito death, one wing was removed with dissecting forceps and mounted on a glass slide using distilled water. Wing length was measured between the alula notch and the wing tip of the seventh venation using a standard ocular micrometer under a dissecting microscope to the nearest 0.01mm. Two measurements were made on the same wing and where there were discrepancies, a third one was taken by an independent entomologist. The tie was broken if the measurement tallied with either of them (Imelda *et al*., 2004). Where the third measurement differed with both, the exercise was repeated and two tallying measurements were taken as the true measurement by consensus.

3.6. Estimation of mosquito longevity

The longevity of the mosquito was calculated by subtracting the date of emergence of an individual mosquito from the date of death in the holding cups as described by Justin *et al* (2011).

3.7 Sample Size Determination

The *analytical Group inc.* free automatic sample calculation software was used to determine the minimum sample size as modified and developed from Cochran's (1977) sample size formula.

$$n = \frac{\frac{z^2 P(1-P)}{d^2}}{1 + \frac{1}{N}\left(\frac{z^2 P(1-P)}{d^2} - 1\right)}$$

Table 3.2- Sample size calculator excel sheet software product (*analytical Group inc.*)

The bolded figures represent the precision.

Sample Size for Given Precision					
Confidence Level:		0.8	0.9	0.95	0.99
z-score:		1.2816	1.645	1.96	2.5758
Precision +/- =	**0.05**				
Population Size =	999,999	Sample size			
Assumed P =	**0.95**	32	52	73	127
Conservative P =	**0.53**	164	270	384/2	662

A factor of 2 is used to account for the male: female ratio in natural population.

Assumptions:

- Infinite population- 999,999 (software specification for infinite populations)
- 95% confidence level

- The common species collected in Mwea in previous studies on *Anopheles arabiensis* (52.5%) (Muturi *et al.*, 2006).
- Required precision of 0.05%

Therefore $n=1.96^2 *0.53*0..47/0.053^2/2$

The minimum sample size 192

3.8 Data management and analysis

To evaluate the effect of agro-chemicals on vector fitness of the emergent adults, the minimum sporogonic incubation period of *Plasmodium falciparum* in the semi-field environment, was estimated at 10 - 12 days (Craig *et al.,* 1999). *Anopheles arabiensis* has a wing length range of 2.8- 3.4mm (Giles, 1972). Data on longevity and wing-length of mosquitoes was compared among different treatments and compared to the respective controls using SPSS version 18. Paired sample t - test analysis was used to calculate significant differences on size and longevity between experiments and control. Field and simulated agrochemical exposed sample results were subjected to similar analysis to determine which agro-chemical combination had a major detrimental effect on vector ‘fitness’ and any statistical correlations between field samples and simulated experiments samples.

3.9 Ethical considerations

The National Council for Science and Technology granted research authorization and approval (annex 1). Paddy owners gave informed consent for use of their paddies in this study (annex 2).

CHAPTER 4: RESULTS

4.1 Mosquito characteristics and proportions in field samples

From September 2010 to January 2011, 1038 pupae were collected in the 3 rice paddies. A total of 269 female *Anopheles arabiensis* emerged. (Table 4.1).

Table 4.1: Mosquito emergence proportions from pupae in the paddy collected samples.

Paddy Name	No. of pupae collected	No. of female *Anopheles arabiensis* emerged	% *Anopheles* mosquitoes in sample
Mbui Njeru	238	56	23.5
Kimbimbi	254	66	25.9
Unit 14	312	70	22.4
Field Control	234	77	33
Total	1038	269	100

4.1.2 Effect of agro-chemicals on egg hatchability rates

Table 4.2 presents the egg hatchability rates in the emergent population of simulated experiments. A combination of Di-ammonium phosphate and Thiophanate methyl had the highest mosquito egg hatchability (70%) and pupae yield.

Table 4.2: Mosquito egg hatchability rates and female *Anopheles* emergence proportions in simulated treatments and their control.

Treatment component	No. of eggs incubated	Total emergence (Males/ Females)	Total female *Anopheles* isolated	% Egg hatchability
T1 All chemicals	150	59	16	39
T2 D	150	93	37	62
T3 DAT	150	42	17	28
T4 SAT	150	73	40	49
T5 DA	150	65	44	43
T6 DT	150	105	64	70
T7 CONTROL	150	95	63	63

Key:	**Chemical component**
T 1 (DSTA)	Di-Ammonium Phosphate, Sulphate of ammonia, Thiophanate methyl and Alpha-cypermethrin
T2 (D)	Di-Ammonium Phosphate only
T 3 (DAT)	Di-Ammonium Phosphate, Alphacypermethrin, and Thiophanate methyl
T 4 (SAT)	Sulphate of ammonium, Thiophanate methyl and Alphacypermethrin
T 5 (DA)	Di-Ammonium Phosphate and Alphacypermethrin
T 6 (DT)	Di-ammonium phosphate and Alphacypermethrin
TT 7 (Control)	Control – No chemicals

4.1.3 Effect of agro-chemicals on longevity and size of emergent adult *Anopheles* mosquitoes in paddy and simulated experiments.

In table 4.3, the pooled mean lifespan of *Anopheles* mosquitoes was 6.5 days in the paddy-exposed vectors compared to 18 days in the control. This longevity was three fold lower compared to the respective control and statistically significant (SD1.1, SEM. 0.6, P = 0.003). Wing-lengths from paddy-exposed vectors did not differ widely from those of the control with averages of 3.1 mm and 3.2 mm respectively (SD 0.5, SEM 0.3, P = 0.60). In the simulated treatments, the pooled mean lifespan was 7.1 days against a mean lifespan of 15 days in simulated experiment control (SD 1.3 S.E.M. 0.5, P = 0.00) which was statistically significant. On the other hand, unlike the paddy-exposed mosquitoes that had similar size in relation to their control, pooled mean wing-length in simulated experiments was at 3.7 mm compared to 3.4 mm in the simulated experiment control (SD 1.7, S.E.M. 0.7 and P = 0.04) which was statistically significant.

Table 4.3: Pooled means of longevity and size of emergent adult *Anopheles* in field and simulated experiments versus respective controls.

Paired Sample Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Average Lifespan –Field Paddies	6.5	3	1.09	.628
	Average Lifespan -Field Control	18	3	.000	.000
Pair 2	Average Wing-Length - Field Paddies	3.1	3	.509	.294
	Average Wing-Length - Field Control	3.2	3	.000	.000
Pair 3	Average Lifespan –Simulated exp.	7.1	6	1.28	.522
	Average Lifespan- simulated exp. Control	15	6	.000	.000
Pair 4	Average Wing Length Treatments	3.7	6	.167	.068
	Average Wing Length Simulated Control	3.5	6	.000	.000

4.1.4 Differences in pooled means in longevity and size of emergent *Anopheles* vectors.

The paddy-exposed vectors had a statistically different mean longevity of 6.5 days in comparison to the 18 days in control (P = 0.003). Wing-length was significantly different in simulated experiment with a P = 0.037 but not significant in paddy-exposed vectors (P = 0.590) (Table 4. 4.)

Table 4.4: Paired t-tests comparison of pooled means of longevity and size of emergent adult *Anopheles* in field and simulated experiments versus their controls.

	Variables	Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	SD	SEM.	Lower	Upper			
Pair 1	Mean LSP. field experiment Mean LSP. field control	-11.0	1.1	.60	-14.2	-8.8	-180	2	.003
Pair 2	Mean WL field experiment Mean WL field control	.187	.51	.30	-1.08	1.5	.640	2	.590
Pair 3	Mean LSP simulated experiment Mean LSP simulated control	-7.60	1.3	.52	-9.00	-6.3	-14.6	5	.000
Pair 4	Mean WL simulated experiment Mean WL simulated control	.190	.17	.068	.02	.37	2.82	5	.037

Key:

WL – Wing length (size of mosquito)

LSP – Lifespan (Longevity of mosquito in days)

4.2 Mean longevity and size of mosquitoes in individual simulated agrochemical combinations.

4.2.1 Treatment 1 – Longevity and wing length of *anopheles arabiensis* in Di-Ammonium Phosphate, Sulphate of ammonia, Thiophanate methyl and Alpha-cypermethrin.

In treatment one, where all the agrochemicals were combined, the mean lifespan was 4.7 days against a mean of 14 days in the control. Mean mosquito wing-lengths in this treatment and control were approximately 3.8 and 3.5 mm respectively (Figure 4.1).

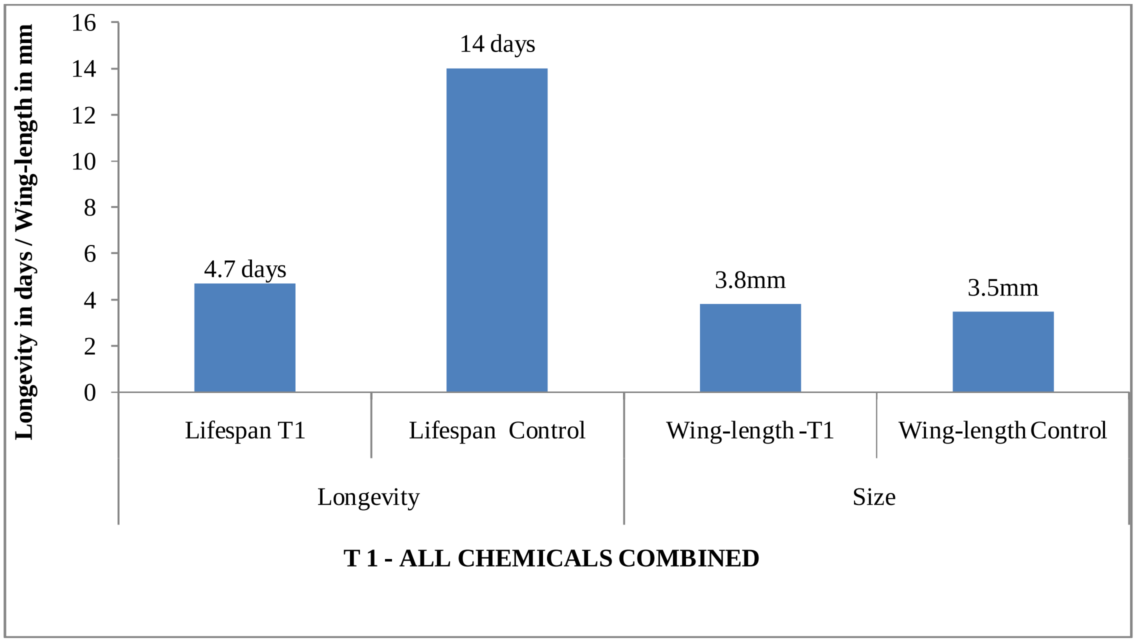


Figure 4.1: Comparison of longevity and size of mosquitoes in Treatment 1 (All chemicals) and the simulated experiment control

In treatment 1 (T1), both the mosquito longevity and wing-length were significantly different at $P < 0.05$ level. Longevity was statistically different from control ($P = 0.000$) while wing length ($P = 0.013$) was likewise significantly different from that of the control (Table 4.5).

Table 4.5: Paired t - tests comparing the differences between mean mosquito longevity and mean wing length in treatment T1 (All chemicals) against the control

		Paired Differences					**t**	**df**	**Sig. (2-tailed)**
					95% CI of the Difference				
Variable		**Mean**	**S. D.**	**S.EM**	**Lower**	**Upper**			
Pair 1	Life span T1 Lifespan Control	-9.30	6.53	1.6	-12.8	-5.80	-5.71	15	.000*
Pair 2	Wing length T1 Wing length Control	.338	.477	.12	.083	.592	2.83	15	.013

*Life span and wing-length difference is significant at P value less than 0.05 level (2-tailed).

4.2.2: Treatment 2 - Longevity and wing length of *A. arabiensis* in Di-ammonium Phosphate fertilizer only

The mean lifespan of mosquitoes in this treatment (T2) was 8 days against 14 days in the control. Wing-length in the treatment was 3.8 mm for the exposed mosquitoes and 3.4 mm for the control (Figure 4.3)

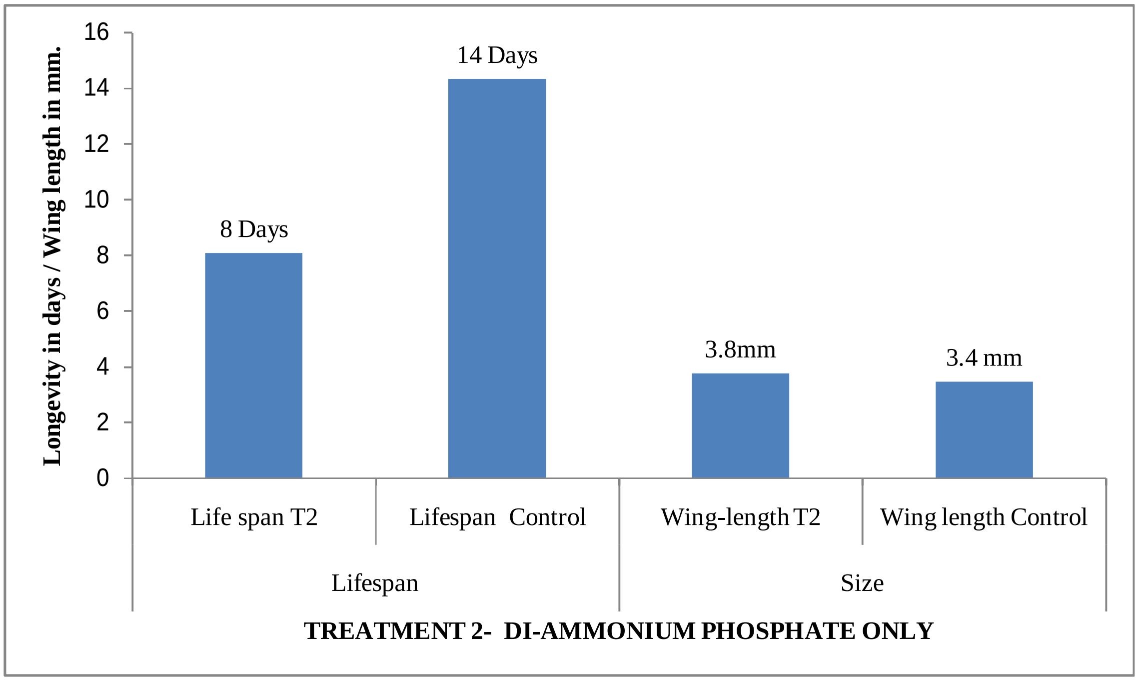


Figure 4.2 Comparison of longevity and size of mosquitoes in T2 (Di- ammonium phosphate only) and the simulated experiment control

4.2.3 Paired t- test on longevity and wing length in treatment T2 Di-ammonium phosphate only

Both longevity and wing length were significantly different (P = 0.00) for comparison of longevity against the control and (P = 0.002) for the wing-length against the control. (Table 4.6.)

Table 4.6: Paired t- test comparing the difference between mosquito longevity and wing-length (size) in treatment T2 (Di-ammonium phosphate only) and the control

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	SD	S.E.M	95% CI of the Difference				
					Lower	Upper			
Pair 1	Life span T2 Lifespan Control	-6.243	8.855	1.456	-9.196	-3.291	-4.289	36	.000
Pair 2	Wing-length T2 Wing-length Control	.2946	.5254	.0864	.1194	.4698	3.410	36	.002

Lifespan and wing-length difference is significant at P value 0.05 level (2-tailed).

4.2.4 Treatment 3 - Di-Ammonium Phosphate, Alpha-cypermethrin, and Thiophanate methyl.

In treatment T3, the average longevity of *A.arabiensis* was 8.2 days against 14 days in the control. Wing-length of mosquitoes in this treatment and the control were at approximately 3.6 mm and 3.4 mm respectively (Figure 4.3).

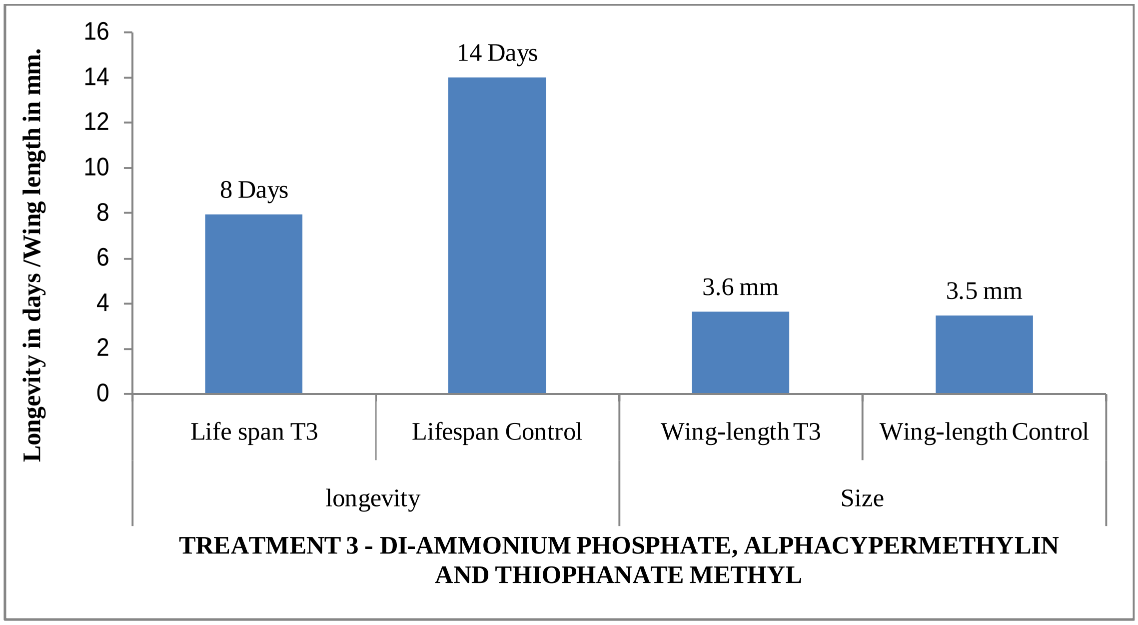


Figure 4.3 Comparison of longevity and size of mosquitoes in T3 (Di-ammonium phosphate,alphacypermethrin and Thiophanate methyl) and the control

4.2.5: Paired-sample t- test on longevity and wing length in treatment T3 - (Di-Ammonium Phosphate, Alpha-cypermethrin, and Thiophanate methyl)

Mosquito lifespan was significantly different in T3 from the control (P = 0.004). However, wing-length was not statistically different from the control (P = 0.229) (Table 4.7).

Table 4.7: Paired t - test comparison of the difference between mosquito mean longevity and Size in treatment T3 (Di-Ammonium Phosphate, Alpha-cypermethrin, and Thiophanate methyl) and the control

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	SD	S.E.M	95% CI of the Difference				
					Lower	Upper			
Pair 1	Lifespan T3 - Lifespan Control	-6.063	7.150	1.788	-9.873	-2.252	-3.391	15	.004
Pair 2	Wing-length T3 – Wing-length Control	.1625	.5188	.1297	-.1140	.4390	1.253	15	.229

Lifespan and wing-length difference is significant at P value 0.05 level (2-tailed).

4.2.6: Treatment 4 – Longevity and wing length of *A. arabiensis* in Sulphate of ammonium, Thiophanate methyl and Alpha-cypermethrin

In treatment 4 the mean lifespan of mosquitoes was 7.8 days compared to 14 days in the control (P = 0.00). The mean wing- length of mosquitoes was 3.6 mm against 3.4 mm in the control (P = 0.38) (Figure 4.4).

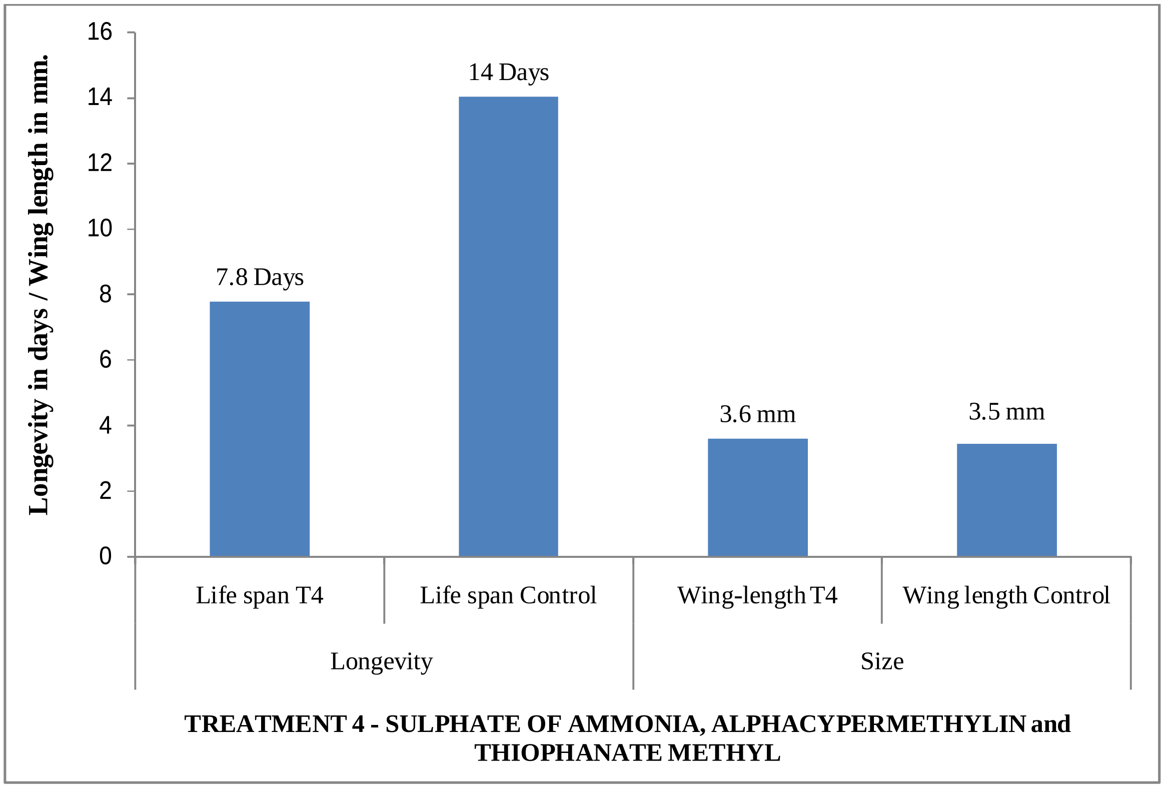


Figure 4.4: Comparison of longevity and size of mosquitoes in Treatment 4 and simulated control

4.2.7: Paired t- test treatment T4 - Longevity and wing length of *A. arabiensis* in Sulphate of ammonium, Thiophanate methyl and Alpha-cypermethrin

Mosquito size showed statistical difference size in comparisons with the control. Experimental mosquitoes had a wing length of 3.8mm against 3.5mm for the control (P = 0.038) longevity was statistically different P = 0.00 (Table 4.8).

Table 4.8: Paired t - test comparison of the difference between *A. arabiensis* mean longevity and Size in treatment T4 (Sulphate of ammonium, Thiophanate methyl and Alpha-cypermethrin) and the control

		Paired Differences					**t**	**df**	**Sig. (2-tailed)**
		Mean	**SD**	**S.E.M**	**95% CI of the Difference**				
					Lower	**Upper**			
Pair 1	Lifespan T4 Lifespan Control	-6.250	7.386	1.168	-8.612	-3.888	-5.352	39	.000
Pair 2	Wing-length T4 Wing length Control	.1650	.4855	.0768	.0097	.3203	2.150	39	.038

Lifespan and wing-length difference is significant at P value 0.05 level (2-tailed).

4.2.8: Treatment 5 - Longevity and wing length of *A. arabiensis* in Di-Ammonium Phosphate and Alpha-cypermethrin.

In treatment T5 a mean lifespan of 7.3 days and wing length of 3.9 mm in size were observed. The control mosquito experiment had 14 days in longevity and 3.4 mm for wing-length. Both longevity and wing length were statistically significantly different (P = 0.000) (Figure 4.5).

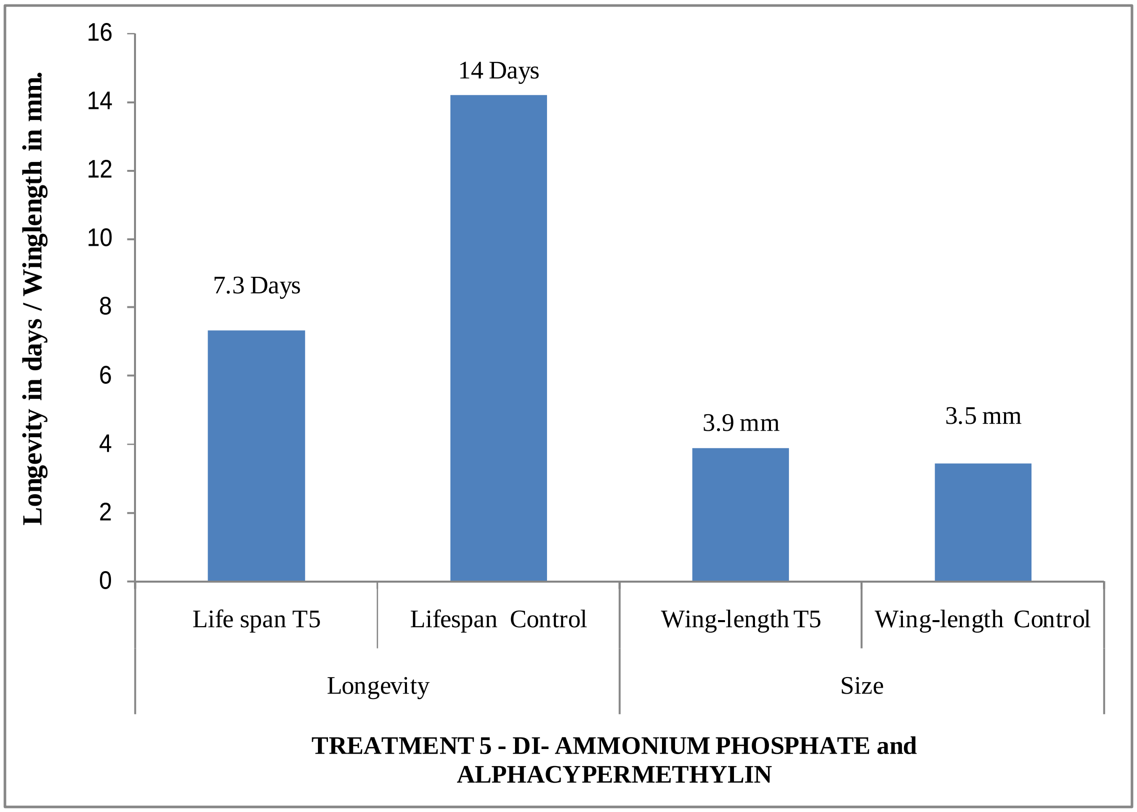


Figure 4.5: Comparison of longevity and size of mosquitoes in T 5 Di-Ammonium Phosphate and Alpha-cypermethrin and the simulated control

4.2.8: Paired t- test treatment for T5- Longevity and wing length of *A. arabiensis* in Di-Ammonium Phosphate and Alpha-cypermethrin

The mean longevity in this trial was 7.3 days compared to 14 days (P = 0.000) which was statistically significantly different. Mosquito size was 3.4mm for the experiment and 3.9 mm for the control (P = 0.000) which is also statistically significant (Table 4.9).

Table 4.9: Paired t - test comparison of the difference between mosquito mean longevity and Size in treatment T5 (Di-Ammonium Phosphate and Alpha-cypermethrin) and control

		Paired Differences					**t**	**df**	**Sig. (2-tailed)**
		Mean	**SD**	**S.E.M**	**95% CI of the Difference**				
					Lower	**Upper**			
Pair 1	Lifespan T5 Lifespan Control	-6.860	5.994	.914	-8.705	-5.016	-7.505	42	.000
Pair 2	Wing-length T5 Wing-length Control	.4500	.4638	.0699	.3090	.5910	6.436	43	.000

4.2.9: Treatment 6 - Longevity and wing length of *A. arabiensis* in Di-Ammonium Phosphate and Thiophanate methyl

The mean mosquito longevity in days for this combination of agrochemicals was 7 days against 14 days in the control which was statistically significant (P = 0.00). However there was no statistically significant difference in wing length with the test having 3.4mm against 3.8 mm (P = 0.276) (Figure 4.6).

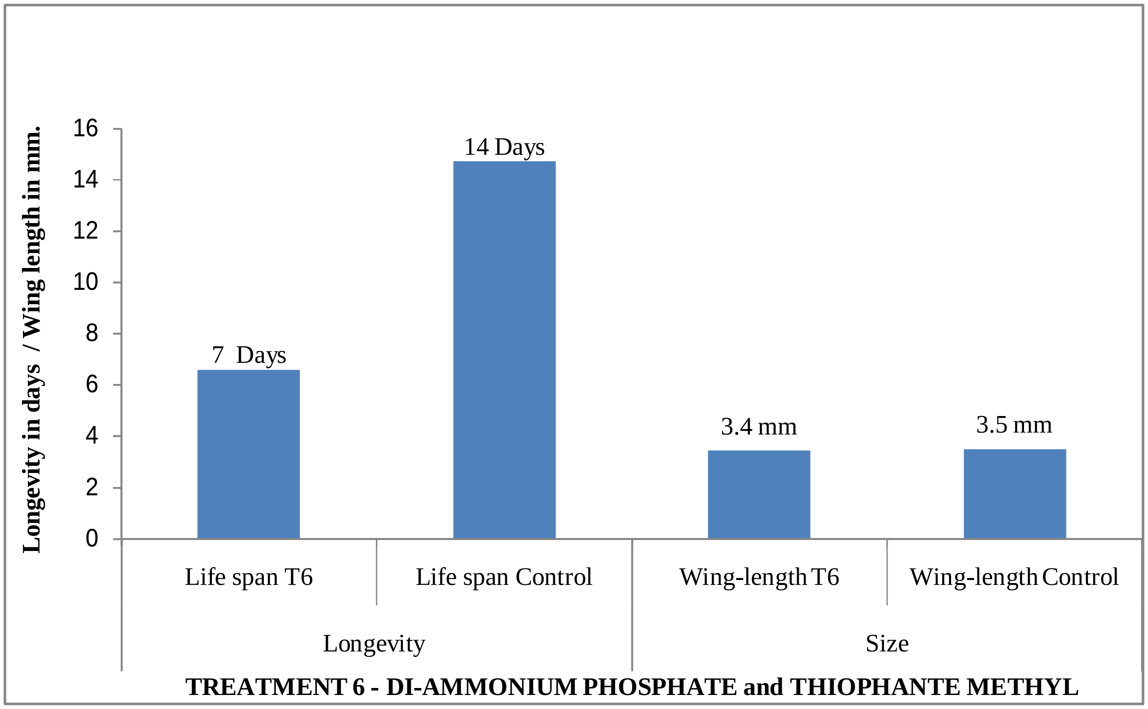


Figure 4.6: Comparison of mean mosquito longevity and size in Treatment 6 and the simulated experiment control

4.2.9: Paired sample t - test treatment T6 - Longevity and wing length of *A. arabiensis* in Di-Ammonium Phosphate and Thiophanate methyl

The results of paired t-tests comparison in this treatment gave statistically significant difference in longevity of mosquitoes (7 days) compared to controls (14 days) with a P- value of 0.000. Mosquito wing-length in this treatment had no statistically significant difference at 3.4 mm in the experiment and 3.5 mm for the control (P = 0.276) (Table 5.0).

Table 5.0: Paired t - test comparison of the difference between mosquito mean longevity and Size in treatment T6 (Diammonium phosphate and thiophante methyl) and the control

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	SD	S.E.M	95% CI of the Difference				
					Lower	Upper			
Pair 1	Lifespan 6 Lifespan Control	-8.127	7.259	.915	-9.955	-6.299	-8.886	62	.000
Pair 2	Wing-length T6 Wing-length Control	-.0714	.5163	.0651	-.2015	.0586	-1.098	62	.276

CHAPTER 5: DISCUSSION, CONCLUSIONS & RECOMMENDATIONS

5.1 DISCUSSION

The findingsof this study revealed reduced longevity of *Anopheles* vectors in paddies and simulated field conditions when exposed to various agrochemicals used in Mwea irrigation scheme either singly or in combination and therefore reduced vectorial ‘fitness’. This finding is corroborated with other related studies on disease vectors. For example, larval and adult nutrition critically affect not only survival, but also flight potential (Nayar and Sauerman, 1971a; 1971b;1975), biting persistence (Nasci, 1991), and thus pathogen transmission by mosquitoes (Nayar and Sauerman, 1975; Foster, 1995).

5.1.1 Effect of agrochemicals exposure on longevity of emergent *Anopheles* vectors in paddy fields and simulated trials.

The mean longevity of agrochemical-exposed *Anopheles* mosquitoes in both paddy and simulated experiments was reduced 2 to 3 fold relative to the controls and this difference was statistically significant (t-test, P= 0.000).This suggests that agrochemical exposed mosquitoes suffered reduced longevity compared to non agrochemical exposed controls. The field control mean mosquito lifespan of 18 days did not compare well with the expected longevity in the wild mosquito lifespan of 34 days as documented by Giles *et al*. (1968). This reduced longevity in study control samples can be explained by rearing laboratory conditions of the vectors from pupae stage to death. Laboratory rearing of mosquitoes does not provide plant sugars common in the field conditions. In similar experiments, mosquitoes lacking natural sugars had shortened lifespan than those in sugar rich fields (Gu *et al.,* 2011). Many physiological attributes, such as body size and nutrient reserves can affect the longevity of adult mosquitoes when reared under field conditions or in the laboratory (Vrzal, 2010). This study established that agrochemical exposed irrigated areas produce short-lived malaria vectors. This finding agrees with the suggestion that rice irrigation support higher densities of malaria vectors, which are short-lived, and therefore less dynamic in malaria transmission (Muriu *et al.*, 2008). Sporogony requires 10-12 days (Gu *et al*., 2011) and the reduced mean longevity of less than 8 days in agrochemical exposed vectors in this study corroborated with this. This suggests few agrochemical exposed vectors survive long enough to transmit *Plasmodium* parasites in irrigated agro-ecosystems.

The vector fitness of malaria transmitting mosquitoes is largely dependent on the size of the mosquito and its longevity (Ameneshewa and Service, 1996). International attention to malaria control and elimination focuses on vector control measures including long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) that target adult mosquitoes. Mosquitoes have the ability to avoid these interventions and bite humans when outside these protected areas. The current intra-domiciliary interventions, of ITNs and IRS remain the most effective means for malaria vector control (WHO, 2009). However, these interventions are not sufficient to meet the goal of malaria elimination, as their benefits are limited to indoor biting mosquitoes while neglecting outdoor biting populations (Pates *et al*., 2005). In this study larvae exposure to agrochemicals resulted in tangible reduction of adult life span. Since mosquitoes at larvae stage in irrigated paddies cannot change or choose their habitat, these findings show that control interventions targeting larvae are still promising in malaria vector control.

This study has revealed that agrochemical interaction and contamination in larvae habitats contribute to reproduction of short-lived emergent adult *Anopheles* mosquitoes with minimal impediment on size. Based on these findings, agrochemical exposed Anophelines pay a 'fitness' cost in longevity. Shortened longevity of agrochemical-exposed mosquitoes compromises sporogony. Studies by Gu *et al*. (2011), demonstrated that sporogony requires a minimum of 10-12 days to complete**.** Consequently, most agrochemical-exposed mosquitoes may not sustain the extrinsic sporogonic cycle of the *Plasmodium* parasites. These findings agree with Woodring *et al.*(1996) that the success of mosquitoes as disease vectors relies heavily on prolonged survival. Prolonged survival allows vectors to feed on multiple hosts and facilitate pathogen development (Woodring *et al.,* 1996).

The diminished longevity of adult *Anopheles* vectors in rice irrigation schemes is attributable to transcendence of particular traits that are dependent on larvae habitats interaction. Agricultural practices such as fertilizer usage affect larval densities (Victor *et al*., 2000,; Mutero *et al.,* 2004). Agro-chemical use modifies the larvae habitats by introduction of new chemical components and affect the physical environment. Introduction of chemical elements via agriculture favor biodiversity and thus improve larvae growth and development by promoting dietary resources available to aquatic organisims (Kenmore *et al.,* 1984). Similarly, agrochemicals may deprive larvae dietary resources if they are noxious to bacteria, algae or other zooplanktons that constitute the variety of food to mosquito larvae. Modification of larval habitats was the key to malaria eradication efforts in the United States, Israel, and Italy (Kitron *et al.,* 1989). Ingestion of these chemicals with larva diets may change the inherent physiological processes of the mosquito immature (Mutero *et al.,* 2004). Duiker *et al.* (2005a) has reported positive relationships in size of larvae in relation to adult life history and even suggested that some fields are simply superior larval habitats to others. In this study, habitat superiority could result from use of agrochemicals; and the direct ingestion of these chemicals by mosquito larvae and or modification of available diets for *Anopheles* larvae may affect the emergent vector ‘fitness’. In a recent related study on female *Culex quinquefasciatus,* Vrzal *et al.* (2010), established that larval diet played an important role in nutritional reserves and size at emergence, which in turn affected adult longevity. Some of the adult cellular traits descend from the larvae tissues. The polyploid larval ileum of *Culex pipiens* undergoes somatic reduction divisions to form the adult ileum (Berger, 1938). Consequently, there is a probability of bioaccumulation of agrochemicals in larvae tissues that can affect the life history of emergent vectors.

5.1.2. Effect of agrochemicals exposure on size of emergent *Anopheles* vectors in paddy fields and simulated trials.

In contrast to the reduced *Anopheles* mosquito longevity found this study, emerging *Anopheles arabiensis* adults exposed to agro-chemicals at larvae stage developed distinctively into bigger or equal sized mosquitoes than their respective non-exposed controls. The pooled mean wing lengths of mosquitoes exposed to agrochemicals were 3.7 and 3.5 mm for the simulated experiment and control respectively. Field paddy exposed mosquito wing length was 3.1 mm compared to 3.2 mm in the field control. *Anopheles spp*. wing length in the field averages 2.8 to 3.4 mm (Giles, 1968). Paired t-test analysis showed no statistical difference in the field agrochemical exposed vectors (P = 0.590). However, statistically significant difference was detected on wing length in the simulated experiment (P = 0.037).

Agro-chemical exposed paddies have adequate resources and produce big sized larvae (Mutero *et al.,* 2010). Mosquitoes exposed to agrochemicals exhibited slightly larger sizes than the controls in simulated trials in this study.

Based on the earlier reported findings of diminished life span of the same vectors in this study, there is a contrast with other studies on size and longevity.

A number of studies uphold that size of an insect may be biologically important and can affect life expectancy (Grimstad and Haramis 1984, Reisen *et al.*, 1984,; Smith, 1972,; Takahashi, 1976). Big sized mosquitoes have enhanced survival (Vrzal *et al.*, 2010). It can be plausible to suggested that other than dietary modification of larvae habitats by agro-chemicals that led to improved mosquito sizes in our study, there could be accumulation of injurious toxic wastes in mosquito immatures that affected survival 'fitness'. This might be through accumulation of chemicals components of the agrochemicals. This suggestion is corroborated with findings of Muturi *et al.* (2007) while determining the survival of larvae in sulfate of ammonia and potash of soda. They suggested that consumption of treated water together with food materials by *Culex* mosquito larvae may interfere with ionic balance of the immature resulting in reduced survival rates. Loss of ionic balance in biological systems leads to poor micronutrient uptake or no absorption at all.

All the agrochemicals used in this study may have toxic inclinations on aquatic habitats and are sometimes carcinogenic in mammalian experiments (MDSS). It has been demonstrated that polyploidy followed by a return to the mitotic cycle may increase the chance that cells will give rise to cancer in *Culex* and *Drosophila spp.* (Donald, 2010). The polyploid ileum of the larvae form part of the adult ileum and therefore if agro-chemicals induce undesired genetic variations in larvae cells like cancerous tendencies (as they have been demonstrated to do in mammals), then we suggest these may be conferred to agro-chemical exposed adults to their detriment.

Sulphur-coated urea is a possible mosquito control agent in rice fields through immature mortality (Roger, 1988). Decomposition of Sulfate of ammonia fertilizer produces sulphur and ammonia in rice paddies. Ammonium salts themselves have a hypothetical risk of ammonia toxicity (MDSS). Phosphate and ammonium salts found in di-amonium fertilizer has serious ionic imbalance in aquatic organisms according to its material data safety sheet (MDSS). Alpha-Cypermethrin is highly toxic to fish, bees and aquatic insects, according to the National Pesticides Telecommunications Network (NPTN 2001, Singh, 2012) and it is used for IRS in malaria vector control while, thiophanate methyl has mortality effects on insects and toxicity on a host of other aquatic organisms.

Another suggestion that may necessitate investigation is based on finding by Vrzal *et al.,* 2010 whilst studying amino acid effect on longevity of female *Culex quinquefasciatus*. They documented that carbohydrates (with or without amino acids) were a critical component of the adult diet, and in their absence, adult mosquitoes died within 3–5 days. Mosquitoes fed on treatments with sugar-adding amino acids to the adult diet of females resulted in a 5% increase in survival of females (Vrizal *et al.,* 2010). These mosquitoes had under gone a larvae diet of either enriched or poor carbohydrate with amino acids. Adult diets lacking sugar (water only or water + amino acids) did not sufficiently support survival, with all individuals living < 5 days. Furthermore, feeding on pollen by female *Aedes aegypti* L. mosquitoes (Eischen and Foster, 1983) or ingestion of amino acids and carbohydrates by various species of male and female butterflies has been reported to enhance longevity, fecundity or both (O'Brien *et al*., 2003; Mevi-Schutz and Erhardt; 2005).

Gu *et al*., (2011) documented that the probability of mosquito survival over the extrinsic incubation period is 4 times greater in sugar rich environment than in sugar poor sites. The poor carbohydrate content of rice straw (Shengying and Hongzhang, 2006), may deprive the supposedly large anopheline females ready energy sources in rice paddies.

5.1.3. Effect of agrochemicals on mosquito egg hatchability and immature survival in simulated trials.

In simulated experiments, combination of Di-ammonium phosphate and Thiosulphate methyl yielded the highest number of mosquito immature stages. Survival in this treatment was highest at 70%. All agro-chemicals combined were most lethal to egg or larvae survival. Higher mosquito yields were found in Di-ammonium phosphate combined with Thiophanate methyl (70%) which was higher than the control (63%) with no agrochemicals. Sunish and Reuben (2002) suggested that fertilizer application results in rapid multiplication of microorganisms upon which mosquito larvae feed on. This outstanding finding can be explained by an assumption of presence of mosquito larvae pathogens that may have been present in the canal water used in the study. Mosquito larvae pathogens *Bacillus thuringiensis* (Bt) *subsp. israelensis* (Bti) and *Bacillus sphaericus* (Bs) have been used in larvae control (Federici *et al.,* 2007) to kill larvae in their habitats. It has also been documented that larvae insects are exposed to many challenges in nature including overcrowding and inadequate nutrition, with larval nutritional deprivation being common in mosquitoes (Day and Van Handel, 1986). It would therefore be reasonable to suggest that there were some larvae/egg predators or pathogens in canal water and rice paddies that might be susceptible to these agrochemicals (esp. alpha-cypermethrin) and which may have been killed by the pesticide at the beginning of the experiment resulting in the higher larvae yield than in the control. Diammonium phosphate fertilizer in this treatment increases the PH of soil on application (Mwangangi *et al.,* 2006) and therefore its use without other combined agrochemicals may have affected the physiology of mosquito larvae. PH is a parameter which fluctuates greatly during the course of rice growing cycle affecting distribution and abundance of mosquito larvae (Muturi *et al.*, 2007). The physio-chemical properties in this treatment seemed to favor mosquito immature with 70% larvae survival compared to the control (63%).

In T1 where all the agro-chemicals were included, a survival rate (39%) was higher than in a combination of di-ammonium sulphate, alpha-cypermethrin and thiophanate methyl in Treatment 3 (28%). Service *et al.* (1977) observed a resurgence of mosquito larvae after initial control with insecticides due to slow rebounding of predator populations after fertilizer application in rice field. The interaction of the three agro chemicals might have produced a more lethal effect to the mosquito immature or an increased reproduction of a pathogen or both and thus the low (28%) survival rate when compared to 63% in the control.

5.1.4 Effects of agrochemicals on mosquito vector 'fitness' in simulated experiments.

Wing-length in T2 and the control were significantly different at means of 3.8mm and 3.4 mm respectively, ($P = 0.02$). The use of di-ammonium phosphate favored development of large mosquitoes and showed a high egg/ larvae survival rate (62%). Heavy chemical loading in habitats may interfere with osmo-regulation of mosquito larvae. Larval anopheline mosquito recta exhibit a dramatic change in localization patterns of ion transport proteins in response to shifting salinity (Smith, 2008). Presence of only one agrochemical must have reduced the physio-chemical burden in this microhabitat and given rise to bigger larvae and subsequently larger mosquitoes. Day and Van Handel, (1986) documented substantial effect of larval nutrition on adult size. Both male and female *Cx.quinquefasciatus* emerged with larger wing lengths, higher dry weights and greater glycogen and lipid reserves when reared on a high-nutrition diet as larvae. Since this study did not quantify food substances available in the habitat, increased or improved food diet is a probable reason for the bigger size of adult mosquitoes (3.8 mm) compared to the control (3.4 mm).

Di-Ammonium Phosphate, Alpha-cypermethrin, and Thiophanate methyl experiment (T3) had mosquito wing length of approximately 3.6 mm against 3.4 mm for the control (P-0.229). *Anopheles spp*. wing length in the field averages 2.8 to 3.4 mm (Giles, 1968). Since this combination was the most lethal to mosquito immature (28% egg hatchability), the small sized adults in comparison to control may be attributed to resource poor habitat in the treatment. The forces that deprived robust immature survival may have had undesired effects on habitat quality. Habitat quality or high competition may result in reduced body size of adults (Day and Van Handel, 1986).

Significant difference in wing length at (P = 0.03) was found where sulphate of ammonia which decomposes to urea upon application in paddy fields was used. Exposure of two snail species to 1–1.25 g/L doses of sulphate of ammonium for 48 h resulted in 100% mortality (Tchounwou, 1991). In this study, the combination may have depressed growth of bigger mosquito adults due to physio-chemical pressure of the toxic sulphur and ammonia. Sulphur-coated urea has also been shown to be a possible mosquito control agent in rice fields. Roger, (1988) suggests that the sulphur component of sulphate of amonium contributes to mosquito larvae mortality. Dietary intake and mosquito size as demonstrated in this study is in harmony with the findings of a study in Mali. In Niño, Mali (Manoukis *et al*., 2006) there was a modest positive relationship between densities of immature and larval size, and a strong relationship between larval and adult size.

5.2 CONCLUSIONS

Anopheles mosquito vector ‘fitness’ is negatively affected by use of agrochemicals in rice agro-ecosystems. Immature *Anopheles arabiensis* vectors when exposed to agrochemicals produce incompetent adults that pay a ‘fitness cost’ with reduced longevity which does not support complete sporogony. However, these adults have an advantage of increased size probably due to availability of resources in larvae habitats enhanced by agrochemical inputs in rice paddies.

Larvae habitat characteristics greatly influence the fitness of emergent anopheles mosquito vector. This study has generated further ecological knowledge on relationships between aquatic and terrestrial stages of malaria vectors on size and longevity. This study confirms interplay of rice irrigation and malaria transmission and that vectors bred in irrigated-agrochemical exposed habitats may have phenotypic variations that reduce their longevity.

This study has benefit to current understanding of malaria epidemiology and gives evidence that agrochemicals if used in synergy with other malaria control strategy can lead to better preventive interventions even in other irrigation based agricultural settings.

5.3 RECOMMENDATIONS

- Further investigations need to be carried out into mechanisms by which agrochemical use contributes to depressed longevity of mosquitoes which can be easily and conveniently exploited for use in malaria vector control.
- There is need for similar research in other types of agro-systems, to demonstrate whether use of agrochemicals would have the same effect on malaria vectors for its possible application as a vector control strategy

- The goal of malaria eradication will require strategic venture into understanding the ecology and evolution of the mosquito vectors that transmit malaria for better control tool development
- Agricultural experts need to collaborate with malaria stakeholders to explore the benefits in protection from malaria vectors that result from use of agricultural chemicals in vector control.

REFERENCES

Albert O M., Lucy W. I., Josephat I S., Ephantus J M., Charles C M., Joseph K N. and John G (2011). Dry season ecology of *Anopheles gambiae* complex mosquitoes at larval habitats in two traditionally semi-arid villages in Baringo, Kenya. *Parasit Vectors.*, **5:** 4-25

Ameneshewa, B. and Service, M. W. (1996). The relationship between female body size and survival rate of the malaria vector *Anopheles arabiensis* in Ethiopia *Med. Vet. Entomol;* **10:** 170–172**.**

Antonio-Nkondjio, C., Tene-Fossog, B., Ndo C., Menze-Djantio B., Zebaze-Togouet S., Awono-Ambene, H., Costantini, C., Wondji, C. and Ranson, H. (**2011).** *Anopheles gambiae* distribution and insecticide resistance in the cities of Douala and Yaoundé (Cameroon): influence of urban agriculture and pollution. *Malar J;* **75:** 10-154.

Ault, S.K. **(1994).** Environmental management: a re-emerging vector control strategy. *Am J Trop Med Hyg;* **50:** 35-49.

Berger, C. (1938). Multiplication and reduction of somatic chromosome groups as a regular developmental process in the mosquito, *Culex pipiens*. *Contrib. Emryol*; **27:** 200–232.

Clements, A. N. (1992). Development, nutrition and reproduction, the biology of mosquitoes Chapman & Hall, New York. Pg 200-226

Clive, S. (2002). Integrated Approach to Malaria Control – *Clin. Microbiol. Rev.***15:** 278–293.

Craig, M. H., Snow, R. W. and le Sueur, D. (1999). A climate based distribution model of malaria transmission in sub-Saharan Africa. *Parasitol Today*; **15:** 105 - 111.

Day, J.F., and Van Handel, E. (1986). Differences between the nutritional reserves of laboratory-maintained and field-collected adult mosquitoes. *J Am.Mosq Contr Assoc*; **2:** 154 - 157.

Dolo, G., Briet OJT, Dao A., Traore S.F., Bouare M., Sogoba N., Niare O., Bagayoko M., Sangare D., Teuscher and T., Toure Y.T. (2004) Malaria transmission in relation to rice cultivaton in the irrigated Sahel of Mali. *Acta Tropica*; **89**:147–159

Donald, T., Fox, J. G. and Spradling A. C. (2010). Error-prone polyploid mitosis during normal Drosophila development. *Genes Dev;* **1:** 2294–2302.

Diuk-Wasser, M.A., Bagayoko, M., Sogoba, N., Dolo, G., Toure, S.F.T. and Taylor, C.E. (2006). Mapping rice field anopheline breeding habitats in Mali, West Africa, using Landsat ETMsensor data, *Intern. J.of Remote Sens.*; **27:** 535–548.

Diuk-Wasser, M.A., Toure´, M. B., Dolo, G., Bagayoko, M., Sogoba, N., Traore´, S. F.T., Manoukis, N. and Taylor. C. E. (2005). Vector abundance and malaria transmission in rice-growing villages in Mali. *Am. J. Trop. Med. Hyg;* **72:** 725-731.

Eischen, F.A, Foster, W.A. (1983). Life span and fecundity of adult female *Aedes aegypti* (Diptera: Culicidae) fed aqueous extracts of pollen. *Ann. of the Entom.Society of Am;*
76: 661–663.

Federici BA, Park HW, Bideshi DK, Wirth MC, Johnson JJ, Sakano and Y Tang M. (2007) Developing recombinant bacteria for control of mosquito larvae. *J Am Mosq Contr Assoc.* **23:**164-175.

Ferreira, A.W. and Cochrane, A.H. (1986). Potential vectors of malaria and their effects on the malaria vector population. *Acta Trop.* **49:** 97-108.

Fillinger, U. and Lindsay S.W. (2006). Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. *Trop Med Int. Health*; **11:** 1629–1642.

Foster, W.A. (1995). Mosquito sugar-feeding and reproductive energetics. *Annu. Rev. Entomol;* **40:** 443- 474.

Foster, W.A. and W. Takken. (2004). Nectar-related vs. human-related volatiles: behavioral response and choice by female and male *Anopheles gambiae* (Diptera:*Culicidae*) between emergence and first feeding. *Bull. Entomol;* **94:** 145-157.

Gillies, M.T., De Meillon, B. and Coetzee, M. (1968). *Anophelinae* of Africa south of the Sahara (Ethiopian Zoogeographical Region). *2nd edn. South African Institute for Medical Research, Johannesburg.* Publication of the South African Institute for Medical Research no. 54.

Giles, M.T. (1972). The *anophelenae* south of Sahara 2nd edition pg 219. South African Institute for Medical Research

Gillies, M., and Coetzee, M. (1987). A Supplement to the *Anophelinae* of Africa South of the Sahara. The South African Institute for Medical Research, Johannesburg.

Greenwood, B. and Mutabingwa, T. (2002). Malaria in 2002. *Nature,* **415:** 670-672.

Grimstad, P. R. and L. D. Haramis. (1984). *Aedes triseriatus* (Diptera: *Culicidae*) and the Crosse virus.III. Enhanced oral transmission by nutrition-deprived mosquitoes. *J. Med. Entomol*; **2t:** 249-256.

Gu W, Müller, G., Schlein, Y., Novak R.J. and Beier, J.C. (2011). Natural plant sugar sources of Anopheles mosquitoes strongly impact malaria transmission potential. *PLoS One. 2011* **6**(*1*):*e15996*. doi: 10.1371/*journal.pone.0015996*

Hargreaves, K., L. Koekemoer, B. Brooke, Hunt, R. H., Mthembu, J. and Coetzee M. (2000). *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. *Med. Vet. Entomol*;. **14:** 181-189.

Hogg, JC., Thomson M.C. and Hurd H. (1996). Comparative fecundity and associated factors for two sibling species of the *Anopheles gambiae* complex occurring sympatrically in The Gambia. *Med. Vet. Entomol*; **10:** 385-391.

IIM, (1996). Proceedings of the Workshop on Irrigation and Vector-Borne Disease Transmission. Colombo, Sri Lanka: *Research Report No. 18*. Colombo, Sri Lanka

Ijumba, J.N., Mwangi, R.W. and Beier, J.C. (1990). Malaria transmission potential of *Anopheles* mosquitoes in the Mwea- Tebere irrigation scheme, Kenya. *Med. Vet. Entomol*; **4:** 425- 32.

Ijumba, J.N. and Lindsay, S. (2001). Impact of irrigation on malaria in Africa: paddies paradox. *Med Vet Entomol*; **15**: 1–11.

Imelda, B., Veronica, B., and Alex, A. (**2004).** Improving the accuracy of malaria-related laboratory tests in Ghana. *Malar. J.* **3:** 3-38.

Jayawardene R.A. (1995). In the Mahaweli: Its social, economic and political implications. Muller HP, Hettige ST, editor. Ratmalana, Sri Lanka, Sarvodaya Book Publishing Services; Healthcare management in Mahaweli Project; pg 205–220.

José. L., Ramirez, Lindsey, S., Garver S. and George D. W. (2009). Challenges and Approaches for Mosquito Targeted Malaria Control Climate change and infectious diseases 103. Chapter 6 2003, *Current Mol. Med.;* **9:** 116-130.

Justin, G., Simon, B., Michael, J., Domingue, M. B., Thomas A. F., Read, T. and Baker, C. (2011). Reduction in host-finding behavior in fungus-infected mosquitoes is correlated with reduction in olfactory receptor neuron responsiveness, *Mal. Journal;* **10:** 2-19.

KARI (2011). Desk information on agrochemical use in rice growing brochure.

Kenmore, P.E, F.O. Carino, C.A. Perez, Dyck, V.A. and A.P. Gutierrez, 1984: Population regulation of the brown plant hopper within rice fields in the Philippines. *jr. Plt. Prot* **1:** 19–37.

Killeen, G., Fillinger, U. and Knols B. (2002). Advantages of larval control for African malaria vectors: low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. *Malar. J*; **1**: 8. doi:10.1186/1475-2875

Kitron, U. and Spielman A. (1989). Suppression of transmission of malaria through source reduction: anti-anopheline measures applied in Israel, the United States and Italy. *Rev. of Infectious Dis;* **11:** 391- 406.

Kittayapong, P., Edman, J.D., Harrison, B.A., Delorme, D.R., Mahidol, B.T., (1992). Female body size, parity, and malaria infection of *Anopheles maculatus* (*Diptera: Culicidae*) in peninsular Malaysia. *J.Med. Entomol;* **29:** 379–383.

Knols, B.G.J., Basilio, N., Njiru, B.N., Mathenge, E.M., Mukabana, R.W., Beier, W.R. and Killeen,G.F. (2002). Malaria Sphere: A greenhouse-enclosed simulation of a natural *Anopheles gambiae* (Diptera: *Culicidae*) ecosystem in western Kenya. *Malar. J*; **1:**19 -28

Lyimo, E.O., Takken, W. and Koella, J.C. (1992). Effect of rearing temperature and larval density on larval survival, age at pupation and adult size of *Anopheles gambiae*. *Entom. Experimentalis et Appl.;* **63:** 265-271.

Macdonald, G. (1951). Analysis of malaria epidemics. *Trop. Dis. Bull;* **50:**871-879.

MacDonald, G. (1957). The epidemiology and control of malaria. London: Oxford University Press Pg 200- 245.

Manoukis, N., Toure, M.B., Sissoko I, Doumbia S., Traore S.F., Diuk-Wasser M.A. and Taylor C.E. (2006). Is vector body size the key to reduced malaria transmission in the irrigated region of Niono, Mali. *Med Entomol;* **43:** 820–827.

Mathenge, E.M., Killeen, G.F., Oulo, and D.O. (2002). Development of an exposure free bednet trap for sampling Afrotropical malaria vectors. *Med and Vet Entomol;* **16:** 67-74.

Mboera, L., Takken, E.W., Chuwa, G. and Picket, J. A. (2000). Oviposition and behavioral response of *culex quinquefaciatus* to skatole and synthetic oviposition pheromone in Tanzania. *J. chem. Ecol.* **26:** 1193-1203

Mboera L.E., Makundi, E.A. and Kitua, A.Y. (2007). Uncertainty in malaria control in Tanzania: crossroads and challenges for future interventions. *Am J Trop Med Hyg;* **77:**112-8.

MDSS – Agrochemical material data safety sheets. Insertion provided by manufacturers.

Mevi-Schutz, J. and Erhardt, A. (2005). Amino acids in nectar enhance butterfly fecundity: a long-awaited link. *Am. Naturalist;* **165:** 411–419.

MOPHS , (2010). Malaria Indicator Survey**.** *www.malariajournal.com/content/11/1/436*

MOPHS, (2009). Kenya Demographic Health Survey Report. Ministry of health report. *www.measuredhs.com/pubs/pdf/SR178/SR178*

MOPHS (2005–2010). National Health Sector Strategic Plan II Kenya *www.scribd.com/doc/*44068450

MOPHS. (2009-2017) Ministry of Public Health and Sanitation Kenya - National Malaria Strategy. *int/.../48311374BCF35DEE492577FA000CE43B* 86 pages.

MOPHS. (2011). Ministry of Public Health and Sanitation Kenya - Division of malaria control Operational plan. *www.measuredhs.com/pubs/pdf/SR178/SR178*

MOPHS. (2012). Ministry of Public Health and Sanitation Kenya - Kenya malaria fact sheet. *www.measuredhs.com/pubs/pdf/MIS7/MIS7*

MOPHS. (2008). Health management information system - annual report 67 pages *www.measuredhs.com/pubs/pdf/MIS5/MIS7*

MOPHS, (2012). Kenya malaria fact sheet. *www.malariajournal.com/content/11/1/436*

Mouchet, J., Manguin, S., Sircoulon, J., Laventure, S. and Faye, O. (1998). Evolution of malaria in Africa for the past 40 years: impact of climatic and human factors. *J Am Mosq Control Assoc;* **14:** 121–130.

Mutero, C., Kabutha C., Kimani, V., Kabuage, L., Gitau, G., Ssennyonga, J., Githure, J., Muthami, L., Kaida, A., Musyoka, L., Kiarie, E. and Oganda, M. (2004). A transdisciplinary perspective on the links between malaria and agro-ecosystems in Kenya. *ActaTrop;* **89:** 171–186.

Muriu, S.M., Muturi J.M.,, Shililu,J.M., Mbogo, C.M., Mwangangi J.M., Benjamin G. J. Irungu, L.W., Mukabana, R.W. Githure, J.I. and Novak, R. (2008). Host choice and multiple blood feeding behaviour of malaria vectors and other *Anophelines* in Mwea rice scheme, Kenya. *Malar J.*; **7:** 7- 43

Muturi, J.M., Jacob, B.G., Shililu, J. and Novak R. (2007). Laboratory studies on the effect of inorganic fertilizers on survival and development of immature *Culex quinquefasciatus* (Diptera: *Culicidae*). *J Vector Borne Dis*; **44:** 259–265.

Muturi, J.E, Burgess, P., and Novak R.J. (2008). Malaria Vector Management: Where Have We Come From and Where Are We Headed? *Am J Trop Med Hyg*; **78 (4):** 536-537

Muturi, J., Shililu. J., Jacob, B., Githure, J., Gu, W. and Novak R. (2006). Mosquito species diversity and abundance in relation to land use in a rice land agro-ecosystem in Mwea, Kenya. *J Vector Ecol;* **31:** 129–137.

Mwangangi, J.M., Shililu,J., Muturi, E.J., Muriu, S., Jacob, B., Kabiru, E.W, Mbogo, C.M, Githure, J. and Novak,R.J (2010). *Anopheles* larval abundance and diversity in three rice agro-village complexes Mwea irrigation scheme, central Kenya. *Malar J;* **9:** 228 doi: 10.1186/1475-2875-9-228

Mwangangi, J.M., Muturi, E.J., Shililu, J., Muriu, S., Jocob B., Kabiru, E.W., Mbogo, C.M., Githure, J. and Novak, R. (2006). Survival of immature *Anopheles arabiensis* (Diptera: *Culicidae*) in aquatic habitats in Mwea rice irrigation scheme, central Kenya. *Malaria J;* **5:** 114 – 121

Nasci, R.S. (1991). Influence of larval nutrition and adult nutrition on biting persistence in *Aedes aegypti*. *J. of Med. Ento;* **28:** 522–526

Natacha, P., Wim Van, B., Niko, S., Van Geertruyden, J.P., Baza, D., D'Alessandro1, U. and Coosemans, M. (2009). Ranking Malaria Risk Factors to Guide Malaria Control Efforts in African Highlands *Plos one* **4:** e8022. ISSN 1932-6203

NPTN, (2001). National Pesticides Telecommunications Network, March 2001 on-line issue of *Horticulture Update.*

Nayar, J.K. and Sauerman, D.M. (1971). Physiological effects of carbohydrates on survival, Florida mosquitoes. *J. Med Entomol.;* **12:** 92–98.

O'Brien, D.M., Boggs, C.L. and Fogel, M.L. (2003). Pollen feeding in the butterfly Heliconius charitonia: isotopic evidence for essential amino acid transfer from pollen to eggs. *P. of the R. Soc. of Lon.;* **270:** 2631–2636.

Okech, B. A., Louis C., G., Killeen, G.F., Bart, G., Knols, J., Kabiru, W.E., Beier, J.C., Yan, G. and Githure, J. (2003). Influence of Sugar Availability and Indoor Microclimate on Survival of *Anopheles gambiae* (Diptera: *Culicidae*) Under Semi field Conditions in Western Kenya. *J. of Med Ento.;* **40:** 657 - 663.

Oladimeji, O., Tona, G.O., Oshiname, F.O., and Titiloye, M.A. (2010). Malaria knowledge and agricultural practices that promote mosquito breeding in two rural farming communities in Oyo State, Nigeria. *Malar J.;* **9:** 91. doi:10.1186/1475-2875-*9-91*

Olliaro PL, Taylor W.R. (2004). Developing artemisinin based drug combinations for the treatment of drug resistant falciparum malaria: A review. *J. Postgrad Med;* **50:** 40 - 44

Omumbo J., Rapuoda J.O., Le Sueur M.H.C. and Snow R.W., (1998). Mapping malaria transmission intensity using geographical information systems (GIS): an example from Kenya. *Ann Trop Med Parasitol* **92:** 7–21.

Pates, H. and Curtis C. (2005). Mosquito behavior and Vector Control. *Annu Rev Entomol;* **50:** 53-70.

Patz, J. A., Githeko, A. K., McCarty, J. P., Hussein,S., Confalonieri, U. N., de Wet., José L. R., Lindsey S., Garver and George D. W. (2009). Challenges and Approaches for Mosquito Targeted Malaria Control Climate change and infectious diseases *Current Mol Med;* **9:** 116-130.

PECA, (2007). Malaria Programmatic Environmental Control Assessment manual *www.malar j.com/content/11/1/21* 111 pages.

Randell, H.F., Dickinson, K.L., Shayo, E.H., Mboera, L.E. and Kramer, R.A. (2010). Environmental management for malaria control: knowledge and practices in Mvomero, Tanzania. *Eco health;* **7:** 507-516.

Reisen, W. K., Milby, M. M. and Bock M. E. (1984). The effects of immature stress on selected events in the life historv of *Culex tarsalis*. *Mosq. News;* **44:** 385-395.

Robert, V., Gazin P., and Boudin, C. (1985). The transmission of malaria in a wooded savannah zone and a rice-growing zone in the vicinity of Bobo Dioulasso (Burkina Faso).*Ann Soc Belg MedTrop*; **65:** 201–214.

Roger, P.A. and Kurihara Y. (1988). Flood water biology of tropical wetland rice fields. Proceedings of the First International Symposium on Paddy Soil Fertility. *Malar J;* **21:** 275–300.

Rogier, C., Toure, Y., and Doumbo, O. (2004). Malaria incidence in relation to rice cultivation in the irrigated Sahel of Mali. *Acta Trop;* **89:** 161–170.

Service, M.W. (1977). Mortalities of the immature stages of species B of *Anopheles gambiae* complex in Kenya: comparison between rice fields and temporary pools, identification of predators, and effects of insecticidal spraying. *J Med Entomol;* **13:** 535–545.

Shengying J.; Hongzhang C.(2006). Superfine grinding of steam-exploded rice straw and its enzymatic hydrolysis. *Bio. Eng. Journ*. **30:** 225 - 230.

Simpson, I. and Roger, P.A. (1991). Impact of agrochemicals on mosquito larvae populations in rice fields. Inter. *Rice Research Newslette*r; **16:** 29-30.

Singh, A.K; Tiwari, M.N., Upadhyay, G., Patel, D.K., Singh, D., Prakash, O. and Singh, M.P. (2012). "Long term exposure to cypermethrin induces nigrostriatal dopaminergic neuro degeneration in adult rats: Postnatal exposure enhances the susceptibility during adulthood". Neurobiology of Aging. *J.neuro. boil. aging;* **33:** 404 – 415.

Smith, K.E., Van Ekeris L.A., Okech, B.A., Harvey, W.R. and Linser, P.J. (2008). Larval *anopheline* mosquito recta exhibit a dramatic change in localization patterns of ion transport proteins in response to shifting salinity: a comparison between *Anopheline* and *Culicine* larvae. *J. of Exp. Biol;* **19:** 3067–3076.

Smith, D. S. (1972). Crowding in grasshoppers II. Continuing effects of crowding on subsequent generations of *Melanopius sanguinipes*. (Orthoptera: *Acrididae*). *Environ. Entomol;* **1:** 314 -317.

Sunish, I. and Reuben, R. (**2002)** . Factors influencing the abundance of Japanese encephalitis vectors in rice fields in India–II:Biotic. *Med Vet Entomol;* **16:** 1 - 9.

Takahashi, M. (1976). The effects of environmental and physiological conditions of *Culex tritaeniorhynthus* on the pattern of transmission of Japanese encephalitis virus. *J. Med. Entomol;* **13:** 275-284.

Tanga M.C., Ngundu W.I., Juditha N., Mbuha J., Tendongfor N., Frédéric S. and S. Wanjia 2010. Climate change and altitudinal structuring of malaria vectors in south-western Cameroon: their relation to malaria transmission. *Trans R Soc Trop Med Hyg* **10:**1016 – 1023.

Tchounwou, P.B., Englande, A.J. and Malek, E.A. (1991). Toxicity evaluation of ammonium sulphate and urea to three developmental stages of freshwater snails. *Arch Environ Contam Toxicol;* **21:** 359–364.

USAID, (2007). Integrated Vector Management Programs for Malaria Vector Control Programmatic Environmental Assessment. RTI: Assesment report. ***usaid**.gov/pdf_docs/PNADI081*

Van der Hoek, W., Konradsen, F., Amerasinghe, P.H., Perera D., Piyaratne, M.K. and Amerasinghe, F.P. (2003). Towards a risk map of malaria for Sri Lanka: the importance of house location relative to vector breeding sites. *Int J Epidemiol*; **32:** 280-285.

Victor, T.J. and Reuben, R. (2000). Effects of organic and inorganic fertilizers on mosquito populations in rice fields of southern India. *Med Vet Entomol;* **14:** 361- 8.

Vrzal, E.M., Allan, S.A. and Hahn D.A.(2010) Amino acids in nectar enhance longevity of female *Culex quinquefasciatus* mosquitoes *J. of Insect Physio*.; **56:** 1659–1664.

WHO, (2006). Geneva, Convention on Use of ITNS and Insecticide Use Report. *WHO/HTM/MAL/*2006.1112

WHO, (2009). World Malaria Report. *who.int/**malaria**/publications/atoz/9789241563901*

WHO, (2011). World Malaria Report. *www.who.int/malaria/world_malaria_report*_2011

William, K. R. and Hugh, D. L. (1999). Effects of sampling design on the estimation of adult Mosquito abundance. *J. of am. Mos. Control Association;* **15(2.):** 105-111

Woodring, J.L., Higgs, S. and Beaty, B.J. (1996). Natural cycles of vector-borne pathogens in biology of disease vectors. University Press of Colorado, *Boulder* **1 :** 51–72.

APPENDIX 1

INFORMED CONSENT FORM

Paddy use for ecological study of mosquito habitats Mwea irrigation scheme

Hello, my name is ………….. …………………and your paddy has been selected for a mosquito ecology survey by a student from Jomo-Kenyatta university of agriculture and technology. The survey will be conducted in partnership with Ministry Of Public Health And Sanitation officers from Kimbimbi Sub - District Hospital, KEMRI and the University student.

Description of the research and participation: Agricultural chemicals used on your farms DAP, sulphate of ammonia, (sukari) and pesticides thoiphanate methyl (Koba) will be applied to a small portion of your plot during rice growing. The investigation team will then start sampling mosquito pupae (*Tugunyu twa rwagi*) as the rice growing continues. The team will not harvest rice from the portion.

The survey will include the following activities:

- Your presence when the team demarcates the plot;
- Rice transplanting on agreed days
- Application of fertilizer and pesticides on the selected plots on agreed days

Benefits of participating:

- Your consent to the use of your land will benefit the government in finding ways to fight Malaria
- You will reap the rice on the plot
- You will not use your money for fertilizing and fumigation of the selected plot

Risks: No new chemical will be used on your plot and the rice straw will be taken care of during sampling to avoid any destruction and therefore there no risk or damage to your land

Protection of confidentiality: This will be an open field exercise for which no confidential information is requested from you.

Voluntary participation: Your participation in this research study is entirely voluntary. You may choose not to allow plot usage

Contact information: If you have any questions contact me, (the interviewer). You may also wish to contact the PS_ MoPHS -0202-567256 or Director,Itromid Jomo Kenyatta university; or Director, KEMRI, Nairobi.

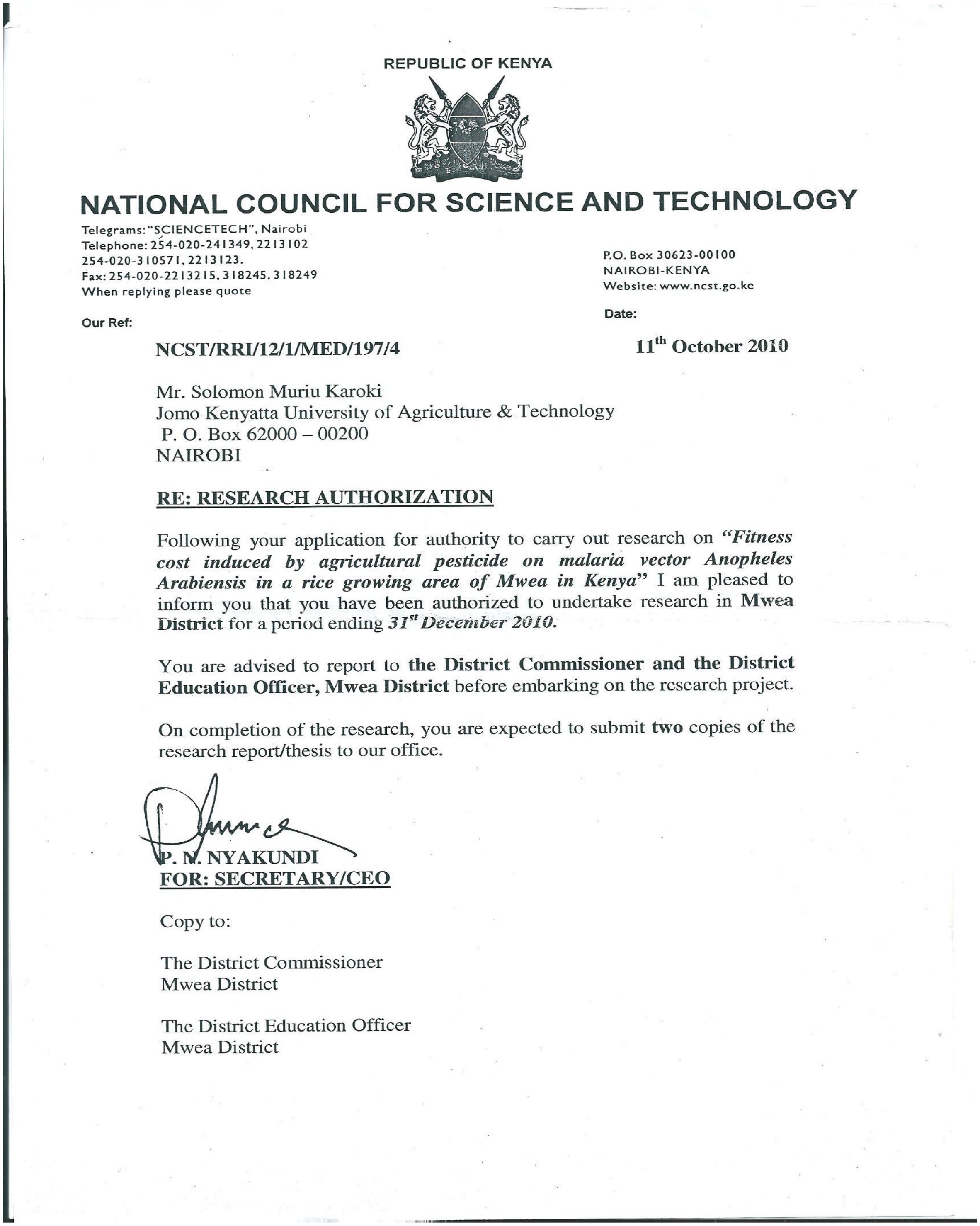
I have read and understood this text and all my questions have been answered. I am aware of my rights and duties and I freely accept that my plot may be used in this study.

Respondent Name: ______________________________ Date: ________________________

Signature: _________________________ Right Thumb Print______________________

Plot number:________ Interviewer:__________________ Date:___ /___/2012

Appendix 2 – Research Authorization Letter.



REPUBLIC OF KENYA

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Date:
11th October 2010

Mr. Solomon Muriu Karoki
Jomo Kenyatta University of Agriculture & Technology
P. O. Box 62000 – 00200
NAIROBI

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Fitness cost induced by agricultural pesticide on malaria vector Anopheles Arabiensis in a rice growing area of Mwea in Kenya"*** I am pleased to inform you that you have been authorized to undertake research in **Mwea District** for a period ending ***31st December 2010.***

You are advised to report to **the District Commissioner and the District Education Officer, Mwea District** before embarking on the research project.

On completion of the research, you are expected to submit **two** copies of the research report/thesis to our office.

P. N. NYAKUNDI
FOR: SECRETARY/CEO

Copy to:

The District Commissioner
Mwea District

The District Education Officer
Mwea District

Appendix 3 - Conference presentation, Amman , Jordan- 2012

THE ROLE OF AGRICULTURAL CHEMICALS ON MALARIA VECTORS' FITNESS IN A RICE AGRO-ECOSYSTEM IN KENYA

Karoki S[1]., Ng'ang'a Z[2]., Mathenge E[3].

Introduction

Background

- An estimated 655,000 persons died of malaria world
- 91% of these malaria deaths occurred in the WHO African Region.
- 25 million out of a population of 39 million Kenyans are at risk of (MoPHS, 2011).
- Currently ITNs and IRS remain the most effective means for malaria vector control in Africa(WHO, 2009).
- Current vector control interventions are not sufficient to meet the goal of malaria elimination as benefits are limited to indoor biting vectors only.
- Replacement of indoor biting malaria vectors by outdoor dwelling mosquitoes and successful malaria transmission has been reported(Hargreaves *et al.*, 2000; Pates *et al.*, 2005).
- Increased knowledge on the ecology and behavior of malaria vectors in their habitats is necessary to design new control measures(Oketch *et al.*, 2003).
- Malaria vector 'fitness' is the ability of a mosquito to successfully transmit malaria and is largely dependent on mosquito size and longevity.
- Mosquitoes must live beyond 12 days, which is required for sporogony to succeed as malaria vectors while size determines the distance it may cover searching a blood meal.

- Malaria is still one of the most serious parasitic diseases with an enormous socioeconomic impact in tropical Africa.
- Malaria transmission in rice agro-ecosystems is constant despite increased vector prevalence during rice growing seasons(Ijumba *et al.*, 2001; Muturi *et al.*, 2009).
- Malaria control programs have so far relied on controlling the mosquito vectors to reduce the disease burden(José *et al.*, 2009).
- Malaria control in the future may be hindered by vector and parasite resistance to available options (Olliaro *et al.*, 2004).
- It is important to continually develop new and innovative malaria vector control strategies based on sound understanding of vector biology and ecology.
- Studying the implication and role of agro-chemical use in rice agro-ecosystems and their effect on vector fitness' is a valuable asset for multi-faceted approaches to effective malaria control.

Figure 1 Sampling pupae in the mwea irrigation scheme rice field paddy experiments (2010)

Problem statement

- In Africa, disease and food insecurity pose major obsta
- Malaria weakens the human labour force working in ag
- Unpredictable climatic conditions have made rain fed a
- The use of irrigation in agriculture is perceived as one way of enhancing food security and promoting economic development in Africa.
- Irrigation especially flooding of land in rice cultivation, has been associated with an increase in the number of disease vectors, and in certain cases, a corresponding increased health burden due to malaria.
- Fertilizer application in rice fields promotes mosquito productivity by stimulating microbial growth and probably by acting as an oviposition cue.
- No information is available on the effects of agricultural chemicals on vector immature and the effect that may transcend the emerging adults and the associated 'fitness' cost in malaria transmission.

Justification

Methods

STUDY SITE

This study was carried out in Mwea rice irrigation scheme located about 120 km North East of Nairobi, Kenya. Mwea occupies the lower altitude zone of Kirinyaga county in an expansive low-lying area. Geographically, Mwea lies between latitude 1 ˚ S of the equator, longitude 37.5 ˚ E and an altitude of 1900M above sea level. 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 - 29.5°C.

The Mwea rice irrigation scheme is located in the west central region of Mwea district and covers an area of about 13,640 ha. More than 50% of the scheme area is under rice cultivation. The remaining area is used for subsistence farming, grazing and community activities.

▪**Figure 2. Map of Mwea district (courtesy open access)**

STUD

▪The the role of agricultural chemicals on mosquito vector 'fitness' with emphasis on fertilizers (*Sulphate of ammonia and Di- ammonium phosphate*) in combination with pesticides (*Thiophanate Methyl and Alpha-cypermethlin)* for their potential application in malaria vector control in paddy and simulated field conditions.

▪ **Sample size** - 192 females anopheles mosquitoes

▪**Field (paddy experiments) study**

▪In the field experiments 3-quarter acre paddy blocks were transplanted with rice seedlings in September 2011 and top dressed and fumigated according to planned rice growing and manfaturers agrochemical use guidelines.

▪Four days post topdressing, pupae were sampled in the 3 selected paddies on a weekly basis until the desired sample size of 192 female *Anopheles* mosquitoes was attained.

▪- Pupae sampled from paddies as rice growing progressed with routine fumigation and fertilization of the blocks.

▪ **Simulated experiment**

▪ 50 Mosquito ova was incubated in three replicate bowls and agro-chemicals in 6 different combinations of introduced as routinely used by farmers in rice growing. Control replicates had no agrochemicals.

▪Anopheles pupae sampled from all experiments were reared in laboratory conditions until death in mosquito holding cups, and wing length meted.(Mathenge *et al* 2003).

▪**Table 1- Agrochemical combination and abbreviations in simulated experiments**

TREATMENT	AGROCHEMICAL COMPONENT
[redacted]	[redacted]
TREATMENT 2 (D)	Di-Ammonium Phosphate only
[redacted]	[redacted]
TREATMENT 4 (SAT)	Sulphate of ammonium, Thiophanate methyl and Alphacypermethylin
[redacted]	[redacted]
TREAMENT 6 (DT)	Di-ammonium phosphate and Thiophanate methyl
[redacted]	[redacted]

Results

Longevity of mosquitoes in paddy and simulated experiments

▪ Emergent Anopheles adults had a statistically significant difference in mean longevity at 6.5 days in paddies compared to control mosquitoes that had a mean longevity of 18 days (p - 0.003).

▪In simulated trials longevity was statistically different with a mean lifespan of 7.1 days compared to a mean longevity of 15 days (p -0.000).

Figure 3 Mean longevity *Anopheles ar* ectors in padd and simulate ents

Figure 4 Effects of di ent com ations ro-che s on A *elesar* nsis ve longev

Wing length (size) of mosquitoes in paddy and simulated trials

▪The pooled mean wing lengths of mosquitoes exposed to agrochemicals were statistically significant at 3.7 and 3.5 mm for the simulated experiment and control respectively (p- 0.037).

▪Field paddy exposed mosquito wing length was 3.1 mm compared to 3.2 mm in the field control and not statistically significant (P - 0.590).

▪ *Anopheles arabiensis* wing length in the field averages 2.8 to 3.4 mm (Giles 1968).

Figure 6. Mean wing length in s of *Anophel* sis vectors in osed and sim periments

Figure5. Effects of ent com ons of a hemical Anophel biensis r size

▪Bibliography
1. Field Epidemiology and Laboratory Training Programme - Kenya
2. Jomo Kenyatta University of Agriculture and Technology
3. Kenya Medical Research Institute Nairobi

Conclusions

▪Mosquito vector 'fitness' is affected by use of agrochemicals in rice agro-ecosystems.

▪ Immature *Anopheles arabiensis* vectors when exposed to agrochemicals produce incompetent adults that pay a 'fitness cost' with reduced longevity.

▪Reduced mosquito longevity cannot support completion of sporogony and is a probable factor of low malaria transmission in rice agro ecosystems despite increased vectors.

▪Mosquito exposure to agrochemicals in rice paddies gives an advantage of increased size probably due to enhanced nutrition in agrochemical exposed habitats.

▪ Larvae habitat characteristics greatly influence the fitness of emergent vectors in malaria transmission fitness.

Recommendations

agrochemical use contributes to reduced longevity which can be easily and conveniently exploited for use in malaria vector control.

▪Extension of similar research to other agro-ecosystems, to demonstrate whether use of agrochemicals would have the same effect on malaria vectors for its possible application as a vector control strategy.

▪Fig

▪References

Manoukis,N. and Taylor. C. E. (2005a). Vector abundance and malaria transmission in rice-growing villages in Mali. Am. J. Trop. Med. Hyg; 72:725731

Gillies, M.T., De Meillon, B. and Coetzee, M. (1968). *Anophelinae* of Africa south of the Sahara (Ethiopian Zoogeographical Region). 2nd edn. South African Institute for Medical Research, Johannesburg. Publication of the South African Institute for Medical Research no. 54.

Hargreaves, K., L. Koekemoer, B. Brooke, Hunt, R. H., Mthembu, J. and M. Coetzee. (2000). *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. *Med. Vet. Entomol*;. 14:181-189.

Clive, S. (2002). Integrated Approach to Malaria Control – Clinical Microbiology Revl; 15(2): 278–293.doi: 10.1128/CMR.15.2.278-293.2002 PMCID: PMC118067

Ijumba, J. and Lindsay, S. (2001). Impact of irrigation on malaria in Africa: paddies paradox. *Med Vet Entomol*; 15: 1–11.

José. L., Ramirez, Lindsey, S., Garver and George D. W. (2009). Challenges and Approaches for Mosquito Targeted Malaria Control Climate change and infectious diseases 103. Chapter 6 2003 *Current Molecular Medicine*;9, 116-130.

Mathenge, E.M., Killeen, G.F., Oulo, D.O. (2002). Development of an exposure free bednet trap for sampling Afrotropical malaria vectors. *Medical and Veterinary Entomology*;16 (1), 67-74

Muturi, J., Shililu. J., Jacob, B., Githure, J., Gu, W. and Novak R. (2006). Mosquito species diversity and abundance in relation to land use in a rice land agro-ecosystem in Mwea, Kenya. *J Vector Ecol*; 31: 129–37.

Okech, B. A., Louis C., G., Killeen, G.F., Bart, G., Knols, J., Kabiru, W.E., Beier, J.C., Yan, G. and Githure, J. (2003). Influence of Sugar Availability and Indoor Microclimate on Survival of *Anopheles gambiae* (Diptera: *Culicidae*) Under Semifield Conditions in Western Kenya. *Journal of Medical Entomology*; 40(5): 657663.

Olliaro PL, Taylor WR (2004). Developing artemisinin based drug combinations for the treatment of drug resistant falciparum malaria: A review. *J Postgrad Med*;50:40-4

WHO report 2011