EFFECTS OF INSECT GROWTH REGULATOR PYRIPROXYFEN ON DRAGONFLY NYMPHS AS PREDATORS OF *ANOPHELES* MOSQUITOES AT MAHANGA, VIHIGA COUNTY, KENYA

AMEKA, CALEB MIKHALI (BSC)

I56/CE/15393/2008

A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Agricultural Entomology) in the School of Pure and Applied Sciences of Kenyatta University

November 2015

DECLARATION

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

Signature---------- Date ---------- 6/11/2015

Ameka Caleb Mikhali (I56/CE/15393/2008)

Department of Zoological sciences

SUPERVISORS

We confirm that the work reported in this thesis was carried out by the candidate under our supervision as the university supervisors.

Signature ---------- DATE: 5/11/2015

Prof. Elizabeth Kokwaro

Department of Zoological Sciences

Kenyatta University

Signature ---------- Date ---------- 19/10/2015

Dr. Bryson Ndenga

Centre for Global Health Research

KEMRI, Kisumu

DEDICATION

This work is dedicated to my wife, Pamela and children, Elsey, Alvin, Fortune and Quintone for their endurance, prayers and moral support during the time of this study. Special dedication to my late father Clement Ameka who was my source of inspiration during childhood.

.

ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude to several people for the role they played to enable me undertake and accomplish this study. I extend my deepest appreciation to my university supervisor Prof. Elizabeth Kokwaro for her unwavering support, constructive criticisms, guidance, patience and availability. Special thanks to Dr. Bryson Ndenga of Kenya Medical Research Institute (KEMRI) who introduced me to the world of mosquitoes and Medical Entomology. His technical, scientific input and guidance throughout the course of research, data analysis and thesis writing was of great help. I am also grateful to Dr. Ulrike Fillinger (ICIPE) for her critical evaluation of the study design and implementation of the work.

I sincerely thank Oscar Mbare, Joan Shiroko, Nicholas Mulaya, Peter Aswani, Michael Ombajo, Geofrey Awesi and David Kituyi for their technical support and tremendous help in the execution of field and laboratory data collection work. My sincere gratitude goes to Dr. John Vulule for granting me an opportunity to use the KEMRI facilities at Kisian, Kisumu. This research study received financial support from the European Commission through the African Vector Control: New Tools (AVECNET) Project and National Commission for Science, Technology and Innovation (NACOSTI).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDIX

ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
CGHR	Centre for global health research
ESRI	Environmental Systems Research Institute
G	Granules
GEE	Generalized Estimation Equations
FAO	Food and Agricultural organization
GPS	Geographical Positioning System
ITN	Insecticide Treated Nets
ICIPE	International Centre of Insect Physiology and Ecology
IGR	Insect Growth Regulator
KEMRI	Kenya Medical Research Institute
NACOSTI	National Commission for Science Technology and Innovation
PCPB	Pest Control Product Board
PPM	Parts Per Million
SSA	Sub Saharan Africa
UNICEF	United Nations Children Education Fund
WHO	World Health 0rganization
WHOPES	World Health Organization Pesticide Evaluation Scheme

ABSTRACT

Malaria in sub-Saharan Africa is transmitted mainly by *Anopheles gambiae* Complex mosquitoes. One way of controlling these vectors is by targeting their aquatic stages, which is anticipated to cause significant reduction in adult vectors, hence in malaria transmission. Use of insect growth regulator, Pyriproxyfen, is one potential way of controlling malaria vectors. This study set out to determine the nymphocidal activity of Pyriproxyfen on non-target aquatic dragonfly nymphs during its application to control malaria vectors in western Kenya highlands. In this study, validation of dragonfly nymphs as predators of malaria vectors was done and impact of Pyriproxyfen on these nymphs was determined in Mahanga Village of Vihiga County in western Kenya highlands. One hundred dragonfly nymphs were exposed to third instar larvae of *A. gambiae* to determine their predation efficiency by counting the number of larvae remaining after predation. Eighty 5th instar dragonfly nymphs were exposed to Pyriproxyfen (Sumilarv0.5 G) at concentrations of 0.01ppm, 0.05ppm, 0.1ppm, and filtered tap water as control. The experiment was replicated four times and repeated in ten rounds in the laboratory. Observations were made at 24 hour intervals and data collected on mortality of dragonfly nymphs. One gram of Pyriproxyfen was applied in the 10 randomly selected mosquito breeding habitats at Mahanga once every month. Control experiments in *An. gambiae* breeding habitats were done at Muluhoro study site located 10Km away from Mahanga to avoid contamination by Pyriproxyfen (Sumilarv 0.5G). Percentage predation of 95% was obtained in 24 hours of exposure of *An. gambiae* larvae indicating that the dragonfly nymphs are efficient predators of *A. gambiae*. The insect growth regulator Pyriproxyfen (Sumilarv 0.5G) had nymphocidal activity on the dragonfly nymphs at a concentration of 0.05ppm and 0.1ppm in laboratory assays. Abundance of dragonfly nymphs in *An. gambiae* breeding habitat was determined by comparing the abundance of dragonfly nymphs in the intervention sites at Mahanga and non intervention site at Muluhoro. The results indicated that the dragonfly nymphs were present in both sites over the 11month period. Analysis using Generalized Estimation Equations (GEE) showed that the abundance of dragonfly nymphs was significantly different ($p<0.01$) and there was insignificant nymphocidal activity of Pyriproxyfen (Sumilarv 0.5G) on the dragonfly nymphs in both the intervention and non intervention ($p>0.05$). The findings of this study have shown that Pyriproxyfen (Sumilarv 0.5G) had insignificant nymphocidal activity on dragonfly nymph when used as a larvicide at lower concentrations of 0.05ppm ($p>0.05$). The study recommends that dragonfly nymphs should be included in mosquito control programs as they are predators of mosquito larvae. Additionally, Pyriproxyfen should not be used at higher dosages of more than 0.05ppm as it affects non target dragonfly nymphs.

CHAPTER ONE

INTRODUCTION

1.1 Background information.

Malaria poses a tremendous public health problem worldwide. In 2013, there were 3.4 billion malaria episodes, leading to approximately 627,000 deaths (WHO, 2013). According to WHO (2007 and 2013), more than 2.4 billion of world's population residing in 100 countries is at risk of malaria and this results in an estimated 300-500 million clinical cases each year, 90% of which occur in sub- Saharan Africa. In Kenya, it threatens lives of 25 million out of the country's total population of 39 million people (Pascaline *et al.*, 2010). In Western Kenya malaria is prevalent in Kisii, Nyamira, Nandi, Bomet, Kakamega, Vihiga, Kisumu, and Homabay Counties with many clinical cases reported (Hay, 2002).

Malaria is transmitted by the *Anopheles gambiae* mosquitoes which can be controlled using insecticide treated bed nets, residual sprays, and application of larvicides. Irrespective of these control methods, malaria still remains a problem in many areas within the Sub Saharan Africa (Bogitish and Thomas, 1990). Larvicides in use have the potential to control the immature stages of mosquitoes. The continued uses of larvicides however have setbacks in that some are harmful to aquatic arthropods that co-exist with mosquitoes in their natural breeding habitats (Hanafi–Bajd *et al* ., 2006). In aquatic ecosystems mosquito larvae coexist with a number of aquatic insects such as dragonfly nymphs (*Annax imperator)* backswimmers (*Notonecta unifasciata)* and water beetles (*Dytiscus marginalis)* (Blaustein and Jonathan, 2009). Most of these insects are

predators of mosquito larvae and are beneficial. Consequently they should not be affected by the application of larvicides in the natural habitats. Previous studies have shown that diving beetles are predators of mosquito larvae and hence can be used to reduce mosquito populations (Lundkvist *et al*., 2003).

Pyriproxyfen (Sumilarv 0.5G) (Sumitomo chemical Co. Ltd., Japan), molecular formula $C_{20}H_{19}NO_3$ and chemical name 4-Phenoxyphenyl(RS)-2-(2-Pyridyloxy) Propylether, is a broad–spectrum Insect Growth Regulator of insecticidal activity against public health insect pests and agricultural pests (FAO/WHO, 1999; Yapabandara, 2005). It is recommended at the dosage of 0.01mg/l for controlling mosquitoes in drinking water containers (WHO, 2007). Pyriproxyfen affects the physiology of morphogenesis, reproduction and embryogenesis of insects by interfering with hormonal control of mosquito growth and development (Kawada *et al.*, 1988, Invest and Lucas 2008, and Yapabandara, 2005). Pyriproxyfen is a new generation of IGR. It functions as an insecticide by overloading hormonal systems of the target insect, ultimately affecting its egg production, brood care and other social interactions, and inhibiting its growth. Pyriproxyfen is reported to exhibit 95% inhibition of the emergence of mosquito larvae and its effects on mosquito larvae having lasted for two months after application (Chen *et al*., 2008; Mbare *et al.*, 2013). Although the treated mosquito larvae continue to pupate, their emergence is inhibited by the action of pyriproxyfen. The main effect of IGRs is the inhibition of adult emergence, reproduction and ecdysteroid production failure in surviving females (Lee, 2002).

In their study in North America, Quiroz- Martinez and Aradna (2007) observed that many aquatic insects such as dragonflies, backswimmers, damselflies, and water beetles feed on mosquito larvae. In a pilot study conducted at Iguhu in Kakamega County, research findings confirmed that backswimmers are predators of *Anopheles gambiae* larvae (Munga *et al*., 2006). From these results it is evident that aquatic arthropods are important components in aquatic ecosystems and thus need to be protected from any adverse effects of broad spectrum larvicides aimed at controlling mosquito larvae. Field surveys conducted in the western Kenya highlands documented that dragonfly nymphs were found in large proportions within western Kenya highlands (Ndenga *et al.,* 2011). The present research work investigated predation efficiency of dragonfly nymphs on *Anopheles gambiae* larvae. Furthermore, nymphocidal activity of insect growth regulator Pyriproxyfen (Sumilarv 0.5G) on dragonfly nymphs in laboratory bioassays and mosquito breeding habitats at Mahanga in Vihiga County, Western Kenya, was investigated.

1.2 Statement of the problem

Pyriproxyfen (Sumilarv 0.5G), an Insect Growth Regulators (IGR), applied in aquatic habitats to control mosquito larvae has the potential of affecting dragonfly nymphs which are predators of mosquito larvae. Pyriproxyfen (Sumilarv 0.5G) is being used mostly in the control of *Aedes* mosquitoes (Invest and Lucas, 2008, Lee *et al.*, 2005). Currently, there is increasing need to evaluate its potential to control malaria vectors. Hence, it is important to evaluate its impact on non-target organisms within aquatic habitats in the field. Therefore, this study aimed to evaluate the nymphocidal activity of

Pyriproxyfen (Sumilarv 0.5G) against one non-target organism commonly found in aquatic habitats in western Kenya, the dragonfly nymphs.

1.3 Justification of the study

Pyriproxyfen (Sumilarv 0.5G) is an insect growth regulator which affects the insect growth processes and can be combined with other existing mosquito control tools to reduce malaria vectors. The study targeted the dragonfly nymphs because they undergo the same developmental stages like the mosquito during the aquatic phase. Earlier studies on mosquitoes revealed that Pyriproxyfen had an inhibition of emergence of 98% at 0.1ppm but its effect on dragonflies was not investigated. This study therefore was done to address the non target effects of Pyriproxyfen (Sumilarv 0.5G) on dragonfly nymphs which are predators of mosquito larvae.

1.4 Research questions

a) What predation activity does dragonfly nymphs have on third instar *An. gambiae* larvae reared in the laboratory?

b) What nymphocidal activity do different concentrations of insect growth regulator Pyriproxyfen (Sumilarv 0.5G) have on dragonfly nymphs in laboratory bioassays?

c) What are the effects of applying insect growth regulator Pyriproxyfen (Sumilarv 0.5G) on dragonfly nymphs in *An. gambiae* breeding habitats at Mahanga?

1.5 Hypotheses

a) Dragonfly nymphs have no predation activity on third instar *An. gambiae* larvae reared in laboratory.

b) The application of different concentrations of insect growth regulator Pyriproxyfen (Sumilarv 0.5G) has no nymphocidal activity on dragonfly nymphs in laboratory bioassays.

c) The application of insect growth regulator Pyriproxyfen (Sumilarv 0.5G) has no effect on abundance of dragonfly nymphs in *An. gambiae* breeding habitats at Mahanga.

1.6 Objectives of the study

1.6.1 General objective

To determine the effect of insect growth regulator Pyriproxyfen on dragonfly nymphs as predators of *An. gambiae* in mosquito breeding habitats at Mahanga Vihiga County, Kenya.

1.6.2 Specific objectives

a) Determine predation activity of dragonfly nymphs on third instar *An. gambiae* larvae reared in the laboratory.

b) Determine nymphocidal activity of different concentrations of Pyriproxyfen(Sumilarv 0.5G) application on dragonfly nymphs in the laboratory

c) Determine effects of insect growth regulator Pyriproxyfen (Sumilarv 0.5G) on abundance of dragonfly nymphs in *An. gambiae* breeding habitats at Mahanga.

1.7 Significance and anticipated output.

Increased incidences of malaria parasites developing resistance to majority of existing drugs calls for alternative effective approaches that can be integrated with existing ones to reduce mosquito populations. Use of predators has potential as these are able to reach vectors in habitats that may be difficult to control with other measures. Among the major mosquito predators are dragonfly nymphs which should be protected from adverse effects of insect growth regulators by applying effective dose rates. Insect growth regulators have greater persistence and could affect beneficial predators that co-exist with mosquitoes if its effective levels and specificity are not determined. This study determined nymphocidal activity of insect growth regulator Pyriproxyfen (Sumilarv 0.5G) on dragonfly nymphs at Mahanga in Western Kenya. The predation efficiency of dragonfly nymphs that could reduce *Anopheles* mosquito populations in their habitat was also determined. Findings of this study will be used to recommend Pyriproxyfen (Sumilarv 0.5G) dosages that have no toxic effects on dragonfly nymphs. Additionally, predatory efficiency of dragonfly nymphs on *Anopheles* larvae will provide understanding on the ecological interactions of dragonflies and mosquitoes to guide conservation programmes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria transmission and control

Malaria transmission is influenced by climatic conditions which often coincide with the rainy season when breeding sites are available with high numbers of anopheline mosquitoes (Craig *et al.,* 1999). Malaria is a life threatening disease in SSA with more than 90% deaths (WHO, 2007). Control measures have been put in place and they include chemotherapy, chemoprophylaxis, vector control and development of a vaccine (Bogitish and Thomas, 1990).

2.1.1 Malaria vectors

There are about 430 species of *Anopheles* and only 30-50 transmit malaria (Clements, 1999). Principal malaria vector in the SSA is *Anopheles gambiae* complex (Howell *et al.,* 1998). There are seven sibling species of this complex which include *Anopheles arabiensis, Anopheles bwambae*, *Anopheles quadriannulatus* species A and *Anopheles quandriannalatus* species B (yet to be assigned a scientific name), *Anopheles comorensis* and salt-water tolerant coastal species *Anopheles melas* and *Anopheles merus* (Obbard *et al.,* 2009). Malaria in western Kenya highlands is transmitted primarily by *Anopheles gambiae sensu stricto* and *Anopheles funestus* (Minakawa *et al.*, 2002). In order to reduce malaria incidences, the primary option is to control these

vectors. Vector control strategies include chemical control, biological control and physical control.

2.2. Life cycle of *Anopheles* mosquitoes

Anopheles mosquitoes have four distinct stages in their life cycle: egg, larvae, pupa and adult. The adult *Anopheles* mosquito is an active flying insect while the other stages are aquatic. Mosquitoes mate during flight and female searches for a blood meal. Female mosquito then seeks out a resting place to digest her meal. Eggs are laid on surface of water and when freshly laid are white, changing to brown or black and are boat shaped. Viable eggs hatch into larvae within 2-3 days in the tropics and 4-7 days or longer in cooler temperatures (Clements, 1999). The first instar larva breaks egg shell and becomes free in water. Resulting larva lies parallel to water surface and is essentially a surface feeder.

Larva undergoes four moults within 6-9 days to reach pupa stage. Pupa lasts 2-3 days depending on the temperature (Clements, 1999). Pupae do not feed but are capable of active movements by using their abdominal muscles and the paddles. Pupa has conical shape and a distinctive characteristic of *Anopheles* pupa is presence of short peg like spines situated laterally near the distal margins of abdominal segments. Adults emerge after the pupa skin splits dorsally during late evening and are able to fly within minutes. Newly emerged adult inflates its wings, separates and grooms its head appendages before flying away (Kettle, 1992). Female *Anopheles* mosquito is a malaria vector. Both male and female mosquitoes feed on nectar and damaged fruits, but only females feed on animal's blood to provide proteins for their eggs. Adult mosquito survives for

between one week (in natural habitat) and one month in captivity. Despite its wide range and variable ecology, a combination of traits allows it to maintain its position as an efficient vector in Sub-Saharan Africa.

2.2.1 Anopheline breeding sites

Anopheles gambiae and *Anopheles funestus* are common vectors of human malaria in SSA. Productions of adults occur in small temporary sunlit, turbid pools of water (Fillinger and Lindsay, 2006). Habitats are often created by human or animal activity where larvae are found in small depressions such as foot or hoof prints, edges of bore holes, burrow pits, roadside puddles formed by tyre tracks, irrigation ditches, ponds, and drainage channels in valley bottoms. In western Kenya highlands majority of the Anopheline habitats are confined to valley bottoms where there is continuous flow of water throught the year. Homesteads are not far away from breeding habitats thus humans provide a ready blood meal to female *Anopheles* mosquitoes.

2.2.2 Mosquito larval source management

Larval source management involves use of a combination of techniques aimed at reducing mosquito larvae in the breeding habitats. Larval source management has been used to control the immature stages of mosquitoes (Walker and Lindsay, 2007). Larval source management is currently being undertaken by many countries in Africa where malaria is endemic (Fillinger *et al.,* 2009). Recent field evaluations under various eco-

epidemiological conditions showed that larviciding reduced exposure to malaria transmission by 70-90% in sites where breeding habitats were well defined (Fillinger and Lindsay, 2006). Vector control programmes are being encouraged to develop integrated vector management strategies for the control of malaria (WHO, 2007).

2.2.3 Insecticides

Insecticides play an important role in reducing malaria vector populations. Some insecticides have been used and good results obtained though with serious environmental setbacks (Hanafi-Bajd *et al.*, 2006). Malathion is an organophosphate that is globally applied and a frequently used insecticide for controlling agricultural pests and mosquitoes (Kesavaraju *et al*., 2010). (Kalysnasundaram *et al.,* 2002) reported that organophosphates such as Durban and Redden were used to control mosquitoes. The Organophosphates were applied in larval habitats by spraying a diluted solution of 1000ppm. Reldan was effective in causing more than 80% reduction in larval density while Durban caused 90% reduction in larval density of *Culex quinquefasciatus* (Kesavaraju *et al.,* 2010). Despite the good results of the two organophosphates they have been associated with serious ecological impacts such as causing severe illnesses, death due to acute poisoning of humans and animals. Indoor residual spray using lambda-cyhalothrin insecticide has been carried out in targeted houses, in Iguhu village, Kakamega district of western Kenya (Zhou *et a*l., 2010). Larvicides have also been used to control immature stages of mosquitoes; they include Aquatain Monomolecular film and Spinosad (Bukhari *et al.,* 2011).

2.2.3.1 Personal protection

Personal protection involves the use of methods which are aimed at repelling the adult mosquitoes away from an individual. Insecticide treated bed nets (ITNS) are mosquito control tools which are erected and covered fully on beds. They have been impregnated with insecticides to prevent mosquitoes reaching their hosts (Zhou *et al.,* 2010). To date use of ITNS alongside other control interventions has reduced malaria significantly. In Kenya, the government has initiated a mass distribution of nets to pregnant mothers and children below age of five years. Use of bednets reduced malaria incidences from 78.1% in 2004 to 67.3% in 2006 in malaria endemic areas in Kenya. The combination of the insecticides and irritant effect of the Pyrethroids with the physical barrier of the bed net was found to reduce vector density, sporozite rates and malaria parasite prevalence (Lindblade *et al*., 2015). Insecticide-treated mosquito nets (ITNs) used for protection against mosquito bites are practically, highly effective, and cost-effective intervention against malaria. Evidence of public health impact of ITNs, supporting their wide-scale use in Africa, is drawn from areas of stable malaria transmission where *Plasmodium falciparum* infection prevalence in communities is often over 40%. Community-based randomized controlled trials (RCT) in these regions have documented average reductions of 20% in all causes of mortality in children under 5 years old (Atieli *et al*., 2010).

Although ITN distribution has been massively expanded in most parts of malaria endemic African countries , there is limited information on community based actual use of nets owned, area specific reasons for non-use, and possible impact of the variations in use on malaria vector densities and transmission in the Kenyan highlands. Despite the campaign and the sensitization on the use of ITNs, there is low utilisation of ITNs among pregnant women in Kilifi County which is an endemic malaria zone (Onyango *et al.,* 2013). Long lasting insecticidal nets have been impregnated with Olyset to control malaria vectors and are being used worldwide (Atieli *et al*., 2010; Lindblade, 2006; Geissbuhler *et al*., 2009; N`guessan *et al*., 2010). Mass distribution of free ITNs has resulted in universal household ownership, but use of bed nets is still very poor. Proper health education is required to encourage consistent use of bednets, even during hot nights, with low mosquito activity.

2.2.3.2 Physical measures

Researchers have been searching for physical methods which are environmentally safe and to which no resistance will appear. Among these acoustic devices, some of which have been designed as attractants and others as repellers for mosquitoes (Dugassa *et al.*, 2013). Another form of using acoustic waves for controlling haemetophagous mosquitoes is the use of simple electronic circuit that generates a frequency between 2000 and 2500Hz. This signal emitted by a loud speaker was used to elicit repulsion in mosquito females. Electric devices and traps have also been used to trap gravid female *Anopheles gambiae* in their breeding habitats. (Dougasa et al., 2014)

2.2.3.3 Environmental management

Environmental management are measures aiming to create a permanent or long-lasting effect on land, water, or vegetation to reduce vector habitats. The installation and maintenance of drains has been done to destroy mosquito breeding sites (Kibe *et al*., 2006). Communities within western Kenya highlands brick making is an income generating activity that leaves big pits which are filled with water. The local communities are being encouraged to rehabilitate these pits by refilling or planting trees to prevent stagnant water which are breeding grounds for mosquitoes. Simple modifications of typical rural house designs can be effective and relatively inexexpensive method of reducing indoor mosquito densities and consequently decreasing malaria transmission (Atieli *et al*., 2009).

2.2.4 Biological vector control

Biological control involves the use of living organisms to control mosquitoes. Biological vector controls are efficient and sustainable methods. There is growing interest in attacking the aquatic stages of malaria vectors with microbial larvicides (Dickman 2000; Merrit *et al.,* 2005; Fillinger and Lindsay, 2006; Poopathi and Abidha, 2010; Carquet *et al*., 2011). Water dispersible and granule formulations of the commercial strains of *Bacillus thuringiensis var israelensis* was applied in western Kenya highlands (Fillinger *et al.,* 2009). Larviciding using *Bti* has also been evaluated in urban areas of Tanzania and it was found that malaria dropped from 17.6 % to 7.1% (Geissbuhler *et al.,* 2009). The fish *Poecilia reticulate* has been successfully used in the control of *Culex quinquefasciatus* and *Anopheles gambiae* in rivers and lakes in Cuba,

Sri Lanka and French Polynesia (Lima *et al*., 2011). Use of fish *Gambusia affins* was evaluated as a potential predator of *Anopheles gambiae* at Iguhu in Kakamega County (Kweka *et al*., 2011). In another study larvivorous fish *Oreochromis nilotica* was introduced in Ponds in Kisii County and assessment of mosquito larval and pupal densities was done. It was recorded that ponds which had *Oreochromis nilotica* had low larval densities, than those that did not. Benefits of larvivorous fish is that mosquito larvae cannot build up physiological resistance and also fish populations are self sustaining and do not depend on the presence of larvae. The use of spiders to predate on mosquitoes has been evaluated and the results documented that spider webs should not be destroyed as they trap outdoor and indoor mosquitoes which are later eaten by the spiders (Berticat *et al.,* 2004). Spider species such as *Tegenaria domestica, Neoscona oaxascencis* and *Tetragnatha elongat*e have been reported to predate adult mosquitoes (Quiroz-Martinez *et al.,* 2007).

2.2.4.1 Biotechnology in Mosquito control

The potential for the use of genetics against mosquito-borne infection has recently been considered in vector control programs (Kumar and Jiang, 2005). Recent techniques to modify genes of mosquitoes are believed to be an appropriate interventional remedy against malaria and dengue fever. Their main purpose is to produce a genetically modified strain of mosquito in the laboratory which does not serve as a carrier of disease and which is competitively superior in natural habitats such that wild mosquitoes will eventually be replaced after the release of genetically altered

mosquitoes in nature (Kumar and Jiang, 2005). However, there are several problems with this approach, such that it is taking time to turn the prospects of this technology into practical tools.

2.3 Predators in mosquito control

There are diverse natural enemies of mosquitoes and each has played a significant role in regulating mosquito densities (Kumar and Jiang, 2005). Many aquatic insects belonging to orders Coleoptera, Diptera, Hemiptera and Odonata prey upon mosquito larvae (Shallan and Canyon, 2009). Mosquito predators feed on many species (polyphagous), while some feed on only one species (monophagous). Majority of documented mosquito predators are polyphagous (Shallan and Canyon, 2006). Problems associated with resistant mosquitoes and effects of non target species by chemicals evoke a reason to find alternative methods to control mosquitoes like use of natural predators. Studies on mosquito predators have demonstrated availability of potential biological resources for controlling malaria vectors (Kweka *et al.,* 2011). Predation is recognized as an important factor in the organization of many ecological communities including aquatic communities. Predators may also not only indirectly affect their prey, but also the trophic level below their prey (Blaustein and Jonathan, 2009). Mosquito ecologists have increasingly taken the approach that understanding how a predator , directly and indirectly affects community structure is also important for understanding under what set of environmental conditions a predator will be effective in reducing mosquito populations.

2.3.1 Dragonflies as mosquito predators

Dragonfly nymphs constitute a well known order of insects that are widely distributed all over the world (Arulprakash and Gunathilagaj, 2011). They are abundantly found in temporal water bodies particularly in tropical countries due to their dispersal ability. Dragonflies are found in the order Odonata which has three major suborders namely; Anisoptera, Zygoptera, and Anisozygoptera. Dragonflies *Annax imperator* are conspicuous predators of mosquitoes during both larval and adult stages (Quiroz-Martinez *et al.,* 2007). In community structure of fresh water ecosystems, the dragonfly nymphs are very important predators of mosquito larvae (Miura and Schaefer, 1998). Experiments carried out by Sebastian on the efficacy of dragonfly as mosquito control agents indicated that dragonflies are potential candidates in controlling mosquitoes (Sebastian *et al*., 1990). All odonates are predatory in all instars, mostly on organisms much smaller than themselves and are important natural control agents of mosquitoes and other aquatic insects.

2.3.2 Predators of dragonflies

In the aquatic nymphal stage the primary predators are ducks, amphibians such as toads and newts, fish and bigger damselflies. When predators clutch onto them, nymphs can escape by allowing their limbs to fall off. These limbs grow back during moulting (Dudgeon and Watt, 1986). The adult dragonflies escape from predators by flying fast. Some species have skin that change to look like the environment to evade predation. Carnivorous plants such as sundew seize dragonflies then promptly digest then to take their nutrients. The plants retrieve dragonflies with the help of tentacles which are

equipped with glands that secrete a gooey substance that secures the dragonfly nymphs when they make their way onto the surface of plant leaves (Howell *et al.,* 1998). Tentacles together with plant leave surround the trapped insects on all sides initiating the digestion process.

2.3.3 Backswimmers as mosquito predators

Backswimmers *Notonecta glauca* of the order Hemiptera have predatory habits (Gittelman, 1975, Quiroz-Martinez *et al*., 2007). In a study to evaluate the predatory activities of the backswimmers at Iguhu in Kakamega county, Munga *et al* (2006) showed that backswimmers are predators of mosquito larvae and thus important in regulating immature mosquito populations.

2.3.4 Aquatic beetles as mosquito predators

Aquatic beetles inhabit permanent and temporary ponds. Beetles of genera *Dytiscus, Laccophilus, Agabus,* and *Rhantu*s have been reported as potential agents of biological control (Charles *et al.*, 1998). Based on studies conducted by Culler and William, 2009) at Jacklane, U.S.A, several species of Dytiscidae and Hydrophilidae are considered good biological control agents due to their predation ability on mosquito larvae. Water beetles play an integral part of the biotic component of any water body or wetland. Aquatic beetles are a diverse group and are excellent indicators of habitat quality, age and naturalness. They are indicators of ecological diversity and habitat characteristics (Thakare and Zade, 2011).

2.4 Effects of larvicides on mosquito predators

A wide range of larvicides have been used to control the immature stages of mosquitoes. These larvicides have significantly lowered malaria vectors in the endemic areas. Continued uses of larvicides however have setbacks in that some are harmful to arthropods that co-exist with mosquitoes in their natural breeding habitats (Xue *et al.*, 2000; Hanafi–Bajd *et al*., 2006). Dragonfly nymphs are predators of mosquito larvae and this makes them beneficial towards regulation of the vector population. However the non target effects of larvicides has not received much attention.

2.5 Biology of dragonflies

Life cycle of dragonflies includes an aquatic larval phase and an adult terrestrial phase (Dudgeon and Watt, 1986). Adults spend time away from water as they forage for insects. Both sexes are polygamous and males are able to remove sperm from previous matings before depositing their own sperm (Howell *et al*., 1998). To ensure sperm precedence, males of many dragonflies maintain the tandem position for long periods after insemination and in some species males remain in tandem until eggs have been deposited. Eggs are laid in or near water, larvae hatch from the eggs in less than a month. Dragonfl y nymphs oviposit endophytically and their eggs suffer low mortality (Resh and David, 1984). Larva or nymphs grow rapidly feeding on small aquatic organisms, other smaller dragonfly nymphs and mosquito larvae (Howell and Alexander, 1998). There are twelve nymphal stages which have a life span ranging from 3-6months. Dragonfly nymphs move to surface of the pond water and start to breathe air. Nymphs climb up the stems of emergent vegetation and swallow air causing their

skin to split down the back and the adult emerges (Aruprakasha and Gunathilagaj, 2011).

2.5.1 Predator escape mechanisms in mosquitoes

Many oviparous insects avoid breeding sites where there is a high risk of predation to their offspring. Mechanisms for predator detection by insects may involve tactile, visual or chemical cues (Kumar and Jiang, 2005). *Anopheles gambiae* actively selects habitats favourable for oviposition (Munga *et al*., 2007). Some mosquito species avoid ovipositing in habitats with predators and competitors. Understanding oviposition behavior of mosquitoes is a vital tool in targeting malaria vectors which can reduce the incidences of malaria in endemic areas.

Proximate mechanisms that mediate avoidance behaviors are chemical exudates released by predators that are called kairomones. Chemically mediated avoidance is an adaptation used by prey to detect and evade predators (Kats and Dill, 1998). Kairomones or semiochemicals emitted from predators are normally used by prey to detect a predator's presence in the environment, and prey can thereby minimize such encounters (Kerfoot and Sih, 1987). Responses to chemicals from predators include an increased use of refugia marked changes in intensity of movements (Chivers and Smith 1998; Rebora and Piersanti, 2004), reduced foraging, reduced courtship behavior, predator avoidance, and increased expansion rates. Gravid females are known to spend some time flying around a water body apparently evaluating it as an oviposition site. Criteria in such a selection process are the presence of competitors, predators, and or kairomones (Blaustein and Kotler, 1995; Kumar and Jiang 2005) and container size.

Females of Culex spp, oviposit on mud or water surface after sampling the chemical composition of the substrate using receptors on the tarsi, antennae, and tips of the proboscis (Clements, 1999).

Bentley and Day, (1989) reported that mosquitoes use chemosensory information to assess several parameters that reflect habitat quality for the offspring, including the availability of nutrients, competitors and predators, and the overall quality and permanence of the water body. It has been reported that mosquitoes reduced ovipositing rates in experimental pools that contained caged sunfish (*Lepomis*) that were not visible to ovipositing females. Studies have shown that adult mosquitoes have the ability to sense the presence of *Gambusia*, and that mosquitoes reduce egg-laying rates in pools containing the odour of mosquito fish (Kumar and Jiang, 2005)

2.5.2 Intraguild predation

Intraguild predation is the consumption of one predator by another predator. Intraguild predation has been found to diminish effectiveness of biological control by minimizing the top down effects (Muiruri *et al.*, 2013). Elucidating the impact of a common predator that feeds across several trophic levels is essential for predicting the strength of top down forces and revealing the potential for the natural enemy to suppress pest population. Studies have shown that, attack rates of generalist predators on mosquito larvae may be changed by availability of alternative prey. Although predators lack prey specifity, they can exert substantial predation impact on a given prey group or prey species (Muiruri *et al*., 2013). One important factor opposing this impact is the intraguild predation which includes cannibalism, predation, predator avoidance

behavior, and predator-predator competition. Intraguild predation can therefore lead to an enhancement of the concerned prey population due to predator –predator antagonism (Debora *et al*., 1988; Synder and Wise, 2001). On the other hand, combination of several predator species can also increase the overall negative impact on a given prey population. More information on multiple predators in regard to biological control is needed in order to separate the single and combined impacts of involved predators on the targeted pests (Andreas and Evans, 2004).

Living things exist within webs of interaction with other living creatures most important of which involves eating or being eaten (Huffaker and Gutierrez, 2004). Interactions between predators and their food produce a negative feedback at population level and hence important in stabilizing population dynamics. Predation is recognized as an important factor in organization of many ecological communities including aquatic communities. Mosquito ecologists have increasingly taken the approach that understanding how a predator directly or indirectly, affects a community structure is important for understanding under what set of environmental conditions a predator will be effective in reducing mosquito populations. Species sharing the same trophic levels as mosquito larvae affects predation intensity on mosquitoes (Chesson, 1989; Blaustein and Jonathan, 2009). Mosquito numbers are naturally regulated by a variety of factors, including adverse climatic conditions, limited food supply, competition, parasites or pathogens and predators. Importance of any of these factors in different environments is poorly understood, thus affecting proper understanding of the factors that affect production of adult populations. Due to the fact that every form of biological control functions only under certain environmental conditions, it is necessary to devote

considerable attention to the ecological details of mosquito larvae and their predators in the aquatic ecosystem (Blaustein and Kotler, 1995).

2.6 Insect growth regulators

Insect Growth Regulators (IGR) are third generation insecticides and are also effective tools for control of a variety of insect pests and disease vectors (Yapabandara, 2005). They show an exceptionally high level of activity against mosquitoes and several other groups of noxious and vector insects. Insect growth regulators in general have low mammalian toxicity, are quite safe to fish, birds and most non-target biota (Kono *et al*., 1997). However, some IGRs have shown a high level of activity against some crustacean groups, inducing a variety of morphological changes. In mosquito control, most IGRs are evaluated and applied against the immature aquatic stages of mosquitoes. Mosquito larvae are ideal targets for IGRs, most of which have delayed activity, including mortality or morphogenetic anomalies in stages beyond the one treated. The insect growth regulators do not induce immediate mortality in the larval stage.

Insect growth regulators are selective insecticides and nontoxic to humans and other vertebrate. Insect growth regulators are chemical substances that may adversely affect insects by regulating or inhibiting specific biochemical pathways or processes essential for insect growth and development (Invest and Lucas 2008). Some insects exposed to such compounds may die due to abnormal regulation of hormone – mediated cell or organ development. Insect growth regulators therefore are promising tools in integrated pest and vector management and can be incorporated in mosquito control programmes (Mbare *et al.*, 2013).

2.6.1 Diversity of insect growth regulators

Use of insect growth regulators is gaining popularity with many insect control programmes. Some IGRs include Diflubenzuron, Methoprene, Chlorfluazuron and Diflubenzuron have been evaluated against *Aedes aegypti* and results indicated reduction in the reproductive potential of mosquito adults that emerged from these treatments (Mortimer and Heather, 1995). It was also reported that there was decrease in egg production of 25.5% for diflubenzuron (Thavara *et al.,* 2007). These results shed light on the extended biological effects of IGRs on mosquitoes and encourage further testing of IGRs for wider use in the control of *Ae. aegypti* and other vectors. Insect growth regulators such as Chlorfluazuron, Methoprene, and juvenile hormone mimics or chitin synthesis inhibitors have been reported to be effective control agent for flea larvae (Kawada *et al*., 1988). Despite all the successes of insect growth regulators, their effects on aquatic mosquito predators need to be determined. Methoprene, which is a juvenile hormone analogue, is an effective additional tool of public health and veterinary importance for controlling pests and disease vectors (Pinkey *et al*., 2000; Kono *et al*., 2006). Methoprene is in granular or pellet form and is applied directly to the water where mosquito larvae are found. When mosquito larvae are exposed to methoprene, their life cycle is disrupted and they are prevented from reaching maturity or reproducing. In field studies, Methoprene has shown high level of activity against many species of mosquitoes and related groups (Butler *et al.,* 2006). Dimilin is an insect growth regulator belonging to the benzoyl ureas class of insecticides that inhibits the synthesis of chitin and, interferes with molting; its active ingredient is Diflubenzuron, which is mainly a stomach poison and, to a lesser extent, a contact poison and acts by

disturbing the molting process of all stages of larvae instars of mosquitoes and other flies. Deviation from normal moulting process ultimately leads to the death of the larvae (Msangi *et al*., 2011). Dimilin has very low toxicity to mammals, birds, fish, honey bees, and most aquatic invertebrates with the exception of small crustaceans (water fleas, and others), and, hence, its effect on the environment is minimal. Diflubenzuron has successfully passed the WHO's pesticide evaluation scheme for mosquito larviciding and is one of the WHO recommended compounds for control of mosquito larvae (Najera and Zain 2002). Pyriproxyfen (Sumilarv 0.5G) acts by binding to juvenile hormone receptors in the immature form of an insect (Dell, 2003; Rachid *et al*., 2009). However, it is of great significance to evaluate effects of IGR Pyriproxyfen on dragonfly nymphs that co-exist with mosquito larvae so as to ascertain the effectiveness and target specificity of the IGR.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The study area

Collection of dragonfly nymphs was conducted in two areas, Mahanga and Muluhoro, both in Vihiga County (Figure 3.1). Pyriproxyfen was applied only at Mahanga, whereas Muluhoro, which is approximately 10 km away from Mahanga, was used as the non-intervention control site for comparison. These areas are characterized by flat bottomed valley with many swampy sections with flowing streams surrounded with hills (Ndenga *et al*., 2011). These areas were selected because of their rich Anopheline larval productivity and abundance of dragonfly nymphs (Ndenga *et al*., 2011). Laboratory experiment on predation efficiency and effect of the insect growth regulator Pyriproxyfen on dragonfly nymphs was carried out at KEMRI-CGHR at Kisian in Kisumu.

3.1.2 Community of Mahanga.

Mahanga village is found in Maragoli a sub-tribe of Luhya ethnic group. They are subsistence farmers and grow maize, tea, beans, sweet potatoes, and bananas. Their farms border the adjacent river valleys where the *Anopheles gambiae* breeding habitats are found. Their houses are semi permanent with mud walls and corrugated iron sheet roofs. There is one health centre and several clinics which local residents receive treatment for minor ailments. There are public and private schools which have large population of children who are at high risk of malaria. Muluhoro village is found in

Bunyore just neighbouring the Maragoli. Their main economic activity is Subsistance farming and small scale businesses. The area has many rivers, streams and ponds which makes it an ideal habitat for mosquito breeding. The area is densely populated with a large proportion being children below ten years.

3.1.3 Mapping of the study areas

Study sites were mapped at the beginning of the study. Coordinates (latitude and longitude) readings and altitude of aquatic habitats, houses, major roads and streams were taken in Mahanga and Muluhoro once using a Geographical Positioning Station (GPS) unit (GPS 12 XL, 15 meters accuracy, Garmin Ltd. 2003, Olathe, Kansas, USA). Coordinates were then imported into ArcMap in ArcGIS (Environmental Systems Research Institute (ESRI) Redlands, California, USA) where the maps were made.

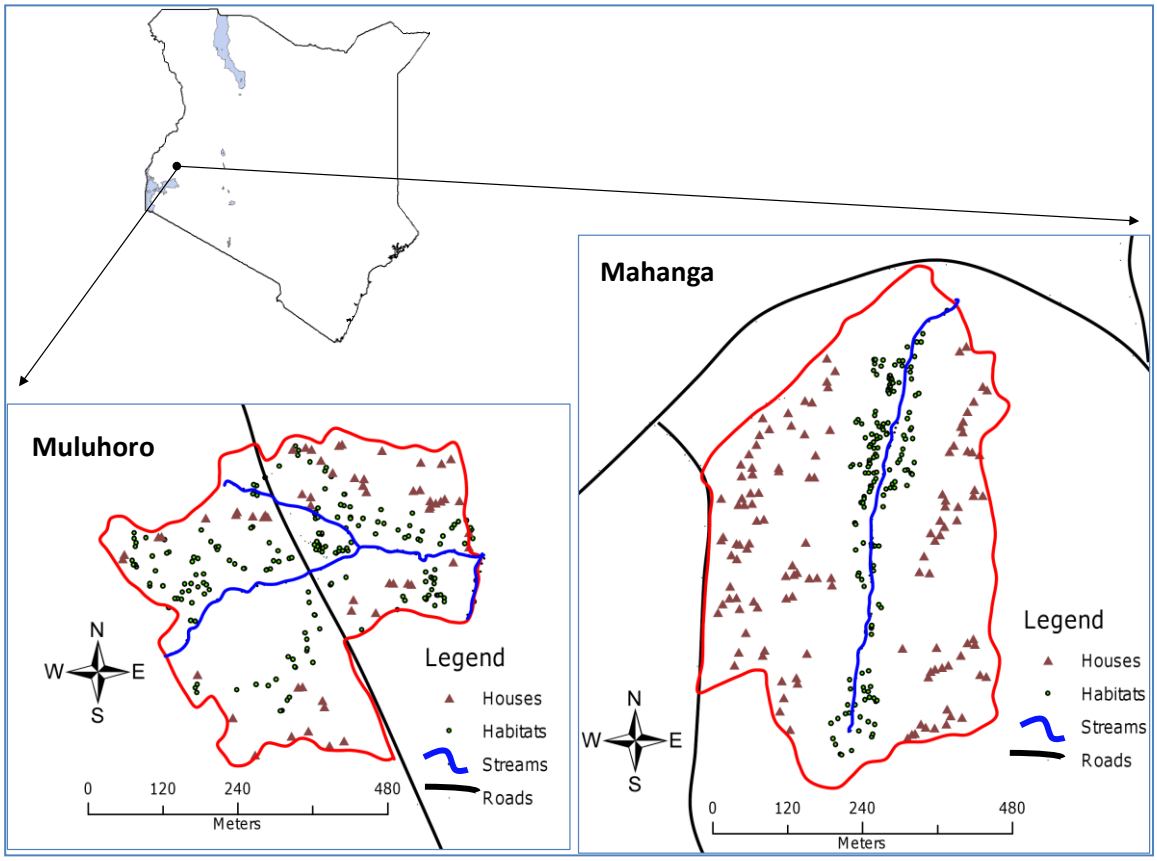


Figure 3.1: Map showing Anopheline breeding habitats in the study area. Source: (Environmental Systems Research Institute (ESRI) Redlands, California, USA)

3.2 Collection of dragonfly nymphs

Dragonfly nymphs were collected from selected mosquito breeding habitats at Mahanga within western Kenya highlands. Dragonfly nymphs were collected a day before laboratory bioassay then transported to KEMRI-Kisian. Collection of fifth instar dragonfly nymphs was done by sweeping the habitats with a pond net and contents of the pond net emptied onto a plastic basin (Figure 3.2). Live individuals of 5th instar dragonfly nymphs were removed using a sieve and placed in plastic jars containing water from mosquito breeding habitats. Plastic jars were covered with a net and transported to the laboratory the same day. On arrival at KEMRI-Kisian dragonfly

nymphs were poured into a plastic tub half filled with water. Two hundred laboratory reared *Anopheles gambiae* third instar larvae were introduced in the tub using pipettes to provide food for dragonfly nymphs. This was done so as to have the dragonfly nymphs get used to the laboratory conditions.

Figure 3.2: Collection of dragonfly nymphs in *Anopheles gambiae* breeding habitats at Mahanga.

3.3 Predation of dragonfly nymphs on *Anopheles gambiae ss* (Kisumu strain) larvae

Laboratory experiments on predation used insectary reared third instar larvae of *An. gambiae* ss Kisumu strain. Mosquito larvae were reared in rectangular tubs (diameter 60cm long and 30cm wide) filled with(Figure 3.3) water 15cm high from Kajulu

springs. Mosquito larvae were fed with fish food (Tetramin © baby) twice daily. Third instar mosquito larvae were selected from different tubs so that larvae were of similar size in each test container. Mosquito larvae were reared in an insectary with average daily temperature of 28° C, natural light and average 76% relative humidy. Twenty plastic containers one litre capacity measuring 20cm length 15cm width and 12 cm height were filled with filtered tap water. One dragonfly nymph of the fifth instar developmental stage was placed in each container. Ten third instar larvae of *An. gambiae* were introduced in each container using a pipette. Third instar larvae were used since they are large and easily captured by predators.

In this experiment ten third larval instars were used because from field surveys it was found that between 8-10 larvae were collected per habitat. Turfts of nut grass were introduced in each container to provide refugia for the dragonfly nymphs and the container covered with a net to prevent dragonfly nymphs from escaping. The number of *An. gambiae* larvae predated upon by dragonfly nymphs was counted after every 24 hours after which new batches of ten third instar larvae was added each day for three days. The experiment was repeated ten times.

Figure 3.3: The predation experiment design of dragonfly nymph on *An. gambiae* larvae.

3.4 The effects of Pyriproxyfen (Sumilarv 0.5G) on the growth of dragonfly nymphs in the laboratory

Five grams of Pyriproxyfen (Sumilarv 0.5G) was weighed on an electric balance and crushed into fine powder using a mortar and pestle. Pyriproxyfen (Sumilarv 0.5G) powder was diluted in 500mls of filtered tap water to form a stock solution of 50ppm and was kept in a volumetric flask then covered using aluminum foil. This stock solution was agitated for one hour using a mechanical agitator and left overnight to allow active ingredients to diffuse in the solution since Pyriproxyfen (Sumilarv 0.5G) is a slow release formulation. Serial dilutions of 0.1ppm, 0.05ppm, and 0.01ppm were made from the stock solution. Dilution of 10ppm was done by, measuring 200mls of the stock solution using a measuring cylinder and emptied in 800mls of filtered tap

water. Pyriproxyfen (Sumilarv 0.5G) concentration of 0.1ppm, 0.05 ppm and 0.01 ppm were prepared by measuring 100mls of 1ppm, 0.1ppm and 0.05ppm which was diluted in 900mls of water respectively. Laboratory bioassays were conducted using sixteen plastic containers measuring 20cm length and 15cm width and 12cm height. There were four experimental treatments and a control which was replicated four times. Control experiment was filtered tap water. Each container was filled with one litre of the diluted concentration of Pyriproxyfen (Sumilarv 0.5G). Twenty dragonfly nymphs of the 5^{th} instar were introduced in each plastic container having the test concentrations by gently pouring. The dragonfly nymphs was introduced into the test containers starting from controls then followed by 0.01ppm, 0.05ppm and 0.1ppm. One gram of brewer's yeast was added in each container using a dip stick to provide feed for the dragonfly nymphs every day.

Ten *An. gambiae s.s.* Kisumu strain in their second instar developmental stage was introduced to provide feed for the dragonfly nymphs in each container every day. A tuft of nut grass was immersed in the container to provide refugia for the dragonfly nymphs. The containers were covered using a net to prevent the dragonfly nymphs from crawling out of the container. Mortalities of the dragonfly nymphs were recorded daily for ten days after which a new set up of the experiment was repeated. This experiment was repeated ten times and in each set up fresh test solutions was made and a new batch of sixteen dragonfly nymphs was introduced. Sample size of sixteen was chosen basing on the size of the habitat and average number of dragonflies swept per habitat

3.5. The abundance of dragonfly nymphs in mosquito breeding habitats

Dragonfly nymphs were sampled weekly in ten randomly selected habitats four months after application of Pyriproxyfen (Sumilarv 0.5G) in the habitats at Mahanga. Sampling was also done at Muluhoro which was used as a control site and was located 10km away to a void contamination by Pyriproxyfen (Sumilarv 0.5G). Sampling involved dipping a pond net in the habitats then the contents were emptied on a white plastic basin. Counts were made on the numbers of dragonfly nymphs collected.

3.6 Effects of Pyriproxyfen (Sumilarv 0.5G) on the abundance of dragonfly nymphs in mosquito breeding habitats at Mahanga

The amount of Pyriproxyfen (Sumilarv 0.5G) to be applied in the aquatic habitats was determined before it was done in the field. The preparation of 0.05 ppm active ingredient (0.05 ppm a.i) was done as follows:

0.05ppm a.i = 0.05mg a.i /L = 5 mg a.i / 100L

1m x 1m =1 m^2 = 100L (assuming a water depth of 10cm)

If 5 mg a.i /100L = 5 mg a.i / m^2

But Sumilarv 0.5G contains 0.5% active ingredient

Hence 0.5 % = 5 mg

100% = (100/0.5) x 5mg

= 1000 mg of Sumilarv (1 g Sumilarv 0.5G)

Therefore 5 mg a.i /m^2 = 1 g Sumilarv 0.5G /m^2.

Hence, one gram of Sumilarv 0.5G was applied per one metre square of water surface area by broadcasting in the habitats. Sampling was done for four months before application, four months during the application of Pyriproxyfen in habitats and also continued for four months after Pyriproxyfen application was terminated. In the control habitats at Muluhoro no treatment of Pyriproxyfen was done and sampling continued.

3.7 Data Management and analysis

Laboratory data on predation efficiency and mortalities of the dragonfly nymphs in each concentration was analysed using SPSS version 17. Data on the presence of the dragonfly nymphs in mosquito breeding habitats was analysed using Generalised Estimation Equation (GEE) basing on habitat type, site code and Pyriproxyfen application.

3.8 Ethical clearance

Permission to use Pyriproxyfen for research purposes was obtained from the Pest Control Product Board of Kenya (PCPB) (Appendix i) and Kenyatta University authorised the research to be done (Appendix ii).

CHAPTER FOUR

RESULTS

4.1 Validation of dragonfly nymph predation on larvae

Out of the 200 *Anopheles gambiae* 3rd instar larvae that were exposed to predation by dragonfly nymphs, 181 (95.3%) were predated upon within 24 hours whereas 189 (95.0%) were predated upon within 48hrs and 190 (95.0%) within 72 hours (Figure 4.1).

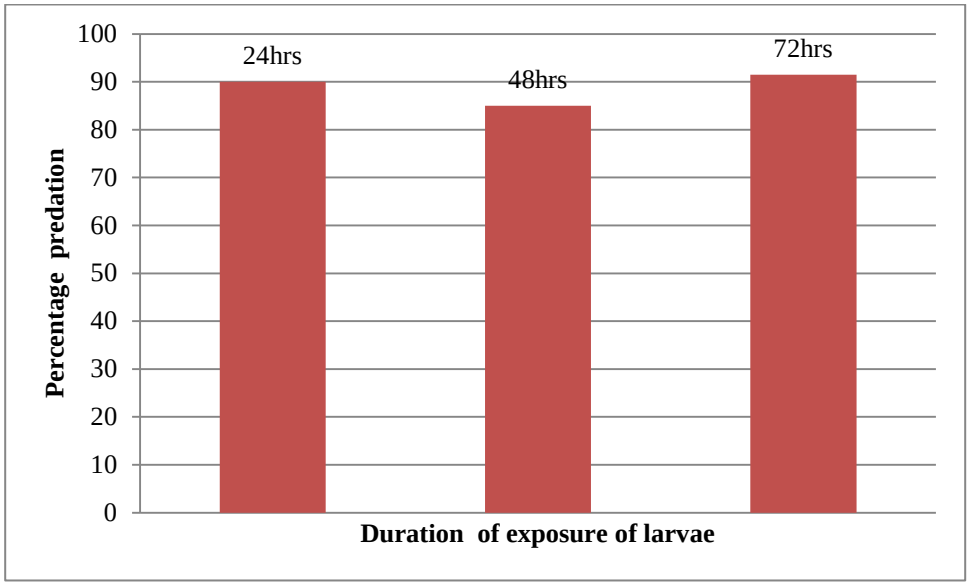


Figure 4.1: Percentage predations on third instar *Anopheles gambiae* larvae.

4.2 Effects of Pyriproxyfen (Sumilarv 0.5G) concentrations on dragonfly nymph in the laboratory

On the 10th day of treatment with 0.01ppm Pyriproxyfen (Sumilarv 0.5G), 1.25% mortality was observed among the dragonfly nymphs population (Figure 5). Pyriproxyfen (Sumilarv 0.5G) dosage of 0.05ppm, and 0.1 ppm had mortalities of 8.75% and 15 % respectively.

The exposure of dragonfly nymphs to Pyriproxyfen (Sumilarv 0.5G) at different concentrations had mortalities recorded. At 0.1ppm, 15% of the dragonflies died after an exposure of 10days. The concentration had the highest mortality. A concentration of 0.05ppm recorded dragonfly nymphal mortality of 8.75% while 0.01ppm recorded mortality of 1.25% of the dragonfly nymphs (Figure 4.2).

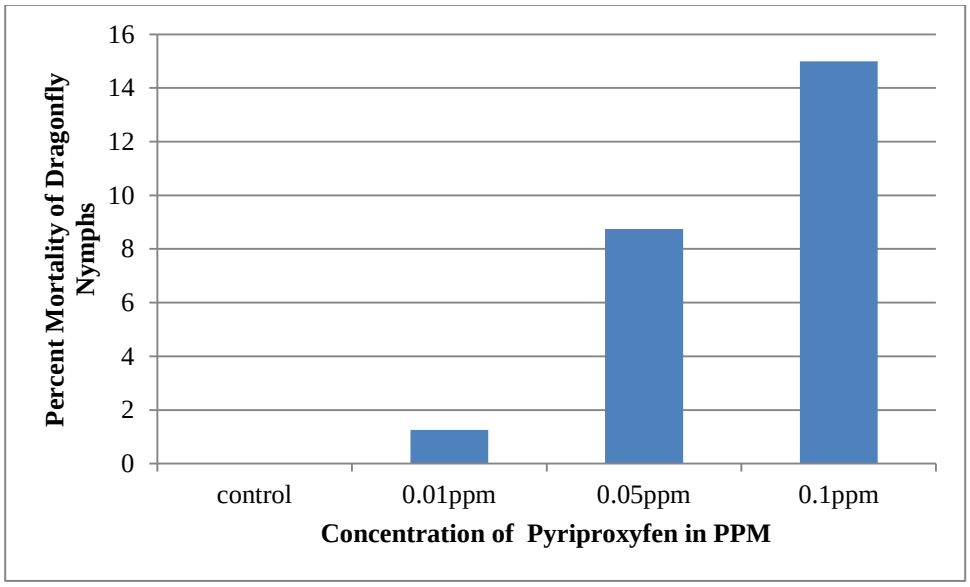


Figure 4.2: Percentage mortality of dragonfly nymphs at different concentrations of pyriproxyfen in parts per million.

4.3 Effects of Pyriproxyfen (Sumilarv 0.5G) on dragonfly nymph abundance under field conditions based on the habitat types

Habitat types in Mahanga where Pyriproxyfen was applied were burrow pits, open drains and natural swamps (Table 4.1). A total of 1,060 sampling visits for dragonfly nymphs were made during the entire study period. These were: 187 (17.6%) from burrow pits, 865 (81.6%) from open drains and 8 (0.8%) from natural swamps. Mean abundance of the dragonfly nymphs ranged from 1.0 - 1.5 per habitat in all the three habitat types (Table 1). The abundance of the dragonfly nymphs was 1.2 times higher but not statistically significant in open drains compared to the natural swamps (Table 4.1). Similarly, the abundance of the dragonfly nymphs was 1.5 times higher but not statistically significant in burrow pits compared to the natural swamps (Table 4.1).

Table 4.1: Summary of abundance of dragonfly nymphs on the basis of habitat type

Parameter	Occasions	Mean (95% CI)	OR (95% CI)	P
Burrow pits	187	1.5 (0.9 - 2.6)	1.5 (0.5 - 4.5)	0.465
Open drains	865	1.3 (1.1 - 1.5)	1.2 (0.4 -3.6)	0.682
Natural swamps	8	1.0 (0.4 - 2.8)	1.0	

4.3.1 Comparison on the abundance of dragonfly at Mahanga and Muluhoro.

Mean abundance of dragonfly nymphs at Mahanga was 1.2(1.0- 1.4) while Muluhoro was 1.4(1.1 – 1.9) (Table 4.2). Mahanga was the intervention site and was compared to Muluhoro which was the non intervention site. The means between Mahanga and

Muluhoro was not statistically significant p> 0.35. The mean abundance of dragonfly nymphs in aquatic habitats at Mahanga (the intervention site) was 0.8 times lower but not significantly different (P= 0.350) compared to Muluhoro (the non-intervention site).

Table 4.2: Comparisons of the abundance of dragonfly nymphs at Muluhoro and Mahanga.

Parameter	Occasions	Mean (95% CI)	OR (95% CI)	P – value
Mahanga	530	1.2 (1.0 - 1.4)	0.8 (0.6 - 1.2)	0.350
Muluhoro	530	1.4 (1.1 - 1.9)	1.0	

4.3.2 Comparison on the abundance of dragonfly nymphs based on Pyriproxyfen application

The reference point was 1.0 which was pre-application period. In comparing the application period (March –June 2012) to pre application period (November 2011-February 2012), the chances of finding dragonfly nymphs were 1.2 times higher but not statistically significant while the post application period(July- October 2012) was 1.7 times higher (Table 4.3). The odds ratio during the pre application period was 1.0 and during application it was 1.2 therefore the chances of finding dragonfly nymphs after application of Pyriproxyfen was higher. When comparing the application period and post application period the odds ratio were 1.2 and 1.7 respectively. This implies that the chances of finding dragonfly nymphs after pyriproxyfen application were

higher. The sampling during the post application period shows that abundance of dragonfly nymphs was statistically significant $p<0.001$.

Table 4.3: Comparisons between Pyriproxyfen pre-application, application and post- application period.

Parameter	Occasions	Mean (95% CI)	OR (95% CI)	P - value
Post-application period	360 (34.0)	1.7 (1.4 - 2.0)	1.6 (1.2 - 2.0)	<0.001
Application period	340 (32.0)	1.2 (0.8 - 1.8)	1.2 (0.8 - 1.7)	0.480
Pre-application period	360 (34.0)	1.0 (0.8 - 1.3)	1.0	

CI =95% Confidence interval

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This study was envisaged to determine predation efficiency of dragonfly on mosquito larvae. Predation experiment demonstrated that dragonfly nymphs are efficient predators of *An. gambiae* larvae. Dragonfly nymphs are found in swamps characterized with grasses such as nut grass, papyrus, with slow moving to still water. The Predation percentages of more than 90% indicate that dragonfly nymphs can consume mosquito larvae thus reducing the immature vectors of malaria. In search for alternatives in mosquito control, biological control could provide ecologically acceptable reductions if suitable biocontrol agents become available (Quiroz-Martinez *et al*., 2007). Previous studies on mosquito predators have demonstrated that species sharing same trophic level as mosquitoes may strongly influence mosquito populations either alone or interacting other predators (Blaustein and Jonathan, 2009). Results on the abundance of dragonfly nymphs indicated that the dragonfly nymphs were present at both Mahanga and Muluhoro.

The mean abundance were Mahanga 1.2 while Muluhoro 1.4. Dragonfly nymphs are generalists and voracious predators that detect their prey using visual cues and mechanoreceptors; they suddenly capture their prey with the labium or palps. Mechanical stimuli have a predominant role in predation thus enabling the predator consume more of prey thereby regulating the prey densities. In the study the dragonfly nymphs consumed large proportions of mosquito larvae within 24 hour period. Adult

dragonfly nymphs actively pursue adult mosquito and voraciously feed on them. Dragonfly nymphs are efficient predators; they capture prey by rapidly extending the labium and seizing prey between two movable hooks at its tip. They are thus useful by consuming large numbers of mosquito larvae and are excellent indicators of fresh water quality (Blaustein and Kotler, 1995). Third instar larvae of *An. gambiae* were easily detected hence captured with ease. Individuals of smaller body size are more, vulnerable to predation by larger larval Odonates (Giacomini *et al*., 2008).

The findings of the present study suggest the use of dragonfly nymphs can be a potential biological resource in regulating larval populations of *An. gambiae.* Mosquito ecologists have increasingly taken the approach that understanding how predator , directly or indirectly , affects a community structure is important for understanding under what set of environmental conditions a predator will be effective in reducing mosquito populations (Blaustein and Jonathan, 2009). Predators are important organizers of community structure. Mortality of dragonfly nymphs might have been caused by the interaction between the dragonfly nymphs and mosquito larvae. Mortality of immature stages of *Anopheles* mosquitoes has been shown to be caused by a variety of factors including adverse climatic conditions, limited food supply, competition, parasites and pathogens, but predation is probably the most limiting factor causing high level of mortality (Muiruri *et al*., 2013). Apart from the natural regulation of mosquito numbers, predators and pathogens have raised interest due to their potential for manipulation for biological control as part of integrated pest management. Use of dragonfly nymph can be integrated in Biological control efforts as they are efficient

predators. Alternative predators like the dragonfly nymphs can be explored for use as biological resources for controlling Anopheline larval stages.

Laboratory assays conducted with different concentrations of Pyriproxyfen thus 0.01ppm, 0.05ppm, and 0.1ppm indicated that at concentration of 0.01ppm, only 1.25% dragonfly mortality was recorded, while 0.05ppm, 8.75% mortality was recorded while 0.1ppm 15% mortality was recorded. The results of the present study showed that Pyriproxyfen had nymphocidal activity at 0.01ppm and 0.05ppm but higher nymphocidal activity was felt at 0.1ppm after ten day exposure. This implies that at dosage rates of 0.01ppm and 0.05ppm are safe to use as they had less than 10% mortality of dragonfly nymphs. Pyriproxyfen had very low mortality of 8.75% on the population of dragonfly nymphs at concentration of 0.05ppm when applied in mosquito breeding habitats.

This study shows that during the eleven month application period of Pyriproxyfen (Sumilarv 0.5G) the mortality of dragonfly nymphs was low at dosages of 0.05ppm. The abundance of the dragonfly nymphs at Mahanga which was the intervention site had mean abundance of 1.2, while compared to Muluhoro the non intervention site was 1.4. The results indicate that Pyriproxyfen had insignificant nymphocidal activity on dragonfly nymphs. Pyriproxyfen (Sumilarv 0.5G) has delayed effects and prevents the insect from moulting from one stage to another. Additionally Pyriproxyfen has long residual effects which if applied in high doses can kill the beneficial predators such as the dragonfly nymphs (Mbare *et al*., 2013). Experiments using Pyriproxyfen (Sumilarv 0.5G) to control mosquito larvae, offered a very good efficacy against *Culex*

quinquefasciatus (Mbare *et al.,* 2013). One ecological interest in mosquito control programs is for control agents to be sufficiently target specific that they do not cause damage to non target species or food webs. The result of the experiments show that Pyriproxyfen (Sumilarv 0.5G) had insignificant toxic effects on predatory dragonfly nymphs except when applied at a very high concentration of 0.1ppm. The low nymphocidal activity of Pyriproxyfen (Sumilarv 0.5G) to dragonfly nymphs makes the juvenile hormone a safe larvicide to use at Mahanga where malaria is endemic.

The dragonfly nymphs are ideal aquatic predators for testing using IGR since they are still undergoing developmental stages just like the mosquito larvae. From earlier experiments, it was proved that dragonfly nymphs are efficient predators of mosquito larvae and can thus be used in mosquito control programs (Kweka *et al., 2011*). Results of this study, are evident that pyriproxyfen (Sumilarv 0.5G) has no effects when applied at recommended dosages of 0.01ppm in water. Previous studies done on the effects of Pyriproxyfen on third instar larvae of *An. gambiae* and *An. arabiensis* at ICIPE-Mbita showed that there was 98% and 96% inhibition of emergence respectively (Mbare *et al* ., 2013). From these reports, Pyriproxyfen (Sumilarv 0.5G) significantly inhibits emergence mosquito larvae at 0.01ppm thus reducing malaria vectors. When Pyriproxyfen was evaluated on mosquito predator the dragonfly nymph, at 0.01ppm only 1.25% mortality was recorded. Field evaluation on the nymphocidal activity of pyriproxyfen on dragonfly nymphs' showed that the IGR had no significant impact on dragonfly nymphs. In all the habitats where sampling and collection was done, it was found that the numbers of live dragonfly nymphs was high in intervention site and control site. In comparison to earlier studies by Invest and Lucas (2008) they

recommended that at 0.01ppm Pyriproxyfen is applied in water tanks to control mosquito larvae. Therefore pyriproxyfen (Sumilarv 0.5G) is safe to humans and plants at very low dose rates.

The study had targeted time of four months sampling before application and eight months after application. When comparisons were made it showed that population of dragonfly nymphs did not reduce even after Pyriproxyfen (Sumilarv 0.5G) application in the targeted eight months. The results showed that the mean of dragonfly nymphs was high after Pyriproxyfen application meaning that there was rapid buildup of dragonfly nymphs after Pyriproxyfen application Therefore Pyriproxyfen (Sumilarv 0.5G) had insignificant effect on the dragonfly nymphs in mosquito breeding habitats at Mahanga. The insect growth regulator Pyriproxyfen (Sumilarv 0.5G) is a novel larvicide which does not kill insects directly but affects their development. The use of insect growth regulators which are also third generation insecticides is not wide spread in Kenya. Most vector control strategies have been using Organophosphate insecticides, and organochlorides (Kesavaraju *et al*., 2010). From this study Pyriproxyfen is a larvicide which can be used in combination with other methods like use of nets, repellents, and trapping to bring down malaria in the western Kenya highlands. A good larvicide should be selective, and attack the intended vector but have no activity on non-target organisms. The outcomes show that Pyriproxyfen (Sumilarv 0.5G) has insignificant nymphocidal activity on dragonfly nymphs at a concentration of 0.05ppm in mosquito breeding habitats and at the same time persists in the environment thus inhibiting mosquito larvae emerging to the pupal stage.

The results of this study indicate that IGR Pyriproxyfen (Sumilarv 0.5G) has insignificant impact on non target dragonfly nymphs at concentrations of 0.01ppm and 0.05 ppm. This is an indicator that Pyriproxyfen (Sumilarv 0.5G) is a safe compound to use as a larvicide. The activity of IGR shows delayed effects in the development of insects (Lee, 2002). It is anticipated that when Pyriproxyfen is applied, the dragonfly larvae will not emerge into adults. During sampling period large proportion of dragonfly nymphs were collected from burrow pits. The burrow pits had good conditions that favoured growth of dragonfly nymphs as they had more water and were more permanent throughout unlike the drains and natural swamps. Shallan and Canyon, 2009) documented that dragonfly nymphs prefer fresh water ecosystems. The drains had passing water therefore during heavy rains the dragonfly nymphs were swept thus lowering their population. The swamps had lowest abundance due to their seasonality and human interference paving way for cultivation.

Dragonfly nymphs colonize natural habitats which also are good breeding grounds for *Anopheles gambiae* that transmit malaria. Similarly abundance of dragonfly nymphs was same before Pyriproxyfen application and after Pyriproxyfen (Sumilarv 0.5G) application at Mahanga. These results imply that the insect growth regulator Pyriproxyfen had statistically significant impact on dragonfly nymphs in intervention site $p<0.001$. Dragonfly nymphs have long aquatic life and this makes them successful predators of mosquito larvae and more susceptible to larvicides which have long residual effect. Pyriproxyfen (Sumilarv 0.5G) has long residual effects of more than three weeks (Invest and Lucas 2008; Mbare *et al*., 2013).

5.2. Conclusions

i. The dragonfly nymph is an efficient predator of *An. gambiae*. The use of natural enemies is an important component for use in integrated vector management. Dragonfly nymphs can thus be integrated into other existing biological mosquito control measures.

ii. The insect growth regulator Pyriproxyfen (Sumilarv 0.5G) had insignificant nymphocidal effect on dragonfly nymph in mosquito breeding habitats at Mahanga at a concentration of 0.05 ppm The abundance of dragonfly nymphs after application of the larvicide was high and this showed that the larvicide was ideal for controlling mosquito larvae without affecting the non target dragonfly nymphs.

5.1.3 Recommendations

This study therefore recommends the following.

i. Researchers should integrate the use of dragonfly nymphs as biological resources for reducing *An. gambiae* populations which leads to a subsequent reduction in malaria transmission.

ii. The use of IGR Pyriproxyfen (Sumilarv 0.5G) should be applied at dosage rates of below 0,05ppm in mosquito breeding habitats.

5.1.4 Limitations of the research study.

i. The laboratory rearing conditions were different from the conditions in the natural mosquito breeding habitats hence could have contributed to the mortality of dragonfly nymphs.

ii. Dilutions of the pyriproxyfen by rain water could have had an impact on the concentration of Pyriproxyfen in mosquito breeding habitats thus could have led to increase in the abundance of dragonfly nymphs.

5.1.5 Suggestions for further research

i. Further research using insect growth regulator Pyriproxyfen should be done on other non target organisms such as beetles, backswimmers, fish, frogs and its effect on plants.

ii. Research should be done on mass rearing of dragonfly nymphs in insectaries and releasing them in mosquito breeding habitats then monitor their predation activity.

REFERENCES.

Andrea, G. and Evans, C. (2004) Temporal limitation on social learning of novel predators by Indian mynah *Acridotheres Tristis* **32**: 45-59.

Arulprakash, R. and Gunathilagaj, K. (2011) Abundance and diversity of Odonata in temporary water bodies of Coimbatore and Salem districts in Tamil nadu. *Journal of Threatened Taxa* **45**: 1099-1102.

Atieli, H.; Menya, D.; Githeko, A and Scott, T (2009) House design modifications reduce indoor resting Malaria vector densities in rice irrigation scheme area in western Kenya. *Malaria Journal* **8**: 108.

Atieli, H.; Munga, S.; Ofulla, A.; and Vulule, J. (2010). Wash durability and optimal drying regimen of four brands of long- lasting insecticide- treated nets after repeated washing under tropical conditions. *Malaria Journal* **9**:342

Berticat, C.; Duron O.; Hyse D.; and Raymond M. (2004) Insecticidal resistance genes confer a predation cost on mosquitoes, *Culex pipiens*. *Journal of Genetics* 27: 467-500.

Bentley, M. D, and Day, J (1989). Chemical ecology and behavioral aspects of mosquito oviposition. *Annual Reviews of Entomology* **34**: 401-421.

Blaustein, L. and Kotler, B. (1995). Oviposition habitat selection by the mosquito, *Culiseta lon-giareolata*: effects of conspecifics, food, and green toad tadpoles. *Journal of Ecology and Entomology* **18:** 104-108.

Blaustein, L. and Jonathan, M. (2009). Interactions between mosquito larvae and species that share the same trophic level. *Annual Review of Entomology*. **52**: 489-505.

Bogitish, B. and Thomas, C. (1990). The Global Approach to Malaria Control and Eradication. *Human Parasitology Saunders* college publication; Philadelphia 1990 p 843.

Bukhari, T.; Takken, W.; Githeko, A.; and Koenraadt, C. (2011). Efficacy of Aquatain, a monomolecular Film, for the Control of Malaria Vectors in Rice Paddies. *PLOS ONE* **6**(6): 234-327.

Butler, M.; Lebrun, R.; Ginsberg, S.; and Gettman, A. (2006). Efficacy of Methoprene for mosquito control in storm water catch basins. *Journal of American Mosquito Control Association*, **22:**333-338.

Carquet, T.; Marc, R.; Pierre, L. G.; and Laurent, L. (2011). Effects of repeated field application of two formulations of *Bacillus thuringiensis var israelensis* on non

target salt marsh invertebrates in Atlantic coastal wetlands. *Journal of Ecotoxicology and Environmental Safety* **74** (5):1122-1130.

Charles, L.; Charles, K.; Rodriguez, A.; Quiroz-Martinez .D.; Solis, C.; and Badii. M. H. (1998). Prey Selectivity of dams *Laccophilus fasciatus* Aube (Coleoptera: Dytiscidae). *Vedalia* **5**:47-49.

Chen, C.; Andy-Tan, W.; Loke, S.; and Sofian –Azirun, M. (2008). Effectiveness of Pyriproxyfen controlled release block against larvae of *Aedes aegypti* in Kula Lumpur. *Dengue Bulletin* 32: 564.

Chesson, J. (1989). The effect of alternative prey on the functional response of *Notonecta boffmani*. *Ecology* **70**. 1227-35.

Chivers, D. and Smith, J (1998). Chemical alarm signaling in aquatic predator/prey interactions: a review and prospects. *Ecoscience* **5:** 338-352.

Clements, A. N (1999). The biology of mosquitoes: Sensory reception and behavior Chapman and Hall Vol. **2**. Pp 645.

Craig M.; Ricardo .G; and Martin. B. (1999). Seasonal forecasts, climate change and human health. Health and climate. *Trends in Parasitology* **15** (3): 105-111.

Culler, L. and William, O. L. (2009). Selective predation by larval *Agabus* (Coleoptera: Dytiscidae) on mosquitoes: Support for conservation –based mosquito suppression in constructed wetlands. *Freshwater Biology* **54**. 2003-2014.

Debora L, F. Robert, D. and Claudio G (1988) Intraguild predation relaxes enemy impacts on herbivores. Pp 45.

Dell, B. A. C. (2003). Horizontal transfer of the Insect Growth Regulator Pyriproxyfen to larval micrococosims by gravid *Aedes albopictus* and *Ochlerotatu*s *triseriatus* mosquitoes in the Laboratory. *Medical and Veterinary Entomology* **17**: 211-220.

Dickman, M. (2000). Impact of mosquito selective pesticide, *Bti* on the macro invertebrates of a subtropical stream in Hongkong. *Chemosphere*.**41**: 209-217.

Dudgeon, D. and Watt, M. C. (1986) Life cycle and diet of *Zygonyx iris insignis* (Insecta: Odonata: Anisoptera) in Hong Kong running waters. *Journal of Tropical Ecology* **2**:73-85.

Dugassa, S.; Lindh, M.; Torr, S; Lindsay, S.; and Fillinger, U. (2014) Evaluation of influence of electrical nets on the behavior of oviposition site seeking *Anopheles gambiae*. *Malaria Journal* **7**: 272.

Dugassa, S.; Lindh, J.; Oyieke, F.; Mukabana, W.; and Lindsay S. (2013). Development of a gravid trap for collecting live malaria vectors. *PLOS ONE* **8** (7): 948.

Food Agriculture Organisation/ Word Health Organisation (1999). Joint meeting of the FAO Panel of experts on pesticide residues in food and the environment and the WHO core assessment group: Pyriproxyfen.*FAO*, Rome. P 987.

Fillinger, U. and Lindsay, S. (2006) Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. *Journal of Tropical Medicine and International Health* **11**(11):1629-1642.

Fillinger, U.; Ndenga, B.; Githeko, A.; and Lindsay, S. (2009). Integrated malaria vector control with microbial larvicides and insecticide – treated nets in western Kenya: a controlled trial. *Bulletin of World Health Organization* **87**: 655- 665.

Geissbuhler, Y.; Kennedy, K.; Pius, P.; Mayegaya, V.; Kiama, M.; Mtasiwa, D.; Mshinda, H.; Lindsay, S.; Tanner, M.; Fillinger, U.; and Killeen, G. (2009). Microbial larvicide application by large scale, community - based program reduces malaria infection prevalence in urban Dar es salaam, Tanzania. *PLOS ONE* **4**(3): 5107.

Giacomini, H. and De marco Jr, P. (2008). Larval eco-morphology of 13 libellulidae (Anisoptera, Odonata) of the middle Rio Doce valley, Minas Gerais, *Brazilian Journal of Biology* 68:1.

Gittelman, S. (1975). The ecology of some Costa Rican Backswimmers Hemiptera: notonectidae). *Annals of Entomological Society of America* **68** (3):56-62.

Hanafi-Bajd, A.; Vatandoost H.; and Jafari, R. (2006). Susceptibility status of *Anopheles dhali* and *Anopheles fluviatilis* to commonly used larvicides in an endemic focus of malaria, Southern Iran. *Journal of Vector Borne Disease* **43** (8): 897-943.

Hay, L. (2002). The intersectoral response to the 2002 malaria outbreak in the highlands of western Kenya. *Lancet Infectious Diseases* **4**(6):327-36.

Howell, D.; John, D.; and Alexander, P. (**1998).** Introduction to insect biology and diversity. Oxford University press Newyork 355-361.

Huffaker, B and Gutierrez Andrew (2004). Dynamics of insect predator prey interactions. *Ecological Entomology* **3** (5): 346-378.

Invest F.J.; and Lucas, J.R. (2008). Pyriproxyfen as a mosquito larvicide. Proceeding of the sixth international conference on urban pests. Pp90.

Kalysnasundaram, I.; Jambuligan, S.; Bhoopathi, D., Dominic A.; and Dass, P(2002). Efficacy of two organophosphorus insecticides, Reldan and Dursban against the larvae of *Culex quinquefasciataus*. *Indian Journal of Medical* **117** : 25-29.

Kats, L.B. and Dill, L. (1998.) The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* **5:** 361-394.

Kawada, H.; Dohara, K.; and Shinjo, G. (1988). Laboratory and field evaluation of an insect growth regulator, 4-phenoxyphenyl (RS)-2(2-pyridyloxy) Propylether, as mosquito larvicides. *Japanese Journal of Sanitary Zoology* **39**:339-346.

Kerfoot, C. and Sih, A. (1987). Predation: direct and indirect impacts on aquatic communities. Hanover, NH: Univ. Press of New England, p793.

Kesavaraju, B.; Alto, B.; Afify, A.; and Gaugler, R. (2010). Malathion influences competition between *Aedes albopictus* and *Aedes japonicas*. *Journal of Medical Entomology* **47**(6): 1011- 1018.

Kettle, D. (1992). Medical and veterinary entomology.*CAB international*. Pp754

Kibe, L.; Mbogo, C.; Keating, J.; and Beier, J (2006) Community based vector control in Malindi, Kenya. *African Health Science* **6**(4) :240-246.

Kono, Y.; Keiko, M.; and Takahashi, M. (1997). Changes insusceptibility to Pyriproxyfen a JH mimic, during late larval and pupal stages of *Culex pipiens*. *Journal of Medical Entomology* **48** (2) 478-490.

Kumar, R. and Jiang –S, H. (2005). Larvicidal efficiency of aquatic predators: A perspective for mosquito biocontrol. *Zoological Studies* **45** (5):321-356

Kweka, J .E.; Goufa, Z.; Thomas, M.; Gilbreath, Y.A.; Mramba, N.; Githeko, A.; and Guiyan, Y.; (2011). Predation efficiency of *Anopheles gambiae* larvae by aquatic predators of western Kenya highlands. *Parasite and Vectors **4**: 128.*

Lee, D. (2002). Field evaluation of insect growth regulator, Pyriproxyfen against *Aedes togoi* larvae in Korea. *Journal of Vector Ecology* **26**: 39 -42.

Lee, Y.; Zairi, J.; Yap , H.; and Adanan , C. (2005) Integration of Bacillus thuringiensis H-14 formulation and Pyriproxyfen for the control of larvae of Aedes aegypti and Aedes alboticus. *Journal of American Mosquito Control Association*. 21 (1): 84-89.

Lima, P.; Paira, M.; and Arajuo, A. (2011). Insecticide resistance in *Aedes aegypti* populations from Ceara, Brazil. *Parasite and Vectors.* **4**:65-67.

Lindblade, K.A., Gimning, J.E., Kamau, L., and Vulule, J.M (2006). Impact of sustained use of insecticide–treated bednets on malaria vector species distribution and Culicine mosquitoes. *Journal of Medical Entomology* **43**; 428-432.

Lundkvist, E.; Landin, J.; Jackson, M.; and Svensson, C. (2003). Diving beetles (Dytiscidae) as predators of mosquito larvae (Culicidae) in field experiments and in laboratory tests of prey preference. *Buletin of Entomological Resesearch* **66** (3): 56-97.

Lindblade , K.; Mwandama, D.; Themba , M.; Laura , S.; John , M.; Monica ,S.; and Mathanga ,D.; (2015) A cohort study of the effectiveness of insecticide treated bednets to prevent malaria in an area of moderate Pyrethroid resistance ,Malawi. *Malaria Journal* **14**: 31.

Mbare ,O.; Lindsay, S.; and Fillinger , U (2013) Dose-response tests and semi field evaluation of lethal and sub-lethal effects of slow release Pyriproxyfen granules (Sumilarv 0.5G) for the control of malaria vectors *Anopheles gambiae sensu lato* . *Malaria Journal* **12**: 94.

Merritt, W.; Richard, J. L.; Lessard, K. J.; Wessel. **O. H.; Martin, B.; Berg, J. R. W.; John, A. N.; John, R.; and Brett, W. M. (2005).** Lack of effects of *Bacillus sphericus* on non target organism in mosquito control programme in south eastern Wisconsin: A 3 –year study. Pp789.

Minakawa, N.; Sonye, G.; Mogi, M.; Githeko, A.; and Yan, G. (2002). The effects of climatic factors on the distribution and abundance of malaria vectors in Kenya. *Journal of Medical Entomology* **39**: 833- 841.

Minakawa, N.; Munga, S.; Atieli F.; Zhou G.; Githeko, A; and Yan, G. (2005). Spatial distribution of anopheline larval habitats in western Kenya highlands; effects of land cover types and topography. *American Journal of Tropical Medicine and Hyg*iene. **73**(1): 157-65.

Miura, T. and Schaefer, H. **(1990).** Chemical persistence and effects of S-31183, 2-(1-Methyl- 2-(4-phenoxy) ethoxy) pyridine, on aquatic organisms in field tests. *Journal of. Economic Entomology* **83** (5): 359-89.

Mortimer, R.M. and Heather, F.C. (1995). Acute toxic effects of (S) – Methoprene and Temephos to some Australian none target aquatic invertebrates crustacean species. *Australian Journal of Ecotoxicology* **1**:111.

Msangi, S.; Lyatu, E.; and Kweka, E. (2011). Field and laboratory evaluation of bioefficiency of an insecticide growth regulator (Dimilin) as larvicides against mosquito and housefly larvae. *Journal of Tropical Medicine* **25** (4): 543-578.

Muiruri, S.; Mwangangi, J.; Calson, J,; Kaburi, E.; Kokwaro, E and Beir, J (**2013).** Effects of predation on Anopheles larvae by five sympatric insect families in coastal Kenya. *Journal of Vector Borne Disease* **50**(2):324-367.

Munga, S.; Noburu, M.; Goufa, Z.; Okeyo- owour, B.; Andrew, G.; and Guiyun, Y. (2006). Effects of larval competitors and predators on oviposition site selection of *Anopheles gambiae* ss. *Journal of Medical Entomology* **43**(2):221-224.

Najera, J and M. Zaim, (2002). "Malaria vector control. Decision making criteria and procedures for judicious use of insecticide," in WHO, World Health Organization Pesticide Evaluation Scheme (WHOPES), 2002, WHO/CDS/WHOPES/2002.5.Pp94.

Ndenga, B.; Simbauni, J.; Mbugi, P.; Githeko, A.; and Fillinger, U (2011) Productivity of Malaria Vectors from different habitat types in the western Kenya highlands. *PLOSE ONE* **6** (4): 426-445.

Ndenga, B.; Githeko, A.; Omukunda, E.; Munyekenye, G.; Atieli, H.; and Wamai, P.; (**2006).** Population dynamics of malaria vectors in western Kenya highlands. *Journal of Medical Entomology* **43**:200.

N'Guessan, R.; Boko, P.; Odjo, A.; Chabi, J.; Akogbeto, M.; and Rowland, M (2010). Control of pyrethroid and DDT- resistant *Anopheles gambiae* by application of indoor residual spraying or mosquito nets with long lasting organophosphate insecticide, chlorpyrifos- methyl. *Malaria Journal* **9:** 44.

Obbard, D.; Welch, J.; and Little, T. (2009). Infering selection the *Anopheles gambiae* species complex: an example from immune related serine protease inhibitors. *Malaria Journal* **8**: 117.

Onyango , S.; Kitron , U.;Mungai ,; Muchiri , E.; Kokwaro , E and Mutuku , F.; (2013). Monitoring Malaria vector control interventions : Effective of five different Adult mosquito sampling methods . *Journal of Medical Entomology* **50** (5): 1140-1151.

Pascaline, J. Mutai.; and Ouma, G. (2010). An inventory of medicinal plants used to treat malaria. Proceedings of the third international e-conference in Agricultural Biosciences P 30-31.

Poopathi, S. and Abidha, S. (2010) Mosquitocidal bacterial toxins (*Bacillus sphaericus*
and *Bacillus thuringiensis var israelensis*); mode of action , cytopathological effects and mechanisms of resistance.*Journal of Physiology and Pathophysiology* volume **1**(3): 346-358.

Pinkney, E.A.; Peter, C. M.; Daniel, R. M. T.; Peter, L.; Donald, W. S.; and Leonard, C. F. (2000). Effects of the mosquito larvicides Temephos and Methoprene on insect populations in experimental ponds. *Environmental Toxicology and Chemistry* **19** (3) : 678-684.

Quiroz-Martinez, H.; and Ariadna, R. C. (2007). Aquatic insects as predators of mosquito larvae. *Journal of American Mosquito Association.* **7** (23): 781-865.

Rachid, R.; Saci, F.; Berrebbah, H.; and Djebar, M. (2009). Toxic effects of combined molecule from Novaluron and Diflubenzuron on *Paramecium caudatum. American-Eurasian Journal of Toxicological Sciences* **2**: 74-80.

Rebora, M. and Piersanti, G. E (2004). Visual and mechanical cues used for prey detection by the larvae of *Libeiiula depressa* (Odonata: libellulidae) **16** (2):133-144.

Resh, V. and David, R. (1984). The ecology of aquatic predators. Rosenberg Praeger publishers. Newyork.Pp563.

Sebastian, A.; Sein. M.M.; and Thu, M. (1990). Suppression of *Aedes aegypti* (L) (Diptera: Culicidae) using augmentative release of dragonfly larvae with community participation in Yangon. *Bulletin of Entomological Research* **89**: 223-232.

Shallan, E. and Canyon, D. (2009). Aquatic insect predators and Mosquito control. *Tropical Biomedicine* **26**(3): 223-261.

Shallan, E. and Canyon, D. (2006). A review of aquatic insect predators and their use in mosquito control. *Tropical Biomedicine* **27** (3):654-67.

Snyder W. E, and David Wise (2000). Experiments on the responses of rejector species to threats of predation. *Environmental Entomology.Pp*90 .

Sohail, A.; Mohamed, K.; and Shahid, N. (2004). Efficacy of monomehypo 5G and chloropyrifos 40EC against insect pest and their effects on natural enemies in rice ecosystem. *Pakistan Entomol*ogy **26** (4): 365-378.

Symondson, W. (2002) Can generalist predators be effective**.** *Annual Review of Entomology* **47**. 561-594.

Thakare, V.G. and Zade, S.V. (2011) Diversity, abundance and species composition of water beetles (Coleoptera: Dytiscidae, Hydrophilidae and Gyrinidae) in Kolkas region of Melghat Toger reserve, Central India. *Academic Journal of Entomology* **4**(2): 64-71.

Thavara, U. A.; Tawatsin, C.; Chansang, P.; Asavadachanukorn, M.; Zaim, M. and S. Mulla, (2007) "Simulated field evaluation of the efficacy of two formulations of diflubenzuron, a chitin synthesis inhibitor against larvae of *Aedes aegypti* (L.) (*diptera*: *Culicidae)* in water-storage containers," *Southeast Asian Journal of Tropical Medicine and Public Health*. **38** (2): 269–275.

Waiker, K. and Lindsay, M. (2007) Contributions of Anopheles larval control to malaria suppression in tropical Africa: review of achievements and potential. *Medical and Veterinary Entomology.* **21**(1): 2-21.

Webb, C. and Richard, C. R. (2008). A laboratory investigation of the mosquito control potential of the monomolecular film Aquatain mosquito formula against immature stages of *Aedes aegypti* and *Culex quinquefasciatus* . *Journal of American Mosquito Control Association* 25:106.

World Health Organisation. (2007). Pyriproxyfen in Drinking-water: Background document for development of WHO Guidelines for Drinking-water Quality, World Health Organization. WHO/SDE/WSH/07.01/10 Pp124.

World Health Organisation. (2011) Report Pp78.

World Health Organisation.(2013) Report pp456.

Xue, R.; Donald, R. B.; and Arshad, A. (2000). Laboratory toxicity of three mosquito oviposition repellents to six non target aquatic invertebrates. *Journal of Environmental Entomol*ogy **29** (3):437-457.

Yapabandara, A. (2005). Review of the efficacy and persistence of Pyriproxyfen 0.5% granules on *Anopheles* sp. and *Aedes* sp. breeding sites in Sri Lanka. Proceeding of the fifth internatinal conference on urban pests.Pp243.

Zhou, G.; Githeko, A.; Minakawa, N.; and Yan, G. (2010). Community wide benefits of targeted indoor residual spray for malaria control in the western Kenya highlands. *Malaria Journal* **9**: 67.

APPENDIX

Appendix i Permit to use Sumilarv 0.5G

PEST CONTROL PRODUCTS BOARD

(A Statutory Organization of Government)

Tel: 254 - 020 - 4446115 / 4450242 / 4441804
254 - 020 - 4444137 / 144
Fax: 254 - 020 - 4449072

WAIYAKI WAY (KARI-NAL)
P.O. BOX 13794
NAIROBI, WESTLANDS - 00800, KENYA
Email: pcpboard@todays.co.ke /
md@pcpb.or.ke
website: www.pcpb.or.ke

Your Ref: .....
Our Ref: PCPB/I11/REG/VOL.I/11/22

Date: 26th January, 2011

Centre Director,
Centre for Global Health Research,
Kenya Medical Research Institute,
P.O. Box 1578-40100,
KISUMU

Attn: B.A. Ndenga

RE: AUTHORITY FOR RESEARCH AND IMPORTATION OF SUMILARV 0.5G

Reference is made to your application for a research permit dated 11th January 2011 for Sumilarv 0.5G which contain 5% Pyriproxyfen. It is noted that supportive technical information provided in support of the application have been provided.

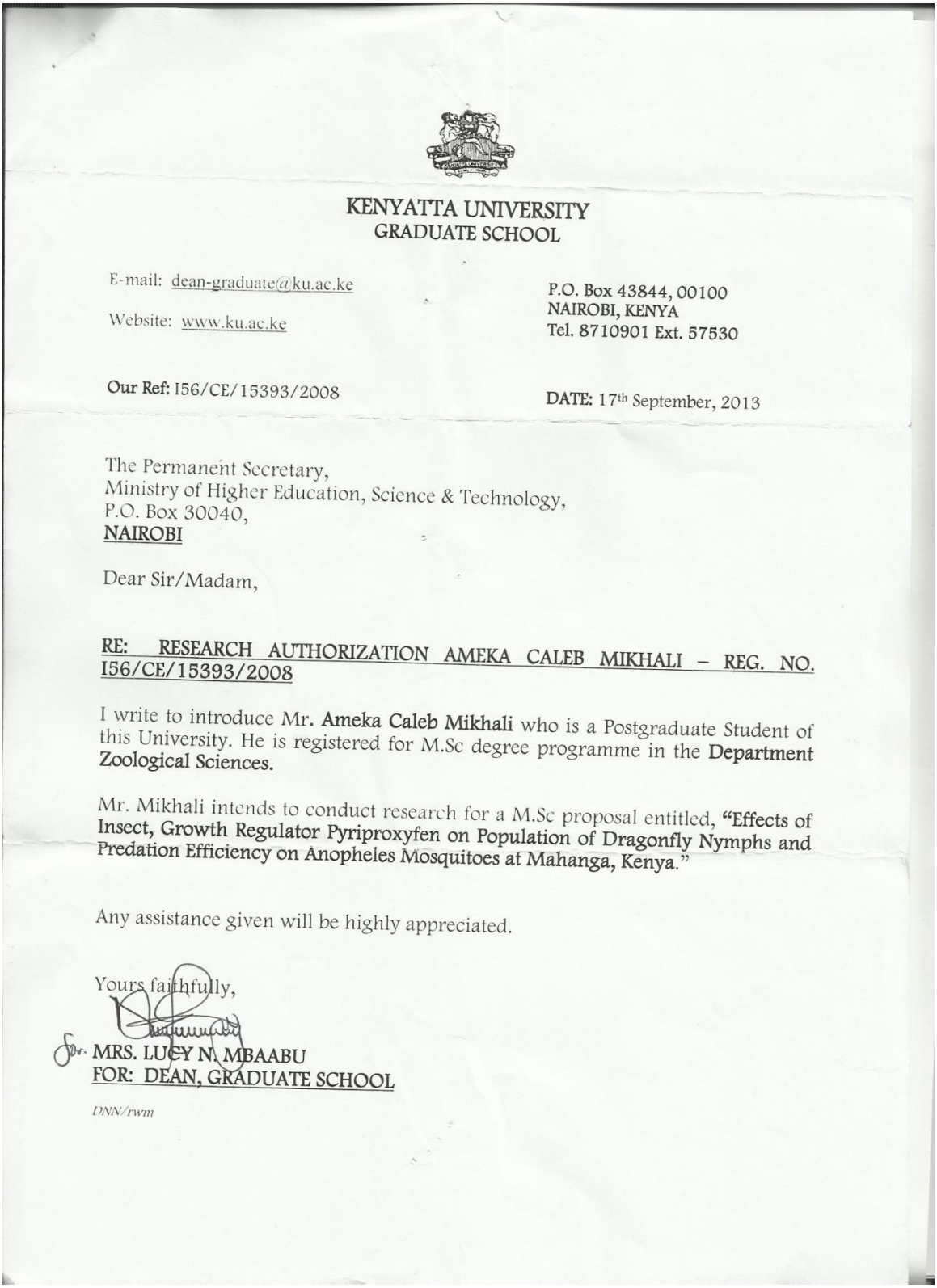
The Board therefore hereby authorizes the importation of 60Kgs of Sumilarv 0.5G for research purposes.

Kindly note that you shall be required to submit a copy of the trial report to the Board. It is important to inform PCPB of the exact trial location and sites for monitoring purposes and submit regular progress reports.

Thank you.

Gladys N. Maina
MANAGING DIRECTOR/SECRETARY

APPENDIX ii Research authorization from Kenyatta University.



KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke

Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: I56/CE/15393/2008

DATE: 17th September, 2013

The Permanent Secretary,
Ministry of Higher Education, Science & Technology,
P.O. Box 30040,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION AMEKA CALEB MIKHALI – REG. NO. I56/CE/15393/2008

I write to introduce Mr. **Ameka Caleb Mikhali** who is a Postgraduate Student of this University. He is registered for M.Sc degree programme in the **Department Zoological Sciences.**

Mr. Mikhali intends to conduct research for a M.Sc proposal entitled, **"Effects of Insect, Growth Regulator Pyriproxyfen on Population of Dragonfly Nymphs and Predation Efficiency on Anopheles Mosquitoes at Mahanga, Kenya."**

Any assistance given will be highly appreciated.

Yours faithfully,

for. MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

DNN/rwm