EFFECT OF EXTRACTS FROM SELECTED *Aloe* PLANT SPECIES ON THE *Anopheles gambiae sensu stricto* AND *Aedes aegypti* MOSQUITOES

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DECLARATION AND RECOMMENDATION

DECLARATION

I hereby declare that this thesis is my original work and has not been submitted for award in any institution of learning to the best of my knowledge.

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RECOMMENDATION

We confirm that this thesis has our approval to be presented for examination as per the Egerton University regulations.

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DEDICATION

This work is dedicated with love to my entire family members, my husband Henry Chore; and my dear children Allan Amiani, Kelly Everia, Tony Kahi and Mercy Cheredi. Your tireless support, endurance and belief in me carried the day for me.

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ABSTRACT

Malaria control through insecticide application has significantly been compromised by the advent of resistance to the insecticide in the *Anopheles* mosquito vector. These developments have necessitated the need to bio prospect for and understand the mode of action of novel insecticide introduction. Larvicidal potential in extracts from *Aloe* plants on *Anopheles gambiae sensu stricto* and *Aedes aegypt*i third instar (two days old) larvae were evaluated. Extracts were obtained from *Aloe turkanensis, Aloe ngongensis and Aloe fibrosa* plants using classical solvent extraction technique. Larvicidal potential of the extract were evaluated through bioassay of the extract on third instar *An. gambiae* s.s and *Ae. aegypti* larvae following 24 h exposure. The dose-response data obtained were analyzed by probit analysis to establish the median lethal concentration (LC_{50}) of the extract to the larvae. The LC_{50} responses against third instar larvae of *An. gambiae s. s.* and *Ae. aegypti* were 0.08 mg / ml and 0.11mg / ml, respectively at 95% intervals. All the *A. ngongensis* plant extracts had larvicidal activity. Only the methanol, acetone and hexane plant extracts of *A. fibrosa* species showed larvicidal activities at LC_{50} concentrations ranging between 0.66 and 3.90 mg / ml at 95% confidence interval. Phytochemical tests showed presence of flavonoids, tannins and saponins in *Aloe turkanensis* plant extract. Apart from the hexane and chloroform plant extracts of *Aloe ngongensis*, those of methanol, acetone and ethyl acetate showed presence of flavonoids. All the *Aloe fibrosa* plant extracts of hexane, acetone and methanol showed presence of flavonoids and tannins. However, only the acetone and methanol plant extracts of *Aloe fibrosa* showed presence of saponins. None of the *Aloe* plant extracts gave positive test for phlobatannins, terpenoids and steroids. The phenolic functional group was prevalent in most of the plant extracts. The leased significant difference (LSD) of the means of the developmental stages of the *Aloe* exposed *Ae. aegypti* were significantly different from those of the negative controls at 5% level. The leased significant means of the egg deposits, egg viability and mortality were significantly different in the *Aloe* exposed *Ae. aegypti* at 5% level The findings of this study will contribute to current *Anopheles* vector control programs by providing information on potential additional anti-*Anopheles* compound(s) and molecular process (es) of subtractive hybridization with bioinformatics, that can be exploited in development of novel insecticides.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AFA	*Aloe fibrosa* acetone extract
AFH	*Aloe fibrosa* hexane
AFM	*Aloe fibrosa* methanol extract
ANA	*Aloe ngongensis* acetone extract
ANC	*Aloe ngongensis* chloroform extract
ANE	*Aloe* ngongensis ethyl acetate extract
ANH	*Aloe ngongensis* hexane extract
ANM	*Aloe ngongensis* methanol extract
ATE	*Aloe turkanensis* ethyl acetate extract
DDT	Dichloro diphenyl trichloroethane
EPI	WHO Expanded Programme on Immunization
FAO	Food for Africa Fund
Kdr	Knockdown resistance
LC_{50}	Concentration required to kill 50% of the test organism
LC_{90}	Concentration required to kill 90% of the test organism
LC_{99}	Concentration required to kill 99% of the test organism
IPM	Integrated pest management
IGRs	Insect growth regulators
IRS	Indoor residual spraying
MOH	Ministry of Health
RBM	Roll back malaria
UNICEF	United Nations Childres's Fund

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE
INTRODUCTION

1.1 Background Information

The mosquitoes are a family of small, midge-like flies: the *Culicidae* (Foot and Cook, 2005)**.** Although a few species are harmless or even useful to humanity, most are a nuisance because they consume blood from living vertebrates, including humans (Clements, 1992 and Apperson *et al*., 2002). In feeding on blood, various species of mosquitoes transmit some of the most harmful human and livestock diseases. Effective management of the malaria and arbovirus vectors by current classes of mosquitocides is challenging, and necessitates prospecting for novel insecticides with unique modes of action, different from the current ones. Current adulticide classes (organochlorines, organophosphates, carbamates, pyrethroids and DDT) are limited to three different modes of action (little target-site diversity), with two different target sites (AchE or voltage gated sodium channel) and a single component (central nervous system) of the vector. It is not therefore surprising that there is cross-resistance to the insecticides in the vector to insecticides within the classes, phenomena currently widespread across Sub-Sahara Africa (Chandre *et al.,* 2000; Enayati and Hemingway, 2010). The resistance to pyrethroids presents a real and immediate challenge to efficacy of otherwise successful insecticide treated nets (ITN) based malaria control intervention against adult vectors (Etang *et al.,* 2004). Resistance development has also hampered larvae control (Diabate *et al.,* 2003), a viable alternative control of the vector populations. This initiative has further been compounded by development of resistance in the larvae to new classes of larvicides (IGRs, bacterial endotoxins and even *Bacillus thuringiensis* (Bti)) (Amy, *et al*., 2005 and Wirth *et al*., 2005).

Recent phytochemical research has begun to reveal a variety of blend effects in the bioactivities of plant natural products. Two principal blend effects have been demonstrated: (1) enhanced biological activity resulting from synergistic or other additive effects of moderately active or individually inactive compounds to give mixtures that are more active than a linear summation of individual activities (Berenbaum and Zangeri, 1987; Isman *et al*., 2008; Romeo *et al.,* 1996; Bekele and Hassanali, 2001); and (2) mitigating effects of structurally related or unrelated compounds against rapid resistance development that characterizes most single-component bioactive compounds (Feng and Isman, 1995; Isman *et al.,* 1996) (characteristic of current mosquitocide classes). Most plants produce a variety of secondary metabolites, which

may or may not be structurally related, with multiplicity of defense and non-defense functions against different pathogens and herbivores. This phytochemical and functional diversity has undoubtedly arisen from sustained selective forces in response to succession of attack by pathogens and herbivores and other selective pressures over evolutionary time. Moreover, phytochemical blends rarely demonstrate acute toxicity. They are often subtle and have longer-term growth-disrupting effects. This project explored the larvicidal effect of *Aloe* plant extracts on *Anopheles gambiae* and *Aedes aegypti* and the adverse influence on their parental (FO) and first generation (F1) developmental stages.

1.2 Statement of the problem

Anopheles gambiae and *Ae. aegypti* are the principal Afro tropical vectors of malaria and arbovirus respectively. These vectors have developed resistance to most conventional insecticides, exposing most of the populations in Sub Saharan African and most of the tropics at risk of contracting the diseases they vector. Adult mosquitoes are highly mobile flying insects that can easily detect and avoid many intervention measures as compared with the larvae that are confined within relatively small aquatic habitats. Malaria control needs multiple initiatives given that plasmodium falciparum has developed resistance to most drugs, including artemisinin-based combination therapy. Resurge in mosquito-borne diseases due to inter-annual and inter-decadal climate variability calls for collaborative intervention measures to contain epidemiology.

1.3 Objectives of the study

1.3.1 General Objective

To determine the effect of extracts from *Aloe* plant species on the immature stages of *Ae. aegypti* (insecticidal) and the mortality of *An. gambiae* s. s. and *Ae. aegypti* mosquito larvae.

1.3.2 Specific Objectives

1. To determine toxicity of organic extracts of *A. turkanensis, A. ngongensis* and *A. fibrosa* plant species to *An. gambiae* s.s and *Ae. aegypti* larvae.
2. To determine phytochemical composition of *A. turkanensis, A. ngongensis* and *A. fibrosa* plant extracts which are toxic to *An. gambiae* s.s and *Ae. aegypti* larvae.
3. To determine effect in *An. gambiae* s.s and *Ae. aegypti* larvae to *A. turkanensis, A. ngongensis and A. fibrosa* plant extracts.

1.4 Hypotheses

1. Organic extracts of *A. turkanensis, A. ngongensis* or *A. fibrosa* plant species are not significantly toxic to *An. gambiae* s.s and *Ae. aegypti* third instar larvae.
2. *Aloe turkanensis, A. ngongensis* and *A. fibrosa* plant extract phytochemical composition do not significantly influence their toxicity to *An. gambiae* s.s and *Aede*s *aegypti* third instar larvae.
3. There are no specific effects of *Aloe* plant extracts of *A. turkanensis*, *A.fibrosa* and *A. ngongensis* on *An. gambiae* and *Ae. aegypti* third instar larvae.

1.5 Justification

Malaria control in the tropics and Africa South of the Sahara is based on insecticide-treated nets) ITNs), indoor-residual spraying (IRS) with insecticides and prompt and effective treatment of clinical malaria. Malaria control initiatives such as U.S. Presidents Malaria Initiative, Roll Back Malaria, Global Strategic Framework for Integrated Vector Management and other partnerships have been involved in mosquito-borne decease control campaigns. Many believe integrated vector management (IVM), targeting both larval and adult mosquitoes, is the future for malaria control .While ITNs are currently the priority strategy, there is growing interest in attacking the aquatic stages of malaria vectors with microbial larvicides, in conjunction with environmental management. Biological vector control makes use of the predatory mosquito larvae (*Toxorhychites spp.*), the mermethid or mosquito-attacking nematode, *Romanomermis culcivorax* and/or the mosquito-eating fish, *Gambusa affinis*, killfish (fundulidae), minnows (cyprinidae), and/or tilapia and or guppies, e.g., *Legister reliculatus* wherever possible. Insect growth regulators (IGRs) such as methoprene (altosid) are used to stop mosquito maturity to adult but insect resistance has been reported in this class of larvicides, just as in the convectional insecticides.

Extracts from *Aloe* plant species have secondary metabolites with insecticidal potential. If effective against *An. gambiae* and *Ae. aegypti* these extracts can contribute to the management of these malaria, arboviruses and/or filarial worm vectors by integrating into the current and existing control methods. Additionally, application of the phytochemicals in vector control will also be more environmentally friendly than application of conventional synthetic insecticides. The use of *Aloe* phytochemicals is potentially useful in the control of the juvenile stages of the vector, particularly larvae, which has been overlooked in many vector control

programs. The use of *Aloe* plant extracts against larvae confined to water habitats will be more efficient than the convectional use of insecticides against the more elusive adult stage of the mosquitoes. It is therefore prudent to evaluate the larvicidal potential of extracts (phytochemicals) from *Aloe* plant species on the larvae of the vectors and identify physiological responses in the vector to the phytochemicals that can be exploited in developing novel biolarvicides.

CHAPTER TWO
LITERATURE REVIEW

2.1 The mosquitoes

Malaria remains one of the most devastating diseases occurring in the world today. It is estimated that about 350 - 500 million clinical cases occur every year with approximately 1-3 million deaths in tropical Africa alone (WHO and UNICEF, 2003). This represents at least one death in every thirty seconds (WHO, 1996). Majority of the cases occur in children under five years and pregnant women are also especially vulnerable. Approximately 40% of the world's population lives in regions where malaria transmission is endemic (Agyepong, 2012). It has been established that 90% of the global malaria morbidity and mortality occurs in sub-Saharan Africa (UNICEF, 2010). In Kenya, over 28 million Kenyans are at risk of malaria infection and an estimated 34,000 children less than five years of age die annually due to malaria (Mugo, 2012). It accounts for 30-50% of all outpatient attendance and 20% of all admissions to health facilities. An estimated 170 million working days are lost to the disease each year (MOH 2001). Malaria is also estimated to cause 20% of all deaths in children under five (MOH 2006). The economic burden in Kenya due to malaria related cases was 2.3 billion shillings (Kemri, 2012).

Malaria transmission in Kenya is by all the four species of human *Plasmodium*: *P. falciparum, P. malariae, P. ovale* and *P. vivax*. *P. falciparum* which causes the severest form of the disease accounts for 98 percent of all malaria infections. The major malaria vectors in Kenya are members of *An. gambiae* complex and *An.funestus* (Lyimo *et al* 1992 and Keating *et al*., 2005). Kenya has four malaria epidemiological zones: endemic, seasonal malaria transmission, Malaria epidemic prone areas of western highlands of Kenya and low risk malaria areas (Kemri, 2012). The malaria endemic zone lies at the altitudes ranging from 0 to 1300 meters around Lake Victoria in western Kenya and in the coastal regions. Rainfall, temperature and humidity are the determinants of the perennial transmission of malaria. The vector life cycle is usually short with high survival rate due to the suitable climatic conditions (Jean-Michel *et al*., 2003). Transmission is intense throughout the year with annual entomological inoculation rates between 30 and 100 (Kemri, 2012). Samples of blood taken from mosquitoes across section of Kenyans have shown seroprevalence to the arbovirus in both humans and mosquitoes (Luke *et al*., 2011 and Desiree *et al*., 2011). Kenya has had multiple arbovirus outbreaks in the past two decades resulting in economic and public health distress, including

yellow fever in 1992 and 1995), chikungunya fever in 2004, and Rift Valley fever (RVF) in 1997 and 2006 (Bird *et al.,* 2008 WHO, 2011). Yellow fever is a viral hemorrhagic fever which strikes an estimated 200 000 persons world-wide each year and causes an estimated 30 000 deaths (WHO, 1992). Yellow fever virus is the prototype of the family *Flaviviridae*, which currently contains over 70 viruses, of which most are arthropod-borne, including the dengue viruses (Monath, 1991 and Kuniholm *et al*., 2006). Dengue fever virus, the most common of the viruses, is transmitted by the bite of an *Aedes* mosquito (Stacy, 2012). Infections can be spread among humans via the mosquito vector. Approximately 2.5 billion people worldwide are at risk of infection with dengue fever in over 100 countries are at risk of infection and 20 million infections are reported annually (WHO, 2010). Patients present with fever, headache, eye, and back pain. The main vector of yellow fever within village and urban settlements is female *Aedes (Stegomyia) aegypti* (only females feed on blood to obtain protein for egg production) Gubler, (1988). The virus is transmitted when a mosquito bites an infected human and then, after an extrinsic (in the mosquito) incubation period of 12-21 days, bites a susceptible human. There is no specific treatment for dengue/ severe dengue, but early detection and access to proper medical care lowers fatality rates below 1% (WHO, 2012). Dengue prevention and control solely depends on effective vector control measures. *Ae. aegypti* breeds readily in all types of domestic and peridomestic collections of fresh water, including flower vases, water drums, tin cans, broken coconut shells, old tyres and gutters (WHO, 1998). Apart from *Brugia malayi* and *Wuchereria bancrofti, Ae. aegypti* also transmits the filarial worm. Lymphatic filariasis is estimated to affect more than 120 million people worldwide, 98% of who live in the tropical and subtropical regions of Africa and Asia (Michael *et al*., 2001 and Braga *et al.,* 2003). There are 72 countries with endemic lymphatic filariasis and a total population of more than 1.3 billion at risk for infection (Jamshaid, 2006). Approximately one third of infected individuals have physical manifestations of elephantiasis. Physical disabilities due to elephantiasis and other chronic organ damage result in the loss of nearly 6 million lives per year. The greatest impact is in Asia followed by Africa (WHO, 2006).

Mosquitoes belong to the order Diptera, a group of insects that only have one pair of wings located on the methothorax. There are about 3200 species of mosquitoes belonging to the family *Culicidae* (Service and Ashford, 2001). Most species of medical importance fall within the sub-families Anophelinae (of which the most important genus is *Anopheles* (Figure 2) and *Culicinae* (which comprises of *Aedes* (Figure 1), *Culex* and *Mansonia*) (Conn, 1997). There are many species of non-biting gnats and midges, which resemble mosquitoes in several

features but do not have the long forwardly- projecting proboscis (Burgess and Cowan, 1993). Both male and female mosquitoes feed on plant fluids and nectar (Carpenter and Walter, 1974). However only the female mosquito typically requires a blood meal from a warm –blooded animal before a viable batch of eggs can be laid. It is the female mosquito only that is capable of sucking blood (Lehane, 2005). The internal male mouthparts are short and only extend about a quarter of the length of the proboscis. In contrast, the female has long, needle-like mouthparts, which are capable of piercing animal tissue (Kong and Wu, 2009). Male mosquitoes have a pair of long bushy (plumose) antennae, (Figures 3 and 4) whereas the antennae of the female are sparsely haired or pilose (Burgess and Cowan, 1993).

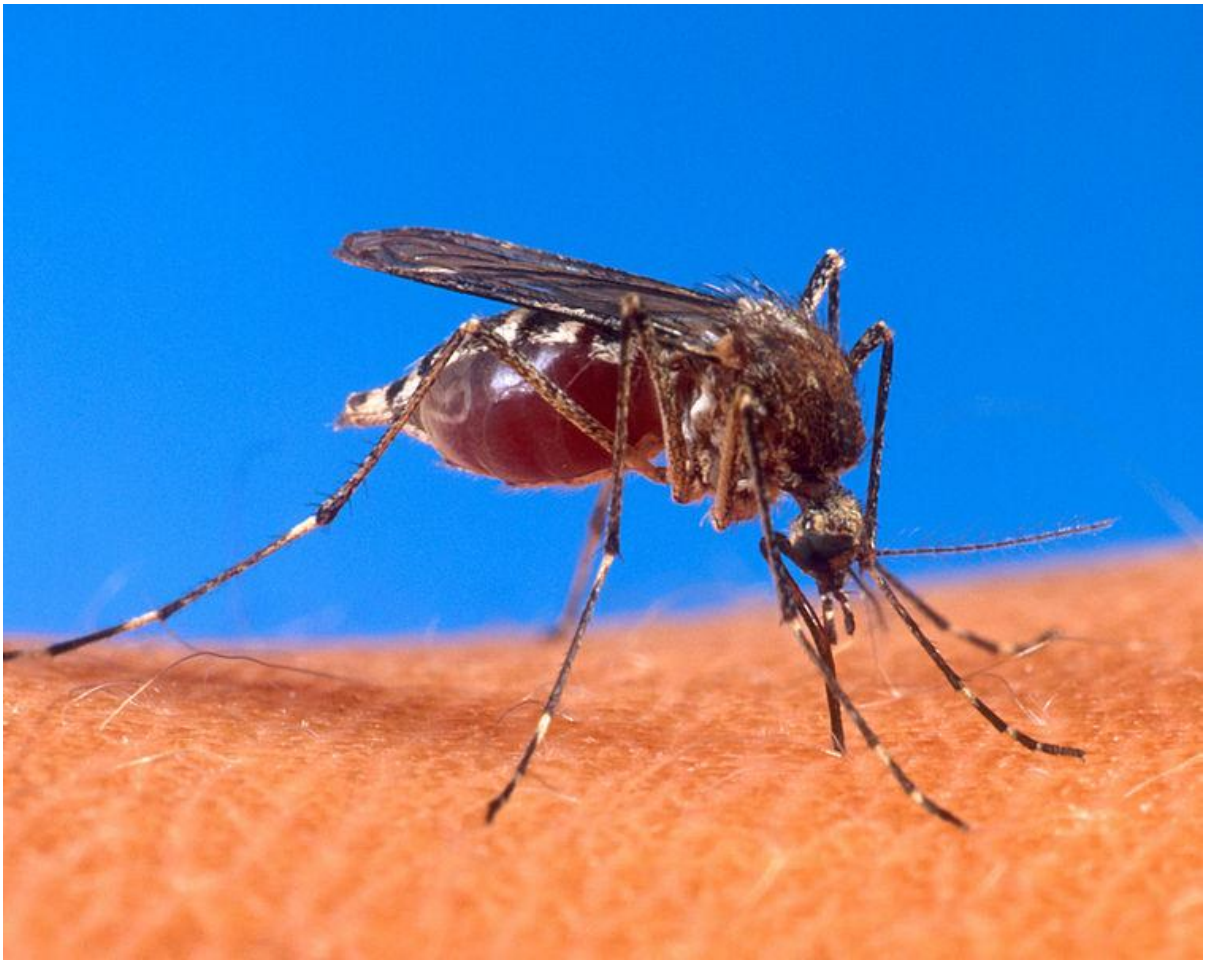


Figure 1: Female *Ae. aegypti* feeding on human flesh (Wikipedia, 2012)

The female *An. gambiae* is distinguished from other mosquitoes by the palpi, which are as long and straight as the proboscis, while palpi of female Culicinae are considerably shorter **(Figure 2)**. Unlike other mosquitoes, the Anopheles have discrete blocks of black and white scale on their wings (Das *et al*., 2007) Adult *Anopheles* can also be identified by their typical resting position: males and females rest with their abdomens sticking up in the air rather than parallel to the surface on which they are resting. One important behavioral factor is the degree to which an Anopheles species prefers to feed on humans (anthropophagy) or animals such as cattle (zoophilic) Howell and Knols, 2009).

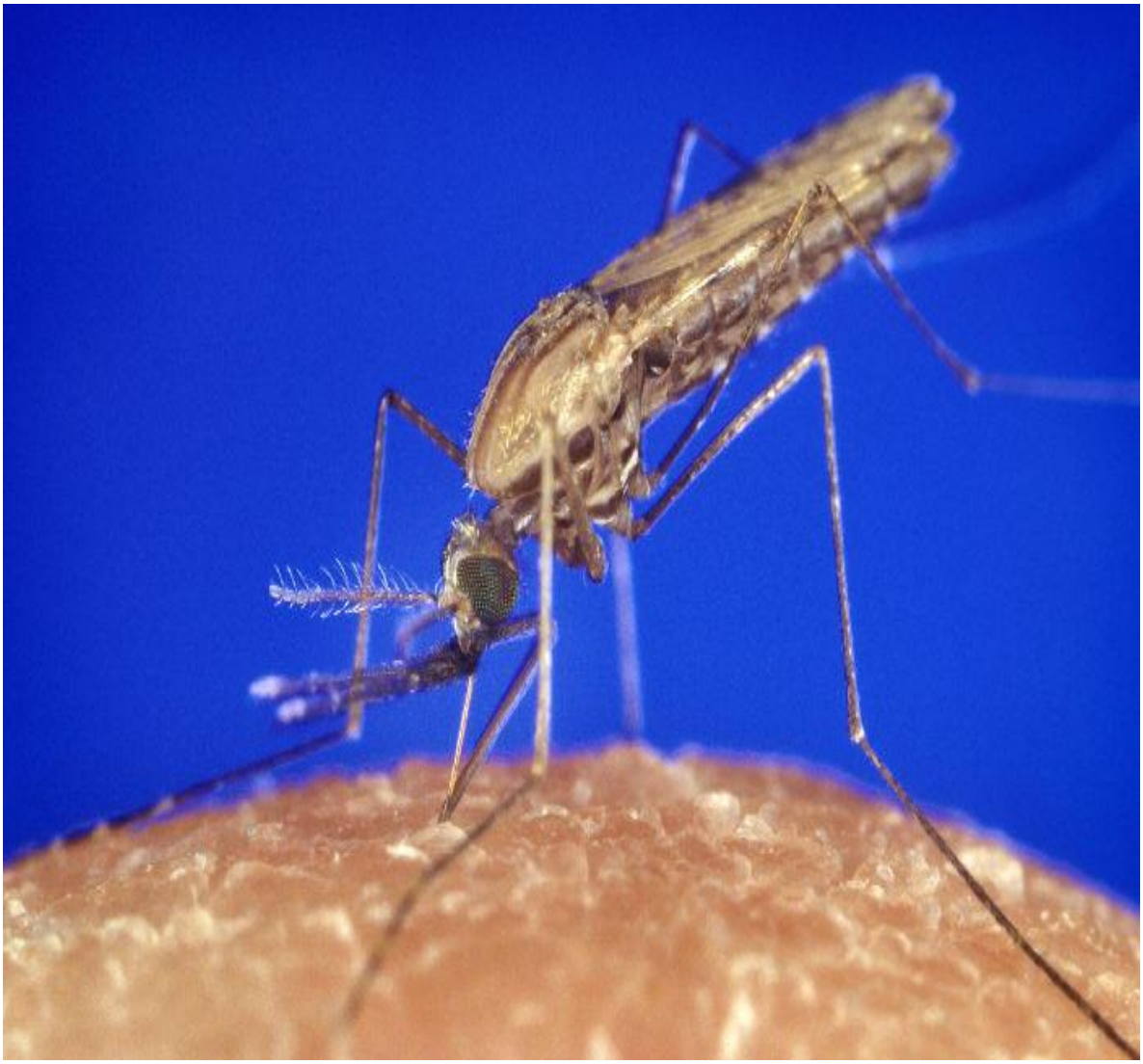


Figure 2: Female *An. gambiae* feeding on human flesh (Wikipedia 2012)

Generally the following features can be used to differentiate a male mosquito from a female mosquito: male mosquitoes tend to hatch before the females, and also generally have shorter lifespan.

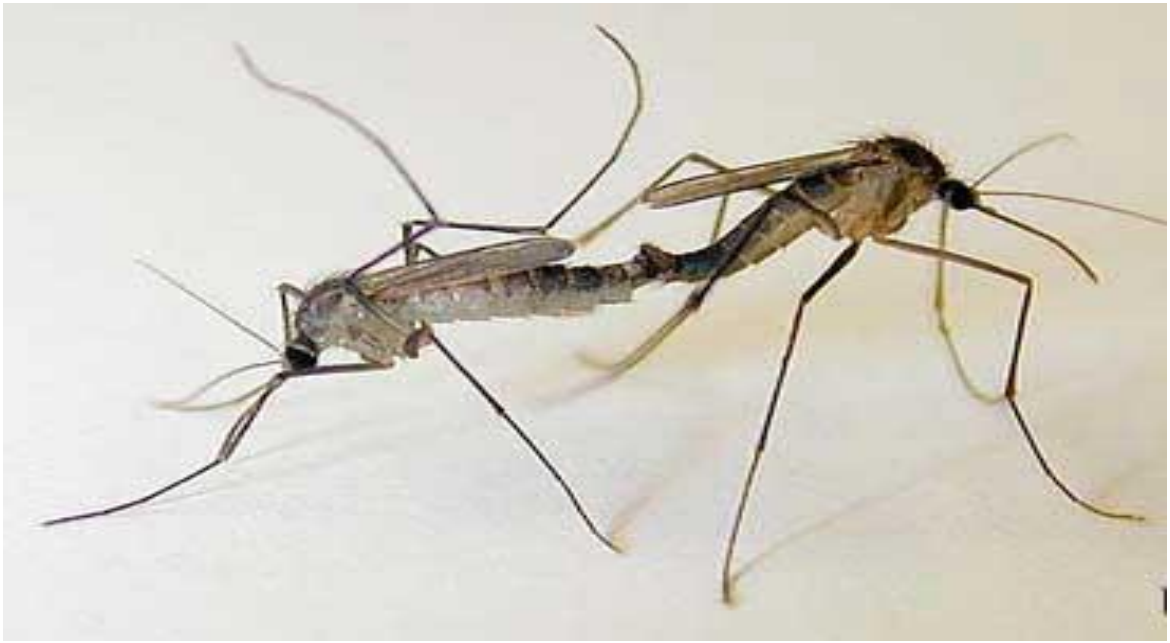


Figure 3: Distinctive proboscis male mosquito (right) and female mosquito (left) (Constant and Lam, 2012)

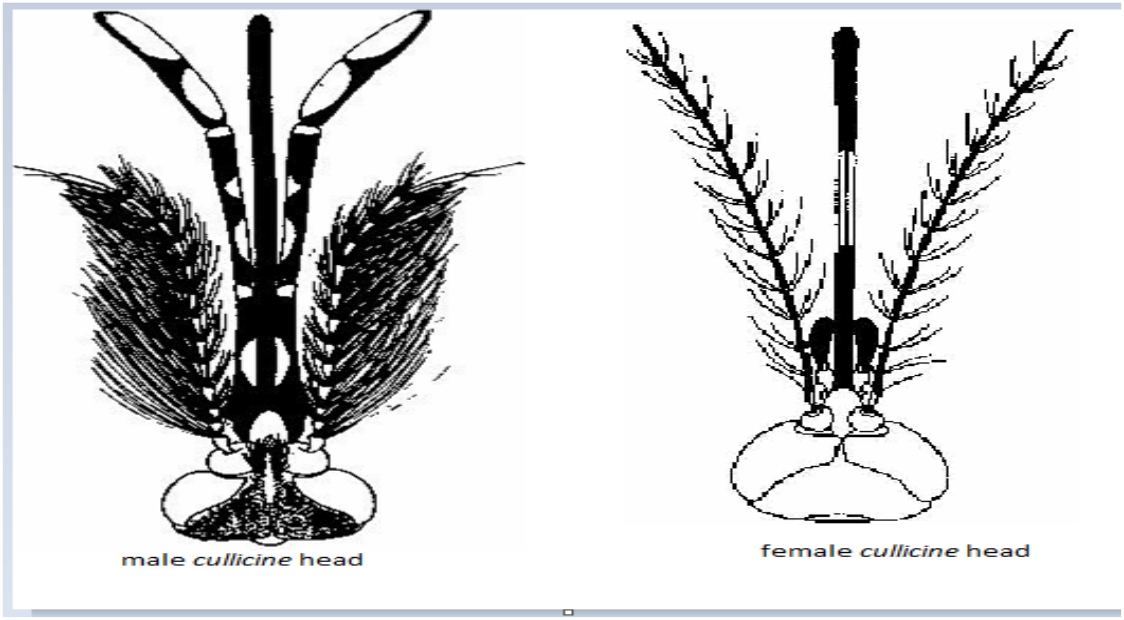


Figure 4: The hairy (plumose) male antennae and the thinly haired (pilose) antennae of female Culicinae mosquito (Clements, 2002)

Both sexes have one pair of compound eyes (Gao *et al.,* 2007). Mosquitoes undergo complete life cycle: egg, larva and pupa before becoming adult (Schäfer and Lundström. 2006). The immature stages are always associated with water which may occur in a ward range of locations (Ulricke *et al.,* 2004). The mosquito egg hatches into a small worm-like larva which feeds on microorganisms in the water or on the water surface using paired mouth brushes on the head (Lehane, 2005). Vision is rudimentary but larvae react rapidly to changes in light intensity, moving actively with a wriggling of darting motion through water (Burgess and Cowan, 1993). The bulky, thoracic part of the larva often has long bristles or hairs, which assist in achieving balance. The larva passes through four stages or instars before molting to the pupa stage (Nishiura *et al.,* 2003).

The pupa is coma shaped; the head and thorax having fused to form cephalothorax, with the abdomen hanging down from it. The pupa stage is actively mobile using a pair of paddles located on the hind end of the abdomen to progress in a tumbling motion through the water (Schäfer and Lundström, 2006). It does not feed but comes to water / air interface to obtain oxygen through a pair of dorsal trumpets on the cephalothoraxes. The adult mosquito can be seen developing through the papal skin. When the adult mosquito is fully developed, the pupa comes out to the surface, and splits across the dorsum, the adult emerging to stand on the water surface, while the exoskeleton hardens and dries. Males will typically emerge first and swarm in the air over the breeding site (Leon and Steven, 2002). When the females emerge mating will take place. The female mosquito stores sperm from a single mating in the sperm theca and these will be used to fertilize eggs from alternate ovaries when required (Burgess and Cowan, 1993). Thus the female mosquito needs to be fertilized once in a lifetime but may lay up to ten or so batches of eggs (Clements, 1992). The female will typically feed in subdued light, especially during the night, but some species will readily feed during the day (Lehane, 2005). Depending on the species, female mosquitoes may rest indoors or outside before or after feeding (WHO, 2006). Identification of the species is thus essential in order that correct control measures are carried out.

2.2 Classification and Distribution of *Anopheles gambiae* complex

There are 460 species of the *Anopheles* mosquito, and about 100 of these are able to transmit malaria and 30-40 are notorious vectors in many parts of the world (Lehrer, 2010). *Anopheles gambiae* is one of the best known because of its predominant role in transmitting of the most dangerous malaria parasite, *Plasmodium falciparum* (Snow et al., 2005). *An. gambiae*

mosquitoes belong to the family *Culicidae*, order *Diptera* in the phylum *Arthropoda* of the animal Kingdom (Leunita and Ogbunugafor, 2008). They constitute a complex of seven sibling species. *An. gambiae s. s.* is the prolific African malaria vector and has expanded its niche into polluted habitats. Recent sdudies found *An. gambiae* larvae thriving in a variety in a variety of anthropogenic urban water bodies, which contained pollution from domestic and/or industrial sewage (Awolola *et al*., 2007). It comprises several chromosomal forms like Bamako, Mopti and Savanna. *Anopheles arabiensis* and *An. gambiae s s.* are isolated reproductively by mainly pre –mating barriers though both occur in sympatry in tropical Africa. Their larval stages inhabit mainly fresh water habitats (Muirhead-Thompson, 1945). *Anopheles melas* Theobald and *Anopheles merus* Donitz are salt-water species found on the West and East coasts of Africa respectively (Gillies and *de* Meillon, 1968). *Anopheles bwamba* White is found in hot water springs in the Ugandan Bwamba County (White, 1985). It breeds in brackish water .*Anopheles quadriannulatus* species B is found in Ethiopia and is considered not as an important malaria vector due to its preference for cattle. *Anopheles quandriannulatus* Theobald is a *Plasmodium falcipurum* vector in South Africa (Walker *et al*., 2007). *Anopheles gambiae sensu lato* is the main vector transmitting *P. falciparum* south of the Sahara. The sibling's species belonging to *An. gambiae* complex are morphologically indistinguishable. Differences between them have been detected using various techniques including the use of banding patterns of polytene chromosomes, enzyme variations banding sequences of mitotic sex chromosomes, DNA and RNA probes (Colluzi *et al.*, 2002 and Fanello *et al*., 2002).

2.3. Life cycle of *Anopheles gambiae* and *Aedes aegypti*

Mosquitoes act as vectors for many human diseases including malaria, West Nile, yellow fever, encephalitis, and dengue (Service, 2001 and WHO, 2006). To understand a disease like dengue fever it is essential to understand the life cycle of the mosquito host, the human host, and the virus, and also to understand the various environmental factors that can support disease transmission including potential epidemics and pandemics (Gubler, 1998). *Anophelines* and *Aedes* mosquitoes go through complete metamorphosis of four stages in their life cycle: egg, larva, pupa and adult (Service, 1993) (**Figure 5**). The first three stages are aquatic and last 5-14 days, depending on the species and the ambient temperatures. Adult female *Anopheles* mosquitoes act as vectors for the malaria parasites and filarial worms (Gillies and De Meillon, 1968). The adult females can live up to a month (or more in captivity) but most probably do not live more than 1-2 weeks in nature (Leunita and Ogbunugafor, 2008). The adult female lays 50-200 eggs per oviposition. Eggs are laid singly directly on water and

are unique in having floats on either side. Each egg is protected by an egg shell, which in many species is elaborately sculpted. Eggs are not resistant to drying and hatch within 2-3 days, although hatching may take up to 2-3 weeks in colder climates (Clements, 1992). Mosquito larvae have a well-developed head with mouth brushes used for feeding, a large thorax and a segmented abdomen. In contrast to other mosquitoes, *Anopheles* larvae lack a respiratory siphon and position themselves so that their body is parallel to the surface of the water (Gillies and De Meillon, 1968). Larvae breathe through spiracles located on the 8th abdominal segment and therefore must come to the surface frequently. The larvae spend most of their time feeding on algae, bacteria, and other microorganisms in the surface micro layer (Clements, 1992). They dive below the surface only when disturbed. Larvae swim either by jerky movements of the entire body or through propulsion with the mouth brushes. Larvae develop through four stages, or instars, after which they metamorphose into pupae (Goma, 1959). At the end of each instar, the larvae molt, shedding their exoskeleton, or skin, to allow for further growth. Larvae of mosquitoes occur in a wide range of habitats but most species prefer clean, unpolluted water (Clements, 1999).

Larvae of *Anopheles* mosquitoes have been found in fresh or salt-water marshes, mangrove swamps, rice fields, grassy ditches, the edges of streams and rivers, and small, temporally rain pools (Gilbert and Calderone, 2007). The pupa is comma shaped when viewed from the side. The head and surface of the cephalothorax splits and the adult mosquito emerges (Clements, 1992). The whole process from egg to emergence of the adult from the pupa takes not more than a week at tropical temperatures (Gillies and De Meillon, 1968). Adults of *An. gambiae* emerge during the late afternoon, and once mature, mate during a twenty-minute period at dusk. When a female has matured a batch of eggs, she takes to the wings and responds to stimuli from suitable oviposition sites. For most mosquitoes, the oviposition site is a water body with particular characteristics: odor, taste, flow and shade; all known to influence different species (Clements, 1999). Within an hour of completing one gonotrophic cycle; a female may commence another. At warmer temperatures, like in the tropics, *Anopheles* oviposits regularly every two or three days (Service, 1993). Many species prefer habitats with vegetation. Others prefer habitats that have none. Some breed in open, sun-lit pools while others are found only in shaded breeding sites in forests. A few species breed in tree holes or the leaf axils of some plants (Blackwell and Johnson, 2000). The *Aedes aegypti* mosquito can be recognized by white markings on legs (Womack, 1993) (**Figures 1 and 5**). The mosquito originated in Africa but is now found in tropical and subtropical regions throughout the world.

The immature stages of the mosquitoes are always associated with free water of some sort but the type and location vary considerably (Munga *et al*., 2005). Some breeding places for *Aedes aegypti* are artificial water containers, such as the odd plastic bucket, flowerpot "saucer", or discarded bottle or tires. *Aedes* females generally drop their eggs singly, much as *Anopheles* do, but not as a rule into water (Wikipeadia, 2012). Instead, they lay their eggs on damp mud or other surfaces near the water's edge. Such an oviposition site commonly is the wall of a cavity such as a hollow stump or a container such as a bucket or a discarded vehicle tire. The eggs generally do not hatch until they are flooded, and they may have to withstand considerable desiccation before that happens (Nelson, 1984). They are not resistant to desiccation straight after oviposition, but must develop to a suitable degree first (Service, 1993). Once they have achieved that, however, they can enter diapause for several months if they dry out. Clutches of eggs of the majority of mosquito species hatch as soon as possible, and all the eggs in the clutch hatch at much the same time (Clements, 1992). In contrast, a batch of *Aedes* eggs in diapause tends to hatch irregularly over an extended period of time. This makes it much more difficult to control such a species than those mosquitoes whose larvae can be killed all together as they hatch (Wikipedia, 2012). Some *Anopheles* species do also behave in such a manner, though not to the same degree of sophistication (Huang *et al*., 2006).

One method of classifying mosquitoes, which is important in the control of the larval stage, is by the type of habitat in which the eggs are laid. Such species that lay eggs singly on the moist soil usually near the edge of temporary pools of water are known as flood water mosquitoes. These eggs only hatch after they have been flooded by water. *Psorophora*, *Aedes*, and *Ochlerotatus* mosquitoes are floodwater mosquitoes which are most abundant shortly after spring rainfall (Oklahoma, 2012). Those species that lay eggs on the surface of the water, either clumped in rafts or as single floating eggs, are known as permanent water mosquitoes. *Anopheles*, *Culiseta*, and *Culex* are permanent water mosquitoes. Eggs of *Aedes aegypti* are long, smooth, ovoid shaped, and approximately one millimeter long. When first laid, the eggs appear white, but within minutes they turn shiny- black. In a warm climate such as the tropics, the eggs may develop in as little as two days, whereas in cooler temperatures development can take up to one week (Foster and Walker, 2002). Anopheline and Culicine larvae can be distinguished in the field by their different resting positions in the water (Clements, 1992). *Culicine* larvae have siphons through which they obtain air from the surface, enabling it to feed below the surface .*Aedes aegyp*ti larvae also have no palpate hairs. The males of *Aedes aegypti* feed on nectar while the females almost exclusively feed on blood (Wikipedia, 2012). This

explains how the arboviruses are transmitted by the *Aedes aegypti* mosquito as the female mosquito spreads the yellow fever, dengue fever and filarial worms while in search of blood (Linquist *et al*., 2012). *Aedes aegypti* also becomes a vicious vector of the arbovirus and filarial worms due to the female habit to take blood meals more than once in a life time. The adult Aedes aegypti is a small to medium sized mosquito, approximately 7 millimeters .Aedes adults have white scales on the dorsal (top) surface of the that basal bands forming the shape of violin or lyre (Carpenter and La Casse, 1985).

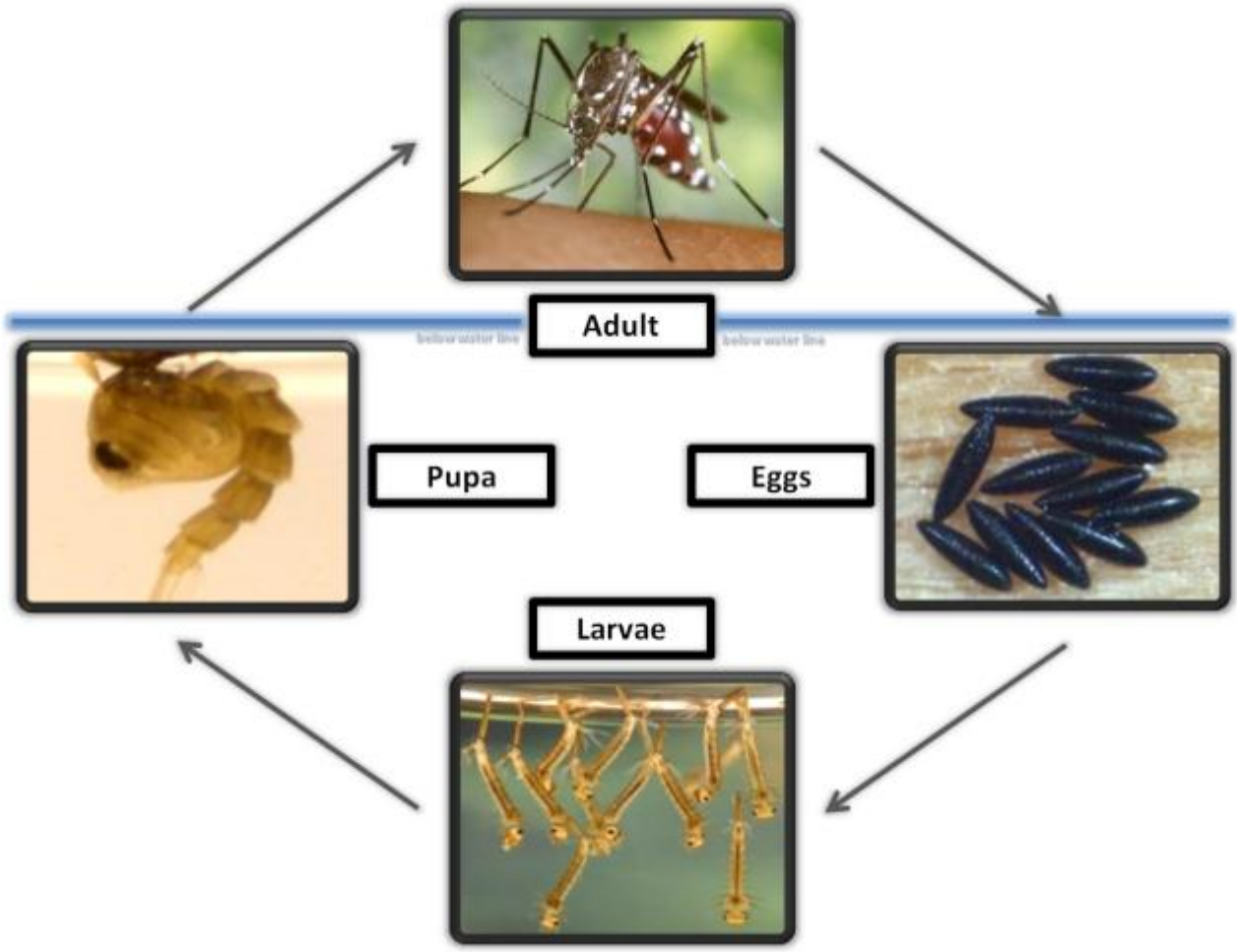


Figure 5: The complete life cycle of a mosquito (Oklahoma University, 2012)

2.4 Control Measures for mosquitoes

Efforts to eradicate malaria have failed and parasitic resistance to the most commonly used and affordable anti- malarial drugs is developing rapidly (Leunita and Ogbunugafor, 2008 and WHO, 2011). Insecticide resistance in the vector is also an evolving problem, (Hargreaves *et al*, 2003). A malaria vaccine is the subject of most research but its testing is incomplete and

full development remains a distant goal (Francine *et al*., 2003). The use of residue pyrethroids to treat bed nets became more fashionable as a means of controlling adult stage malarial vectors for the following reasons: first, most *Anophelines* bite indoors late at night and bed nets thus intercept mosquitoes as they approach sleepers in search of blood (Clements, 1999). Secondly, in many countries there has been a tendency to replaster walls as soon as they have been sprayed covering up insecticide deposit (WHO, 1996). While insecticide treated nets (ITNs) are currently the priority strategy in containing the adult stage of the mosquito, there is growing interest in attacking the aquatic stages of malaria vectors with microbial larvicides in conjunction with environmental management (Utzinger *et al*., 2002) This initiative that originally started in Tanzania, was meant to spread to other countries under the Presidents' Malaria Initiative (Utzinger *et al.,* 2002). Global initiatives to fight malaria and other vector borne diseases so as to manage and control them to tolerable levels include: Roll Back Malaria (RBM), Medicines for Malaria Venture (MMV), Malaria Vaccine Initiative (MVI), Mapping Malaria Risk in Africa (MARA), Multilateral Initiative on Malaria (MIM), The Presidents Malaria Initiative (PMI) and other global partners (WHO, 2012). These initiatives are responsible for the distribution of insecticide treated bed nets, use of treatment based on combination therapy (Mugo, 2012), concise delivery of insecticides and drugs (RBM), development of new antimalarial drugs (MMV) the development of chemical testing of malaria vaccines (MVI), the mapping of malaria risk (MARA) and the promotion of malaria research and capacity building (MIM). A marked increase in malaria has recently been noted in the African highlands, largely due to the rise of drug-resistant strains of *Plasmodium falciparum* parasites (Bøcker *et al*., 2000). The ecological features of the western highlands of Kenya support stable parasite transmission, and increasing population pressure has led to the clearance of natural swamps, massive deforestation and crop cultivation in the valley bottoms (Munga *et al* 2006). Because of these agricultural changes, many water bodies are now exposed to the sun and provide ideal conditions for vector proliferation and increased malaria transmission (Briet *et al*., 2003). Malaria control in these highlands is based on insecticide-treated nets (ITNs), indoor-residual spraying (IRS) with insecticides and prompt and effective treatment of clinical malaria Many believe integrated vector management (IVM), is the way forward in malaria control (Role Back Malaria, 2005 and Nagera *et al*., 2011). Integration of larval source management into ongoing programmes is likely to be most effective when transmission is moderate or low where mosquito breeding sites are contained and well defined (Kitron *et al*., 1989). This will work mainly in urban and peri urban areas. Larval control can be attained

through environmental management, large space coverage and community participation (Utzinger, 2001). This can be achieved by use of chemical or biological control.

Mosquito larvae basically have the nature to breed everywhere in small amounts of water on the surface of the ground and hence their control can be acceptable only under suitable mapping and characterization of breeding sites. Vector control should target all stages of the mosquito life cycle yet for the last 50 years it has focused on the adult mosquito control (WHO, 1999). While ITNs have saved live in Africa, their use is limited as adult mosquitoes feed outside houses and before sleeping hours. Hence larval source management targeting both indoor and outdoor vector populations should contribute to greater reductions in transmissions than ITNs alone (Kitron and Spielman, 1989).There has been reduced efficacy of insecticide treated nets and indoor residue spraying for malaria control due to pyrethroids resistance (N′ Guessan *et al.,* 2007). Also there have been reports of emergence of resistance in field populations of tropical *Culex quiquefasciatus* to the microbial agent *Bacillus sphaericus* (Mulla *et al*., 2003). Integrated vector management (IVM) programme consists of standard water management, open marsh-water management, biological control, larval and adult mosquito control. These techniques are based on an understanding of the mosquito life cycle. Water management exploits the fact that the larvae are vulnerable to removal of water they need to survive on. Biological control uses fish and other predators to eat the larvae. Water management and biological control are combined in open marsh water management. Larval control targets mosquito's larvae using highly specific materials such as bacterial pesticides and insect growth regulators (Amy, 2006). Bacterial larvide (*Bacillus thuringiensis* var.i*sraelensis*) is highly specific to mosquito larvae and is an environmentally friendly product (WHO, 2005).

A new bacterial granular larvicide with the trade name Vectolex ®CG with live B*acillus sphaericus* as its active ingredient is used in larval control programme. This provides a new form of cost effective long term control in areas that continually hold water and breed mosquitoes such as drainage ditches and swamps (Gilbert and Calderone, 2007). A juvenile growth hormone with the trade name Altosid prevents the mosquito from molting from the larval stage to adult. Larvicidal effects of a neem (*Azadiracta indica*) oil formulation on the malaria vector control has been explored and other phytochemicals explored for larvicidal activity include extracts from *Aloe* species and cashew nut shell in their possible use in malaria control (Amy, 2006). Protection against mosquito bites includes closing the doors and windows in the evenings to prevent entry into human dwellings, using mosquito repellant lotions, creams, mats or coils (WHO, 2006). Local infestations can be managed using hand held or

truck mounted sprayers. Adult control is accomplished using ground or aerial applications of ultra- low-volume (ulv) aerosols of materials that rapidly degrade in the environment (Gilbert and Calderone, 2007 and Nobert *et al*., 2010). Travelers to endemic areas and high-risk individuals should be started on anti- malarial drugs to suppress malaria (Kakkilaya 2006). WHO responds to dengue in the following ways: supports countries in the confirmation of outbreaks through its collaborating network of laboratories; provides technical support and guidance to countries for the effective management of dengue outbreaks; provides training on clinical management, diagnosis and vector control at the regional level with some of its collaborating centers; formulates evidence-based strategies and policies; develops new tools, including insecticide products and application technologies; gathers official records of dengue and severe dengue from over 100 Member States and publishes guidelines and handbooks for dengue prevention and control for Member States WHO, 2012). The Joint WHO/UNICEF Technical Group on Immunization in Africa recommended in 1988 incorporation of yellow fever vaccine in routine child immunization programmes of countries at risk for yellow fever, and the World Bank's 1993 World Development Report also strongly endorsed adding yellow fever vaccine to the EPI of the at-risk countries (Lhuillier et al., 1989 and WHO, 1998).Measures of yellow fever immunization monitoring and fast-tracking of positive viral load cases has to be adhered to at the country level (Roger *et al*., 2009 and Kemri, 2012).

2.5 Use of plants in mosquito control

The use of plant and plant-derived products to control pests in the developing world is well known and prior to the discovery of synthetic pesticides, plant or plant-based products were the only pest-managing agents available to farmers around the world. There are repots of medicinal plants with insecticidal including antifeedant, larvicidal, ovicidal and repellence activities against mosquitoes. Such plants include those of *Annonaceae*, *Papiloinaceae*, *Meliaceae*, *Mimosaceae* and *Lamiacea*e genera (Kalyana *et al,* 1985). The pool of plants possessing insecticidal substances is enormous. These have generated extraordinary interest in recent years, as potential sources of natural insect control agents. Today over 2000 species of plants are known that possess some insecticidal activity (Jacobson, 1975, 1989). Biochemical pesticides include plant-derived pesticides (botanicals) that can interfere with the growth, feeding or reproduction of pests or insect pheromones applied for mating disruption, monitoring or attract-and-kill strategies. Antioviposition and insecticidal activity of *Imperta cylindrical* (Graminaceae) plant in Ethiopia has been documented Mohsen and *et al*., 1995) Insect vector management is facing the economic and ecological challenge worldwide due to

the human and environmental hazards caused by majority of the synthetic insecticide chemicals (FAO, 1992).

Identification of novel effective insecticidal compounds is essential to combat increasing resistance rates that have been associated with synthetic convectional insecticides. Plant extracts have been found to be advantageous for use in field mosquito programs (Abebe and Ayehu, 1993). Secondary metabolites present in plants apparently function as defense (toxic), which inhibits reproduction and other processes (Rattan, 2010). The botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms including man. They are also biodegradable and harmless to the environment. Furthermore, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise an array of chemical compounds which act concertedly on both behavioral and physiological processes (Adeyemi, 2010). Thus the chances of insecticides developing resistance to such bio insecticides are unlikely. Among the phytochemicals found to have insecticidal potential are the triterpenoids, diterpenes, monoterpenes, alkaloids, flavonoids and steroidal saponins (Isman and (Machial, 2006). Use of botanical involves the development of the green technology using oil-in-water micro-emulsions as a nano-insecticide delivery system to replace the traditional emulsifiable concentrated oil. This in essence reduces organic solvents and increases the dispersity, wettability and penetration properties of the droplets (Koul *et al* 2008). The antifeedant behavior of the plant triterpenoids secondary metabolites prevent the insects from feeding but do not immediately kill them hence the effective results can be accomplished by integrating them with more creative strategies. Such a strategy can be incorporating them with insect growth regulators (Adeyemi, 2010). Larvicidal activity of the leaf plant extracts of *Paullina pinnata Linn* against *An.gambiae* has been attributed to the presence of alkaloids, tannins and saponins in it Jaiyesimi *et al*., 2011. The plant extracts of *Hemidesmus indicus*, *Gymnema sylvestre* and *Eclipta prostrate* have been found to have larvicidal potential against *Culex quinquefasciatus* mosquito larvae (Gopiesh *et al*., 2007). Phytochemical constituents of Ethiopian medicinal plants were screened and found to have larvicidal activity against *Ae. aegypti*, *Ae. africanas* and *Culex quinqufasciatus* third and fourth instar larvae (Asfaw *et al.,* 2007). Essential oils from *Striga hermothica, Hyptis spicigera* and *Ocimum basilicum* leaf extracts have been found to have mosquito repellency activity against *An. gambiae* and *Culex quinquefastus* (Gabi *et al* 2012).The use of plant secondary metabolites in the fight against the diseases vectored by mosquitoes will be more

cost effective compared to the synthetic derived chemical that has to be imported hence more uneconomical (Adeyemi, 2010).

2.6 Mode of Action of Insecticides

To understand the mode of action of insecticide, it is necessary to understand how the targeted systems in pests normally function. This would help prevent development of pesticide resistance in the target pests. Using pesticides with similar mode of action, contributes to the problem of killing susceptible pests and leaving only those with resistance to the entire class of pesticides. Development of pest resistance can be avoided or delayed by rotating pest control chemicals that work through different modes of action (Amy, 2006). Insecticides and miticides generally target the nervous system, growth and development or energy production of the pest. Both human and insects have many different neurotransmitters that work at different sites through the nervous system. Of the many neurotransmitters that both insects and humans have, acetylcholine (ACh) and gamma-amino butyric acid (GABA) are important targets of some insecticides. Acetylcholine can either excite or inhibit its target neurons depending on the particular neuron and the receptors at the receptor sites. Acetylcholine can cause particular neurons to "fire" continuing the nerve impulse transmission, or it can cause the nerve impulse to stop at that particular site. In contrast, GABA is an inhibitory neurotransmitter. When GABA is the neurotransmitter activated at the synapse, the nerve impulse stops. Some insecticides interfere with the normal action of these neurotransmitters. Organophosphate and carbamate insecticides are known as cholinesterase inhibitors (Amy, 2006). They bind to the enzyme that is normally responsible for breaking down acetylcholine after it has carried its message across the synapse. This causes over stimulation of the nervous system and the insect dies. Although cholinesterase inhibition by carbamates can be reversible, organophosphate poisoning is irreversible. Electrically charged ions move along neurons in channels which include the sodium, calcium, potassium and chloride channels. Many of the channels have gates that open or close in response to a certain stimulus, which is an important mechanism through which some insecticides work. Avermectins derived from a soil microorganism act by binding to the chloride channel causing an inhibitory effect that causes the death of the pest. Organochlorine insecticides of the cyclodiene type affect the chloride channel by inhibiting the GABA receptor, (Dong, 1997). Pyrethrins, which are naturally-occurring compounds derived from the *Chrysanthemum* family are sodium channel modulators. They have a quick knockdown effect against insects, but are unstable in the environment and therefore they do not last long enough to kill the pest.

Pyrethroids are synthetic versions of pyrethrins and act in tiny channels through which sodium is pumped to cause excitation of neurons, resulting in continued nerve impulse transmission, tremors and eventually death (Amy *et al*; 2005). *Bacillus thuringiensis* (Bt) which produces crystalline inclusions composed of endotoxins, referred to as crytoxin Bt crystals, and must be ingested in the alkaline insect midgut, where they dissolve into protoxins, which are cleaved by proteases into active toxins. When Bt is eaten by a larva, it attacks the lining of the insect's midgut and causes it to stop feeding and die. Some insecticides such as organotin miticide inhibit oxidative phosphorylation directly, while pyrroles work by uncoupling oxidative phosphorylation from electron transport. In either case, the cell is unable to produce ATP as a source of its energy. Studies of the biophysics and biochemistry of receptors and enzymes associated with insecticide targets elucidates potential binding sites and illustrate that these proteins are dynamic molecules that interact in various conformation with their antagonists and agonists (Amy,2006). Some insecticides target the insect growth and development processes by interfering with hormones and others by blocking the production of structural components of the exoskeleton. Juvenile hormone (JH) analogue insecticides act as JH agonists and generally show the highest toxicity when applied at the onset of metamorphosis (Thomas, 2004). A physiological basis for the toxicity and morphogenetic effects of these insecticides has been suggested by linking their effects with interference of the expression or action of certain genes especially the Broad- complex (BR-C) transcript gene. *Azadirachtin*, derived from neem oil, interferes with the insect development hormone, prothoracicotromic hormone. The active principle from the seed kernel, the tetranortriterpenoid (limonoid), has the insecticidal effect (Tomlin, 2003). There are numerous methods for studying gene expression which include the substractive reverse transcriptase chain reaction (RT-PCR) of pests exposed to sub lethal doses of the insecticides. Because of the potential effect of insecticides on the expression of certain genes, it is important to establish the mode of action of insecticides by evaluating gene expression profiles

2.7 Vector Resistance to Insecticides

A mechanism of pest resistance to insecticides is either through mutation within the target site of the insecticides or alteration in the rate of insecticide detoxification. Three enzyme systems: glutathione s-transferase, esterases and monooxygenases are involved in the detoxification of the major insecticide classes. These enzymes act rapidly, metabolizing the insecticides to non-toxic products or turning over the insecticide (sequestration). Gene splicing, amplification and regulation plays a major role in mosquito insecticides (Chouaibou *et al.*,

2008) Replacement of alanine 302 in the RDL containing GABA receptor not only interacts directly with the drug binding site within the ion channel pore but also allosterically destabilizes the preferred desensitized state of the insecticide receptor. In the para gated sodium channel, individual mutations in different channel polypeptides combine to cause mutations similar to those found in the same polypeptide, suggesting that each mutation plays a unique role in affecting channel function (Dong, 1997). In the *para*-voltage gated channel, amino acid replacements are confined to only two positions (Guerrero *et al*., 1997). The first, associated with the original kdr strain, is in S6 hydrophobic segment of homology domain II, and the second is associated with another more resistant allele, termed-super-Kdr. This "kdr"- like replacement is similar in the housefly, horn fly, cockroach the tobacco budworm Helothis *virescens*, the aphid and the mosquito *An. gambiae* (Amy *et al*; 2005). Acetyl cholinesterase encoding gene Ace, undergoes a limited subset of amino acid replacement (Weill *et al*., 2000 and Weill *et al*., 2004). This causes insecticidal resistance despite the widely differing sizes and structure of different organophosphate and carbamate insecticides (Amy, 2006). Resistance to dieldrin and fibronil has been associated with chromosome inversion 2La in *An. gambiae*. Pyrethroid insecticides' resistance has also been reported in *Anopheles funestus* in South Africa (Hargreaves *et al.,* 2003).

Since the kdr mutation was first detected in *An. gambiae* from Africa in 1998, molecular monitoring has been intensified world over. This mutation was found exclusively in the S molecular form of the *An. gambiae* "group" in West and Central Africa (Weill, 2004). The mutation was also found in Southern Benin, Cote-d' Ivoire and Burkina Faso in the M molecular form. A different Kdr type mutation (Leucine-phenylalanine) has been found in *An. gambiae* in Western Kenya. Metabolic pyrethroid resistance has been found in *Anopheles sundaicus* found in Southern Vietnam, a vector of local importance in this area (WHO, 2006). A mutation confirming resistance to carbamates and organophosphates has been detected in West Africa in *An. gambiae*. From the forgoing literature, management of resistance to insecticides by insects relies on proper monitoring of the resistance. This activity has to be integrated as a component of any malaria vector control program.

2.8 Characteristics of *Aloe* species

There has been a concerted effort worldwide to screen traditional medicinal plants for their insecticidal potential (Asfaw *et al*., 2007). The active constituents have been found to be antiprotozoal, antifungal, antibacterial, antinematode and effect on behavioral and physiological responses on target organisms (Adeyemi, 2010). The Aloe species has active

phytochemical ingredients that need to be investigated for their potential use in biprospecting for new botanicals in mosquito control measures (Isman and Machial, 2006). *Aloe*, in the family of *Aloaceae* , is composed of herbaceous, shrubby or perennial, xerophytic, succulent plants found in tropics and Southern African regions (Van Damme, 1991). The genus *Aloe* has nearly 400 species confined to Africa. It is common in Kenya with about 60 taxa recognized. Most of the taxa have a restricted distribution with *Aloe secundiflora* var *secundiflora* being widespread in the country (Baillie *et al*., 2004). *Aloe vera* is one of the species which originated from Africa and is a plant of dry sandy places and hilly sides. Its fleshy cactus like appearance distinguishes from other herbs (Beentje and Smith; 2001). The diversity pattern in Kenya indicates a high concentration of *Aloe* in three areas that are known as *Aloe* hot spots and thus of high priority for conservation of the genus. These areas are; the Kulal-Nyiro-Ndotos-Marsabit areas in the north, the Taita-Shimba Hills zone to the South- East and the Naivasha-Baringo area in the Rift Valley. *A. turkanensis* occurs in north western Kenya and in the Karamajong District of Uganda. *Aloe turkanensis* (**Figure 6**) grow on stony, sandy soil or lava/ usually in the shades of shrubs in arid areas at 600-1,250m altitude (Wabuyele *et al*., 2006). *Aloe* plant species have been used all over the world for their various medicinal properties. Treatment of streptocin induced-diabetic rats with *Aloe barbadensis* (*A. vera),* increases antioxidant enzymes like superoxide dismutase and it also decreases lipid peroxidation product levels (Nwanjo, 2006). Cinnamoyl, P-coumaroyl, feruloyl, caffeoyl, aloesin and related compounds have been isolated from *Aloe* species. The anti-inflammatory and antioxidative activities of these compounds have been examined based on the structure-activity relationship (Akira, 2008).

Investigation using the contact hypersensitivity response indicates a preventive effect of aloesin on the UV-B-Induced immune suppression (Chaz, 2008). Aloesin inhibits tyrosine hydroxylase and dihydrophenylalanine (DOPA) oxidase activities of tyrosinase from normal human melanocyte cell lysates. This makes aloesin a positive pigment-altering agent for cosmetic application (Yagi and Takeo, 2003). The fact that *Aloe vera* can alter skin pigmentation points to the fact that its constituents could have physiological and physical effect on target organisms, including mosquitoes (Adeyemi, 2010).

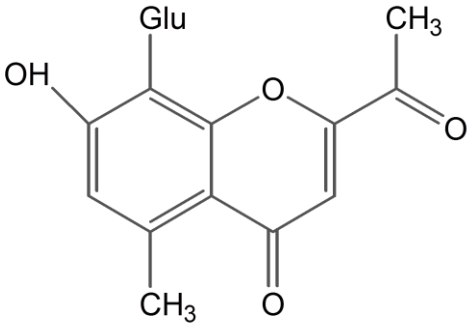
Figure 6: *Aloe turkanensis* plant prevalent in North Western Kenya and the Karamajong District of Uganda (Bosch, 2006).

Neutral polysaccharides such as aloemannan and acemannan have shown anti tumor, anti-inflammatory and immunosuppressive activities (Singh *et al.*, 2000). Glycoprotein fractions with body kinindegrading and cell proliferation-stimulating activities have been identified from the nondialysate fraction of the gel of *Aloe* species (Tan and Vanitha 2004). Aloesin has also been found to stimulate the proliferation of cultured human hepatoma SK-Hep 1 cells, with other related and high molecular-weight materials such as verectin and aloemannan could exert therapeutic properties for wounds, burns and inflammation (Yakugaku, 2003). *Aloe* plant species have been considered to have a hypoglycemic effect and many diabetic patients take the gel because of its hypoglycemic effect (Tiwari and Rao, 2002). *Aloe vera,* one of the most important species of *Aloe* is considered to have hypotensive,

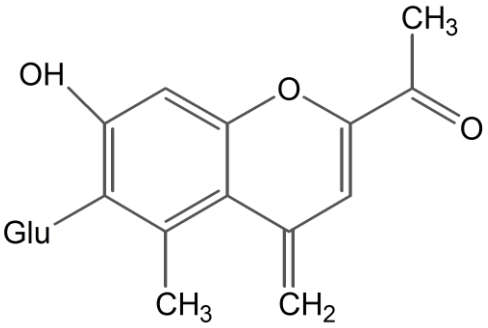
hepatoprotective and blood purification property (Nwanjo, 2006). Chaz, (2008) asserts that *Aloe* plant species have the property to boost the immune system, avert allergies, sinusitis and bronchitis. The polysaccharides in *Aloe* species lowers down the body's serum lipids by lowering triglycerides, low-density lipoprotein levels and increasing high-density lipoproteins (Yagi; Takeo, 2003). Several compounds from the *Aloe* –based alcoholic beverages have been separated and identified (Tawfik *et al*., 2001). Among the noticeable compounds include; aloesin, aloeresin A, hydroxyaloin, aloin A and B and aloinoside A and B. Among other constituents isolated from *Aloe* vera, phenolic compounds containing alkaloidal groups have insecticidal activities (Tan and Vanitha, 2004).

2.8 *Aloe* species Phytochemicals

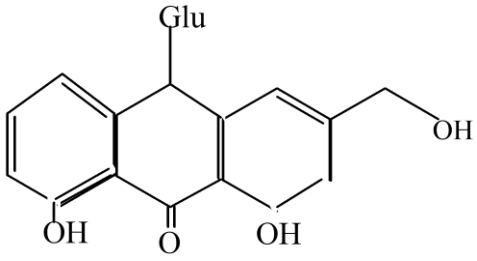
The chemical composition of *Aloe* leave exudates has been investigated (Nauwinger, 2000). The compounds that have been identified can be classified into two main groups namely chromones and anthraquinones. In some cases both types are present, and in others only one. Many of the major constituents such as isoaloesin (**1**) aloesin (**2**) and aloin (**3**) of **Figure 7** occur in chemo-taxonomically distinct species. *A. vera's* mucilaginous pulp contains 93-96% water and the rest are solids that contain active compounds for its biological activity. The mucilage of the leaves also contain various sugars and amino acids.



1. Isoaloesin



2. Aloesin



3. Aloin

Figure 7: The chemical formulae of the phytochemicals found to be prevalent in the *Aloe* plant species (Dagne et al., 1996)

2.9 Phytochemistry and plant pharmacology screened medicinal plants

Plants are nature's chemical factories" providing the richest source of organic chemicals on earth. The use of plants , whether herbs, shrubs or trees in parts or in whole treatment management of diseases and disorders date back to pre-historic days (Egwaikhide, 2007). The detection of active principles in medicinal plants plays a strategic role in phytochemical investigation of crude plant extracts and is very important in regards to their potential pharmacological effects (Majaw and Moirangthem, 2009). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavanoids, and phenolic compounds. (Edoega *et al*., 2005). In the recent years the emphasis to control the mosquito populations has shifted from the use of convectional chemicals towards more specific and environmentally friendly materials, which are generally of botanical origin (Matasyoh *et al.*, 2008). For this reason a lot of phytochemicals extracted from various plant species have been tested for theirs larvicidal and repellent actions against mosquitoes (Ciccia *et al*., 2000)

Terpenoids exert their effect as anti- bacteria, anti-fungi, anti-viral, anti- protozoan, anti-allergens and anti-neoplastic agents (Maiyo *et al*., 2010). Membrane disruption may be the mode of action employed by terpenoids on target organisms. Flavanoids are structural derivatives of flavones. They contain conjugated aromatic systems often bound to sugar (s) as glycosides. Flavanoids are water soluble. Flavanoids act as anti- microbials by complexing extracellular proteins, soluble proteins and bacteria cell wall. Terpenoids form a large and structurally diverse family of natural products derived C5 isoprene units. Typical structures contain carbon skeletons represented by $(C_5)n$ and are classified as hemiterpenes (C5), monoterpenes (C_{10}), sesquiterpenes (C15), diterpenes (C20), sesterpenes (C_{25}), triterpene (C_{30}) and tetrapenes (C_{40}) (Rohmer M. 1999). Terpenoids exert their anti- bacteria, anti-fungi, anti-viral, anti- protozoan, anti-allergens and anti-neoplastic agents (Maiyo *et al*., 2010). Membrane disruption may be the mode of action employed by terpenoids on target organisms and microbial cell membranes (Gadelha *et al.,* 2005). Saponins are surface active agents. They have soapy like properties. They can be detected by their ability to cause foaming and to haemolyse red blood cells (Harboune, 1991). Steroidal saponins have been known to have anti-protozoa activity by reacting with cholesterol in the protozoal cell membrane (McAllister, 2001). *Aloe* plants from arid area of South Africa were found to contain secondary phenol metabolites (Gutterman and Chausen, 2007). Phenolic compounds have been found to have allelopathic potential on other vegetation (Anne *et al.,* 2005). Tannins are astringent, bitter plant polyphenols. They bind, precipitate or shrink proteins (Amy, 2006)

CHAPTER THREE
MATERIALS AND METHODS

3.1Sampling of test *Aloe* species

Aloe plant species *A. turkanensis*, *A. ngongensis* and *A. fibrosa* were collected from Turkana, Ngong and Kajiado regions of Kenya respectively. With the assistance of a taxonomist, plant materials were identified and a voucher specimen was deposited at the Department of Biological Sciences of Egerton University herbarium. The *Aloe* species were designated labels and further identification of the samples subjected to the sequential organic solvents was carried out:

Aloe species	Voucher specimen Identification Code	Solvent of Extraction	Laboratory Identification Code
A. turkanensis	AT	Ethyl acetate	ATE
A. ngongensis	AN	Ethyl acetate	ANE
A. ngongensis	AN	Hexane	ANH
A. ngongensis	AN	Chloroform	ANC
A. ngongensis	AN	Acetone	ANA
A. ngongensis	AN	Methanol	ANM
A. fibrosa	AF	Hexane	AFH
A. fibrosa	AF	Acetone	AFA
A. fibrosa	AF	Methanol	AFM

3.2 Test Insect

Mosquito colony of *An. gambiae s.s.* was obtained from the Kenya Medical Research Institute (KEMRI), Kisumu, Kenya. This colony was originally collected from Mbita field station (00025'S, 34013'E), South Nyanza, Kenya in December, 2000 where *An. gambiae s.s.* are abundant. At the time of this work, the colony was in the 35th filial generation post field sampling, away from any possible selection pressure by *Aloe* plant species extracts. A laboratory mosquito colony of *Ae. aegypti* was also obtained from Pyrethrum Board of Kenya, Nakuru that had not been under any possible selection pressure by *Aloe* plant species

3.2.1 Rearing and maintenance of mosquito colonies

The insectary strain of *An. gambiae s.s.* was reared in KEMRI insectary, Kisumu, in the Republic of Kenya. The *Ae. aegypti* insectary strain was reared at the Pyrethrum Board of Kenya insectary. The standard procedure for rearing *Anopheles* and *Culicine* mosquitoes was followed (Ford and Green, 1972 and WHO, 2005). All life stages were reared in the insectary (28 ± 2°C, 75 – 80 % Relative Humidity and 12L: 12D photoperiod) of Animal Rearing and Quarantine Unit (ARQU) of KEMRI Kisumu, Kenya and the Pyrethrum board of Kenya, Nakuru. From the day of emergence, adult mosquitoes were provided with 10% sugar solutions soaked in cotton wools. The adult mosquitoes were sampled using an aspirator connected to vacuum Three-day-old female mosquitoes were allowed to feed on anaesthetized mice. The female mosquitoes were singled out due to their conspicuous thinly haired antennae and forwardly projecting proboscis, as opposed to the males that have bushy antennae. Approximately 2-3 days later, oviposition dishes were placed in the cages containing gravid females. The eggs were placed on distilled water and were surrounded with floating wax papers, which served to keep eggs from becoming stranded on the sides of the hatching trays. Approximately 30 mg pulverized Tetramin fish food (Tetra GmbH, Melle, Germany) per pan were sprinkled on the surface of the water twice daily. Pupae were collected daily and transferred to breed cap bowls containing distilled water and placed inside emergent cages covered with nets. A small moist filter paper wrapped in a conical shape is put in the breed cap bowl containing distilled water and kept inside the cage overnight for the mosquito to lay eggs. The bowls were placed in emergent cages for adult emergence.

3.3 *Aloe* leaf homogenization and subsequent solvent extraction of phytochemicals from plant material

The leaf plant materials were cut into small pieces, and macerated in a blender. The homogenates were extracted sequentially with solvents of increasing polarity as follows: hexane, chloroform, ethyl acetate, acetone and methanol. The extracts were filtered through active charcoal to remove the chlorophyll matter. The solvents were removed by roter evaporation under pressure to give five extracts for each species. The yields were noted for each extract.

3.4 Larvicidal tests

Bioassay was performed according to WHO procedure (WHO, 2005). Third instar larvae of *An. gambiae* was sourced from Kenya Medical Research Institute laboratories (KEMRI) Kisumu and bioassay in response to *Aloe* extracts carried out in their laboratories. The *Ae.*

aegypti were sourced from the Pyrethrum Board of Kenya where the bioassay to *Aloe* plant extracts was conducted. Minimum and maximum ranges were determined by carrying out bioassays at concentrations that could kill 10% and 95% of the third instar larvae respectively. The *Aloe* plant extracts were solubilized in analytical grade dimethyl sulphoxide (DMSO) and distilled water added to give serial dilution concentrations. Twenty larvae were placed into each 50 ml disposable cups containing 15 ml of *Aloe* plant extract solution. Larvae were fed with tetramin fish food during all testing. Serial dilutions of *Aloe* plant extracts ranging from 0.05-2 mg/ ml were made. Three replicates were used for each concentration level. Control test (negative control) was carried out in parallel using DMSO and water for comparison. DMSO was kept at a concentration of not more than 1%. A conventional insecticide (pyrethrum based larvicide, pylarvex) was used as a positive control sample at 0.05 mg / ml. Larvae mortality in treated and control cups was recorded after 24 hours exposure. The dead larvae were counted and the median lethal concentrations (LC_{50}) determined by probit analysis (Finney 1971). The dead larvae in the four replicates were pooled and expressed as percentage mortality for each concentration. Observation was made on the behavior of the larvae to ascertain mortality by gently pricking them using end of a pipette to provoke arousal.

3.5 Physiological Responses

3.5.1 Selection for *Aloe* species tolerance

Susceptible strains of third instar *Ae. aegypti* larvae were separately placed under selection pressure with the extracts from *Aloe* plant species at concentrations that caused 50% mortality (LC_{50}). *Aloe* plant samples in which survivors failed to emerge as adults in sufficient numbers were discarded. One thousand five hundred larvae were selected for the parental and first generations and type of *Aloe* plant extract (n = 3) in three replicates each consisting of 1500 larvae in 1500 ml water in polypropylene cylindrical pans with radius and height of 10.5, 24.14 cm respectively. The susceptibility levels of *Ae. aegypti* to each *Aloe* plant extract in successive generations were monitored by determining the LC_{50} values. A control colony was reared simultaneously in a separate room and handled in the same manner through all manipulations but was not exposed to *Aloe* plant extracts. The larvae were not fed during the 24 h exposure. Survivors were normally propagated.

3.5.2 Physiological Resistance Diagnostic Test and Controls

Toxicity range tests (24 h) of *Aloe* plant extracts were conducted on each generation using third instar *An. gambiae s.s.* and *Ae. aegypti* larvae. After determining the upper and

lower toxicity ranges of each *Aloe* plant extract, 24 h acute toxicity tests were conducted. Three replicates (n = 25 per replicate) were exposed to five logarithmically separated *Aloe* plant extract concentrations within the established toxicity response ranges (Finney 1971), in 400 ml of distilled water in the polypropylene cylindrical pans. Larval mortalities were evaluated 24 h post exposure and LC_{50} determined by probit analysis (Finney 1971).

3.6 Phytochemical and functional group tests of the *Aloe* plant extracts

The *Aloe* plant extracts were assayed for the presence of the following phytochemical groups: flavonoids, saponins, tannins and polyphenols, phlobatannins, steroids and terpenoids. The *Aloe* plant extracts were also assayed for the presence of unsaturation, alcohol groups, phenolic groups, aldehyde groups and carboxylic functional groups. The phytochemical test for flavonoids was carried out by adding about 5 ml of dilute aqueous ammonia was added to a portion of filtrate of the plant extract, followed by addition of concentrated sulphuric acid. A positive test was confirmed by the formation of a yellow coloration that disappeared instantly (Edeoga *et al.,* 2005).Test for saponins was done by adding three drops of olive oil was to 0.1 g of extract and shaking vigorously. Formation of an emulsion indicated a positive test for saponins **(**Egwaikhide, 2007). Tannins and polyphenols were tested for by adding few drops of 1% ferric chloride to 0.2 g of extract previously dissolved in 10 ml of water. A brownish- green or blue- black coloration was indicative of a positive test for either a polyphenol or tannin presence (Edeoga *et al*., 2005).The test for phlobatannins was carried out by adding about 1ml of 1% ferric chloride to 0.1g of extract. Presence of depositions of a red precipitate was positive for phlobatannins (Majaw and Moirangthem, 2009). To test for steroids, about 2 ml of acetic anhydride and 2ml of sulphuric acid were added to 0.5g of extract. Change of color from violet to blue was indicative of the presence of steroids (Egwaikhide, 2007). Lastly the terpenoids phytochemical group was tested for by the Salkowski test where bout 3 ml of concentrated sulphuric acid and 2 ml of chloroform were added to 5 ml of extract to form a layer. A red coloration at the interface was indicative of the presence of terpenoids (Majaw and Moirangthem, 2009). The functional groups in the *Aloe* plant extracts were assayed. Presence of unsaturation was done by adding about 2ml of extract drop wise to 2ml of potassium permanganate while shaking. Discoloration of the 1M potassium permanganate solution was indicative of the presence of unsaturation in the extract. The dissolution of extract in a solution of sodium hydroxide indicated presence of phenolic groups. The rate at which the *Aloe* plant extracts reacted to form cloudiness with Luca's reagent was indicative of the presence of either the primary, secondary or tertiary alcohols. The presence of the aldehyde functional group was

tested by adding about 3-5drops of Tollen's reagent to 2ml of plant extract. Formation of a silver mirror indicated presence of aldehydes. (Harboune, 1991). The presence of carboxylic acid group was assayed by addition of 3-5 drops of 1m sodium hydrogen carbonate to 2 ml of sample extract. Solubility and effervescence of the sample was confirmation of presence of carboxylic functional group (Harboune, 1991).

3.7 Data analysis

Acute mortality responses were corrected by Abbot's formula (Busvine 1971) and then transformed to Probits (Finney, 1971) for linear regression analyses and 50 % lethal concentration (LC_{50}) determination. Data sets with more than 10% control mortality were not considered for analysis (Finney, 1971). Patterns of larval mortality, pupae formation and mortality as well as male and female adult eclosion between the treatments were conducted through cumulative frequency distribution. Probit analysis of concentration mortality data was conducted to estimate the LC_{50}, LC_{90} and LC_{99} values and associated 95 % confidence limits. The effects of *Aloe* species selection on egg viability/hatchability, larval and pupa mortalities, adult mortalities and female and male emergence were evaluated by one-way analysis of variance (ANOVA) on three datasets. The 1% dimethyl sulphoxide (DMSO) and distilled water were used as negative controls. Results that were significantly different were analyzed using GENSTAT Version 10.s (Hemel Hempstead, GENSTAT software analysis developer), (Jawara *et al*., 2009). The least significant difference was identified using Waller – Duncan statistical procedures (Steel and Torrie, 1980).

CHAPTER FOUR
RESULTS

4.1 Larvicidal results

Larvicidal results for the preliminary assays of fifteen extracts from *A. turkanensis*, *A. ngongensis* and *A. fibrosa* against third instar larvae of *An. gambiae* and *Ae. aegypti* showed that only nine were active that could cause 60 % mortality at 2 mg/ml. Only the ethyl acetate extract of *Aloe turkanensis*, hexane, ethyl acetate, acetone, chloroform and methanol extracts of *A. ngongensis* and the hexane, acetone and methanol extracts of *A.fibrosa* showed activity. The ethyl acetate soluble extract of *A. turkanensis* showed larvicidal activity where 99 % mortality was achieved at a concentration of 0.25 mg/ ml within the lower and upper limits of 0.19-0.41 mg / ml respectively against third instar larvae of *An. gambiae s.s* (**Table 1**). In comparison, *A. turkanensis* ethyl acetate plant extract exhibited 99 % mortality larvicidal activity against third instar *Ae. aegypti larvae*, within the range of 0.25-0.56 mg / ml. upper and lower limits with a median value of 0.33mg / ml (**Table 2**). The LC_{50} responses against third instar larvae of *An. gambiae s.s.* and *Ae. aegypti* using the ethyl acetate extract of *Aloe turkanensis* were 0.08 mg / ml and 0.11mg / ml, respectively. The *A. ngongensis* plant extracts in acetone, ethyl acetate, hexane, and methanol and chloroform all showed larvicidal activity against third instar larvae of the *Anopheles* and the *Aedes* mosquitoes species, exhibiting LC_{50} values tabulated in tables 1 and 2. Among the assayed *A. ngongensis* plant extracts, the hexane extract comparably showed larvicidal activity of LC_{50} concentration of 0.11 mg /ml against third instar larvae of *Ae. aegypti* mosquito species. Only the methanol, acetone and hexane plant extracts of *A. fibrosa* species showed larvicidal activities at LC_{50} concentrations ranging between 0.66-3.90 mg / ml against third instar larvae of *An. gambiae* and *Ae. aegypti* mosquito species.

Table 1

Probit analysis results for the concentrations (mg/ml) of selected *Aloe* plant extracts against *An. gambiae s. s.* third instar larvae

***Aloe* species**	**Extraction solvent**	**LC50 (95CL)**	**LC90 (95CL)**	**LC99 (LC95)**	**Slope (β ± SE)**	**; χ2≠**
A.turkanensis	Ethyl acetate	0.08 (0.07-0.1)	0.15 (0.13-0.21)	0.25 (0.19-0.41)	4.89 ±0.78	1.24
A.ngongensis	Ethyl acetate	0.66(0.52-0.84)	2.11(1.49-3.90)	5.45(3.15-15.24)	2.5±0.41	0.31
A.ngongensis	Hexane	0.52(0.41-0.65)	1.54(1.11-2.72)	3.75(2.24-9.79)	2.7±0.44	0.36
A.ngongensis	Chloroform	0.81(0.65-1.03)	3.51(2.27-8.33)	6.43(3.59-21.07)	2.5±0.47	1.21
A.ngongensis	Acetone	1.21(0.98-1.55)	3.65(2.53-7.41)	8.91(5.01-28.90)	2.68±0.48	0.95
A.ngongensis	Methanol	2.67(2.40-3.17)	4.92(3.90-8.18)	8.04(5.59-18.37)	4.8±0.10	3.44
A. fibrosa	Methanol	2.74(1.9-5.53)	14.64(6.68-128.69)	57.32(17.04-1787.74)	1.76±0.44	0.23
A.fibrosa	Acetone	2.70(2.41-3.15)	4.99(3.97-8.18)	8.23(5.74-18.58)	4.80±0.97	0.80
A.fibrosa	Hexane	2.13(1.84-2.57)	4.62(3.50-8.21)	8.66(5.60-22.34)	2.82±0.73	0.42

95 CI = 95% confidence interval;

SE = standard error,

LC 50=lethal concentration of *Aloe* plant extract that kills 50% of the *An. gambiae* larvae

LC 90=lethal concentration of *Aloe* plant extract that kills 90% of the *An. gambiae* larvae

LC 99=lethal concentration of *Aloe* plant that kills 99% of the *An. gambiae* larvae

$\chi 2^{\neq}$ = Greek small letter Chi squared

β ± is the Greek sign beta-plus or minus.

Table 2

Probit analysis results for concentrations (mg/ml) of selected *Aloe* plant extracts against *Ae. aegypti third* instar larvae

Aloe species	Extraction solvent	LC50 (95CL)	LC90 (95CL)	LC99 (LC95)	Slope (β ± SE)	; χ2≠
A.turkanensis	Ethyl acetate	0.11(0.09-0.12)	0.20(0.16-0.27)	0.33(0.25 0.56)	4.7±0.76	0.42
A.ngongensis	Ethyl acetate	0.15(0.13-0.17)	0.32(0.25-0.5)	0.62(0.41-1.43)	3.7±0.70	1.21
A.ngongensis	Hexane	0.11(0.08-0.15)	0.48(0.29-1.24)	1.67(0.76-7.94)	1.0±.0.34	1.03
A.ngongensis	Chloroform	0.33(0.28-0.37)	0.62(0.52-0.86)	1.05(0.79-1.84)	4.58±0.76	3.02
A.ngongensis	Acetone	0.77(0.67-0.89)	1.57(1.26-2.42)	2.82(1.97-5.82)4	4.11±0.73	0.09
A.ngongensis	Methanol	0.39(0.34-0.45)	0.79(0.63-1.22)	1.42(0.99-2.95)	4.11±0.73	2.70
A. fibrosa	Methanol	3.90(3.37-4.47)	7.84(6.36-11.54)	9.56(7.43-15.48)	4.23±0.73	1.95
A.fibrosa	Acetone	0.66(0.53-1.02)	1.76(1.1-6.41)	3.94(1.91-29.60)	2.99±0.73	0.06
A.fibrosa	Hexane	2.39(2.07-2.89)	5.15(4.60-13.06)	9.64(6.21-25.12)	3.84±0.75	1.18

95 CI = 95% confidence interval;

SE = standard error,

LC 50=lethal concentration of *Aloe* plant extract that kills 50% of the *Ae. aegypti* larvae

LC 90=lethal concentration of *Aloe* plant extract that kills 90% of the *Ae. aegypti* larvae

LC 99=lethal concentration of *Aloe* plant that kills 99% of the *Ae. aegypti* larvae

$\chi 2^{\neq}$ = Greek small letter Chi squared

β ± is the Greek sign beta-plus or minus.

4.2 Phytochemical qualitative analysis results of selected larvicidal *Aloe* plant extracts

The qualitative analysis of the ethyl acetate extract of *A. turkanensis* gave positive test results for saponins, tannin and polyphenol test and also the flavanoids test (**Appendices 19, 20** and **22**). Apart from the hexane and chloroform extracts of *A. ngongensis* plant extract, the others of methanol; acetone and ethyl acetate gave positive flavanoids results (**Appendix 19**). All the *A. fibrosa* plant extracts exhibited presence of flavonoids (**Table 3**). Tannins were present in all the analyzed *Aloe* plant extracts except those of the hexane, ethyl acetate and the chloroform extracts of the *A. ngongensis* plant extract (**Appendix 20**). Apart from the ethyl acetate and the chloroform extracts of *A. ngongensis* and also the hexane extract of *A. fibrosa* all the other assayed *Aloe* plant extracts showed the presence of saponins (**Appendix 22**) .All the analyzed plant extracts exhibited absence of steroid, phlobatannins and terpenoid phytochemicals (Appendices **17, 18** and **21**).

Table 3

Summary of the qualitative phytochemical constituents of the selected *Aloe* plant extracts

***Aloe* species**	**Solvent of extraction**	**Terpenoid test**	**Steroid test**	**Flavonoid test**	**Saponin test**	**Tannin/Polyphenol test**	**Phlobatannin test**
ATE	Ethyl acetate	-	-	+	+	+	-
ANE	Ethyl acetate	-	-	+	-	-	-
ANA	Acetone	-	-	+	+	+	-
ANC	Chloroform	-	-	-	-	-	-
ANH	Hexane	-	-	-	+	-	-
ANM	Methanol	-	-	+	+	+	-
AFA	Acetone	-	-	+	+	+	-
AFM	Methanol	-	-	+	+	+	-
AFH	Hexane	-	-	+	-	+	-

(+) Positive for test reaction
(-) Negative for test reaction
ATE *Aloe turkanensis* ethyl acetate plant extract
ANE *Aloe ngongensis* ethyl acetate plant extract
ANA *Aloe ngongensis* acetone plant extract
ANC *Aloe ngongensis* chloroform plant extract
ANH *Aloe ngongensis* hexane plant extract
ANM *Aloe ngongensis* methanol plant extract
AFA *Aloe fibrosa* acetone plant extract
AFM *Aloe fibrosa* methanol plant extract
AFH *Aloe fibrosa* hexane plant extract

4.3 Analysis of chemical functional groups in *Aloe* extracts

All the *Aloe* plant extracts analyzed exhibited absence of unsaturated bonds in their structures .Only the ethyl acetate plant extract of *A. ngongensis* showed presence of tertiary alcohols while the *A. fibrosa acetone* extract showed presence of secondary alcohol functional group. The other *Aloe* plant extracts showed possibility of presence of primary alcohols. Apart from the chloroform plant extract of *A. ngongensis*, all the analyzed plant extracts showed presence of the phenolic radical. Also predominantly present in all the assayed *Aloe* plant extracts was the aldehyde functional group whose presence was detected by the reduction of ionized silver to elemental silvery mirror. All larvicidal *Aloe* plant extracts showed presence of carboxylic acid functional group apart from the chloroform extract of *A. ngongensis* plant. The analysis of the functional groups in the assayed *Aloe* plant extracts is illustrated in **Table 4**.

Table 4

***Aloe* species functional groups**

***Aloe* plant extract**	**Solvent of extraction**	**Unsaturation test**	**Carboxylic group test**	**Alcohol group test**	**Aldehyde group test**	**Phenol group test**
ATE	Ethyl acetate	-	+	-	+	+
ANE	Ethyl acetate	-	+	Tertiary alcohol	+	+
ANA	Acetone	-	+	-	+	+
ANC	Chloroform	-	-	-	+	-
ANH	Hexane	-	+	-	+	+
ANM	Methanol	-	+		+	+
AFA	Acetone	-	+	Secondary alcohol	+	+
AFM	-	-	+	-	+	+
AFH	-	-	+	-	+	+

(+) Positive for reaction test
(-) Negative for reaction test
ATE *Aloe turkanensis* ethyl acetate plant extract
ANE *Aloe ngongensis* ethyl acetate plant extract
ANA *Aloe ngongensis* acetone plant extract
ANC *Aloe ngongensis* chloroform plant extract
ANH *Aloe ngongensis* hexane plant extract
ANM *Aloe ngongensis* methanol plant extract
AFA *Aloe fibrosa* acetone plant extract
AFM *Aloe fibrosa* methanol plant extract
AFH *Aloe fibrosa* hexane plant extract

4.4 The effect of *Aloe* plant extracts on *Ae. aegypti*

The effects of the Aloe species plant extracts on the egg, larvae, pupa and adult stages of the mosquito *Ae*. *aegypt*i are summarized in **Table 5** and **Appendices 1 to 16**. During this study, both the parental (F0) and the first generation (F1) *Aloe* exposed groups were adversely affected in their developmental stages (**Figures 7 and 8**). While the mean larval mortality in the control groups in F0 was 2.67% and 9.33% in distilled water and DMSO respectively, the *Aloe* plant exposed larvae exhibited mortalities of 48.60%, 51.55% and 51.55% with LSD of 62.5 and coefficient of variation of 7.1% with F value of < 0.01.The mean larval mortality in the F1 *A. ngongensis* ethyl acetate plant extract exposed larvae was 14.38%compared to 2.9% in the untreated control(**Appendix 1**) While the pupae mortality in the controls was between 1.03% and 2.13%, that in the *Aloe* exposed group ranged between 12.12% and 13.10% in F0 generation (**Appendix 2**). The ability of the *Aloe* exposed mosquitoes to lay eggs was equally hampered as evidenced in **table 3** (there were strong negative correlations with egg deposits of 4000 per treatment and fecundity of 133 (**Appendices 6** and **16**)). In comparison the water and DMSO control mosquitoes deposited 7333 and 6030 eggs respectively (hence fecundity of 244. and 201).

The egg viability in the F1 generation group exposed to *Aloe* plant extracts was adversely affected ranging between 19.8% and 49.3% of the possible 1500 exposed eggs. This is in contrast to the control group which had more eggs hatching of up to 94.6% and 96.7% out of the batch of 1500.eggs (**Appendix 10**). The age specific studies on the 28th day revealed there were between 14 and 16 males in the control group as opposed to greatly reduced numbers in the *Aloe* plant exposed mosquitoes (averagely 2-6 (**Appendices 7, 8** and **9**). On the same day the females in the control group were between 24 and 25 in contrast with the *Aloe* exposed female mosquitoes that were between 7 and 22 out of the original number of 30. Consequently the ratio of surviving males and females on the 28th day was 0.47 and 0.44 in the water and DMSO control environment mosquitoes. This is opposed to the lower ratios of 0.35, 0.27 and 0.20 in the respective *Aloe* exposed mosquito batches (**Appendix 8**). Also profoundly affected were the rates of both the female and male mosquito emergence (53-224). This is in contrast to the *Aloe* unexposed mosquitoes that showed male and female emergence of between 647 and 628 from the possible exposed 1500 egg batch (**Appendices 3** and **4**).

Table 5

The effect of *Aloe* plant extracts on 1500 third instar larvae of *Ae. aegypti* parental generation (F0) and first generation (F1) and the subsequent developmental stages

ATTRIBUTES	CONTROL	1%DMSO	AFA	ANE	ATE	LSD	F	%CV
F0 larvae mortality	40.0[a]	140.0[b]	729.0[c]	773.3[c]	779.7[c]	62.5	<.001	7.1
F1 larvae mortality	44.0[a]	143.0[b]	183.7[c]	215.7[d]	145.0[b]	21.3	<.001	7.7
F0 Pupae mortality	15.3[a]	26.7[a]	88.7[b]	92.0[b]	101.3[b]	28.4	<.001	23.3
F1 Pupae mortality	28.3[a]	25.3[a]	79.3[b]	61.3[c]	24.3[a]	13.3	<.001	16.1
F0 egg deposits	7394.0[a]	6030.0[b]	4357.0[c]	4210.0[c]	4181.0[c]	775.	<.001	5.5
F1 Egg deposits	7033.0[a]	7615.0[b]	7083.0[c]	4948.0[d]	4597.0[d]	416.4	<.001	3.5
F1 Egg viability	1453.0[a]	1419.0[a]	749.0[b]	700.0[b]	297.0[c]	79.3	<.001	4.6
F0 ♂ Age specific	16.7[a]	14.7[a]	6.33[b]	3.33[b]	2.33[b]	5.2	<.001	11.6
F0 ♀ Age specific	25.7[a]	24.3[a]	21.7[a]	10.8[b]	7.8[b]	5.4	<.011	15.8
Survivorship ♂ and ♀	0.47[a]	0.44[a]	0.35[b]	0.27[c]	0.20[d]	0.06	<.001	82
FI ♂ emergence	647.0[a]	628.3[b]	224.3[c]	193.7[d]	62.0[e]	12.2	<.001	1.8
F1♀emergence	624.3[a]	607.7[b]	220.0[c]	186.7[d]	53.3[e]	13.2	<.001	2.1

LSD = Least Significant Difference of means (5% level)
CV = coefficient of variation
DMSO = 1% solution of dimethyl sulphoxide
ATE = Ethyl acetate plant extract of *Aloe turkanensis*
ANE = Ethyl acetate plant extract of *Aloe ngongensis*
AFA = Acetone plant extract of *Aloe fibrosa*
F0 and F1 = parental and first filial generations respectively
The symbols ♂ and♀ mean the male and the female mosquitoes respectively
The means with the same letter superscript on the same row are not significantly different ((P<.0.05)

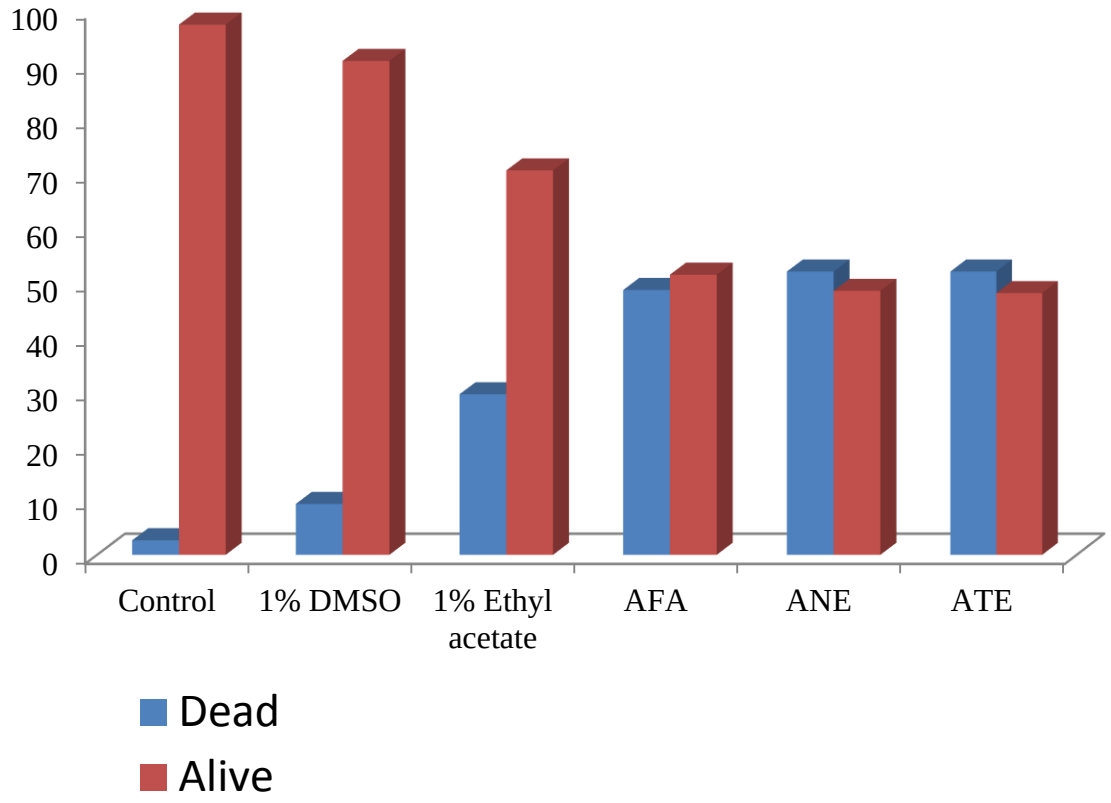


Figure 8: The responses of third instar larvae post 24 hour exposure to selected *Aloe* plant extracts and the negative controls

(a) Control= Distilled water
(b) 1% DMSO= 1% Dimethyl sulphoxide solution
(c) 1% ethyl acetate =1% ethyl acetate solution
(d) ANE= LC50 *Aloe ngongensis* ethyl acetate plant extract
(e) ATE= LC50 *Aloe turkanensis* ethyl acetate plant extract
(f) AFA=LC50 *Aloe fibrosa* plant extract
(g) Dead larvae
(h) Surviving larvae

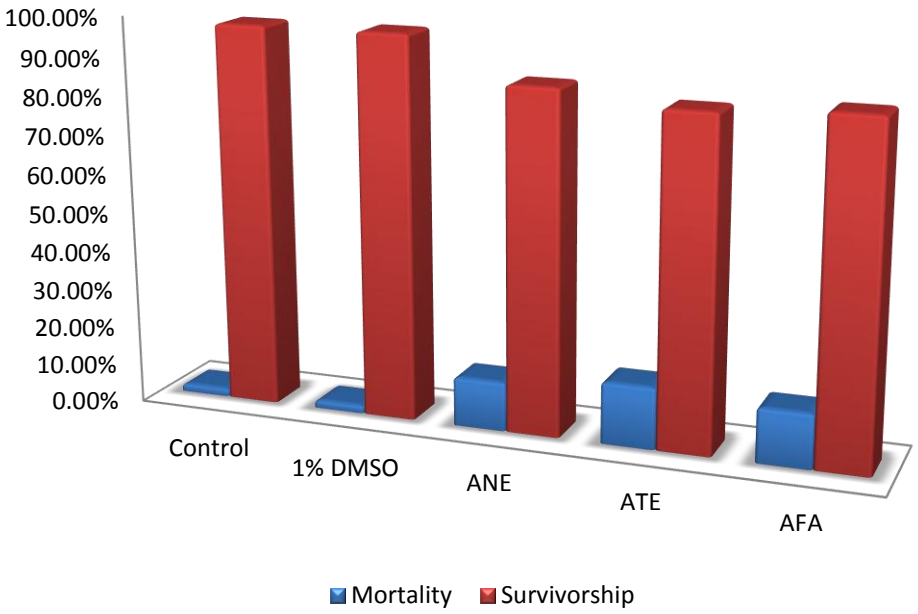


Figure 9: The effect of *Aloe* plant extracts on the pupae of *Ae. aegypti*

(a) Control= Distilled water
(b) 1% DMSO= 1% Dimethyl sulphoxide solution
(c) ANE= LC50 *Aloe ngongensis* ethyl acetate plant extract
(d) ATE= LC50 *Aloe turkanensis* ethyl acetate plant extract
(e) AFA=LC50 *Aloe fibrosa* plant extract
(f) Dead pupae
(g) Surviving pupae

CHAPTER FIVE
DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

This study has confirmed that plant extracts can be used as larvicides and deterrents against mosquitoes. The *A. turkanensis* leaf extract effected 99% mortality against *An. gambiae* at 0.25mg/ml within the lower and upper ranges of 0.19mg/ml and 0.41mg/ml respectively. This is comparable to the larvicidal effect of *Lantana camara* Linn against mosquito species *Ae. aegypti* and *Culex quinquefasciatus* where 100% mortality was attained at 1.0mg/ml and 3.0mg/ml respectively (Sathish and Maneemegalai, 2008) .There are reports of medicinal plants with insecticidal activity including ovicidal, antifeedant, larvicidal and repellent activities (Asfaw *et al*., 2007). Mosquito larvicidal and cytotoxic activities of three *Annona* species against third instar *Culex quinquefasciatus* and brine shrimp larvae have been reported (Magdula *et al*., 2009). Also the developmental stages of the bio assayed *Ae. aegypti* were markedly affected by exposure to *Aloe* plant extracts. This observation correlates with that of *Imperta cylindrical* (Graminaceae) which showed antioviposition and insecticidal effects against mosquitoes (Mohsen *et al.,* 1995). In this study flavonoids were present in most of the assayed *Aloe* species.

Flavonoids have been found to have insecticidal activity against wooly apple aphid, *Eriosoma lanigerum* (Mazen, 2012). Sathish and Maneemegalai (2008) found saponins, flavonoids, terpenoids and cardiac glycosides as secondary metabolites present in plant extracts with insecticidal potential. It is therefore hypothesized that larvicidal activity could be due to the disruption of the larval cell membrane (Mayo *et al*., 2010). This is because the mode action of flavonoids is by disrupting the cell membrane. That all the assayed *Aloe* larvicidal extracts gave a positive Tollen's test suggests presence of potentially free aldehyde groups. This is collaborated by the fact that flavanoids are structural derivatives of flavones containing conjugated aromatic systems often bound to sugar(s) as glycosides (Berenbaum and Zangeri, 1987; Harboune, 1991). The toxicity of benzaldehyde, an aromatic aldehyde, against the immature and adult stages of *Ae. aegypti* and *Culex quinquefasciatus* has been reported (Paulraj *et al*., 2011). In this study the *A. turkanensis* ethyl acetate plant extract exhibited the highest larvicidal activity against third instar larvae of *Ae. aegypti* and *An. gambiae.* This extract exhibited presence of flavonoids, saponins and the tannins and polyphenol group. Polyphenols have previously been isolated from *A. vera* leaf plant extracts (Dagne *et al*., 1998).Screening

of some Ethiopian medicinal plants with larvidal activity revealed constituent presence of saponins, polyphenols, alkaloids and glycosides

All the three species of analyzed larvicidal *Aloe* plant extracts depicted absence **of** terpenoids and steroids thus probably suggesting that the larvicidal metabolites not derived from the isoprene units. That larvicidal activity in assayed *Aloe* plant extracts is not attributed to terpenoids concurs with Phytochemistry analysis of the *Meliaceae* plant in which very minimal insecticidal activity was reported (Isman *et al* 1996). Also profoundly absent in all the larvicidal plant extracts of *Aloes* under study were the phlobatannins. Most of the phytochemicals extracted from various plant species have indeed been tested and found to exhibit larvicidal and repellent action against mosquitoes (Ciccia *et al*., 2001; Innocent *et al*., 2010). The larvicidal activity of *Aloe* plant extracts assayed in this study against *An. gambiae* and *Ae. aegypti* larvae corroborates earlier results achieved for plant extracts as alternative larvicides for mosquito control (Berenbaum *et al.,* 1991, Matasyoh *et al*., 2008). The physiological responses of all the developmental stages of *Ae. aegypti* were profoundly affected by the *Aloe* plant extracts. The larval mortality in the *Aloe* exposed treatments was higher than in the control experiments. This was also depicted in survivorship studies where the untreated mosquitoes outlived those exposed to *Aloe* plant extracts. The same trend was observed in the egg deposits whereby the *Aloe* plant extract exposed mosquitoes had lower fecundity as compared to the control group. The egg viability in the control groups was higher in comparison to the *Aloe* exposed groups .The females in this study tended to outlive their male counterparts. This concurs with the established norm of the mosquito life span (Hurd *et al* 1995). That plant extracts affect the physiology of mosquitoes is also evidenced by the repellent effect of constituents of essential oils of *Suregada zanzibariensis* (Agiospermae Euphobiaceae) leaves to the mosquito *An. gambiae s.s* (Innocent *et al*., 2010). It has been established that there is insecticidal, repellent and oviposition deterrent activity of selected essential oils against *Anopheles stephensi, Ae. aegypti* and *Cu. quinquefasciatus* (Veena *et al.,* 2011). Despite the above observation it is generally held that the efficacy of plant derived phytochemicals is a function of the environmental conditions (Berenbaum and Zangeri, 1987).

While vector control is one of the options employed to contain malaria epidemics, there is continuous increase in resistance of mosquitoes to convectional insecticides (Chandre *et al*., 1999. Diabate *et al.,* 2003 and Hunt, *et al.,* 2010). There have also been reports of chemical insecticide residuals deposited in food (FAO, 1992). This has necessitated the need to bio prospect for secondary metabolites of botanical origin (Adeyemi 2010), that could be

environmentally friendly. The plant- derived natural products as larvicides have the advantage of being harmless to beneficial non-target organisms and the environment (Pitasawat *et al.*, 2007). Hence the larvicidal effect of the bioactive *Aloe* plant extracts shall be used alongside other convectional vector control methods to break *Ae. aegypti* and *An. gambiae* mosquito breeding cycles. None of the extracts exhibited the presence of steroids. Hence possibly phytosteroids may not be the active agents responsible for their larvicidal effects. That flavanoids in this plant extracts could possibly be larvicidal is supported by work done using essential oils of chloroxylon *Swietenia* DC against *Ae. aegypti* and *An. stephensi* (Kiran *et al* 2006). The major constituents in the members of *Aloeceae* are typically chromones and anthraquinones, or anthrones, and so possibly phenolic group could be responsible for the larvicidal effect on mosquitoes (Matasyoh *et al*., 2008).

Tannins have been known to have histopathological effects on the midgut epithelium of *Papilio polyexenes* and *Papilio glaucus* (family of white and black swallow tail butterflies) (Berenbaum and Steinly, 1986). Hence it is hypothesized that the prevalence of the phenolic group in the assayed *Aloe* plant extracts could play a role in physiological and larvicidal responses against the mosquito. This study confirmed that *Ae. aegypti* survivorship, fecundity and egg viability were significantly reduced due to exposure to the selected *Aloe plant* extracts. Effect on the oviposition and embryo toxicity of plant extract of *Indigofera suffruticosa* on early development of *Ae. aegypti* has been reported (Cardoso *et al*., 2011). The fact that natural products can have inhibitory effect on an organism has been established (Flavi *et al*., 2005). Vector borne diseases are among the major causes of illness and death in many developing countries affecting substantial portion of the productive force. Medicinal plants with larvicidal properties have paramount importance for the local control of the mosquito. With the advent of drug resistance to the most commonly used drugs against the malaria parasite; integrated vector control approach with global initiatives is the way to contain vector-borne diseases (Adeyemi, 2010).

5.2 Conclusion

(1)The organic extracts of *A. turkanensis, A. ngongensis* and *A. fibrosa* species are toxic to third instar larvae of *Ae. aegypti* and *An. gambiae* and hence their larvicidal activity can be used in mosquito control.

(2) The phytochemical composition *A. turkanensis, A.ngongensis* and *A. fibrosa* influence their toxicity to third instar larvae of *An. gambiae* and *Ae. aegypti*.

(3) The extracts of *A. turkanensis*, *A. fibrosa* and *A. ngongensis* have deterrent effect on the developmental stages of *An. gambiae* and *Ae. aegypti* mosquitoes. hence they can be integrated in existing vector control programmes to control the mosquito vector.

5.3 Recommendations

(1) Extracts of *A. turkanensis*, *A. ngongensis* and *A. fibrosa* with larvicidal activity should be used as alternative larvicides against mosquitoes.

(2) The respective larvicide-extract responsive genes should be established through comparative analysis of gene transcripts from sub lethal dose (LC50) survivors of the extract exposure and non-exposed control mosquito larvae, through subtractive hybridization molecular approaches coupled to bioinformatics analysis.

(3) Chemical purification of the extracts should be carried out to aid in definite identification of the constituent larvicides in the *Aloe* plant larvicides.

(4) An attempt should be made to establish the mode of action of the larvicides for future incorporation in formulations to counter vector resistance to insecticides.

REFERENCES

Abebe D. and Ayehu A. (1993). *Medicinal plants and enigmatic health practices of Northern Ethiopia, Addis Ababa* BSPE, pp. 511

Adeyemi M. M. (2010). The potential of secondary metabolites in plant material as deterrents against insect pests. *African Journal review of Pure and Applied Chemistry*. **4**:243-246.

Agyepong N., Mak-Mensah E. E. and Brown C. A. (2012). Prevalence of *Anopheles gambiae* s. s and their pyrethroids knock down resistance pattern in communities in Kumasi metropolis using polymerase chain reaction (PCR). *European Journal of experimental Biology*. **2**:304-310.

Asfaw D., Aseyed T., Abebe D., Kissi M., Daniel M. and Girum T. (2007). Screening of some Ethiopian medicinal plants of for mosquito larvicidal effects and phytochemical constituents. *Journal of Pharmacology online*. **3**:231-243

Akira Yagi (2008). The chemistry of low molecular weight chromones and glycoproteins from *Aloe vera*. Accessed online 01/08/2008 at file://E: \chemistry of *Aloe*

Amy L., Ranson H., McCall P.J., Randile N.P., Black C. W., Walker L. D. and Donlley M. J. 2005). A simplified high through method for pyrethroids knock down resistance (Kdr) detection in *Anopheles gambiae. Journal of Malaria* **4**: 1475-2875.

Amy E.B, (2006). Mode of action of insecticides and related pest control chemicals for production agriculture, ornamentals and turf. Pesticides information Leaflet No.43. http:// pesticides. UMD.EDUC. Accessed 22/09/2008.

Anne B., Sophie L., Christine R., Stephane G., Sylvie D. and Catherine F. (2005). Allelopathic potential of *Medicago arborea,* a Mediterranean invasive shrub. *Journal of Ecology,* **15**: 193-198.

Apperson C, S., Harrison B.A., Unnasch T. R., Hassan H. K., Irby W.S., Savage H.M., Watson D. W., Rueda L. M., Engber B. R. and Nasci R. S. (2002). Host-feeding habits of Culex and other mosquitoes (Diptera: Culicidae) in the Borough of Queens in New York City, with c characters and techniques for identification of Culex mosquitoes. *Journal of Medical Entomology*. **39:** 777-785.

Awolola T. S., Oduola A.O., Obansa J. B., Chukwurar N. J and Unyimundu J. P.(2007). *Anopheles gambiae s.s* breeding in polluted water in urban Lagos, southwestern Nigeria. *Journal of Vector Borne Diseases.* **44**:241-244.

Baillie J. E. M. B., Hilton-Taylor C. and Stuart S.N. editors. (Eds). 2004) (. IUCN Red List of Threatened Species: A global Species Assessment IUCN. Gland & Cambridge.

Beentje H. and Smith S. (2001). FTEA and after. In Robbrecht E; Degreef J. and Friis I editors. (Eds). *Journal of Plant Systematics and Phytogeography for the Understanding of African Biodiversity. Proceedings of the XVIth AETFAT Congress* Systematics and Geography of Plants. **71**:2265–2290

Bekele J. and Hassanali A. (2001). Blend effects in the toxicity of the essential constituents of *Ocimum kilimands-charium* and *Ocimum kenyense* (*Lambiatae*) on two post harvest insect pests. *Journal of Phytochemistry*. **57**: 385-391.

Berenbaum R. and Steinly N. (1986). Histopathological effects of tannins on the midgut epithelium of *Papilio polyexenes* and *Papilio glaucus*. *Journal of Entomology and experimental Applications*.**39**: 3-9.

Berenbaum. and Zangeri A. R. (1987). Furanocoumarins in Wild Parsnip: effect of photosynthetically active radiation, ultraviolet light and nutrients. *Journal of Ecology*. **68**: 516-570.

Berenbaum R., Nitao J. K. and Zangeri A. R. (1991). Adaptive significance of furanocoumarins diversity in *Pastinaca sativa* (Apiaceae). *Journal of Chemical Ecology*. **20**: 207-215.

Bird B. H., Githinji J. W., Macharia J. M., Kasiiti J .L., Muriithi R. M and Gacheru S.G. (2008) Multiple virus lineages sharing recent common ancestry were associated with a large Rift Valley fever outbreak among livestock in Kenya during 2006–2007. *Journal of Virology*.**82**:11152–66.

Blackwell A. and Johnson S.N. (2000). Electrophysiological investigation of larval water and potential chemo-attractants for *Anopheles gambiae* s.s *Journal of Annals of Tropical Medicine and Parasitology***. 94**: 389-398.

Bødker R., Kisinza W., Malima R., Msangeni H. A. and Lindsay S.W. (2000). Resurgence of malaria in the Usambara Mountains, Tanzania is due to an epidemic of drug-resistant parasite. *Journal of Global Change*. **43**:1-134.

Bosch C.H. (2006). *Aloe turkanensis* Christian [Internet]. Record from Protabase. Schmeizer G. H. and Curib-Fakim A. (Editors). Prota (Plant Resourses of Tropical Africa/Resourseshttp;//database.prota.org/search.htm>ACC

Braga C., Dourado M. I., Ximenes R. A., Alves L., Brayner F., Rocha A. and Alexander N. (2003). Field evaluation of the whole blood immunochromatographic test for rapid bancrofti filariasis diagnosis in the northeast of Brazil. *Journal Review of Institute Medical Tropics*. **45**:125–129.

Briet O.J., Dossou-Yovo J., Akodo E., van de Giesen N. and Teuscher T.M. (2003). The relationship between Anopheles gambiae density and rice cultivation in the savannah zone and forest zone of Cote d'Ivoire .*Journal of Tropical Medicine in International Health*.**8**: 43-48.

Busvine J. R. (1971). *A critical Review of the Techniques for testing Insecticides,* CAB, London, pp.: 263-288.

Burgess N. R.H. and Cowan G. O. (1993). *Color Atlas of Medical Entomology*. pp. 10.

Carpenter S. J. (1955). *Mosquitoes of North America* (*North Mexico).* University of California Press, Berkeley, CA. pp. 360.

Carpenter S. J. and Walter J. L. (1974). *Mosquitoes of North America (north of Mexico*) p. 1

Chaz M. (2008).' Get an immune boost with *Aloe vera'* "Ezzine Articles" 20 April 2008, 02 May 2008.<http// e-zine articles.com Get-an-immune-system-boost-with-*Aloe-vera.*

Cardoso V. J.R., Roberto M. P.L., Daniela do Amaral F. N., Everson M. B. and Sonia P. L. (2011). *Oviposition and embryo toxicity of Indigofera suffruticosa on early development of Aedes aegypti (Diptera: Culicidae).* Academic editor Diethand Wahner-Roedler volume 2012 pp. 1-5.

Chandre F., Darriet F., Manguin S., Brengues C., Carnevale P. and Guillet P. (1999). Pyrethroids cross-resistance spectrum among populations of *Anopheles gambiae* s.s. from Cote D' Ivoire. *Journal of the American Mosquito Control Association*. **15**: 53-59.

Chandre F., Darret F., Duchon S. (2000). Modifications of pyrethroids effects associated with kdr mutation in *Anopheles gambiae*. *Journal of Medical and Veterinary Entomology*. **8**:63-72.

Chouaibou M., Etang J., Brevault T., Nwane P., Hinzoumbe C. K., Mimpfoundi R. and Simard F. (2008). Dynamics of insecticide resistance in the malaria vector A*nopheles gambiae s. l.* from an area of extensive cotton cultivation in Northern Cameron. *Journal of Tropical. Medical International Health*. **13**:476-486

Ciccia G., Coussio J. and Mongelli E. (2001). Insecticidal repellent activity against *Aedes aegypti* larvae of some medicinal South American plants. *Journal of Ethno pharmacology and Pharmacy.* **71**:267.

Clements A.N. (1992). The *Biology of Mosquitoes Development, Nutrition and Reproduction,* Chapman and Hall, London.pp.536

Clements A. N. (1999). *The Biology of Mosquitoes Sensory Reception and Behavior,* CABI, UK.pp.756.

Clements A.N. (2002). *The Biology of Mosquitoes*. Volume 3 Chapman and Hall. London. pp. 1

Colluzi M., Sabatia A., Della Tore A., Deco M.A. and Petracca U. (2002). A polytene chromosome analysis of the *Anopheles gambiae* species complex. *Journal of Science.* **298**:1415-1417.

Conn J. E. (1997). Systematics of Mosquito Disease Vectors (Diptera, Culicidae): Impact of Molecular Biology and Cladistics Analysis .*Journal of Annual Review of Entomology***. 42**: 351-369

Constant B. and Lam R. (2012). Mosquito life cycle. Accessed online 23/7/2021

Dagne E., Wyk B., Stephenson D., and Steglich W. (1996). Three oxanthrones from *Aloe littoralis* .*Journal of Phytochemistry*. **42**: 1683-1687.

Dagne E., Bisrat D., Codina C. and Bastida J. (1998). A. C. O-diglucosylated oxanthrone from *Aloe littoralis*. *Journal of Phytochemistry* **48**: 903-905.

Das S., Garver L. and Dimopoulos G. 2007). Protocol for mosquito rearing (*An. gambiae*). *Journal of Visions Experiments.* **5**: 221.

Desiree A. L., Sutherland L.J., Muiruri S., Muchiri E, M., Gray L. R., Zimmerman P. A., Hise A. G. and Charles King H .(2011). Arbovirus Prevalence in mosquitoes, Kenya. *ELD Journal* Volume **17**:2.

Diabate A., Baldet T., Chandre C., Dabire K. R., Kengne P., Gulguemde T. R., Simmard F., Guillet P., Hemingway J. and Hougard J. M. (2003). Kdr mutation, a Genetic Marker to Assess events of introgression between the molecular M and S forms of *Anopheles gambiae* (Diptera: Culicidae) in tropical Savannah area of West Africa. *Journal of Medical Entomology.* **40***:*1905-1906.

Dong K. (1997). A single amino acid change in the para sodium channel protein is associated with knockdown resistance (kdr) to pyrethroids resistance in German cockroaches *Journal of Insect Biochemistry and Molecular Biol*ogy **27**:93-100.

Edoega H.O., Okwu D. E. and Mbaebie B. O. (2005). Phytochemical constituents of some Nigerian plants. *African Journal of Biotechnology*. **4**: 685-688.

Egwaikhide P. A. (2007). Analysis of the phytochemical content and activity of *plectranthus glandulosis* whole plant. *Journal of Middle East Scientific Research* **2**: 135-138.

Enayati A. and Hemingway J. (2010). Malaria management past, present and future. *Journal of Annual Review and Entomolology*.**55**:569-591

Etang J., Manga L., Jean-Claude T., Gullet P., Fondjo.E. and Chandre F. (2004). Spectrum of metabolic based resistance to DDT and pyrethroids in *Anopheles gambiae s.l.* populations from Cameroon. *Journal of Vector Ecology*. **32**: 1.

Fanello C., Santolamazza F. Della Torre A. (2002). "Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCR-RFLP". *Journal of Medical and Veterinary Entomology* **16**: 461.

FAO (1992). Pesticide residues in food. Report, 116-146.

Feng R. and Isman M. B. (1995). Selection for resistance to Azadiracta in the green peach aphid, *Myzus persicae*. *Journal of Cell Molecular Life Science*.**51**:831-833.

Finney D.J. (1971). *Probit analysis-a statistical treatment of the sigmoid response curve, 3rd edition.* Cambridge, United Kingdom.

Flavia C., Maskia L., Tamara R.C., Wagner V. and Iracilda Z.C. (2005). Inhibition of hydrogen peroxide, nitric oxide and TNF for production in peritoneal macrophages by ethyl acetate fraction from *Alchornea glandulosa*. *Journal of Biological and Pharmaceuticals Bulletin.* **28**: 1726

Ford H. R. and Green E. (1972). Laboratory rearing of *Anopheles albimanus*. *Journal of Mosquito News*. **32**:509-513.

Foster W. A. and Walker C. B. (2002). Mosquitoes (*Culicidae*). *Journal of Medical and Veterinary Entomology* **597**: 203-262.

Foote R. H. and Cook R. D. (2005) *Mosquitoes of Medical Importance*. pp. 168.

Fraincine H., Abdoulage A., Djimde W.M. and Thomas C. (2003). The importance and future of malaria in Africa. Accessed 1/8/2008.

Gabi B., Lawal A. O. and Hauwa B. S. (2012). Mosquito Repellent activity and phytochemical characterization of essential oils from *Striga hermonthica*, *Hyptis spicigera* and *Ocimum basilicum* leaf extracts. British Journal of Pharmacology and Toxicology **3**:43-48.

Gadelha A.P.R., Vidal F., Castro T.M., Lopez C.S., Alberella N., Coelho N., Figueiredos. F.L. and Monteiro-leal L.H. (2005). Susceptibility of *Giandia lamblia* to *Hoveria dukis* extracts. *Journal of Parasitology Research*. **97**:399-407.

Gao X., Yan X., Yao X., Xu[1] L., Zhang K., Zhang J., Yang B. and Jiang L. (2007). The dry-style antifogging properties of mosquito compound eyes and artificial analogues prepared by soft lithography. *Journal* of *Advanced Materials*. **19**:2213-2227.

Gilbert A. and Calderone, (2007). Mosquito control. Suffolk county Government. Accessed 3/9/2007@www.suffolkcounty.gov/health/suffolk.vectorplan/ pdf/final/appendix

Gillies M.T. and De Meillon. (1968). The *Anophelinae* of Africa South of the Sahara. (Ethiopian zoogeographical region) *South African Journal. Institute for Medical Research, Johannesburg*, 343

Goma L. K. H. (1959). Periodic pupation in *Anopheles gambiae* Giles. *Journal of Entomological Society of South Africa*. **22**:275-276

Gospiesh V. K. and Kannabiran K. (2007). Larvicidal effect of *Hemidesmus indicus*, *Gymnema sylvestre* and *Eclipta prostrate* against *Culex* q*uinquefasciatus* mosquito larvae .*African Journal of Biotechnology*. **6**: 307-311.

Gubler D. J. (1998). Resurgent vector –borne diseases as a global health problem. *Journal of Emergent Infectious Diseases*. **4**: 442-450

Guerrero F. D., Jamroz. R. C., Kammaiah D. and Kunz S.E. (1997). Toxicological and molecular characterization of pyrethroids-resistant *Haematobia irritants*: Identification of kdr and super-kdr point mutations. *Journal of Insect Biochemistry and Molecular Biology*. **27**:745-755.

Gutterman Y. and Chausen V. (2007). Secondary phenol metabolites (SPHMS) distribution and content of some *Aloe* species originated from arid zones of South Africa: A review. *Journal of Food Technology*. **2**: 555-569.

Harboune J. B. (1991). *Phytochemical Method*s. *A guide to Modern Techniques of Plant Analysis.* Chapman and Hall. New York, Tokyo. Melbourne, Madras. Second edition. Textbook, pp. 120.

Hargreaves K., Hunt R.H., Brooke B.D., Muthembu J., Weeto M. M., Awolola T.S. and Coetzee M. (2003). *Anopheles arabiensis* and *An. quadriannulatus* resistance to DDT in South Africa. *Journal of Medical and Veterinary Entomology*.**17**:417-422.

Huang J. Walker, Edward D, Vulule J. Miller J. R. (2006) Daily temperature profiles in and around Western Kenyan larval habitats of Anopheles gambiae as related to egg mortality. *Malaria Journal*. **5**:87.

Hunt R. H., Edwardes M.and Coetzee M. (2010). Pyrethroids resistance in Southern African *Anopheles funestus* extends to Likoma Island in Lake Malawi. *Journal Parasites and Vectors* **3**: 122

Hurd H., Hogg C. J. and Renshaw M. (1995). Interactions between blood feeding, fecundity and infection in mosquitoes. *Journal of Parasitology Today*. **11**:411-416.

Innocent E., Cosam C. J. and Gikonyo N. K. (2010). Constituents of the essential oil of *Suregada zanzibariensi*s leave are repellent to the mosquito *Anopheles gambiae* s.s *Journal of Insect Science*. **10**: 1-8.

Isman M.B., Matsuura H., Mackinnon S., Durst T., Towers G.H.N. and Arnason J. T. (1996). Phytochemistry of the *Meliaceae*: so many terpenoids, so few insecticides. In Phytochemical diversity and redundancy in ecological interactions, Plenum Press, New York, London pp. 155-178.

Isman M. BB. and Machial C. M. (2006). Pesticides based on plant essential oils: from traditional practice to commercialization. In Rai M. and Carpinella M. C. (Eds.). Naturally occurring Bioactive Compounds. *Journal of Elsevier, BV.***11**: 29-44

Isman M. B., Akhtar Y. and Yeoung Y. K. (2008). Comparative bioactivity of selected extracts from *Meliaceae* and some commercial botanical insecticides against two noctuid caterpillars, *Trichoplasia ni* and *Pseudaletia unipunota. Journal of Phytochemistry Review.* **7**: 77-78.

Jacobson M. C. (1975). *Insecticides from plants: A review of the literature, 1954-1971:* Agricultural Handbook, US Department of Agriculture, Washington D. C., pp. 462:138.

Jacobson M. C. (1989). Botanical pesticides, past, present and future. Insecticides of plant origin (Ed. Arnason, J. T.). Proceedings of the Chemical Society, Washington. D. C., pp. 1 -10.

Jaiyesimi A. A. and Anthony O. (2011). Larvicidal properties of *Paullina pinnata* Linn leaf against *Anopheles gambiae*. *Journal of Pharmacognosy Communication*.**1**:1

Jamshaid I. (2006). Determination of the prevalence of lymphatic filariasis among migrant workers in Kuwait by detecting circulating filarial antigen. *Journal of Medical Microbiology*. **55**: 401-405

Jawara M., Smallegange R. C., Jeffries D., Nwakurima D. C., Awolola T. S. Bart G., Knols W. T, and Conway D. J. (2009). Optimizing Odor Baited Traps Methods for collecting mosquitoes during the malaria season in the Gambia. *Journal of PLoS ONE,* **4**: 8167.

Jean-Michel L. P. T., Jean-Bernard D., Laurence M., Patrick R., Gilbert L. G., Voahirana G. and Vincent R. (2003). Distribution of the species of the *Anopheles gambiae* complex and first evidence of *Anopheles merus* as a malaria vector in Madagascar. *Malaria Journal*. **2**:33.

Kakkilaya B.S. (2006). Control of malaria: scandalously scarce resource *Journal of Nature.* **3**:417-419.

Kalyanasundaram M. and Das P. K. (1985). Larvicidal and synergist activity of plant extracts for mosquito control. *Indian Journal of Medical Resources*. **82**:19-23

Keating J., Mbogo C.M., Mwangangi J., N Zovu J. G., Guiyun Y., Githure J. I. and Beier J. (2005). *Anopheles gambiae s.l.* and *Anopheles funestus* mosquito Distributions at 30 villages along the Kenyan coast. *Journal of Medical Entomology*. **43**: 241-246.

Kemri. (2012). Kenya Malaria Fact Sheet. Malaria in Kenya at a glance. Accessed online on 23/7/2012 at http//www.org/inclee.php/aboutkemri/board-of-management

Kiran S.R., Bhawan K., Devis P. S., Rajeswara R. and Reddy J. K. (2006). Composition and larvicidal activity of leaves and stem essential oils of chloroxylon *Sweetenia* DC against *Aedes aegypti* and *Anopheles stephensi*. *Journal of Bioscience Technology,* **97**: 2481-2484.

Howell P.I.and Knols B. G J. (2009). The Biology of mosquito mating. *Malaria Journal*. **2:**8

Kitron U. and Spielman A. (1989). Suppression of transmission of malaria though source reduction: antianophelline measures applied in Israel, The United States and Italy. *Journal Review of Infectious Diseases*.**11**:391-406.

Kohl O., Walia S. and Dhaliwal G. S. (2008). Essential oils as green pesticides: Potential constraints. *Journal of Biopestic International*. **4**: 63-84.

Kuniholm M. H., Wolfe N.D., Huang C. Y., Mpoudi-Ngole E., Tamoufe U., Le Breton M., Burke D.S. and Gubler D. J. (2006). Seroprevalence and distribution of *Flaviridae, Togaviridae,* and *Bunyaviridae* arboviral infections in rural Cameroonian adults. *American Journal of Tropical Medicine and Hygiene.* **75**: 371

Kong X. Q. and Wu C. W. (2009). Measurement and Prediction of Insertion Force for the Mosquito Fascicle Penetrating into Human Skin. *Journal of Bionic Engineering.* **6***:* 143-152.

Lehane M. J. (2005). *The biology of blood-sucking in insects.* Cambridge University Press. pp. 58

Lehrer S. (2010). *Anopheles* / mosquito transmission of brain tumor. *Journal of Medical Hypotheses*. **74**:167-168.

Leiriao P., Rodrigues C.D., Sonia S.A. and Mota M.M. (2004). Survival of protozoan intra cellular parasites in host cells. *Journal of EMBO reports* **5**:1142-1147

Leon B. and Steven S. (2002). Why Study Ecology in Temporary Pools? *Journal Israel of Zoology* **47:** 4

Leunita A, S. and Ogbunugafor C. B. (2008). Behavioral evidence for the existence of a region-specific oviposition cue in *Anopheles gambiae s.s Journal. of Vector Ecology.* **33**:321-324.

Lhuillier M., Mazzariol M. J., Zadi S., Le-Cam N., Benteja M. C., Adamowiez L., Marie F. N. and Fritzel B.(1989). Study of combined vaccination against yellow fever and measles in infants from six to nine months. *Journal of Biology standing*. **17**:9-15.

Luke E. M., Rodney L C., Lillian A M., Trish P., Fredrick O., Victor O. O., Randal J S., Cindy A R. and Nicholas A. (2011). Seroprevalence and distribution of arboviral infections among rural Kenyan adults: A cross-sectional study. .*Journal of Virology* **8**:371.

Linquist M., Kramer Laura and Dennis L. (2012). Mosquito reference manual. Accessed online on 25/7/2012

Lyimo E.O., Taakken W. and Koella J.C. (1992). Effect of rearing temperature and larval density on larval survival, age at pupation and adult size of *Anopheles gambiae. Journal of Experimental Entomolog*y. **63**:265-271.

Magdula J. J., Innocent E. and Otieno J.N. (2009). Mosquito larvicidal and cytotoxic activities of 3 *Annona* species and isolation of active principles. *Journal of Medicinal Plants Research*.**3**:674-680.

Maiyo Z.C., Ngure R.M., Matasyoh J.C. and Chepkorir R. (2010). Phytochemical constituents and antimicrobial activity of leaf exudates of three *Amaranthus* plant species. *African Journal of Biotechnology*. **9**: 3178-3182.

Majaw S. and Moirangthem E., (2009). Qualitative and quantitative Analysis of *Clerodendron colebrookiarium* Walp. Leaves and *Zingibe cassumunar* Roxb. Rhizomes. *Journal of Ethno botanical Leaflets*, **13**: 578-589.

Matasyoh J.C., Wathuta E. U., Kariuki S. T., Chepkorir R. and Kavulani J. (2008). *Aloe* plant extracts as alternative larvicides for mosquito control. *African Journal of Biotechnology*. **7**: 912-915.

Mazen A. (2012). Impact of flavonoids against woolly apple aphid, *Eriosoma lanigerum* (Hausman) and its sole parasitoid *Aphelinus mali* (Haid). *Journal of Agricultural Science*. **4**:2.

Michael E., Simonsen P.E., Malecela M., Jaoko W. G., Pedersen E. M., Mukoko D., Rwegoshora R. T. and Meyrowitsch D. W. (2001). Transmission intensity and the immunoepidemiology of bancrofti filariasis in East Africa. *Journal of Parasite Immunology*. **23**: 373-388.

Mcllister T.M., Anneh C.B., Cockwill CL., Olsorim E., Wang Y. and Cheeke P.R. (2001). Studies on use of *Yucca schidigera* to control giardiasis. *Journal of Veterinary Parasitology*. **97**: 85-89.

Mohsen Z. H., Jawad A. M. al Saad M. and Al Naib A. (1995). Antioviposition and insecticidal activity of *Imperta cylindrical* (Graminaceae).*Journal of Medica*l *Veterinary Entomology*.**9**: 441-442

MOH (2001) Kenya malaria Fact Sheet. Malaria in Kenya at a glance. Kemri report. Accessed online 22/7/ 2012.

MOH (2006). Kenya malaria Fact Sheet. Malaria in Kenya at a glance. Accessed online22/7/2012.

Monath T. P. (1991) Yellow Fever: Victor, Victoria? Conqueror, Conquest Epidemics and Research in the Last Forty Years and Prospects for the Future. *American Journal of Tropical. Medicine and Hygiene*: **45**:1-43.

Mugo B. (2012).The Impact of the Global Fund: "The will to win: how the global fund has changed millions of lives in Kenya". Remarks by Hon. Beth Mugo E.G.H., M.P, during the Global Fund Session at the European Parliament, Brussels, Belgium. Malaria News 11TH April, 2012. Accessed online on 22/ 7/2012.

Mulla M. S., Thavara V., Tawatsin A., Chopsri J. and Su T. (2003). Emergence of resistance management in field populations of tropical *Culex quiquefasciatus* to the microbial agent *Bacillus sphaericus*. *Journal of American Mosquito Control Association.* **19**:39-49.

Muirhead–Thompson, R.C.N. (1945). Studies on breeding places and control of *Anopheles gambiae* in the Coastal district of Sierra Leone. *Journal of Bulletin of Entomological Research*, **36**:185-252.

Munga S., Minakawa N., Zhou G. B., Okeyo-Owuor J., Githeko A. K. and Yan G. (2005). Oviposition Site Preference and Egg Hatchability of *Anopheles gambiae*: *Journal of Effects of Land Cover Types*. **42**: 993-997.

Munga S., Minakawa N., Zhou G., Mushinzimana E., Barrack O. O and Githeko A. K. (2006). Association between land cover and habitat productivity of malaria vectors in Western. *American Journal of Tropical Medicine and Health*. **74**:75-69.

Najera J. A., Kouznetsov R, L. and Delacollette C. (2011). Malaria control forecasting and prevention.Accessed27/1/2012@http//www.rollbackmalaria.org/docs/najeraepedemics/najll.htm

Nauwinger H. D. (2000). *African traditional medicines: a dictionary of plant use and application.* Med Pharm Scientific; Stuttgart, Germany. pp. 589.

Nelson M. J. (1986). *Aedes* mosquitoes: Biology and Ecology. Pan American Health Organization, Washington, D. C.

N′Guessan R., Corbel V., Akogbeto M. and Rowland M. (2007). Reduced efficacy of insecticide treated nets and indoor residue spraying for malaria control in pyrethroids resistance area, Benin. *Journal of Emerging Infectious diseases*. **13**:199-206

Nishiura J., Polly T. and Ray K. (2003). Methoprene Interferes with Mosquito Midgut Remodeling During Metamorphosis *Journal of Medical Entomology*. **40**:498-507.

Nobert B., Dusan P. and Marija Z. 2010. *Mosquitoes and their control*. European mosquito Bulletin. Springer Publishers. pp. 25.

Nwanjo H. U (2006). Antioxidant activity of the exudates forms *Aloe barbadensis* leaves in diabetic rats. *Journal of Bouline International*. **18**: 77-81.

Oklahoma University (2012). Entomology and Plant Pathology. Media News. Fax 405.744.6039. Accessed online 21/7/2012.

Paulraj M.G., Reagan A. D. and Ignacimuthu S. (2011). Toxicity of benzaldehyde and propionic acid against immature and adult stages Ae .aegypti (Linn) and Culex quinquefasciatus (Say) (Diptera: Culicidae). *Journal of Entomology* **8**: 539-547.

Pitasawat B., Chapakaew D., Choochote W., Jitpakdi A., Chaithong U., Kanjanapothi R., Tippawangkosol P., Riyong D., Tuetun B. and Chaiyasit D. (2007). Aromatic plant derived essential oil: An alternative larvicide for mosquito control. *Journal of Fitoterapia* **78**: 205 - 210.

Rattan R.S.(2010). Mechanism of action of insecticidal secondary metabolites of plant origin: Crop protection.29:913-920.

Roger W. (2009). *Communicable disease epidemiology and control: A global perspective*.CA BI, pp. 178.

Rohmer M. (1999). The discovery of the mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. *Journal. Natural Products Rep*ort. **16**. 565-574

Roll Back Malaria Partnership .*Roll Back Malaria: global strategic plan.* 2005-2015. Geneva: RBM; 2005.

Romeo J. T., Saunders J.A. and Barbosa P. (1996). (Eds) Recent advances in Phytochemistry, Vol. 30. Phytochemical diversity and redundancy in ecological interactions Plenum press, New York, London pp. 155-178.

Royston M. R., Gilbert J. C., Lynn B. R. and Allan S. W. (1974). *An introduction to Modern Experimental Organic Chemistr*y. Second edition, pp. 440

Sathish K. and Maneemegalai. (2008). Evaluation of larvicidal effect of *Lantana camara* Linn against *Aedes aegypti* and *Culex quiquefasciatus*. *Journal of Advances in Biological Research***.2**:39-43.

Schäfer M.L. and Lundström J.O. (2006). Different responses of two flood - water mosquito species, *Aedes vexans* and *Ochlerotatus sticticus* (Diptera: Culicidae), to larval habitat drying. *Journal of Vector Ecology*. **31**:123-128.

Service, M. W. (1993). *Mosquito Ecology: field sampling methods*, Chapman and Hall, London. pp. 988.

Service M. W. and Ashford R. W. (2001). *Encyclopedia of Arthropod Infections of Man and Domesticated Animals*. Chapman and Hall, London pp. 319.

Singh M., Dhanalakshmi E. and Rao M. (2000). Chemo modulatory action of *Aloe vera* on the profiles of enzymes associated with carcinogen metabolism and antioxidant status regulation in mice .*Journal of Phytomedicine* **28**:317-324.

Snow R.W., Guerra C. A., Noor A. M., Myint H. Y. and Hay I.S. (2005). The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Journal of Nature* **434**: 214-217

Steel R. G. D. and Torri J. H. (1980). *Principles and procedures of statistics. A Biometrical approach*. Second edition. pp. 170-176

Stacy A. G.(2012) 33-Year-Old Liver Transplant Recipient Returns From Hunting Trip in Sudan With Intermittent Fevers and Gross Hematuria. *Journal of Laboratory Medicine*: 43:3-6

Tan B.K.H. and Vanitha J. (2004). Immunomodulatory and antimicrobial effects of some traditional Chinese herbs: A review. *Journal of .Current Medicinal Chemistry*, **11**: 1423-1430

Tawfik K. M. Sheteawi S. A. and El-Gawad Z. A. (2001). Growth and aloin production of Aloe vera exudates under different ecological conditions. *Ethiopian Journal of Biology (Botany).* **3**: 149-159.

Tiwari A.K. and Rao M. (2002). Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects. *Journal of Current Science* **83**: 30-38.

Thomas A.W. (2004). The molecular site of action of juvenile hormone and juvenile metamorphosis: How these compounds kill insects. *Journal of Insect Physiology*. **50:**111-121.

Tomlin C.D. S. (2003). *The pesticide manual*. British crop protection council Publication 13 edition Pg. 37

UNICEF (2010). Fact of the week-90: Of all malaria deaths worldwide, currently 90 per cent occur in Sub- Sahara Africa. Millennium Development Goal 4: Reduce Child Mortality.Updated:11 October 2010. http:// www.chilinfo.org./malaria.htm1

Ulrike F., George S.., Gerry F., Killeen B. G. J., Knolls G.J. and Norbert B. (2004). The practical importance of permanent and semi permanent habitats for controlling aquatic stages of *Anopheles gambiae sensu lato* mosquitoes: operational observations from a rural town in western Kenya. *Journal of Tropical Medicine and International Health.* **9** :1274–1289

Utzinger A., Tozah T. and Singer B.H. (2001). Tropical medicine and international health Blackwell Synergy. Efficacy and cost effectiveness of environmental management for malaria control. *Journal of Medical International Health*. **6**:677-687

Utzinger J., Tanner M., Kammen D, M., Killeen G. F. and Singer B. H. (2002).Integrated programme is key to malaria control. *Journal of Nature* **4**: 419-431.

Van Damme P. (1991). Plant Ecology of the Nabib desert. *Journal of Africa focus*, **7**: 355-400.

Varavikova E. and Theodore H.T. (1998). The new public health. *Journal of Emergent Infectious Diseases.* **4**:442-450

Veena P., Tripathi A.K., Aggarwal K.K. and Khanuja S. P. S. (2011). Insecticidal, repellent and oviposition deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus. Journal of Bioresource Technology*, **96**: 1749- 1757

Wabuyele E., Bjorå C. S., Nordal I. and Leonard E. N. (2006). Distribution, Diversity and conservation of the genus *Aloe* in Kenya. *Journal of East African Natural History*. **95**:213-225.

Walker E. D., Thibault A.R., Thelen A. P., Bullard B. A., Huang J., Odiere M. R., Bayoh N.M., Wilkins E. E. and Vulule J. M. (2007). A Review Distribution of African malaria mosquitoes belonging to the Anopheles gambiae complex. *Journal of Malaria* **27**: 60: 23.

Weill, M., Chandre F., Brengues C., Manguin S., Akogbreo M., Pasteur N., Guillet, P. and Raymond, M. (2000). The unique mutation in ace-1 giving high insecticide resistance is easily detected in mosquito vectors. .*Journal of Insect Molecular Biology*. **9**: 451-455.

Weill M., Malcolm C., Chandre F., Mogensen K., Bertholomiew A. S., Marquina B. and *Raymond* M. (2004). The unique mutation in ace-1 giving high insecticide resistance is easily detected in mosquito vectors. *Journal of insect Molecular Biology.* **3**: 1-7.

Wikipedia, the free Encyclopedia (2012). Mosquito. Accessed online 20/7/2012 at http://en.wikipedia.org/wiki/mosquito#mw-head

White G. B.(1985) .*Anopheles bwambae* sp., a malaria vector in the Semliki Valley, Uganda, and its relationships with other sibling species of the *An.gambiae* complex (Diptera: Culicidae). *Journal of Systematic Entomology*. **10**:501-522.

Wirth M. C., Hyun-Woo P., Walton W. E. and Federiri B. A. (2005). Cyt1A of *Bacillus thuringiensis* delays evolution of resistance to Cry11A in the mosquito *Culex quinquefasciatus Journal of Applied Environmental Microbiology.* **71**: 1185-1189.

WHO, 1992. Division of Epidemiological Surveillance and Health Situation and Trend Assessment. Global Health Situation and Projections: Estimates. Geneva, Switzerland.

WHO, (1998). Division of emerging and other communicable diseases surveillance and control global programme for vaccines and immunization expanded programme on immunization.

WHO. (1996). Investing in health research for development. Report of the Ad Hoc Committee on Health Research Relating to Future Intervention Options; 1996 Report No. TDR/Gen/96.1. Geneva: The organization

WHO and UNICEF (2003). The Africa malaria Report WHO/CDS/MAL/2003.1093. Retrieved 27th April 2003 from http://mosquito.who.int/amd2003/

WHO (2005). Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization communicable disease control, prevention and eradication. WHO pesticide evaluation scheme.

WHO. (2006). Malaria vector control and personal protection. Report of WHO study Group WHO Technical Report Series 936.

WHO,(2009). Integrated malaria vector control with microbial larvicides and insecticide treated nets in Western Kenya: a controlled trial. Bulletin of the World Health Organization. **87**: 645-732

WHO. (2010). First WHO Report on neglected diseases 2010: working to overcome the global impact of neglected tropical diseases.

WHO (2011). Urgent action essential to protect malaria therapies says WHO

WHO (2011) .Global plan for artemisinin resistance containment. pp 1-87.

WHO (2011). Global Alert and Response (GAR). Yellow fever: a current threat. Accessed 27th January 2011 @ http://www.who.int/csr/disease/yellowfev/impactl/en/index.html

WHO (2012). Dengue and the severe dengue. Fact sheet No. 117.

Womack M. (1993). "The yellow fever mosquito, *Aedes aegypti*. *Journal of Wing Beats* **5**: 4

Yagi A. and Takeo S. (2003). Anti-inflammatory constituent's aloesin and aloemannan in *Aloe* species and effects of tanshinon VI in *Salvia miltiorrhiza* on heart accessed online 08/08/2012. yagi @fupharm.fukuyama-u.ac.jp.

Yakugaku Z. (2003). Ant-inflammatory constituents of aloesin and aloemannan in *Aloe* species. *Journal of Public Medicine.* **123**:517-532.

APPENDICES

Appendix 1

F0 Post 24 hour exposure to Aloe extracts

Treatment	Rep. 1		Rep. 2		Rep. 3		Mean Mortality	% Mortality	Mean Survivorship	% Survivorship
	Dead	Alive	Dead	Alive	Dead	Alive				
Control	25	1475	55	1445	40	1460	40	2.67	1460	97.33
1%Dimethyl Sulphoxide	150	1350	115	1385	155	1345	140	9.33	1360	90.67
1% ethyl acetate	495	1005	390	1110	440	1060	442	29.44	1058	70.56
LC_{50} *A. ngongensis*	835	665	760	740	725	775	773.33	51.56	726.67	48.44
LC_{50}.*A. turkanensis*	759	741	775	725	805	695	779.67	51.98	720.33	48.02
LC_{50} *A.fibrosa*	730	770	720	780	737	763	729	48.6	771	51.4

Appendix 2

Pupae emergence

Treatment	Rep. 1		Rep. 2		Rep. 3		Mean Mortality	% Mortality	Mean Survivorship	% Survivorship
	Dead	Alive	Dead	Alive	Dead	Alive				
Control	15	1445	5	1455	26	1434	15.336	1.05	1444.67	98.95
1%Dimethyl Sulphoxide	10	1350	30	1330	40	1320	26.67	1.96	1333.33	98.04
LC_{50} *A. ngongensis*	107	620	77	650	92	635	92	12.65	635.00	87.35
LC_{50}.*A. turkanensis*	101	619	112	608	91	629	101.33	14.07	618.67	85.93
LC_{50} *A.fibrosa*	104	667	90	681	72	699	88.67	11.5	682.33	88.50

Appendix 3

Adult Male Emergence

Treatment	Rep. 1	Rep. 2	Rep. 3	Mean	%
Control	671	668	663	667.33	88.98
DMSO	659	663	669	663.67	88.49
LC_{50} *A. ngongensis*	323	319	326	322.67	43.02
LC_{50} *A. turkanensis*	309	312	299	306.67	40.89
LC_{50} *A. fibrosa*					

Appendix 4

Adult female emergence

Treatment	Rep. 1	Rep. 2	Rep. 3	Mean	%
Control	641	646	635	640.67	85.42
DMSO	633	640	637	636.67	84.89
LC_{50} *A. ngongensis*	310	315	312	312.33	41.89
LC_{50} *A. turkanensis*	309	305	286	300	40
LC_{50} *A. fibrosa*	346	352	351	349.67	46.62

Appendix 5

Male: female sex ratio (Male/Total)

Treatment	Sex ratio
Control	0.51
1%Dithyl Sulphoxide	0.51
LC_{50} *A. ngongensis*	0.51
LC_{50} *A. turkanensis*	0.51
LC_{50} *A. fibrosa*	0.50

Appendix 6

Egg deposits (oviposition) studies on 30 males+30 females

Treatment	Rep. 1	Rep. 2	Rep. 3	Mean ED	Fecundity
Control (dist. Water)	6461	8121	7600	7394.00	246.67
1%Dimethyl Sulphoxide	5947	6300	5844	6030.33	201.01
LC_{50} *A. ngongensis*	4100	4230	4300	4210.60	140.33
LC_{50} *A. turkanensis*	4428	3996	4120	4181.33	139.37
LC_{50} *A. fibrosa*	4500	4670	4800	4656.67	155.22

Appendix 7:

Age specific survivorship of adults for males (F0) where; x=yx/y0

Treatment	Rep. 1	Rep. 2	Rep. 3	Mean	x=yx/y0 (28th day)
Control	42	46	40	42.66	0.71
1% Dimethyl sulphoxide	39	37	42	39.33	0.67
LC_{50} *A. ngongensis*	14	17	12	14.33	0.23
LC_{50} *A. turkanensis*	10	13	9	10.67	0.18
LC_{50} *A. fibrosa*	25	29	31	28.33	0.47

Appendix 8

Age-specific survivorship of female adults (F0)

Treatment	Rep. 1	Rep. 2	Rep. 3	Mean	x=yx/y0 (28th day)
Control	23	29	25	25.67	0.85
1% Dimethyl sulphoxide	25	28	20	24.33	0.81
LC_{50} *A. ngongensis*	10	9	13	10.67	0.36
LC_{50} *A. turkanensis*	8	6	9	7.67	0.26
LC_{50} *A. fibrosa*	19	24	22	21.67	0.72

Appendix 9:

Male Age specific mortality studies (F1)

Treatment	Rep. 1	Rep. 2	Rep. 3	Mean	x=yx/y0 (28th day)
Control	19	14	17	16.67	0.56
1% Dimethyl sulphoxide	14	11	19	14.67	0.49
LC_{50} *A. ngongensis*	4	5	1	3.33	0.11
LC_{50} *A. turkanensis*	2	4	1	2.33	0.08
LC_{50} *A. fibrosa*	9	4	6	6.33	0.21

Appendix 10:

F1 Egg Hatchability 48 hours post *Aloe* extracts exposure of 1500 eggs of *Aedes aegypti*

Treatment	Rep. 1	Rep. 2	Rep. 3	Mean Viability	% viability
Control	1452	1437	1470	1453	96.87
1% Dimethyl sulphoxide	1398	1460	1400	1419	94.6
LC_{50} *A. ngongensis*	750	630	721	700	46.67
LC_{50} *A. turkanensis*	300	250	340	297	19.8
LC_{50} *A. fibrosa*	800	690	757	749	49.93

Appendix 11

Larvae emergence

Treatment	Rep 1		Rep. 2		Rep. 3		Mean Mortality	% Dead	Mean Alive	% Alive
	Dead	Alive	Dead	Alive	Dead	Alive				
Control	43	1410	50	1403	39	1414	44	3.03	1409	96.97
1%DMSO	152	1267	141	1278	136	1283	143	10.8	1276	89.92
LC_{50} *A. ngongensis*	217	483	200	500	230	470	216	30.86	484	69.14
LC_{50} *A. turkanensis*	134	163	147	150	154	143	145	48.82	153	51.52
LC_{50} *A. fibrosa*	172	577	180	569	199	550	184	24.56	565	75.43

Appendix 12

Pupae emergence

Treatment	Rep. 1		Rep. 2		Rep. 3		Mean Mortality	% Mortality	Mean Survivorship	% Survivorship
	Dead	Alive	Dead	Alive	Dead	Alive				
Control	32	1377	29	1380	24	1385	28.33	2.01	1380.67	97.98
1%Dimethyl Sulphoxide	26	1250	34	1242	16	1260	25.33	1.98	1250.67	98.01
LC_{50} *A. ngongensis*	63	421	54	430	67	417	61.33	12.67	422.66	87.32
LC_{50}*.A. turkanensis*	21	132	24	129	28	125	24.33	15.9	128.67	84.09
LC_{50} *A.fibrosa*	80	485	72	493	86	479	79.33	14.04	485.67	85.96

Appendix 13

Adult male emergence

Treatment	Rep 1	Rep. 2	Rep. 3	Mean	%
Control	648.00	654.00	639.00	647.00	86.27
1%DMSO	627.00	635.00	623.00	628.33	83.77
LC_{50} *A. ngongensis*	192.00	189.00	200.00	193.66	25.82
LC_{50} *A. turkanensis*	57.00	60.00	69.00	62.00	8.27
LC_{50} *A. fibrosa*	218.00	230.00	225.00	224.33	29.91

Appendix 14

Adult female emergence

Treatment	Rep 1	Rep. 2	Rep. 3	Mean	%
Control	628.00	628.00	617.00	624.33	83.24
1%DMSO	603.00	622.00	598.00	607.67	81.02
LC_{50} *A. ngongensis*	186.00	182.00	192.00	186.67	24.88
LC_{50} *A. turkanensis*	55.00	57.00	48.00	53.33	7.11
LC_{50} *A. fibrosa*	215.00	224.00	221.00	220.00	

Appendix 15

Male and female sex ratio

Treatment	Sex ratio
Control	0.51
1%Dithyl Sulphoxide	0.51
LC_{50} *A. ngongensis*	0.51
LC_{50} *A. turkanensis*	0.51
LC_{50} *A. fibrosa*	0.50

Appendix 16

***Aedes aegypti* F1 oviposition**

Treatment	Rep. 1	Rep. 2	Rep. 3	Mean oviposition	Fecundity
Control (dist. Water)	7300.00	7000.00	6800.00	7033.00	234.43
1%Dimethyl Sulphoxide	7342.00	7742.00	7760.00	7614.66	253.82
LC_{50} *A. ngongensis*	4847.00	4997.00	5000.00	4948.00	164.93
LC_{50} *A. turkanensis*	4500.00	4390.00	4900.00	4596.66	153.22
LC_{50} *A. fibrosa*	6934.00	7200.00	7114.00	7082.66	236.08

Appendix 17

Qualitative analysis of terpenoids from selected larvicidal Aloe plant extracts

Extract	Observation	Inference
ATE	A dark brown coloration at the interface	Absence of terpenoids
ANH	A dark brown coloration at the interface	Absence of terpenoids
ANE	A light brown coloration of interface	Absence of terpenoids
ANC	A brown coloration of interface	Absence of terpenoids
ANA	A brown of interface	Absence of terpenoids
ANM	A dark brown coloration of interface	Absence of terpenoids
AFH	A dark brown coloration of interface	Absence of terpenoids
AFA	A dark brown coloration of interface	Absence of terpenoids
AFM	A reddish brown coloration at interface	Presence of terpenoids

Appendix 18

Qualitative analysis of steroids of selected larvicidal *Aloe* plant extracts

EXTRACT	Observation	Inference
ATE	Formation of a brownish yellow solution was observed	Absence of steroids
ANH	A brownish colored solution was formed that was stable on standing	Absence of steroids
ANE	A yellow colored solution was formed	Absence of steroids
ANC	A brown colored solution was formed	Absence of steroids
ANA	A brown colored solution was formed	Absence of steroids
ANM	A light brown colored solution was formed	Absence of steroids
AFH	A reddish brown colored solution was formed	Absence of steroids
AFA	A reddish brown colored solution was formed	Absence of steroids
AFM	A dark brown solution formed that is stable on standing	Absence of steroids

Appendix 19

Qualitative analysis of flavanoids of selected larvicidal Aloe plant extracts

Extract	Observation	Inference
ATE	A yellow coloration was formed	Presence of flavanoids
ANH	A clear solution was formed	Absence of flavanoids
ANE	A yellow coloration was formed	Presence of flavanoids
ANC	A colorless solution	Absence of flavanoids
ANA	A yellow coloration was formed	Presence of flavanoids
ANM	A faint yellow coloration was observed	Presence of flavanoids
AFH	A faint yellow coloration was observed	Presence of flavanoids
AFA	A yellow coloration was formed	Presence of flavanoids
AFM	A yellow coloration was formed	Presence of flavanoids

Appendix 20

Qualitative analysis of tannins and polyphenols of larvicidal selected *Aloe* plant extracts

Extract	Observation	Inference
ATE	Formation of a brownish green solution	Presence of tannins
ANH	Formation of an orange solution	Absence of tannins
ANE	Formation of an orange solution	Absence of tannins
ANC	Formation of a light orange solution	Absence of tannins
ANA	Formation of a brown green solution	Presence of tannins
ANM	Formation of a blue black solution	Presence of tannins
AFH	Formation of a blue black solution	Presence of tannins
AFA	Formation of blue black solution	Presence of tannins
AFM	Formation of blue black solution	Presence of tannins

Appendix 21

Phlobatannins analysis in selected Aloe plant extracts

Extract	Observation	Inference
ATE	A clear solution forms	Absence of phlobatannins
ANH	A clear solution forms	Absence of phlobatannins
ANE	A clear solution forms	Absence of phlobatannins
ANC	A clear solution forms	Absence of phlobatannins
ANA	A clear solution forms	Absence of phlobatannins
ANM	A clear solution forms	Absence of phlobatannins
AFH	A clear solution forms	Absence of phlobatannins
AFA	A clear solution forms	Absence of phlobatannins
AFM	A clear solution forms	Absence of phlobatannins

Appendix 22

Qualitative saponin test results for selected larvicidal *Aloe* plant extracts

Extract	Observation	Inference
ATE	Formation of an emulsion	Presence of saponins
ANH	Formation of an emulsion	Presence of saponins
ANE	Formation of an emulsion	Presence of saponins
ANC	Formation of two immiscible layers	Absence of saponins
ANA	An emulsion was observed	Presence of saponins
ANM	Formation of two immiscible layers	Absence of saponins
AFH	Two immiscible layers were formed	Absence of saponins
AFA	Formation of an emulsion	Presence of saponins
AFM	Formation of an emulsion	Presence of saponins