EFFECTS OF COW DUNG AND SELECTED MEDICINAL PLANTS ON ANOPHELES SPECIES AS A STRATEGY FOR MALARIA VECTOR CONTROL IN AHERO RICE IRRIGATION SCHEME, KENYA

BY

NGUGI, MICHAEL KAHATO (B.ED. SC. (Hons.))
Reg. No. 156/13492/05

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DECLARATION

Candidate

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

NGUGI MICHAEL KAHATO

Signature..........................

Date.........................02/04/2009

Supervisors

We confirm that the candidate under our supervision carried out the work reported in this thesis. We have read and approved this thesis for examination.

Prof. Elizabeth Kokwaro

Department of Zoological Sciences,
Kenyatta University.

Signature.......................... Date.2/04/09

Dr. Michael Gicheru

Department of Zoological Sciences,
Kenyatta University.

Signature.......................... Date.2-4-2009
DEDICATION

This work is dedicated to my loving wife Beth Kahato, our son Lance Calvin and my mentor Rev. Fr. Dr. Lance P. Nadeau.
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ABBREVIATIONS AND ACRONYMS

ACT: Artemisinin - based Combination Therapies
CHMC: Cyclohexamethylene carbamide
DALYs: Disability Adjusted Life Years
DDT: Dichlorodiphenyltrichloroethane
DMP: Dimethyl phthalate
GDP: Gross Domestic Product
GOK: Government of Kenya
IPT: Intermittent Preventive Treatment
IRS: Indoor Residual Spraying
ITNs: Insecticide Treated Nets
KEMRI: Kenya Medical Research Institute
LLINs: Long Lasting Insecticidal Nets
MOH: Ministry of Health
NIAID: National Institute of Allergy and Infectious Diseases
NIB: National Irrigation Board
SP: Sulphadoxine - pyrimethamine
WHO: World Health Organization
ABSTRACT

The control of malaria is complex and has previously focused on the use of drugs and chemicals targeting the parasite and the vectors respectively. However, this has been a challenge because the parasite and the vectors have developed resistance. However, integrated approaches that incorporate use of cow dung and plants products could increase the chances of malaria control. Deliberate organic contamination as a measure against mosquitoes has been shown to have a larvicidal effect but the findings have not been tested in semi-field conditions. For a long time, neem has been reported to possess larvicidal properties against mosquitoes. However, the combined effect of cow dung and neem on immature stages of *Anopheles* has not been investigated. This research was therefore designed to investigate the effect of cow dung alone and a combination of cow dung and neem on the immature stages of *Anopheles* mosquitoes. Despite the wide use of *Artemisia annua* in manufacture of malarial drugs little is known about its effect on the adult *Anopheles* species, the vector of malaria. This study was therefore undertaken to investigate the effects *A. annua* on adult *Anopheles* mosquitoes. In the laboratory experiments, 10 individuals of each immature stage were placed in bowls containing either cow dung, neem alone or neem and cow dung combined. Mortality was recorded after 24 hours. Semi-field experiments were conducted in a screen house where sub-plots measuring 1.5 x 0.5 m were created. Cow dung, neem alone or cow dung combined with neem was added. 50 individuals of each immature stage were then introduced in each sub-plot and then mortality was recorded. In testing the effects of *A. annua* on adult *Anopheles* mosquitoes, crude extracts of *A. annua* were smeared on hands of human volunteers and subjected to 30 starved females. The number of mosquitoes that landed on hand with and without *A. annua* were counted and recorded after every 5 minutes for a period of 1 hour. Also the number of mosquitoes whose abdomens appeared reddish and dilated were counted and recorded at the end of the experiment. Analysis of variance tests was performed to determine the effect of cow dung, neem alone and a combination of neem with cow dung on the immature stages of *Anopheles* species in both the laboratory and the semi-field conditions. In evaluating the effect of *A. annua* against adult *Anopheles* mosquitoes, t-tests were performed to compare mean numbers of mosquitoes that landed and the means of fed and unfed mosquitoes. Results for laboratory experiments indicated that cow dung and neem individually caused significant mortality of immature stages of mosquitoes. Also, a combination of cow dung and neem individually caused higher mortality than cow dung, neem alone but the difference was not significant. In semi-field experiments, there was a significant difference in mortality between all the immature stages in sub-plots containing cow dung alone. The mortality was higher in sub-plots applied with a combination of cow dung and neem but as reported in the laboratory experiments the difference was not significant. In evaluating the effect of *A. annua* on *Anopheles* mosquitoes, the results showed that higher number of females landed and fed on the hands without *A. annua* than on hands applied with *A. annua*. However, efficacy of the latter reduced as time progressed. The findings of this study have demonstrated that cow dung, neem alone and a combination of neem and cow dung caused mortality of the immature stages and can thus be used to reduce abundance of larval stages in the mosquito breeding sites. It was also evident that, *A. annua* reduced the landing and feeding response of mosquitoes and could therefore be used to reduce human–vector contact.
CHAPTER ONE
INTRODUCTION

1.1 Malaria epidemiology

Globally malaria deaths are responsible for almost 3% of the world’s daily adjusted life years (DALYs). Despite the enormous investment in control efforts this disease continues to be a major cause of morbidity, mortality and associated economic losses in tropical and sub-tropical countries of the world (Malaney et al., 2004). According to World Health Organization (WHO, 2007) malaria kills between 1.5 and 2.7 millions people each year in the world and between 300 and 500 million others fall ill from it often severely. Over a million of these deaths are in children under 5 years of age and women in their first or second pregnancy (Bardaji et al., 2008). Reports by Breman and Hollway (2007) indicated that, globally in each year, approximately 41% of the world population is at risk as many thousands of malaria cases are imported into malaria free countries from endemic sites. This has translated to an increase of close to 10% over the past decade (Breman et al., 2004).

In Africa, malaria is still a major complex public health problem. The disease is responsible for over two million deaths, mainly in children under five years of age especially in remote areas with poor access to health facilities (WHO, 2006). Pregnant women are four times more likely to suffer malaria attacks due to their low malaria immunity leading to low birth weights and still births, thus endangering the health of the women and prospects for the new born (Lindsay et al., 2000). In all the global reported malaria cases, more than 95% of them occur in Africa. Outside tropical Africa, malaria
deaths occur mainly among non-immune new comers to endemic areas, for example among agricultural workers, miners and settlers in newly colonized areas (WHO, 2000). Malaria is generally endemic in tropics, with extension into sub-tropics. It is still responsible for up to 50% of outpatient cases and 20% of hospital admissions. In total, malaria is estimated to cost Africa about US$12 billion annually with an average annual reduction of 1.3% per year in economic growth through families spending a significant portion of their income on malaria treatment (WHO, 2007). In terms of distribution, an estimated 74% of the population in the African region lives in areas that are highly endemic for malaria and 19% in epidemic prone areas. Only 7% of the region’s population lives in low risk or malaria free zones (WHO, 2006).

Malaria is one of the leading causes of death in Kenya. Approximately, 29 million people (78%) of Kenya’s population live in areas of risk for malaria transmission. Transmission occurs throughout the year along the border with Uganda near Lake Victoria. Elsewhere, seasonal transmission occurs, with duration varying greatly from locality to locality and some areas experiencing two transmission seasons. Malaria impacts on economic growth and productivity, and it has been estimated that nearly 170 million working days are lost annually due to this disease (WHO, 2006). Malarial impacts are high especially in Western and Coastal Kenya. This menace is approximated to cause 26,000 deaths of children in Kenya annually. It is responsible for 30% of all outpatients’ attendance and 19% of attendance of all admissions to the health facilities (MOH, 2001). Estimates of infant and child mortality on the Kenyan coast show that at least 58 infants per 1000 life births and 12 children per 1000 children aged between one and four years die each year (Snow et
Primary school students miss 11% of school days and secondary students miss 4.3% of school days because of malaria (Sachs and Malaney, 2002). Epidemics of *Plasmodium falciparum* malaria have been observed in high altitude areas and reasons attributed to the increased malaria incidence in these areas of unstable malaria transmission include changes in land use patterns, increase in vector population, breakdown in provision of health services and pesticide resistance (Shanks *et al.*, 2002). Studies by Brooker *et al.* (2004) in western Kenya highlands indicated that the epidemics normally peaked one month after the onset of long rainy season usually from May to August. In the lowland areas around the Lake Victoria and Kenyan coast malaria is endemic, with the transmission season mainly peaking during the rainy season (Githeko and Ndegwa, 2001).

1.2 Malaria vectors

Out of more than 400 described species of mosquitoes by White (1977) some 45 of them are implicated in the transmission of malaria worldwide. Different species of *Anopheles* are responsible for the transmission of malaria in specific geographical regions. The density of mosquito population is dependent on larval ecology. Irrigation schemes especially those used for rice growing are preferred breeding sites for *An. gambiae* s.l and *An. funestus*. *Anopheles balabacensis* and *An. dirus* are abundant in the forested areas (Muirhead-Thomson, 1951). *Anopheles merus* and *An. melas* have extensive breeding sites within the tidal limits of the Kenyan coastal line (Bryan, 1983). The malaria vectors play an important role in the transmission of protozoan parasites *plasmodium falciparum*, *P. ovale*, *P. vivax* and *P. malariae*. In Kenya, the malaria parasites are the *P. falciparum*
forms. The malaria vectors generally cause high parasite inoculation rates and are also remarkably stable in a wide range of bio-ecological and seasonal conditions hence appears to be very flexible, both in exploiting new man made habitats and their response to malaria control activities (Coluzzi et al., 1984).

1.3 Statement of the problem and justification of the study

Malaria is one of the most serious disease that causes morbidity and mortality, creating a significant barrier to economic development worldwide. It is one of the major vector borne diseases affecting the poor rural communities especially in rice growing areas. The use of irrigation to flood agricultural land for rice cultivation has over the years been associated with an increase in disease vectors, and in certain cases, a corresponding increased burden of malaria and other vector and water borne diseases (Lacey and Lacey, 1990; Ijumba and Lindsay, 2001). The problem of the disease is made worse by mosquito resistance to insecticides (Snow et al., 1998). At present there is no drug that offers foolproof protection against malaria and some of the drugs used to treat the disease have severe side effects on a small percentage of people who use them (Hastings and Mackinnon, 1998).

In Kenya the parasite has become 80% resistant to quinine. This level of resistance is said to be the highest globally (WHO, 1987). Also effective drugs for treating malaria are unaffordable to majority of the rural population. Some agricultural activities such as flooding of rice paddies and broadcasting of nitrogenous inorganic fertilizers in rice paddies have been shown to enhance mosquito breeding and malaria transmission (Victor
and Reuben 2000). Knowledge on environmental management and mosquito breeding has been used in the past to control malaria in irrigation systems. Organic matters such as pig droppings and horse manure have previously been used with success to control Culicine mosquitoes such as Cx. vishnui, Cx. pseudovishnui and Cx tritaeniorynchus mosquitoes in India (Hackett et al., 1938). The method has also been used effectively against An. fluviatilis and An. maculatus in India and Malaysia (Beales and Gilles, 2002). However, no studies have been conducted to test the effectiveness of cow dung in semi-field conditions. Neem (Azadirachta indica) products have been shown to exhibit a wide range of effects that are potentially useful for mosquito control (Okumu et al, 2007) these effects are frequently attributed to the azadirachtin contents of the products. However, the combined effects of the cow dung and neem as a means of Anopheles control have not been investigated. Also in any control measure, not all the targeted individual will be affected by the measures applied and therefore a small percentage of the emerged adults will need an intervention measure that reduces human-vector contact. Artemisia annua is known to provide Artemisinin, a chemical for making anti-malarial drugs. However little is known about the effect of A. annua against adult Anopheles species. With resistance of malaria parasite to drugs and the vector to chemicals, alternative control methods must be sought. This study therefore investigated the effects of cow dung, neem separately and in combination with neem on immature stages of Anopheles species in Ahero rice irrigation scheme. The study also investigated the effects of A. annua on the behavior of adult Anopheles species.
1.4 Research questions

i. What is the effect of cow dung on survival of immature stages of *Anopheles* mosquitoes?

ii. What is the effect of combination of cow dung and neem on survival of immature stages of *Anopheles* mosquitoes?

iii. What is the effect of *Artemisia annua* on feeding behaviour of adults of *Anopheles* mosquitoes?

1.5 Hypotheses

i. Cow dung has no effect on survival of immature stages of *Anopheles* mosquitoes.

ii. A combination of cow dung and neem has no effect on survival of immature stages of mosquitoes.

iii. *Artemisia annua* has no effect on feeding behaviour of adults of *Anopheles* mosquitoes.

1.6 Objectives of the study

1.6.1 General objective

To determine the effect of cow dung, neem and *A. annua* on *Anopheles* species of mosquitoes as a strategy for malaria vector control in Ahero rice irrigation scheme.
1.6.2 Specific objectives

i. To investigate the effect of cow dung on survival of immature stages of *Anopheles* mosquitoes.

ii. To investigate the effect of a combination of cow dung and neem on survival of immature stages of *Anopheles* mosquitoes.

iii. To determine the effect of *A. annua* on feeding behaviour of adults *Anopheles* mosquitoes.
CHAPTER TWO
LITERATURE REVIEW

2.1 Distribution of *Anopheles* mosquitoes

Malaria in Africa is mainly transmitted by three mosquito species namely: *An. gambiae*, *An. arabiensis* and *An. funestus*. The primary malaria vectors in Kenya are *An. funestus* and three members of *An. gambiae* complex: *An. gambiae* sensu stricto, *An. arabiensis* and *An. merus* (Collins *et al.*, 1988; Beier *et al.*, 1999). *Anopheles gambiae* s.s and *An. arabiensis* are mostly closely associated with humans and represent the major vectors of malaria (Muirhead-Thomson, 1951; Highton *et al.*, 1979). The distribution of these two species overlaps and they occur sympatrically in large areas of tropical Africa such as Kenya, Ethiopia, Sudan, Tanzania, Nigeria among other areas. *An. gambiae* sensu lato and *An. merus* are predominantly found along the Kenyan coast and Western Kenya around Lake Victoria. The distribution of potential and predominant malaria vector is shown in the figure that follows (Fig. 2.1)
Figure 2.1: The distribution of dominant or potentially important malaria vectors (Kiszewski et al., 2004).

2.2 The life cycle of Anopheles vectors of malaria

Like all mosquitoes, anophelines go through four stages in their life cycle: egg, larva, pupa and adult. Figure 2.2 shows the life cycle of Anopheles mosquitoes. The first three stages are aquatic and last for 5-14 days depending on the species and the ambient temperature. After mating and blood feeding, a female Anopheles mosquito lays 50-200 eggs per oviposition that are 1 mm long and brown or blackish boat shaped eggs on the water surface. Eggs are laid singly directly on water and are unique in having floats on either side. The eggs are not resistant to drying and if viable, eggs hatch into larvae within 2-3 days in the tropics. However, in colder climates hatching may take up to 2-3 weeks (Service, 1980).
Figure 22: Schematic illustration of the life cycle of Anopheles vectors of malaria (After Service, 1980).
Anopheles larvae lack a respiratory siphon and for this reason, in water they position themselves so that their body is parallel to the surface of the water to allow water intake and surface feeding. At mean water temperature of 25 - 28°C the larvae undergo four molts within 6 - 9 days to reach the pupal stage, which lasts 2 - 3 days depending on the temperature. Thus, the minimum duration for one generation may be as long as 10 - 15 days. The breeding habitats vary from large and usually permanent collections of water, such as rice fields, fresh or salty water marshes, mangrove swamps, borrow pits, grassy ditches, the edges of streams and rivers to smaller collections of temporary water such as gulleys, small pools of rain water, hoofs prints, car tracks, ditches, drains and puddles. The pupae are comma shaped and bear respiratory trumpets that are short and broad distally thus appearing conical (Fig. 2.2). The most distinctive characteristic of Anopheles pupae is the presence of short peg-like spines situated laterally near the distal margin of the abdominal segments. After a few days as a pupa, the pupal skin splits dorsally and the adult emerges.

The duration from eggs to adults varies considerably among species and is strongly influenced by ambient temperature. In tropical conditions this takes 10-15 days (Service, 1993). After emerging, careful movements are needed to ensure that the adult mosquito does not fall sideways and be trapped in the surface film. This danger is particularly acute when the adult is largely out of the pupal exuviae but the terminal appendages are still not free. Finally the legs become free and spread on the water surface giving the newly emerged adult some stability. The newly emerged adult inflates its wings, and separates and grooms its head appendages before flying away (Kettle, 1992).
2.3 Feeding and resting behavior of *Anopheles* mosquitoes

Mosquitoes within the *An. gambiae* species complex are the most important vectors of malaria in sub-Saharan Africa (Kiszewski *et al.*, 2004). The infective bites of these mosquitoes is in large part responsible for the more than 500 million clinical attacks of malaria reported worldwide each year, resulting in more than one million deaths (Snow *et al.*, 2005). Most *Anopheles* mosquitoes are crepuscular (active at dusk or dawn) or nocturnal (active at night). Eighty percent of female adult *Anopheles* feed on any large mammal that is available (Gilles, 1972). The host preference by a particular species of mosquitoes is also likely to be influenced by environmental conditions. Some of the mosquitoes are strictly zoophilic while others are anthropophilic. Of the three species of *An. gambiae* complex, *An. arabiensis* and *An. merus* are partially zoophilic and partially endophilic (prefer to rest indoors) (Mosha *et al.*, 1983).

Studies in Western Kenya have showed that *An. arabiensis* has a lower proportion in terms of frequency of human meals, which reflect a high degree of exophily (prefer to rest out doors) (Githeko *et al.*, 1994). Petrarca *et al.* (1991) found out that a significant proportion of *An. arabiensis* fed on cattle but were also collected indoors. *Anopheles arabiensis* is generally diverted to cattle feeding than *An. gambiae* s.s (Githeko *et al.*, 1994). *An. gambiae* is primarily endophilic (rest indoor) and endophagic (feed indoors) whereas *An. arabiensis* and *An. merus* show some degree of partial exophily and zoophagy (Coluzzi *et al.*, 1979; Gilles and Coetzee, 1987).
Blood feeding by females is essential for transmission of malaria parasites and of this characteristic behavior can have major implications in the epidemiology of the disease. Briegel and Horler (1993) showed that anopheline mosquitoes take multiple blood meals within the gonotrophic cycle. Since biting is one of the factors determining the vectorial capacity of the mosquito species, multiple feeding has a profound effect on the rate of malaria transmission. The probability that an infected mosquito will survive until it is able to transmit malaria parasites will increase when it takes a blood meal early in life thereby increasing the vectorial capacity (Garret, 1964).

2.4. Gaseous exchange by developmental stages of mosquitoes

Larvae live in the water but are required to 'break' the water surface to breathe. Most species such as Culicines and Aedes have specialized breathing siphons at the 'tail' end of their bodies. However, most species of larvae hang, upside down, breathing with siphons and filtering food with their specialized mouthparts. Mosquito larvae have evolved different strategies of obtaining air ranging from breathing in atmospheric air to breathing in dissolved oxygen (Manna et al., 2008). Most mosquito larvae except Anopheles breathe atmospheric air through a breathing tube called a siphon. Anopheles larvae breathe throughspiracles located on the eight abdominal segments (Kettle, 1992). Larvae that possess respiratory siphons (for instance, culicine mosquitoes) hang down from the surface film to breath and feed. Those species that lack respiratory tubes (such as anopheline larvae) lie parallel to the surface film to breathe atmospheric air. Some such as Coquillettidia perturbans attach to aquatic plants and breathe through the plants stalks. The ability of Anopheles larvae to adhere to the surface film depends on the level of
oxygen tension of water (Nayar and Ali, 2003). While in water of low oxygen tension, *Anopheles* larvae that are not able to adhere to the surface film often suffocate (Reiter and McMullen, 1978). This makes *Anopheles* larvae more susceptible to organic pollution, a factor that is thought to reduce the oxygen tension of water (Rafatjah, 1988).

2.5 Foraging by mosquito larvae

Mosquito larvae feed on aquatic microorganisms such as bacteria, yeast, protozoa and other detritus material (Wallace and Merritt, 2004). Growth of microbial community is generally maintained by decomposing organic matter (Wotton *et al.*, 1997). However, during the decomposition of certain types of organic matter, toxic compounds are released which if consumed by mosquito larvae may cause death (Pautou *et al.*, 2000). For instance, fresh water breeders are adversely affected by nitrogenous derivatives that are produced by decomposing organic matter (Williamson, 1928). Therefore, pollution of water with organic matter may also be used for controlling mosquito larvae.

2.6 Patterns of growth and development in mosquito larvae

Mosquito larvae develop through four instar stages after which they metamorphose to pupae. The rate of larval development is influenced by ambient water temperature (Bayoh and Lindsay, 2003) and the availability of food (Gimnig *et al.*, 2002). There is a critical relationship between temperature and the mosquito life cycle. Temperature affects the rate of larval development and adult size. Low temperatures lead to slow rates of larval development and big adult body sizes while high temperatures are associated with faster larval development and smaller adult body sizes (Rueda *et al.*, 1990). Temperature
influences growth rate by modulating the rate of feeding, high temperatures lead to faster feeding rates (Rashed and Mulla, 1989). High temperatures also increase the rate of organic decomposition that promotes growth of microorganisms used as food by mosquito larvae. Food shortages increase mortality and reduce the body size of resulting adults (Gimnig et al., 2002). Cow dung has been used as a source of food for mosquito larvae in recent studies (Gimnig et al., 2002).

2.7 General locomotion in mosquito larvae

Two types of locomotion involving mosquito larvae include jerking of the body or propulsion of the mouth brushes. Mosquito larvae often leave the water surface while escaping from predators and in response to stimuli such as light, temperature, contact, and chemicals (Bates, 1949). Mosquitoes dive when searching for food; they dive more frequently when food is scarce (Linley, 1995). Change in light intensity and mechanical disturbances also cause the larvae to dive to the bottom of the water (Workman and Walton, 2003; Duhrkopf and Benny, 1990). Migration from one habitat to another is also common especially as a strategy of evading unfavorable conditions. For instance, in response to desiccation mosquito larvae are capable of relocating to other adjacent aquatic habitats (Koenraadt et al., 2003). Polluting water with cow dung can also cause mosquito larvae to migrate away from the source of such pollution (Simonet et al., 1978).

2.8 Malaria control strategies

The strategy of malaria control is based on breaking the chain of transmission of the parasites between humans and mosquitoes. Generally, control has been achieved through chemotherapy and vector control but vaccine development has also been attempted.
2.8.1 Malaria vaccine

Over the past decade, substantial progress has been made in search for malaria vaccine. The three major vaccines being developed are: anti-sporozoite vaccine, which is designed to prevent infection (Franke et al., 1999), anti-asexual-blood state vaccine designed to prevent severe manifestations of the disease and the transmission blocking vaccine designed to arrest the development of the parasite in the mosquito (WHO, 1998). A vaccine that was developed in Britain and tried in Gambia offered 47% protection (D'Alessandro et al., 1995), and researchers hope that a more effective vaccine will be available by the year 2010. The development of such vaccines has been complicated by the parasite ability to change their immunological identity, thereby concealing themselves from the immune response that might be otherwise stimulated by a vaccine (Bojang et al., 2001). Vaccine development may still not be easy due to the different set of genes which are probably switched on and off at each of the four stages of the parasite complex life cycle.

2.8.2 Chemotherapy

Chemotherapy in malaria control was started as early as 1930s with the discovery of aetiology and mode of transmission of malaria (Hackett, 1937). When anti-vector measures failed to significantly reduce the effects of the disease in endemic areas despite some early success, it became evident that there was heavy reliance on chemotherapy. Quinine was the drug that was widely used at that time but had undesirous side effects. Thus the advent of the new and effective therapeutic drugs particularly the 4-aminoquinolines in the 1940s provided for the first time, reliable and acceptable means of
treating malaria (Jeffery et al., 1985). Chloroquine became the antimalarial treatment of choice because it was safe, inexpensive and highly effective against susceptible malaria parasites (WHO, 1997). Thus malaria chemotherapy assumed a major role in primary health care because of the rationale of preventing mortality and curbing morbidity.

However the efficacy of antimalarial chemotherapy has been compromised with the development of resistance to the drug by malaria parasites (Bjorkman and Philip, 1990). In Africa resistant strains of *P. falciparum* were first recorded in Kenya and Sudan in 1978 (WHO, 1987) and more recently in West Africa (Cheesbrough, 1991). In many parts of Africa, the drug was never used alone for therapy (Brasseur et al., 1998).Chemotherapy has been compromised further by the development and spread of resistance to other antimalarial drugs such as sulphadoxine - pyremethamine (Fansidar® Tm) (Watkins and Mosobo, 1993), which is known to be heavily influenced by a combination of factors such as human migration, heavy antimalarial transmission, severe infection, immune response to malaria and sustained and haphazard use of drug. Currently, drug combination therapy is recommended for malaria control in areas where drug resistant is highly prevalent. Extensive resistance to chloroquine, sulphadoxine-pyrimethamine (SP) and amodiaquine monotherapy has prompted malaria treatment policy change to more expensive combinations, especially artemisinin-based combination therapies (ACTs) such as Coartem®. Widespread use of artemisinin-based combination therapies has been shown to decrease malaria transmission in Zanzibar, South Africa and Thailand (Zikusooka et al., 2008).
2.8.3 Vector control

Vector control remains the most generally effective measure to prevent malaria transmission (WHO, 2006) and it is an important part of the global malaria control strategy (Muturi et al., 2008). Presently, vector control is thought of as one of the best approaches in the war against malaria, especially in the absence of a vaccine and an effective chemotherapeutic agent. Control of malaria vector can be achieved using insecticides, larvicides, insect growth regulators (IGRs), traps baited with semiochemicals (attractants or pheromones) and the use of repellents. Insecticide resistance is now a major problem facing malaria vector control programs in most African countries with all three important vector species, *An. gambiae*, *An. arabiensis* (of the *A. gambiae* complex), and *An. funestus*, showing resistance to one or more of the insecticide classes used in vector control.

In Africa, malaria vector control has a long history. Prior to the Second World War, control was based mainly on antimalarial measures and source reduction, while pyrethrum spray as an adulticide was tried on small scale in certain areas with variable results (Zahar, 1984). During the 1940's when the organochlorine insecticide Dichlorodiphenyltrichloroethane (DDT) became available during the second world war, malaria control by house spraying was initiated on small scale in certain countries and on large scale in Madagascar, Mauritius, South Africa, Swaziland and Zimbabwe (Bruce-Chwatt, 1963). When the global malaria eradication programme was initiated by the WHO in 1955 (WHO, 1999), eradication pilot projects were circumscribed in areas in several countries from mid 1950s to the early 1960s.
These projects, initiated by the WHO Malaria Eradication Program were aimed at complete interruption of malaria transmission as a pre-requisite for malaria eradication but were later abandoned after it was realized eradication was impossible. The main approach was residual house spraying using DDT with or without chemoprophylaxis. In some areas there was a drop in parasite rates and malaria incidences following the use of DDT (WHO, 1983). Malaria was successfully eradicated in large areas such as South America, Southern Europe and some territories in India. The disease persisted in Latin America, most Asian countries and Africa. In the dry savanna of West Africa complete failure to control malaria was encountered (Haworth, 1981). In 1968, DDT resistance in *An. gambiae* s.l was reported in areas in West Africa where DDT was used in house spraying. A report of resistance to other classes of insecticides by malaria vectors has further compromised the vector control. Some of the vector control strategies that have an impact include biological control agents, microbial larvicides, oils and films, insecticides and bed nets, repellents, and use of plant materials.

2.8.3.1 Biological control agents

Biological control or "biocontrol" is the use of natural enemies to manage mosquito populations. These control methods are once again being given much research focus for malaria vector control. This is largely due to the emerging threat of strong mosquito resistance to insecticides. Service (1983) found that biological control agents could effectively reduce mosquito numbers, but a controversy existed then about its value. However, Legner and Sjogren, 1984; Howard et al., 2007) illustrated successful control by several fish species such as *Gambusia affinis* and *Oreochromis niloticus* (mosquito-eating fish) species. These can be reared in large numbers and released into mosquito-
breeding sites where they feed on mosquito larvae and pupae. Mosquito fish have to be released annually in habitats that do not have year-round water or where the water freezes. Larvivorous fish offer advantages in that they are generally self-sustaining, so in most cases do not require repeated applications.

The advantage of fish is that they feed on mosquito pupae, a stage that is not affected by most chemicals and bio-larvicides (Mohamed, 2003). One disadvantage is that larvivorous fish can only be used under certain conditions conducive to their survival. Currently, almost 200 fish species are known to feed on mosquito larvae and have been used in mosquito control for over 100 years (Bay, 1967). Other biological control organisms include mosquito larvae (such as *Toxorhynchites* spp.) that feed on other container-breeding mosquito larvae. However, inoculate releases of *Toxorhynchites* spp. with the purpose of colonization and effective predation have been conducted without establishing control (Schreiber and Jones, 1994). Microcrustaceans (predaceous copepods, for example *Macrocyclops longisetus*) also feed on mosquito larvae. Tietze *et al.* (1994) showed that Mesocyclops if integrated with biorational larvicides extended its effectiveness. Mermithid nematodes (for example *Romanomermis* spp.) also have shown larvicidal successes. Microsporidia parasites *Edhazardia aedis* are common pathogens of *Aedes aegypti* mosquito larvae (Becnel and Johnson, 2000). The fungus *Lagenidium giganteum* has been developed as a mosquito biological control organism (Roberts and Panter, 1985). Mosquito viruses include mosquito iridescent viruses and cytoplasmic polyhedrosis viruses (cytoplasmic polyhedrosis virus), these viruses are awaiting future developmental technology to enhance their efficacy (Federici, 1985).
2.8.3.1.1 Microbial larvicides

Larval control of mosquitoes either by source reduction, use of larvicides or a combination of these is a preferred method for reducing adult mosquitoes in many areas of the world. In arid regions where the extent of mosquito developmental sites is limited, larval control through the use of chemical and microbial larvicides has been the method of choice (Mulla et al., 2001). Microbial larvicides such as *Bacillus thuringiensis* var. *israelensis* (Bti) and *Bacillus sphaericus* are highly selective for mosquito larvae. *Bacillus thuringiensis* (Bti) was isolated from *Culex pipiens* in Israel in 1976 (Goldberg and Margalit, 1977) and designated serovar H-14. Since then, several mosquitocidal strains have been isolated and classified. However, microbial larvicide resistance has been documented in the literature (Rodcharoen and Mulla, 1996: Zahiri et al., 2002; Paul et al., 2005).

2.8.3.2 Oils and films

Use of oils in controlling *Anopheles* larvae dates back to as early as 1899, when Ronald Ross applied kerosene on anopheline larval breeding sites in Sierra Leone (Bockarie et al., 1999). This method still remains an approach with great potential for future malaria vector control. Oils and films such as kerosene and light fuel oil were reported by Herrick (1901) to be effective larvicides and pupicides when either sprayed or poured on the water. During the 1960s through early 1980s, oils and films were considered useful larvicide alternatives. These larvicides reduce surface tension, making it difficult for the larva, pupa, or emerging adults to attach to the surface of the water and hence they drown. Surface oils and films now are highly refined petroleum products that are virtually
odorless and colorless. Both are highly effective but the oils are short-lived in the environment (usually less than 12 h), whereas the films can persist effectively for 10-14 days. They present a low hazard to non-target organisms.

2.8.3.3 Insecticides and bed nets

Insecticides have played an important role in the control of insect vectors of diseases since early 20th century (Greenwood et al., 2008). However, management of malaria vector using synthetic chemicals has failed because of insecticide resistance, vector resurgence and environmental pollution. Consequently, intensive efforts have been made to find alternative methods of malaria vector control (Service, 1989). An important innovation during the past decade was the widespread introduction of pyrethroid treated mosquito nets for protection against malaria transmission (Chavasse et al., 1999) and indoor residual spraying (IRS). Insecticide treated nets were first used in Russia in 1930s and by American and German forces during the 2nd world war. Bednets have been used against nuisance mosquitoes in China, Thailand, Latin America and Africa. Insecticide treated nets (ITNs) have effectively reduced malaria morbidity and mortality in many different epidemiological settings (Lengler, 1999). Their use has benefited not only those who actually sleep under ITNs but when used by the majority of the community, people who sleep without treated nets may also receive fewer bites of infective Anopheles mosquitoes (Maxwell et al., 1999).

In Africa and South Asia, ITNs and IRS have been very effective (Curtis et al. 1999; Rowland and Nosten, 2001). Their effectiveness has been enhanced by the advent of long-
lasting insecticidal nets (LLINs) which resist washing and greatly extend the effective life of the insecticide (N'Guessan et al., 2001; Graham et al., 2005). The use of impregnated bednets and curtains with pyrethroids seems to be the most promising available method of controlling malaria in endemic tropical countries. Studies carried out in Senegal (Alonso et al., 1991), China (Cheng et al., 1995) demonstrated the efficiency of ITNs for reducing infant mortality. In Kenya, reports by Okiro et al. (2007) indicates there are indications that malaria morbidity and mortality is on a decline as a result of scaled use of insecticide treated nets. These findings have been confirmed by subsequent large scale multicenter studies in six countries across Africa including Ghana (Nevill et al., 1996). Since most Anopheles species bite at night, it has been assumed that nets should reduce the chances of contracting malaria (Lindsay and Gibson, 1987).

In Gambia (Alonso et al., 1993), Guinea Bissau (Joenson et al., 1994), and other areas like Sudan (Curits et al., 1987), introduction of insecticide impregnated nets in the communities remarkably reduced parasite prevalence and malaria incidences (Marbiah et al., 1998). Malaria control using bed nets treated with pyrethroid insecticides have proven difficult to implement correctly because of problems related to equity, accessibility and user compliance (Casmiro et al., 2006). For example, in western Kenya, the most important reasons for non-adherence to use of ITNs was the disruption of sleeping patterns due to visitors, funerals, house constructions and other events. Other concerns included fear of the insecticide, which is thought by some, to be a toxic drug used for family planning purposes (Allaji et al., 2003). Also, there have been reports of the emergence of pyrethroids resistance in the Anopheles vectors. Some cases of such
resistance are known, notably in *An. gambiae* in West Africa where the knockdown type of resistance has been noted probably due to the use of pyrethroids in cotton production (Zahar, 1984). Moreover in Ivory Coast, Doannio *et al.* (1999) reported that despite the known repulsion effect of permethrin treated bednets on mosquitoes, the use of ITNs had no significant impact on transmission.

2.8.3.4 Repellents

Repellents are chemical substances that protect animals, plants or materials such as fabrics, grain and timber from insect attacks by rendering them unattractive, unpalatable or offensive (Metcalf and Flint, 1962).

2.8.3.4.1 Synthetic repellents

A commonly advocated approach for preventing arthropod attack is personal protection, a suite of avoidance techniques that includes the use of topical (skin) repellents (Barnard 2000). With the recent evolution of pyrethroid resistance in African anophelines (Guessan *et al.* 2006) and the prospect of reduced efficacy of insecticide-treated bed nets for preventing disease highlights, there is need for strengthening the available arsenal of personal protection measures for public health reasons. The substance which may not be poisonous or mildly toxic, are rarely effective against all kinds of insects.

The practical problem of repellency is essentially a behavioral one. To be effective a repellent compound must first be capable of stimulating some sensory system other than that which mediates attraction. The repellent must also act upon a system which has some
influence on locomotion or feeding since the response of the organism depends upon which sensory system has been stimulated, and which reflex arcs are placed in operation. Vapor repellents act in the gaseous phase and are most often stimulants of the olfactory receptors (Dethier et al., 1960). The search for new repellent during the second world war led to the establishment of criteria for a good repellent against blood sucking insects. These were: effective protection of the treated area for several hours, on all types of subjects and under all climatic conditions, complete freedom from toxicity and irritation when regularly applied to human or animal skin, cosmetic acceptability, including freedom from unpleasant odour, taste, touch and harmlessness to clothing, protection against wide variety of biting insects, low cost and availability. No compound so far has been found that meets all these requirements satisfactorily (Metcalf et al., 1962; Kirk and Orthmer, 1992).

During the second world war there was need to search for new repellents that could be used by the military. Almost 7000 synthetic organic compounds were screened for repellency against mosquitoes on human skin and clothing in the USA (Knipping, 1949). Dimethyl phthalate (DMP) and n-butylmesityloxide oxalate are some of the earliest repellents synthesized in the laboratory. Each showed differences in repellency which was found to be specific for various mosquito species. Differences disappeared and effectiveness was enhanced when they were mixed. The mixture was effective against a wide range of mosquito species. The search for new and longer acting repellent resulted in the discovery of diethyl-m-toluamide (DEET) and cyclohexamethylene carbamide (CHMC) which are the most potent of the modern synthetic repellents. DEET was
introduced in the 1950s and shown to be more effective than DMP (Kirk and Orthmer, 1992) but these two chemicals are still available in some insect repellent preparation. However, important disease vector species such as *An. pulcherrimus* (Zhogolev, 1968), *An. albimanus* and *An. gambiae* have been reported to be less susceptible than *Aedes aegypti* (Curtis *et al.*, 1987). Most of the synthetic organic repellents act as solvents for lacquers and should not be applied to watch crystals, spectacle frames, synthetic fibres, paints and varnishes. This is a major undoing for synthetic repellents formulations. Disadvantages of synthetic repellents in mosquito control include: development of resistance, toxicity to other animals, high cost and environmental pollution (Stinecipher *et al.*, 1997). Pyrethrin is an excito-repellent chemical with moderate repellency of up to 7 hrs. Furthermore, mosquitoes not repelled by pyrethrin die after biting. Mosquito coils have been produced from pyrethrum flowers for protection against indoor mosquito bites.

Studies on repellency of mosquito coils in Tanzania and Papua New Guinea revealed a protection of between 40-80% (Charlwood and Jolly, 1984). A coil containing 0.5% natural pyrethrins, reduced the landing rates of *An. gambiae* by 40% while knock down began after 2 minutes, but a few took some blood and otherwise recovered (Curtis and Hill, 1988). A synthetic derivative of Citronella has been used as an ingredient for commercial repellents. A fresh application of this derivative was found to be as effective as DEET against mosquitoes (Rutledge *et al.*, 1985).
2.8.3.4.2 Plant-derived repellents

Insect repellents play an important role in reducing man-vector contact (Allaji et al., 2003). Recently, the environmentally friendly and biodegradable natural insecticides of plant origin have been receiving attention as an alternative measure of control of arthropods of public health importance (Nathan et al., 2005). Repellents of plant origin have been used for medicinal purposes for a long time because they do not pose hazards of toxicity to human or domestic animals and are easily biodegradable (Das et al., 2003). Thus, thousands of plants have been tested as potential sources of insect repellents (Sukumar et al., 1991). However, none of the plant-derived chemicals tested to date demonstrate the broad effectiveness and duration of DEET, but a few show repellent activity. Compared to other synthetic compounds, natural products are presumed to be safer for human use (Chogo and Crank, 1981) justifying therefore a broad search for eco-friendly biological materials to be used for the control of vectors of medical importance.

The repellent action of different plant parts or oil extracts has been evaluated against Afro-tropical mosquitoes (Palsson and Jaenson, 1999). Essential oils of some plants have mosquito repellents properties. These have been observed in the oils of cassia, camphor citronella, lemon grass, clove, thyme, geranium, bergamot, pine, winter green, bay laurel, pennyroyal and eucalyptus (Waka et al., 2006), cedar, verbena, lavender, cajeput, cinnamon, rosemary, basil, allspice, garlic, and peppermint (Brown and Hebert, 1997). These oils have been the basis for most commercial natural repellents and many different varieties have been produced and tested, until the advent of synthetic compounds such as DEET. Completely unrelated plants in most cases share some of the repellent constituents.
Citronella oil is a popular repellent in India and is still used as a repellent in Europe and North America. The main constituent of citronella oil is citronella, which is found to be as effective against mosquitoes as DEET and DMP when freshly applied. Citronella oil has a lemony scent and was originally extracted from the grass plant *Cymbopogon nardus*. Citronella candles have been promoted as an effective way to repel mosquitoes. One study compared the ability of commercially available 3% citronella candles, 5% citronella incense, and plain candles to prevent bites by *Aedes* mosquitoes under field conditions (Lindsay *et al.*, 1996). Persons near the citronella candles had 42% fewer bites than controls, who had no protection. However, burning ordinary candles reduced the number of bites by 23%. The efficacy of citronella incense and plain candles did not differ significantly. The ability of plain candles to decrease biting could have resulted from their action as a decoy source of warmth, moisture, and carbon dioxide. The citrosa plant (*Pelargonium citrosum*) has been marketed as being able to repel mosquitoes through the continuous release of citronella oils. Unfortunately, when tested, these plants offered no protection against bites (Matsuda *et al.*, 1996; Cilek and Schreiber, 1994).

Plant products have been used in many parts of the world for killing or repelling mosquitoes either as extracts or as whole plant (Okumu *et al.*, 2007). Burning of certain herbs such as *Artemisia* and *Calamus* species is still practiced in remote villages in China to repel mosquitoes (Curtis, 1991). Before the advent of synthetic chemicals, people have used plants and their derived substances to repel or kill mosquitoes. The Ainu people Hakkaido, Japan and Indians (Curtis, 1991) wore leggings of sedges, bark or cloth to reduce insect biting nuisance, which is concentrated around the leg. Ancient Chinese
people had many prescriptions of repellents against mosquitoes and other blood sucking insects. Herbs of the basil family (Labiatae) have many traditional medicinal uses in Africa and Asia. In East and West Africa they are also used as mosquito repellents (Kokwaro, 1993). In Northern Tanzania, basil like herbs (*Ocimum* spp and *Hyptis soaveolens*) and neem (*A. indica*) leaves have been used for repelling mosquitoes. The juice of *Ocimum* spp when applied on legs reduced biting by caged *An. gambiae* (Hassanali, 1996). Later the repellency was attributed to eugenols (Hassanali, 1996) which are the main components of clove and other essential oils with repellent properties. The woods or resins of aromatic trees are widely sold in the markets of Gambia for burning as mosquito repellents. Dry smouldering sticks of thyme (*Thymus serphyllum*) were reported to give 85-90% protection for 60-90 minutes in the open air (Philip *et al*., 1945). In India, women who smeared their bodies with turmeric and gingili or mustard oil before bathing with soap had much reduction in bites of *An. fluviatilis*. Flowers from *Lantana camara* have shown repellence activities against *Aedes aegypti* (Dua *et al*., 1996).

Repellents do not all share a single mode of action, and surprisingly little is known about how repellents act on their target insects (Davis, 1985). Moreover, different species of mosquitoes may react differently to the same repellent (Rutledge *et al*., 1985). To be effective, a repellent must show an optimal degree of volatility, making it possible for an effective repellent vapor concentration to be maintained at the skin surface without evaporating so quickly that it loses its effectiveness. Many factors play a role in how effective any repellent is, including the frequency and uniformity of application, the number and species of the organisms attempting to bite, the user's inherent attractiveness
to blood-sucking arthropods, and the overall activity level of the potential host (Schreck, 1995). Abrasion from clothing, evaporation and absorption from the skin surface, wash-off from sweat or rain, higher temperatures or a windy environment all decrease repellence effectiveness (Maibach et al., 1974, Gabel et al., 1976, Khan et al., 1972). Each 10°C increase in temperature can lead to as much as a 50% reduction in protection time. The repellents currently available must be applied to all exposed areas of skin; unprotected skin a few centimeters away from a treated area can be attacked by hungry mosquitoes (Schreck, 1995). However, efforts to find such a compound have been hampered by the numerous variables that affect the inherent repellency of any chemical. Thus, the search for the perfect topical insect repellent continues. This ideal agent would repel multiple species of biting arthropods, remain effective for at least 8 hours, cause no irritation to the skin or mucous membranes, cause no systemic toxicity, be resistant to abrasion and rub-off, and be greaseless and odorless. At the moment, there is no available insect repellent that meets all of these criteria.

2.8.3.5 Control of immature stages of Mosquitoes

Source reduction through management of larval habitats has been the key to malaria eradication efforts in the United States, Italy and Israel (Kitron and Spielman, 1989). The suppression and even eradication of malaria in these regions has been attributed to effective large-scale programs that targeted the immature stage of vector mosquitoes or reduced the extent of suitable habitats in proximity to vulnerable human populations (Killeen et al., 2002). Thus a renewed interest in mosquito larval control has been the central theme in recent studies exploring the feasibility of reducing malaria vector
population through environmental and agro-ecosystem management approaches (Keiser et al., 2002; Kileen et al., 2002). The control of immature mosquito populations is often advantageous because the larvae are usually concentrated, relatively immobile, and occupy minimal habitat area compared with adults that can rapidly disperse over large areas and escape intervention measures. Effective larval control minimizes the use of adulticides and is environmentally friendly (Floore, 2006). Larval control can be done using source reduction which is the elimination of mosquito production sites, and it is often an effective and long-term solution for mosquito control. Howard et al. (2007) discussed the drainage of swamps and screening of rain barrels to eliminate breeding sites of mosquitoes. However, mosquitoes often develop in economically important wetlands such as rice agro-ecosystems and thus, habitat modification may not always be an option. Mosquitoes around the home can be reduced significantly by disposal of plastic containers, discarded pools, tyres or similar water-holding containers. This method eliminates ovipositional opportunities for floodwater species and effectively reduces their populations. Conversely, these locations may be drained before adult mosquitoes can emerge.

2.8.3.6 Deliberate water contamination to control mosquitoes

Deliberate water pollution using organic matter is a naturalistic method of malaria vector control (Hackett et al., 1938; Beales and Gilles, 2002; Lindsay et al., 2004). The technique has been used successfully to control An. fluviatilis and An. maculatus in India, Malaysia and Singapore (Lindsay et al., 2004). On decomposition, the dung of most herbivores may deter species that prefer breeding in fresh water such as An. gambiae from
ovipositing. It has been reported that, eggs laid in polluted water usually tend to keep together in clumps and thus their viability is reduced (Muirhead-Thomson, 1951). It is thought that an olfactory response causes the gravid mosquitoes to avoid the polluted water or the lowering of the surface tension caused by organic pollution may have a negative effect on the aquatic stages of mosquitoes by preventing them from attaching to the surface film from where they breathe atmospheric oxygen (Rafatjah, 1988). The success of this method mainly depends on the type of the material used to pollute mosquito breeding habitats. On decomposition, organic materials produce toxic substances, which if consumed by mosquito larvae are likely to cause death (Pauto et al., 2000). Such harmful substances have been identified as ammonium salts and nitrite (Beattie, 1932).

2.8.3.7 Botanicals used in the control of mosquitoes

Plants extracts have also been used against mosquito larvae. Plants alkaloids such as nicotine, anabasine, methylanabasine and lupinine extracted from the Russian weed Anabis aphylla killed the larvae of Cx. pipiens, Cx. territans, Cx. Quinquefasciatus (Amer and Mehlhorn, 2006). Neem (A. indica) has a wide range of effects that are potentially useful for malaria control and include antifeedancy, ovicidal activity, fecundity suppression, insect growth regulation and repellency. Recent studies have also demonstrated neem-induced effects on vitellogenesis and severe degeneration of follicle cells during oogenesis in mosquitoes. Neem has several formulations, which include emulsifiable concentrates (ECs), wettable products (WPs), suspension concentrates, ultra low volume (ULV) and granular formulations. These effects are frequently attributed to
the azadirachtin contents of the products (Okumu et al., 2007). Neem larvicides have shown potency against anophelines, notably *An. culicifacies, An. arabiensis, An. gambiae* and *An. stephensi* (Sharma et al., 1993). Neem-based products are relatively safe towards non-target biota, with only minimal risk of direct adverse effects on aquatic macro invertebrates resulting from contamination of water bodies with neem-based insecticides (Dunkel and Richards, 1998). In addition, the products are less likely to induce resistance due to their multiple modes of action on insects (Okumu et al., 2007).
CHAPTER THREE
MATERIALS AND METHODS

3.1 Description of the study area

The studies reported in this thesis were conducted in the laboratories, insectary and semi-field conditions within National Irrigation Board (NIB) Ahero (Fig. 3.1). Ahero Rice Irrigation Scheme is located in Nyando District, Nyanza province, approximately 400km west of Nairobi and 24km East of Kisumu. Ahero lies between latitude 0° 10’S and longitude 34° 55’E (Fig. 3.1). The area experiences a mean annual temperature of 23°C, mean annual rainfall 1300mm and mean relative humidity of 76%. These conditions render the area highly vulnerable to malaria transmission. According to 1999 census, the population of the district is 61,556 (GoK, 1999). Majority of the inhabitants belong to the Luo ethnic group. Anopheles gambiae s.l and An. arabiensis are the most important malaria vectors in this area. Mosquito populations rapidly increase during the rainy season while rice irrigation fields act as mosquito breeding grounds during the dry season. A high malaria incidence in the area makes it an important target for studies on the vector control.
Figure 3.1: Map of Kenya showing Ahero rice irrigation scheme
3.2 Mosquito rearing for experiments

*Anopheles* mosquitoes were used in all the experiments. Mosquito rearing was done in an insectary within NIB Ahero. Climatic conditions within the insectary were maintained at 24 - 30°C temperature, 60 -80% relative humidity and 12L: 12D photoperiod. Wild, indoor-resting, blood fed *Anopheles* mosquitoes were collected from 0600hrs to 0800hrs from houses in Ahero irrigation scheme, Nyando District, Western Kenya, by means of aspiration. They were immediately transported to the field laboratory at Ahero NIB for sorting out. Identification was done by the use of naked eye, magnifying lens or microscope where necessary in order to distinguish gravid female *Anopheles* spp. from males and other species of no medical importance. Males and species of no medical importance were discarded while the selected gravid females were transferred into standard mosquito cages measuring each 30 x 30 x 30 cm.

The gravid female mosquitoes from the field were provided with oviposition cups to lay eggs. The laid eggs were dispensed in larval rearing trays (measuring 31 x 21 x 8 cm) each containing 1 litre of clean distilled water in which they hatched and underwent development from the first instar larval stage (L1) all through to pupation. The emerged larvae were fed on approximately 0.04mg of Vipan® fish food per tray containing 100 larvae. The water in which larvae were bred was replaced after every 2 days. To accomplish this, larvae were sieved using a plastic sieve and transferred into trays containing fresh distilled water. This process was continued until development from the first instar larvae (L1) through to pupae was completed. Pupae were collected using plastic droppers and transferred into clean bowls containing distilled water. Bowls
containing pupae were put in adult standard mosquito holding cages (30 x 30 x 30 cm) and covered with mosquito netting. The bowls were left to stay in the cages until adult mosquitoes emerged. After emergence, adults were fed on 6% sucrose solution for 3 days using folded paper towel in form of wicks while water was provided using soaked cotton wool placed on top of the cages. Adult female mosquitoes aged between 3 - 4 days were starved for 6 hours and then allowed to feed on the fore-arm of a human volunteer as a source of blood meal for egg laying. Feeding was done under complete darkness between 0700hrs and 0800hrs continuously for the 3 days. The feeding process lasted for 10 – 15 minutes. Gravid female mosquitoes were provided with clean tap water in half full Petri dishes in which to lay eggs. Laid eggs were collected, dispensed in larval rearing trays and the process continued. The resultant larvae were used in the subsequent experiments.

3.3 Cow dung

Fresh cow dung was collected from Freshian cows. The cows were kept in a zero grazing unit in NIB Ahero. The cow dung was green and coarse in texture at the time of collection. The main diet of the cow was mainly Napier grass and commercial feeds. The cow dung was collected from the same cows every day between 0800 and 0900hrs. It was placed in plastic paper bags and immediately transported to the laboratory at NIB Ahero. This was used in the laboratory and semi-field experiments to test the effects of cow dung and a combination of neem and cow dung on the immature stages of Anopheles mosquitoes.
3.4 Laboratory experimental procedures.

Experiments aimed at determining the effect of cow dung and cow dung combined with neem on immature stages of *Anopheles* mosquitoes were carried out for a period of 4 weeks. The experiments were undertaken in the laboratory at NIB Ahero using bowls. The bowls measured 19 cm x 9 cm each and were orange in colour.

![Experimental layout of bowls in the laboratory.](image)

**Figure 3.2:** Experimental layout of bowls in the laboratory.

3.4.1 Experimental procedure on effect of cow dung on immature stages of *Anopheles* mosquitoes.

The effect of cow dung on the immature stages of *Anopheles* mosquitoes was carried out in the laboratory for a period of 4 weeks. The larvae and pupae of *Anopheles* spp. mosquitoes used in this experiment were reared as described in section 3.2. The term 'immature stages' as used in this section refers to early instar larvae (L1 and L2), late instar larvae (L3 and L4) or pupae. Ten grams of fresh cow dung was put in each of five
bowls each measuring 19 cm x 9 cm. Thirty milliliters of water from rice canal was added and the mixture was stirred using a glass rod and then placed on the laboratory benches for 2 hours to allow the floating debris to settle down. Ten individuals of each immature stage (L1, L2, L3, L4 and pupae) were introduced into the bowls (each bowl contained a separate immature stage). Rice canal water without addition of cow dung served as the control. The experiment was replicated 30 times. Each bowl containing the pupae was put inside an adult mosquito holding cage to prevent the emerging adults from escaping. Larvae in each bowl were fed on approximately 0.04 mg of Vipan® fish food per day. Each day individuals in each bowl were removed using plastic pipettes. They were then placed in petri dishes containing distilled water. Counting was done and their number recorded separately according to whether they were dead or alive. Dead individuals were discarded after counting and the emerged adults transferred to the adult mosquito cages. Similar experiments were done using 1g of neem cake powder as the treatment.

3.4.2 Experimental procedure on effects of cow dung and neem combined on immature stages of *Anopheles* mosquitoes in the laboratory.

Experiments were carried out to investigate the larvicidal activity of cow dung and neem against immature stages of *Anopheles* spp of mosquitoes. *Anopheles* spp larvae and pupae used in this experiment were reared as described in section 3.2. One gram of neem was mixed with 1 litre of water from rice canal. The mixture was stirred and left for 12 hours. Thirty milliliters of this solution was mixed with 10 g of fresh cow dung in bowls measuring each 19 cm x 9 cm. The mixture was stirred using a glass rod and placed on the laboratory benches for 2 hours for the floating debris to settle. Ten individuals of each
immature stage (L1, L2, L3, L4 and pupae) were introduced. Rice canal water in bowls served as the control. This experiment was replicated 30 times. Each day individuals in each bowl were removed using a plastic pipette and placed in petri dishes. They were counted and recorded on whether they are dead or alive using the procedure explained for cow dung experiments conducted in the laboratory (section 3.4.1).

3.5 Experimental sub-plots

Similar experiments were carried out in semi-field conditions conducted in sub-plots that were inside a screen house at NIB, Ahero. The screen house measured 4.5 m x 1.5 m, was wooden and covered with mesh wire. The land inside the screen house was sub-divided into 5 sub-plots. Each sub-plot measured 0.5 m x 1.5 m. Between any two sub-plots, a 0.5 m wide and 5 cm high mud walled band was created to prevent mixing of treatments (Figs. 3.3 and 3.4).

Figure 3.3: A screen house which was used for semi-field experiments
3.5.1. Experimental procedure on effect of cow dung on *Anopheles* mosquitoes in semi-field conditions.

The effect of cow dung on immature stages of *Anopheles* spp. mosquitoes in semi-field was investigated. *Anopheles* spp larvae and pupae used in this experiment were reared as described in section 3.2. Each of the 5 sub-plots measuring 1.5m x 0.5m (Fig. 3.3) was filled with 6 litres of water from rice canal and two hundred grams of fresh cow dung.
added. The mixture was stirred using a glass rod and left for 2 hours for the floating debris to settle. In each of the 5 sub-plots, 50 individuals of each immature stage (L1, L2, L3, L4 and pupae) were introduced. Rice canal water (6 litres) alone was used as the control. The experiment was replicated 30 times. In sub-plots containing pupae, a mosquito cage was mounted on top to prevent emerging adults from escaping. Larvae in each sub-plot were fed with approximately 200 mg of Vipan® fish food daily. Individual immature stages were removed using a pipette and placed in petri dishes. They were counted and those that were dead or alive recorded. Similar experiments were done using 10g of neem cake powder as the treatment.

3.5.2 Experimental procedure on effects of cow dung combined with neem, on Anopheles mosquitoes in semi-field conditions.

To determine the effect of combining cow dung and neem, each sub-plot was filled with 6 liters of water from rice canal. Anopheles spp. larvae and pupae used in this experiment were reared as described in section 3.2. Two hundred grams of fresh cow dung and 20 g of neem powder were added. The mixture was stirred thoroughly and left for 2 hours for the floating debris to settle. In each sub-plot, 50 individuals of each immature stage (L1, L2, L3, L4 and pupae) were introduced. Rice canal water (6 litres) alone was used as the control. The experiment was replicated 30 times. In sub-plots containing pupae, a mosquito cage was mounted on top to prevent emerging adults from escaping. Larvae in each sub-plot were fed with approximately 200 mg of Vipan® fish food daily. Individual immature stages were removed, they were counted and those that were dead or alive recorded.
3.5.3 Control experiments in semi-field conditions

Control experiments were carried out in plastic troughs measuring 42 x 16 cm and orange in colour. They were half filled with soil from rice field and 10 litres of rice canal was added. The mixture was stirred using a glass rod and the mixture left for 2 hours for the floating debris to settle. In each trough, 50 individuals of each immature stage (L1, L2, L3, L4 and pupae) were introduced. The experiment was replicated 30 minutes. Feeding of larvae and monitoring of survival was done as earlier described (Section 3.4.1).

3.6 Experimental procedure to determine effects of *A. annua* on the feeding response of adult female *Anopheles* mosquitoes

Experiments to investigate the effect of *A. annua* on adult female *Anopheles* mosquitoes were carried out within a period of 4 weeks in a dark room with red lights as the only source of illumination. The mosquitoes used in these experiments were obtained from a colony that was reared as earlier described (Section 3.2). The room temperature and humidity were set using a heater and a humidifier respectively to mimic the host feeding conditions for the female *Anopheles* mosquitoes (temperature 27 - 35°C and relative humidity 60 - 80%). Feeding female mosquitoes were selected by placing a human hand on the side of a mosquito cage. Then females attracted to the hand were aspirated into a cup to make batches of 30 for each replicate trial. Two human volunteers were used in testing effects of *A. annua* on *Anopheles* species. They were not allowed to use lotions, perfumes, oils and perfumed soaps on the day of the tests. The experiments were
conducted in mosquito cages measuring 30 x 30 x 30 cm in the laboratory. Before the application of *Artemisia* paste, hands of the volunteer were washed with bar soap, rinsed with tap water and then allowed to dry for 15-20 minutes.

All tests were carried out using 5-7 days old female *Anopheles* that had been starved for 6-8 hours, but previously fed on 6% glucose solution. Thirty pre-starved female mosquitoes were introduced into the mosquito cages. Five fresh leaves of 3 months old *A. annua* plant were crushed using mortar and pestle, ten milliliters of distilled water was added to form a paste. Three milliliters of *A. annua* was used to smear on the right hands of the two human volunteers from the wrist to the elbow. The other remaining part of the hand was covered with a glove to make it unattractive to the mosquitoes. Three milliliters of distilled water was dispensed on the left hands to act as a control. Each hand was put inside its own cage containing 30 female *Anopheles* mosquitoes. The number of mosquitoes that landed on the hands after every 5 minutes was recorded for a period of 1 hour. Also, the number of fed mosquitoes was recorded after 20 minutes duration. Mosquitoes whose abdomens appeared reddish and distended were considered fed. These experiments were replicated 30 times.

### 3.7 Data management and analysis

All data obtained from this study were statistically analyzed using Statistical Analysis System® (2002) statistical package. Analysis of Variance (ANOVA) tests was used on data collected to evaluate the effects of cow dung alone and a combination of neem and cow dung on the immature stages of *Anopheles* mosquitoes both in laboratory and semi-
field experiments. Significantly different means (<0.05) were separated using Student-Newman-Keuls (SNK). The effects of *A. annua* against adult *Anopheles* species of mosquitoes were analyzed using Student’s t-tests. The results were termed significant if P value was < 0.05.

3.8 Ethical clearance

The research protocol was reviewed at Kenyatta University, Department of Zoological Sciences Board of Postgraduate Committee and ethical clearance was obtained. The research permit was given by the Ministry of Education Science and Technology approval number MOST/001/37C 145/2
CHAPTER FOUR

RESULTS

4.1 Effects of cow dung on immature stages of *Anopheles* mosquitoes in the laboratory.

Out of the 1200 larvae and 300 pupae used to test the effect of cow dung, it was observed that all the immature stages reared in rice canal water containing cow dung had the highest mortality in the first instar larval stage (mean mortality was $7.23 \pm 0.23$). This was followed by the second instar larvae with mean mortality of $6.65 \pm 0.05$. Third and fourth instar larvae had a lower mortality of $6.00 \pm 0.04$ and $5.53 \pm 0.04$ respectively while the lowest mortality was in the pupal stage ($1.37 \pm 0.05$; Fig 4.1). In the controls (rice canal water) the mortality was low in all the stages when compared to mortality in cow dung. The highest mortality was recorded in the 4th instar stage ($1.67 \pm 0.06$) and the lowest in pupae stage ($1.37 \pm 0.05$) Fig. 4.1
A significant difference in mortality was recorded between all the immature stages ($F$=88.22; $P<0.0001$) that were in bowls containing fresh cow dung (Fig 4.1). It was also observed that there was a significant difference in mortality of the first instar larval stage that were in bowls containing cow dung compared to those placed in the control (rice canal water; $F$=215.27; $P<0.0001$). Likewise for L2, L3 and L4 instar stages that were in cow dung, there was a significant difference in mortality in comparison to those placed in rice canal water ($F$=88.22; $P<0.0001$). It was also observed that there was a significant difference in mortality between the early instar larvae (L1 and L2) and the fourth instar larvae placed in bowls containing cow dung ($F$=88.22; $P<0.0001$; Fig. 4.1). There was no significant difference in mortality of the pupal stage between those placed in bowls.
containing cow dung and those in bowls containing rice canal water (control; \( F = 0.74; P = 0.529 \)).

4.2 Effects of cow dung on Anopheles mosquitoes in semi-field conditions.

Mortality of 1\textsuperscript{st} instar larvae was observed to be the highest (35.87 ± 0.01) for the larvae placed in sub-plots containing cow dung. The late instar larvae (L3 and L4) had a lower mortality (29.17 ± 0.01 and 28.00 ± 0.03 respectively) than the early instar larvae (L1 and L2) (Fig 4.2). The lowest mortality was observed in the pupal stage with a mean mortality of 1.97 ± 0.10. All immature stages that were in sub-plots containing rice canal water (control) had a lower mortality compared to those in cow dung (Fig. 4.2). In these sub-plots the highest mortality was in 2\textsuperscript{nd} instar larvae (2.93 ± 0.06) while the lowest (2.03 ± 0.06) was noted in the 3\textsuperscript{rd} instar larvae (Fig. 4.2).
In sub-plots with cow dung there was a significant difference in mortality between all the immature stages \((F=560.09; P<0.0001)\). A significantly higher mortality \((F, 560.09; P, <0.0001; \text{Fig } 4.2)\) was noted in the early instar with a mean mortality of 35.87 ±0.01 and 32.30 ± 0.01 respectively. Among the experimental groups there was no significant difference \((F=560.09; P>0.05)\) in mortality between 3\textsuperscript{rd} (mean mortality 29.17 ± 0.01) and 4\textsuperscript{th} instar larvae \((28.00 ± 0.03)\). It was observed that, in all the immature stages in cow dung there was a higher mortality compared to those in rice canal water \((F=535.16; P<0.0001; \text{Fig. } 4.2)\).
4.3 Effects of neem on immature stages of *Anopheles* mosquitoes in the laboratory.

A significant difference in mortality was recorded between all the immature stages \((F=45.54; P<0.0001)\) that were in bowls containing neem (Fig 4.3). The highest mortality was observed in the first instar \((7.43 \pm 0.03)\) followed by the second instar \((7.00 \pm 0.04)\). However the difference in mortality between L1 and L2 was not significant \((F=45.54; P, >0.05; \text{Fig. 4.3})\). It was also observed that there was no significant difference in mortality of the first and second instar larval stage compared to the third instar stage which had a mean mortality of \(6.00 \pm 0.05 \ (F= 45.54; P, >0.05; \text{Fig. 4.3})\). A significant difference was observed in mortality of the 4th instar \((3.88 \pm 0.08)\) compared to 1st, 2nd and 3rd instars in bowls containing neem. Pupae that were reared in bowls containing neem alone had the lowest mortality (mean mortality \(1.50 \pm 0.11\)) the difference was significant \((F= 45.54; P, < 0.0001; \text{Fig. 4.3})\).

In comparing the mortality in the experimental and the control, a significant difference \((F= 215.27; P, <0.0001)\) in the first instar \((7.43 \pm 0.03)\) was noted compared to the control which had a mean mortality of \(1.66 \pm 0.05\). Similarly there was a significant difference in mortality between the second instar placed in neem compared to those in rice canal water \((F= 99.33; P, <0.0001; \text{Fig. 4.3})\). In the third instar placed in neem, there was a significant difference in mortality compared to those in the rice canal water only \(F= 140.41; P, < 0.0001; \text{Fig. 4.3}\). A similar mortality was noted in the fourth instar whereby the experimental had significantly higher mortality than the control \((F= 55.72; P, 0.0001; \text{Fig. 4.3})\) There was no significant difference \((F= 0.74; P, 0.529)\) in mortality of the pupal
stage between those placed in bowls containing neem (mean mortality 1.50 ± 0.11) and those containing rice canal water (1.37 ± 0.05).

Figure 4.3: Mean mortality of immature stages of *Anopheles* species after being placed in bowls containing neem and rice canal water. Standard error bars of the mean number of dead individuals are shown.

4.4 Effects of neem on immature stages of *Anopheles* mosquitoes in the semi-field.

The highest mortality was noted in the first instar placed in sub-plots containing neem (37.03 ± 0.02) this was followed by the second instar larvae with a mean mortality of 36.03 ± 0.02. The difference in mortality between the L1 and L2 was not significant (*F*, 757.19; *P*, > 0.05; Fig. 4.4). The mortality was lower in the late instar stages (mean mortality of 31.13 ± 0.03 and 29.13 ± 0.03 respectively) compared to the first and second instars. The difference in their mortality was significant (*F* = 757.19; *P* < 0.0001; Fig 4.4).

In comparing the experimental and the control, a significant higher mortality was noted in the first instar placed in sub-plots containing neem than those in the rice canal water. (*F* =
615.97; \( P, 0.0001 \)). In second instar, the mortality was significantly higher \( (F = 535.15; P, < 0.0001) \) in the experimental \( (37.03 \pm 0.02) \) than in the control \( (2.67 \pm 0.06) \). In the late instar (L3 and L4), a similar significantly higher mortality was noted \( (F = 535.1; P, < 0.0001 \) and \( F = 567.39, P, < 0.0001 \) respectively). In the pupal stage the difference in mortality was not significant between the experimental and the control \( (F = 10.25 ; P, > 0.05) \).

![Figure 4.4: Mean mortality of immature stages of *Anopheles* species after being placed in sub-plots containing neem and rice canal water. Standard error bars of the mean number of dead individuals are shown.](image)

4.5 Effects of cow dung combined with neem on immature stages of *Anopheles* mosquitoes in the laboratory.

The highest mortality was observed in the early instar larvae (L1 and L2) reared in cow dung and neem (mean mortality 8.11 ± 0.03 and 7.82 ± 0.03 for L1 and L2 respectively) while the lowest mortality was observed at pupal stages in a similar treatment (1.04 ±
0.07; Fig. 4.5). In the control (rice canal water) the mortality of all immature stages was lower compared to that of those in cow dung and neem. The highest mortality in the control (rice canal water) was in the 4th instar stage (1.67 ± 0.06) while the lowest was also in pupal stage 1.37 ± 0.05 (Fig 4.5).

![Diagram showing mean mortality of immature stages of Anopheles species](image)

**Figure 4.5:** Mean mortality of immature stages of *Anopheles* species after being placed in bowls containing a combination of cow dung with neem and rice canal water. Standard error bars of the mean number of dead individuals are shown.

In all the immature stages placed in bowls containing neem combined with cow dung a significant difference in mortality was observed ($F= 252.68; P<0.0001$; Fig. 4.5). For all immature stages (L1 - L4 and pupae) that were placed in bowls containing a combination of neem and cow dung a significantly higher mortality was observed than in those placed in bowls containing rice canal water ($F= 252.68; P<0.0001$; Fig.4.5). The late instar larvae (L3 and L4) placed in bowls containing cow dung combined with neem revealed a
significantly lower mortality than the early instars (L1 and L2) placed in a similar treatment ($F=55.72; P<0.0001$). However, there was no significant difference in mortality between the pupae that were in bowls containing cow dung and neem and those in bowls containing rice canal water ($F=0.74; P>0.529$; Fig. 4.5).

4.6 Effects of cow dung combined with neem, on *Anopheles* mosquitoes in semi-field conditions.

A higher mortality (mean mortality $38.97 \pm 0.01$ and $37.30 \pm 0.02$) was observed in the early instar larvae (L1 and L2 respectively) compared to the late instar larvae (mean mortality of $34.00 \pm 0.02$ and $32.00 \pm 0.02$ respectively). Pupal stage showed the lowest mortality $4.63 \pm 0.06$ (Fig. 4.6). Among the immature stages in the control, 4th instar larvae had the lowest mortality $2.03 \pm 0.06$. 
Figure 4.6: Mean mortality of immature stages of *Anopheles* species after being placed in sub-plots containing a combination of cow dung and neem or rice canal water. Standard error bars of the mean number of dead individuals are shown.

In sub-plots containing a combination of neem and cow dung, there was no significant difference in mortality between 1st, 2nd and 3rd instars. However, a significant difference was observed between 1st and 4th instars stage ($F=701.97; P<0.0001$; Fig. 4.6). The pupae in cow dung combined with neem had a significantly low mortality ($F=10.25; P<0.0001$; Fig 4.6) than those in the control. There was a significant difference in mortality between immature stages placed in sub-plots containing cow dung and neem combined compared with those in rice canal water ($F=701.97; P<0.0001$; Fig 4.6). Pupae in cow dung combined with neem had significantly higher mortality than pupae in the rice canal water ($F, 10.25; P, <0.0001$)
4.7 Comparison of the effects of cow dung and neem combined compared to cow dung alone in the laboratory

A comparison between the effect of cow dung combined with neem on the immature stages and cow dung alone was done using the data from the laboratory experiments. In the first instar larvae higher mortality (8.11 ± 0.03) was recorded in cow dung and neem than in cow dung alone (7.23 ± 0.24; Fig. 4.7). A similar trend was recorded in the second instar larvae, higher mortality was recorded in larvae placed in a combination of cow dung and neem (7.82 ± 0.03) while mortality of larvae in cow dung had a lower mean mortality of 6.65 ± 0.05 (Fig. 4.7). Third instars larvae placed in a combination of cow dung and neem also had a higher mortality compared to those placed in cow dung alone (6.33 ± 0.03 and 6.00 ± 0.04 respectively; Fig. 4.7). Similarly in the fourth instar larvae in a combination of cow dung and neem, mortality was higher (5.91 ± 0.04) than in cow dung alone (5.53 ± 0.04; Fig. 4.7). However, there was no significant difference in mortality between all the larval instars placed in a combination of cow dung and neem in comparison to those that were placed in cow dung alone (F,140.44; F>0.05; Fig. 4.7).
4.7 Developmental stages

Figure 4.7: Mean mortality of immature stages of Anopheles species after being placed in cow dung combined with neem compared to cow dung alone in the laboratory. Standard error bars of the mean number of dead individuals are shown.

4.8 Effect of *A. annua* on landing responses of *Anopheles* mosquitoes

There was no landing of mosquitoes on the hands applied with *A. annua* during the first 10 minutes of the experiment. Landing of mosquitoes started on the 15th minute and increased as time progressed and maximum number of landing mosquitoes recorded at the 30th minute (5.5 ± 0.08 mosquitoes). The number of landing mosquitoes started to reduce after 35th minute (5.4 ± 0.07; Fig. 4.8). The lowest mean (0.43 ± 0.12 mosquitoes) of landing mosquitoes was recorded at the 50th minute. On the hand without application of *A. annua* (control), a higher number of mosquitoes landed at the beginning of the experiment (5-15 minutes) with the highest mosquitoes landing at the 5th minute (5.4 ± 0.08 mosquitoes). The number reduced as time progressed with the lowest recorded landing response being at the 50th minute (Fig. 4.8)
On the hand applied with *A. annua* there was a significant difference on crude *Artemisia* extracts efficacy over time ($F= 44.94; P< 0.0001; \text{Fig. 4.8}$). The results revealed a significant difference in the number of landing mosquitoes between hands applied with *A. annua* and hands without application of *A. annua* across all the tested time intervals ($F= 44.94; P< 0.0001; \text{Fig. 4.8}$). On the hands without application of *A. annua* (control), there was a significant difference in the number of landing mosquitoes over time ($F= 59.63; P < 0.001; \text{Fig. 4.8}$).

**Figure 4.8:** Variation in the mean number of mosquitoes landing on hands applied with *A. annua* and hands without *A. annua* over a period of time (Mean ± SE).
4.9 Anti-feedancy effect due to *A. annua* against *Anopheles* mosquitoes

The results revealed a higher number of fed mosquitoes on the hands without *A. annua* with a mean of 7.0 ± 0.28 mosquitoes while mean number of fed mosquitoes was lower in the hands applied with *A. annua* 2.6 ± 0.30 mosquitoes (Fig. 4.9). There was a significant difference in the number of fed mosquitoes between the hands applied with *A. annua* and those without *A. annua* application (*F* = 12.20; *P* < 0.0001; Fig. 4.9).

![Figure 4.9: Mean number of mosquitoes that fed on hands applied with *A. annua* and hands without application of *A. annua* over a period of time (Mean ± SE).](image-url)
CHAPTER FIVE
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Currently, malaria vector control is thought to be one of the best approaches in controlling the high malaria incidences. Different studies have clearly demonstrated that it is far less costly and more effective to control mosquito populations as larvae, before they mature and disperse into the environment. Kitron and Speilman (1989) suggested that larval control is a more effective way of reducing the malaria vector. In this study the role played by cow dung and plant material *A. indica* and *A. annua* in the control of malaria vector was investigated since these materials are less costly, easily applicable and biodegradable.

In this study, experiments on the effects of cow dung on immature stages of *Anopheles* mosquitoes have shown that, deliberate contamination of water with cow dung results in mortality of the immature stages of *Anopheles* spp. This was supported by the higher mortality of the larvae reared in cow dung than in the control (rice canal water). Previous studies by Aly and Dadd (1989) suggested that larvae imbibe micronutrients in the larval habitat in the process of filter feeding thereby ingesting harmful products that then results to their death. The lowest mortality was observed in the pupal stage indicating that pupae are less susceptible to contamination with organic materials such as cow dung. These could be attributed to the non feeding nature of pupae therefore avoiding ingesting of lethal materials.
In semi-field conditions, higher mortality in the immature stages in cow dung than those in rice canal water was recorded showing the potential of cow dung as a contaminant that can be used to reduce immature stages of mosquitoes. Early instar larvae (L1 and L2) were affected more than the late instar larvae (L3 and L4). These could be attributed to the fact that early instars larvae are more susceptible to mortality than the late instars larvae. Early instar larvae feed on the available organic material indiscriminately unlike the late instar larvae which specialize on feeding on particular organic matter (Beales and Giles, 2002). These could have caused the higher mortality of early instars than the late instar. The result of mortality induced by cow dung was in accordance with the findings of a study reported by Lee et al. (2003). This author reported that the dung of herbivores exhibited larvicidal activity against immature stages of Anopheles mosquitoes. Further studies have also shown that the normal larval breeding habitats become unfavorable if they are accidentally or artificially polluted with animal wastes or organic matter (Muirhead-Thomson, 1951). On decomposition, cow dung reduces the amount of oxygen content available in water. Low oxygen tension then reduces the survival of mosquito immature stages therefore rendering the habitats unfavorable.

High larval mortality was observed in larval stages that were placed in bowls and subplots containing neem than in the rice canal water. Early instar larvae had a significantly higher mortality compared to the late instar in both the laboratory and semi-field experiments. This finding suggests the high toxicity of neem to the different developmental stages of Anopheles mosquitoes. Studies conducted in Nigeria using Anopheles larvae found a similar trend where by all larvae died within 12 hours after
neem extracts were applied in water (Aliero, 2003). The neem cake powder had greatly reduced-sized particles and was evenly mixed within the water with a few suspended particles on the water surface. The spread of these fine particles probably increased the efficacy of the neem since *An. gambiae* s.s. are small particle surface feeders (Gianotti *et al.*, 2008). When ingested, the neem product particles induce anti-feedancy in larvae either by altering the insect's chemoreception or by reducing the food intake due to its toxicity (Howard *et al.*, 2009).

Mordue and Blackwell (1993) reported that azadirachtin, a botanical pesticide derived from the neem tree is generally considered less harmful to the environment than other commonly used pesticides. Cow dung and neem (*A. indica*) are biodegradable and therefore after decomposition they not only kill mosquito larvae but also act as fertilizers. For instance, neem has been shown to have anti-larval properties and also act as a fertilizer (Rao *et al.*, 2008). Therefore neem has a dual purpose in that once applied in rice fields it could reduce mosquito immature stages and also act as a fertilizer (Victor and Reuben, 2000). When cow dung was mixed with neem and its effect investigated against the immature stages, a higher mortality of all the immature stages occurred in the water contaminated with cow dung and neem than in the rice canal water. Furthermore, this study revealed that the highest mortality was recorded in early larval instars (L1 and L2) than the late larval instars (L3 and L4). These observations revealed that cow dung and neem are efficacious against mosquito larvae and supports the findings by Killeen *et al.* (2002) and Minakawa *et al.* (2002) who reported that larval control is a more effective strategy for reducing mosquitoes. In a combination of neem and cow dung, mortality was
lowest at pupal stage. These findings can be attributed to the fact that pupa is a non-
feeding stage thus they do not ingest the contaminants resulting from neem and cow dung
that were used in this experiment. Other studies have also confirmed that pupal stage has a
casing that prevents their contact with most of the contaminants thus conferring them
protection against possible control strategies (Mulla et al., 2001). This implies that any
control measure which targets the developmental stages of mosquitoes should target the
larval stage and especially the early instars since they were more susceptible than late
instars and pupae. When the combination of cow dung and neem was tested in semi-field
conditions, a significantly higher mortality compared to rice canal water was noted.
Studies conducted by Okumu et al. (2007) in Kenya showed that neem is an effective
larvicide which is highly toxic to larvae of Anopheles mosquitoes.

In this study, the high larval mortality indicates that the combination of cow dung and
neem is toxic. Early larvae instars also had higher mortality than the late larval instars. At
this stage of growth, the larvae are still adapting to the environment and therefore contact
with contaminants is likely to make the habitat unfavorable (Kettle, 1995). These could
also be attributed to the indiscriminate ingestion of any suitably sized particles especially
by the early instars larvae (Kettle, 1995). These findings of decreased mortality with
increase in age support the reports by Boschitz and Grunewald (1994) who found out that
the sensitivity of larvae to neem seed kernel decreased with increase in larval age and thus
the late instar larvae were less susceptible to the larvicidal activity of neem. From this
study, a combination of cow dung and neem caused mortality of the different stages of the
mosquito larvae. Both cow dung and neem had earlier been reported to be larvicidal (Lee et al., 2003; Okumu et al., 2007).

Plant products have been used in many parts of the world for killing or repelling mosquitoes either as extracts or as whole plant (Kweka et al., 2008). They usually play an important role in reducing human-vector contact which is crucial in minimizing malaria transmission. *Anopheles* spp. the mosquito species used for these tests is an important malaria vector not only in Ahero rice irrigation scheme but globally. A significant higher number of mosquitoes landed on the hand which had not been smeared with *A. annua* than on the hand that had been smeared with *A. annua*. This could be attributed to the repellency effect of *A. annua* to the adult *Anopheles* mosquitoes. As time progressed, the efficacy of *A. annua* reduced. When crude extracts of *A. annua* was evaluated against *Anopheles* spp. it gave protection for 30 minutes, but this declined after 35 minutes. The results of reduced efficacy of *A. annua* with time are comparable to the findings of Govere et al. (2000) who reported that the efficacy of citronella reduced with increase in time. In this study, *A. annua* was found to be effective in reducing the human vector contact for 30 minutes. These could probably be attributed to the reduced effectiveness of the active ingredients of *A. annua* with increase in time.

Similar experiments with *A. annua* revealed that a significant higher number of mosquitoes fed on the hand without *A. annua* while very few mosquitoes fed on the hand with *A. annua*. These demonstrated that *A. annua* has an anti-feedancy effect on adult *Anopheles* mosquitoes and have potential in reducing the human vector contact. The
results of anti-feedant effect due to *A. annua* are comparable with those of Kant and Bhatt (1994) who reported that neem prevented *Anopheles* mosquitoes from feeding.

5.2 Conclusions

From the results of this study, the following conclusions can be made:-

i. Both cow dung and a combination of cow dung and neem cause significant mortality of all larvae instars of *Anopheles* species and are thus efficacious against these stages.

ii. *Artemisia annua* applied on human hands reduced the landing rates of adult mosquitoes on human hands.

iii. The application of *A. annua* reduced feeding of *Anopheles* mosquitoes. Therefore, use of *A. annua* crude leaf extracts as an adult mosquito repellent against *Anopheles* spp. may have potential in reducing the vector contact though for a short time.

5.3 Recommendations

i. There is a need to identify the active components of cow dung that affect the malaria vector.

ii. Experiments should be conducted to establish whether the techniques of polluting water with cow dung and neem for mosquito control can work in the breeding sites.

iii. More research is necessary to establish the active components of *A. annua* causing the repellency and anti-feedancy effects on adult *Anopheles* spp.
REFERENCES


