EFFECT OF IVERMECTIN ON THE OVIPOSITION SITE SELECTION, EGG HATCHABILITY AND ATTRACTABILITY OF*CULEX QUINQUEFASCIATUS* SAY 1823 (DIPTERA: CULICIDAE)

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NIGERIA.

JUNE 2014

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MSc/Scie/03935/2010-11

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER DEGREE IN BIOLOGY.

DEPARTMENT OF BIOLOGICAL SCIENCES,

FACULTY OF SCIENCE

AHMADU BELLO UNIVERSITY, ZARIA

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JUNE, 2014

DECLARATION

I hereby declare that the work in this thesis titled "Effect of ivermectin on the oviposition site selection, egg hatchability and attractability of*Culex quinquefasciatus* Say" has been carried out by me in the Department of Biological Sciences, under the supervision of Prof. I. H. Nock and Prof. I. S. Ndams.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any institution.

AIYEDUN Emmanuel Adonmosi

Signature Date

CERTIFICATION

This thesis titled "EFFECT OF IVERMECTIN ON THE OVIPOSITION SITE SELECTION, EGG HATCHABILITY, AND ATTRACTABILITY OF*CULEX QUINQUEFASCIATUS* SAY" by AIYEDUN, EMMANUEL ADONMOSI meets the regulations governing the award of the degree of Master of Science (M.Sc.) in Biology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ACKNOWLEDGEMENTS

My wholehearted gratitude go to my Lord and Savior Jesus Christ for grace and divine enablement to complete this study. Lord I can't thank you enough but deep down in my heart, I want to say Lord I love you.

So much thanks to the best Dadin the whole world, Mr. G. A. Elegbe, who out of nothing was able to finance this worthy study from start to finish. May the Almighty God bless and increase you Sir. I love you lots.

My profound gratitude goes out to my supervisors, Prof. Ishaya, H. Nock and Prof. Iliya, S. Ndams for their great supervision and contributions towards the success of this study. Their invaluable corrections and constructive criticisms have made this research credible and insightful. May the Almighty God bless and increase you and members of your family in Jesus name.

My sincere thanks also go out to Mr. David Adebote, Mr. G. J. Sow, Ademola, Zulaihat, and Peace for their immense contributionsin various capacities towards the success of this study. I love you all dearly.

I wouldn't fail to sincerely appreciate my darling Uncles, Prof. Joseph Ajanusi, Prophet Moses Aiyedun, Dr. Emmanuel Aiyedun and my dearest sisters, Mrs. Joyce Taire and Mrs. Faith Oshafunmi for their love and kind support towards the success of this study. May God bless you all richly.

And last but not the least; I want to hugely appreciate my best friend, my sister, my best companion, Miss Deborah Adepoju, for being with me from the beginning to the end of this study. Your kind advice and encouragement motivated me to carry on even when I faced difficult situations. God bless you. I love you dearly.

ABSTRACT

This study was conducted to evaluate the effects of ivermectin on oviposition site selection, egg hatchability, attractabilityand larval mortalityof*Culex quinquefasciatus* Say 1823 (Diptera:Culicidae).Oviposition site selection was evaluated by placing 10 gravid female *Cx. quinquefasciatus* in a wooden cage provided with graded dilutions of ivermectin solutions using distilled water in plastic cups as control. Oviposition deterrent test and effective repellency (ER) were used to evaluate the oviposition deterrent activity of ivermectin.Groups of 50 freshly laid eggs were exposed to graded dilutions of ivermectin for 18 hours, and the hatchability (%) of these eggs was calculated. Groups of 50 freshly laid eggs were exposed to graded dilutions of ivermectin solution for 5, 15, 30 and 60 minutes. Graded dilutions of ivermectin were mixed with 10% sucrose solution in plastic cups with a cotton wick and placed in cages to test for its efficiency as attractive toxic sugar bait. High concentrations of ivermectin was observed to deter*Cx. quinquefasciatus*females while lower concentrations showed "attract and kill" effect on them: oviposition activity index valuesfor the test species were 0.6, 0.3, 0, 0, -1, -1 and -1 for ivermectin at concentrations of1, 5, 10, 20, 40, 80 and 100ppm respectively. 93.3% of the female*Cx. quinquefasciatus* died (out of which 67.9% were still gravid and 32.1% oviposited) in ivermectin treated cups, while 6.7% survived in the control. The 50% egg hatching inhibition concentration (IC_{50}) was noted at 26.81ppm. Time of exposure and ivermectin concentration influenced egg hatchability.Ivermectin was highly toxic to adult *Cx. quinquefasciatus* as an attractive sugar bait.Ivermectin showed $LC_{50}$of 3.78ppb and $LC_{95}$of 7.47ppb for 1st instar larvae and $LC_{50}$of 11.49ppb and LC_{95} of 35.20ppb against 3rd instar larvae of*Cx. quinquefasciatus* as determined by probit analysis.It is concluded that ivermectin serves as a potent insecticide against *Cx. quinquefasciatus*, thereforeholding promise to suppress vector population therebyreducing its biting nuisance

and nocturne discomfort while adversely influencing the transmission of its associated disease pathogens.

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ABBREVIATIONS, DEFINITIONS, GLOSSARY AND SYMBOLS

ABU – Ahmadu Bello University

Ae. – Aedes

AGP - Antigenaemia Prevalence

AMCA- American Mosquito Control Association

An. – Anopheles

ANOVA – Analysis of Variance

ATP – Annual Transmission Potential

BLU - Bluetongue disease

C. - Culicoides

Cx. – Culex

CNS – Central Nervous System

DDT - Dichlorodiphenyltrichloroethane

DEC - Diethylcarbamazine

EC – Effective Concentration

ED – Effective dose

EFS - Embryonal Fyn-associated Substrate

ER – Effective Repellency

ERAPharm - Environmental Risk Assessment of Pharmaceuticals

EU – European Union

FDA – Food and Drug Agency

GABA - Gamma-AminoButyric Acid

GMI - Geometric Mean Intensities

IC – Inhibition Concentration

ICT - Immunochromatographic Test

IPM - Integrated Pest Management

ISO - International StandardizationOrganization

IVR - Ivermectin

LC – Lethal Concentration

MDA – Mass Drug Administration

MFP - Microfilaraemia Prevalence

NOEC – No Observed Effect Concentration

OAI – Oviposition Activity Index

OECD - Organization for Economic Co-operation and Development

OWMM - Open water marsh management

PECs – Predicted Environmental Concentrations

P-gp – P-glycoprotein

PRC - Principal Response Curve

RIM- Rotational Impoundment Management

TMEs - Terrestrial Model Ecosystems

VG - Vitellogenin

VICH – Veterinary International Conference on Harmonization

WHO – World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

The distribution of mosquito larvae depends mainly on the selection performed by the adult femalefor an appropriate oviposition site called oviposition site selection, animportant phenomenon in mosquitoes. The factors involved in oviposition site selection by vector mosquitoes have become a focal point of interest in theconcept of integrated vector control management (Graham-Bryce, 1987). Oviposition site selection by gravid mosquitoes is oneof the crucial events in non-parental organisms (Huang*et al.,* 2005; Aravand Blaustein, 2006).In mosquitoes, the reproductive success depends onthe ability of a mosquito to search for the suitableoviposition site that will ensure the survival of theprogeny (Bentley*et al.,* 1976;Bentley and Day, 1989). The decision of where and how many eggs to deposit in breeding sites is critical for the fitness of juveniles of animals like mosquitoes that are unable to move to more suitable habitats if the local site is unfavourable (Onyabe and Roitberg, 1997). It has been proposed that habitat choice is determined by the ability of gravid females to determine habitats where the fitness of their offspring is optimized (Mangel, 1987). In general, given the heterogeneity of natural environments, fitness is a complex function of exogenous (e.g., food sources) and endogenous factors (e.g. intra-specific competition). Therefore, habitat colonization will follow, under ideal conditions, a pattern where average fitness differences are similar across a variety of habitats where females can make oviposition choices (Fretwell and Lucas, 1970; Ellis, 2008). Several factors have been identified and known toproduce highlyselective oviposition attractancy for vector mosquitoes. These ranges from purely chemical cues (Ikeshoji*etal*., 1966; Dadd and Kleinjan, 1973; Bentley and Day,

1989; Millar *et al*., 1994; Braks *et al*., 2007), substances originating from the egg (Bruno and Laurence, 1979), larva (Kalpage and Brust, 1973) and pupa(Andreadis, 1977), the presence of microorganisms (Rockett, 1987; Bentley and Day, 1989; Beehler *et al*., 1994; Poonam *et al*., 2002), conspecific individuals(Suleman and Shirin, 1981; Madder *et al*., 1983;Wilmot *et al*., 1987; Dhileepan, 1997;Onyabe and Roitberg, 1997; Zahiri *et al*., 1997) and oviposition medium quantity and quality(Reiskind and Wilson, 2004; Harrington *et al*., 2008). Therefore natural selection should favour female mosquitoes that select habitats which best support the development of their offspring (Onyabe and Roitberg, 1997).

Oviposition is a critical stage in the mosquito lifecycle,especially for *Culex* mosquitoes, which arethought to lay a single clutch of eggs in a single habitatper gonotrophic cycle (Clements, 1999).Variousstudies have suggested that mosquito oviposition behaviorand suitable larval habitat availability influence theabundance, distribution, biting rate and pathogentransmission ability of such vectors (Patz *et al*., 1998; Carter *et al*., 2000; Spencer*et al*., 2002). Thus, a better understanding ofmosquito reproductive ecology could lead to interventionsaimed at reducing risk of malaria and other mosquito-borne diseases (Reiskind and Wilson, 2004).

Mosquitoes may detect chemicals from the airwhen the chemical possesses sufficient volatility or, in thecase of low volatility chemicals, by a gustatory mechanisminvolving direct contact with the water (Clements, 1999). Gravid mosquitoes are often attracted to water but the decisionto lay eggs maydepend on additional olfactory signalsor other stimuli received when the insect lands on thewater surface (Huang*et al.,* 2005).Habitat related chemical cues havebeen shown to play an important role in oviposition site selection by gravid mosquitoes (Braks *et al.,* 2007; Burkett-Cadena and Mullen, 2007).Microorganisms inhabiting mosquitobreeding sites have been found to play a majorrole in the decomposition of detritus and other nutrientspresent in these habitats, leading to the productionof several metabolites

emanating differentchemical cues (Androsov*et al.*, 1986). Natural chemical cues also influence mosquito oviposition behavior (Geetha *et al*., 2003). 3-methylindole was shown to be the most attractive chemical togravid *Culex* females under both laboratory and field conditions (Millar *et al*., 1992; Mordue*et al*., 1992; Blackwell *et al*., 1993; Beehler *et al*., 1994). Inaddition, the influence of some fatty acids on the oviposition behaviors of *Culex quinquefasciatus* and *Cx. tarsalis* Coquillett has also been evaluated underlaboratory condition (Hwang *et al*., 1980; 1982). However, chemical cues andwater color do not act independently in natural situations, as they typicallyinteract with other factors at the oviposition site for mosquito species (Dhileepan, 1997; Mboera *et al*., 1999; Olagbemiro *et al*., 2004).Therefore, extrinsic micro-environmental factors such as water quality affect mosquito oviposition site selection, hatching of eggs and larval development. In view of these, interruption ofoviposition site selection and egg hatchability for mosquitoes may lead topopulation growth reduction and have a positiveimpact in control programmes.

Mosquito control involves careful consideration of thebiology of the mosquitoes that areforming the nuisance or constitute adisease threat.The need to control major diseases such as malaria, filariasis and dengue is widely accepted, but many challengesremain unmet. These challenges encompasstechnical issues, funding and regulatory constraintsthat influence how rapidly new control tools can bedeveloped, anticipated economic, environmental,health and social trade-offs associated with theuse of different control methods and social andcultural issues expected to influence acceptanceof these methods.Mosquito control is a vital public-health practice throughout the world and especially in the tropics because mosquitoes spread many infectious diseases (Walker and Lynch 2007). Mosquito-control operations are targeted against three different problems:

1. Nuisance mosquitoes bother people around homes or in parks and recreational areas;

2. Economically important mosquitoes reduce real estate values, adversely affect tourism and related business interests, or negatively impact livestock or poultry production;
3. Public health is the focus when mosquitoes are vectors or transmitters of infectious diseases.

In the past, mosquitoes (and, consequently, malaria) have been controlled by environmentaland chemical means that reduce mosquito survival and/or population expansion. These methodshave been successful in many areas of the world,yet there are roadblocks that prevent these classicalapproaches from effectively controlling mosquitoes. Somelimitations come from the infrastructure of endemicareas, while others, such as insecticideresistance, are purely biological.Insecticide use still plays a significant rolein malaria control programs involving the use of insecticide-treated bednets and indoor residual spraying (WHO, 2006). Insecticide-treated bed nets alone have been regarded as anexcellent tool for reducing malaria transmission in highlyendemic countries,especially by reducing child mortalityand morbidity (WHO, 2007). It has been noted that in order to achievea greater efficacy in Africa, insecticide-treated bed netsneed to be widely distributed among the populationand insecticide re-impregnation services have to beprovided at a relatively low cost if continuous protectionis to be maintained.

Understanding vector biology and its relationship with *plasmodium* spp malariabiology has opened the door to new control strategies.Most new ideas in this field make use of moleculardata and methods to add to the insights of entomologistsfrom the pre-genomic era and explain themolecular mechanisms behind vector-parasite interactionsand mosquito biology and behavior. The genomicanalyses made available by the sequencing of the *Anophelesgambiae* genome have provided significant insight intothe vector's biological processes that can potentially bedisrupted or exploited in an effort to control

malaria.Understanding of the molecular basis of mosquito resistance to insecticides isof importance in maximizing the efficacy of the currently available insecticides employed in malaria controlprogrammes (José*et al*., 2009).In recent times, the data generatedfrom molecular analysis are the result of a multifacetedapproach that strives to understand not only themolecular mechanisms governing mosquito biologyand mosquito-malaria interactions but alsohow the insects can be manipulated to reduce malariatransmission and how these mechanismsand manipulations play out across populations. Itis the synergy of these areas of molecular entomologythat has revealed the possibility of applying vector-basedmalaria control strategies that has given newhope to efforts aimed at malaria eradication. These new strategies,however, also face challenges that will need tobe addressed before they can be implemented in the field (José*et al*., 2009).

Research has also shown that human odour is the principal cue employed by the mosquitoes to locate their blood host (Takken and Knols, 1999).Currently, work is in progress to elucidate component of human odour that are responsible for the attractiveness of humans to malaria mosquitoes (Zwiebel and Takken, 2004). It is anticipated that this research will lead to the development of powerful odour bait that can be used to lure mosquitoes away from human dwellings and trap them into a killing device.

Recently there have been reports on the ability of an anti-helminthic drug called ivermectin to kill adult mosquitoes during a mass drug administration (MDA). In a report by Kobylinski *et al.*(2011), laboratory studies indicated that *Anophelesgambiae* (s.s.) mosquitoes can be killed by ivermectin present in human blood after a standard ivermectin dose. Kobylinski and his colleagues also reported that ivermectin MDA for lymphatic filariasis provided to humans in Senegalese villages significantly reduced the proportion of *Plasmodium falciparum* infected *An. gambiae* (s.s.) relative to those caught from nearby control villages for up to two weeks post administration (Kobylinski *et al.*, 2011). Mass drug administration of ivermectin to

humans has been a safe and effective control strategy for onchocerciasis and lymphatic filariasis. These suggest that ivermectin MDA, provides an alternative control method currently used to interrupt malaria parasite transmission. A unique delivery method is employed which targets the mosquito vectors irrespective of their feeding habits.

Ivermectin has been used extensively as part of the World Health Organisation's Onchocerciasis Eradication Program. Ivermectin has aunique mode of action. Compounds of the class bind selectively and with high affinity to glutamate-gatedchloride ion channels which occur in invertebrate nerve and muscle cells. This leads to an increase in thepermeability of the cell membrane to chloride ions with hyper polarization of the nerve or muscle cell,resulting in paralysis and death of the parasite (FDA, 2011).

Among the arthropod parasites claims of ivermectin efficacy have been established for the biting fly *Haematobia irritans,* the screw-worm *Chrysomyia bezziana,* larvae of the oestrid flies *Hypoderma* spp*., Dermatobia hominis, Oestrus ovis* and *Gastrophilus* spp, lice, mange mites and the ticks (*Boophilus* sppand *Ornithodorus savignyi).* Previous studies have shown that the avermectins are lethal for immature and adult stages of some insect orders (Strong and Brown, 1987). They are also efficient for the control of larval and adult stages of *Cx. quinquefasciatus*(Freitas *et al.*,1996; Alves *et al.*,2004). In Diptera and Coleoptera, there are reports about water accumulation in the individuals due to avermectin action, suggesting that the drug has interference in the Malpighian tubules and hormones that act on water balance (Strong, 1993).In addition, mortality and paralysis caused by ivermectin have been observed in adults of *Aedes fluviatilis, Ae.aegypti, An. quadrimaculatus* and other mosquito species (Tesh and Guzman, 1990; Gardner*et al*., 1993;Consoli and Oliveira, 1994).

Two avermectins (MK-933 and MK-936) were shown to be effective as mosquito larvicide and induce mortality when fed to adult female mosquitoes in sucrose solution or in blood.

Ivermectin has been shown to have significant effect on the survival and fecundity of *Cx. pipiens*(Nasr *et al.,* 1996).

One of the possible strategies of malaria control is to identify local vector species and then attack water bodies that contain their immature stages (**Arnaud***et al.,* 2005).

It has been observed that a water body containing ivermectin was devoid of mosquito larvae for over five months during the raining season (Nock, pers com, 2011). It could be that the mosquitoes laid viable eggs that lost their hatchability and did not hatch to the larval stages, or that these mosquitoes did not even ovipositat all in the water due to the presence of ivermectin. The goal of this project therefore, was to assess the effects of ivermectin on oviposition site selection,egg hatchabilityand as an attractive toxic sugar baitto*Cx. quinquefasciatus.*

1.1 Statement of Research Problem

It has been shown that ivermectin has adulticidal and larvicidal effects on mosquitoes, but its effect on oviposition site selection, egg hatchability and as an attractive toxic sugar bait to *Cx. quinquefasciatus*remains unknown to our knowledge.

1.2 Justification

Information derived from this study might provide a more cost effective mechanism of control against *Cx. quinquefasciatus in the study area,* therebyreducing its biting nuisance and nocturne discomfort while adversely influencing the transmission of its associated disease pathogens.More research is needed in this field to complement existing mosquito control strategies to alleviate the burden of malaria, filariasis and other diseases to which mosquitoes are vectors.

1.3 Aim

To further evaluate the potential of ivermectin as a cost effective tool in the control of mosquito population growth.

1.4 Research Objectives

i. to determine the effect of ivermectin on oviposition site selection of*Cx. quinquefasciatus.*

ii. to determine the effect of ivermectin on egg hatchability in *Cx. quinquefasciatus.*

iii. to determine the effect of ivermectin as an attractive toxic sugar bait to *Cx. quinquefasciatus.*

iv. *to determine the* LC_{50}-LC_{95} *of ivermectin against* 1^{st} *and* 3^{rd}*larval instars ofCx. quinquefasciatus.*

1.5 Research (Null) Hypotheses

i. Ivermectin has no significant effect on oviposition site selection of*Cx. quinquefasciatus.*

ii. Ivermectin has no significant effect on egg hatchability in *Cx. quinquefasciatus.*

iii. Ivermectin has no significant effect as an attractive toxic sugar bait to *Cx. quinquefasciatus.*

v. There is no significant difference in the LC_{50} *and* LC_{95} *of ivermectin against* 1^{st} *and* 3^{rd} *instar larvae of Cx. quinquefasciatus.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mosquito Control

Mosquito control manages the population of mosquitoes to reduce their damage to human health, economies and enjoyment. Mosquito control is a vital public-health practice throughout the world and especially in the tropics where mosquitoes spread many diseases, such as filariasis and malaria. The acquisition of knowledge concerning proper control of mosquitoes and mosquito borne diseases requiresgood knowledge of the biology, physiology, anatomy,genetics, taxonomy and ecology of this insect. Insecticide resistance, biological, chemical and integrated control must be investigated (Gerberg, 1970).Research on mosquitocidal chemicals may have far-reaching consequences in the control of mosquitoes and mosquito-bornediseases.

2.2 General Mosquito Control Methods

Depending on the situation, source reduction, biocontrol, larviciding or adulticiding may be used to manage mosquito populations. These techniques are accomplished using habitat modification, pesticide, biological-control agents and trapping (Walker, 2002).

2.2.1 Source reduction

Since many mosquitoes breed in standing water, source reduction can be as simple as emptying water from containers around the home. This is something that homeowners can accomplish. For example, homeowners can eliminate mosquito breeding grounds by removing unused plastic pools, old tires, or buckets, by clearing clogged gutters and repairing leaks around faucets, by regularly changing water in bird baths and by filling or draining puddles, swampy areas and tree stumps. Eliminating such mosquito breeding areas can be an

extremely effective andcheap way to reduce mosquito populations without resorting to insecticides (FCCMC, 1998).

Open water marsh management (OWMM) involves the use of shallow ditches, about 4 feet (1.2 m) wide and 2 feet (0.61 m) deep, to create a network of water flow within marshes and to connect the marsh to a pond or canal. The network of ditches drains the mosquito habitat and lets in fish which will feed on mosquito larvae. This reduces the need for other control methods such as pesticides. Simply giving the predator access to the mosquito larvae can result in long-term mosquito control (FCCMC, 1998). Rotational impoundment management (RIM) involves the use of large pumps and culverts with gates to control the water level within an impounded marsh. RIM allows mosquito control to occur while still permitting the marsh to function in a state as close to its natural condition as possible. Water is pumped into the marsh in the late spring and summer to prevent the female mosquito from laying her eggs on the soil. The marsh is allowed to drain in the fall, winter and early spring. Gates in the culverts are used to permit fish, crustaceans and other marsh organisms to enter and exit the marsh. RIM allows the mosquito-control goals to be met while at the same time reducing the need for pesticide use within the marsh.

2.2.2 Biocontrol

Biological control or "biocontrol" is the use of natural enemies to manage mosquito populations. There are several types of biological control including the direct introduction of parasites, pathogens and predators to target mosquitoes. Effective biocontrol agents include predatory fish that feed on mosquito larvae such as mosquitofish (*Gambusia affinis*) and some cyprinids (carps and minnows) and killifish. Tilapia also feed on mosquito larvae(MacKay, 1995). Other predators include dragonfly naiads, which feed on mosquito larvae in the breeding waters and adult dragonflies, which eat adult mosquitoes. Some other

biocontrol agents that have had lesser degrees of success include the predator mosquito *Toxorhynchites* and predator crustaceans - Mesocyclops, copepods, nematodes andfungi(Marten and Reid, 2007).Some public agencies also employ other predators such as birds, bats, lizards and frogs, but evidence of effectiveness of these agents is only anecdotal.

Like all animals, mosquitoes have their own set of diseases. Invertebrate pathologists study these diseases in the hope that some of them can be utilized for mosquito management. Microbial pathogens of mosquitoes include viruses, bacteria, fungi, protozoa, nematodes and microsporidia (Durden and Mullen, 2002).

Integrated pest management (IPM) is the use of the most environmentally appropriate method or combination of methods to control pest populations. Typical mosquito-control programs using IPM first conduct larval and adult surveys, in order to determine the species composition, relative abundance and seasonal distribution of adult and larval mosquitoes and only then are the best and most effective methods of control utilized (FCCMC, 1998).

2.2.3 Larviciding

Control of mosquito larvae can be accomplished through the use of contact poisons, growth regulators, surface films, stomach poisons (including bacterial agents) and biological agents such as fungi, nematodes, copepods and fish(Walker andLynch, 2007). A chemical commonly used is methoprene, considered slightly toxic to larger animals, which mimics and interferes with natural growth hormones in mosquito larvae, preventing their further development

2.2.4 Adulticiding

Control of adult mosquitoes is the most familiar aspect of mosquito control to most of the public. It is accomplished by ground-based applications or via aerial spraying of chemical

pesticides. Generally modern mosquito-control programs in developed countries use low-volume applications of pesticides, although some programs may still use thermal fogging.

DDT was formerly used throughout the world for large area mosquito control, but it is now banned in most developed countries.Controversially, DDT remains in common use in many developing countries, which claim that the public-health cost of switching to other control methods would exceed the harm caused by using DDT. It is sometimes approved for use only in specific, limited circumstances where it is most effective, such as application to walls.The role of DDT in combating mosquitoes has been the subject of considerable controversy. While some argue that DDT deeply damages biodiversity, others argue that DDT is the most effective weapon in combating mosquitoes and hence malaria(Chevillon *et al.,* 1999). Notwithstanding, DDT-resistant mosquitoes have started to increase in numbers, especially in the tropics due to genetic mutations, reducing the effectiveness of this chemical. DDT has not been in use for over 20 years.

Another approach to killing mosquitoes in a non-toxic way is to use a device that burns propane, thus generating carbon dioxide, warmth and water vapor. These three elements, often coupled with a chemical attractant heated in this process, draws the mosquitoes toward the propane flame, where they are then sucked into a net or holder where they collect (Whitaker, 1994).

Some newer mosquito traps or known mosquito attractants emit a plume of carbon dioxide together with other mosquito attractants such as sugary scents, lactic acid, octenol, warmth, water vapor and sounds. By mimicking a mammal’s scent and outputs, the trap draws female mosquitoes toward it, where they are typically sucked into a net or holder by an electric fan where they are collected. According to the American Mosquito Control Association (AMCA), "these devices will, indeed, trap and kill measurable numbers of mosquitoes," but

their effectiveness in any particular case will depend on a number of factors such as the size and species of the mosquito population and the type and location of their breeding habitats. They are useful in specimen collection studies to determine the types of mosquitoes prevalent in an area but are typically far too inefficient to be useful in reducing mosquito populations.The advantage of non-toxic methods of control is that they can be used in Conservation Areas(Whitaker, 1994).A traditional approach in controlling mosquito populations is the use of lethal ovitraps by providing artificial breeding spots for the mosquitoes but destroying the developing larvae.The latest approach is the automatic lethal ovitrap which works like a traditional ovitrap but automates all steps needed to provide the breeding spots and to destroy the developing larvae (Service, 1993).Scientists have created male mosquitoes which were spermless and are experimenting with methods of introducing the sterile males into the environment in the hope of reducing overall mosquito numbers (Carpenter, 2011).

2.3 Mosquito Life Cycle

When adult mosquitoes emerge from aquatic stages, the males and females mate. The female seeks a blood meal to obtain the protein necessary for the development of her eggs. Different species lay their eggs in a variety of stagnant water sources that range from small containers to vast expanses of marshland. The females of a few species may produce a first batch of eggs without this first blood meal. After the blood meal is digested and eggs are laid, the female mosquito again seeks a blood meal to produce a second batch of eggs. Depending on her stamina and the weather, she may repeat this process many times without mating again. The male mosquito does not take a blood meal, but may feed on plant nectar. It lives for only a short time after mating. The larval stage is always aquatic and shuttles from the subsurface where it filter-feeds on microorganisms, to the surface to obtain oxygen through a snorkel-

like breathing apparatus. The pupal stage does not feed but unlike most insect pupae, it is extremely active. The adult (Fig. 2.1) emerge from the pupal case using air pressure and assumes a terrestrial existence.

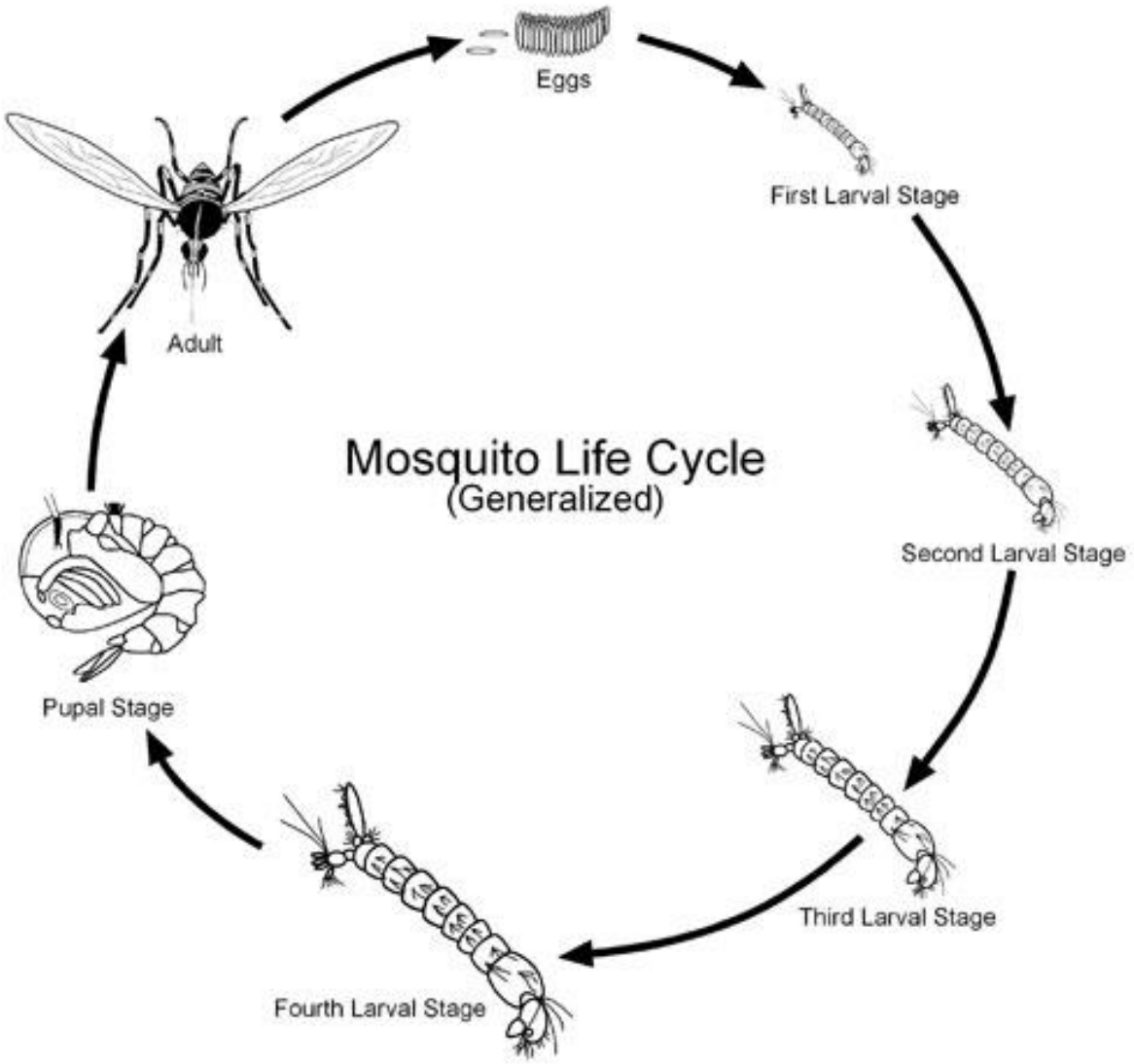


Figure 2.1. Generalized life cycle of a mosquito
Source:Mosquito Information website (www.mosquitoworld.net)

2.4 *Culex quinquefasciatus* – The Southern House Mosquito

Culex quinquefasciatus Say, commonly known as, the southern house mosquito (www.mosquitoworld.net) is amedium-sized brown cosmopolitan mosquito that is highly anthropophilic and completely adapted to urban conditions with a larval stage capable of developing in highly polluted water collections. This buzzing and biting mosquito is responsible for great nocturne discomfort and allergic responses (Malafronte *etal*., 2003). Additionally, *Cx. quinquefasciatus* transmits pathogens such as *Wuchereriabancrofti*, one of the agents of lymphatic filariasis and is a competent vector of neurotropic viruses (Gaunt *et al.*, 2001). The St. Louis and Japanese encephalitis viruses, eastern and western equine encephalomyelitis viruses, Rift Valley virus and West Nile virus are also efficiently transmitted by *Cx. quinquefasciatus* (Vaidyanathan and Scott, 2007; Hamer *etal.*, 2008).

Gravid *Cx. quinquefasciatus* females fly during the night to nutrient-rich standing water where they lay their eggs. They oviposit in waters ranging from waste water areas to bird baths, old tires, or any container that holds water. If the water evaporates before the eggs hatch or the larvae complete their life cycle, they die (Mosquito Information Website, 2009). The larvae feed on biotic material in the water and require between five to eight days to complete their development at 30°C (Gerberg *etal*., 1994). The larvae progress through four larval instars and towards the end of the fourth instar they stop eating and molt to the pupal stage. Following 36 hours at 27°C the adults emerge from the pupal stage (Gerberg *etal*., 1994). The time of development under natural conditions for all stages is variable and dependent on temperature.

Both males and females take sugar meals from plants. Following mating, the female seeks a blood meal. *Culex quinquefasciatus* are opportunistic feeders, feeding on mammals and/or birds throughout the night. Males survive only on sugar meals, while the female will take multiple blood meals. After a female mosquito digests the blood meal and the eggs develop,

she finds a suitable place to lay her eggs (Plate V) and the cycle begins again. A single female can lay up to five rafts of eggs in a lifetime (Gerberg *etal*., 1994). The number of eggs per raft varies with climatic conditions.

2.4.1 Distribution

Culexquinquefasciatus is a sub-tropical mosquito species usually found within the latitudes 36° N and 36° S, which include Africa, North America, South America, Australia, Asia, the Middle East and New Zealand (Cutwa-Francis and O'Meara, 2008).

2.4.2 Description

2.4.2.1 **Adults**

Adult *Cx. quinquefasciatus*(Plate I) vary from 3.96 to 4.25 mm in length (Lima *etal*., 2003). The mosquito is brown with the proboscis, thorax, wings and tarsi darker than the rest of the body. The head is light brown with the lightest portion in the center. The antennae and proboscis are about the same length, but in some cases the antennae are slightly shorter than the proboscis. The flagellum has thirteen segments that have few to no scales (Sirivanakarn and White, 1978). The scales of the thorax are narrow and curved. The abdomen has pale, narrow, rounded bands on the basal side of each tergite. The bands barely touch the basolateral spots taking on a half-moon shape (Darsie and Ward, 2005).

2.4.2.2 **Eggs**

Common to the *Culex* genus, the eggs of *Culex quinquefasciatus* are laid in oval rafts (Plate II) loosely cemented together with 100 or more eggs in a raft which will normally hatch 24 to 30 hour after being oviposited (Bates, 1949). The eggs possess thick, hard chorion.

2.4.2.3 **Larvae**

The larval head is short and stout becoming darker towards the base (Plate III). The mouth brushes have long yellow filaments that are used for filtering organic materials. The abdomen consists of eight segments, the siphon and the saddle. Each segment has a unique setae pattern (Sirivanakarn and White, 1978). In *Cx. quinquefasciatus,* the siphon is on the dorsal side of the abdomen and is four times longer than it is wide with multiple setae tufts (Darsie and Morris, 2000). The saddle is barrel shaped and located on the ventral side of the abdomen with four long anal papillae protruding from the posterior end (Sirivanakarn and White, 1978).

2.4.2.4 **Pupae**

Similar to other mosquito species, *Cx. quinquefasciatus* pupae are comma shaped (Plate IV) and consists of a fused head and thorax (cephalothorax) and an abdomen. The cephalothorax color varies with habitat and darkens on the posterior side. The trumpet, which is used for breathing, is a tube that widens and becomes lighter in color as it extends away from the body. The abdomen has eight segments. The first four segments are the darkest and the color lightens towards the posterior. The paddle, at the apex of the abdomen, is translucent and robust with two small setae on the posterior end (Sirivanakarn and White, 1978).



Plate I. Adult female *Cx. quinquefasciatus* Say, southern house mosquito, feeding
Source: Photograph by James Newman, University of Florida



Plate II.Eggs of *Cx. quinquefasciatus* Say,the southern house mosquito
Source: MR4 - Behavior and Physiology of Anophelines in the Laboratory



Plate III. Larva of *Cx. quinquefasciatus* Say, the southern house mosquito
Source: Photograph by Stephanie Hill, University of Florida



PlateIV. Pupa of *Cx. quinquefasciatus* Say, the southern house mosquito
Source: Photograph by Stephanie Hill, University of Florida



Plate V. Female *Cx. quinquefasciatus* Say, southern house mosquito (oviposition)
Source: Photograph by Sean McCann, University of Florida

2.5 Ivermectin

The drug ivermectin (**Mectizan; Stromectol**) is a semi-synthetic, anti-helminthic agent. Ivermectin was derived from the avermectins (a series of 16-membered macrocyclic lactone derivatives), a class of highly active broad-spectrum, anti-parasitic agents isolated from the fermentation products of the fungus*Streptomyces avermitilis*(Elgart and Meinking, 2003).

2.5.1 Chemistry of ivermectin

Ivermectin contain a mixture of at least 90% 22,23-dihydroavermectin B_{1a} and 10% or less of 22,23-dihydroavermectin B_{1b}(Merck & Co., Inc., 2010). The structural (Fig 2.2) and empirical molecular formulas are indicated below

Molecular Formula: $H_2B_{1a}\ C_{48}H_{74}O_{14}$ ($R=C_2H_5$)
$H_2B_{1b}C_{47}H_{72}O_{14}$($R = CH_3$)

Molecular Weight: H2B1a 875.10 ($R=C_2H_5$)
H2B1b 861.07 ($R= CH_3$)

2.5.2 Properties of ivermectin

Ivermectin is available in tablets / chewables for heartworm prevention, topical solution for ear mite treatment, or as oral or injectibles solution for other parasite problems. Ivermectin also comes as a tablet to take orally. It is usually taken as a single dose on an empty stomach with water (FDA, 2011).The ivermectin tablet is a white to yellowish-white, non-hygroscopic, crystalline powder with a melting point ofabout 155°C. It is insoluble in water but is freely soluble in methanol and soluble in 95%ethanol. Ivermectin contains the following inactive ingredients: microcrystalline cellulose, pregelatinized starch, magnesium stearate, butylated hydroxyanisole and citric acid powder (anhydrous).

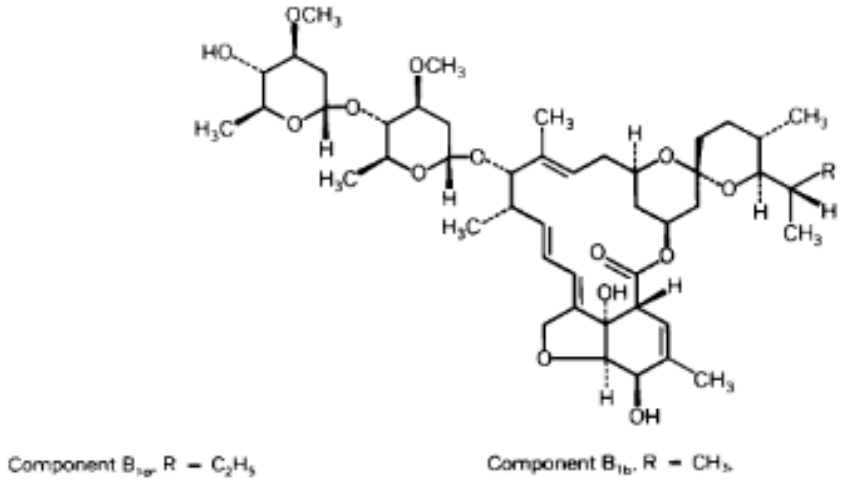


Figure 2.2: Structural formula of ivermectin
Source: Merck Sharp & Dohme (NZ) Ltd

2.5.3 Pharmacokinetics

With 12 mg single oral doses of ivermectin administered as tablets, the mean peakplasma concentration of the major component (Ha) was 46.6 (± 21.9) ng/mL atapproximately 4 hours after dosing.Plasma concentration increases with increasing dose in a manner approximately proportional to the dose. Ivermectin is metabolised in humans. Ivermectin and/or itsmetabolites are excreted almost exclusively in the faeces over an estimated 12 hours withless than 1% of the administered dose being excreted in the urine. The plasma half-life ofivermectin in man is about 12 hours and that of the metabolites is about 3 days.

A multiple-dose (30 to 120 mg [333 to 2000 mcg/kg]) clinical safety study has beenconducted to assess the safety and pharmacokinetic properties of ivermectin in healthyvolunteers. Subjects received oral doses of 30 to 120 mg (333 to 2000 mcg/kg) ivermectinin a fasted state or 30 mg (333 to 600 mcg/kg) ivermectin following a standard high-fat(48.6 g of fat) meal. Administration of 30 mg ivermectin following a high-fat meal resultedin an approximate 2.5-fold increase in bioavailability relative to administration of 30 mgivermectin in the fasted state. However, there were no indications of central nervoussystem toxicity associated with either regimen.

2.5.4 Mode of action

Ivermectin was introduced for the control of ectoparasites and endoparasites of sheepand cattle (Gunn and Sadd, 1994). It is a member of a family of compounds, theavermectins, a class of chemicals active against a broad spectrum of nematode and arthropod parasites of animals. Ivermectin is a derivative of avermectin B_1, ahigh-molecular-weight fermentation product of microbial cultures of the soil-dwellingactinomycete,*Streptomycesavermitilis* (Campbell *et al*., 1983).Avermectin B_1acts by blocking signal transmission from interneurons toexcitatory motor neurons, specifically that of the gamma-aminobutyric acid(GABA)

neurotransmitter (Campbell *et al.*, 1983). In invertebrates such asnematodes and arthropods, GABA-mediated nerves primarily control theperipheral muscles. Administration of ivermectin results in paralysis anddeath of the parasite either through starvation or detachment from the host(Gunn and Sadd, 1994). However, ivermectin is not active against protozoa,flukes, or tapeworms, presumably because neurotransmission in these groups isnot GABA-mediated (Campbell *et al.*, 1983). In mammals, GABA-mediated nervesoccur only in the central nervous system and therefore ivermectin should have alimited effect provided it does not cross the blood–brain barrier.Ivermectin may be administered orally, via subcutaneous injection, or as a pour-onformulation (Bloom and Matheson, 1993).

Inhumans, ivermectin is used in the treatment of

- intestinal strongyloidiasis (anguillulosis).
- proven suspected microfilaraemia in patients with lymphatic filariasis caused by *Wuchereria bancrofti*.
- sarcoptic scabies after prior treatment has failed. Treatment is justified when the diagnosis of scabies has been established clinically and/or by parasitological examination. Without formal diagnosis, treatment is not justified in case of pruritus.

2.5.5 Dosage and Administration

A) Treatment of intestinal strongyloidiasis

The recommended dosage is one single oral dose of 200 micrograms of ivermectin per kg body weight.For guidance, the dose, as determined by the patient's weight, is as follows:

BODY WEIGHT (Kg)	DOSE Number of 3 mg tablets
15 – 24	One
25 – 35	Two
36 – 50	Three
51 – 65	Four
66 – 79	Five
≥ 80	Six

B) Treatment of microfilaraemia caused by *Wuchereria bancrofti*

The recommended dosage for mass distribution for the treatment of microfilaraemia caused by *Wuchereria bancrofti* is a single oral dose once every 6 months designed toprovide approximately 150 to 200 µg/kg of body weight.In endemic areas where treatment can only be administered once every 12 months, therecommended dosage is 300 to 400 µg/kg of body weight to maintain adequatesuppression of microfilaraemia in treated patients. For guidance, the dose, as determined by the patient's weight, is as follows:

BODY WEIGHT(kg)	DOSE when given once every 6months Number of 3 mg tablets	DOSE when given once every 12months Number of 3 mg tablets
15 to 25	One	Two
26 to 44	Two	Four
45 to 64	Three	Six
65 to 84	four	Eight

Alternatively and if no scales are available, the dose of ivermectin for use in masschemotherapy campaigns may be determined by the patient's height as follows:

HEIGHT (cm)	DOSE when given once every 6months Number of 3 mg tablets	DOSE when given once every 12months Number of 3 mg tablets
90 to 119	One	Two
120 to 140	Two	Four
141 to 158	Three	Six
> 158	Four	Eight

2.5.6 Precautions

Efficacy and dosing regimen of ivermectin in immunocompromised patients being treatedfor intestinal strongyloidiasis have not been established by adequate clinical studies. Therehave

been reported cases which show the persistence of infestation following a singledose of ivermectin particularly in these types of patients.

Ivermectin is not a prophylactic therapy of infection with filariae, there areno data available demonstrating the efficacy of ivermectin either for killing or preventingthe maturation of infective larvae in humans.Ivermectin has not been shown to have any activity against the adult worm of any species of filariae.Ivermectin has not been shown to have any beneficial effect on tropical pulmonaryeosinophilia syndrome on lymphadenitis or lymphangitis observed in case of infection withfilariae.

Following administration of ivermectin, the intensity and severity of adverse experiencesare probably related to the pre-treatment microfilarial density particularly in the blood. Inpatients co-infected with *Loa loa*, microfilarial density, particularly in the blood, is mostoften high which predisposes the treated patients to an increased risk in the occurrence ofserious adverse experiences.

2.5.7 Overdosage

There are reports of accidental overdosage of ivermectin, but no fatalities have been attributable to ivermectin overdosing. In accidental intoxication with unknown quantities of veterinary formulations of ivermectinin humans, either by ingestion, injection, exposure to body surfaces, the followingsymptoms have been reported: rash, contact dermatitis, oedema, headache, vertigo, asthenia, nausea, vomiting, diarrhoea and abdominal pain. Other adverse effects that have been reported include: seizures, ataxia, dyspnoea, paresthesia and urticaria. In case of an accidental poisoning, symptomatic treatment and supervision in specialised care unit with fluid replacement and hypertensive treatment if necessary.

2.5.8 Effect of ivermectin on water viscosity

Ivermectinis insoluble and unstable in water and it solubilizes by the formation of colloidal particles, called micelles, with surface active agents as solubilizers and stabilized by using cosolvents and/or appropriate substrates in the aqueous formulation. The liquid formulations are suitable for use as parenteral or oral administration for the treatment of parasitic infections.Lo and Williams (1983) stated that ivermectin aqueous solution avoids all of the disadvantages of non-aqueous formulations while retaining the required attributes of a parenteral or oral formulation. The solution is stable, both chemically and physically; it is low in viscosity, therefore its syringability is excellent; it does not cause any irritation or tissue damage at the injection site; its taste is not objectionable upon oral administration; at most concentrations, the solution is totally dilutable with water without precipitating the avermectin; the avermectin is rapidly absorbed; and the solution is produced at low cost.

2.5.9 Absorption and biodegradation of ivermectin

The distribution of ivermectin within the environment determines the levelof exposure to organisms(Tremblay and Wratten, 2002). Ivermectin has a vapour pressure of approximately1.5 × 10 mm Hg, indicating that it is not very volatile and therefore would beunlikely to disperse into the atmosphere (Bloom and Matheson, 1993). It has a lowsolubility in water and a high affinity for organic matter and therefore will tend to be stronglyabsorbed to soil and sediments (Bloom and Matheson, 1993). Ivermectin degrades tomore polar, less toxic metabolites in the environment. Biodegradation is largelytemperature-dependent, with half-lives ranging from 1–2 weeks for soil/manuremixtures in outside summer temperatures to 35 weeks for soil/manure mixturesstored at 22°C in the laboratory and in the dark (Halley *et al.*, 1989). Ivermectincan be photodegraded near the surface of open waterbodies, with half-lives of12h in summer and 39h in winter (Bloom and Matheson, 1993).

2.5.10 Environmental risk assessment of ivermectin

Concerns over potential environmental side-effects of ivermectin usage exist, asa large percentage of the drug is excreted unmetabolised in faeces of treatedanimals (Halley *et al.*, 1989; Gunn and Sadd, 1994). For example, 39%, 69% and43% of the initial dilutions of radioactive ivermectin were recovered in faecalsamples of steer, sheep and swine treated via subcutaneous or intraruminalinjections at levels of 50%, 50% and 33% above recommended dosages,respectively (Halley *et al.*, 1989). In a worst-case scenario of feedlot wastecollected after a 7-day period, the calculated concentration of unmetabolisedivermectin from cattle treated with subcutaneous injections of ivermectin at200 mg/kg body weight was approximately 351 µg ivermectin/kg faeces wetweight. If this waste was to be used as fertilizer (spread at a rate of 15 ton/acre(5.5 t/ha) and ploughed to a depth of 15 cm), soil concentrations of ivermectinwere estimated as 5.1 µg/kg of soil (Bloom and Matheson, 1993).

Ivermectin was selected as a case study compound within the project ERAPharm (Environmental Risk Assessment of Pharmaceuticals). Based on environmental data generated within ERAPharm and additional literature data, an environmental risk assessment (ERA) was performed mainly according to international and European guidelines. For the environmental compartments of surface water, sediment and dung, a risk was indicated at all levels of the tiered assessment approach (Liebig *et al*., 2010). Only for soil was no risk indicated after the lower tier assessment. However, the use of effects data from additional 2-species and multispecies studies resulted in a risk indication for collembolans. Although previously performed ERAs for ivermectin revealed no concern for the aquatic compartment and the transient effects observed on the dung-insect populations were not considered as relevant. However, the present ERA clearly demonstrates unacceptable risks for all investigated environmental compartments and hence suggests the necessity of reassessing

ivermectin-containing products. Based on this case study, several gaps in the existing guidelines for ERA of pharmaceuticals were shown and improvements have been suggested. The action limit at the start of the ERA, for example, is not protective for substances such as ivermectin when used on intensively reared animals. Furthermore, initial predicted environmental concentrations (PECs) of ivermectin in soil were estimated to be lower than refined PECs, indicating that the currently used tiered approach for exposure assessment is not appropriate for substances with potential for accumulation in soil.

As part of the risk assessment of veterinary pharmaceuticals, the potential impact of chemicals on soil ecosystems has to be determined according to European law. However, almost no data from standardized laboratory tests are available(Römbke*et al.*, 2010). Therefore, in the EU FP6 ERAPharm, the effects of the widely used veterinary pharmaceutical ivermectin were studied in chronic laboratory tests performed according to OECD (Organization for Economic Co-operation and Development) and ISO (International Organization for Standardization) guidelines. In detail, three soil invertebrate species-the earthworm *Eisenia fetida*, the springtail *Folsomia candida* and the predatory mite *Hypoaspis aculeifer*-were tested. The nominal concentrations of ivermectin mixed into the test substrate artificial soil was verified using residue analysis, which indicated that the test substance is persistent for at least up to 28 days. As expected when considering the mode of action of this substance, survival and reproduction of collembolans were clearly affected [LC_{50} = 8.4 mg/kg soil dry weight (dw); NOEC (repro) = 0.3 mg/kg soil (dw)]. Predatory mites reacted less sensitively [LC_{50} = 31.6 mg/kg soil (dw); NOEC(repro) = 3.2 mg/kg soil (dw)]. Earthworm survival and reproduction were affected in the same order of magnitude as the predatory mites [LC_{50} = 10 mg/kg soil (dw); NOEC(repro) = 2.5 mg/kg soil (dw)]. These results are in good agreement with the few data known from non-standardized tests for the same or related

soil invertebrate species. The results of these tests indicate that the effects of ivermectin on soil invertebrates, (collembolansin particular) cannot be excluded at field-relevant concentrations, as determined in a risk assessment according to VICH guidelines. More sophisticated higher-tier tests (e.g., in multispecies or semi field test systems) are recommended in order to assess the potential risk more accurately.

The effect of ivermectin on soil organisms was assessed in Terrestrial Model Ecosystems (TMEs) by Förster*et al.* (2011). Intact soil cores were extracted from a pasture in England and kept for up to 14 weeks in the laboratory. Ivermectin was applied to the soil surface via spiked cow dung slurry at seven concentration rates ranging from 0.25 to 180 mg/TME, referring to concentrations of 0.19-227 mg ivermectin/kg soil dry weight in the uppermost (0-1 cm) soil layer. After 7, 28 and 96 days following the application soil cores were destructively sampled to determine ivermectin residues in soil and to assess possible effects on microbial biomass, nematodes, enchytraeids, earthworms, micro-arthropods and bait-lamina feeding activity. No significant effect of ivermectin was found for microbial respiration and numbers of nematodes and mites. Due to a lack of dose-response patterns no effective concentrations could be determined for the endpoints enchytraeid and collembolan numbers as well as total earthworm biomass. In contrast, EC_{50} values for the endpoint feeding rate could be calculated as 0.46, 4.31 and 15.1 mg ivermectin/kg soil dry weight in three soil layers (0-1, 0-5 and 0-8 cm, respectively). The multivariate Principal Response Curve (PRC) was used to calculate the NOEC(community), based on earthworm, enchytraeid and collembolan abundance data, as 0.33 and 0.78 mg ivermectin/kg soil dry weight for day 7 and day 96, respectively. The results obtained were in line with laboratory data, indicating in general low to moderate effects of ivermectin on soil organisms. As shown by the results of

the bait-lamina tests, semi-field methods such as TMEs are useful extensions of the battery of potential test methods since complex and ecologically relevant endpoints can be included.

2.5.11 Toxic and side effects of ivermectin

Ivermectin has raised attention due to its high toxicity against nematodes and arthropods and very specific toxic side effects that it may produce in hosts. Dominant clinical symptoms of adverse effects and toxicity of ivermectin in animals are tremor, ataxia, CNS depression and coma which often results in mortality (Trailovic *et al*., 2010). Ivermectin is considered a very safe drug; however, there are reports of toxic effects in particularly sensitive populations or due to accidental overdose (Trailovic and Nedeljkovic, 2011).

Trailovic and Nedeljkovic, (2011) conducted a study to further characterize the central and peripheral toxic effects of ivermectin in animals and to determine possible therapeutic strategies for use in cases of ivermectin poisoning. They tested the effects of experimental dilutions of ivermectin previously reported to cause various intensities of CNS depression. It was observed that ivermectin at 2.5, 5.0 and 7.5mg/kg intravenous did not produce visible CNS depression in rats and 10mg/kg resulted in sleepiness and staggering 10 to 40 min after application, while a dose of 15mg/kg caused CNS depression very similar to general anesthesia. Ivermectin dose-dependently potentiates thiopentone-induced sleeping time in rats. Flumazenil (0.2 mg/kg), the benzoDiasepine antagonist, did not affect the action of thiopentone; however, it significantly reduced sleeping time in rats treated with a combination of ivermectin and thiopentone. Ivermectin causes an increase in the tonus and contraction amplitude of isolated guinea pig ileum, very similar to GABA, but without the initial relaxation period. These effects are dose-dependent and sensitive to atropine. Their results confirm the central and peripheral GABAergic properties of ivermectin in mammals and also indicate involvement of the cholinergic system in its toxicity. In addition the results

suggest that flumazenil and atropine have potential clinical roles in the treatment of ivermectin toxicity.

In another study on ivermectin effects on motor coordination and contraction of isolated rat diaphragm, Trailovic *et al*. (2010) observed that increasing intravenous dilutions of ivermectin, (6 or more times higher than therapeutic dose: 1.25, 2.5, 3.75, 5.0, 6.25 and 7.5mg/kg), caused dose-dependent disturbance of motor coordination in treated rats. The median effective dose (ED_{50}) that was able to impair the rota-rod performance in rats treated 3min before testing was 2.52mg/kg. This effect weakens over time, while in the rats treated 60min before the rota-rod test, ED_{50} of ivermectin was 4.21mg/kg. Whereas, all tested dilutions of ivermectin did not cause any other clinical symptoms of toxicity. They concluded that ivermectin has no effect on the contraction of isolated diaphragm caused by the embryonal fyn-associated substrate (EFS), which effectively blocked mecamylamine (100µM) and pancuronium (1 and 2µM). Effect on motor coordination is the first detectable clinical symptom of ivermectin toxicity and apparently is a result of its central effects.

Neurological side effects consistent with ivermectin toxicity have been observed in dogs when high dilutions of the common heartworm prevention agent ivermectin are coadministered with spinosad, an oral flea prevention agent (Dunn *et al*., 2011). Based on numerous reports implicating the role of the ATP-binding cassette drug transporter P-glycoprotein (P-gp) in ivermectin efflux in dogs, an in vivo study was conducted to determine whether ivermectin toxicity results from a pharmacokinetic interaction with spinosad. Beagle dogs were randomized to three groups treated orally in parallel: Treatment group 1 received 60µg/kg of ivermectin, treatment group 2 received 30mg/kg of spinosad and treatment group 3 received both ivermectin and spinosad. Whereas spinosad pharmacokinetics was unchanged in the presence of ivermectin, ivermectin plasma pharmacokinetics revealed a statistically significant increase in the area under the curve when ivermectin was coadministered with

spinosad. The majority of the interaction is proposed to result from inhibition of intestinal and/or hepatic P-gp-mediated secretory pathways of ivermectin. The findings suggests that spinosad, acting as a P-gp inhibitor, increases the risk of ivermectin neurotoxicity by inhibiting secretion of ivermectin to increase systemic drug levels and by inhibiting P-gp at the blood-brain barrier.

2.5.12 Effects of ivermectin on terrestrial and aquatic invertebrates

The toxicity of ivermectin to a selected number of terrestrial and aquaticinvertebrates has been assessed in the laboratory. Toxicity to the earthworm*Eisenia foetida* has been determined in two studies. Halley *et al*. (1989) derivedan LC_{50} of 315 mg/kg while Gunn and Sadd (1994) obtained an LC_{50} of 15.8 mg/kg. The large discrepancy is likely to be explained by differences inexperimental designs: Halley*et al.* (1989) used pure technical-grade ivermectinand conducted the experiments at a high soil pH, while Gunn and Sadd (1994)used the formulated drug (i.e. ivermectin in the presence of stabilisers,emulsifiers, etc.), which may make it more readily absorbed by the earthworm.The results of Gunn and Sadd (1994) are a more appropriate indication of a realsituation where technical grade ivermectin was applied. Gunn and Sadd (1994)found that earthworm growth was affected by ivermectin, with an EC of 4.7mg/kg and that cocoon production was reduced at concentrations of 4 mg/kg.Concentrations higher than these reported effect-levels have been observed infaeces of cattle 17 days after their treatment with ivermectin drench orinjectable ivermectin (Sommer *et al.,* 1992). However, Bloom and Matheson's(1993) estimate of soil ivermectin concentrations following spreading of faecalmaterial from ivermectin-treated cattle as fertilizer is 5.1 µg/kg, which is wellbelow levels that affect earthworms.

Unmetabolised ivermectin in treated animal faeces can affect dung-dwellinginvertebrates and insect species under some circumstances (Wall and Strong, 1987; Halley e*t al.*, 1993; Floate,

1998). This biocidal action could be consideredbeneficial for the control of pest insects such as stable flies and the larvae ofanimal parasites; however, there is also an effect under some experimentalregimes on non-pest invertebrates such as dung beetles (Wall and Strong, 1987).The consequent influence of ivermectin on dung decomposition has been debated.Some authors, who have observed decreased populations of dung-dwellinginvertebrates and insects in the faeces of animals treated with ivermectin,suggest this also reduces the rate of dung decomposition (Wall and Strong, 1987;Floate, 1998). Similarly Gunn and Sadd (1994) suggest that the impact ofivermectin on the growth and fecundity of earthworms, which are closelyassociated with cowpats and important to their degradation, may explain theretardation of cowpat decomposition in pastures of ivermectin-treated cattle, asobserved by other authors. While an increase in the time for decomposition ofdung from treated animals was observed under laboratory conditions, thenumber of earthworms found in dung pats from control cattle and ivermectin-treatedcattle was similar (Halley *et al.,* 1993). These authors suggest that thevarietyof physical, chemical and biological factors (e.g. weather, climate, soiltype,soil fauna) that influence dung decomposition are more significant andthat,under field conditions, the presence of ivermectin residues has nosignificanteffect on dung degradation rates (Halley *et al.,* 1993).

Concerns about the effect of ivermectin on dung-nutalising insect populationsand the potential forconsequent persistence of dung on pastures have beenraised. However, Wratten *et al.* (1993) published the results of a two-year studyof the rate of decomposition of cattle dungpats exposed to normalenvironmental influences. The cattle had been treated at therapeutic levels witheither an injectable or a sustained-release bonus formulation of ivermectin. Therate of decomposition of the dungpats, the extent of their avoidance by thecows, the organic matter content of the soil and the populations of earthwormsin the pastures did not differ between treated cattle and controls. The model ofSherratt *et al.* (1998)

was a detailed simulation model which provided estimatesof insect mortality in cattle farms under realistic sets of assumptions. Theimpact of avermectins on dung insect populations was highly dependent on arange of environmental factors and it was concluded that in typical farmingsystems the maximum cumulative insect mortality in a given season wasunlikely to be greater than 25%. A wider analysis carried out by Wratten and Forbes (1996) in a major review of the literature concluded that even underconditions of relatively high levels of avermectin use in cattle, the impact onnon-target insect populations and their ecological function is limited.

The water flea (*Daphnia magna*) is the most sensitive freshwater invertebratecrustacean species tested in the laboratory. However, ivermectin has alow solubility in water and a high affinity for organic matter and, in thepresence of sediment and/or suspended solids, toxicity was significantly reduced.The marine annelid worm, *Arenicola marina*, was the most sensitive sedimentdwellingspecies tested. Ivermectin was also used to control sea lice infarmedsalmon and the predicted environmental concentration of ivermectin inmarinesediment around a salmon farm was estimated at 33.4 µg/kg,with a half-lifegreater than 100 days (Davies *et al.*, 1998). These authors concluded thattheremight be a significant risk to some sediment-dwelling species around thecagesof farmed salmon treated with ivermectin. This was further confirmed byscientistsin the UK, who concluded that ivermectin had the potential to affectthe European marine environment (Codling *et al.*, 1998).

2.5.13 Effects of ivermectin on freshwater fisheries

Fish are approximately 100 times less sensitive to ivermectin than *Daphniamagna*, with bluegill sunfish and rainbow trout showing 96-h LC values of 5.3 and 3.3µg/L, respectively (Bloom and Matheson, 1993). In addition,ivermectin was shown to be rapidly depurated from

bluegill sunfish and to notaccumulate in fish (Halley *et al.*, 1993). Beyond these laboratory tests, there isnothing published on the effects of ivermectin in freshwater systems.

2.5.14 Effects of ivermectin on insects

Ivermectin has demonstrated a broad spectrum of activity against parasitic infections in vertebrates (Campbell, 1989). It has been shown to affect haematophagous arthropods (Campbell, 1989) and has also been found to have negative effects on beneficial coprophagous fauna (Rosales *et al.*, 2012). At higher dosages, effects have been paralysis and death, reduced adult survival and reduced survival of adults of the subsequent generation; lower dosages have produced blood digestion without ovarian development and decreased fecundity and egg hatch (Focks *et al.*, 1991, Mahmood *et al.*, 1991).

Strong *et al*. (1996) studied the effects of faecal drug residues following the administration of anthelmintics in the form of sustained-release boluses, on dung-colonising Coleoptera and Diptera. In blind field trials, pats of standard weight and size were prepared from the dung of cattle treated with an ivermectin or a fenbendazole sustained-release bolus and from a third control group of cattle that received no treatment. Pats were recovered after 7, 14, 21 and 42 days in the field and searched for invertebrates. There were no differences in the numbers of adult beetles found in the pats from the three treatment groups. Pats made from the dung of ivermectin-treated animals contained no larval Diptera Cyclorrhapha and significantly fewer larval Scarabaeidae than pats made from the dung of the other two groups. Furthermore, larval Scarabaeidae in the ivermectin pats were inhibited in their development. The pats from fenbendazole-treated animals contained similar numbers of larval Scarabaeidae and Diptera as the pats from untreated animals throughout the trial. At 42 days, the solid matter of the control and fenbendazole-containing cow pats were reduced to a crumbling, granular texture, while the pats from the ivermectin-treated animals were solid and compacted. Pitfall trapping,

using traps baited with dung from the three groups, showed no significant difference between the numbers of adult Scarabaeidae attracted, though a trend towards higher numbers attracted to the dung of both anthelmintic-treated groups was evident. The results provide evidence of the toxic effects of excreted ivermectin on key dung-colonising families of insects and show that fenbendazole lacks such toxic effects (Strong *et al*., 1996).

2.5.15 Effects of ivermectin on the dung beetle (*Euoniticellus intermedius*)

The dung beetle performs an important ecological activity within cattle pasture land (Nichols *et al.,* 2008) because they bury cattle dung to nest and feed on and in this way, they prevent the proliferation of flies and some cattle parasites while returning nutrients to the soil (Edwards and Ascherborn, 1987; Chirico *et al*., 2003). Rosales and colleagues showed that survival, fecundity and fertility of *Euoniticellus intermedius* were reduced under laboratory conditions when ivermectin was added to cattle dung at three different concentrations (Rosales *et al.,* 2012). The design included two controls (spiked dung) and the following product concentrations: 0.01, 0.1 and 100ppm which were homogenized with wet cattle dung. The survival of all specimens, the fertility of 20 females and the gonadal maturity of 17 males were verified. The highest ivermectin concentration was toxic at 1.0ppm, the survival of adults was reduced to almost half and at 100ppm, total mortality was observed. The effects on specimens' reproductive systems included, the ovary was not affected, the testicle size increased, the fecundity and weight of brood-masses were reduced. Pre-imaginal development increased 0.5 times at 0.01ppm concentration and the width of the cephalic capsule in third instar larvae diminished. The prolongation of development time may cause a phase lag in their field activity cycle; this lag may reduce the number of *E. intermedius* and the efficiency of the environmental services that they provide.

Ivermectin is widely used as an antiparasitic drug for reindeer *(Rangifer tarandus* (L.). Most of the ivermectin injected in the animal is excreted unchanged in the faeces (Nilsen *et al.,* 1999). Several reports show that ivermectin in cattle dung disrupts colonization and survival of beneficial dung breeding insects. Nilsen and colleagues (1999) investigated the effect of ivermectin on the reindeer dung fauna. Four reindeer calves (males, 6 months of age) were injected subcutaneously with standard dilutions of ivermectin (0.2 mg/kg body weight). The daily produced faeces were collected until day 30 after treatment and the concentration of ivermectin was determined by high pressure liquid chromatography (HPLC) with fluorescence detection. The highest concentration measured (mean 1632ng/g faeces (dry weight), range 907 to 2261ng/g among the animals) was on day 4 after treatment. The concentration decreased gradually to 28ng/g (range 6 to 58ng/g) on day 30 (Nilsen *etal*., 1999).

The most important coprophilous beetles *(Apbodius* spp.) and flies *(Scatbophaga* spp.) were not detected in this winter dung whether it contained ivermectin or not, probably because of the dry consistency and small size of the pellets. On the other hand, these insects (larvae and imagines) were common in summer dung, which had been deposited naturally in the field and later placed together with the ivermectin-containing winter dung for comparison. The summer dung has a softer and lumpy consistency. Treatment in autumn or early winter implies that the bulk of the ivermectin from the animal will be present in faeces with winter consistency, since this bulk portion is excreted during the first 30 days after treatment. These dry and pelleted faeces is not utilized by the important coprophilous insect species and the current practice of treatment of reindeer with ivermectin in autumn or early winter is therefore the regime representing the least danger of harmful influence on the coprophilous fauna and their contribution to the dung decomposition process (Nilsen *etal*., 1999)..

2.5.16 Effects of ivermectin on the biting midge (*Culicoides variipennis*)

Holbrook and Mullens (1994) reported the effects of ivermectin in blood on the mortality, fecundity and egg hatchability of *Culicoides variipennis*. Bluetongue (BLU) disease of ruminants is widespread in the USA (Metcalf *et al*., 1981), with the biting midge *Culicoides variipennis* (Coq.) the principal vector of the BLU viruses (Price and Hardy, 1954). Recommendations for the control of this disease in North America have been directed primarily against the vector, with emphasis on water and waste management and the use of insecticides (Holbrook, 1988; Mullens and Rodriguez, 1990).

The survival of *C. variipennis* female for 7 days post-engorgement on blood meals with or without ivermectin, eggs produced/living female/day and daily hatch rates were assessed. Eggs were deposited during days 3-7, with eggs produced by females at all dosages below 1.0µg/ml. However, there were such high mortality rates during the egg-laying period at dilutions above 0.2µg/ml that the numbers of eggs were insufficient for statistical analyses. The slower rate of egg development reported in female *Aedes aegypti* (Linn) treated with ivermectin (Mahmood *et al.*, 1991), was not evident in *C. variipennis*, because both untreated flies and those treated with 0.2, 0.4, or 0.8µg/ml began producing eggs on day 3. Nevertheless, at dose rates of 0.2-1.0µg/ml of blood, the total number of eggs, the number of eggs/living females produced and the egg hatch rate decreased significantly as the rate of ivermectin increased. It appeared that 200 µg of ivermectin /kg of body weight would not have a meaningful effect on the reproductive capacity of *C. variipennis*, because the highest plasma concentration (0.0444 µg/ml at 24 h post inoculation) was still 4.5 times less than the lowest concentration of ivermectin (0.2 µg/ml) used in this test. Neville (1978) proposed placing cattle, a preferred host for *C. imicola* Kieffer, with sheep to differentially attract host-seeking females and thus reduce the attack rates on the sheep. Treating those cattle with very high concentrations of ivermectin might significantly reduce the vector population. Dosages

up to 6,000 µg/kg of body weight (30 times the recommended rate) produced an estimated peak blood concentration of 1.3µg/ml and have no obvious ill effects in cattle (Pulliam and Preston, 1989). With a biological half-life of 3 days (Fink and Porras, 1989), the concentration of ivermectin should be high enough for a few days to significantly affect mortality, egg production and egg fertility in *C. variipennis sonorensis* females.

2.5.17 Effects of ivermectin on phlebotomine sand flies

Mascari and Foil (2010) carried out a study to evaluate the use of boric acid, imidacloprid, ivermectin, or abamectin incorporated into sugar baits as oral toxicants for adult phlebotomine sand flies. Variable toxicity of insecticide-sugar bait solutions to adult male and female sand flies were demonstrated, based on male/female median lethal concentration values. Complete control of sand flies could not be achieved with as high as 40g/L of boric acid in sugar bait solution. Incorporation of imidacloprid, ivermectin, or abamectin into sugar bait did not reduce the effect whether adult male and female sand flies fed on these sugar baits. They then proposed that imidacloprid, ivermectin, or abamectin could be used to control adult sand fly populations with targeted use of insecticide-treated sugar baits.

2.5.18 Effects of ivermectin on ticks

Following an infestation of dogticks in kennels housing dogs used for long-term studies of the pathogenesis of Chagas disease, Dias and colleagues examined the effect of ivermectin treatment on the dogs, ticks, trypanosome parasites and also on triatomine vectors of Chagas disease (Dias*et al.*, 2005). Ivermectin treatment was highly effective in eliminating the ticks, but showed no apparent effect on the dogs or on their trypanosome infection. Triatominae fed on the dogs soon after ivermectin treatment showed high mortality, but this effect quickly declined for bugs fed at successive intervals after treatment. Dias and colleagues concluded that although ivermectin treatment may have a transient effect on peri-domestic populations

of Triatominae, it is not the treatment of choice for this situation. The study also showed that although the dogticks could become infected with *Trypanosoma cruzi*, this only occurred when feeding on dogs in the acute phase of infection and there was no evidence of subsequent parasite development in the ticks.

Jackson and Chesterman (1989) showed that sub-lethal effects seen amongst *Rhipicephalus appendiculatus* feeding on ivermectin-treated rabbits were diverse and dependent both on drug dose, pharmacokinetics and tick feeding patterns: changes in drug formulation, the time of infestation relative to treatment and the tick instar used, profoundly influenced acaricidal activity (Jackson and Chesterman, 1989). Death was a sequel to paralysis only if tick feeding was interrupted for sufficient time to produce irreversible dehydration. Concurrent pharmacokinetic investigations revealed that, for the larvae of *R. appendiculatus*, the mean critical lethal dose of ivermectin imbibed over a 5-day engorgement period was 3500 micrograms/kg. This quantity of ivermectin was achieved in the blood-meals of larvae feeding on rabbits treated subcutaneously with a single dose of Ivomec injection greater than or equal to 800 micrograms/kg, provided infestation took place within 24 hour of treatment. At lower drug dilutions, or if larval infestations were delayed for greater than 24 hour post-treatment, the quantity of circulating ivermectin (and thus imbibed by the tick larvae) fell below 3500 micrograms/kg and an increasing percentage of larvae successfully engorged and detached. More than 90% of such larvae moulted to the nymphal stage. Nymphae and larvae exhibited similar susceptibility to ivermectin on treated rabbits which could be explained by similar feeding patterns. However, adult female and male ticks were markedly less susceptible and interpretation of ivermectin-induced effects was more complex.

Morsy and Haridy (2000) also Ivermectin (Ivomec) proved to be effective in controlling *R. sanguineus*. All brown dog ticks infesting the treated dogs (subcutaneous injections) with 1.5 ml or 2 ml/50 kg dropped off within 4 or 3 days respectively. The dropped off ticks were

completely inactive and died within one day or two. Ticks exposed to filter papers impregnated with 100 micrograms/ml (66.67%) died within four days. A dose of 1.5-2 microliters/50 kg eradicated *Toxocara canis* infection in four dogs and caused shrinkage of *Dipylidium caninum* gravid segments in three.

2.5.19 Effect of ivermectin on ectoparasites

The ectoparasites that cause scabies, *Sarcoptes scabiei* and *Pediculosis pubis* are common causes of skin rash and pruritus worldwide. They are transmitted primarily by person-to-person spread and are generally associated with low morbidity. The preferred treatment for scabies has generally been topical agents such as lindane and permethrin. Recently, ivermectin has demonstrated good efficacy in the treatment of scabies and it may be of particular use in institutional outbreaks and in communities in which scabies is endemic.Ivermectin is used in veterinary practice against many ectoparasites and endoparasites and is the drug of choice for treatment of human onchocerciasis. Youssef *etal*. (1995)carried out a study to investigate the effect of topical application of this drug against human ectoparasites (*Sarcoptes scabiei* and *Pediculus humanus capitis*). Ivermectin was found to have a curative effect on head lice after a single topical application. In patients with scabies, the drug was also found to be effective after a single application. However, in 50% of the cases, another application was needed five days later (Youssef*et al.,* 1995).

Anecdotal reports of improvements in patients who suffered from infestation with the mites *Sarcoptes scabiei* suggest that the ectoparasitic disease might be treated with ivermectin. Meinking *etal*. (1995) conducted an open–label study in which ivermectin was administered in a single oral dose of 200µg/kg to 11 otherwise healthy patients with scabies and to 11 patients with scabies who were also infected with the human immunodeficiency virus (HIV), 7 of whom had the acquired immunodeficiency syndrome. They reported that none of the 11

otherwise healthy patients had evidence of scabies four weeks after a single dose of ivermectin (Meinking *et al.*, 1995). Ten of the eleven patients with HIV (91%) had no evidence of scabies four weeks after their first treatment with ivermectin. They concluded that ivermectin given in a single oral dose is an effective treatment for scabies in otherwise healthy patients and in many patients with HIV infection.

2.5.20 Use of ivermectin in the control of onchocerciasis

Onchocerciasis is a vector-borne parasitic disease caused by the filarial worm *Onchocerca volvulus*. The disease is endemic in Central and South America and the Yemen but 99% of the disease occurs in sub Saharan Africa, where it causes blindness and skin disease (WHO, 1995). It is a disabling disease that causes significant morbidity, psychosocial problems and reduced work, especially reduced agricultural productivity in populations affected by the disease. About 37 million people in tropical Africa and 140,000 others in Latin America are infected with *O. volvulus* (WHO, 1995; Remme *etal*., 2006). In many endemic countries including Nigeria, onchocerciasis constitutes a major public health and socio-economic problem because of its dermal and ocular manifestations. The main strategy for control in endemic countries is by mass ivermectin (Mectizan) distribution. Following the availability of ivermectin and its donation free of charge by Merck and Co. to all who need it for as long as necessary (Colatrella, 2008), the control programmes in Africa adopted mass ivermectin distribution using community-directed treatment as its main control strategy (Amazigo, 2008).

Onchocerciasis can be effectively controlled as a public health problem by annual mass drug administration of ivermectin, but it was not known if ivermectin treatment in the long term will be able to achieve the elimination of onchocerciasis infection and interruption of transmission in endemic areas in Africa. A study in Mali and Senegal provided the first

evidence of elimination after 15-17 years of treatment. Following this finding, the African Programme for Onchocerciasis Control (APOC) started a systematic evaluation of the long-term impact of ivermectin treatment projects and the feasibility of elimination in APOC supported countries. **Tekle***etal.,* (2012) reported the first results for two onchocerciasis foci in Kaduna, Nigeria.

In 2008, the epidemiological evaluation using skin snip parasitological diagnostic method was carried out in two onchocerciasis foci, in Birnin Gwari Local Government Area (LGA) and in the Kauru and Lere LGAs of Kaduna State, Nigeria. The survey was undertaken in 26 villages and examined 3,703 people above the age of one year. The result was compared with the baseline survey undertaken in 1987. The communities had received 15 to 17 years of ivermectin treatment with more than 75% reported coverage. For each surveyed community, comparable baseline data were available. Before treatment, the community prevalence of *Onchocerca volvulus* microfilaria in the skin ranged from 23.1% to 84.9%, with a median prevalence of 52.0%. After 15 to 17 years of treatment, the prevalence had fallen to 0% in all communities and all 3,703 examined individuals were skin snip negative (**Tekle***etal.,* 2012).The results of the surveys confirm the finding in Senegal and Mali that ivermectin treatment alone can eliminate onchocerciasis infection and probably disease transmission in endemic foci in Africa. It is the first of such evidence for the APOC operational area.

2.5.21 Use of ivermectin in the control of filariasis and mosquitoes

Ivermectin is one of the most frequently used substances for parasite control (Rosales *et al*., 2012). The global initiative to eradicate bancroftian filariasis currently relies on mass treatment with four to six annual dilutions of anti-filarial drugs. The goal is to reduce the reservoir of microfilariae in the blood to a level that is insufficient to maintain transmission by the mosquito vector. *Wuchereria bancrofti* transmitted by *Culex quinquefasciatus*

accounts for over 90% of the global burden of lymphatic filariasis (Das and Subramanian, 2002). Recent advances in diagnostic and control tools and a better epidemiological understanding of the disease have led to hope that lymphatic filariasis is eradicable. The World Health Organization has helped a number of member countries to lunch nationwide programmes of mass treatment with antifilarial drugs such as diethylcarbamazine, albendazole and ivermectin, for the elimination of this disease. In other to make rational decisions about control strategies, reliable predictions of the long-term impact of such treatment and of alternative interventions, need to be made and these can only be based on a sound, quantitative understanding of the population biology of the parasites. Mathematical models have proven valuable in gaining quantitative insights into the population dynamics of the parasites and may be used to make credible predictions of the likely outcomes of various control strategies.

In nearly 2500 residents of Papua New Guinea, Bockarie *et al*. (2002) prospectively assessed the effect of four annual treatments with a single dose of diethylcarbazine plus ivermectin or diethylcarbazine alone on the incidence of microfilariae-positive infections, the severity of lymphatic disease and the rate of transmission of *Wuchereria bancrofti* by mosquitoes. Random assignment of treatment regimens was carried out according to the village of residence and villages were categorized as having moderate or high rates of transmission. The proportion with microfilariae-positive infections decreased by 86 to 98%, with a greater reduction in areas with a moderate rate of transmission than in those with a high rate. The rate of transmission by mosquitoes decreased substantially and new microfilariae-positive infections in children were almost completely prevented over the five-year study period. Annual mass treatment with drugs such as diethylcarbazine can virtually eliminate the reservoir of microfilariae and greatly reduce the frequency of clinical lymphatic abnormalities due to bancroftian filariasis. Eradication may be possible in areas with

moderate rates of transmission, but longer periods of treatment or additional control measures may be necessary in areas with high rates of transmission.

Richards*et al.* (2005) conducted a prospective entomological survey in four sentinel villages in central Nigeria from 1999-2000, to assess the impact of annual, single-dose, mass drug administrations (MDA), with a combination of ivermectin and albendazole, on transmission of *Wuchereria bancrofti*. The four villages received annual MDA based on ivermectin alone for 7 years prior to the addition of albendazole. Resting *Anopheles gambiae* s. l., *An. funestus* and *Culex* species were collected from 92 sequentially sampled households and dissected. Mosquitoes harboring any larval stage of *W. bancrofti* were classified as 'infected' and those containing the third-stage larvae of the parasite were classified 'infective'. The base-line data, from dissections performed before the addition of albendazole to the MDA, showed high prevalences of mosquito infection and infectivity, despite apparently good treatment coverages during the years of annual ivermectin monotherapy. Only the Anopheline mosquitoes were found to harbor *Wuchereria bancrofti* larvae. After the third round of MDA with the ivermectin-albendazole combination, statistically significant decrease in the prevalence of mosquito infection and infectivity were observed. It was then concluded that the combination of ivermectin and albendazole appear to be superior to ivermectin alone for reducing the frequency of *Wuchereria bancrofti* infection in mosquitoes.

The resurgence in filarial transmission after withdrawal of mass drug administration and the relationship between antigenaemia and microfilaraemia was studied by Sunish *et al*. (2002). In this longitudinal study, seven village units endemic for filariasis were assigned randomly into three arms with different intervention strategies in the years 1995 and 1996. Villages in Group A received two annual mass drug administrations (MDAs) of diethylcarbamazine (DEC) plus ivermectin (IVR). Group B received the same MDAs in combination with vector control; in Group C only placebo was administered. Post-treatment evaluation in 1997

revealed marked reductions in microfilaraemia prevalences (MFP) and geometric mean intensities (GMI). Subsequently, vector control in Group B was continued by the community. Group A and B received no MDAs between 1997 and 1999 and were re-evaluated in 1999. During this evaluation, antigenaemia prevalence (AGP) was estimated along with MFP, using immunochromatographic test (ICT) kits. The gains of the MDAs were sustained in Group B, while resurgences occurred in Group A, where annual transmission potential (ATP) rose and MFP doubled. Group C continued to have high ATPs, while Group B had very low ATPs. It was then concluded according to the data obtained, that vector control would be useful as an adjuvant to chemotherapy to prevent resurgences.

Sunish *et al*. (2007) conducted a study to determine the role of vector control in further decreasing the transmission of bancroftian filariasis achieved by mass drug administration and the long-term impact on filariometric indices. Three rounds of annual mass drug administration, with diethylcarbamazine and ivermectin, were complemented by vector control (mainly using polysterene beads) in villages of Tirukoilur, south india, during 1995-99. Subsequently, drug administration is being carried out with diethylcarbamazine and albendazole or diethylcarbamazine alone. They evaluated the impact of mass drug administration used alone or in conjunction with vector control (1995 to 2005) on vector transmission indices (such as transmission intensity index, monthly biting rate, monthly transmission potential and annual transmission potential). Vector density greatly decreased in villages where vector control was used as an adjunct to mass drug administration and almost no infective mosquitoes were found in the small numbers still remaining. Filarial antigenaemia was low and continue to decrease significantly in the age group 15-25 years in villages receiving mass drug administration with vector control in contrast to villages receiving only mass drug administration. The gains of mass drug administration were sustained only with the integration of vector control measures (Sunish *et al*., 2007). Sunish *et*

al. (2007) therefore advocated the incorporation of vector control in the Global Programme to Eliminate Lymphatic Filariasis as it can potentially decrease the time required for eliminating lymphatic filariasis.

In a study conducted by Amuzu *et al*. (2010), two lymphatic filariasis endemic communities Mampong and Hwida in Ghana were regularly monitored for impact on transmission after annual mass drug administration (MDA) with albendazole and ivermectin. *Anopheles gambiae* s.s was the main vector at Mampong and was found to possess significantly more ciberial teeth than *An. melas,* the principal vector at Hwida. They postulated that the different impact observed after 6 MDAs may be due to *An. gambiae* s.s exhibiting 'facilitation' at Mampong and at Hwida *An. melas,* the main vector exhibits 'limitation'. Thus it may be necessary to compliment MDA with vector control to achieve interruption of transmission in areas where *An. melas* may exhibit limitation.

2.5.22 Effect of ivermectin on adult mosquitoes

Ivermectin has a broad-spectrum activity against nematodes and ectoparasites and is widely used in mass treatment campaigns against onchocerciasis (Foy *et al.,* 2011). It has recently received much attention because of the reduced lifespan of malaria-transmitting mosquitoes after feeding on humans and cattle treated with ivermectin (Foley *et al.,* 2000; Fritz *et al.,* 2009; Chaccour *et al.,* 2010; Sylla *et al.,* 2010). This makes ivermectin a potentially attractive component of malaria control efforts, where it could be part of a drug combination that not only treats malaria patients but also reduces onward transmission.

The effect of Ivomec, a formulation of ivermectin administered by various routes: through biological membrane and feeder animal, was assessed by Iakubovich *et al.* (1989). Higher insecticidal effect was obtained by feeding mosquitoes with blood containing ivermectin through biological membrane. *Anopheles stephensi* females were the most sensitive of 3

species under study. Their total death after feeding was observed at1ppm, while for *Aedes aegypti* the lethal dose was 2.5ppm and for *An. sacharovi*, 50ppm. Subcutaneous injection of the drug to rabbits at the dose 0.34mg/kg wt with further feeding on it of 3 *Anopheles* species demonstrated the highest drug sensitivity of *An. stephensi* mosquitoes. Death rates among the females fed on the rabbit 4-6 days after injection of the drug were 93, 70 and 79% respectively. Death rates among *An. sacharovi* and *An. atroparvus* differed only slightly from that of the control group. In some experiments, ivermectin effect on fertility and larvae hatching from ova can be traced. Probably, increasing the preparation dose, administered to an animal-feeder, may change significantly characteristics of the malaria carrier natural population. Moreover, further use of ivermectin against helminths and mosquitoes in zootechnical practice may produce a side-effect on mosquitoes, feeding recurrently on domestic animals, thus increasing the effect of malaria zoological control.

The possible release of energy for the metabolization of insecticide may be the cause of the decrease in the number of egg mass found in females arisen from ivermectin exposed larvae. This is because during vitellogenesis, fat body cells suffer changes, passing of storage cells for cells with a high protein synthesis (Raikhel and Lea, 1983). The vitellin is the major protein of yolk being produced from another protein, the vitellogenin (VG). The synthesis of VG occurs in a short period of time, involving extensive cytological changes of the fat body and ovaries. Clements (1996) have pointed out that some increase in cellular size occurs with the accumulation of lipids and glycogen, while the storage of proteins is associated with a considerable increase in the volume of the fat body cells. Decrease in egg production was also observed in *Rhodnius prolixus* females exposed to ivermectin (De Azambuja *et al.,* 1985).Involvement of vitellogenesis with the ivermectin can also affect polyamines synthesis in *C. quinquefasciatus,* because this synthesis is one of the first events of the yolk formation in *Aedes aegypti* (Kogan and Hagedorn, 2000). When the polyamine synthesis

suffers reduction, induced experimentally, there are decline in the levels of nucleic acid and proteins, causing abnormal ovary development.

Kobylinski *et al.* (2010) examined concentration of drugs that follow human pharmacokinetics after MDA and that matched with mosquito feeding times, for effects against the anthropophilic mosquito vectors *Anopheles gambiae* s. s. and *Aedes aegypti*. According to this study, ivermectin was the only human-approved MDA drug that affected mosquito survivorship and only *Anopheles gambiae* s. s. were affected at concentrations respecting human pharmacokinetics at indicated dilutions. Ivermectin was also found to delay *Anopheles gambiae* s. s. re-feeding frequency and defecation rates and two successive ivermectin-spiked blood meals following human pharmacokinetic concentrations compounded mortality effects compared to controls. These findings suggest that ivermectin MDA in Africa may be used to decrease malaria transmission if MDAs were administered more frequently. Such a strategy would broaden the current scope of polyparasitism control already afforded by MDAs and which is needed in many African villages simultaneously burdened by many parasitic diseases.

Pesticide resistance has parallels with multi-drug resistance syndrome of tumours in clinical medicine, which has been linked to an ATP-dependent pump, p-glycoprotein (P-gp) (Buss *et al*., 2002). P-gps pump drugs out of the cell, thereby reducing cellular concentrations of the chemical. P-gps have been found in several invertebrate species and have been shown to provide a defense against environmental xenobiotics, including pesticides. This study used a model cell culture system to investigate the interaction of pesticides with P-gp. Ivermectin and endosulfan were shown to be strong inhibitors of dye transport out of cells, which is a standard measure of P-gp modulation. Buss *et al.* (2002) then investigated the action of a P-gp inhibitor, verapamil (calcium channel blocker), on insecticide toxicity to fourth-instar mosquito larvae of the *Culex pipiens* L. complex (Diptera: Culicidae). Verapamil increased

toxicity to examples of three insecticide classes (cypermethrin, endosulfan, ivermectin), but not to chlorpyrifos (organophosphate). The discovery of a novel protective mechanism in mosquitoes, with a wide substrate range, has implications for the control of important pest and vector species.

Nasr *et al*. (1996) evaluated the effect of two different dilutions of ivermectin on the survival and fecundity of *Cx. pipiens*. Female mosquitoes (50 in each group), same age and generation, were fed once on blood from ivermectin-treated rabbits. Comparative treatments consisted of two different dilutions of ivermectin (0.1mg or 0.4mg/kg of body weight) and mosquito groups were fed on the rabbits at 3 days and 10 days post-treatment respectively. Mosquitoes were maintained on 10% sucrose solution, at 25degrees C and 70% relative humidity. Observations were made at 7, 10, 15, 20, 25 and 30 days post feeding. Their results provide new information about the effect of ivermectin on the vector of lymphatic filariasis and add a new dimension to the use of ivermectin in filariasis control.

Fritz and colleagues showed that addition of ivermectin to bovine blood that is directly fed to *An.gambiae*s.s. and *An. arabiensis* mosquitoes reduced their survivorship and fecundity (Fritz *et al.*, 2009) and that ivermectin is already lethal to *An. arabiensis* at low concentrations (LC_{50} of 7.9ppb) (Fritz *et al.*, 2012). Work in rodents and cattle suggest a long-acting effect of ivermectin on anophelines; affecting mosquito survival rates as long as 10 days after feeding on an ivermectin-treated animal (Iakubovich *et al.*, 1986; Fritz *et al.*, 2009). Chaccour and colleagues directly fed *An. gambiae* mosquitoes on healthy human volunteers 1 and 14 days post-ivermectin administration and observed an increased mortality at only the first time-point (Chaccour *et al.*, 2010). In a study from Papua New Guinea, blood-fed mosquitoes were collected from the huts of people 1-3 days and 28 days after an ivermectin mass treatment campaign. *An. punctulatus* or *An. koliensis* caught 1-3 days post-treatment showed 70% mortality within 24 hours after capture compared to 2% caught 28 days post-

treatment (Bockarie *et al.,* 1999). A study from Senegal similarly determined survival in blood-fed *An. gambiae* s.s. mosquitoes sampled from houses after ivermectin mass treatment campaigns and found that survival rates were lower for mosquitoes that were caught 1-6 days post treatment; the effect for individual days was not reported (Sylla *et al.,* 2010). A study where *An. farauti*mosquitoes were allowed to feed on a single Indonesian volunteer 0, 7, 10, 14, 26 and 44 days after administration of 250µg/kg ivermectin suggested that the mosquitocidal effect may last for 14 days (Foley *et al.,* 2000).

Bastiaens, *et al.* (2012) suggested that the duration of the mosquitocidal effect of ivermectin iskey to its potential role as an adjunct malaria therapy. They determined mortality rates of *Anopheles stephensi* mosquitoes that took a blood meal on Swiss mice, Wistar rats and Cynomolgus monkeys that received ivermectin orally at 200-400µg/kg. Mosquito feeding assays were performed on five consecutive days after ivermectin administration. Mosquito mortality was determined in the first 72 hours after feeding. Results showed that mosquito mortality was 70-100% when mosquitoes fed on any of the animals 1-2 days after the last ivermectin administration. After this time-point the mosquitocidal effect was still evident in some animals but became more variable. This finding of a pronounced but short-lived mosquitocidal effect makes the timing of ivermectin administration crucial to form a useful addition to anti-malarial drugs.

Mortality was investigated in *Anopheles farauti* mosquitoes, a major coastal malaria vector in the south-west Pacific, fed on a volunteer who had taken a 250 micrograms/kg dose of ivermectin. High mortality was recorded in mosquitoes feeding during the first week after treatment of the volunteer, for instance 80-100% failed to survive three days. A long term residual effect of ivermectin in the blood was indicated by a small but significantly higher mortality in mosquitoes fed six weeks after ivermectin was taken (Foley *et al.,* 2000). These effects were included in malaria transmission models that incorporated host choice and host-

induced mortality parameters. For the zoophilic *An. farauti*, ivermectin treatment of animals resulted in a greater reduction in malaria than ivermectin treatment of humans alone, whereas for an anthropophilic vector, treatment of humans was more important. This study suggests that ivermectin treatment of animals could have an important role in malaria control where *An. farauti* is the vector. Improvement in the health of humans and domestic animals through control of parasitic worms and mites might encourage community participation in strategies involving ivermectin.

2.5.23Effect of ivermectin on mosquito larvae

Floating layers of polystyrene beads suffocate mosquito larvae and pupae and inhibit egg laying (Curtis *et al.*, 2002). The layers are very durable in breeding sites with water contained within walls, as in wet pit latrines and soakage pits which constitute an important breeding site for *Cx. quinquefasciatus.* Trials have been conducted in communities in Zanzibar, Tanzania and in Tami Nadu, India, where such mosquito populations were the vectors of *Wuchereria bancrofti.* In each case, treatment of all the pits with polystyrene beads was integrated with mass treatment of people with antifilarial drugs – in Zanzibar in 1988 with diethylcarbamazine (DEC) and in India in the 1990s with DEC plus ivermectin. The results were compared with those in communities with the mass drug treatment alone and with control communities with neither treatment. The polystyrene-bead treatments greatly and sustainably reduced the vector populations. Comparison of the communities after drug treatment ceased showed that this form of vector control contributed markedly to the prevention of a resurgence of filarial infection. Pampiglione *et al.*(1985) reported that two avermectins were shown to be effective as mosquito larvicides or when fed to adult female mosquitoes in sucrose or in blood. Larval LC_{50} values of compounds MK-933 and MK-936, expressed as parts per billion, were found to be 3.94 and 2.42 for *Cx. pipiens*, 5.85 and 2.90

for *An. stephensi and* 23.4 and 10.4 for *Ae. aegypti.* When fed to adult females of *An. stephensi,Ae. Aegypti*or *Cx. quinquefasciatus*, MK-933 at 2.8mg active ingredient per litre in sucrose solution caused complete mortality within 60 hours. When female mosquitoes engorged from mice injected subcutaneously 12 hours previously with MK-933 at 82mg a.i./kg, mortality after 36 hours were 100% for *An. stephensi*, over 60% for *Ae. aegypti* and over 50% for *Cx. quinquefasciatus*. Thus the systemic insecticidal effect was greater against anopheline than against culicine female mosquitoes.

Alves *et al.* (2010) described morphological alterations in the fat body and midgut of *Cx. quinquefasciatus* larvae following exposure to different insecticides. Both third and fourth instars of *Cx. quinquefasciatus* larvae were exposed for 30 and 60 min to organophosphate (50 ppb), pyrethroids (20 and 30 ppb) and avermectin derivates (1.5 and 54 ppb). Following incubation, pH measurements of the larvae gut were recorded. The fat body and midgut were also analyzed by light and transmission electron microscopy. These studies demonstrate a decrease in the pH of the larvae anterior midgut following exposure to all the tested insecticides. Histochemical tests revealed a strong reaction for neutral lipids in the control groups and a marked decrease in the group exposed to cypermethrin. Furthermore, a weak reaction with acidic lipids in larvae exposed to deltamethrin, temephos, ivermectin and abamectin was also observed. Insecticide-exposed larvae also exhibited cytoplasm granule differences relative to control larvae. They also noted a small reduction in microvilli size in the apex of digestive cells, although vesicles were found to be present. The destructive changes in the larvae were very similar regardless of the type of insecticide analyzed. The data suggest that alterations in the fat body and midgut are a common response to cellular intoxication.De Freitas *et al*. (1996) observed the effect of ivermectin on *Cx. quinquefasciatus* larvae. Toxic effects and mortality evaluation were carried out after 5, 15, 30 and 60 minutes of exposure to 1, 5 or 10ppm of ivermectin solutions. Observations were

made 24 and 48 hours after the beginning of the experiment and loss of mobility, progressive paralysis and high mortality of larvae were recorded. They concluded that the observed effects of ivermectin on the mosquito larvae are probably correlated with chloride channel activation on cell membranes.

2.5.24 Attractability of ivermectin

Soil may become contaminated when veterinary medicinesare dispersed via slurry or deposited on land bygrazing animals (Kolar *et al.,* 2008). Ivermectin undergoeslittle metabolic change and most of the dose given to theanimal is excreted, relatively unaltered, primarily in thefeces (Halley *et al.,* 1989). Avermectins in the feces of cattletreated with sustained-release bolus, pour-on or subcutaneousinjection formulations are lethal for dung-breedi/iginvertebrates and can also negatively affect earthworms.Concentrations of ivermectin above 18.5 mg/kgin thedung can lead to death of earthworms (Sun *et al.,* 2005).Halley *et al,* (1989) reported the 28-day lethal concentration(LC) of ivermectin mixed in artificial soil for earthworm*Eisenia fetida*.Earthworms are often used in standardized ecotoxicological tests (ISO 1993). Anavoidance test reflects behavioral properties of earthworms,which is based on the stimulation of sensory cells (Schaefer, 2003). Such a test is quick and easy to perform andisknown to be sensitive to a wide range of chemicals.

2.5.25 Ivermectin Attracts Earthworms

Changes in the behavior of earthworms (for exampleavoidance of a particular substrate) can influence the soilecosystem(Torkhani *et al*., 2010). Torkhani and colleagues carried out a study to determine whether the earthworms*Eisenia fetida* and *Lumbricus terrestris* are able to avoid ivermectin. A standard avoidance test with earthwormswas conducted using

standardized Lufa 2.3 soil and sandy soil provided by Cinkarna Celje. A two-chamber system of avoidancetest as described in ISO guideline(ISO 2008)wasapplied. They investigated the substrate preference of *E. fetida*and *L. terrestris* after 48 h at ivermectin concentrations of 8, 32, 64 and 256 mg/kgdry soil.No deterrent effects were observedfor both species and either soil types. *E. fetida* in Lufa 2.3soil was clearly attracted to ivermectin. More than 70% ofthe earthworms preferred soil with ivermectin concentrations of 8, 64 and 256 mg/kg. They concluded that earthworms, as it wasobserved in *E. fetida* in Lufa 2.3 soil treated withivermectin, may be attracted by some chemicals.

2.5.26 Ivermectin attracts dung beetles

Holter *etal*. (1993) studied the effect of ivermectin treatment of cattle on the attractiveness of dung to scarabaeid dung beetles by pitfall trapping in Denmark, Tanzania and Zimbabwe. Traps were baited with dung collected at intervals after heifers were treated with ivermectin by subcutaneous injection (0.2 mg kg-1 body weight). In one Danish trial, beetles preferred control dung from untreated cattle, whereas no preference was found in two other experiments. In Tanzania, the overall tendency for beetles was also to prefer control dung. In Zimbabwe, two species (*Euoniticellus intermedius* and *Liatongus militaris*) were particularly attracted to dung from treated cattle, whereas three others did not discriminate between dung types. It is concluded that at least in some cases the attractiveness of cattle dung to scarabaeid beetles is affected by avermectin therapy.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in the main campus of Ahmadu Bello University located in Samaru, a suburb of Zaria in Kaduna state, Nigeria. Zaria is located in the northern Guinea savannah vegetation belt of Nigeria (ABU website). Samaru is characterized by an open wooded savanna with the total and mean annual rainfall falling between June and October, being approximately 974mm and 81.1mm respectively (ABU website). The temperature ranges from 15.2ºC to 37.4ºC with relative humidity range of 56% to 66%. Ahmadu Bello University main campus is situated on the latitude 11º 10^{1} 4^{11} N and longitude 07º $39^{1}1^{11}$ E (Google Earth), at an altitude of 550-700 meters. The mosquitoes were collected from the postgraduate hostels (Akenzua and Suleiman Halls) of Ahmadu Bello University Samaru Zaria, Nigeria.

3.2 Sample Collection and Rearing

Adult resting fully engorged (i.e. dark and swollen abdomen filled with blood) female mosquitoes werecarefully caught in student hostels using a test tube. They weregently transferred into separate colorless plastic containers (218cm^3) and taken to the Parasitology and Entomology Research Laboratory, Department of Biological Sciences, Ahmadu Bello University, Zaria. The mosquitoes were held in separate wooden cages (30x30x30cm) (Plate VI) for pure line breeding and provided with ovitraps for egg laying(oviposition). Theywere provided continuously with 10% sucrose solutionin a plastic cup with a cotton wick as a source of carbohydrate. The containers wereleft in the cages overnight and observation for mosquito eggs raftwas after 24-72hoursusing a magnifying lens. Each egg raft was thentransferred to aseparate rearing plates containing distilled water using a wet filter paper.



PlateVI.Wooden cages for adultmosquitoes and plastic plates for larval rearing

After 24-48hours, hatched larvae werekept at room temperature and fed with fine powder of fish flakes 2-3 times daily (WHO, 1975a; 1975b). Care was taken to avoid overcrowding. Developing pupae wereseparated from larvae daily and placed in a container with fresh clean water and the container placed in the cages for adult emergence. The emerged adults were maintained on10% Sucrose solutionin a plastic cup soaked with a cotton wick.

3.3 Identification of mosquitoes (larvae and adult)

Larval Identification: The developing larvae collected from rearing plates were identified up to genus level using morphological featuresas described byRoy and Brown, (1971), Patel, (2002) and Sumit, (2008) as seen under a dissecting microscope (Plate vii b).

Adult identification: Some emerged adults were collected in plastic cups (218 cm^3). They were knocked down using drops of acetyl acetate on a large Whatman's filter paper which was used to cover the cups. Immobilized adults were identified up to species level using the morphological features (Figure 3.1)described by,Roy and Brown, (1971), Patel, (2002),Sumit, (2008) andVarnado *et al.* (2012)as observed under the x40 magnification in a dissecting microscope (Plate vii a).

3.3.1 Summary of keys for adult identification

- Abdominal segments with pale bands or pale lateral patches not forming a straight line.
- Second marginal wing cell at least as long as vein R_{2+3}.
- Basal pale bands of abdominal terga rounded posteriorly, with marked sub lateral constrictions,narrowly joined to lateral pale patches.
- Proboscis without pale band
- Hind femur with pale stripe on anterior surface
- Abdominal tergite segments with narrow pale band

- Tarsal segments are without basal pale bands

- Legs are dark brown, pale posteriorely

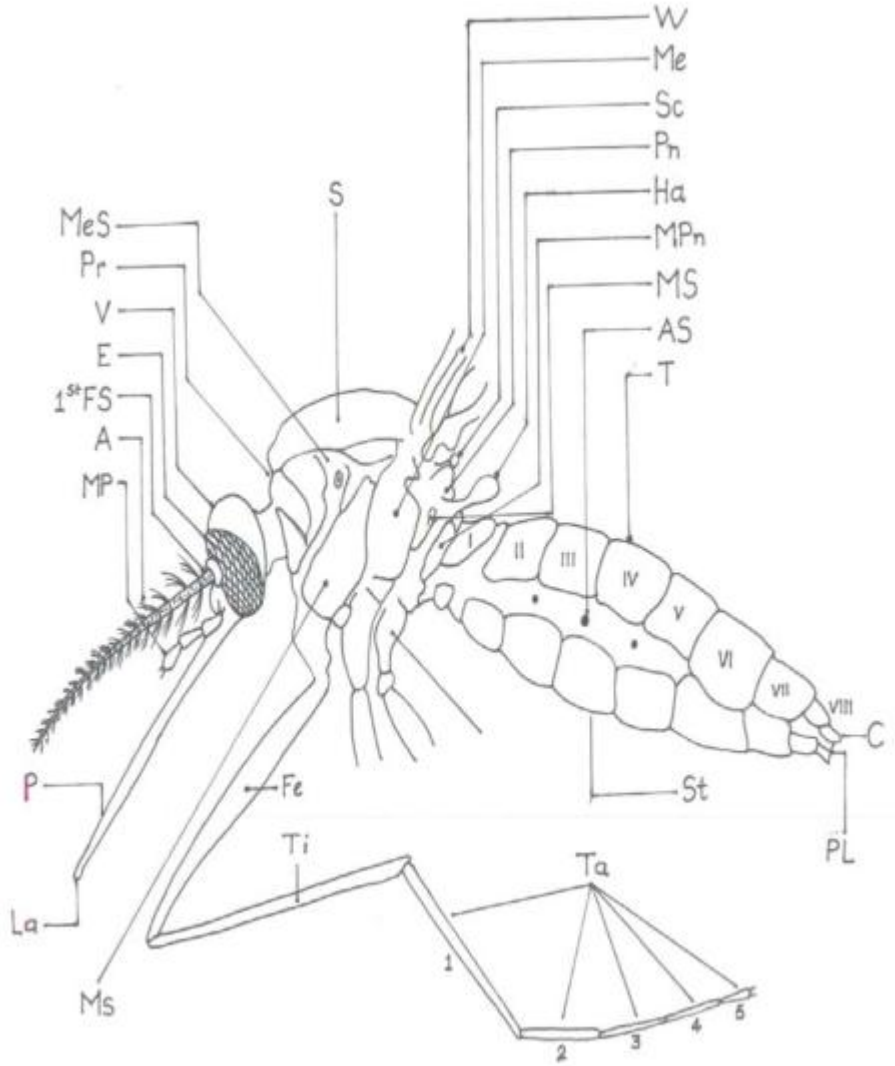


Figure 3.1 Morphological features for the adult *Culex* female mosquito identification.
Source: Patel, (2002)

A - Antenna, AS - Abdominal Spiracle, C – Cercus, E – Eye, Fe – Femur, 1st FS – 1st Flagellar Segment, Ha– Haltere, La – Labellum, Me – Mesopimeron, MP – Maxillary Palp, MPn – Meso Postnotum, MS –Metathoracic Spiracle, Ms – Mesosternum, P - Proboscis PL – Postgenital Lobe, Pn – Postnotum, Pr –Pronotum, S – Scutum, Sc – Scutellum, St – Sternite, T – Tergite, Ta – Tarsus, Ti – Tibia, V – Vertex, W –Wing

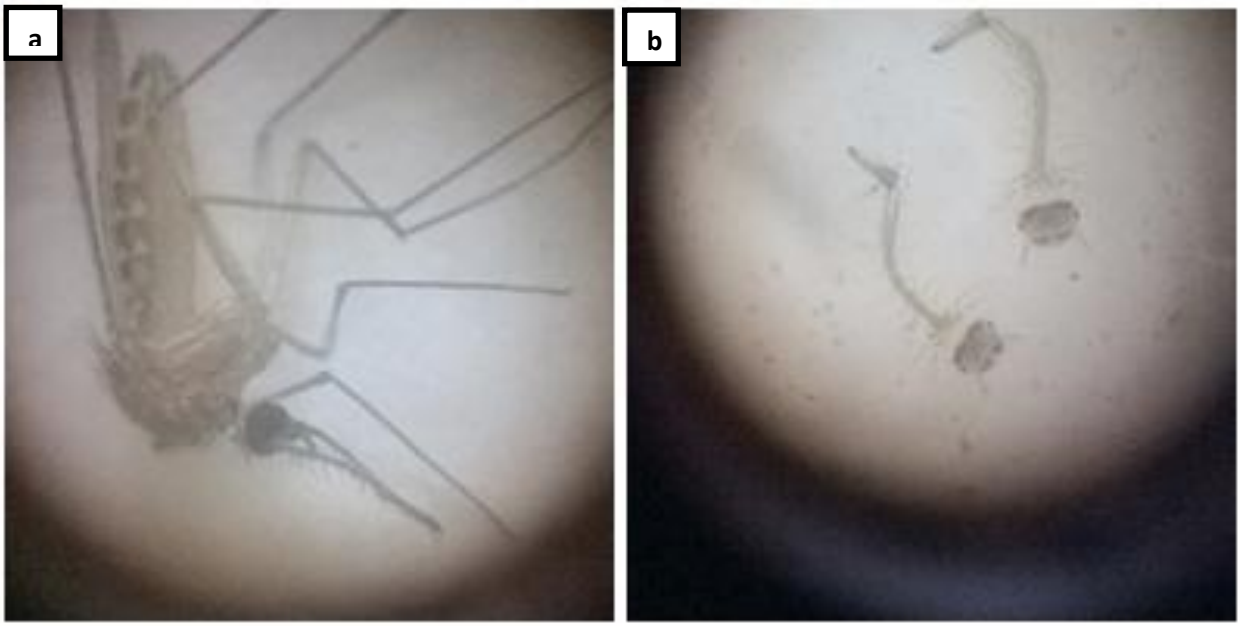


Plate VII. (a) Adult female *Cx. quinquefasciatus* (b) 3rd instar larvae of *Cx. quinquefasciatus* Magnification (x40)

- Scutum without pale-scaled spots.
- Spiracular and post spiracular setae absent.
- Palpi short, .abdomen blunt or rounded
- Thorax, proboscis, tarsi and wing scales entirely dark scaled.
- Mid-lobe of scutellum with patch of long pale scales.
- Abdominal segments with broad basal pale bands (bands narrow in width laterally)
- Abdominal segments 7–8 covered two-thirds or more with dark scales, with no copper scales.
- Abdomen with black and white scales on bottom side(ventral).
- Proboscis without pale band.
- Postspiracular area without pale scale patch.
- Tarsal segments are without basal pale bands.
- Hind femur with pale stripe on anterior surface.
- Legs are dark brown, pale posteriorly.

3.3.2 Summary of keys for larval identification.

- Siphon (air tube) present, no palmate hairs.
- Siphonal saddle index less than 3.45; seta 1-III, IV usuallysingle.
- Saddle shorter than siphon.ecten not extending to apex of siphon.
- No basal tuft on siphon.
- 3 or more pairs of siphonal tufts.
- Spiracular apparatus at apex of siphon unmodified, comprised of 5 lobes.
- Siphon with pecten.
- All scales of comb evenly fringed at sides and apex.
- Distal pecten spines with 2-5 denticles of different sizes arising proximally.

- Comb scales arising from unsclerotized integument.
- Siphon with at least 3 pairs of seta l-S, usually more.
- Seta 1-S longer than diameter of siphon at point of attachment, usually in 4 pairs.
- Seta 6-iv normally double.
- Seta 1-C unpigmented with sides smooth.
- Seta i-ii-iv of abdomen usually single.

3.4 Blood feeding of female *Cx. quinquefasciatus*

Emerged females of *Cx. quinquefasciatus* were kept with males to mate and a blood meal from a restrained quail was provided for the females 4-5 days after emergence. Prior to blood feeding, mosquitoes were starved for 24hours by removing sucrose solution from the cages to ensure that proper blood meal was obtained. Since *Cx. quinquefasciatus* prefers to feed during the night (Gerberg, 1970), the quails were left in cages overnight. 24hours after blood feeding,gravid female *Cx. quinquefasciatus* were used for oviposition experiment.

3.5 Demectin – Manufacturers details

1% Demectin (R) injectibles solution (a multiple-dose, rubber-capped bottle) was bought from a reputable veterinary pharmacy outfit in Zaria. Each ml of Demectin contains 10mg ivermectin.

3.5.1 Computation and preparations of ivermectin concentrations

The 1% Demectin (R) has a concentration of 10mg/ml and its concentration in ppm (parts per million) was calculated as below:

1% = 1/100 = 0.01 x 1,000,000 = 10,000 ppm

The various dilutions were made by obtaining the appropriate ratios as shown in Appendix XI.

3.6 Experimental procedures

The hypotheses were tested in the following experiments.

3.6.1 Experiment 1: Effect of ivermectin on oviposition site selection

Four days after blood feeding, 10 gravid females of *Cx. quinquefasciatus* were placed in a 30 x 30 x 30cm wooden cage fitted with wire mesh where seven experimental concentrations (1, 5, 10, 20, 40, 80 and 100ppm) of ivermectin solution in plastic cups (218cm^3) were placed. Distilled water without ivermectin was used as controland the experiment was replicated three times.The cups were placed in a completely randomized manner. The positions of the cups were alternated betweenthe different replicates so as to nullify any effect of positionon oviposition.The females were fed with 10% sucrose solution in a plastic cup with a cotton wick. After 24hours, the number of egg rafts laid in the treated and control cups was recorded. Containers with egg rafts were replaced to eliminate cueing effect. The result of the oviposition experiment were expressed as mean number of egg rafts and the percentage effective repellency (%ER) for ivermectin concentrations was calculated using the formula:-

$$ER\ (\%) = \frac{NC - NT}{NC}\ x\ 100$$

Where *ER* = percent effective repellency; *NC* = number of eggs in control; and *NT* = number of eggs in treatment(Murugan *et al.*, 2012).

Oviposition activity index (OAI) was calculated using the formula:-

$$OAI = \frac{NT - NS}{NT + NS}$$

Where *NT* is the total number of eggs in the test solution and *NS* is the total number of eggs in the control solution. Oviposition activity Index values lie within the range of +1 to -1

Positive OAI values indicate that more eggs were deposited in the test than the control and that the test solutions were attractive. Conversely, negative values indicate that more eggs were deposited in the control than in the test and that the test solutions were deterrent (Murugan *et al.*, 2012).

3.6.2 Experiment 2: Effect of ivermectin on egg hatchability

Freshly laid eggs were collected by providing ovitraps in mosquito cages from 10.00 to 16.00 hours for collecting 0-6h old eggs. Egg rafts were placed on a wet filter paper and carefully teased with a fine brush to separate the eggs (Su and Mulla, 1998). Groups of 50 eggs werecounted under a dissecting microscope and exposed to graded dilutions of ivermectin (25, 50, 100, 150, 200, 250 and 300ppm) in triplicatesfor 18hours before they were transferred to different cups containing distilled water using a filter paper.

Distilled water was used as control. Hatchability was assessed in percentage on the basis of hatched eggs with opened opercula 72hours post treatment using the formula:-

$$Hatchability\ (\%) = \frac{Number\ of\ larvae\ hatched}{Total\ number\ of\ eggs\ in\ treated\ water}\ x\ 100$$

To test for the effect of time of exposure on hatchability, the same procedure above (3.4.2) was followed. Groups of 50 eggs were exposed to graded dilutions of ivermectin (150, 200, 250 and 300ppm) for 5, 15, 30 and 60 minutes after which the eggs were rinsed by placing them on a wet filter paper and then carefully dipped into series of distilled water containers to rinse off the ivermectin. The experiment was replicated 3 times (Plate VIII) for each exposure time. Distilled water was used as control. Egg hatchability was assessed by observing the state of the opercula (opened opercula) 72hours post treatment (Su and Mulla, 1998).

3.6.3 Experiment 3 – Effect of ivermectin as an attractive toxic sugar bait to *Cx.quinquefasciatus*

Attractive toxic sugar bait (ATSB) test using ivermectin mixed with sucrose was conducted to test its “attract and kill” potential for mosquito control. The fact that mosquitoes are dependent onplant-derived sugar and are selectively attracted to sugar sources has practical implications tocontrol(Schleinand Müller, 2010). 50mL each of the experimental

concentrations (1, 5, 10, 20, 40, 80 and 100ppm) were separately mixed with 50mL of 10% sucrose solution. Each of the ivermectin plus 10% sucrose mixture were poured into separate plastic cups with a cotton wick and placed randomly in a screened cage. 10% sucrose without ivermectin was used as control.



Plate VIII. Experimental setup for the effects of ivermectin and time of exposure on *Cx. quinquefasciatus* egg hatchability

A water container with 30 pupae was then placed in the cage so that as adults emerged, their choice for source of sugar was observed and recorded accordingly. The setup was replicated 3 times and the positions of the cups were alternated in the replicates.

3.6.4 Experiment 4: Larvicidal effect of ivermectin on *Cx. quinquefasciatus* Larvae

Groups of 20 eggs (obtained as in 3.4.2 above) were placed in distilled water in plastic containers for hatching. The resulting first instar larvae were then sieved out using a large Whatman's filter paper. The first instar larvae were exposed to graded concentrations of ivermectin (16, 8, 4, 2, 1, 0.5, 0.25ppb and 0ppm) in triplicates.After 72hours, the number of dead/alive first instar larvae in each treatment was counted. The percentage mortality was calculated on the basis of the number of larvae that died.

The larvicidal potential of ivermectin was also evaluated against third instar larvae of *Cx. quinquefasciatus* using WHO protocol (2005) with slight modification. Groups of 20 third instar larvae were placed on wet filter paper using a pipette and then transferred into plastic containers containing ivermectin in varying concentrations of 62, 31, 15.5, 7.75, 3.86, 1.94 and 0.97ppb. Control groups were placed in plastic cup containing distilled water. The experiment was done in triplicates. Observations were made 24-72hours after the beginning of the experiment. Loss of mobility and mortality of larvae were recorded andpercentage mortality was calculated on the basis of the number of larvae that died (WHO, 2005) following the formula:-

$$\%Mortality = \frac{Number\ of\ dead\ larvae}{Number\ of\ larvae\ in\ treated\ \ water} x\ 100$$

3.7 Data Presentation and Analysis

One way ANOVAwasperformed followed by Turkey's test usingSPSSsoftwareto test the significant difference in the choice of oviposition site of gravid female *Cx. quinquefasciatus*.

Pvalues less than0.05 were considered to indicate statisticalsignificance.Two wayANOVAwas used to test the effect of exposure time and concentration on egg hatchability. Anova was used to determine difference in dose-dependent attraction amongst mixtures. Probit values were determined according to Greenberg (1980) to obtain the concentration of ivermectin that kills 50% and 95% of the $1^{st}$and 3^{rd}instar larvae (LC_{50} and LC_{95}). Chi square analysis was used to associate LC_{50} and LC_{95} in first and third instar larvae.

CHAPTER FOUR

4.0 RESULTS

4.1 Oviposition Site Selection Test

Table 4.1 shows the effect of ivermectinconcentration on oviposition site selectionbygravidfemales of *Cx. quinquefasciatus*.The Table shows the mean number of egg raft(s) laid in each site from which oviposition activity index (OAI) and effective repellency were derived. The result of the experiment wasexpressed as mean number of egg raft(s) laid, oviposition activity index (OAI) andpercentage effective repellency (%ER).Egg deposition was absent in sites 100, 80 and 40ppm, but was present in sites 20, 10, 5 and 1ppm including the control (distilled water).Sites with concentration 40ppm and above werenot selected by female *Cx. quinquefasciatus* for oviposition.

The oviposition activity index (OAI) and percentage effective repellency (%ER) values from this study, shows that ivermectin at40, 80 and 100ppmhas ahighoviposition deterrency and effective repellency against female*Cx. quinquefasciatus*. 44.5%of the eggs were deposited in 1ppm, 22.2%in 5ppm and 11.1%each, in 20ppm, 10ppm and distilled water (control).The most selected oviposition site was 1ppm (Plate ix), while the least selected sites were 100ppm, 80ppm and 40ppm without any egg raft in them.The result showed that ovipositing female*Cx. quinquefasciatus* preferred to lay their eggs in 1ppm and 5ppm ivermectin concentration than in the control cup.

One-way analysis of variance (Appendix I) showed significant difference in number of egg rafts laid per treatment ($p<0.05$). *Turkey's test* at $p<0.05$ showed significant difference in the number of egg laid per treatment (Appendix II). Although statistically there was no significant difference in the effect of 1ppm, 5ppm, 10ppm, 20ppm and distilled water (control) on oviposition site selection by gravid females, 1ppm performed significantly different from all the remaining concentrations.

Table 4.1:Effect of ivermectin on oviposition site selection of *Cx. quinquefasciatus*

Oviposition sites (ppm)	No. of females exposed	Mean number of egg rafts ± SD	Effective repellency (%)	Oviposition activity index (OAI)
100	10	0 ± 0.0000	100	-1
80	10	0 ± 0.0000	100	-1
40	10	0 ± 0.0000	100	-1
20	10	1 ± 0.3333	0	0
10	10	1 ± 0.3333	0	0
5	10	2 ± 0.3333	-100	0.3
1	10	4 ± 0.3333	-300	0.6
0	10	1 ± 0.3333	-	-

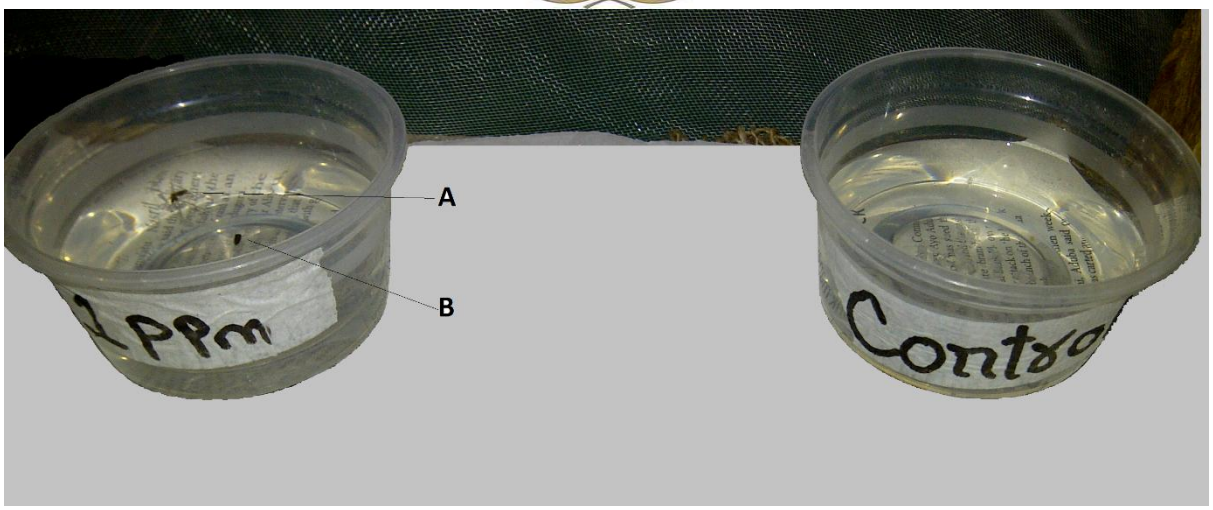


Plate IX. Selection of 1 ppm oviposition site by a female *Culex quinquefasciatus* (A) with an egg raft (B)

The OAI value of ivermectin at 40, 80 and 100ppm was -1. The OAI values increased from -1 to 0.6, showing that repellency decreased with decrease in concentration of ivermectin. The highest attraction was recorded at the 1ppm with an OAI value of 0.6.

An incidental observation was made during the oviposition experiment in 4.1.1 above. Mortality was observed in gravid and ovipositing females within 2-5 days of the experiment. 93.3%of the females died by drowning while only 6.7% survived. Out of the total number of females that died (i.e. 93.3%) in the oviposition sites, 67.9% oviposited (laid egg) while 32.1% were still gravid. Out of the gravid females that died, 32.1% drowned in 20ppm, 28.6% in 10ppm, 14.3% in 5ppm and 3.6% each in 1ppm and 80ppm. Drowning was not observed in both 100ppm and distilled water (control).Highest gravid female mortality was observed in 20ppm.

Figure 4.1 shows that mortality of oviposited females of *Cx. quinquefasciatus* increased sharply from 0ppm (control) to 1ppm. The mortality then reduced as concentration of ivermectin increased from 1ppm to 40ppm. No oviposited female was found dead in 40, 80 and 100ppm oviposition sites.The results also showed higher mortality in ovipositing females than in gravid females.

One-way analysis of variance (Appendix iii) showed that there was a significant difference in the mortality of female *Cx. quinquefasciatus* between the various ivermectin concentration ($p<0.05$). *Turkey's test* at $p<0.05$ showed significant difference in the mean number of female mosquitoes dead per treatment (Appendix iv). Although there was no significant difference in adult mortality between 10ppm and 20ppm, mortality effect on gravid mosquitoes was greater at 20ppm compared to the remaining concentration. There was no significant

difference in the mortality between 40ppm and 10ppm even though more female mosquitoes died in 10ppm.

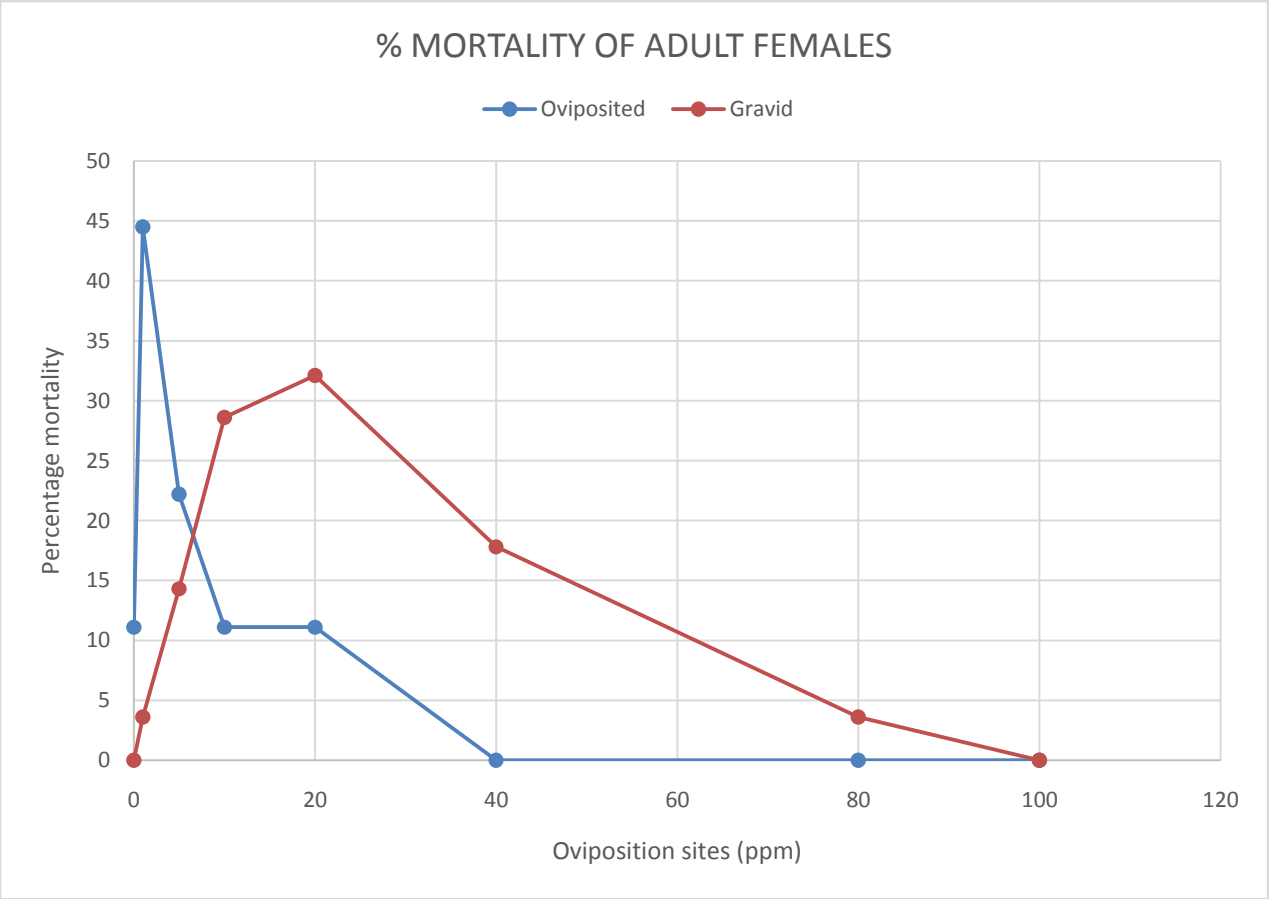


Figure 4.1: Percentage mortality of gravid and oviposited females for eachoviposition site

There was no significant difference in mortality between 5ppm and 40ppm, although more mortality was recorded in 40ppm. There was no significant difference in mortality of adultmosquitoes (gravid and oviposited) in 5ppm, 80ppm and 1ppm, even though more death was observed in 5ppm. There was no significant difference in mortality of adult mosquitoes in 80ppm, 1ppm, 100ppm and the control (distilled water).

4.2 Egg Hatchability Test

This study on the effect of ivermectin on egg hatchability showed that hatchability of eggs exposed to various concentrations of ivermectin were significantly lower than those of the control (Table 4.2). After 72hour post-treatment, 96.0% of the control group eggs hatched while none of the eggs in 300ppm and 250ppm ivermectin-treated cups hatched. The effect of ivermectin on hatching increased with increase in concentration. The highest hatch rate was recorded in the control (96.0%). Varying degrees of mortality was observed in the first instar larvae hatching from the eggs as ivermectin concentration decreased. The 50% egg hatching inhibition concentration (IC_{50}) was noted at 26.81ppm (Appendix x).

The result on theeffect of time of exposure to ivermectin on*Cx. quinquefasciatus* egg hatchability is summarized in a table (Appendix xii).Figure 4.2 shows that all eggs exposed to 300ppm of ivermectin solution lost their hatchability at all the periods of exposure. The state of the opercula of all the eggs collected from each treatment at each time of exposure indicated that some eggs were still viable in ivermectin concentrations lower than 300ppm, however, total mortality was observed among the first instar larvae hatching from all the eggs.Figure 4.2 shows that at higher concentrations of ivermectin (250-300ppm) hatchability was low irrespective of the time of exposure. Complete loss of hatchability was observed in 300ppm. Loss of hatchability also increased with increase in time of exposure.

Table 4.2:Effect of ivermectin concentration on egg hatchability of *Cx. quinquefasciatus*

Ivermectin conc. (ppm)	No. of eggs exposed	Mean no. of eggs hatched±SD	Hatchability (%)
300	50	0 ± 0.0000	0
250	50	0 ± 0.0000	0
200	50	10.0 ± 1.7667	20.0
150	50	28.0 ± 1.7667	56.0
100	50	44.6 ± 1.7667	89.3
50	50	46.6 ± 1.7667	93.3
25	50	45.7 ± 1.7667	91.4
0	50	48.0 ± 1.7667	96.0

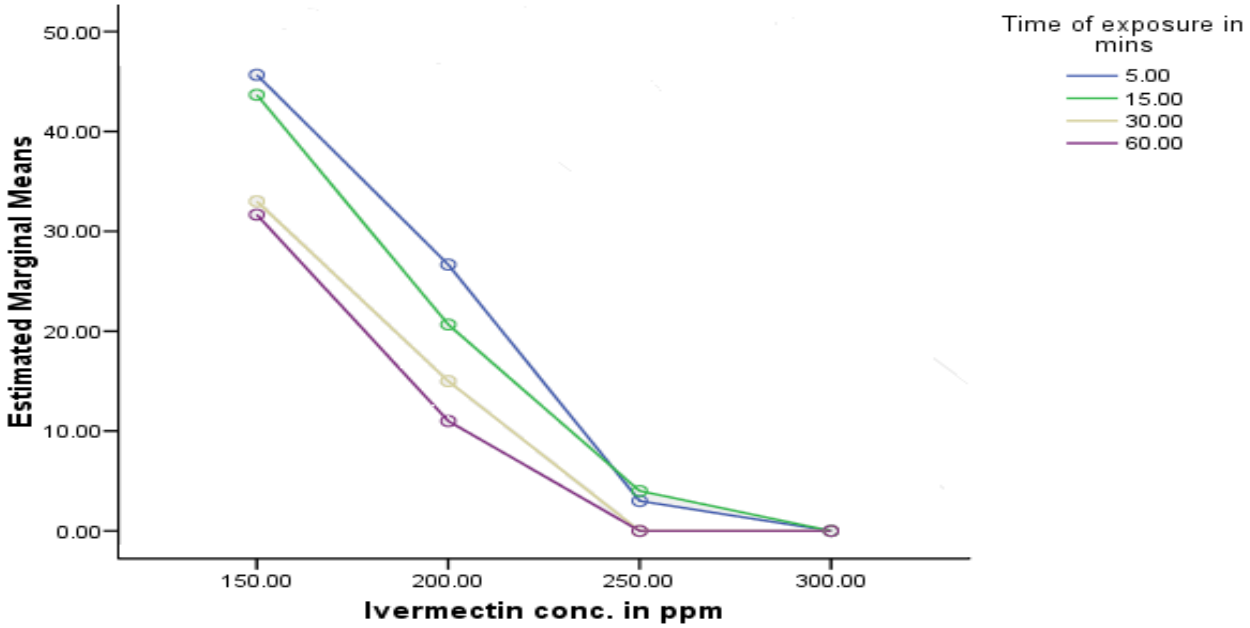


Figure 4.2: Estimated means of ivermectin concentration and exposure time

The highest hatchability was recorded at the lowest time of exposure (5min) while the lowest hatchability was recorded at the highest time of exposure (60min). 150ppm showed a hatch rate of 91.3%, 87.3%, 66% and 63.3% for 5, 15, 30 and 60min respectively.

Two-way analysis of variance showed that there is a significant difference in the effect of ivermectin concentration and exposure time on mosquito egg hatchabilityat $p<0.05$ (Appendix v and vi). Anova also showed that concentration had a significant effect on mosquito egg hatchability at $p<0.05$. *Turkey's test*at $p<0.05$ showed significant difference among concentrations (Appendix vii). There was no significant difference in the effect of 300ppm and 250ppm of ivermectinon mosquito egg hatchability. The effect of 250ppm and 300ppm of ivermectin on egg hatchability of*Cx. quinquefasciatus*was significantly different from those of 200ppm and 150ppm. Time of exposure of eggs to ivermectin concentrations was also significant ($p<0.05$). Turkey's test at $p<0.05$ showed that there was no significant difference in the effects of 60min and 30min time of exposure to ivermectin on mosquito egg hatchability (Appendix viii). The effects of 15min and 5min time of exposureon mosquitoegg hatchability was also not different from each other. There was no significant interactionbetween ivermectin concentrations and exposure time on mosquito egg hatchability ($p<0.05$).

4.3 Attractive Toxic Sugar Bait Test

Result obtained after 72h is as summarized in Table 4.3. The Table showed that all treatments had complete mortality effect on mosquitoes that fed on them after 72h. A high proportion (17% each) of mosquitoes was attracted to 100ppm, 40ppmand 10ppm ivermectin-baited sucrose-soaked cotton pads, while 1ppm ivermectin-baited sucrose-soaked cotton pad (Plate x) recorded the lowest attraction at 6%. Although all mosquitoes that obtained sugar from the

mixtures were killed, Anova showedthat there is no statisticallysignificant difference in the attraction of mosquitoes at $p<0.05$ among mixtures (Appendix ix).

Table 4.3:Attractive effect of ivermectin as a toxic sugar baitto*Cx. quinquefasciatus*

Ivermectin (ppm) + sucrose (10%)	No. exposed	No. attracted	Proportion (%)
100 + Sucrose	30	5	17
80 +Sucrose	30	4	13
40 + Sucrose	30	5	17
20 + Sucrose	30	3	10
10 + Sucrose	30	5	17
5 + Sucrose	30	3	10
1 + Sucrose	30	2	6
Sucrose	30	3	10

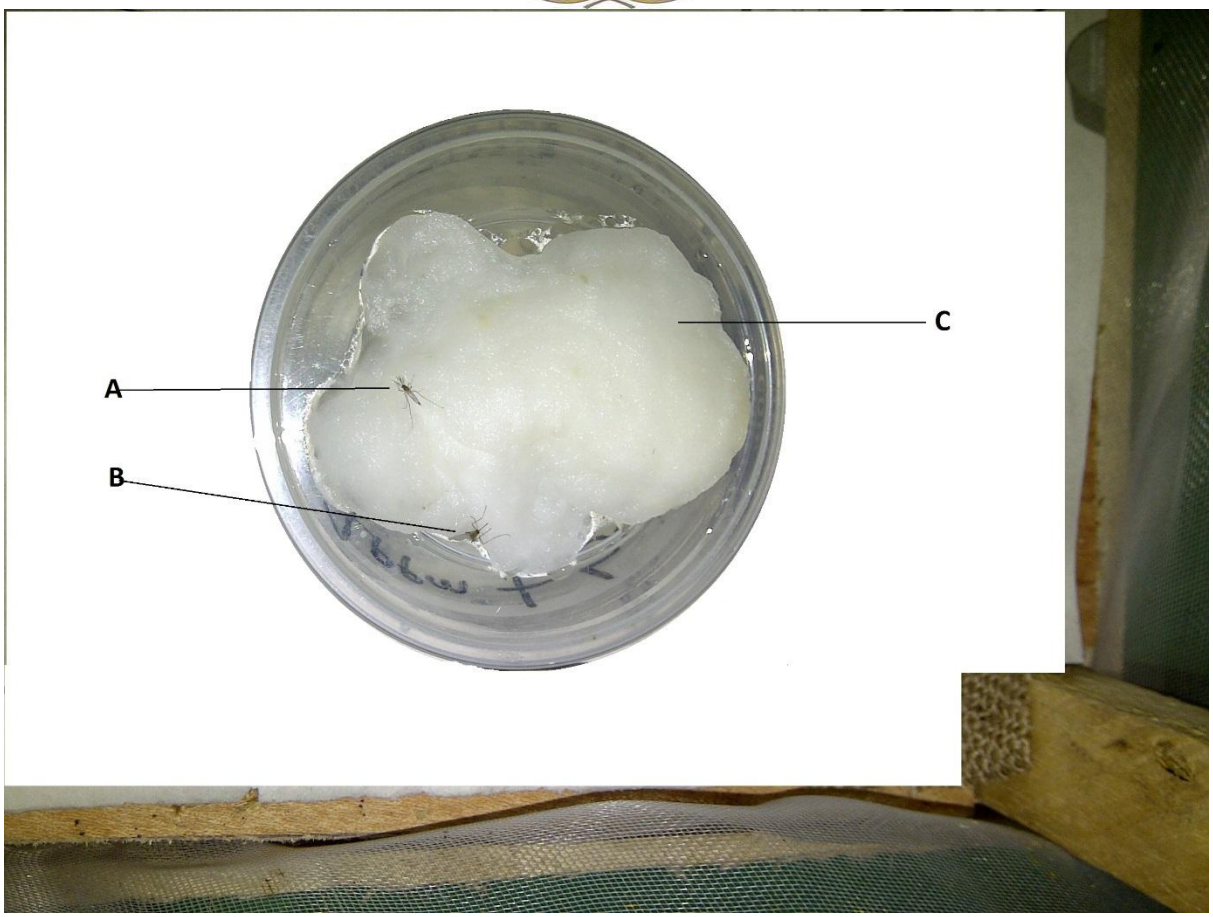


Plate X. Adult male (A) and female (B)*Cx. quinquefasciatus* attracted to feed on 1ppm ivermectin + sucrose soaked cotton pad (C)

Experiment 4.4 Larvicidal effect on first and third instar larvae

Table 4.4 shows the result obtained when groups of 20 first instar larvae were exposed to graded doses of ivermectin concentrations (16, 8, 4, 2, 1, 0.5 and 0.25ppb). Varying degree of dose-dependent mortality of these larvae was observed after 48h post-hatching. Complete mortality (100%) was recorded in 16 and 8ppb after 48h post-hatching, while 65% of the first instar larvae died in 4ppb. The remaining lower concentration did not show any larvicidal property on larval 1st instars.

Table 4.4 showed that there was no first instar larval mortality at 0.25ppb, 0.5ppb, 1ppb and 2ppb, but mortality rose sharply to 65% as concentration increased to around 4ppb. At concentration of 8ppb and 16ppb, mortality was constant at 100%.

Using percentage probit and the logarithm of concentration (Figure 4.3), the LC50 and LC95 evaluated for ivermectin was 3.78ppb and 7.47ppb respectively for 1st instar larvae of *Cx. quinquefasciatus*(Figure 4.3).

With respect to 3rd stage instars, total (100%) mortality was recorded in 62ppb, 95% in 31ppb, 63% in 15.5ppm, 30% in 7.75ppb and 15% in 3.86ppb. No mortality was observed in 1.94ppb, 0.97ppb and in the control after 72h (Table 4.5).

The LC50 and LC95 for the 3rd stage instars were 11.49ppb and 35.20ppb respectively (Figure 4.4). Chi square shows that there was no significant difference in the LC50 and LC95 of 1st and 3rd instars of *Cx. quinquefasciatus*larvae (Table 4.6).

Table 4.4:Larvicidal effect of ivermectin againstfirst instar larvae of *Cx. quinquefasciatus*

Treatments (ppb)	No. of 1st instar larvae exposed	No. dead	Mortality (%)
16	20	20	100
8	20	20	100
4	20	13	65
2	20	0	0
1	20	0	0
0.5	20	0	0
0.25	20	0	0
0	20	0	0

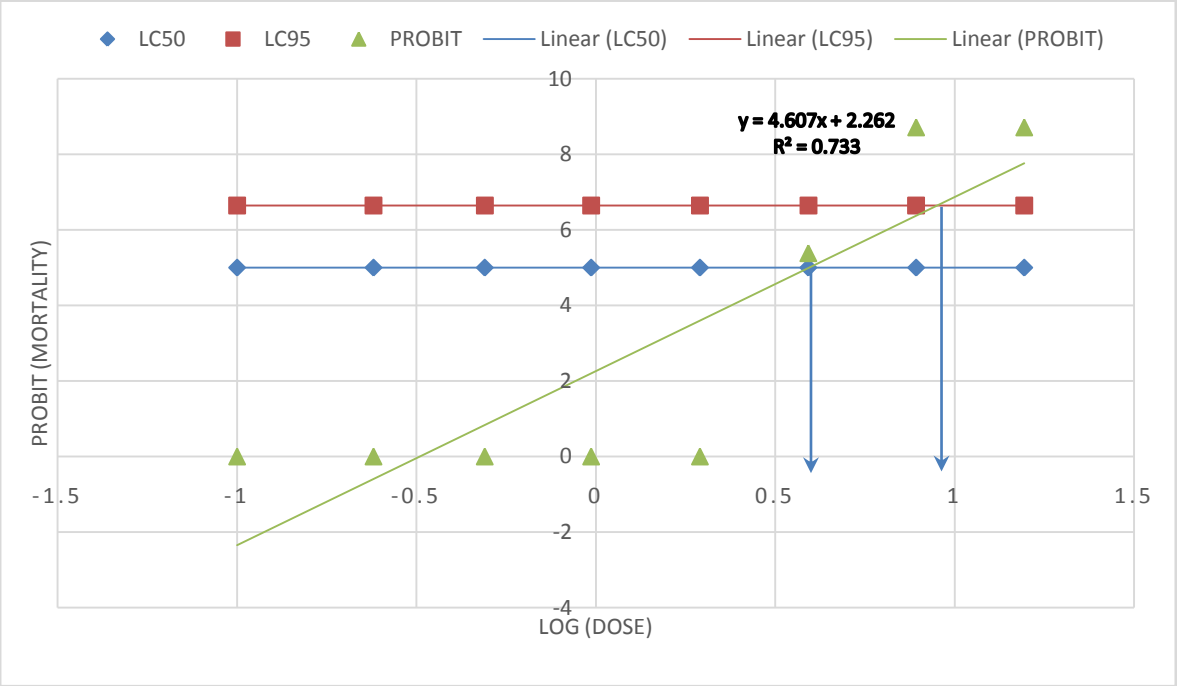


Figure 4.3:Larvicidal effect of ivermectin against 1st instar larvae of *Cx. quinquefasciatus* expressed as linear regression

Table 4.5: Larvicidal effect of ivermectin against third instar larvae of *Cx. quinquefasciatus*

Treatments (ppb)	No. exposed	No. dead	Mortality (%)
62	20	20	100
31	20	19	95
15.5	20	13	63
7.75	20	6	30
3.86	20	3	15
1.94	20	0	0
0.97	20	0	0
0	20	0	0

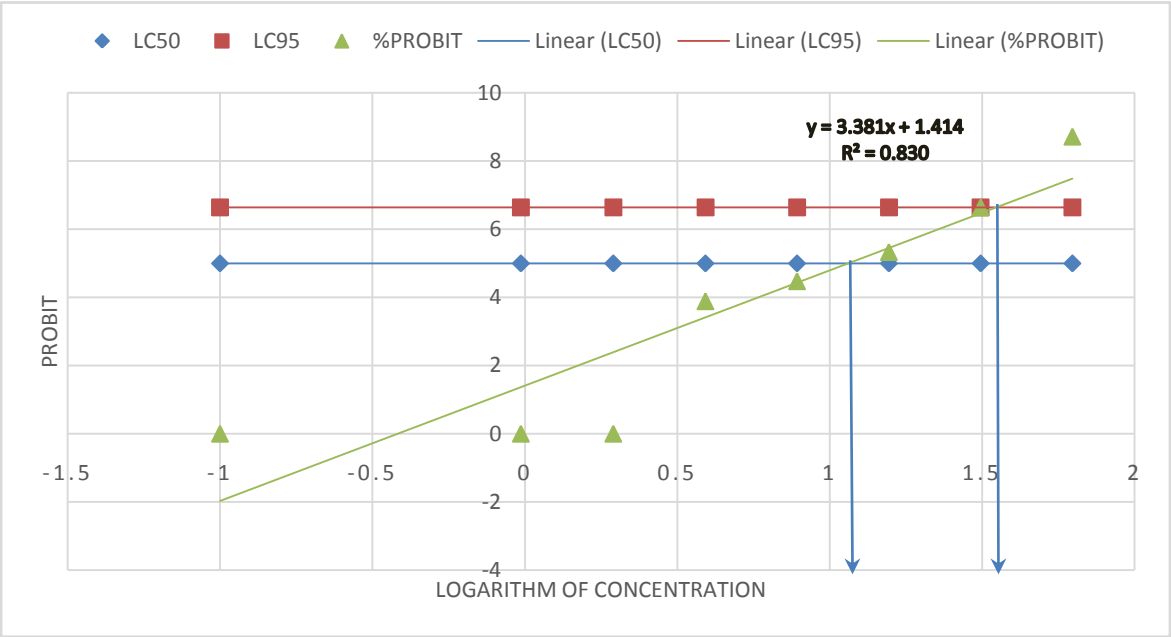


Figure 4.4:Larvicidal effect of ivermectin against 3rd instar larvae of *Cx. quinquefasciatus* expressed as linear regression

Table 4.6:Evaluation of Chi Square to associate LC_{50} and $LC_{95}$for first and third instar larvae of *Cx. quinquifasciatus*

	LC 50	LC 95	χ^2	P value
1st Instar	3.78	7.47	0.391151	0.531695
3rd Instar	11.49	35.47		

CHAPTERFIVE

5.0 DISCUSSION

In this study, results obtained fromthe oviposition site selection studysuggests possible reduced surface tension in the oviposition sites because of the presence of ivermectin.Another suggestion is on possible contact toxicity on gravid and oviposited females alighting on the treated oviposition sites. At higher concentrations, it is suggested that ivermectin gives off a strong olfactory signal to repel gravid *Cx. quinquefasciatus* from ovipositing in those sites. The results of this study are favorably supported by the findings ofClements(1999), who stated that mosquitoes can detect chemicals from the airwhen it possesses sufficient volatility or, in thecase of low volatility chemicals, by a gustatory mechanisminvolving direct contact with water.Huang*et al.* (2005) also stated that gravid mosquitoes are often attracted to water but the decisionto lay eggs maydepend on additional olfactory signalsor other stimuli received when the insect lands on thewater surface.Braks *et al.* (2007) andBurkett-Cadena and Mullen (2007) stated that habitat related chemical cues havebeen shown to play an important role in the oviposition site selection by gravid mosquitoes. Previous studies conducted by Strong and Brown (1987) and Freitas *et al.* (1996) have shown that the avermectins are lethal for immature and adult stages of some insect orders. Strong (1993) concluded that it is not necessarily the ingestion of ivermectin, because the contact with the same already is effective in the majority of the cases.The attractiveness of ivermectin treated cups to gravid and ovipositing mosquitoes as observed in this study is supported by the previous study results of Torkhani *et al*.(2010) who concluded that earthworms, (*Eisenia fetida* and *Lumbricus terrestris*in soils treated with ivermectin), may be attracted by some chemicals. Holter and colleagues (1993) alsostated that at least in some cases the attractiveness of cattle dung to scarabaeid beetles is affected by avermectin therapy;beetle discrimination between dung types is probably attributable to some unknown side effect of

the ivermectin treatment rather than being a direct response to the drug itself.The oviposition deterrent effect at higher concentrations and toxic-attractive effect on gravid and ovipositing adults at lower concentrationsof ivermectin against *Cx. quinquefasciatus*as shown in his study, makes ivermectin a potent alternative to other insecticides in mosquito control programs.

Results from the present study shows that ivermectin has a potent effect on egg hatching which may translate to possible loss of hatchability of *Cx. quinquefasciatus* eggs. The treatment of eggs with various dilutions of ivermectin may have caused embryonic death, resulting in failure of eggs to hatch. In this present study, the mode of action of egg hatching inhibitionactivity of ivermectin was not studied. However, previous studies have shown that the egg shell is made ofthree chorionic layers(to protect the embryo) as well as chitin. Ivermectin may inhibit the hatchability of the eggs by interfering with their chorion. Theaction of lipophilic substances upon eggs may be by causingindurations of the egg shell and interference in water andgas exchange or by penetration into the egg and impedingenzymatic reactions as well as hormonal activities whichdisturb the embryogenesis process (Miura *et al.*, 1976).

The 100% loss of hatchability observed in higher concentrations (300ppm) suggests that the effect of ivermectin on hatchability is dose-dependent. Also, eggs exposed to ivermectin for longer periods showed higher mortality, revealing that the time of exposure of eggs to ivermectin is crucial to egg hatchability.Results from the present study shows that a short duration of exposure of *Cx. quinquefasciatus* eggs to ivermectin was not as potent as longer exposure period in halting egg hatching. This result agrees with the work of Miura *et al.*(1976) who stated that the exposure time and the age of the embryos played a crucial role in stopping eggsfrom hatching.Miura and his colleagues (1976) showed that the ageof the

embryos at the time of treatment played a crucialrole with regard to the effectiveness of the chitin synthesisinhibitor,Dimilin to *Cx. quinquefasciatus*.The efficiency of ivermectin as an ovicide to act on the embryo inside the egg shell depends on its ability to penetrate the egg shell, which in turn is influenced by the exposure period (Grosscurf *et al.,* 1977). The total inhibition of egg hatching observed at high concentration of ivermectinmay be indicative ofincreased penetration of the chemical inside the egg shell, which subsequently affected embryogenesis. Longer exposure periods also facilitated increased penetration of the compounds into the cells, thus increasing their effectiveness (Smith and Sakeled, 1966).There was an inverse relationship between concentration and the magnitude of the hatching rate. In general, the hatching rates increased with time from 5min to 60min, but hatching rate decreased with an increase in concentration. Thisresult is comparable with earlier studies offoliage extract from*Ricinuscommunis* L. against*Cx. quinquefasciatus*and *An.Arabiensis*(Elimam*et al.,* 2009) andleaf extract of*Solanum trilobatum*against eggs with age groupranging from0-18hours of*An.Stephensi*Liston(Rajkumar and Jebanesan, 2005).This result on the ovicidal effect of ivermectin is also comparable toα-amyrin acetate(Kuppusamy and Murugan, 2012) and *Vetiveria zizanioides* L. root extracts (Aarthi and Murugan, 2011) which caused 56.88% and 55% ovicidal activity against the malarial vector *An.Stephensi*Liston.

Although this study showed that ivermectin was highly toxic as an attractive sugar bait when mixed with 10% sucrose solution, it did not show any significant dose-dependent attraction to adult mosquitoes. The result means that *Cx. quinquefasciatus* could not detect the presence of ivermectin in the sugar bait, making it a good poison and an excellent tool for control.

Also in this study, ivermectin was found to possess an excellent larvicidal activity against *Cx. quinquefasciatus*. According to this study, ivermectin gave anLC_{50}- LC_{95} of 3.78 - 7.47ppb

and 11.49 – 35.20ppb against 1st and 3rd instar larvae of *Cx. quinquefasciatus* respectively.Although the concentration of ivermectin needed to kill 1st instars of*Cx. quinquefasciatus*was lower than that needed to kill 3rdinstars, there was no significant difference between these concentrations. This implies that the same concentration of ivermectin is needed to kill 50% or 95% of either first or third instar larvae of *Cx. quinquefasciatus.* The result also revealed that within the same instar larvae (e.g. first instar larvae) of *Cx. quinquefasciatus*, the concentration needed to kill 95% of the test population is higher than the concentration needed to kill 50% of the same population, although Chi square did not show any significant association. The different larval instars susceptibilityresults agree with the findings of Freitas *et al*. (1996) and Alves *et al*. (2010), who reported that susceptibility of *Cx. quinquefasciatus* larvae to ivermectin decreases from 1st to 4th instars, with the 4th instars being the least susceptible. Report from previous study confirmed that2ndinstar larvae of*An. arabiensis*were more susceptible than3rdinstar and the later wasmore susceptible than4thinstar larva to *Coccinia indica* essential oil (Rajkumar, *et al*., 2011). The LC_{50}andLC_{95} of this work are larval age dependent. This may support the ideas of Kumar and Dutta (1987) who stated that insect age andphysiological status of larvae plays animportant role ininfluencing susceptibility.The mortality values of ivermectin obtained in this research are comparable with that of Alves *et al*. (2010). Results obtained by Alves *et al*. (2010) showed that ivermectin in a concentration of 1.5 ppb caused paralysis in 3rd instars of*Cx. quinquefasciatus* larvae with a mortality rate of 73.38%, mobilization of substances stored in the fat body and reduction of the number of egg laid in the adult stage.

In the present study, the majority of the larvae exposed to the 7.81ppb solution of ivermectin presented ataxia, followed by death, suggesting binding of this insecticide to the GABA with consequent lethal action for *C. quinquefasciatus*, which agreed with results obtained by

Freitas *et al*. (1996). For high concentrations (31.25 and 62.5ppb) tested during this experiment, the *Cx. quinquefasciatus* larvae affected by ivermectin displayed progressive paralysis. Alves *et al.* (2004) observed thativermectin caused paralysis in *Cx. quinquefasciatus*larvae. The high mortality rate observed for larvae exposed to ivermectin was in agreement with previous results found for *Cx. quinquefasciatus* larvaeby Alves *et al*. (2004),for *Chrysomya bezziana* and *Calliphora vomitoria* by Strong (1993) who found inhibition of pupation and adult development in exposed to sub-lethal dilutions of ivermectin.

This study showed that ivermectin caused high level of larval mortality and decreased reproductive performance of gravid and ovipositing adults, suggesting that ivermectin is a drug that could be used for the control of mosquito population growth.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONAND RECOMMENDATION

6.1 Summary

In this study it was observed that, in addition to attraction of *Cx. quinquefasciatus* to ivermectin, ivermectin showed larvicidal and egg hatching inhibitionactivity against *Cx. quinquefasciatus*. It was also observed that at lower concentrations, ivermectin did not show oviposition deterrence, but rather acted as bait where mosquitoes are attracted to lay their eggs. The gravid adults will either drown in the water due to reduced surface tensionor lay eggs that may hatch but the first instars hatching out will all be killed. The biological and broad spectrum activities of ivermectinagainst all stages of the mosquito life cycle makes it a potent arsenal for vector management, especiallyin areas where mosquitoes have developed resistance toconventional insecticides.

The values presented in the present study indicate that chemical treatment using ivermectin is viable. The results confirm the need for preventive strategies and alternative control methods that might diminish the selection of resistance after the massive use of temephos in dengue control programs. The constant use of pesticides worldwide has brought a general tolerance for these chemicals and the outcome of resistance. Ivermectin rises as an alternative for the future substitution of temephos in mosquito control programs whereby it has shown effectiveness in killing *Cx. quinquefasciatus* and *Ae. aegypti* larvae under laboratory conditions. Even some other drugs used by mosquito control programs as cypermethrin has already shown signs of resistance in Brazil (Luna *et al.*, 2004), suggesting a need for immediate replacement of these insecticides by some other product of chemical or biological origin like ivermectin.The present findings suggest that ivermectin provided an excellent potential for controlling *Cx. quinquefasciatus* mosquito at earlier stage of their life cycle or

even as adults since they do not detect the presence of ivermectin when mixed with sugar, hence a reduced chance of developing any form of resistance to it.

6.2 Conclusion

i. Ivermectin had a significant effect on oviposition site selection of*Cx. quinquefasciatus. Gravid females were effectively deterred at high concentrations and attracted at lower concentrations; therefore the null hypothesis is rejected.*

ii. Ivermectin had a potent effect on egg hatchability of*Cx. quinquefasciatus.Eggs exposed to ivermectin treatment showed a concentration and time of exposure dependent loss of hatchability;therefore the null hypothesis is rejected.*

iii. Although ivermectin was highly toxic as an attractive sugar bait, it did not show any significant dose-dependent attraction to adult *Cx. quinquefasciatus;therefore the null hypothesis is rejected.*

vi. There was no significantassociation between the LC_{50} *and* LC_{95} *of ivermectin in* 1^{st} *and* 3^{rd} *instar larvae of Cx. quinquefasciatus. Therefore the null hypothesis is accepted.*

The present study therefore conclusively shows that three things might have happened with respect to the initial observation made. Since the concentration of ivermectin in the water body was not known, the following could have happened;

- *The initial ivermectin concentration was high enough to deter gravid mosquitoes so that oviposition did not take place.*
- *The onset of rain reduced the concentration of ivermectin but the concentration was still enough to cause mortality in gravid and ovipositing females and also cause loss of hatchability and hatchability in the oviposited eggs.*

- *At lower concentrations, the gravid females were attracted to oviposit but all the first instars resulting from the eggs died out such that mosquito breeding was absent in the water body for five months of the rainy season.*

6.3 Recommendations

i. The use of ivermectin in the control of *Cx. quinquefasciatus*in the study area is recommended to alleviate the biting nuisance and high nocturne discomfort that it constitutes.

ii. The use of ivermectin in thecontrol of mosquito population growth, especially the immature stages should be incorporated into MDA's for lymphatic filariasis in areas where the disease is of public health importance.

iii. Similar research using the malaria vector is recommended in the study area for the control of immature stages of *Anopheles*mosquitoes in order to reduce malaria mortality and morbidity.

iv. Ivermectin still holds more promise in the control of other menacing arthropods. It is recommended that more research be carried out on the effects of ivermectin on other arthropods of medical and economic importance in an attempt to further evaluate the potentials of this drug.

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APPENDIX I

One way anova of oviposition by concentration

Test of Homogeneity of Variances

Number of egg rafts oviposited

Levene Statistic	df1	df2	Sig.
6.857	7	16	.001

Anova

Number of egg rafts oviposited

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	4.500	7	.643	3.086	.029
Within Groups	3.333	16	.208		
Total	7.833	23			

APPENDIX II

Turkey's Test - One way anova of oviposition by concentration

Homogeneous Subsets

Number of egg rafts oviposited

concentration of ivermectin (ppb)	N	Subset for alpha = .05	
	1	2	1
40000.00	3	.0000	
80000.00	3	.0000	
100000.00	3	.0000	
.00	3	.3333	.3333
10000.00	3	.3333	.3333
5000.00	3	.6667	.6667
20000.00	3	.6667	.6667
1000.00	3		1.3333
Sig.		.635	.197

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

APPENDIX III

One way anova of adult mortality by concentration

Anova

Number of dead adults

	Sum of Squares	Df	Mean Square	F	Sig.
---------- ---Between Groups	30.000	7	4.286	20.571	.000
Within Groups	3.333	16	.208		
Total	33.333	23			

APPENDIX IV

Turkey's Test - One way anova of adult mortality by concentration

Homogeneous Subsets

Number of dead adults

Ivermectin concentration (ppm)	N	Subset for alpha = .05				
	1	2	3	4	5	1
.00	3	.0000				
100.00	3	.0000				
1.00	3	.3333	.3333			
80.00	3	.3333	.3333			
5.00	3		1.3333	1.3333		
40.00	3			1.6667	1.6667	
10.00	3				2.6667	2.6667
20.00	3					3.0000
Sig.		.982	.197	.982	.197	.982

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

APPENDIX V

Analysis of variance

Between-Subjects Factors

		N
Ivermectin conc. in ppm	150.00	12
	200.00	12
	250.00	12
	300.00	12
Time of exposure in mins	5.00	12
	15.00	12
	30.00	12
	60.00	12

Levene's Test of Equality of Error Variances(a)

Dependent Variable: hatchability

F	df1	df2	Sig.
7.248	15	32	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
a Design: Intercept+concentration * exposurettime+concentration+exposurettime

Tests of Between-Subjects Effects

Dependent Variable: hatchability

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	12483.646(a)	15	832.243	44.436	.000
Intercept	10296.021	1	10296.021	549.732	.000
concentration * exposurettime	366.854	9	40.762	2.176	.051
Concentration	11561.063	3	3853.688	205.759	.000
Exposurettime	555.729	3	185.243	9.891	.000
Error	599.333	32	18.729		
Total	23379.000	48			
Corrected Total	13082.979	47			

a R Squared = .954 (Adjusted R Squared = .933)

APPENDIX VI

Estimated marginal means

Grand Mean

Dependent Variable: hatchability

Mean	Std. Error	95% Confidence Interval	
Lower Bound	Upper Bound	Lower Bound	Upper Bound
14.646	.625	13.373	15.918

APPENDIX VII

Post hoc

Concentration in ppm

Multiple Comparisons

Dependent Variable: hatchability
Turkey HSD

(I) Ivermectin conc. in ppm	(J) Ivermectin conc. in ppm	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
		Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound
150.00	200.00	20.1667(*)	1.76678	.000	15.3798	24.9535
	250.00	36.7500(*)	1.76678	.000	31.9631	41.5369
	300.00	38.5000(*)	1.76678	.000	33.7131	43.2869
200.00	150.00	-20.1667(*)	1.76678	.000	-24.9535	-15.3798
	250.00	16.5833(*)	1.76678	.000	11.7965	21.3702
	300.00	18.3333(*)	1.76678	.000	13.5465	23.1202
250.00	150.00	-36.7500(*)	1.76678	.000	-41.5369	-31.9631
	200.00	-16.5833(*)	1.76678	.000	-21.3702	-11.7965
	300.00	1.7500	1.76678	.756	-3.0369	6.5369
300.00	150.00	-38.5000(*)	1.76678	.000	-43.2869	-33.7131
	200.00	-18.3333(*)	1.76678	.000	-23.1202	-13.5465
	250.00	-1.7500	1.76678	.756	-6.5369	3.0369

Based on observed means.
* The mean difference is significant at the .05 level.

Homogenous Subset

hatchability

Turkey HSD

	N	Subset		
Ivermectin conc. in ppm	1	2	3	1
300.00	12	.0000		
250.00	12	1.7500		
200.00	12		18.3333	
150.00	12			38.5000
Sig.		.756	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 18.729.
a Uses Harmonic Mean Sample Size = 12.000.
b Alpha = .05.

APPENDIX VIII

Time of exposure in mins

Multiple Comparisons

Dependent Variable: hatchability
Turkey HSD

(I) Time of exposure in mins	(J) Time of exposure in mins	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
		Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound
5.00	15.00	1.7500	1.76678	.756	-3.0369	6.5369
	30.00	6.8333(*)	1.76678	.003	2.0465	11.6202
	60.00	8.1667(*)	1.76678	.000	3.3798	12.9535
15.00	5.00	-1.7500	1.76678	.756	-6.5369	3.0369
	30.00	5.0833(*)	1.76678	.034	.2965	9.8702
	60.00	6.4167(*)	1.76678	.005	1.6298	11.2035
30.00	5.00	-6.8333(*)	1.76678	.003	-11.6202	-2.0465
	15.00	-5.0833(*)	1.76678	.034	-9.8702	-.2965
	60.00	1.3333	1.76678	.874	-3.4535	6.1202
60.00	5.00	-8.1667(*)	1.76678	.000	-12.9535	-3.3798
	15.00	-6.4167(*)	1.76678	.005	-11.2035	-1.6298
	30.00	-1.3333	1.76678	.874	-6.1202	3.4535

Based on observed means.
* The mean difference is significant at the .05 level.

Homogenous Subsets

hatchability

Turkey HSD

	N	Subset	
Time of exposure in mins	1	2	1
60.00	12	10.6667	
30.00	12	12.0000	
15.00	12		17.0833
5.00	12		18.8333
Sig.		.874	.756

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 18.729.
a Uses Harmonic Mean Sample Size = 12.000.
b Alpha = .05.

APPENDIX IX

Anova;

Completely Randomized

Anova: Single Factor

SUMMARY

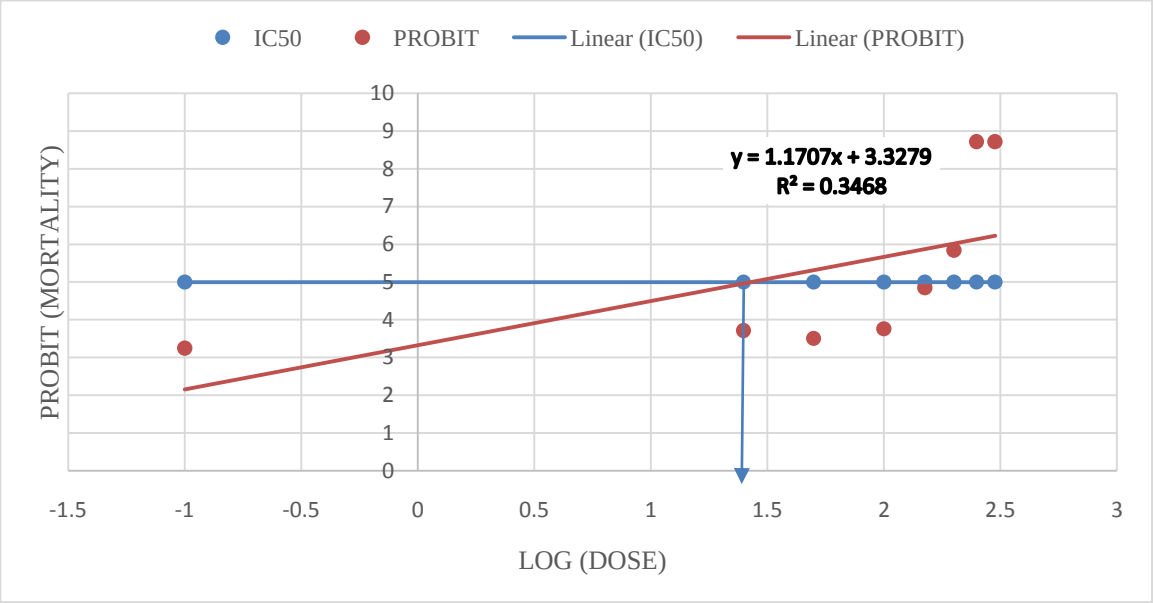
Groups	*Count*	*Sum*	*Average*	*Variance*
100000 + Sucrose	3	15	5	1
80000 + Sucrose	3	12	4	4
40000 + Sucrose	3	15	5	4
20000 + Sucrose	3	9	3	1
10000 + Sucrose	3	15	5	0
5000 + Sucrose	3	9	3	4
1000 + Sucrose	3	6	2	1
Sucrose	3	9	3	9

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	28.5	7	4.071429	1.357143	0.288178	2.657197
Within Groups	48	16	3			
Total	76.5	23				

APPENDIX X

50% egg hatching inhibition concentration



APPENDIX XI

Dilution Ratios

- 10,000ppm was diluted to 40ppm by obtaining the ratio40/10,000= 1/250
  2mL of the10,000ppm Demectin was then poured into a 500mL measuring cylinder and distilled water added until the water level reached the 500mL mark on the cylinder. Solution was then poured into a clean jerry-can and labeled appropriately.
- 10,000ppm was diluted to 20ppm by obtaining the ratio 20/10,000= 1/500
  1mL of the 10,000ppm Demectin was then poured into a 500mL measuring cylinder and distilled water added until the water level reached the 500mL mark on the cylinder. Solution was then poured into a clean jerry-can and labeled appropriately.

Same procedure was followed in the preparation of the remaining concentrations ranging from 10ppm – 0.0002ppm. Ppm values were converted to ppb (parts per billion) by multiplying by 1,000.

APPENDIX XII

Effects of exposure time and ivermectin concentration on *Cx. quinquefasciatus* egg hatchability

	Hatched eggs at			
Ivermectin conc.	**5min**	**15min**	**30min**	**60min**
300ppm	0	0	0	0
	0	0	0	0
	0	0	0	0
250ppm	3	4	0	0
	4	5	0	0
	2	3	0	0
200ppm	25	19	17	13
	34	20	12	6
	21	23	16	14
150ppm	48	47	20	25
	50	38	40	33
	39	46	39	37