EFFICACY OF *BACILLUS THURINGIENSIS VAR ISRAELINSIS (BTI)* ON *CULEX* AND *ANOPHELINE* MOSQUITO LARVAE IN ZOMBA

MSc (ENVIRONMENTAL SCIENCES) THESIS

By

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Submitted to the Faculty of Science, Department of Biological Sciences, in partial fulfilment of the requirements for the degree of Master of Science (Environmental Sciences)

UNIVERSITY OF MALAWI
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FEBRUARY, 2016

DECLARATION

I declare that this thesis is my own original work and effort and has not been submitted to any other institution for similar purposes. Where other people's ideas have been used, proper acknowledgments have been made.

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CERTIFICATE OF APPROVAL

We the undersigned declare that this thesis is a result of the candidate's own work and effort and has been submitted with our approval.

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To my mother Francess Rexa Mphande, brothers Stanley and Lister Chiumia

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisors Mr D. Pemba and Dr. C. Chilima for the untiring guidance and supervision during the research project. I would like to thank the Chemicals and Marketing Company for providing the *Bti* which I used in my studies. My special thanks should go to the Ministry of Education for granting me a study leave.

I am greatly indebted to the Department of Biological Sciences, Chancellor College for allowing me to use the laboratory at any time I needed to do my laboratory work. I also recognise the following for their assistance: Dr. Themba Mzilahowa, Dr. Standwell Nkhoma, Augustine Choko, Felix Kumwenda, Raphael Kondwani, Twalibu Tandwe, Alfred Kambwiri, Zipangani Munideranji and all those who assisted me in time of need.

My cordial thanks goes to the academic and technical staff of the Faculty of Science, Chancellor College, University of Malawi, for the academic and technical help they gave me throughout the period of my studies and research work. I also thank the entire team of Malaria Alert Centre (College of Medicine) for their untiring support during my studies.

Special thanks should go to my mother, Frances Rexa Mphande, for withstanding all the hardships when I was at school, and by providing support during my studies.

Most importantly I thank God, as He is the controller and foreseer of everything. Without Him this could not have been possible.

ABSTRACT

Laboratory-based experiments were conducted using *Bacillus thuringiensis var israelinsis (Bti)* to establish the efficacy of *Bti* on *Anopheles* and *Culex* mosquito larvae collected within Zomba district. The study evaluated two formulations of *Bti* namely VectoBac® WG and VectobaBac® 12AS against selected species of mosquito larvae. Mosquito samples were collected using two methods; Adult blood-fed female mosquitoes and larvae from their breeding sites. Adult mosquitoes laid eggs and hatched into larvae. While those collected from the field as larvae were allowed to emerge into adults and got blood-fed with *Rattus norvegicus Albinus*, then laid eggs and hatched into larvae. Third in star larvae were used in all experiments. During this study, six different concentrations of *Bti* were set and 360 mosquito larvae were exposed to these different concentrations and results were observed hourly for 10 hours, then after 24 hours and 48 hours. The experiment was replicated three times. Results show that the lower effective dosage that can be used to control *Culex* mosquito larvae in Zomba after 48 hours of exposure is 47.73g/ha of *Bti*, the LT_{50} and LT_{90} being 7.5 hrs and 24.3 hrs, respectively. On the other hand, *Anopheles* mosquito larvae required 103.41g/ha of *Bti* which is more than double as much as that required by *Culex.* The LT_{50} and LT_{90} for *Anopheles* were 6.2 hrs and 18.5 hrs respectively. In addition, it was observed that when *Culex* and *Anopheles* mosquito larvae were exposed to the same dosage of liquid formulation of *Bti* (0.001ml/L) there was no significant difference in their mortalities. Both liquid and granular *Bti* have shown to be effective against mosquito larvae, hence this has a direct impact in reducing populations of adult mosquitoes and consequently a reduction in the transmission of Malaria, Lymphatic filariasis and other diseases that are spread by mosquito bites.

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LIST OF ABBREVIATIONS AND ACRONYMS

Bti *Bacillus thuringiensis var. Israelinsis*

Bt *Bacillus thuringiensis*

DDT Dichlorodiphenyl-trichloroethane

DEC Diethyl-Carbamazine

ICT Immunochromatographic Test

IRS Indoor Residual Spraying

ITNs Insecticide Treated Mosquito Nets

IVM Integrated Vector Management

LA Lumefantrine – Artemether

LC_{50}, LC_{90}, LC_{95} Lethal concentration that will kill 50%, 90% and 95% respectively of organisms exposed to it within a specified duration

LT Lethal Time

LT_{50}, LT_{90}, LT_{95} Lethal time at which 50%, 90% and 95% of the organisms will be dead respectively

LLIN Long Lasting Insecticidal Net

MDA Mass Drug Administration

MF Microfilaria

MoH Ministry of Health

MOP Malaria Operational Plan

NGOs Nongovernmental Organisations

SP Sulfadoxine–pyrimethamine

USA United States of America

WDG Water Dispersible Granular

WHO World Health Organisation

LIST OF SYMBOLS

cm	Centimetre
^{0}C	Degrees Celsius
^{0}F	Degrees Fahrenheit
EC	Effective Concentration
g	Gram
ha	Hectare
Hrs	Hours
Km	Kilometre
L	Litre
µL	Micro litre
ml	Millilitre
mins	Minutes
%	Percent
RH	Relative humidity

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Malaria is ranked the highest killer disease in Africa (CDC, 2014) and Malawi has not been spared from this challenge. Malaria kills over one million victims every year and infects another 300 million worldwide (WHO, 1992). The disease affects mostly people from developing countries. The most vulnerable to malaria are pregnant women and under five year old children (Phillips, 2003).

The disease is transmitted by female *Anopheles* mosquitoes, for example *Anopheles gambiae* and *Anopheles funestus*. Therefore, one of the ways of protecting ourselves from the disease is through protection against disease vectors. This can be achieved by getting rid of female *Anopheles* mosquitoes.

Lymphatic filariasis is another disease that is transmitted by mosquitoes in Malawi. This disease is caused by *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* and is spread through bites of *Anopheles, Culex, Mansonia,* and *Aedes* mosquitoes (WHO, 2014). The worms invade lymphatic vessels and lymphoid tissue, causing chronic swelling of the lower extremities and other parts of the body (WHO, 2014). Lymphatic filariasis is the leading type of lymphodema world wide; it affects an estimated population of 120 million people. Over 40 million people are seriously

incapacitated and disfigured by the disease. This disease is mainly found in the tropical regions of the world, for example, South East Asia, the Indian subcontinent, Africa and areas of South America (Das, Subramanyam & Pan, 2002).

Lymphatic filariasis infection is spread when an infected female mosquito bites a person. One of the mosquitoes responsible for the transmission of filariasis is *Culex* mosquito (Turell, O'Quinn, & Jones, 2001). It injects microfilaria into the blood where it reproduces and spread throughout the bloodstream, and can live for many years. Symptoms of this disease take a very long time to appear after the infection. As parasites accumulate in the blood vessels, they restrict circulation and cause fluids to build up in surrounding tissues. Some of the signs indicating the infection are excessive enlargement of arms, legs, genitalia, and breasts (Das et., 2002).

The Ministry of Health (MoH) and some non-governmental organisations (NGOs) have tried their best to combat malaria and filariasis by employing several methods, ranging from vector to parasite control. As regards vector control, the following methods have been tried in Malawi: use of Insecticide treated mosquito nets (ITNs), Indoor residual spraying (IRS) using Perimiphosmethyl (Actellic), Fendona and DDT. However, each and every method stated has its own shortfalls. For example, use of Insecticide treated bed nets is very effective when you are in bed. In addition, use of Insecticide treated nets and spraying of insecticides in houses is highly effective but is also vulnerable to the development of resistance and behaviour change of vectors (Killeen, Fillinger, Kiche, Gouagna, & Knols, 2002; Vulule, Beach, Atieli, Roberts, Mount & Mwangi, 1994). A recent study also shows high level of resistance of *Anopheles funestus* to Pyrethroids in Malawi after mass net distribution and IRS

programmes were conducted (Mzilahowa, 2013). The over reliance on insecticides to control mosquitoes has led to physiological resistance of mosquito vectors including *Anopheles gambiae* (Koekemoer, Spillings, Christian, Lo, Kaiser, Norton & Coetzee, 2011; Koffi, Alou, Adja, Kone, Chandre, & N'Guessan, 2012), *Culex papiens* (Labbe, Berthomieu, Berticat, Alout, Raymond, Lenormand & Weill, 2007 ; Liu, Zhang, Qiao, Lu, & Cui, 2011) and *Aedes aegypti* (Dusfour, Thalmensy, Gaborit, Issaly, Carinci & Girod, 2011 ; Kamgang, Marcombe, Chandre, Nchoutpouen, Nwane, Etang & Paupy, 2011; Lima, Paiva, de Araujo, da Silva, da Silva, de Oliveira & de Melo Santos , 2011). The use of drugs to destroy the pathogen (*Plasmodium*) has also resulted into much more complex and resistant *Plasmodium* (Tjitra, Anstey, Sugiarto, Warikar, Kenangalem, Karyana, Lampah *et al.*, 2008*).*

Since effective control of mosquito-borne diseases is under threat from drug and insecticidal resistance, mosquito larvae control has recently received improved attention by the international scientific community and recent attempts to develop integrated vector management (IVM) strategies for different eco-epidemiological settings re-consider mosquito larva control as one of the tools to reduce malaria and filariasis transmission.

Although IRS can effectively control the *Anopheline* mosquito populations, this method is less effective in controlling *Culicine* mosquitoes. The reason is that Malawi is still using Pyrethroids in IRS programmes to which *Culicine* are resistant. Larval control, whether by insecticides, biological control agents, habitat modification or elimination remains a useful method for reducing *Culicine* populations (Silver, 2007).

In some countries *Bacillus thuringiensis var israelinsis (Bti)* is used as a means of controlling mosquitoes at larval stage. *Bti* is a naturally occurring rod-shaped soil bacterium. In the environment, it rests in a dormant stage as a spore up until it is ingested by an insect. Once it gets exposed to the alkaline environment and enzymes found in the gut of an insect, it gets activated. Then endotoxins are released which degrade the insect's gut lining and eventually the host dies (Suom & Smith, 2008). This is the mode of action for *Bti*. Although larval source reduction has been successful in Italy, Israel, United States, and parts of Brazil, as a tool for eradicating malarial vectors over a large scale (WHO, 1998; Killeen *et al.*, 2002) no attention has been given to larval control and environmental management as a means of reducing mosquito vector populations, and consequently mosquito-borne diseases, in Malawi.

1.2 Significance and Justification of the Study

This research is important because it will establish the optimal dose of *Bti* that can be used in controlling mosquito larvae in Malawi since different soils have different amounts of naturally occurring *Bti*. Therefore, the data generated will assist Malawian institutions which need to use *Bti* in controlling malaria, filariasis and any other disease that is transmitted by mosquito bites. Secondly, this is economically significant because cost will be reduced through the reduction of wastage of useful and expensive *Bti*.

No research has been conducted to determine the lowest amount of *Bti* that could be used in controlling mosquito larvae in Malawi. Because of the stated reasons, a research was conducted on the minimum efficacy dose of *Bacillus thuringiensis var israelinsis* to control *Anopheles* and *Culex* mosquito larvae in Zomba, Malawi.

1.3 Research Objectives

The main objective of this study was to evaluate the efficacy of *Bti* on mosquito larvae collected around Zomba, Malawi by

(i) Determining the lower effective dosage (minimum efficacy) of *Bti* on *Anopheles* and *Culex sp* mosquito larvae

(ii) Comparing the mortality rates of *Anopheles* and *Culex sp* mosquito larvae exposed to similar dosage of liquid *Bti*.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

It has been observed that the main methods used in controlling mosquitoes in Malawi are chemical in nature. However, insecticides are toxic and adversely affect the environment by contaminating soil, water and air (Kalu, Ofoegbu, Eroegbusi, Nwachukwu & Ibeh, 2010).Therefore, there is need to find alternatives to use of insecticides. Some insecticides pose a health risk to animals, for example DDT, when used in aquatic environment. DDT bio-accumulates as it goes up the food chain and eventually organisms at the very tip are exposed to high concentrations of DDT, with adverse effects. Therefore, it is of paramount importance that these biological control measures should be put in place.

Recent studies have shown that the use of larval control is a very effective method of reducing malaria transmission intensity, however, this method is being under-utilized (Killeen, McKenzie, Foy, Schieffelin, Billingsley & Beier, 2000).The use of larvicides is essential because mosquitoes are controlled in premature stages before dispersing and attaining the potential to transmit diseases (Killeen *et al*., 2002).

Furthermore, larvae control is advantageous over adult control because larvae are usually relatively immobile, concentrated and occupies a small habitat as compared to adult mosquitoes, therefore, easy to control (Floore, 2006).

Becker, Zgomba, Ludwig, Petric, & Rettich (1992) conducted an experiment to find out environmental factors influencing the effectiveness of microbial control agents in mosquito control programmes. Four factors were studied with *Bacillus thuringiensis var.israelensis* and these were water temperature, larvae density, sunlight and the effect of associated filter feeders. The study was conducted in Europe under laboratory and semi-field conditions using different instars of *Aedes vexans, Ae.aegypti* and *Culex pipiens*. The results indicated that the efficacy of *Bti* decreased in linear manner with increasing larval density. Sunlight reduced the effectiveness of *Bti*. Competition in food intake by filter feeders like the Daphnia resulted in lower mortality of mosquito larva after *Bti* application.

Chen, Lee, Nazni, Seleena, Lau, Daliza & Mohd (2009) conducted a study to find out the impact of larvaciding using a *Bti* (*Bacillus thuringiensis israelensis*) formulation (VectoBac WG) against *Aedes aegypti* larvae in earthen jars containing aquatic plants. Aquatic plant species used included *Pistia stratiotes* (L.) (Liliopsida: Araceae) and *Saggittaria* sp. (Liliopsida: Alismataceae) were placed inside earthen jars filled with 50 litres tap water. *Bti* formulation at 8g/1000L was used in treating all earthen jars. Untreated jars with and without aquatic plants were also set up as controls. Fifty laboratory-bred 2nd instar larvae were introduced into each earthen jar. All earthen jars were observed on daily basis. The results of this experiment indicated that the treated earthen jars containing *P. stratiotes* and *Saggitaria* sp showed significant residual

larvicidal effect of up to 7 weeks, in comparison to untreated control ($p < 0.05$). The larva mortality ranged from 77.34 to 100% for jars with aquatic plants vs. 80.66% to 100% for jars without aquatic plants. Earthen jars treated with *Bti* without aquatic plants also exhibited significantly longer residual larvicidal activity of up to 10 weeks ($p < 0.05$). The larval mortality ranged from 12.66% to 100% for jars with aquatic plants vs. 59.34% to 100% for jars without aquatic plants. Thus, earthen jars without aquatic plants exhibited longer residual larvicidal effect than those with aquatic plants. It is therefore concluded that containers with aquatic plants for landscaping must be treated more frequently with *Bti* because of the shortened residual activity.

Boisvert (2005), states that there are a number of factors affecting *Bti* activity against mosquitoes. Firstly, **mosquito species:** different species among mosquitoes exhibit different levels of susceptibility to the same *Bti* preparation. For example *Culex* and *Aedes* larvae are more susceptible than *Anopheles* larvae (Mulla, 1990).

Secondly, feeding **behaviour of mosquitoes**: those species which feed actively up and down the whole depth of shallow water body, for example, *Culex* and *Aedes* mosquito larva are at risk of ingesting lethal dose over a short period of time. On the contrary, *Anopheles* larvae, which feed at the surface-air interface of water, may not be able to ingest a lethal quantity of toxic particles in the relatively short period of time taken by particles that sink from the surface layer (Boisvert, 2005). Furthermore, studies have shown that *Anopheles* larvae ingest ten times less materials than *Aedes* (Mahmood, 1998). This laboratory result is very important in explaining the difference in susceptibility of mosquito larvae to toxic substances.

Thirdly, **Instar susceptibility**: younger instar larvae are more susceptible than older ones. This is so because late fourth instars cease feeding or feed little before pupation hence are much less susceptible to lethal dose (Boisvert, 2005). This was taken into consideration in the current study. That is why 3rd instar larvae were used.

Fourthly, **larval density**: this is one of the biological factors that influence larvicidal efficacy of *Bti*. For example, in an experiment, a given dosage of *Bti* that will control 95-100% of larvae that occur at low density will not produce identical results if larvae density was significantly increased (Boisvert, 2005).

Fifthly, **suspended organic matter:** if there are more organic and inorganic pollution or floating materials, more *Bti* would be required to obtain a given level of mortality because in the presence of all these particles less toxic particles are ingested per unit time than in the absence of these materials. Additionally, the availability of crystals is decreased by their adsorption onto suspended particles (Boisvert, 2005).

Water temperature is another factor that affects the efficacy of *Bti*. Although *Bti* is found to be active at low temperatures, its effectiveness may be reduced in cold water due to cessation or low rate of feeding in some species of mosquito larvae, because the metabolic rate decreases during cold seasons (Boisvert, 2005).

Finally, other factors which are very important in the efficacy of *Bti* are **intensive vegetative cover** and **increased water depth**.

An evaluation of the efficacy of new water-dispersible granular (WDG) formulations of *Bacillus thuringiensis var israelinsis* (*Bti*, VectoBac) and *B.sphaericus* (*Bs* VectoLex, Valent BioScience Corp., Illinois, USA) for the control of larval *Anopheles gambiae sensu lato* Giles mosquitoes was conducted in malarial-endemic areas around Lake Victoria, Western Kenya. WDG and powder formulations were compared in laboratory bioassays followed by efficiency and residual effect assessments of both WDG formulations in open field experiments. LC_{50} and LC_{95} values for the *Bti/Bs* strains and their formulations show high susceptibility of *A. gambiae sensu stricto* under laboratory conditions. The larvae proved more susceptible to *Bs* than to *Bti* and the WDG formulations were slightly superior to the powder formulations. High efficacy was also shown in the open field trials and a minimum dosage of 200 g/ha *Bti* WDG, representing the LC_{95} of the laboratory tests, was sufficient to fully suppress emergence of mosquitoes when applied at weekly intervals. *Bti* WDG did not show a residual effect, irrespective of the concentration applied. The *Bs* WDG formulation, however, showed significant larval reductions up to 11 days post-treatment at application doses of either 1 or 5 kg/ha. It was concluded that the main malaria vector in the study area is highly susceptible to these microbial control agents. Minimum effective dosages to achieve elimination of the larval population in a given habitat are extremely low and environmental impact is negligible. Microbial products for larval control have, therefore, great potential within Integrated Vector Management programmes and may supplement control efforts against adult vector stages, such as the use of insecticide-treated bed nets, in many parts of Africa (Fillinger, Knols & Becker, 2003).

Suom & Smith (2008) carried out an experiment on *Bacillus thuringiensis israelensis* (*Bti*) which is a commonly used larvicide in mosquito control programs. They tested the potency of VectoBac G (*Bti*) after it had been stored away for a year. Third instar *Ochlerotatus abserratus* larvae were subjected to five treatments: two sub-lethal dosages of 2.5 and 5 lbs/acre, minimum, and maximum label rate of 10 and 20 lbs/acre, and untreated control. Larval mortality was recorded at 1hr, 2hrs, 24hrs, and 48hrs post exposure. After 24 hours, more than 92% mortality was observed in larvae exposed to all treatments of *Bti*. 100% mortality was recorded after 48 hours of exposure to all treatments of *Bti*. The untreated control group reported 2% mortality after 48 hours. In conclusion, VectoBac G was still very potent and effective after one year in store.

Kroeger, Horstick, Riedl, Kaiser & Becker (1995) conducted a study on the efficacy of *Bti*. They sprayed *Bti* in breeding places in Pacific coast of Peru and Ecuador and in the Amazon area of Peru. It was found that *Bti* is a powerful larvacide for *Anopheles* larvae, although it sinks quickly, whereas *Anopheles* larvae feed at the water surface. In the two study areas, *Bti* was sprayed weekly over a period of 10 and 7 weeks, and the adult mosquito densities were monitored. The *Anopheles* adult density (bites per person per hour on human baits) was reduced by an average of 70% in one area and by up to 50% in the other. This means that *Bti* spraying can potentially be an important component in reducing Malaria cases.

2.2 Effect of *Bacillus thuringiensis* on Fish and Frogs

Some insecticides are hazardous to aquatic animals hence *Bti* was tried to assess its impact on animals that can associate with mosquito larvae. As such Christensen (1990a, b and c) conducted laboratory studies and found that *Bti* had no effect on blue gill fish (*Lepomis macrochirus*), Sheephead minnow (*Cyprinodon variegates*), and rainbow trout (*Oncorhynchus mykiss*) when exposed to 1.3 -1.7x 10^{10} cfu/g of diet.

WHO (1992) reviewed a number of laboratory and field work, and examined the impact of *Bt* on frogs, newts, salamanders and toads results showed no harmful effects on the organisms tested.

2.3 Advantages and disadvantages of using *Bacillus thuringiensis israelinsis*

Cranshaw (2010) highlighted a number of advantages and disadvantages of using *Bti* and some of them are highlighted below.

2.3.1 Advantages of using *Bacillus thuringiensis israelinsis*

- *Bti* is very specific**.** Therefore, it is regarded as very helpful as compared to other insecticides. *Bti* do not have a broad spectrum of activity; as such, they do not kill other important insects, such as honey bees.
- The greatest advantage is that *Bti* is non-toxic to people (WHO, 1999), wildlife and pets. This high degree of safety makes it easy to be used in food crops. Likewise, this makes it easier to be used in much sensitive areas where use of pesticides can cause undesirable consequence.
- *Bti* has low probability of causing resistance in mosquito vectors (Charles and Nielsen, 2000).

2.3.2 Disadvantages of using *Bacillus thuringiensis israelinsis*

- ***Bti* must be eaten to be effective**: this is a limiting factor in the sense that organisms that do not ingest it may survive despite its availability.
- **High specificity**: this may limit its use especially when used on crops where several pests would attack these plants.
- **Degradation by sunlight**: *Bti* is easily degraded by sunlight energy; as such, there is need of re-spraying after every two weeks which could be somehow involving to the applicant.
- **Shorter shelf life**: products with *Bt* component tend to have shorter shelf life than other insecticides. Generally, there is a reduced effectiveness in these products after they have been stored for more than two years. Their shelf life can be increased by keeping them in cold and dry places, and out of direct sunlight energy.
- The other shortfall with *Bacillus thuringiensis* is the limited treatment window available. Timing is very important during *Bti* application, the reason being that mainly it works well with the 2^{nd} and 3^{rd} instar. First instar larva is susceptible to *Bti;* however, it is not mainly targeted because the hatching is incomplete during this period. Treatment to 4^{th} instar mosquito larvae leads to undesirable results because larvae stop feeding prior to moulting to pupae (Ellis, 2001).
- In general the greatest challenge with the use of biolarviciding system is that mosquitoes may still migrate from neighbouring states to areas which have already been sprayed (Chukwu & Pate, 2011).

2.4 *Bacillus thuringiensis israelinsis* mode of action

When *Bacillus thuringiensis israelinsis* is eaten by mosquito larvae it gets dissolved in the alkaline gut fluids, and midgut proteases cleave the protoxin, yielding the active delta-endotoxin proteins. The binding of endotoxins to specific receptors results in an osmotic in balance across the midgut epithelial cell membranes, causing severe damage to the gut wall and this leads to rapid death (Chilcott, Kalmakoff & Pillai, 1983; Boisvert, 2005).

2.5 Mosquito life cycle

Figure 1 is a general life cycle of a mosquito. Within this life cycle the stage of greatest importance is mosquito larvae because this is the only stage where *Bacillus thuringiensis var israelinsis* works.

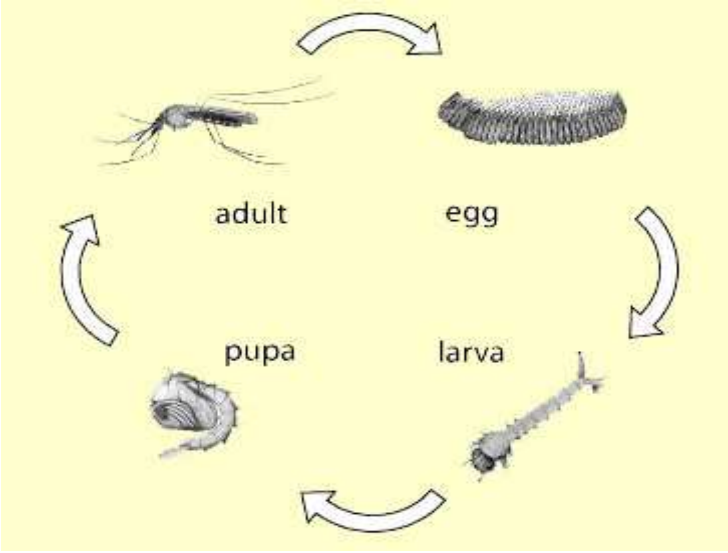

Figure 1: Mosquito life cycle
Adopted from: McCafferty (1983) in Alameda County Mosquito Abatement District (2014)

Egg: Several eggs are laid at a time depending on the mosquito species. The laid eggs float on the surface of water. *Culex* mosquitoes lay their eggs one at a time, sticking them together to form a raft of from 200- 300 eggs. A raft of eggs looks like a speck of soot floating on the water and is about 1/4 inch long and 1/8 inch wide. On the other hand *Anopheles* species do not make egg rafts but lay their eggs singly on the water. Both *Culex* and *Anopheles* mosquitoes lay their eggs on water surface while other species like *Aedes* lay their eggs on damp soil. After 48 hours most mosquitoes hatch into larvae (McCafferty, 1983).

Larva: It lives in water and comes to the surface to breathe. Larvae shed their skin four times during their growth. Most larvae have siphon tubes for breathing and hang from the water surface. *Anopheles* larvae do not have a siphon and they lay parallel to the water surface. The larvae feed on micro-organisms and organic matter in the water. On the fourth moult the larva changes into a pupa.

Pupa: This is a resting stage, also known as non-feeding stage. This is the time the mosquito turns into an adult. It takes about two days before the adult is fully developed. When development is complete, the pupa skin splits and the mosquito emerges as an adult.

Adult: The newly emerged adult rests on the surface of the water for a short time to allow itself to dry and all its parts to harden. Also, the wings have to spread out and dry properly before it can fly (McCafferty, 1983).

The egg, larvae and pupae stages depend on temperature and species characteristics as to how long it takes for development to be completed. For instance, *Culex tarsalis* might go through its life cycle in 14 days at 70 ^{0}F and take only 10 days at 80 ^{0}F. Some species have naturally adapted to go through their entire life cycle in as little as four days or as long as one month (McCafferty, 1983).

2.6 Historical background of Filariasis and Malaria in Malawi

2.6.1 Filariasis

Ngwira, Tambala, Perez, Bowie & Molyneux (2003) conducted a survey in which they targeted 23 districts in which 35 villages were sampled in Malawi. Antigenaemia prevalence [based on immunochromatographic card test (ICT)] ranged from 0% to 35.9%. The study showed that villages found in the western side of the country and distant from the lake tended to have lower prevalence, with the exception of Mchinji district which borders with Zambia; which had a prevalence of 18.2%. However, villages from lake shore districts had a prevalence rate of over 20%, e.g. Salima, Mangochi, Balaka and Ntheu (Bwanje valley) and Phalombe. In addition, districts found along Shire River have high cases of filariasis, for example, Chikhwawa district. In 2009 Malawi embarked on nationwide mass drug administration campaign (MDA) using two drugs, albendazole and ivermectin in order to combat the disease.

A recent study conducted by Reimer, Chiumia, Mzilahowa, Mkwanda & Hope (2013) in Chikhwawa found out that the prevalence of adult filarial worm antigen measured using rapid immunochromatographic test (ICT) cards ranged 4.1 to 38.5% and the prevalence of night blood microfilaria (MF) was estimated to range 0 to 7.5% with less than 10 km between the villages with the highest and lowest MF rates. Median

MF density was 4 MF/20 µL (range: 0.3 to 58.5 MF/20 µL). Self-reported MDA coverage in the fifth round ranged 69.2 to 90.2% and household LLIN coverage ranged 74.5 to 92.2% with approximately 20 km between villages with high and low coverage rates.

2.6.2 Lifecycle of Wuchereria bancrofti

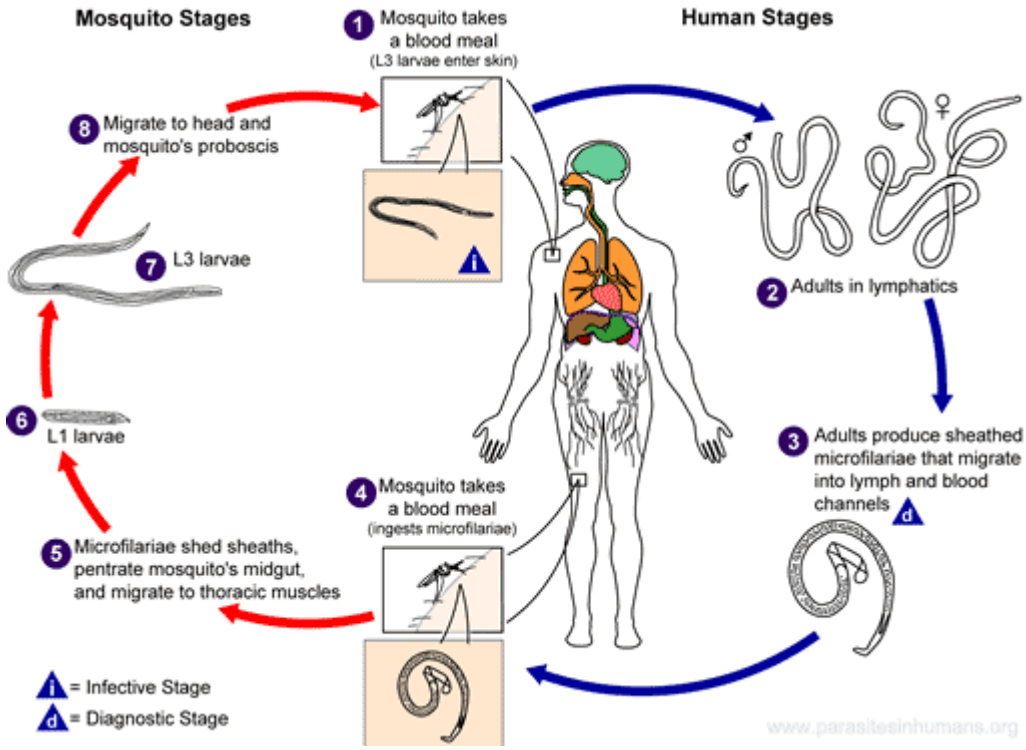

Figure 2: Generalised lifecycle of *Wuchereria bancrofti*
Adopted from Parasites in Humans (2014).

Lymphatic filariasis is spread when a vector mosquito bites a person who has the disease and then bites the other one who does not (Figure 2). Once it bites an infected person it picks up the microfilaria which circulates in the blood of the patient. *Wuchereria bancrofti* are nocturnal in many parts of the earth; they appear in blood only at night (Service, 2008). Their presence during this time coincides with the peak biting of mosquitoes. Once they have entered into the mosquito's stomach the

microfilaria penetrate the gastric wall and migrate to the insect's thoracic muscles where they mature. Thereafter, they migrate to the mosquito's labium (non-biting lower lip) of the proboscis. Mosquito's salivary glands do not play a direct role in the transmission of Lymphatic filariasis (Service, 2008) and this is contrary to malaria parasites.

The process from the time mosquitoes take an infected blood meal to the presence of the infective filarial worms in the labium takes 7-21 days. When the mosquito sucks blood again, the 1.2-1.6mm long infective larvae break through the labium and sneak into a person's skin. Infective larvae enter the human skin through the bite of the wound and travel to the lymph vessels where they develop into adults (Service, 2008). An adult larva stays in the human lymph nodes for 5-7 years, producing many microfilaria. Filarial worms damage the lymphatic system leading to its inappropriate functioning. Consequently, this results in accumulation of fluids and swelling of certain parts of the body, for instance arms, legs, breasts and genitalia as shown in Figure 3.

Figure 3: An individual suffering from Lymphatic Filariasis

Adopted from: http://www.ehow.com (by Wolfe, 2012)

2.6.3 Treatment of lymphatic Filariasis

Lymphatic filariasis can be treated by using the most commonly used drug Diethyl-carbamazine (DEC).This kills both the microfilaria and adult worms. Although this is not licensed for use in the United Kingdom (UK), it can be used on a named patient basis (Palumbo, 2008). Some drugs can be used alone or in combination with DEC. For example, Ivermectin can be used for *Wuchereria bancrofti* alone or in combination with DEC. It is very effective but has adverse reactions once taken, hence the need for close supervision (De Sole, Remme, Awadzi, Accorsi, Alley, Ba & Keita, 1989). Mebendazole and its analogue flubendazole may also be used, and the other possibility is the use of Albendazole. A single dose of Ivermectin with or without albendazole appears to be effective to treat *W. bancrofti* infection (Dunyo, Nkrumah & Simonsen, 2000; Reddy, Gill & Kalkar, 2007).

2.7 Malaria and its cause

There are four species of protozoan parasite of genus *Plasmodium* that cause malaria in humans and these are *P. falciparum, P. vivax, P. ovale* and *P. malariae*. Malaria caused by *P.vivax* is the most common. However, the most lethal is the one caused by *P. falciparum* (WHO, 2014).

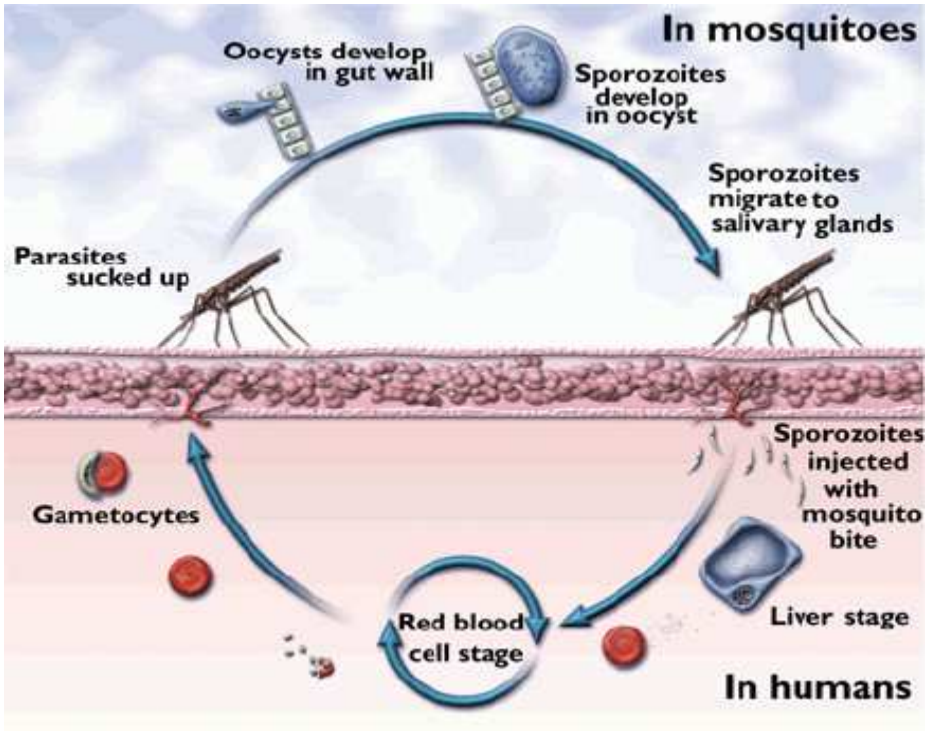

Figure 4: A generalised lifecycle of Plasmodium that causes Malaria
Adopted from physiopedea (2012) - http://www.physio-pedia.com

The bites of female *Anopheles* mosquitoes transmit Malaria. Parasites are injected into the human body through the saliva of a mosquito (Figure 4). Within 30 minutes after the bite, uni-nucleated sporozoites migrate to the liver and invade hepatocytes (liver cells) and develop into Schizonts (McW Healthcare, 2008). Schizonts multiply in liver cells until there is no space left. Within a week of entering the liver cells, mature liver stage Schizonts rupture spilling merozoites into the bloodstream. These merozoites in the blood stream attack circulating erythrocytes and develop into trophozoite, secreting proteins that form knobs on the erythrocyte membrane. With the help of these knobs it attaches itself to the capillary wall affecting the microcirculation. On maturity the erythrocyte ruptures and merozoites spill out from each one which in turn invades other erythrocytes which have been unaffected (McW Healthcare, 2008).

CHAPTER THREE

METHODOLOGY AND RESEARCH DESIGN

3.1 Mosquito collection

Female blood-fed mosquitoes were collected from the field using aspirators. Figure 5 shows an aspirator used in the adult mosquito collection.

Figure 5: Aspirator

Blood meal taken by female mosquitoes is very important because proteins from blood are primarily used in the development of eggs, but it is also used as a source of energy (Smartt, Richards & Anderson, 2009). Collected mosquitoes were placed in collecting cups. Figure 6 is a diagram showing one of the collecting cups that were used during mosquito collections in the field.

Figure 6: Collection Cup

Upon arrival in insectaries, collected blood fed mosquitoes were transferred to cages (Figure 7) where they were reared.

Figure 7: Mosquito cage

The collected adult mosquitoes were fed 10% sugar solution that was soaked in cotton wool and placed on top of the cage. Egg cups were placed in cages when it was observed that the collected mosquitoes were gravid. It took three days for the collected blood-fed mosquitoes to become gravid. Thereafter, moist filter papers were placed in plastic cups with a small amount of water to keep the filter paper moist. Egg cups were left in the cage overnight and the following morning they were removed and eggs collected. These eggs were then placed in plastic containers (24 x 15 cm) with distilled water for hatching. No food was placed in the containers with eggs up until the 1st instar appeared. During early stages, larvae were fed sparingly to avoid

over feeding. Yeast (10%) was used to feed mosquito larvae. Sieving was done once water became dirty. This ensured favourable environment for survival of mosquito larvae. Mosquito larvae were kept up until they reached 3rd instar, which was the time they were ready for testing.

Mosquito samples used in this study were collected from Chikanda, Chilole, Chiliko and Mpwepwe within Zomba district as shown in Figure 8. In the township, that is where *Culex* mosquito samples were found while *Anopheles* mosquito samples were found close to Lake Chirwa hence the choice of these sites.

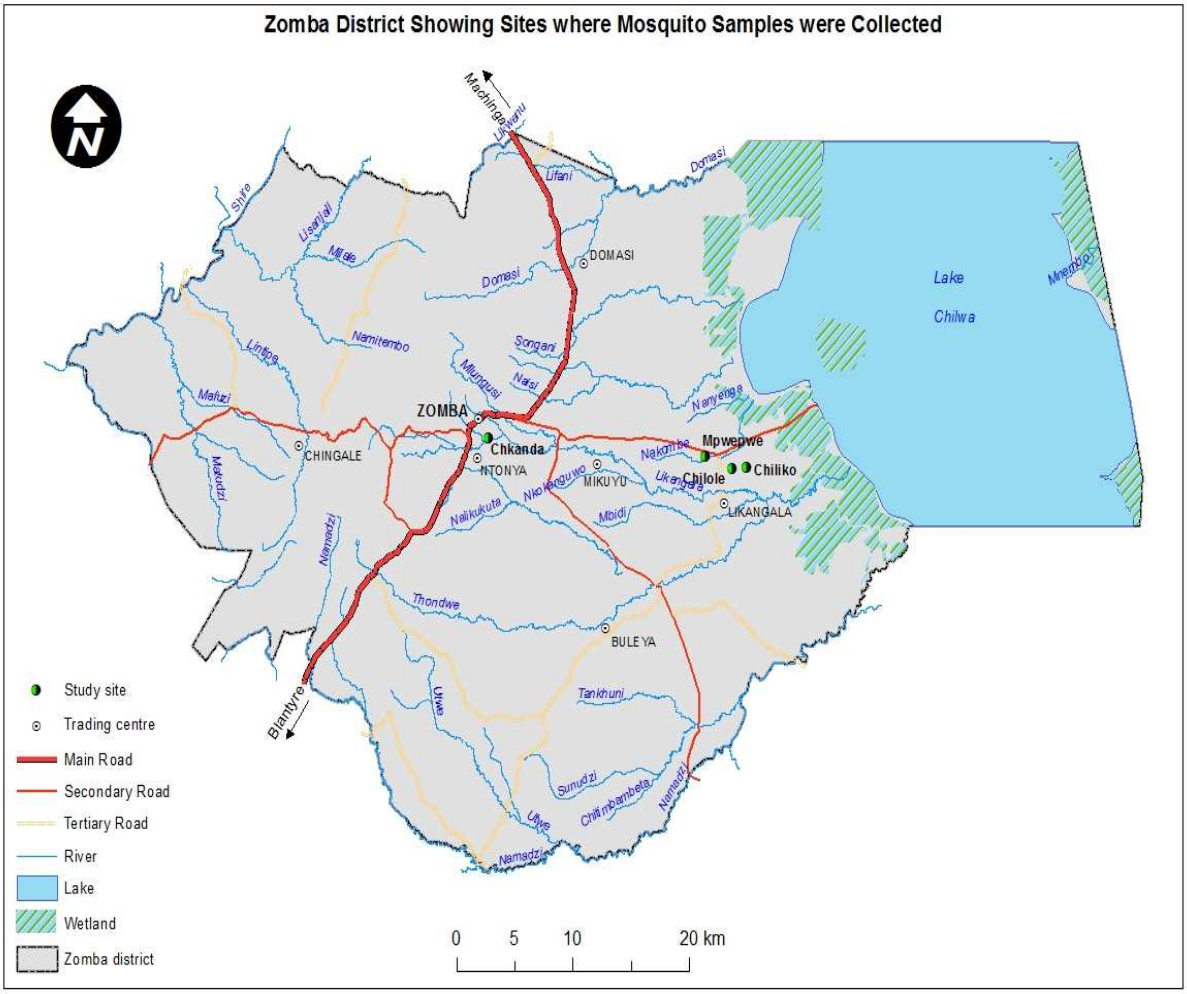

Figure 8: Map of Zomba District showing sites where mosquito samples were collected

3.1.1 Mosquito larvae and pupae collection

Mosquito larvae and pupae were collected from stagnant water. These were collected by using the dip method. In this case, a basin was dipped into stagnant water and if mosquito larvae and pupae were found, a pipette was used to transfer mosquito larvae to collecting bottles.

All pupae were placed in a cage where they emerged into adults, while larvae were placed in containers and were fed yeast up until they became pupae and then eventually adults. Adult mosquitoes were identified into *Anopheles* and *Culex* by using morphological keys (Gillies and Coetzee, 1987). The identified species were placed into separate cages and got blood-fed with albino rats, *Rattus norvegicus Albinus,* to allow egg laying.

3.1.2 Mosquito blood feeding

Firstly, *Rattus norvegicus Albinus* were exposed to Chloroform to get them to sleep. Then their abdominal part was shaved to remove abdominal fur, so that mosquitoes can easily access the skin. Thereafter, the rats were placed on a cage, exposing the shaved belly to mosquitoes for ten minutes and this allowed mosquitoes to suck up blood. During blood feeding all lights were switched off because mosquitoes prefer biting in the dark. Three days after blood feeding, mosquitoes became gravid and ready to lay eggs. Thereafter, a cup with moist filter paper (egg cup) was placed in the cage to allow the mosquitoes to lay eggs. After laying eggs, filter papers were placed in larger containers to allow hatching into mosquito larvae. Larvae were fed 10% concentration of dissolved yeast. The larvae were allowed to grow from 1^{st} to 3^{rd} instar, when they were ready for experiments.

3.2 Preparation of *Bti* solutions

Six different concentrations of *Bti* were made basing on manufacture's recommended concentration as standard. These were 3/2 of the manufacture's concentration, 1 of the manufacture's concentration, 3/4 of the manufacture's concentration, 1/2 of manufacture's concentration, ¼ of the manufacture's concentration and control which was 0 of the manufacture's concentration. Plastic basins of capacity 20 litres each were used in these experiments. Twenty mosquito larvae were used per basin. Since the set up was in triplicate, a total of 320 mosquito larvae were used per experiment. Mosquito larvae were selected randomly from stock containers to compensate for the differences in body mass. Mosquito larvae were fed during the experimental set up to avoid starvation and to initiate the ingestion process of *Bti*. Temperature readings were within the range of 22^0C to 28^0 C and humidity within the range of 72% to 85%.

3.3 How mortality of mosquito larvae was scored

Twenty mosquito larvae were kept in each basin and thereafter *Bti* was introduced**.** A glass rod was used to determine whether the mosquito larvae were dead or not. After every one hour, this rod was dipped into the basin and brought very close to each and every larva. The larva that was still alive would respond rapidly by either bending itself or moving away from the glass rod. The dead larvae could not respond no matter how close the glass rod was brought, and these larvae were, therefore, scored dead. The results were recorded on a data sheet, and subsequently entered into SPSS version 16.0 database for analysis.

During this set up where mortality exceeded 10% in the controls, the experiment was discarded and repeated (Fillinger et al., 2003). Two different types of mosquito larvae

were used in this experiment, namely, *Anopheles* and *Culex*. All these experiments were conducted in Biology laboratory at Chancellor College, Zomba.

3.4 Preparation of granular *Bti* in the experimental set up

The *Bti* used was manufactured by Valent Biosciences, and recommends that 200g of VectoBac®WG be sprayed or used per hectare. However, the experimental set up used plastic basins with a diameter of 40 cm.

Therefore the surface area of the basins was found by using the formula below:

$\Pi r^2 = 22/7 \times 20 \times 20$

$= 1257.142857$

$= \underline{1257.14\ cm^2}$

The basin surface area was calculated as a fraction of the hectare to establish the required amount of *Bti* at manufacturer's dosage.

If 200g $= 100\ 000\ 000\ cm^2$

$1257.142857/100\ 000\ 000 \times 200g = 1257.142857\ cm^2$

$=\underline{0.002514g}$

In this case we wanted to find the lower dosage than the recommended, hence 3/2, 1, 3/4, 1/2, 1/4 and 0 of the recommended concentrations were prepared as indicated in Table 1.

Table 1: Preparation of granular *Bti*

Treatment	Amount of *Bti* in g/cm^2	Amount of *Bti* in mg/cm^2
1	0.0038	3.8
2	0.0025	2.5
3	0.0019	1.9
4	0.0013	1.3
5	0.0006	0.6
6	0 (control)	0 (control)

3.5 Dilutions of liquid *Bti* based on concentrations

Manufactures (VALENT BIOSCIENCES$_{TM}$) of VectoBac® 12AS recommends 0.5 litre per ha in clean water. In this regard liquid *Bti* stock solution was prepared by dissolving 2ml of *Bti* into 20 000ml of water. As such several dilutions were made from this stock as indicated below;

Table 2: Preparation of liquid *Bti*

Treatment	Concentration of *Bti* in ml/L	Concentration of *Bti* in µl/L
1	0.0015	1.5
2	0.001	1.0
3	0.00075	0.75
4	0.0005	0.50
5	0.00025	0.25
6	0 (control)	0 (control)

These different concentrations were applied to mosquito larvae and mortality scored hourly.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

Results collected in this study mainly report on the mortalities of *Culex* and *Anopheles* mosquito larvae exposed to different concentrations of liquid and granular *Bacillus thuringiensis israelinsis*. The data was entered and analyzed by SPSS version 16.0. One way ANOVA was used to compare the means of mortality rates of mosquito larvae exposed to different concentrations of *Bti*. Due to significant differences that appeared between the means, the results were further analysed by Posthoc test. This test was chosen to specifically figure out where the differences occurred. In addition, t-test was used to find out if there were significant differences in mortalities of different mosquito species exposed to a similar dosage of *Bti*. Furthermore, to determine the LT_{50} and LT_{90} the data was analysed using probit analysis and Grafit.

4.2 To find lower effective dosage on *Culex* and *Anopheles* mosquito larvae using granular and liquid *Bti*.

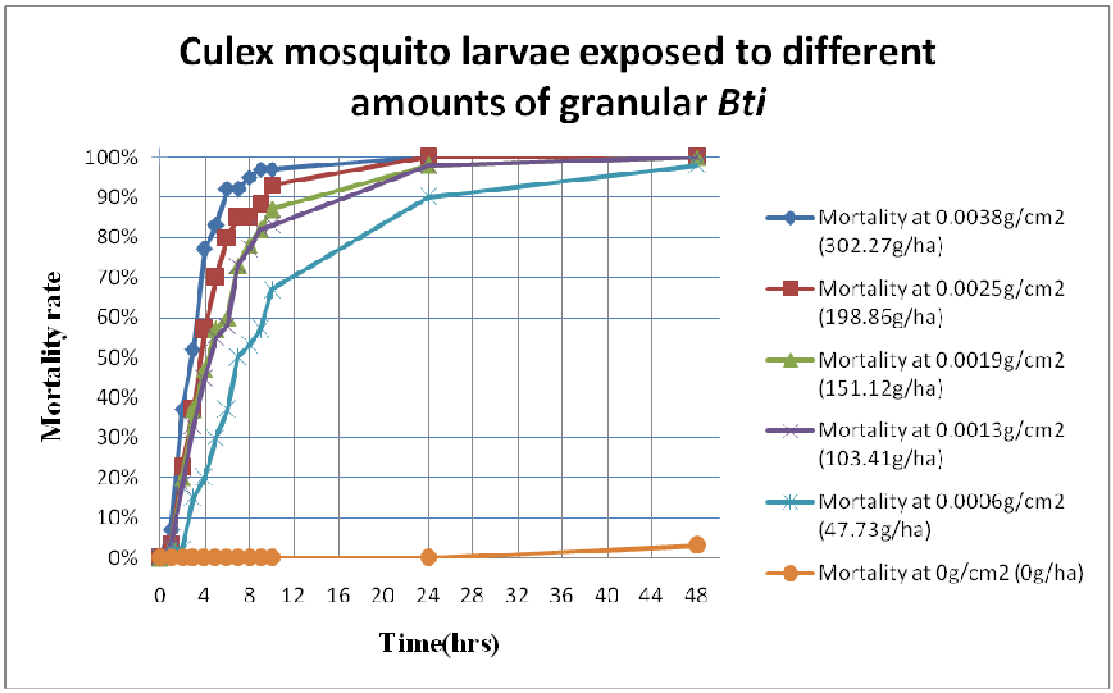

Figure 9: Graph showing mortality of *Culex* larvae exposed to different amounts of granular *Bti*.

Figure 9 (see also Table 8, Appendix1) shows that when Culex mosquito larvae were exposed to granular *Bti* at a concentration of 302.27g/ha and 198.86g/ha, 100% mortality rate was achieved within 24hours, where the initial number of larvae, n=60. When 151.12g/ha and 103.41g/ha were used, 100% mortality was achieved after 48 hours of exposure (n =60), respectively. Finally, using 47.73g/ha achieved the population reduction by 98% (n=59).

Table 3: Data analysed in SPSS (*Culex* mosquito larvae exposed to granular *Bti*)

(I)Concentration of *Bti* used in controlling mosquito larvae	(J)Concentration of *Bti* used in controlling mosquito larvae	Mean difference (I-J)	Std Error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
198.86g/ha	302.27g/ha	0	0.27217	1.000	-0.593	0.593
	151.12g/ha	0	0.27217	1.000	-0.593	0.593
	103.41g/ha	0	0.27217	1.000	-0.593	0.593
	47.73g/ha	0.33333	0.27217	0.244	-0.2597	0.9263
	0g/ha	19.33333*	0.27217	0.001	18.7403	19.9263

Table 3 shows that during the 48^{th} hour, if we use granular *Bti* to control *Culex* mosquito larvae there was no significant difference in using 302.27g/ha, 198.86g/ha, 151.12g/ha,103.41g/ha and 47.73g/ha since the results are at 100% (n=60) mortality except 98% (n=59) and all of them have a p-value which is greater than 0.05. Therefore, we conclude that the lower effective dosage that can be used in controlling *Culex* mosquito larvae in Zomba is 47.73g/ha, considering that our cut off point is 90%. Its LT_{50} and LT_{90} are 7.5 hours and 24.3 hours, respectively (Appendix 3a).

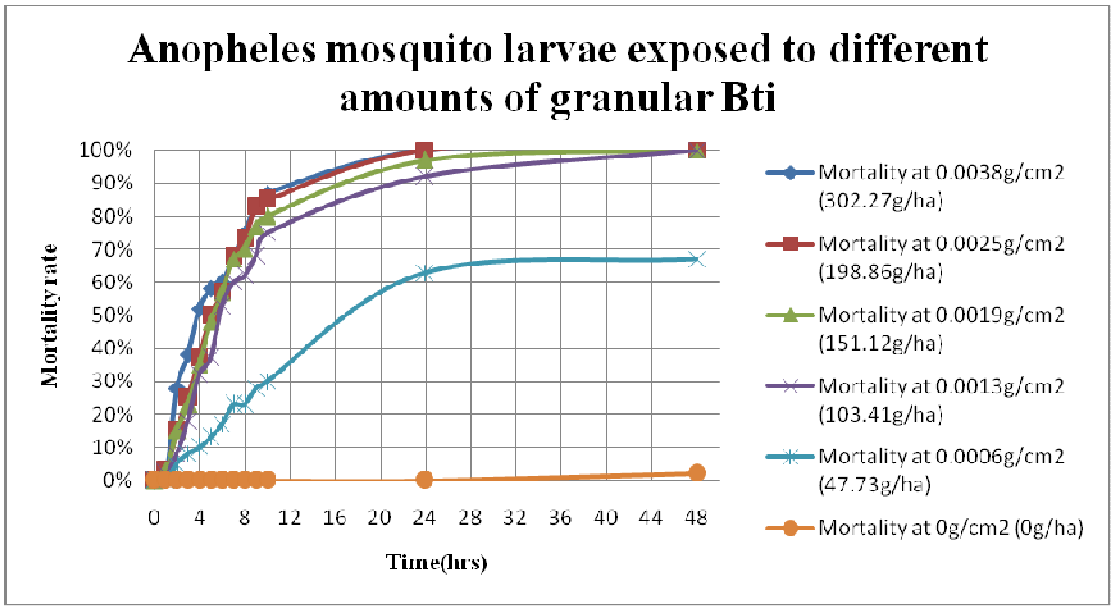

Figure 10: Graph showing mortality of *Anopheles* mosquito larvae exposed to different amounts of granular *Bti*

Figure 10 (see also Table 9, Appendix 1) shows that when *Anopheles* mosquito larvae are exposed to granular *Bti*, it takes 24 hrs to attain 100% mortality if 302.27g/ha and 198.86g/ha are used. If we use 151.12g/ha and 103.41g/ha, it takes 48 hours to attain 100% mortality rate of *Anopheles* mosquito larvae. However, if 47.73g/ha are used and 48hrs of exposure allowed, there is only 67% population reduction which is far below the cut off point of 90%.

Table 4: Data analysed in SPSS (*Anopheles* mosquito larvae exposed to granular *Bti*)

(I)Concentration of *Bti* used in controlling mosquito larvae	(J)Concentration of *Bti* used in controlling mosquito larvae	Mean difference (I-J)	Std Error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
198.86g/ha	302.27g/ha	0	0.98131	1.000	-2.1381	2.1381
	151.12g/ha	0	0.98131	1.000	-2.1381	2.1381
	103.41g/ha	0	0.98131	1.000	-2.1381	2.1381
	47.73g/ha	4.66667*	0.98131	0.001	2.5286	6.8048
	0g/ha	19.33333*	0.98131	0.001	17.1952	21.4714

The information in Table 4 shows that there is no significant difference in controlling *Anopheles* mosquito larvae by using 302.27g/ha, 198.86g/ha, 151.12g/ha, and 103.41g/ha since the following mortalities were achieved 100%(n=60),100%(n=60),100%(n=60) and 100%(n=60) respectively. In addition all have a p-value greater than 0.05. Therefore, *Anopheles* larvae can be controlled by using 103.41g/ha of granular *Bti*, and its LT_{50} is 6.2 hours while its LT_{90} is 18.5 hours (Appendix 3b).

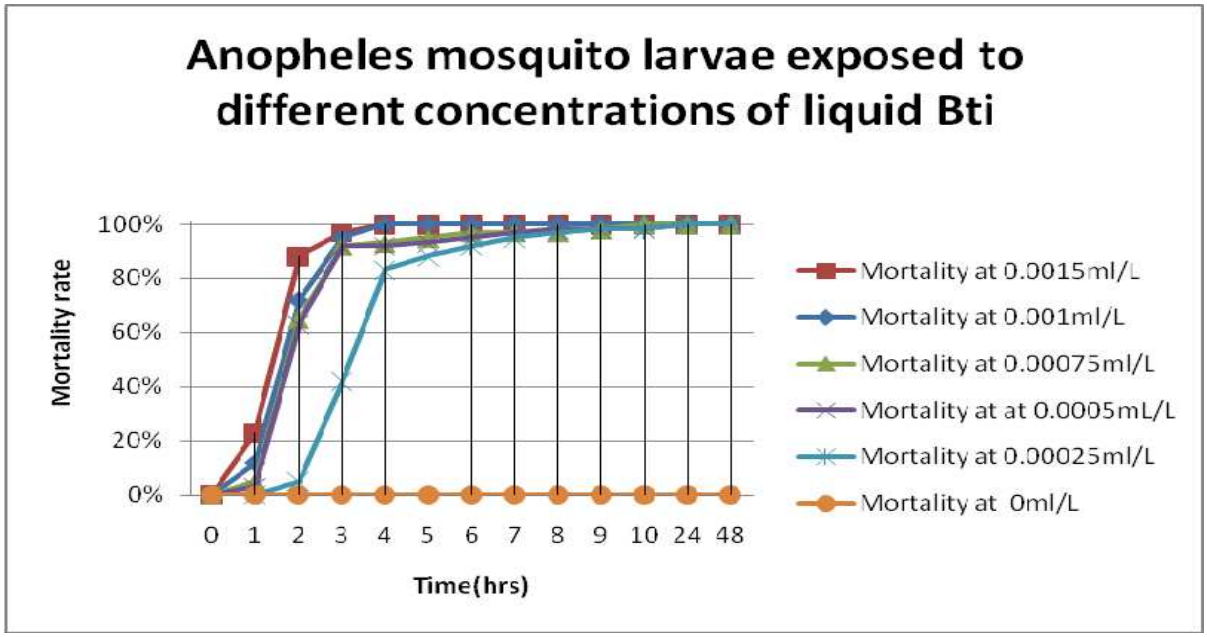

Figure 11: Graph showing mortality of *Anopheles* mosquito larvae exposed to different concentrations of liquid *Bti*

Figure 11(see also Table 10, Appendix 1) indicates that when 0.0015ml/L and 0.001ml/L are used, 100 %(n=60) mortality of *Anopheles* mosquito larvae was achieved within 4 hours. If *Anopheles* mosquito larvae are exposed to 0.00075ml/L of liquid *Bti* 100 %(n=60), mortality was achieved within 10 hours. If exposed to 0.0005ml/L and 0.00025ml/L of *Bti* 100% (n=60) mortality was achieved within 24 hours.

Table 5: Data analysed in SPSS (*Anopheles* mosquito larvae exposed to liquid *Bti*)

Dependent Variable	(I)Concentration of *Bti* used in controlling mosquito larvae	(J)Concentration of *Bti* used in controlling mosquito larvae	Mean difference (I-J)	Std Error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
10 Hours	0.0015ml/L	0.001ml/L	.00000	.27217	1.000	-.5930	.5930
		0.00075ml/L	.00000	.27217	1.000	-.5930	.5930
		0.0005ml/L	.33333	.27217	.244	-.2597	.9263
		0.00025ml/L	.33333	.27217	.244	-.2597	.9263
		0.00000 ml/L	20.00000*	.27217	.000	19.4070	20.5930

Table 5 indicates that there is no significant difference in using 0.001ml/L, 0.00075ml/L, 0.0005ml/L and 0.00025ml/L because all of them result into 100 % (n=60) mortality and all have a p-value greater than 0.05. Therefore, the lower effective dosage that can be used to control *Anopheles* mosquito larvae is 0.00025ml/L and its LT_{50} and LT_{90} are 3.2 hours and 5.5 hours, respectively (Appendix 3c).

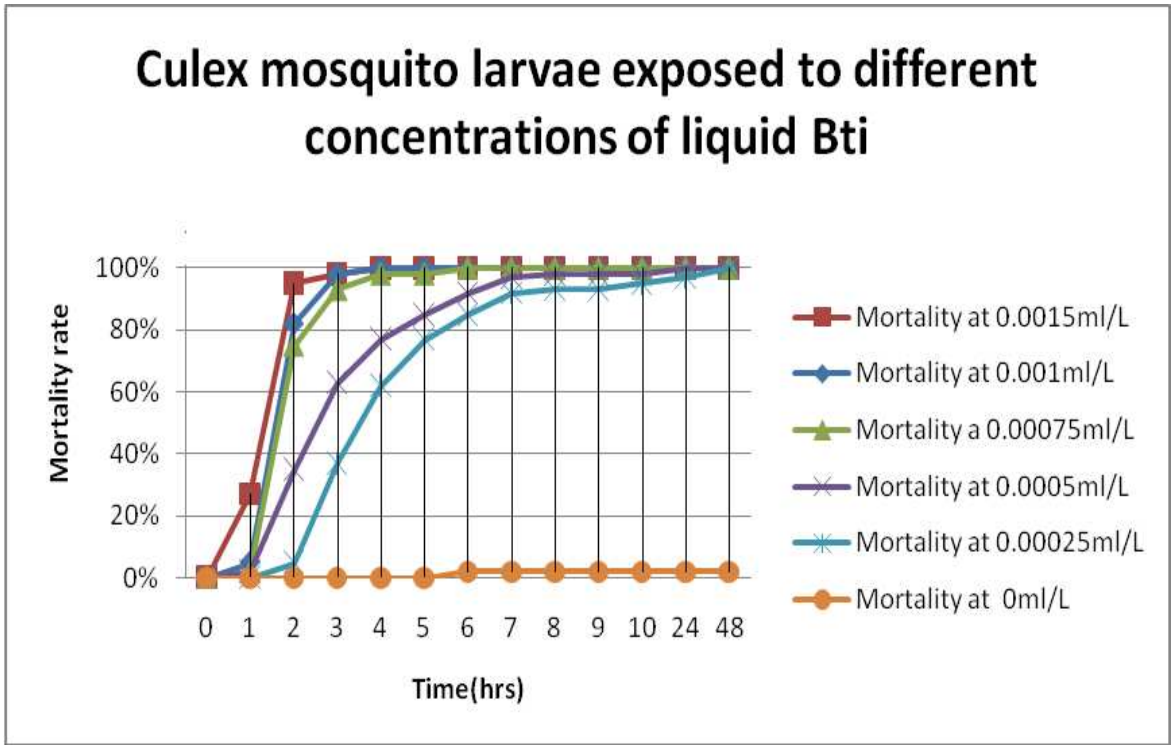

Figure 12: Mortality of *Culex* mosquito larvae exposed to different concentrations of liquid *Bti*

Figure 12 (see also Table 11, Appendix 1) illustrates that the more concentrated the solution of *Bacillus thuringiensi*s is the less the time is required to achieve 100% mortality rate. For instance, it took only four hours for the 0.0015ml/L and 0.001ml/L to kill 100% (n=60) *Culex* mosquito larvae. While for 0.00075ml/L of *Bti* it took six hours to kill 100 % (n=60) *Culex* mosquito larvae. For 0.0005ml/L it took 24 hours to attain a 100% (n=60) mortality rate. Finally, the one with the least concentration of 0.00025ml/L led to100% (n=60) mortality of *Culex* mosquito larvae after 48 hours of exposure. Hence should we need to minimise the wastage of useful *Bti*, it is better to consider the lower effective dosage that would result into the same mortality after 48 hours of exposure. Therefore, 0.00025ml/L would be recommended for the control of *Culex* mosquito larvae, and its LT_{50} and LT_{90} are 3.6 hours and 6.8 hours, respectively (Appendix 3d).

Table 6: Results of exposing *Culex* mosquito larvae to liquid *Bti* for 48 hours

Dependent Variable	(I)Concentration of *Bti* used in controlling mosquito larvae	(J)Concentration of *Bti* used in controlling mosquito larvae	Mean difference (I-J)	Std Error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
48 Hours	0.0015ml/L	0.001ml/L	.00000	.19245	1.000	-.4193	.4193
		0.00075ml/L	.00000	.19245	1.000	-.4193	.4193
		0.0005ml/L	.00000	.19245	1.000	-.4193	.4193
		0.00025ml/L	.00000	.19245	1.000	-.4193	.4193
		0.00000 ml/L	19.66667*	.19245	.000	19.2474	20.0860

Table 6 indicates that after exposing *Culex* to liquid *Bti* for 48 hours, there is no significant difference in using 0.001ml/L, 0.00075ml/L, 0.0005ml/L and 0.00025ml/L. All result into 100% (n=60) mortality and have a p-value of 1 which is greater than 0.05. Therefore, the lower effective dosage required to be used is 0.00025ml/L.

4.3 Mosquito genera response to *Bti*

To achieve the second objective, namely:

> Comparing the mortality rates of *Anopheles* and *Culex sp* mosquito larvae exposed to the recommended dosage of liquid *Bti* (0.001ml/L), and for equal duration

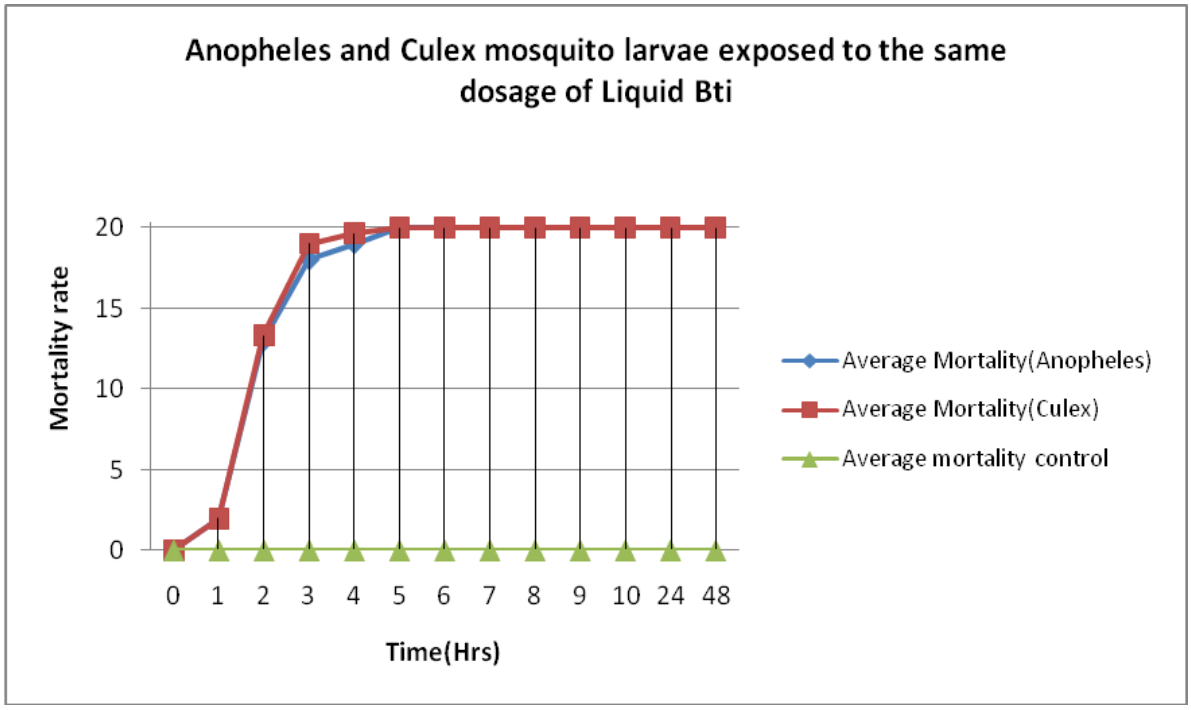

Figure 13: Comparison of mortality rates of *Culex* and *Anopheles* mosquito larvae exposed to the same dosage of liquid *Bti*

Figure 13(see also Table 12, Appendix 1) shows mortality rates for *Culex* and *Anopheles* mosquito larvae when exposed to the same dosage of liquid *Bti* (0.001ml/L). Both species respond in the same way during the 1st hour, but between 2nd and 4th hours *Culex* die faster than *Anopheles* larvae while between 5th to 48th hours the mortalities registered were the same (100%,n=60). Despite that *Culex* mosquito larvae died faster than *Anopheles* larvae, their mortalities were not significantly different p=0.09.

Table 7: Comparison of mortality rate between *Culex* and *Anopheles* larvae using t-test

Mortality rate of mosquito larvae	Type of Mosquito	Mean	Std Deviation	Sig.(2-tailed)
	Culex	16.54	7.15	0.0935
	Anopheles	16.31	7.08	

Table 7 shows that p-value (0.0935) is greater than 0.05. Therefore, we accept the null hypothesis. Hence, we conclude that there is no significant difference between the mortality rate of *Culex* and *Anopheles* mosquito larvae exposed to the same dosage of liquid *Bti* (0.001ml/L). Hence, when an area is infested with mosquito larvae we can apply 0.001ml/L of *Bti* regardless of whether it is *Anopheles* or *Culex* larvae.

4.4 Probit analysis and Grafit

The two methods were used to calculate LT_{50} and the results were not significantly different. For example, when we used probit analysis to find the LT_{50} of *Anopheles* mosquito larvae exposed to liquid *Bti* at 0.00025ml/L the value of LT_{50} was 3.2 hrs, while this was 3.1 hrs using Grafit and the two are not significantly different. However, probit analysis was preferred for use throughout in this paper because it was able to accommodate a number of variables. Refer to Appendix 2 on comparison of using Probit Analysis and Grafit to find $LT_{50.}$

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The study has established the lower effective dosage of granular *Bti* on *Culex* mosquito larvae as 47.73g/ha and its LT_{50} and LT_{90} are 7.5 hours and 24.3 hours, respectively. It has also shown that the minimum effective dosage of granular *Bti* for *Anopheles* mosquito larvae is 103.41g/ha and its LT_{50} is 6.2 hours and its LT_{90} is 18.5 hours.

The results obtained show that the effectiveness of *Bacillus thuringiensis var israelinsis* is higher on the larvae of *Culex* as it requires less amount of *Bti* (47.73g/ha) as compared to *Anopheles* larvae which requires 103.41g/ha within the same period of 48 hours to cause 100%(n=60) mortality. This shows high sensitivity of *Culex* larvae as compared to *Anopheles* larvae. This observation can be explained by physiological and behavioural differences in the species under study. For example, *Culex* larvae feeds actively up and down the whole depth of shallow water body hence at risk of ingesting lethal dose over a short period of time. On the contrary, *Anopheles* larvae, which feed at the surface-air interface of water, may not be able to ingest a lethal quantity of toxic particles in the relatively short period as particles sink from the surface layer. These results are in line with what Boisvert (2005) and Kroeger *et al.*, (1995) observed. Likewise, Aly & Mulla (1987) stated that the larvae of *Anopheles* would show a higher death rate if the crystals of *Bacillus thuringiensis* were delivered under a floating formulation.

The study also established that when *Culex* larvae and *Anopheles* larvae are exposed to the same dosage of liquid *Bti* (0.001ml/L), *Culex* larvae were slightly more susceptible as compared to *Anopheles* larvae. However, there were no significant differences in their mortalities as $p>0.05$. The results also show that liquid *Bti* performs much faster than granular *Bti*. This is in agreement with Foster & Smith (2013) who found out that liquid suspension disperses evenly in water than granular *Bti,* hence performs faster. In addition, granular *Bti* is dry while liquid *Bti* is alive and active in a liquid.

It has been found out that both *Culex* and *Anopheles* mosquito larvae are susceptible to *Bti*. However, when they are allowed to emerge into adult mosquitoes, *Culex* becomes completely resistant to pyrethroids and the emergence of resistance in *Anopheles* species to pyrethroids in Malawi, especially *Anopheles funestus,* is worrisome (Wondji, Coleman, Kleinschmidt, Mzilahowa, Irving, Ndula, Rehman *et al.*, 2012) so *Bti* is more ideal.

In short, *Bti* whether liquid or granular is very important as it significantly reduces the densities of mosquito larvae, and this has a direct impact in reducing populations of adult mosquitoes and consequently a reduction in the transmission of Malaria, Lymphatic filariasis and other diseases that are spread by mosquito bites.

5.2 Recommended areas of further research

Compare the mortality rate of mosquitoes at species level exposed to the same dosage of *Bti*.

Conduct the field trials of *Bti* on mosquito larvae from all the three regions in Malawi

Find out the residual effect of the minimum recommended effective dosage of *Bti*.

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APPENDICES

Appendix 1: Tabular presentation of mosquito larvae mortalities

Table 8: Mortality of *Culex* mosquito larvae exposed to different amounts of granular *Bti*

Time (Hrs)	*Mortality at 0.0038g/cm^2 (302.27g/ha)	*Mortality at 0.0025g/cm^2 (198.86g/ha)	*Mortality at 0.0019g/cm^2 (151.12g/ha)	*Mortality at 0.0013g/cm^2 (103.41g/ha)	*Mortality at 0.0006g/cm^2 (47.73g/ha)	*Mortality at 0g/cm^2 (0g/ha)
0	0%(n=0)	0%(n=0)	0%(n=0)	0%(n=0)	0%(n=0)	0%(n=0)
1	7%(n=4)	3%(n=2)	3%(n=2)	3%(n=2)	2%(n=1)	0%(n=0)
2	37%(n=22)	23%(n=14)	20%(n=12)	18%(n=11)	2%(n=1)	0%(n=0)
3	52%(n=31)	37%(n=22)	37%(n=22)	33%(n=20)	15%(n=9)	0%(n=0)
4	77%(n=46)	57%(n=34)	47%(n=28)	45%(n=27)	20%(n=12)	0%(n=0)
5	83%(n=50)	70%(n=42)	57%(n=34)	55%(n=33)	30%(n=18)	0%(n=0)
6	92%(n=55)	80%(n=48)	60%(n=36)	58%(n=35)	37%(n=22)	0%(n=0)
7	92%(n=55)	85%(n=51)	73%(n=44)	73%(n=44)	50%(n=30)	0%(n=0)
8	95%(n=57)	85%(n=51)	78%(n=47)	77%(n=46)	53%(n=32)	0%(n=0)
9	97%(n=58)	88%(n=53)	82%(n=49)	82%(n=49)	57%(n=34)	0%(n=0)
10	97%(n=58)	93%(n=56)	87%(n=52)	83%(n=50)	67%(n=40)	0%(n=0)
24	100%(n=60)	100%(n=60)	98%(n=59)	98%(n=59)	90%(n=54)	0%(n=0)
48	100%(n=60)	100%(n=60)	100%(n=60)	100%(n=60)	98%(n=59)	3%(n=2)

***Note:** 60 mosquito larvae were exposed to each concentration; n= total number of dead mosquito larvae

Appendix 1(Continued)

Table 9: Mortality rate of *Anopheles* mosquito larvae exposed to different amounts of granular *Bti*

Time (Hrs)	*Mortality at 0.0038g/cm^2 (302.27g/ha)	*Mortality at 0.0025g/cm^2 (198.86g/ha)	*Mortality at 0.0019g/cm^2 (151.12g/ha)	*Mortality at 0.0013g/cm^2 (103.41g/ha)	*Mortality at 0.0006g/cm^2 (47.73g/ha)	*Mortality at 0g/cm^2 (0g/ha)
0	0%(n=0)	0%(n=0)	0%(n=0)	0%(n=0)	0%(n=0)	0%(n=0)
1	3%(n=2)	3%(n=2)	3%(n=2)	2%(n=1)	0%(n=0)	0%(n=0)
2	28%(n=17)	15%(n=9)	15%(n=9)	8%(n=5)	5%(n=3)	0%(n=0)
3	38%(n=23)	25%(n=15)	23%(n=14)	18%(n=11)	8%(n=5)	0%(n=0)
4	52%(n=31)	37%(n=22)	35%(n=21)	32%(n=19)	10%(n=6)	0%(n=0)
5	58%(n=35)	50%(n=30)	48%(n=29)	37%(n=22)	13%(n=8)	0%(n=0)
6	60%(n=36)	57%(n=34)	57%(n=34)	53%(n=32)	17%(n=10)	0%(n=0)
7	68%(n=41)	68%(n=41)	67%(n=40)	60%(n=36)	23%(n=14)	0%(n=0)
8	75%(n=45)	73%(n=44)	70%(n=42)	62%(n=37)	23%(n=14)	0%(n=0)
9	83%(n=50)	83%(n=50)	77%(n=42)	68%(n=41)	28%(n=17)	0%(n=0)
10	87%(n=52)	85%(n=51)	80%(n=48)	75%(n=45)	30%(n=18)	0%(n=0)
24	100%(n=60)	100%(n=60)	97%(n=58)	92%(n=55)	63%(n=38)	0%(n=0)
48	100%(n=60)	100%(n=60)	100%(n=60)	100%(n=60)	67%(n=40)	2%(n=1)

***Note:** 60 mosquito larvae were exposed to each concentration; n= total number of dead mosquito larvae

Appendix 1(Continued)

Table 10: Mortality rate of *Anopheles* mosquito larvae exposed to liquid *Bti*

Time (hrs)	*Mortality at 0.0015ml/L	*Mortality at 0.001ml/L	*Mortality a 0.00075ml/L	*Mortality at 0.0005ml/L	*Mortality at 0.00025ml/L	*Mortality at 0ml/L
0	0%(n=0)	0%(n=0)	0%(n=0)	0%(n=0)	0%(n=0)	0%(n=0)
1	23%(n= 14)	12%(n=7)	5%(n=3)	3%(n=2)	0%(n=0)	0%(n=0)
2	88%(n=53)	72%(n=43)	65%(n=39)	63%(n=38)	5%(n=3)	0%(n=0)
3	97%(n=58)	95%(n=57)	92%(n=9)	92%(n=55)	42%(n=25)	0%(n=0)
4	100%(n=60)	100%(n=60)	93%(n=56)	92%(n=55)	83%(n=50)	0%(n=0)
5	100%(n=60)	100%(n=60)	95%(n=57)	93%(n=56)	88%(n=53)	0%(n=0)
6	100%(n=60)	100%(n=60)	97%(n=58)	95%(n=57)	92%(n=55)	0%(n=0)
7	100%(n=60)	100%(n=60)	97%(n=58)	97%(n=58)	95%(n=57)	0%(n=0)
8	100%(n=60)	100%(n=60)	97%(n=58)	98%(n=59)	97%(n=58)	0%(n=0)
9	100%(n=60)	100%(n=60)	98%(n=59)	98%(n=59)	98%(n=59)	0%(n=0)
10	100%(n=60)	100%(n=60)	100%(n=60)	98%(n=59)	98%(n=59)	0%(n=0)
24	100%(n=60)	100%(n=60)	100%(n=60)	100%(n=60)	100%(n=60)	0%(n=0)
48	100%(n=60)	100%(n=60)	100%(n=60)	100%(n=60)	100%(n=60)	0%(n=0)

***Note:** 60 mosquito larvae were exposed to each concentration; n= total number of dead mosquito larvae

Appendix 1(Continued)

Table 11: Mortality rate of *Culex* mosquito larvae exposed to liquid *Bti*

Time (hrs)	*Mortality at 0.0015ml/L	*Mortality at 0.001ml/L	*Mortality at 0.00075ml/L	*Mortality at 0.0005ml/L	*Mortality at 0.00025ml/L	*Mortality at 0ml/L
0	0% (n=0)	0%(n=0)	0%(n=0)	0%(n=0)	0%(n=0)	0%(n=0)
1	27%(n=16)	5%(n=3)	2%(n=1)	2%(n=1)	0%(n=0)	0%(n=0)
2	95%(n=57)	82%(n=49)	75%(n=45)	35%(n=21)	5%(n=3)	0%(n=0)
3	98%(n=59)	98%(n=59)	93%(n=56)	63%(n=38)	37%(n=22)	0%(n=0)
4	100%(n=60)	100%(n=60)	98%(n=59)	77%(n=46)	62%(n=37)	0%(n=0)
5	100%(n=60)	100%(n=60)	98%(n=59)	85%(n=51)	77%(n=46)	0%(n=0)
6	100%(n=60)	100%(n=60)	100%(n=60)	92%(n=55)	85%(n=51)	2%(n=1)
7	100%(n=60)	100%(n=60)	100%(n=60)	97%(n=58)	92%(n=55)	2%(n=1)
8	100%(n=60)	100%(n=60)	100%(n=60)	98%(n=59)	93%(n=56)	2%(n=1)
9	100%(n=60)	100%(n=60)	100%(n=60)	98%(n=59)	93%(n=56)	2%(n=1)
10	100%(n=60)	100%(n=60)	100%(n=60)	98%(n=59)	95%(n=57)	2%(n=1)
24	100%(n=60)	100%(n=60)	100%(n=60)	100%(n=60)	97%(n=58)	2%(n=1)
48	100%(n=60)	100%(n=60)	100%(n=60)	100%(n=60)	100%(n=60)	2%(n=1)

***Note:** 60 mosquito larvae were exposed to each concentration: n= total number of dead mosquito larvae

Appendix 1(Continued)

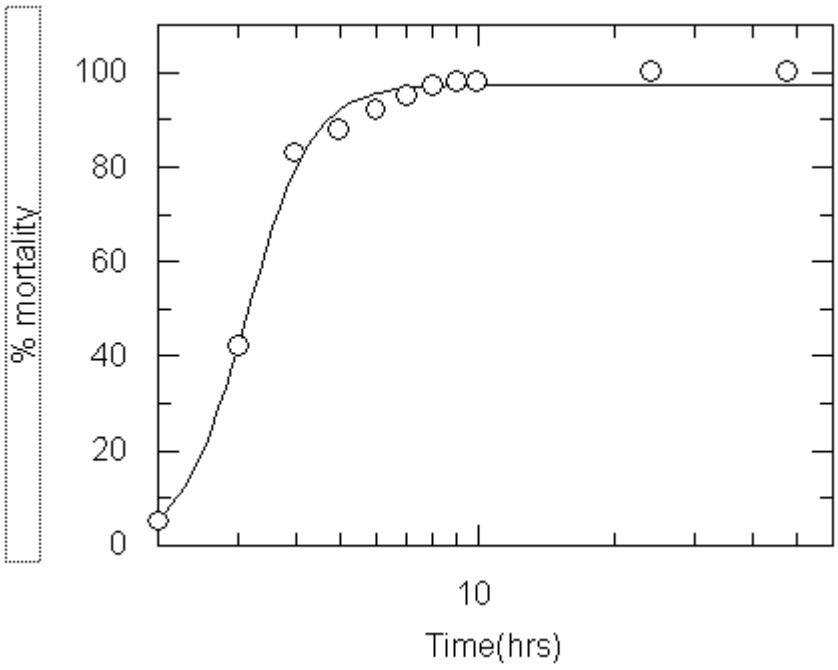
Table 12: *Culex* and *Anopheles* mosquito larvae exposed to the same dosage of liquid *Bti*

Time(Hrs)	***Mortality(*Culex*)**	***Mortality(*Anopheles*)**	***Mortality Control**
0	0%(n=0)	0%(n=0)	0%(n=0)
1	10%(n=6)	10%(n=6)	0%(n=0)
2	67%(n=40)	65%(n=39)	0%(n=0)
3	95%(n=57)	90%(n=54)	0%(n=0)
4	98%(n=59)	95%(n=57)	0%(n=0)
5	100(n=60)	100(n=60)	0%(n=0)
6	100(n=60)	100(n=60)	0%(n=0)
7	100(n=60)	100(n=60)	0%(n=0)
8	100(n=60)	100(n=60)	0%(n=0)
9	100(n=60)	100(n=60)	0%(n=0)
10	100(n=60)	100(n=60)	0%(n=0)
24	100(n=60)	100(n=60)	0%(n=0)
48	100(n=60)	100(n=60)	0%(n=0)

***Note:** 60 mosquito larvae were exposed to each concentration; n= total number of dead mosquito larvae

Appendix 2: Comparison of using Probit analysis and Grafit to find LT_{50}.
Results from the two methods are not significantly different.

(a) Results of exposing *Anopheles* mosquito larvae to 0.00025ml/L of Liquid *Bti*, its LT_{50} using **Grafit** is 3.1 hrs as shown below;



LT50 - Start at 0
Simple weighting

Reduced Chi squared = 7.499

Variable	Value	Std. Err.
Y Range	97.3645	1.0901
LT 50	3.1261	0.0519
Slope factor	-6.1125	0.5774

Appendix 2(Continued) : Comparison of Using Probit analysis and Grafit to find LT_{50}

(b) Results of exposing *Anopheles* mosquito larvae to 0.00025ml/L of Liquid *Bti*, its LT_{50} using **Probit analysis** is 3.2 hrs as shown below;

Study Area:	Year:	Pesticide:
Zomba	2011	Liquid *Bti*

Pesticide conc.		SMI%
2.0		5.00
3.0		42.00
4.0		83.00
5.0		88.00
6.0		92.00
8.0		97.00
10.0		98.00

n =	1	S =	1.5403		2.1662
a =	2.3374	A =	1.1360		2.3305
b =	2.3022	K =	7		3.7197
r =	0.9476	N' =	2		4.6208
c^2 =	0.2480	R =	5		7.4180

EC	Mean	95% Confidence Intervals Lower	Higher
EC_1	1.1573	0.1560	8.5845
EC_{16}	2.0638	0.8855	4.8097
EC_{50}	3.1788	1.3640	7.4084
EC_{84}	4.8963	2.1009	11.4112
EC_{90}	5.5467	1.4911	20.6321
EC_{95}	6.4948	1.4056	30.0109
EC_{99}	8.7318	1.1771	64.7726

***Note:** EC=LT

Appendix 3: Probit Analysis Outcomes for LT_{50} and LT_{90}

(a) Probit Output for granular *Bti* on *Culex* mosquito larvae at 47.73g/ha(LT_{50} and LT_{90})

Study Area:	Year:	Pesticide:
Zomba	2011	Granular *Bti*

Pesticide conc.		SMI%
1.0		2.00
3.0		15.00
5.0		30.00
7.0		50.00
9.0		57.00
24.0		90.00
48.0		98.00

n =	1	S =	2.4866		3.2118
a =	2.7972	A =	1.2659		4.2923
b =	1.0917	K =	7		8.1920
r =	0.9977	N' =	3		11.1825
c^2 =	0.0117	R =	48		22.3103

EC	Mean	95% Confidence Intervals Lower	Higher
EC_1	0.8931	0.0400	19.9243
EC_{16}	3.0246	0.7047	12.9825
EC_{50}	7.5211	1.7522	32.2827
EC_{84}	18.7023	4.3572	80.2756
EC_{90}	24.3278	2.9697	199.2947
EC_{95}	33.9332	3.0345	379.4566
EC_{99}	63.3412	2.8391	1413.1635

***Note:** EC=LT

Appendix 3 (Continued)

(b) Probit Output for granular *Bti* on *Anopheles* mosquito larvae at 103.4g/ha (LT_{50} and LT_{90})

Study Area:	Year:	Pesticide:
Zomba	2011	Granular *Bti*

Pesticide conc.		SMI%
1.0		2.00
3.0		18.00
5.0		37.00
7.0		60.00
9.0		68.00
10.0		75.00
24.0		92.00

n =	1	S =	2.3495		2.6330
a =	2.8834	A =	1.2873		2.8812
b =	1.1642	K =	7		5.1775
r =	0.9924	N' =	5		6.7946
c^2 =	0.0330	R =	24		12.2949

EC	Mean	95% Confidence Intervals Lower	Higher
EC_1	0.8352	0.0679	10.2685
EC_{16}	2.6217	0.9100	7.5536
EC_{50}	6.1599	2.1380	17.7476
EC_{84}	14.4729	5.0233	41.6987
EC_{90}	18.5206	3.5771	95.8905
EC_{95}	25.3033	3.7240	171.9268
EC_{99}	45.4322	3.6952	558.5833

***Note:** EC=LT

Appendix 3 (Continued)

(c) Probit Output for liquid *Bti* on *Anopheles* mosquito larvae at 0.00025ml/L

Study Area:	Year:	Pesticide:
Zomba	2011	Liquid *Bti*

Pesticide conc.		SMI%
2.0		5.00
3.0		42.00
4.0		83.00
5.0		88.00
6.0		92.00
8.0		97.00
10.0		98.00

n =	1	S =	1.5403		2.1662
a =	2.3374	A =	1.1360		2.3305
b =	2.3022	K =	7		3.7197
r =	0.9476	N' =	2		4.6208
c^2 =	0.2480	R =	5		7.4180

EC	Mean	95% Confidence Intervals Lower	Higher
EC_1	1.1573	0.1560	8.5845
EC_{16}	2.0638	0.8855	4.8097
EC_{50}	3.1788	1.3640	7.4084
EC_{84}	4.8963	2.1009	11.4112
EC_{90}	5.5467	1.4911	20.6321
EC_{95}	6.4948	1.4056	30.0109
EC_{99}	8.7318	1.1771	64.7726

***Note:** EC=LT

(d) Probit Output for liquid *Bti* on *Culex* 2 mosquito larvae at 0.00025ml (LT_{50} and LT_{90})

Study Area:	Year:	Pesticide
Zomba	2011	Liquid *Bti*

Pesticide conc.		SMI%
2.0		5.00
3.0		37.00
4.0		62.00
5.0		77.00
6.0		85.00
7.0		92.00
9.0		93.00

n =	1	S =	1.6284		2.3641
a =	2.3634	A =	1.1899		2.1809
b =	2.0397	K =	7		3.9097
r =	0.9779	N' =	3		5.0515
c^2 =	0.1046	R =	4.5		8.7324

EC	Mean	95% Confidence Intervals Lower	Higher
EC_1	1.1643	0.1333	10.1671
EC_{16}	2.2368	1.0256	4.8784
EC_{50}	3.6423	1.6701	7.9437
EC_{84}	5.9310	2.7195	12.9352
EC_{90}	6.8275	1.7463	26.6935
EC_{95}	8.1586	1.6151	41.2126
EC_{99}	11.3946	1.3049	99.5017

***Note:** EC=LT