MALAWI JOURNAL OF SCIENCE AND TECHNOLOGY VOLUME 10: ISSUE 1 | 2014

Efficacy of Bacillus thuringiensis var israelinsis (*Bti*) on *Culex* and Anopheline mosquito larvae in Zomba

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Abstract

Laboratory based experiments were conducted using Bacillus thuringiensis var israelinsis (Bti) to establish the efficacy of Bti on Anopheles and Culex mosquito larvae from Zomba.

The study evaluated two formulations of Bti namely VectoBac® WG and VectobaBac® 12AS against selected species of mosquito larvae. 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 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 48hours of exposure is 47.73g/ha. The LT50 and LT90 being 7.5hrs and 24.3 hrs respectively. On the other hand, Anopheles mosquito larvae require 103.41g/ha of Bti which is almost double as much as that required by Culex. Anopheles LT50 is 6.2 hrs and LT90 is 18.5 hrs. 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. Following the successful results of Bti in controlling mosquito larvae at laboratory level it is our recommendation to ask the Government of Malawi to come up with a policy to allow the use of Bti in controlling mosquito larvae in Malawi.

Introduction

Malaria is ranked the highest killer disease in Africa. Likewise 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 years 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.

Ministry of health (MoH) and other non-governmental organizations (NGOs) have tried their best to combat malaria 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 behavior change of vectors (Killeen et al, 2002; Vulule et al, 1994). A recent study also shows high level of resistance of *Anopheles funestus* to

pyrethroids in Malawi after massive net distribution and IRS programs were conducted (Mzilahowa, 2013). The over reliance on insecticides to control mosquitoes has led to physiological resistance of mosquito vectors including *Anopheles gambiae* (Koekemoer et.al, 2011; Koffi et.al, 2012), *Culex papiens* (Labbe et.al, 2007; Liu et.al, 2011) and *Aedes aegypti* (Dusfour et.al, 2011; Kamgang et.al, 2011; Lima et al, 2011). The use of drugs to destroy the pathogen (*plasmodium*) has also resulted into much more complex and resistant *plasmodium* (Tjitra 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 transmission.

Although IRS can effectively control the *Anopheline* mosquito populations, this method is less effective at controlling *Culicine* mosquitoes. The reason is that Malawi is currently using Pyrethroids in its IRS program 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 larva stage. *Bti* is 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 get activated. Then endotoxins are released which degrade the insects' gut lining and eventually the host dies (Suom et al, 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.

Research objectives

Main Objective

The 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 *Bti*.

Methodology

Mosquito collection

Female blood fed mosquitoes were collected from the field using aspirators. Aspirators were made locally by joining glass tubing to plastic tubing and a net mesh in between.

Blood meal is very important because the proteins from blood are primarily used in the development of eggs, but it is also used as a source of energy (Smartt et.al, 2009). The collected mosquitoes were placed in collecting cups.

Upon arrival in the in sectary all collected mosquitoes were transferred to mosquito cages where they were reared. The collected adult mosquitoes were fed 10% sugar solution; this was soaked in a 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 3 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 were collected. These eggs were then placed in containers (24 × 15 cm) with distilled water for hatching. No food was provided to containers with eggs up until the 1st in star appeared. During early stages larvae were fed sparingly to avoid over feeding. Yeast (10%) was used to feed mosquito larvae. Sieving were conducted once water is dirt, this ensured favorable environment for survival of mosquito larva. Mosquito larvae were kept up until they reached 3rd in star, which was the time they were ready for testing.

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

All pupae were placed in a cage and 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.

The abdominal part of the *Rattus norvegicus Albinus* was shaved to remove abdominal fur so that mosquitoes can easily access the skin. *Rattus norvegicus Albinus* was exposed to Chloroform to get knocked down. Then it was placed on a cage exposing the shaved belly to mosquitoes for 10 minutes and this allowed mosquitoes to suck up blood. During blood feeding all lights were switched off. 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 these mosquitoes lay eggs. After laying eggs, these 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 1st to 3rd in star that is when they were ready for experiments.

Preparation of 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 liters each were used. 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 from stock containers randomly to compensate 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°C to 28°C and humidity within the range of 72% to 85%.

How mortality 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. For the larva that was still alive could respond rapidly by either bending itself or moving away from the rod while for the dead ones no matter how close the rod was brought, there was no response. The results were properly recorded on a data sheet, and then thereafter 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, 2003). Two different types of mosquito larvae were used in this experiment namely; *Anopheles* and *Culex* mosquitoes. All these experiments were conducted in Biology laboratory at Chancellor College, Zomba.

Preparation of *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 hector. 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:

$\pi r2 = {}^{22}/_{7} \times 20 \times 20$

$= 1257.142857$

$= 1257.14\ cm^2$

And thereafter, the basin surface area calculated as a fraction of the hectare to establish the required amount of *Bti* at manufactures dosage.

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

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

$= 0.002514g$

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 below;

Table 1: Preparation of Granular *Bti*

Treatment	Amount of *Bti* in g/cm²	Amount of *Bti* in mg/cm²
1st	0.0038	3.8
2nd	0.0025	2.5
3rd	0.0019	1.9
4th	0.0013	1.3
5th	0.0006	0.6
6th	0 (control)	0 (control)

Dilutions of liquid *Bti* based on concentrations

Manufactures (VALENT BIOSCIENCES$_{TM}$) of VectoBac® 12AS recommends 0.5 liters 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
1st	0.0015	1.5
2nd	0.001	1.0
3rd	0.00075	0.75
4th	0.0005	0.50
5th	0.00025	0.25
6th	0 (control)	0 (control)

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

Results and Discussion

The results collected in this study mainly reports 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 rate of mosquito larvae exposed to different concentrations of *Bti*. Due to significant differences that appeared between the means, the results were further analyzed 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 LT50 and LT90 the data was analyzed using probit analysis and Grafit.

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

Table 3.1: Mortality of *Culex* mosquito larvae exposed to granular *Bti*

Time(Hrs)	Mortality at 0.0038g/cm² (302.27g/ha)	Mortality at 0.0025g/cm² (198.86g/ha)	Mortality at 0.0019g/cm² (151.12g/ha)	Mortality at 0.0013g/cm² (103.41g/ha)	Mortality at 0.0006g/cm² (47.73g/ha)	Mortality at 0g/cm² (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 mosquitoes

Table 3.1 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 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 a mortality of 98 % (n=59).

Table 3.2: 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 3.2 shows that during the 48th hour if we use granular Bti to control *Culex* mosquito larva there is 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 LT50 and LT90 are 7.5 hours and 24.3 hours respectively.

Table 4.1: *Anopheles* mosquito larvae exposed to granular *Bti*

Time (hrs)	Mortality at 0.0038g/cm² (302.27g/ha)	Mortality at 0.0025g/cm² (198.86g/ha)	Mortality at 0.0019g/cm² (151.12g/ha)	Mortality at 0.0013g/cm² (103.41g/ha)	Mortality at 0.0006g/cm² (47.73g/ha)	Mortality at 0g/cm² (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 mosquitoes

Table 4.1 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 after 48hrs of exposure there is only 67% population reduction which is far below the cutoff point 90%.

Table 4.2: 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 4.2 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 LT50 is 6.2 hours and its LT90 is 18.5 hours.

Table 5.1: Summary of mortality rate of *anopheles* 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	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 mosquitoes

Table 5.1 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 5.2: Data analyzed 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 5.2 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 results 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 LT50 and LT90 are 3.2 hours and 5.5 hours respectively.

Table 6.1: Mortality rate of *Culex* mosquito larvae exposed to Liquid *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 mosquitoes

Table 6.1 illustrates that the more concentrated the solution of Bacillus thuringiensis is the less the time is required to achieve 100% mortality rate. For instance, it took only 4hours for the 0.0015ml/L and 0.001ml/L to kill 100 %(n=60) *Culex* mosquito larvae. While for the 0.00075ml/L of *Bti* it took 6hours to kill 100(n=60) *Culex* mosquito larvae. For 0.0005ml/L it took 24hours 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 minimize 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 LT50 and LT90 are 3.6 hours and 6.8 hours respectively.

Table 6.2: Data analyzed in SPSS (*Culex* 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**
		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
48 Hours	0.0015ml/L	0.00000 ml/L	19.66667*	.19245	.000	19.2474	20.0860

Table 6.2 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 results 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.

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)

Table 7.1: *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 mosquitoes

Table 7.1 shows *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 and 48th hours the mortalities registered were the same(100%,n=60). Despite that *Culex* mosquito larvae died faster than *Anopheles* larvae but their mortalities were not significantly different p=0.09.

Table 7.2: Comparison of mortality rate between *Culex* and *Anopheles* larvae using t-test

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

Table 7.2 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 in 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.

Conclusion

The study has established the lower effective dosage of granular *Bti* on *Culex* mosquito larvae as 47.73g/ha and its LT50 and LT90 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 LT50 is 6.2 hours and its LT90 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 behavioral 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); Kroeger et.al (1995) observed. Likewise, Aly et al. (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 et.al, 2013 who found out that liquid suspension disperses evenly in water than granular *Bti* hence performs faster.

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 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. Therefore, it is of paramount importance that the government of Malawi should come up with a policy that would allow the use of *Bacillus thuringiensis israelinsis* as a means of combating malaria and other diseases that are spread by mosquito bites.

Acknowledgements

We acknowledge Mr R Kondawani Department of Biological Sciences for providing technical support in mosqiuito rearing.We acknowledge Chemicals and Marketing company of Malawi for supplying the *Bti* that was used in the study for free. It is stated herein that authors have no conflict on interest in anything related to this work. It was purely an academic study.

7.0 References

Aly, C., & Mulla, M.S. (1987). Effect of two microbial insecticides on aquatic predators of mosquito. *Journal of Applied Entomolgy*, 103, 113-118

Boisvert, M. (2005). Utilization of Bacillus thuringiensis var.israelensis (Bti)-Based on formulations for the Biological Control of Mosquitoes in Canada. Retrieved from http://www.erudit.org/livre/pacrim/2005/000220co.pdf

Dusfour, I., Thalmensy, V., Gaborit, P., Issaly, J., Carinci, R., Girod, R. (2011). Multiple insecticide resistance in Aedes aegypti (Diptera: Culicidae) populations compromises the effectiveness of dengue vector control in French Guiana. *Mem Inst Oswaldo Cruz*, 106, 346-35

Fillinger, U., Knols, B., & Becker, (2003). Efficacy and efficiency of new Bacillus thuringiensis var. israelinsis and Bacillus sphaericus formulations against Afrotropical anophelines in western Kenya. *The journal of Tropical Medicine and International Health*, 8(1), 37-41.

Foster and Smith (2013). Microbe-Lift Liquid Mosquito control. Retrieved from http://www.drsfostersmith.com/product/prod_display.cfm?pcatid=12263

Gillies, M.T. and Coetzee, M. (1987). A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region). Retrieved from http://ww.w.sandflycatalog.org/files/pdfs/190531.pdf

Kamgang, B., Marcombe, S., Chandre, F., Nchoutpouen, E., Nwane, P., Etang, J.....Paupy, C. (2011). Insecticide susceptibility of Aedes aegypti and Aedes albopictus in Central Africa. *Parasit Vectors* 4, 79.

Killeen, G., Fillinger, U., Kiche, I., and Gouagna, L., & Knols, B. (2002). Eradication of Anopheles gambiae from Brazil: lessons for malaria control in Africa. *Lancet Infect Dis*, 2, 618-627

Koekemoer, L.L., Spillings, B.L., Christian, R.N., Lo, T.C., Kaiser, M.L., Norton, R.A..... Coetzee, M. (2011). Multiple insecticide resistance in anopheles gambiae (Diptera: Culicidae) from pointe Noire, Republic of the Congo. *Vector Borne Zoonotic Dis*, 11(8),1193-200 . Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21417925

Koffi, A.A., Alou, L.P., Adja, M.A., Kone, M., Chandre, F., N'Guessan, R. (2012). Update on resistance status of Anopheles gambiae s.s. to conventional insecticides at a previous WHOPES field site, "Yaokoffikro", 6 years after the political crisis in Cote d'Ivoire. *Parasit Vectors* 5, 68.

Kroeger, A., Horstick, O., Riedl, C., Kaiser, A., & Becker, N. (1995). The potential for malaria control with the biological larvicide Bacillus thuringiensis israelinsis (Bti) in Peru and Ecuador. *Acta Trop*., 60(1), 47-57. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8546038

Labbe, P., Berthomieu, A., Berticat, C., Alout, H., Raymond, M., Lenormand, T.,& Weill, M. (2007). Independent duplications of the acetylcholinesterase gene conferring insecticide resistance in the mosquito Culex pipiens. *Mol Biol Evol*, 24, 1056-1067.

Lima, E.P., Paiva, M.H., de Araujo, A.P., da Silva, E.V., da Silva, U.M., de Oliveira, L.N..... de Melo Santos, M.(2011). Insecticide resistance in Aedes aegypti populations from Ceara Brazil. *Parasit Vectors*, 4, 5.

Liu, Y., Zhang, H., Qiao, C., Lu, X., & Cui, F. (2011). Correlation between carboxylesterase alleles and insecticide resistance in Culex pipiens complex from China. *Parasit Vectors*, 4,236.

Mzilahowa, T. (2013). Preliminary data on the impact of combined scale up of indoor residual spray (IRS) and long lasting insecticide treated bed nets (LLINs) on development of resistance in Malawi. Malawi, Blantyre: University of Malawi, College of Medicine, Malaria Alert Centre.

Phillips, K. (2003). Malaria in Experimental Biology. Retrieved from http://jeb.biologists.org/content/206/21/3723.full

Silver, J.B. (2007). Mosquito Ecology: Field sampling Methods. Retrieved from http://books.google.mw/books/about/Mosquito_Ecology.html?id=VM8MA4E_VT8C&redir_esc=y

Smartt, C.T., Richards, S.L., & Anderson, S.L. (2009).The Female Mosquito's Quest for Blood: Implications for Disease Cycles. University of Florida USA. Retrieved from http://edis.ifas.ufl.edu/IN811.

Suom, C., & Smith, J.J. (2008). VectoBac G (Bti) Efficacy for Field Application of Ochlerotatus abserratus Mosquito Larvae. Retrieved from http://www.nmca.org/2008norfolk.pdf

Tjitra, E., Anstey, N.M., Sugiarto, P., Warikar, N., Kenangalem, E. Karyana, M, Lampah, D.A. & Price, R.N (2008). Multidrug-Resistant Plasmodium vivax Associated with Severe and Fatal Malaria: A Prospective Study in Papua, Indonesia. Retrieved from http://www.plosmedicine.org/article/info%3Adoi%2F10.1371%2Fjournal.pmed.0050128

Vulule, J.M., Beach, R.F., Atieli, F.K., Roberts, J.M., Mount, D.L., Mwangi, R.W. (1994). Reduced susceptibility of An.gambiae to permethrin associated with the use of permethrin-impregnated bed nets and curtains in Kenya. *Medical and Veterinary Entomology*, 8, 71-75

Wondji, C.S., Coleman, M., Kleinschmidt, I., Mzilahowa, T., Irving, H., Ndula, M., Rehman, A., Morgan, J., Barnes, K.G., & Hemingway, J. (2012). Impact of pyrethroid resistance on Operational Malaria Control in Malawi. *Proc Natl Acad Sci* USA, 109(47), 19063-70.

World Health Organisation (1998). In Fact Sheet No. 203. Geneva, Switzerland: World Health Organisation.

World Health Organization (1992). Safe Use of Pesticides (Technical Report Series No.813). Geneva: WHO