EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 3
82-89
Year: 2018

EFFICACY OF SELECTED MICROBIAL LARVICIDES ON FIELD-COLLECTED LARVAE OF MOSQUITOES FROM PORT HARCOURT METROPOLIS

Ekerette I. B.*[1] and Ebere N.[2]

[1,2]Department of Animal and Environmental Biology, Faculty of Science, Rivers State University, Port Harcourt.

***Corresponding Author: Ekerette I. B.**
Department of Animal and Environmental Biology, Faculty of Science, Rivers State University, Port Harcourt.

Article Received on 06/01/2018 **Article Revised on 26/01/2018** **Article Accepted on 16/02/2018**

ABSTRACT
The comparative effectiveness of two microbial larvicides: *Bacillus thuringiensis* var *israelensis/Bacillus sphaericus* (*Bti/Bs*) combined and *Bacillus thuringiensis* var *israelensis* (*Bti*) were determined against *Culex quinquefasciatus* and *Anopheles gambiae* complex collected from the field. Various concentrations (0.2, 0.4, 0.6, 0.8, 1.0) mg/l of the two larvicides were tested in the laboratory against third instar of the larval species, to determine their mortality rates, lethal concentration (LC) at 50% and 90%. The field diagnostic concentrations being twice the LC-99.9% value were determined. All values were evaluated using probit analysis. The dose-response lines of *Anopheles gambiae* complex and *Culex quinquefasciatus* showed lethal concentration (LC): LC_{50} – 0.458mg/l; LC_{90} – 1.470mg/l and LC_{50} – 0.392mg/l; LC_{90} 1.109mg/l respectively, after 48 hours, when treated with *Bacillus thuringiensis* var *israelensis/Bacillus sphaericus (Bti/Bs).* Activity with *Bacillus thuringiensis* var *israelensis (Bti)* alone for *Anopheles gambiae* complex and *Culex quinquefasciatus* after 48 hours were LC_{50} – 0.684mg/l; LC_{90} – 3.413mg/l and LC_{50} – 0.622mg/l; LC_{90} – 2.713mg/l, respectively. *Culex quinquefasciatus* was non significantly more susceptible than *Anopheles gambiae* complex for the same concentrations of *Bti/Bs* and *Bti* alone. The diagnostic concentrations after 48 hours exposure of the larvae, had no significant difference between the species, when they were treated with the same larvicide, *Bti/Bs* (*Culex quinquefasciatus* – 5.179mg/l; *Anopheles gambiae* complex – 7.601mg/l); *Bti* alone (*Culex quinquefasciatus* – 18.032mg/l; *Anopheles gambiae* complex – 25.298mg/l). Mortality observed in the control was less than 5%. These results propose the use of *Bti/Bs and Bti alone* in the control of mosquito vectors of many disease agents that plague humans. The combination of *Bti/Bs* appear to be more effective than *Bti* alone in the control of *Culex quinquefasciatus* more than *Anopheles gambiae* complex.

KEYWORDS: *Efficacy, Bacillus thuringiensis var israelensis, Bacillus sphaericus, Mosquitoes, Port Harcourt.*

INTRODUCTION
Mosquito is a vector of many diseases inflicting humans and domestic animals (WHO, 2016a), such diseases include malaria, lymphatic filariasis, dengue and severe dengue, yellow fever, zika virus, West Nile virus and Chikungunya (WHO, 2016b). In Nigeria, malaria is transmitted all over the country and does have 97% of the people being exposed to the danger of the disease (PMI, 2017). Therefore there is great need to control the mosquito vector, consequently, reducing the population at risk to malaria.

Vector control is a fundamental and efficient method for checking the transmission of vector-borne sicknesses, particularly in place where resistance of parasites to treatment is increasing (Poopathi and Tyagi, 2006). Though the current extensively applied strategy for adult mosquito control include use of insecticide through insecticide-treated materials or indoor residual spraying (Le Menach *et al.,* 2007). Larval control at site of breeding is another appropriate alternative (Gu *et al.,* 2006).

Presently, there is a universal spread in resistance, affecting, all classes of chemical or synthetic insecticides used against a lot of invertebrate pests of agricultural and public health importance, causing difficulties in controlling them. In Nigeria, Awolola *et al.,* (2008) reported the resistance of *Anopheles gambiae* sensu strict to pyrethroid insecticides. In another study by Corbel *et al,* (2007) in Benin Republic of West Africa, multiple resistance by *Anopheles gambiae* and *Culex quinquefasciatus* to organochorines, pyrethroids, organophosphate and carbamates has been reported. Ranson *et al.,* (2011) reported that the gene responsible for this resistance have disseminated at an abnormally fast rate across Africa.

Ecologically, there have been negative feedback from the use of chemical insecticides as adulticide sprays for

example, dichlorodiphenyltricholoroethane (DDT) broken down residue have been found in food supply, human blood and these residues have been implicated in public health to cause delay in normal development, inability to produce viable sperms in men, reduced birth weight, destruction of the liver and nervous system, breast and other types of cancers (Yu *et al*., 2011).

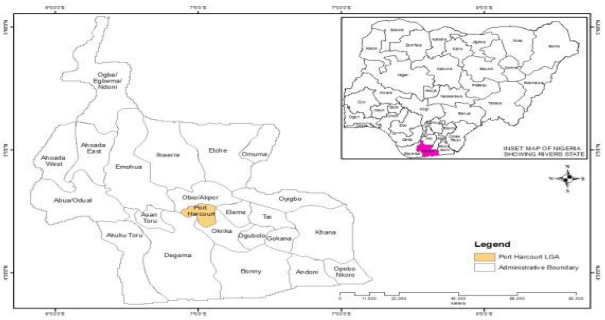
There is therefore the need to find an alternative control measure that is environmental friendly and cost effective. Consequently, *Bacillus sphaericus* (*Bs*) and *Bacillus thuringansis* var *israclensis* (*Bti*) have been experimented as microbial (biological) larvicides, through varying conditions against the chief vectors of malaria and filariasis in Africa. Microbial larvicides in the form of granules are easy and safe to apply, and are capable of being produced locally unlike many of their chemical counterparts, like dichlorodiphenyltrichloroethane (DDT) that have been found to be humanly and environmentally unfriendly (Eskenazi et al., 2009). There are accessible larvicides that are highly efficient, specific in action (Charles and Nielsen-Le-Roux, 2000) and non-toxic to the environment with which humans and unintended organisms are not targets (WHO, 1999).

Larviciding and source reduction promises a primary alternative to vector control because they have principal benefits of checking the population and propagation of mosquitoes when they have not yet been scattered into the environment and have not yet started transferring the disease parasites (Killeen *et al.,* 2002).The aim of this study was therefore to comparatively ascertain which of the selected larvicides, is most effective in the control of the two species of mosquito larvae in the field.

2. MATERIALS AND METHOD

Study Area

The study was carried out in Port Harcourt metropolis, which is the capital and largest city in Rivers State, Nigeria. It is an urban settlement which lies along the upper reaches of Bonny River in the Niger Delta and bounded by coordinates 4°49′27″N 7°2′1″E. According to 2006 census, it holds a population of 1,382,592 with a density of 1,500/km^2 (3,900/sq mi) (Federal Republic of Nigeria, 2007). Field-collected larvae of *Anopheles* and *Culex* species from this area were used for this study. The ambient temperature covering the study period ranged between 27°C and 29^6 C with relative humility of 65% (Chanda *et al.*, 2013.) the study was carried out at the malaria entomology Research unit laboratory of the department of Animal and environmental Biology, Rivers State University, Port Harcourt.



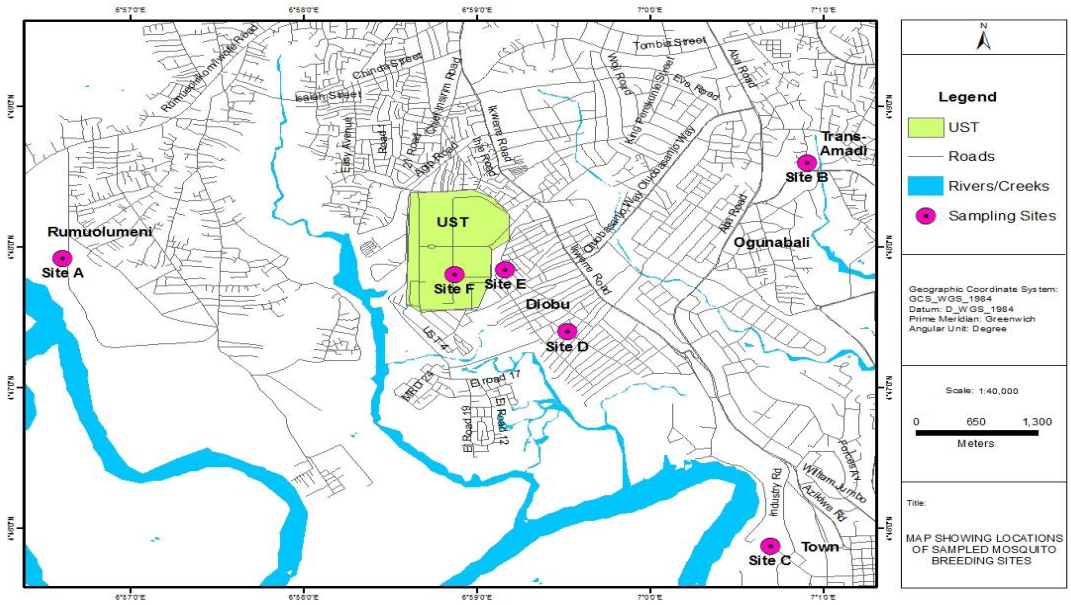


Figure 1: Map Showing Locations of Sampled Mosquito Breeding Sites in Port Harcourt Metropolis.

Larvae collection, Rearing and Identification

larvae of *Anopheles* and *Culex* species from breeding sites were collected using a three hundred and fifty milliliters (350ml) dipper and transported to the laboratory for evaluation assay. The larvae were pooled together and reared in plastic containers. A (three

milliliters size) pipette was used to pick the larvae from the pool into plastic containing distilled water. The larvae were fed with yeast at 10mg\l every 2 days till they attained the third instar stage. The larvae of *Culex* quinquefasciates and *Anopheles gambia* complex were identified using standard keys from Harback and Knight, (1980). The larvae in the control bowl were reared to adult stage under standard laboratory conditions with a relative humility of 70% -80% and temperature range of 25^0C to 28^oC. Mosquitoes were identified using standard keys from Gillet and Smith (1972).

Evaluated Product

Bacillus thuringensis var *israelensis (Bti)* is a biolarvicide used against larvae stages of certain dipterans. *Bti* produces toxins which are effective in killing various species of mosquitoes, fungus gnats and blackflies while having almost no effect on other organisms. The product name is ABG-6511.

Bacillus thuringiensis var *israelensis / Bacillus sphaericus (Bti/Bs)* is a combination of 2 microorganisms to formulate the larvicide. *Bacillus sphaerieus (Bs)* is an obligate aerobe bacterium used as a larvicide for mosquito vector control (USEPA, 2007).

Preparation of the stock solution

Stock solution of 1% was prepared by dissolving 200mg of each evaluated product in 20ml solvent (acetone), according to the method of Chanda *et al.,* (2013). The stock solution was later serially diluted in the solvent 2ml solution to 18ml solvent. The control and test concentrations of (0.0, 0.2, 0.4, 0.6, 0.8 and 1.0) mg/l were obtained.

Laboratory Bioassay

Batches of 25 larvae were introduced in test vessels of 3-litre bowls (0.0, 0.2, 0.4, 0.6, 0.8 and 1.0)mg/l. Third instar larvae were selected for the experiment as they were large enough and could easily be counted and had two stages before becoming adults, thereby, giving investigators enough time to make follow-ups of their development (Chanda *et al.,* 2013). All the test concentrations had four replicates in addition to a control, in bowls which were covered with nets to prevent successfully emerged adults from escaping into the environment (WHO, 2016). All the test containers were held at 25^oC – 28^oC for a photoperiod of 12L:12D. Mortality of the larvae was counted and the dead ones removed daily from the bowls. In recording percentage mortality in each concentration, moribund larvae, dead larvae and pupae, as well as adult mosquitoes not completely separated from the pupae case, were considered as "affected". From the mortality scores of the experiments carried out, lethal concentration (LC_{50} and LC_{90}) and diagnostic concentration of the different larvicides for a period of 1-8days were determined. The experiment stopped when all the larvae or pupae in the controls had died or emerged as adults. The larvae were fed with yeast at a concentration of 10mg/l at two-day intervals. The food powder was suspended in the water, as 2 drops were added per bowl containing 1L distilled water. The various concentrations were replicated four times and there was a control for each concentration.

Data Analysis

The LC_{50}, LC_{90} and $LC_{99.9}$ values of the two microbial larvicides used (*Bti/Bs* and *Bti* alone) were calculated by probit analysis using SPSS version 21 computer software programme. In all the tests, control mortality was less than 5% after larvae were exposed to these larvicides, hence, no correction was necessary using Abbott's formula. Diagnostic or discriminatory concentration of the study larvicides were determined by doubling the $LC_{99.9}$ values generated by the SPSS 21 statistical package using probit regression. The diagnostic concentration is the dosage or amount of the larvicide that will kill 100% of the test vector. WHO (2016) established the diagnostic concentration in the field to be double the minimum concentration that result in 100% mortality in the laboratory, to evade erroneous reporting of resistance in the field.

RESULTS

The percentage mortality of *Culex quinquefasciatus* was higher when treated with *Bti*/*Bs* than with *Bti* alone. At 0.4mg/l concentration of *Bti*, mortality was about 30% while with *Bti*/*Bs* the mortality at the same concentration was about 40%, also at 1.0mg/l concentration, the mortality was above 60% with *Bti* alone, at same concentration of *Bti*/*Bs*, the mortality rose above 90%(Fig 2). A similar trend was observed with *Anopheles gambiae* complex as less than 30% mortality was recorded at 0.4mg/l concentration when treated with *Bti* alone, while at same concentration, a higher value greater than 30% was observed when treated with *Bti*/*Bs*(Fig 3). The analysis of variance for the effect of *Bti/Bs* and *Bti* larvicides on the mortality of the larvae of *Culex quinquefasciatus* and *Anopheles gambiae* complex showed significant difference ($F = 26.24$; $p<0.05$) among the means.

The dose-response lines in Fig. 4 showed the closeness in susceptibility of both *Culex quinquefasciatus* and *Anopheles gambiae* complex to the *Bti*/*Bs* larvicide. At 0.8mg/l concentration there was about 70% mortality for *Anopheles gambiae* complex and less than 80% mortality for *Culex quinquefasciatus*, at same concentration. A rise and fall pattern was observed in the mortality of the test organisms when treated with *Bti* alone. At 0.6mg/l concentration, *Culex quinquefasciatus* recorded just below 40%, while with *Anopheles gambiae* complex, it was just above 40% mortality when treated with *Bti* alone. The opposite was observed in 0.8mg/l and 1.0mg/l concentrations, as the mortality of *Culex quinquefasciatus* was higher than that obtained in *Anopheles gambiae* complex (Fig 5).

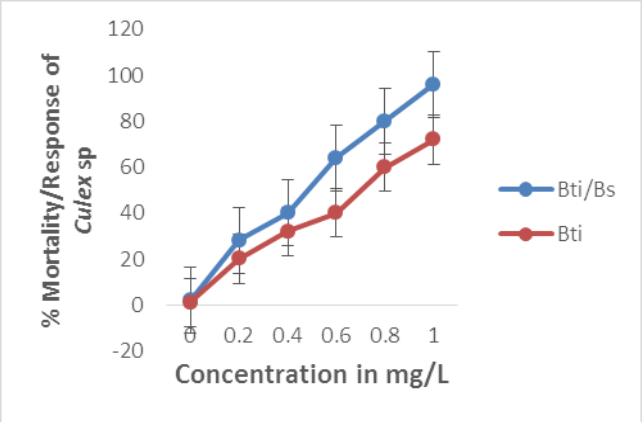


Figure 2: Percentage Mortality of *Culex quinquefasciatus* at Different Concentrations of *Bti/Bs* (mg/l) and *Bti* (mg/l) Larvicides after 48 Hours Exposure.

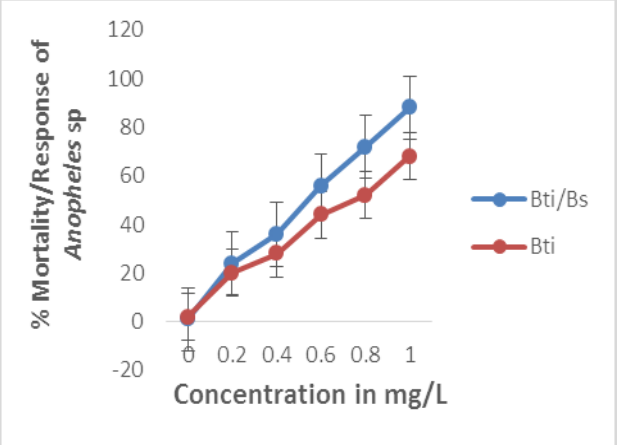


Figure 3: Percentage Mortality of *Anopheles gambiae* Complex at Different Concentrations of *Bti/Bs* (mg/l) and *Bti* (mg/l) Larvicides after 48 Hours Exposure.

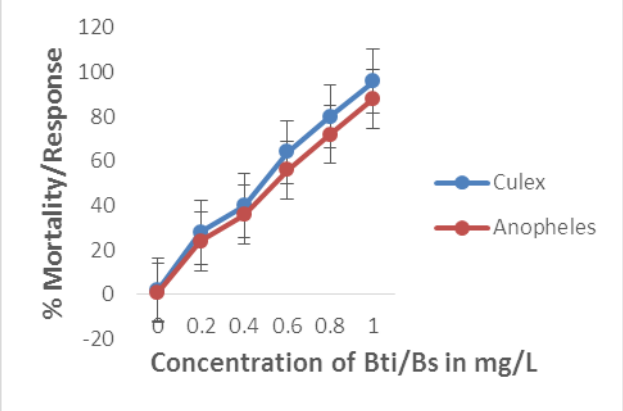


Figure 4. Percentage Mortality of *Culex quinquefasciatus* and *Anopheles gambiae* Complex at Different Concentrations of *Bti/Bs* (mg/l) Larvicide after 48 Hours Exposure.

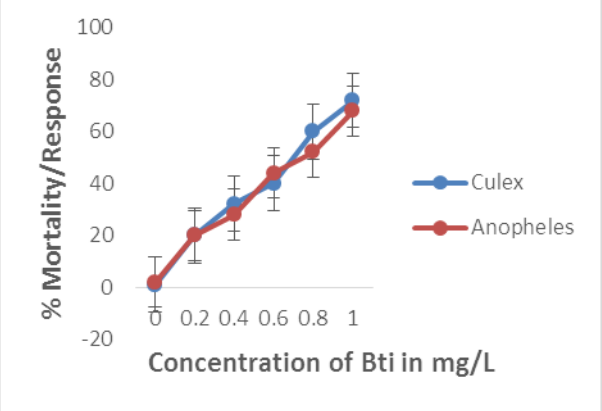


Figure 5: Percentage Mortality of *Culex quinquefasciatus* and *Anopheles gambiae* Complex at Different Concentrations of *Bti* (mg/l) Larvicide after 48 Hours Exposure.

The LC_{50} and LC_{90} values of *Bti/Bs* against *Culex quinquefasciatus* larvae after the second day were 0.392mg/l and 1.109mg/l while that of *Anopheles gambiae* complex after the same period were 0.458mg/l and 1.470mg/l, respectively. The LC_{50} and LC_{90} were observed to reduce as the days increased. On the fifth day, LC_{50} and LC_{90} for *Culex quinquefasciatus* were 0.259mg/l and 0.710mg/l, respectively while those of *Anopheles gambiae* complex for the same period were 0.237mg/l and 0.909mg/l, respectively. By the eighth day, the concentration of *Culex quinquefasciatus* had dropped to 0.114mg/l and 0.290mg/l, respectively while that of *Anopheles gambiae* complex for the corresponding period dropped to 0.133mg/l and 0.411mg/l, respectively (Table 1).

The LC_{50} and LC_{90} of *Bti* alone against *Culex quinquefasciatus* larvae after the second day were 0.622mg/l and 2.713mg/l while that of *Anopheles gambiae* complex after the same period were 0.684mg/l and 3.413mg/l respectively. The LC_{50} and LC_{90} were observed to reduce as the days increased, as was observed with *Bti/Bs*. On the fourth day, LC_{50} and LC_{90} for *Culex quinquefasciatus* were 0.378mg/l and 1.644mg/l respectively while those of *Anopheles gambiae* complex for the same period were 0.360mg/l and 2.442mg/l respectively. By the eighth day, that of *Culex quinquefasciatus* had dropped to 0.160mg/l and 0.500mg/l respectively while that of *Anopheles gambiae* complex for the corresponding period dropped to 0.174mg/l and 0.411mg/l, respectively(Table 2).

Table 1: Lethal Concentration (LC) of *Bti/Bs* for a period of 8 days.

Time (Days)	*Culex quinquefasciatus*				*Anopheles gambiae* complex			
	LC (mg/l) with 95% C.I.		X^2	P-value	LC (mg/l) with 95% C.I.		X^2	P-value
	LC_{50}	LC_{90}			LC_{50}	LC_{90}		
1	0.483 (0.384–0.592)	1.393 (1.017-2.607)	2.49	0.48	0.559 (0.448-0.702)	1.714 (1.186-3.788)	1.36	0.72
2	0.392 (0.300-0.478)	1.109 (0.840-1.882)	4.19	0.24	0.458 (0.352-0.570)	1.470 (1.037-3.082)	2.50	0.48
3	0.329 (0.036-0.533)	0.921 (0.561-0.550)	6.02	0.11	0.359 (0.009-0.629)	1.224 (0.677-3.574)	5.57	0.13
4	0.285 (0.08-0.486)	0.851 (0.498-0.104)	6.57	0.087	0.317 (0.028-0.548)	0.999 (0.571-0.723)	6.37	0.095
5	0.259 (0.174-0.327)	0.710 (0.558-1.091)	9.08	0.028	0.237 (0.128-0.328)	0.909 (0.659-1.875)	5.40	0.145
6	0.175 (0.084-0.241)	0.506 (0.392-0.776)	4.68	0.197	0.186 (0.068-0.273)	0.784 (0.566-1.683)	4.79	0.187
7	0.146 (0.056-0.208)	0.385 (0.293-0.578)	5.19	0.16	0.152 (0.053-0.226)	0.511 (0.384-0.841)	4.33	0.23
8	0.114 (0.016-0.177)	0.290 (0.194-0.449)	1.33	0.72	0.133 (0.017-0.190)	0.411 (0.283-0.678)	2.65	0.45

Table 2: Lethal Concentration (LC) of *Bti* for a period of 8 days.

Time (Days)	*Culex quinquefasciatus*				*Anopheles gambiae* complex			
	LC (mg/l) with 95% C.I.		X^2	P-value	LC (mg/l) with 95% C.I.		X^2	P-value
	LC_{50}	LC_{90}			LC_{50}	LC_{90}		
1	0.860 (0.682-1.318)	2.821 (1.670-11.163)	1.41	0.70	0.862 (0.661-1.469)	3.459 (1.835-22.116)	0.97	0.81
2	0.622 (0.473-0.889)	2.713 (1.531-13.498)	1.55	0.67	0.684 (0.552-1.090)	3.413 (1.733-29.261)	1.09	0.78
3	0.490 (0.343-0.667)	2.390 (1.359-12.416)	2.00	0.57	0.483 (0.301-0.710)	3.272 (1.558-57.113)	1.35	0.72
4	0.378 (0.245-0.494)	1.644 (1.054-5.187)	3.27	0.35	0.360 (0.173-0.506)	2.442 (1.272-28.714)	2.21	0.53
5	0.296 (0.176-0.389)	1.159 (0.811-2.665)	4.87	0.182	0.245 (0.080-0.360)	1.539 (0.922-9.073)	0.98	0.81
6	0.237 (0.122-0.323)	0.909 (0.659-1.875)	5.40	0.14	0.189 (0.027-0.305)	1.420 (0.834-13.373)	1.68	0.64
7	0.193 (0.078-0.276)	0.761 (0.557-1.526)	4.75	0.19	0.186 (0.056-0.274)	0.854 (0.600-2.143)	4.31	0.23
8	0.160 (0.064-0.230)	0.500 (0.380-0.794)	5.37	0.147	0.174 (0.078-0.244)	0.539 (0.413-0.858)	4.86	0.18

The diagnostic concentration is twice the value of $LC_{99.9}$. After the first day, when *Bti*/*Bs* was used, the diagnostic concentration for *Culex quinquefasciatus* and *Anopheles gambiae* complex were 6.601mg/l and 8.547mg/l respectively. This concentration gradually reduced as the days increased for *Culex quinquefasciatus* but for *Anopheles gambiae* complex, it increased on the fifth day to 5.437mg/l, having been at 5.099mg/l on the fourth day (Table 3). A similar trend was observed with *Culex quinquefasciatus* when treated with *Bti* alone, the diagnostic concentration on the first day was 14.862mg/l while that of the second day rose to 18.032mg/l, before dropping to 17.395mg/l which continued in the same trend to eighth day with a value of 2.529mg/l. *Anopheles gambiae* complex treated with *Bti* alone did not also have a trendy reduction from day 1 to day 8, rather after the first day with a diagnostic concentration of 21.475mg/l, there was a sequential increment on the second and third days with 25.298mg/l and 31.114mg/l, respectively before dropping to 23.246mg/l from which it reduced continuously to the eighth day with a value of 2.712mg/l (Table 3).

Table 3: Diagnostic Concentration of *Bti/Bs* and *Bti* when used on *Culex quinquefasciatus* and *Anopheles gambiae* complex larvae.

Time (Day)	*Bti/Bs*				*Bti*			
	Culex quinquefasciatus		*Anopheles gambiae* complex		*Culex quinquefasciatus*		*Anopheles gambiae* complex	
	LC99.9(mg/l) with 95% C.I.	Diagnostic concn. (mg/l)	LC99.9(mg/l) with 95% C.I.	Diagnostic concn. (mg/l)	LC99.9(mg/l) with 95% C.I.	Diagnostic concn. (mg/l)	LC99.9(mg/l) with 95% C.I.	Diagnostic concn. (mg/l)
1	3.301 (1.951-10.083)	6.601	4.273 (2.326-16.894)	8.547	7.434 (3.279-67.416)	14.862	10.138 (3.949-215.476)	21.475
2	2.589 (1.609-6.946)	5.179	3.801 (2.096-14.578)	7.601	9.016 (3.483-141.941)	18.032	12.649 (4.136-482.397)	25.298
3	2.133 (0.970-13035.208)	4.266	3.326 (1.232-516E +12)	6.652	8.698 (3.265-172.348)	17.395	15.557 (4.212-2893.193)	31.114
4	2.076 (0.892-1.470E +15)	4.151	2.550 (1.223-2.406E +16)	5.099	5.447 (2.441-49.984)	10.895	11.623 (3.463-1440.615)	23.246
5	1.616 (1.062-3.961)	3.233	2.719 (1.466-14.041)	5.437	3.521 (1.808-19.906)	7.041	6.875 (2.466-347.646)	13.750
6	1.203 (0.781-3.559)	2.405	2.531 (1.322-17.978)	5.063	2.719 (1.466-14.041)	5.437	7.358 (2.376-1667.333)	14.716
7	0.849 (0.569-2.654)	1.697	1.373 (0.837-5.678)	2.745	2.334 (1.267-13.385)	4.668	3.020 (1.462-32.677)	6.041
8	0.624 (0.417-3.330)	1.249	1.180 (0.703-7.684)	2.360	1.264 (0.795-4.467)	2.529	1.356 (0.853-4.516)	2.712

5. DISCUSSION

Results showing the efficacy of different larvicides (*Bti*/*Bs* and *Bti*) on field-collected vectors (*Anopheles gambiae* complex and *Culex quinquefasciatus*) revealed that higher percentage of vector mortality occurred with increase in concentration (dosage) and exposure period to *Bti/Bs* and *Bti* alone. The dose-response line graph (Fig. 2), generally showed that susceptibility of *Culex quinquefasciatus* to *Bti/Bs* was higher for every concentration, than it was for *Bti* alone. The observed high larvicidal activities of *Culex quinquefasciatus* treated with *Bti/Bs as compared to Anopheles gambiae* complex treated with *Bti* alone agrees with the study of Fillinger *et al.*, (2003) and Majambere *et al.*, (2007). According to Wirth *et al.*, (2004), this could be attributed to endotoxin production by *Bti*/*Bs*, which combined to form a complex, along with the synergistic interaction between the two microbial larvicides, the level of toxic action on susceptible vectors increases and this works to evade resistance, in a manner that *Bti* alone, cannot achieve.

The significant mean differences observed between one larvicide and the larval species it was treated implies that one larvicide-larval species combination is better than the other, in other words, the combination of *Bti-Bs against Culex quinquefasciatus* had a higher biopotency than *Bti alone*.

The percentage mortality of *Culex quinquefasciatus* and *Anopheles gambiae* complex when treated with *Bti/Bs* larvicides, showed *Culex quinquefasciatus* to be more susceptible than *Anopheles gambiae* complex, to the larvicide (Fig. 4) but the margin was quite narrow, such that when the mortality of the two larvae was compared using Students-t test, there was no significant difference between them ($t= 1.845$; $P > 0.05$). A similar observation was made when the percentage mortality of *Culex quinquefasciatus* and *Anopheles gambiae* complex was treated with *Bti* larvicide. The result was a zigzag line graph (Fig. 5), which crossed each other as the two larval species responded, when treated with the larvicide but *Culex quinquefasciatus* was again more susceptible than *Anopheles gambiae* complex, with a close narrow margin, with *Bti* larvicide, as observed previously with *Bti/Bs.* In the case of the *Bti* larvicide, when the percentage mortality of the two larvae were compared using Students-t test there was no significant difference ($t=1.77$; $P > 0.05$). This result also agrees with the findings of Dylo *et al.*,(2014).

The effect of the concentration on the mortality of the larvae showed that, mortality of the larvae increased and there was significant difference in all the comparisons of concentrations. As the means were separated into groups, significant difference was observed from one another in all the concentrations.

The overall significant effect of concentration on the mortality of the larvae species indicates that, it was not only the biopotency of the larvicides that was solely responsible for the mortality result in this research work, but also the different concentrations of the larvicides did impact on the mortality. The mortality mean increased as the concentration increased in all the larvicide – larvae species combinations. The combined effect of both larvicides and concentrations on the mortality of the larvae species was analysed and found to be significant ($F=6.600$; $P < 0.05$), though the effect of this combination on mortality was less than the effect of the larvicides alone, on the mortality of the larval species. It was also observed that the effect of the larvicides alone on the larval species was less than the effect of the different concentrations on the mortality of the larvae species. The time duration (in days) also contributed to the increase in mortality level of the larvae species. It was observed that, mortality across all the concentrations consistently increased as the days increased, which means, the longer the days, the higher the percentage mortality.

6. CONCLUSION

Comparatively, susceptibility of *Anopheles gambiae* complex and *Culex quinquefasiatus* to *Bti/Bs* was higher for all concentrations than it was for *Bti* alone. *Culex quinquefasciatus* treated with *Bti/Bs* had the highest larvicidal activity, while *Anopheles gambiae* complex treated with *Bti* alone, showed the weakest larvicidal activity. *Bti/Bs* was more lethal than *Bti* alone, against *Anopheles gambiae* complex and *Culex quinquefasciatus.* The LC_{50} and LC_{90} (0.392 and 1.109) mg/l respectively of *Culex quinquefasciatus* treated with *Bti/Bs* was lower than LC_{50} and LC_{90} (0.458 and 1.470) mg/l respectively of *Anopheles gambiae* complex treated with *Bti/Bs.*

Culex quinquefasciatus is more susceptible than *Anopheles gambiae* complex, to both microbial larvicides and *Bti/Bs* is more effective than *Bti* alone. The diagnostic concentrations of 5.179mg/l and 7.601mg/l are required for monitoring vulnerability of *Culex quinquefasciatus* and *Anopheles gambiae* complex respectively, in the field, using *Bti/Bs* larvicide. In larviciding activity of *Anopheles gambiae* complex and *Culex quinquefasciatus*, *Bti/Bs* was more effective and should be preferred over *Bti* alone, since both vectors are more susceptible to *Bti/Bs* than they are to *Bti* alone. *Bti/Bs* is preferred in field application for use on *Culex quinquefasciatus*, since it is more susceptible to the larvicide than *Anopheles gambiae* complex.

REFERENCES

1. Awolola, T. S., Oduola, O. A., Strode, C., Koekemoer, L.L., Brooke, B. & Ranson, H. (2008). Evidence of multiple pyrethroid resistance mechanism in the malaria vector *Anopheles gambiae* sensus stricto from Nigeria. *Royal Society Tropical Medicine & Hygiene*, 103(11): 1139 – 1145.
2. Chanda, E., Kandyata, A., Chanda, J. & Baboo, K. S. (2013). Bio-efficacy and persistence evaluation of Dimilin® GR-2% and Mosquiron® 10 EC insect growth regulators against *Anopheles gambiae* s.l. (diptera: Culicidae) larvae. *Journal of Biomedical Science and Engineering*, 6: 11-16.
3. Charles, J. F. & Nielsen-Leroux, C. (2000). Mosquitocidal bacterial toxins: diversity, mode of action and resistance phenomena. *Memorias Do Instituto Oswaldo Cruz*, 95: 201-206.
4. Corbel, V., N'Guessan, R. N., Brengues, C., Chandre, F., Djogbenou, L., Martin, T. & Rowland, M.(2007). Multiple insecticides resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. *Acta Tropical*, 101: 207-216.
5. Dylo, P., Martin, C. & Mhango, C. (2014). Efficacy of *Bacillus thuringiensis* var *israelensis* (*Bti*) on *Culex* and *Anopheline* mosquito larvae in Zomba. *Malawi Journal of Science and Technology*, 10(1): 40-52.
6. Eskenazi, B., Chevrier, J., Rosas, L. G., Anderson, H. A., Bornman, M.S., Bouwman, H. & Stapleton, D. (2009). The Pine River Statement: human health consequences of DDT us. *Environmental Health Perspectives*, 117(9): 1359-67.
7. Federal Republic of Nigeria (2007). Legal Notice on Publication of the Details of the breakdown of the National and State provisional Totals 2006 Census, *Official Gazette.*
8. Fillinger, U., Knols, B.G.J. & Becker, N. (2003). Efficacy and efficiency of new *Bacillus thuringiensis* var *israelensis* and *Bacillus sphaericus* formulations against Afrotropic alanopheline in Western Kenya. *Tropical Medicine and International Health*, 8(1): 37-47.
9. Gillett, J. D. & Smith, J. G. (1972).Common African Mosquitoes and their medical importance. London, England: William Heinemann Medical Books Ltd.
10. Gu, W., Regens, J. L., Beier, J. C. & Novak, R. J. (2006). Source reduction of mosquito larval habitats has unexpected consequences on malaria transmission. *Proceedings of the National Academy of Sciences of the United States of America*, 103(46): 17560-17563.
11. Harback, R.E. & Knight, K.L.(1980). Taxonomist's glossary of mosquito anatomy. Marlton, New Jersey: Plexus Publishing Incorporated.
12. Killeen, G. F., Fillinger, U. & Knols, B.G.J. (2002) Advantages of larval control for African malaria vectors: low mobility and behavioural responsiveness of immature stages allow high effective coverage. *Malaria Journal*, 1(8): 18 -29.
13. Le Menach, A., Takala, S. & Mckenzie, F.E. (2007). An elaborated feeding cycle model for reductions in vectorial capacity of night-biting mosquitoes by insecticide-treated nets. *Malaria Journal*, 6(10): 3-8.
14. Majambere, S., Lindsay, S. W., Green, C., Kandeh, B. & Fillinger, U. (2007). Microbial larvicides for malaria control in the Gambia. *Malaria Journal*, 6(76): 46-53.
15. Poopathi, S. & Tyagi, B. K. (2006). The challenge of Mosquito Control Strategies: from Primordial to Molecular approaches. *Biotechnology and Molecular Biology Review*, 1(2): 51-65.
16. Presidential Malaria Initiative, PMI(2017). Malaria Operational Plan FY 2017. United States Agency for International Development.
17. Randson, H., N'Guessan, R., Lines, J., Moiroux, N., Nkuni, Z. & Corbel, V. (2011). Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends in Parasitology*, 27(2): 91-98.
18. United States Environmental Protection Agency(USEPA). (11th April, 2007). Larvicides for mosquito control. United States Environmental Protection Agency.
19. World Health Organisation WHO (1999). International programme on chemical safety (IPCS): microbial pest control agent *Bacillus thuringiensis. Environmental Health Criteria*, 217: 1-105.
20. World Health Organisation (WHO). (2016a). Fact sheet. Geneva: World Health Organization.

21. World Health Organisation (WHO).(2016b). Fact sheet: World malaria Report 2015. Geneva:World Health Organization.
22. Wirth, M. C., Jiannino, J. A., Federici, B. A. & Walton, W. E. (2004). Synergy between toxins of *Bacillus thuringiensis* subsp *israelensis* and *Bacillus sphaericus. Journal of Medical Entomology*, 41(5): 935-911.
23. Yu, M., Tsunoda, H. & Tsunoda, M. (2011). Environmental Toxicology: Biological and Health Effects of Pollutants (3rd Ed.). New York: CRC Press.