International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319-6718, ISSN (Print): 2319- 670X
www.ijpsi.org Volume 14 Issue 6 ǁ Nov. – Dec., 2025 ǁ PP. 77-82

Larvicidal activities of *Bacillus thuringiensis* (Bt) on larvae of *Culex quinquefasciatus* in lymphatic filariasis vector control in Lokossa district in south-western Republic of Benin, West Africa

Sylvestre Codjia[1], Habib Tamègnon[1], Nazaire Aïzoun[1*] and Daniel Chougourou[2]

[1]*Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET), Normal High School of Technical Teaching (ENSET) of Lokossa, National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM) of Abomey, P. O. Box 133 Lokossa Cotonou, Benin*

[*] ***Corresponding Author:*** *Nazaire Aïzoun*

[2]*Department of Environment Genius, Polytechnic School of Abomey-Calavi (EPAC), University of Abomey-Calavi (UAC), Cotonou, Benin.*

Abstract

Background: *Mosquito control programs are now threatened by the selection of mosquito populations resistant to the chemical insecticides. Thus, alternative vector control methods are necessary.*

Objective: *This study was aimed at investigating on the larvicidal activities of Bacillus thuringiensis (Bt) on larvae of Culex quinquefasciatus in lymphatic filariasis vector control in Lokossa district in Mono department in south-western Benin, West Africa.*

Material and Methods: *Larvae of Culex quinquefasciatus Say mosquitoes were collected from breeding sites using the dipping method from September to November 2024 during the small rainy season in Lokossa district. A batch of 25 larvae of fourth instar were exposed to a mixture of Bacillus thuringiensis with distilled water saturated with oxygen containing in each of five glass jars or test cups of same dimensions with different concentrations of 1mg/l, 2mg/l, 3mg/l, 4mg/l and 5mg/l and one control jar for each serial concentrations containing no trace of Bacillus thuringiensis. Larval mortality was recorded after 24 hours, 48 hours and 72hours exposure.*

Results: *The results showed that Bacillus thuringiensis had acted by poisoning the larvae of four instars which could not breathe and pupate.*

Conclusion: *The use of Bacillus thuringiensis disallows mosquito larvae to acquire tolerance.*

Key words: *Bacillus thuringiensis, siphonal respiration, larvae of Culex quinquefasciatus, Republic of Benin*

Date of Submission: 11-12-2025 Date of acceptance: 22-12-2025

I. Introduction

Mosquitoes are of immense importance because they are able to host and transmit various diseases pathogens species of virus, protozoa and nematodes, till today; they pose a threat to health [1]. The Mosquito born-diseases are prevalent almost in all Africa countries and infecting over millions of people every year globally but less in developed countries. They act as a vector for most of the life threatening diseases like malaria, filariaisis, yellow fever, denque fever, encephalitis, and virus infection and so on, in almost all tropical and subtropical countries and other part of the world.

Killeen *et al*. [2] suggest that the limitations of larval control in sub-Saharan Africa are "practical rather than functional" and that, because of the limited mobility of immature mosquito stages they can be effectively controlled. Several studies have shown that relatively large water bodies harbor mosquito larvae in western Kenya (Lockhart *et al*. [3], Fillinger *et al*. [4], Mutuku *et al*. [5], Howard *et al*. [6], Howard and Omlin [7]) and, presumably, also elsewhere in Africa where rural populations are increasingly putting more pressure on the land.

To prevent proliferation of mosquito born-diseases and to improve quality of environment and public health, control is essential. The control of mosquitoes has received some attention based on the study of ecology and distribution with field trials of pesticides aimed at controlling and eradicating it. The major tools in

mosquitoes control operation are the application of synthetic insecticides such as organochlorine and organophosphate compounds.
Current researches have to explore several alternative avenues of controlling the bites of adult *Cx. Quinquefasciatus*, and one particular approach that appears to be gaining attention is an environmental management strategy that aims to reduce adult vector population by targeting their aquatic immature stages (i.e., mosquito eggs, larvae and pupae).

Very few researches were published on the use of *Bacillus thuringiensis* in the management of larvicide resistance in larvae of *Culex quinquefasciatus* Say mosquitoes in Republic of Benin. Therefore, there is a need to carry out new researches for this purpose.

The goal of this study was to investigate on the larvicidal activities of *Bacillus thuringiensis* (Bt) on larvae of *Culex quinquefasciatus* in lymphatic filariasis vector control in Lokossa district in Mono department in south-western Benin, West Africa.

II. Material and methods

Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Mono. Mono department is located in the south-western Republic of Benin and the study was carried out in Lokossa district (Figure 1). The southern borders of this district are Athiémé and Houéyogbé districts. The northern border is Dogbo district. The eastern border is Bopa district and the western border is Togo republic. Lokossa district covered 260 km^2 .The choice of the study sites took into account the economic activities of populations, their usual protection practices against mosquito bites and peasant practices to control farming pests. We took these factors into account to study the larvicidal activities of *Bacillus thuringiensis* (Bt) on larvae of *Culex quinquefasciatus* in lymphatic filariasis vector control in Lokossa district in south-western Republic of Benin, West Africa. Lokossa has a climate with four seasons, two rainy seasons (March-July and August-November) and two dry seasons (November-March and July-August).The temperature ranged from 25 to 32°C with the annual mean rainfall, which is between 900 et1100 mm.

Figure 1: Map of Republic of Benin showing Lokossa District Surveyed

Mosquito sampling

Culex quinquefasciatus larvae were collected from September to November 2024 during the small rainy season in Lokossa district. Larvae were collected from breeding sites using the dipping method and kept in labeled bottles (Figure 2). The samples were then carried out to the Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) in the Department of Sciences and Agricultural Techniques located in Dogbo district.

Figure 2: *Culex quinquefasciatus* larvae breeding site surveyed in Lokossa district

Purchase of *Bacillus thuringiensis*

Bacillus thuringiensis used in the current study was in wettable powder and selt in some bags of 100g and then carried in the Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) of Department of Sciences and Agricultural Techniques at Normal High School of Technical Teaching (ENSET) of Lokossa. It was then held in refrigerator in Laboratory.

Bioassays

A batch of twenty five (25) larvae of four instars reared in the insectary of the Department of Sciences and Agricultural Techniques was added to each of five glass jars or test cups of same dimensions containing the dilutions of 1.0mg/liter, 2.0mg/liter, 3.0mg/liter, 4.0 mg/liter and 5.0mg/liter respectively of *Bacillus thuringiensis*. These tests cup were covered with small cutting untreated net. At each range of dilutions, there is a corresponding control. The control jars contained no trace of *Bacillus thuringiensis*.

Four replicates were set up and an equal number of controls were set up simultaneously with distilled water. The test containers were held at 25-28°C.

Larval mortality was recorded after 24hours, 48hours and 72hours exposure. Dead larvae were those that could not be induced to move when they were probed with a needle in the siphon or the cervical region. Moribund larvae were those incapable of rising to the surface or not showing the characteristic diving reaction when the water was disturbed.

Statistical analysis

Analysis using t-test was performed with 95% confidence interval in SPSS version 16.0 (SPSS Inc., Chicago, IL). The p-value acquired by t-test for all cases of this study is less than 5%.

III. Results

Evaluation of larvicidal effect of *Bacillus thuringiensis* on larvae of *Culex quinquefasciatus* from Lokossa district

The analysis of figure 3 showed that after the exposure of *Culex quinquefasciatus* larvae of four instars (L4) to *Bacillus thuringiensis*, no dead and moribund larvae were registered in the control plastic cups after 24 hours, 48 hours and 72hours recording, they were all alive. The analysis of the same figure showed that a lot of dead larvae were registered after 24 hours exposure with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l ($P < 0,05$). The recording of 48 hours exposure also showed that a lot of dead larvae were registered in the all test plastic cups with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l ($P <0,05$). In addition, all the tested larvae with concentrations of 3 mg/l, 4mg/l and 5mg/l were dead. The recording of 72 hours exposure showed that more dead larvae were registered in the all test plastic cups with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l ($P <0,05$). Otherwise, the number of dead larvae recorded after 72 hours exposure was higher than that registered after 48 hours exposure. Finally, the highest mortality rate was recorded with the concentrations of 3 mg/l, 4mg/l and 5mg/l (125 dead larvae on a total of 125 tested larvae).

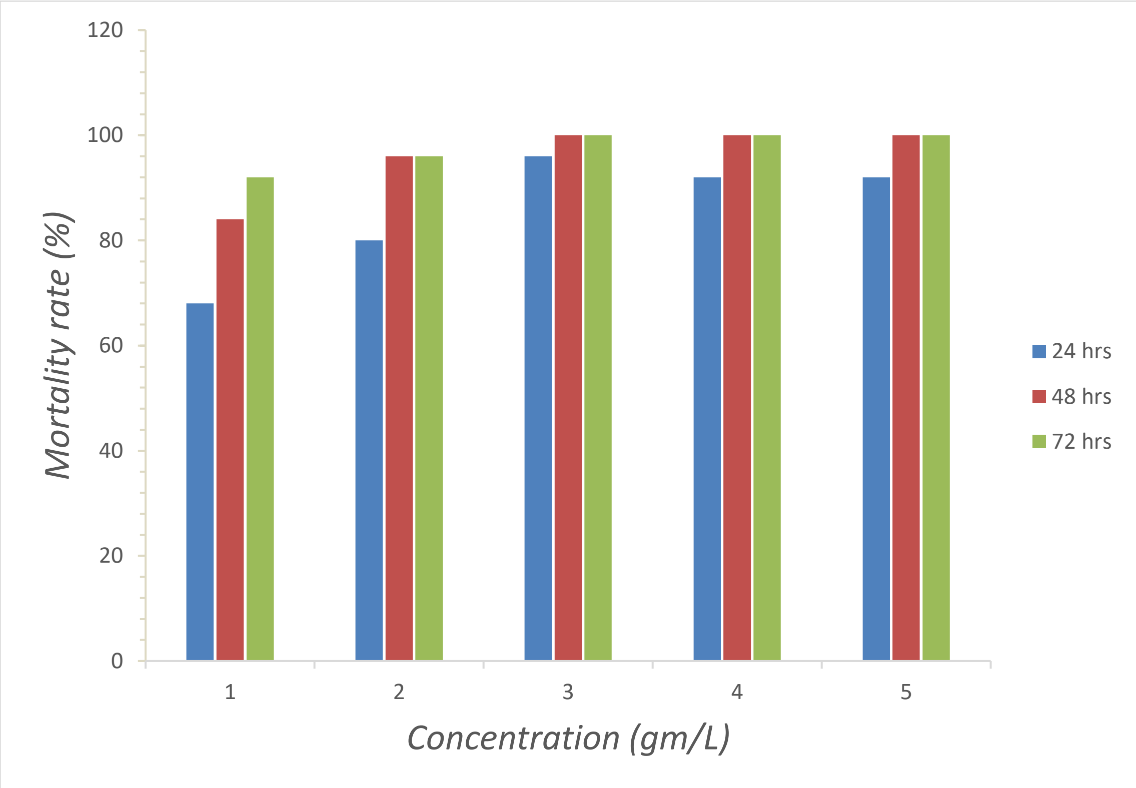

Figure 3: Larvicidal activity of *Bacillus thuringiensis* on larvae of *Culex quinquefasciatus*

Advantages and disadvantages of the use of *Bacillus thuringiensis* against *Culex quinquefasciatus* larvae

The analysis of Table 1 shows that there are many advantages in the use of *Bacillus thuringiensis* to control mosquito larvae. But, also there are very few disadvantages.

Table 1: Advantages and disadvantages of the use of *Bacillus thuringiensis*

Advantages	**Disadvantages**
During sporulation, *Bti* produces a highly specific delta endotoxin, which is only toxic to larvae of mosquitoes, black flies and closely related flies upon ingestion	Expensive for large-scale treatment (is the main disadvantage)
Bti is effective where insects have developed resistance to synthetic and/or biochemical larvicides	

Residual efficacy is dependent on target habitat/species complex and formulation type This bacterium produces insecticidal crystal proteins that kill susceptible larvae within 24h of ingestion At recommended dosages it is harmless to non-target aquatic invertebrates, insects, fish, birds, animals and humans	
It is safe for use in drinking water or on irrigated crops	
Mosquitoes cannot develop resistance to *Bti*	

IV. Discussion

In the current study, after the exposure of *Culex quinquefasciatus* larvae of four instars (L4) to *Bacillus thuringiensis*, no dead and moribund larvae were registered in the control plastic cups after 24 hours, 48 hours and 72hours recording, they were all alive. A lot of dead larvae were registered after 24 hours exposure with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l. The 48 hours exposure also showed that a lot of dead larvae were registered in the all test plastic cups with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l. In addition, all the tested larvae with concentrations of 3 mg/l, 4mg/l and 5mg/l were dead. The 72 hours exposure showed that more dead larvae were registered in the all test plastic cups with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l. Otherwise, the number of dead larvae recorded after 72 hours exposure was higher than that registered after 48 hours exposure. Finally, the highest mortality rate was recorded with the concentrations of 3 mg/l, 4mg/l and 5mg/l. These results showed that *Bacillus thuringiensis* had acted by poisoning the larvae of four instars which could not breathe and pupate. Our results corroborated with those obtained by Lacey and Lacey [8] who had studied the larvicidal activity of *Bacillus thuringiensis* var. israelensis (H-14) against mosquitoes of the central Amazon basin**.** In fact, a standardized air dried spore and crystal preparation of *Bacillus thuringiensis* var. israelensis (IPS-78) produced at the Pasteur institute, Paris was bioassayed under laboratory conditions against late instars of *Culex quinquefasciatus* found in the vicinity of Manaus, Brazil. The LC50 and LC95 for *Culex quinquefasciatus* were 0.042 ppm and 0.33 ppm, respectively. When an LC100 concentration was administered to *Culex quinquefasciatus,* mortality was noticeable after two (02) hours and was complete within twelve (12) hours. Another research carried out by Su [9] had studied the prevention of resistance to spinosad by a combination of spinosad and *Bacillus thuringiensis* subsp. israelensis in *Culex quinquefasciatus* (Diptera: Culicidae). In fact, the Combination of spinosad and *Bti.* negates resistance development to spinosad, as opposed to spinosad alone in *Culex quinquefasciatus*. Moreover, the species tested does not develop resistance to this combination upon repeated exposures, implying the potential for further developing this combination as a viable product for larval mosquito control.

Bti and *L. sphaericus* crystals remain the most powerful and selective insecticidal compounds, available to date, with proven field effectiveness for controlling dipteran species relevant to public health. Recent findings on their mode of action, more specifically on the mechanism of synergistic action of the toxins from both bacteria and the new insights of their interaction with the midgut cells, can be exploited in the future to confer advantages such as broader spectra of action, or to reduce the risk of resistance selection and to improve the persistence under field conditions. Such advancements allied with improved operational practices will allow the evolution of the use of these larvicides from single control agents to their adoption as part of more effective integrated control programs. In addition to the effectiveness of the toxins currently available, these entomopathogenic bacteria also represent opportunities to develop new and/or improved toxins able to display better activities and play an outstanding role in the future of mosquito control.

V. Conclusion

The use of *Bacillus thuringiensis* israelensis (*Bti*) is effective method for disturbing the respiration of *Culex quinquefasciatus* mosquito larvae. However, this study was conducted in laboratory conditions and there is also a need to carry it out in field conditions for better conclusions.

Acknowledgements

The authors would like to thank people from Lokossa district surveyed who had helped us in mosquito collection.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

[1]. Iwuala MOE, and Ejezie G. Control of Arthropod vector of Diseases in Nigeria; A review. Bull Anim Health Prod Afr. 1978; 202-205.

[2]. Killeen G, Fillinger U, and Knols B.. Advantages of larval control for African malaria vectors: low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. Malar J. 2002; 1: 8.

[3]. Lockhart JDF, Highton RB, and McMahon JP. Public health problems arising out of man-made Þsh ponds in the Western Province of Kenya Þsh culture. East Afr Med J. 1969; 46: 471-480.

[4]. Fillinger U, Sonye G, Killeen GF, Knols BGJ, and Becker N. The practical importance of permanent and semipermanent habitats for controlling aquatic stages of *Anopheles gambiae* sensu lato mosquitoes: operational observations from a rural town in western Kenya. Trop Med Int Health. 2004; 9: 1274-1289.

[5]. Mutuku FM, Bayoh MN, Gimnig JE, Vulule JM, Kamau L, Walker ED, Kabiru E, and Hawley WA. Pupal habitat productivity of *Anopheles gambiae* complex mosquitoes in a rural village in western Kenya. Am J Trop Med Hyg. 2006; 74: 54-61.

[6]. Howard AFV, Zhou G, and Omlin FX. Malaria mosquito control using edible Þsh in western Kenya: preliminary Þndings of a controlled study. BMC Public Health. 2007; 7: 199.

[7]. Howard AFV, and Omlin FX. Abandoning small-scale Þsh farming in western Kenya leads to higher malaria vector abundance. Acta Trop. 2008; 105: 67-73.

[8]. Lacey LA and Lacey JM. The larvicidal activity of *Bacillus thuringiensis* var. israelensis (H-14) against mosquitoes of the central Amazon basin**.** *Mosquito news*. 1981; 41 (2): 266-270.

[9]. Su T. Prevention of resistance to spinosad by a combination of spinosad and *Bacillus thuringiensis* subsp. israelensis in *Culex quinquefasciatus* (Diptera: Culicidae). *Pest Manag. Sci*. 2024; 80 (12): 6585-6589.