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

ISSN 2349-8870
Volume: 8
Issue: 9
405-410
Year: 2021

# THE USE OF *BACILLUS THURINGIENSIS* IN THE MANAGEMENT OF LARVICIDE RESISTANCE IN LARVAE OF *ANOPHELES GAMBIAE SENSU LATO* FROM DOGBO DISTRICT IN COUFFO DEPARTMENT IN SOUTH-WESTERN REPUBLIC OF BENIN, WEST AFRICA

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**Article Received on 25/06/2021** **Article Revised on 14/07/2021** **Article Accepted on 2/08/2021**

## ABSTRACT
**Background**: The use of chemical insecticides causes important damages to environment and human health and there is a need to search for alternative solutions. **Objective:** This study aims to investigate on the use of *Bacillus thuringiensis* in the management of larvicide resistance in larvae of *Anopheles gambiae sensu lato* from Dogbo district in Couffo department in south-western Benin, West Africa. **Methodology:** Larvae of *Anopheles gambiae s.l* mosquitoes were collected from breeding sites using the dipping method in June 2020 during the rainy season in Dogbo district. A batch of 20 larvae of four instars 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 contained each 48 ml distilled water saturated with oxygen plus 2 mg of *Bacillus thuringiensis* and one control jar containing no trace of *Bacillus thuringiensis*. Larval mortality was recorded after 24 hours, 48 hours and 72hours exposure. **Results:** The results show that *Bacillus thuringiensis* acts by poisoning the larvae of four instars which cannot breathe and pupate. **Conclusion:** The use of *Bacillus thuringiensis* disallows mosquito larvae to acquire tolerance.

**KEYWORDS:** *Bacillus thuringiensis*, siphonal respiration, larvae of *Anopheles gambiae s.l.*, malaria control, Benin.

## INTRODUCTION
The increase in the use of World Health Organization approved vector control methods between 2000 and 2015 contributed to a large decline in the number of malaria cases globally. But progress against malaria has stalled. There was no significant reduction in global malaria between 2015 and 2017. An estimated 219 million malaria cases were reported in 2017 (versus 213 million in 2015). There were more than 435 000 deaths in 2017.[1]

In Africa, the vector control relies primarily on two effective and complementary tools: long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS).[2,4] Several studies have demonstrated the effectiveness of both tools in reducing the incidence of malaria.[5,6] morbidity and mortality in Africa.[7,10]

In Benin, malaria vector control also relies mainly on the mass distribution of LLINs, and on IRS operations with good results.[11] However, some researches showed that these measures are currently inadequate.[12] and not sufficient to halt malaria transmission and would likely contribute to the eventual emergence of insecticide resistant mosquitoes.[13,15]

Currently researchers have been exploring several alternative avenues of controlling malaria, 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). This strategy is becoming increasingly important in many countries especially in sub-Saharan Africa and involves different species of mosquitoes including those that transmit malaria. The strategy depends on the use of various larvicidal techniques and environmental management practices aimed at reducing larval density and therefore minimizing or reducing vector abundance.[16]

Very few researches were published on the use of *Bacillus thuringiensis* in the management of larvicide resistance in larvae of *Anopheles gambiae s.l.* from Dogbo district in Couffo department in south-western Benin. Therefore, there is a need to carry out new researches for this purpose.

The goal of this study was to determine if there is tolerance to *Bacillus thuringiensis* in *Anopheles gambiae s.l.* larvae from Couffo department in south-western Benin.

## MATERIALS AND METHODS

### Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Couffo. Couffo department is located in the south-western Benin and the study was carried out more precisely in Dogbo district (Fig.1). The southern borders of this district are Lokossa and Bopa districts. The northern border is Djakotomey district. The eastern border is Lalo district and the western border of Dogbo district is Togo republic. Dogbo district covered 475 km2 and belongs to geographic region of ADJA. The choice of the study site 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 use of *Bacillus thuringiensis* in the management of larvicide resistance in larvae of *Anopheles gambiae s.l.* from Dogbo district in Couffo department in south-western Benin**.** Couffo has a climate with four seasons, two rainy seasons (March to July and August to November) and two dry seasons (November to March and July to August). The temperature ranges from 25 to 30°C with the annual mean rainfall between 900 and 1100 mm.

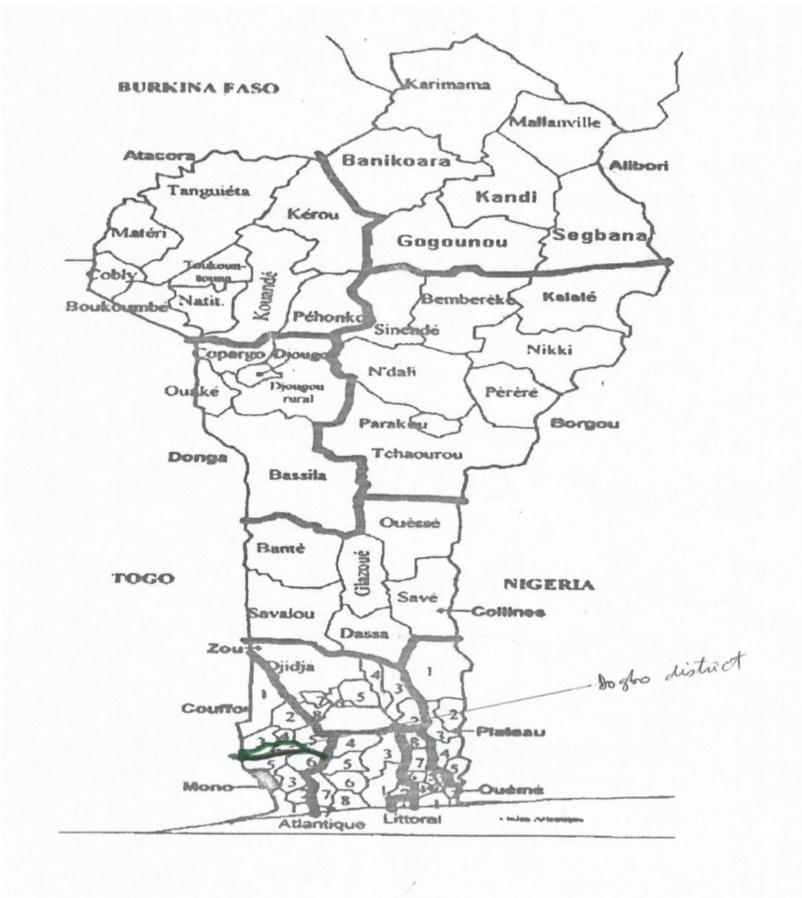


**Fig. 1: Map of Republic of Benin showing Dogbo District.**

**Mosquito sampling**

*Anopheles gambiae s.l.* mosquitoes were collected in June 2020 during the rainy season in Dogbo district. Larvae were collected from breeding sites using the dipping method and kept in labeled bottles (Fig.2). The samples were then carried out to the Laboratory of Applied Entomology and Vector Control (LAEVC) of the Department of Sciences and Agricultural Techniques located in Dogbo district.

**Fig. 2: An *An. gambiae s.l.* larvae breeding site surveyed in Dogbo district.**

**Purchase of *Bacillus thuringiensis***

*Bacillus thuringiensis* used in the current study were bought in a shop in Lomé, capital of Republic of Togo. It was in wettable powder and selt in some bags of 100g and then carried by car to Republic of Benin more precisely in Laboratory of Applied Entomology and Vector Control (LAEVC) of the Department of Sciences and Agricultural Techniques of Normal High School of Technical Teaching (ENSET) of Lokossa. It was then held in refrigerator in Laboratory.

**Bioassays**

A batch of 20 larvae of four instars reared in the insectary of the Laboratory of Applied Entomology and Vector Control (LAEVC) was added in each of five glass jars or test cups of same dimensions contained each 48 ml distilled water saturated with oxygen plus 2 mg of *Bacillus thuringiensis* and one control jar containing no trace of *Bacillus thuringiensis*. Otherwise, the control jar or control cup containing only 50 ml distilled water saturated with oxygen and 20 larve of four instars.

Four replicates were set up and an equal number of controls were set up simultaneously with distilled water. Each test was run three times on different days. The test containers were held at 25-28°C.

Larval mortality was recorded after 24 h, 48 h and 72h exposure. Moribund larvae were counted and added to dead larvae for calculating percentage mortality. 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%. Abbott's formula was not used in this study for the correction of mortality rates in test jars because the mortality rates in all controls was always less than 5%.[17]

## RESULTS

The recording of the number of dead larvae was done after 24hours, 48 hours and 72hours exposure. The analysis of Table 1 shows that no dead larvae was registered in control jar or control cup during the different bioassays. After 24hours exposure, there was no alive larvae in test cups, but three (03), one (01) and two (02) moribund larvae respectively were registered during the bioassay 1, 2 and 3.

**Table 1: Recording the number of dead larvae after 24 hours exposure.**

| Control | | | | Bioassay 1 | | | | Bioassay 2 | | | | Bioassay 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number tested | Alive | Moribund | Dead | Number tested | Alive | Moribund | Dead | Number tested | Alive | Moribund | Dead | Number tested | Alive | Moribund | Dead |
| 20 | 20 | 0 | 0 | 20 | 0 | 3 | 17 | 20 | 0 | 1 | 19 | 20 | 0 | 2 | 18 |

In the same way, the analysis of Table 2 shows that no dead larvae was registered in control jar or control cup during the different bioassays. After 48hours exposure, there still was no alive larvae in test cups, but two (02), one (01) and one (01) moribund larvae respectively were registered during the bioassay 1, 2 and 3. These results show that some of moribund larvae were died after 24 hours exposure to the mixture of *Bacillus thuringiensis* with distilled water saturated with oxygen.

**Table 2: Recording the number of dead larvae after 48 hours exposure.**

| Control | | | | Bioassay 1 | | | | Bioassay 2 | | | | Bioassay 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number tested | Alive | Moribund | Dead | Number tested | Alive | Moribund | Dead | Number tested | Alive | Moribund | Dead | Number tested | Alive | Moribund | Dead |
| 20 | 20 | 0 | 0 | 20 | 0 | 2 | 18 | 20 | 0 | 1 | 19 | 20 | 0 | 1 | 15 |

The same remark was made when we analyze the Table 3. In fact, after 72 hours exposure, there was no alive and no moribund larvae in the test cups of the different bioassays. They were all died.

**Table 3**: **Recording the number of dead larvae after 72 hours exposure.**

| Control | | | | Bioassay 1 | | | | Bioassay 2 | | | | Bioassay 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number tested | Alive | Moribund | Dead | Number tested | Alive | Moribund | Dead | Number tested | Alive | Moribund | Dead | Number tested | Alive | Moribund | Dead |
| 20 | 20 | 0 | 0 | 20 | 0 | 0 | 20 | 20 | 0 | 0 | 20 | 20 | 0 | 0 | 20 |

The analysis of Table 4 shows that there are many advantages in the use of *Bacillus thuringiensis* to control mosquito larvae. But, also there are very few disadvantages.

**Table 4**: **Advantages and disadvantages of the use of *Bacillus thuringiensis.***

| Advantages | Disadvantages |
|---|---|
| During sporulation, *Bacillus thuringiensis* **(***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* | |

## DISCUSSION

The control measures for mosquitoes involve chemical control,[18,20] biological control, environmental management, genetic control, and physical control,[21] In addition, the density of *An. gambiae* larvae were influenced by several physicochemical environmental factors or parameters that are associated with mosquito breeding sites. These environmental factors were functions of the human related activities going on around these breeding sites.

The results obtained in the current study shows that *Bacillus thuringiensis* acts by poisoning the larvae of four instars of *Anopheles gambiae sensu lato* surveyed in Dogbo district in south-western Benin. After the application of *Bti*, the larvae of four instars cannot breathe and also cannot pupate. In fact, the most of them died only after 24 hours exposure to the mixture of *Bacillus thuringiensis* with distilled water saturated with oxygen.

Bacterial larvicides (BL) have a number of advantages in general. They are highly efficacious against malaria vectors, do not harm non-target organisms, are selective in action, are unlikely to produce resistance and are safe for humans to handle.[22] Bacterial larvicides differ from conventional chemicals or biochemicals in that identical strains produced under different conditions by different manufacturers will have varying end-use product quality and biological performance. It is important to use WHOPES-recommended bacterial larvicides and formulations that are linked to specific strain numbers to ensure biological and performance equivalency,[23]

In the current study, many advantages in the use of *Bacillus thuringiensis subsp.* israelensis (*Bti*) can explained the results obtained. In fact, *Bti* is a naturally occurring, spore-forming bacterium found in soil and aquatic environments throughout the world. 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. *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. But, expensive for large-scale treatment is the main disadvantage of the use of *Bti*.

## CONCLUSION

The use of *Bacillus thuringiensis* disallows mosquito larvae to acquire tolerance. It acts by poisoning them. In the current study, the use *Bti* is effective method for disturbing the respiration of mosquito larvae. After the exposure to the mixture of *Bacillus thuringiensis* with distilled water saturated with oxygen, the larvae of four instars cannot breathe and also cannot pupate. However, this study was conducted in laboratory conditions and there is also a need to carry it out in field conditions for better conclusions.

## ACKNOWLEDGEMENT

The authors would like to thank people from locations surveyed who had helped us in mosquito collection. We would also like to thank KOUASSI Prisca for technical assistance in laboratory during the current study.

## CONFLICTS OF INTEREST

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

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