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# EVALUATION OF *Bacillus thuringiensis* FROM DIFFERENT HABITATS FOR LETHALITY AGAINST LARVAE OF SOME SPECIES OF MOSQUITO

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**ABSTRACT**

This work was aimed at evaluating the lethality effects of *Bacillus thuringiensis* isolated from different habitats *against* larvae of some species of mosquito. The isolated and identified microorganism from the different sampling sites were subjected to bioassay against different species of mosquito larvae at 0, $8.0 \times 10^9$, $1.6 \times 10^{10}$, $3.2 \times 10^{10}$, $6.4 \times 10^{10}$, and $7.2 \times 10^{10}$ cells/ ml. The result of the study revealed that *B. thuringiensis* isolated from compost manure had the highest bioactivity of 100% mortality against all the three species of mosquito larvae used in this work at the concentration of $3.2 \times 10^{10}$ cells / ml while those isolated from garden soil, refuse, animal residue and dry leaf residue gave 100% mortality at $6.4 \times 10^9$ cells / ml against the three species of mosquito larvae with the exception of that isolated from animal residue which gave 95% mortality against aedes larvae at $6.4 \times 10^{10}$ cells/mls. Analysis of variance at $P < 0.05$ revealed a significant difference in activity of *B. thuringiensis* isolated against mosquito larvae belonging to different species. This indicated that *Bacillus thuringiensis* had higher lethal activities against culex and anopheles larvae than aedes larva.

**Keywords:** *Bacillus thuringiensis*; Bioactivity; Mosquito; Larvae; Mortality

## INTRODUCTION

Malaria is endemic throughout most of the tropics and over 94% of the disease is seen in Africa (WHO, 2021). The mode of transmission of malaria is through the bite of female Anopheles mosquito. Pregnant women, children under five years of age and immune compromised people are most vulnerable to malaria (Phillips, 2003). Chemical insecticide that used to be effective against mosquito control is facing the problem of its potency. Economically, the rapidly increasing cost for development and synthesis of petro chemically derived insecticides together with the reducing effectiveness as a result of widespread insect resistance; the chemical pesticide industries continue to develop new and more expensive compounds with increase in price. The chemically synthesized pesticides pose toxicity risks to the environment. It is estimated that only a minute fraction of the insecticides applied would be required for suppression of the target pest. The remainder, more than 99% enters the environment through soil, water and food cycles (Killeen *et al.*, 2002).

Alternative method which involves the use of microbial insecticides for insect management offers adequate level of pest control and poses fewer hazards. *Bacillus thuringiensis* (Bt) is a spore forming, gram-positive rod bacterium of ubiquitous occurrence. It produces proteinaceous crystal (cry) toxin. There proteinaceous inclusions of *Bacillus thuringiensis* are called as crystal protein or delta endotoxins (Tabashnik *et al.*, 1991). These delta endotoxins are activated by proteases in the alkaline condition of the midgut. These activated toxins binds with the receptor on the brush border membrane vesicle of the midgut epithelium and perforate the cell membrane, which leads to ionic imbalance and insect death. Therefore, this work is aimed at evaluation of *Bacillus thuringiensis* isolated from various habitats as bio pesticides against larvae of some species of mosquito with a view to eradicate the malaria vector.

## MATERIALS AND METHODS

### Sampling site and Sample Collection

The soil samples were collected in Gwale local government area of Kano State Nigeria. The samples were collected with a sterile spatula and placed inside sterile polythene. Five (5) samples from garden soil, refuse, compost, dry leafy residues and animal residues were collected. The soil sample of five (5) grams each was collected aseptically from top to a depth of 10cm after scrapping off the surface materials with a sterile spatula and placed immediately inside polythene and stored at room temperature and processed within one week from the date of collection.

**Isolation of *Bacillus thuringiensis* from the Samples**

One gram of each soil sample was suspended in 10 mls of Luria's broth buffered with 0.25 sodium acetate at pH of 6.8. These samples were then incubated at 50$^{O}$C for four (4) hours. The sodium acetate delays the germination of *Bacillus thuringiensis* spores. After incubation, the samples were then subjected to heat treatment at 80$^{o}$C for 3 minutes.

**Identification of *Bacillus thuringiensis* Isolates**

Identification of *Bacillus thuringiensis* from the Samples was carried as described by Jyothi and Priya (2018). *Bacillus thuringiensis* isolated from five (5) different habitats were smeared thinly on a clean glass slide, dried in air and heat fixed. The smear was covered with crystals violet and kept for one minute. This was then followed by washing the slide and then covered it with iodine solution, allowing it to stand for one (1) minute. The slide was then briefly decolorized with acetone. Serial dilution (up to $10^{-4}$) was carried out on each of the five (5) samples (i.e soil, refuse, compost manure, dry leaf residues and animal residues), concomitantly. About 20 µL of each diluent was spread on Luria's broth (LB) agar and incubated at 37$^{o}$C in an incubator. Colonies with the appearance of a fried egg on the plate were identified as *Bacillus thuringiensis* (BT).The slide was washed with water, counter stained with safranin and allowed to stand for ten 10 minutes before it was washed with water, blot dried and examined under the microscope first with × 40 objective to check the staining and to see the distribution of materials, and then with the oil immersion objectives × I00. Heat treatment was given at the end of four (4) hours incubation to kill other species of *Bacillus thuringiensis* that might germinate ahead of *Bacillus thuringiensis* spore.

**Biochemical Characterization of *Bacillus thuringiensis* Isolates**

Endospore Test was carried out microscopically. Motility test, Methyl Red Test, Voges-Proskauer Test, Citrate Utilization Test, Indole Test, Citrate Utilization Test, Indole Test, Urease test, catalase Test and Starch Hydrolysis Test were carried out as described by Cheese-brough (2000).

The bacterial cell was smeared on a clean glass slide and allowed to dry in air and heat fixed.

**Collection of Mosquito Larvae**

The larvae of the mosquitoes were collected by using white enamel pan which was swept through the water until half full, while the larvae within it were removed. For the small inaccessible locations of tree holes, a tube was used to remove water and larvae together and transferred to a pan (Bravo *et al.,* 2011).

**Identification of the Different Species of Mosquito Larvae**

**Anopheles Larvae:**

Anopheles mosquito has a well-developed head with mouth brushes used for feeding. It has a large thorax and a nine segmented abdomen, absence of legs, in contrast to other mosquitoes. First stage larva is about one (1nm) in length, fourth stage larva is normally 5-8mm in length. The process from egg-laying to emergence of the adult is temperature dependent, with a minimum time of seven days (Bossi *et al.,* 1989).

**Culex Larvae**

Larvae of culex have a broad head and a long antenna with a large tuft at the ends. On the ventral side of the larva head, an oval gill is positioned at the base of the antenna. They have an up curved siphon and a curved preapical spine at the end of the siphon. There are eight pairs of long tuft of setae on the siphon, lomb scales on the eight abdominal segment in a single row that appear long and pointed. And also possesses gills of two different lengths (Aly *et al.,* 1988).

**Aedes Larvae**

Egg of aedes are smooth, long, avoid shaped and roughly 1mm long. When firs laid, eggs appear white but within minutes turn shiny black. In warm climate, egg may develop in as few as two days, where as in cooler temperature climate, eggs development can take up to a week. Laid eggs can survive for a very long period in a dry state, often for more than a year. However, they hatch immediately once submerged in water. This makes the control of dengue virus mosquito very difficult (Aguilar-Meza, 2010).

**Mortality Determination**

Twenty mosquito larvae (culex, aedes and anopheles larva) each where kept in pan and subjected to different concentrations of the *Bacillus thuringiensis* isolates. A glass rod was used to determine whether the larvae were dead or not. After every one hour, this rod was dipped into the basin and brought very close to each and every larva. The larvae that were still alive could respond rapidly either bending itself or moving away from the rod while dead ones no matter how close the road was brought, there was no response. The results obtained for motility were recorded (Costechareyre *et al.,* 2010).

**RESULTS AND DISCUSSION**

Table 1 presented the microscopic and biochemical characteristics of Isolates of *Bacillus thuringiensis* . The isolates which were of compost manure, animal, residue dry leafy residue, garden soil and refuse were tested for bioactivity at different concentrations of 0, $8.0 \times 10^{9}$, $1.6 \times 10^{10}$, $3.2 \times 10^{10}$, $6.4 \times 10^{10}$, and $7.2 \times 10^{10}$cells/ml against culex, anopheles and aedes larvae where $8.0 \times 10^{8}$ Cell / ml ($OD_{600}$ of 1.0 ). The results revealed that *Bacillus thuringiensis* Isolated from garden soil, dry leafy residue soil, animal residue and compost manure exhibited varying bioactivity against the three species of mosquito larvae with the peak activity observed at a concentration of $6.4 \times 10^{10}$ cells/ml where it results in 100% mortality against all the three species of larvae (Table 2, 3,4 and

6). Table 5 also showed *Bacillus thuringiensis* isolated from animal residue exhibited varying bioactivity against culex and anopheles larvae with the peak activity observed at a concentration of $6.4 \times 10^{10}$ cells / ml where it results in 100 % mortality on culex and anopheles larva but with mortality of 95 % on aedes larva. Certain toxins which are ingested by susceptible mosquito larvae may result in the death of the target mosquito larvae. This is consistent with the report by Beegle and Pankaraft, (1992). Mortality of 95% that was recorded on aedes larvae from animal residue may be due to the application of the isolate to the third in star of the aedes larvae. This is consistent with the report by Lima *et al*. (2007). At a lower concentration of 40 cells / ml, 100% mortality was obtained. This may due to development of structural adaptational change that compost manure isolates might have undergo thus making it highly sensitive. This result is comparable with the report where *Bacillus thuringiensis* research was stimulated by progress in biotechnology by cloning a crystal toxic gene leading to improved target spectra and discovered more infectious strains of *Bacillus thuringiensis* (Fillinger, *et al*., 2003).

**Table 1: Microscopic and Biochemical characteristics of *Bacillus thuringiensis* from isolates**

| S/NO | TEST | RESULT |
|---|---|---|
| 1. | Gram staining | + |
| 2. | Spore staining | + |
| 3. | Mortality test | + |
| 4. | Indole test | + |
| 5. | Voges-Proskauer test | + |
| 6. | Methyl Red test | – |
| 7. | Starch hydrolysis test | + |
| 8. | Citrate utilization test | + |
| 9. | Catalase | + |
| 10. | Urease | + |

**Key**
+ = Positive result
- =Negative result

**Table 2 : Effect of *Bacillus thuringiensis* Concentration Isolated from garden soil on Culex, Anopheles and Aedes Larva . (N=20)**

| Larvae | Concentrations/Mortality (cells/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0** | **$8.0 \times 10^9$** | **$1.6 \times 10^{10}$** | **$3.2 \times 10^{10}$** | **$6.4 \times 10^{10}$** | **$7.2 \times 10^{10}$** | **Mean± SD** |
| Culex | 0(0%) | 15(75%) | 17(85%) | 19(95%) | 20(100%) | 20(100%) | 15.17±6.68 |
| Anopheles | 0(0%) | 15(75%) | 16(80%) | 17(85%) | 20(100%) | 20(100%) | 14.67±7.47 |
| Aedes | 0(0%) | 10(50%) | 11(55%) | 13(65%) | 20(100%) | 20(100%) | 12.33±7.45 |

**Table 3 : Effect of *Bacillus thuringiensis* Concentration Isolated from Refuse on Culex, Anopheles and Aedes Larva .** (N=20)

| Larvae | Concentrations/Mortality (cells/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0** | **$8.0 \times 10^9$** | **$1.6 \times 10^{10}$** | **$3.2 \times 10^{10}$** | **$6.4 \times 10^{10}$** | **$7.2 \times 10^{10}$** | **Mean± SD** |
| Culex | 0(0%) | 16(80%) | 18(90%) | 19(95%) | 20(100%) | 20(100%) | 15.50±7.74 |
| Anopheles | 0(0%) | 18(90%) | 18(90%) | 18(90%) | 20(100%) | 20(100%) | 15.67±7.74 |
| Aedes | 0(0%) | 10(50%) | 13(65%) | 14(70%) | 20(100%) | 20(100%) | 12.83±7.44 |

**Table 4 : Effect of *Bacillus thuringiensis* Isolated from Dry Leafy Residue on Culex, Anopheles and Aedes Larva . (N=20)**

| Larvae | Concentrations/Mortality (cells/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0** | **8.0 x $10^9$** | **1.6 x $10^{10}$** | **3.2 x $10^{10}$** | **6.4 x $10^{10}$** | **7.2 x $10^{10}$** | **Mean± SD** |
| Culex | 0(0%) | 17(85%) | 18(90%) | 19(95%) | 20(100%) | 20(100%) | 15.67±7.76 |
| Anopheles | 0(0%) | 16(80%) | 16(80%) | 17(85%) | 20(100%) | 20(100%) | 14.83±7.49 |
| Aedes | 0(0%) | 14(70%) | 12(60%) | 13(65%) | 20(100%) | 20(100%) | 13.17±7.33 |

**Table 5 : Effect of *Bacillus thuringiensis* Isolated from Animal Residue on Culex, Anopheles and Aedes Larva .**
(N=20)

| Larvae | Concentrations/Mortality (cells/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0** | **8.0 x $10^9$** | **1.6 x $10^{10}$** | **3.2 x $10^{10}$** | **6.4 x $10^{10}$** | **7.2 x $10^{10}$** | **Mean± SD** |
| Culex | 0(0%) | 10(50%) | 14(70%) | 17(85%) | 20(100%) | 20(100%) | 13.5±7.64 |
| Anopheles | 0(0%) | 11(55%) | 14(70%) | 16(80%) | 20(100%) | 20(100%) | 13.5±7.48 |
| Aedes | 0(0%) | 10(50%) | 10(50%) | 14(70%) | 19(95%) | 20(100%) | 12.17±7.33 |

**Table 6 : Effect of *Bacillus thuringiensis* Concentration Isolated from Compost Manure on Culex, Anopheles and Aedes Larva .**
(N=20)

| Larvae | Concentrations/Mortality (cells/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0** | **8.0 x $10^9$** | **1.6 x $10^{10}$** | **3.2 x $10^{10}$** | **6.4 x $10^{10}$** | **7.2 x $10^{10}$** | **Mean± SD** |
| Culex | 0(0%) | 19(95%) | 20(100%) | 20(100%) | 20(100%) | 20(100%) | 16.5±8.09 |
| Anopheles | 0(0%) | 18(90%) | 19(95%) | 20(100%) | 20(100%) | 20(100%) | 16.17±7.96 |
| Aedes | 0(0%) | 10(50%) | 12(60%) | 20(100%) | 20(100%) | 20(100%) | 13.67±8.04 |

## CONCLUSION

All the isolates of *Bacillus thuringiensis* from different habitats showed efficacy for the control of culex and anopheles larvae at 6.4 x $10^{10}$ cells/ml. *Bacillus thuringiensis* isolated from compost manure has the highest activity at 3.2 x $10^{10}$cells / ml while *Bacillus thuringiensis* from animal residue recorded lowest activity against the aedes larva at 6.4 x $10^{10}$ cells/ml.

## RECOMMENDATION

The use of *Bacillus thuringiensis* as insecticides should be encouraged for combating malaria because it gives high mortality against malaria vectors.

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