Effect of Local Isolate of *Bacillus Thuringiensis* on *Aedes Aegypti* Linn. and *Culex Quinquefasciatus* Say Larvae

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Abstract - Ten millions more are killed and debilitated by a host of mosquito-borne diseases, including filariasis and dengue. One alternative measure of control involves the use of entomopathogen, *Bacillus thuringiensis* (Bt)—a gram positive spore-forming soil bacteria that produces δ-endotoxins, which make-up the crystalline inclusions as part of its metabolic process. In this study the local isolate of Bt was identified through its morphological and biochemical characteristics and was tested for toxicity against *Aedes aegypti* and *Culex quinquefasciatus* larvae. With concentrations 0.5, 1.0, and 3.0% of Bsore-crystal complex, samples were subjected to 24, 48, and 72h of exposure. In both species, the 72 h of exposure showed a mean difference of significance at the 0.05 level. Since the crystal proteins bind specifically to certain receptors in the insect’s intestine, certain processes require a longer span of time to exhibit their effects on the mosquito species. Hence, the activity of Bt with respect to span of incubation had a significant effect on *A. aegypti* and *C. quinquefasciatus* larvae. Also, the results differed with respect to the species of tested mosquito.

Keywords : crystal proteins, dengue, endotoxin, entomopathogen, filariasis.

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Effect of Local Isolate of Bacillus Thuringiensis on Aedes Aegypti Linn. and Culex Quinquefasciatus Say Larvae

Jessil Ann L. Pajar *, Jing R. Bautista * & Franco G. Teves *

Abstract - Ten millions more are killed and debilitated by a host of mosquito-borne diseases, including filariasis and dengue. One alternative measure of control involves the use of an entomopathogen, Bacillus thuringiensis (Bt)—a gram positive spore-forming soil bacteria that produces δ-endotoxins, which make-up the crystalline inclusions as part of its metabolic process. In this study the local isolate of Bt was identified through its morphological and biochemical characteristics and was tested for toxicity against Aedes aegypti and Culex quinquefasciatus larvae. With concentrations 0.5, 1.0, and 3.0% of Btspore-crystal complex, samples were subjected to 24, 48, and 72h of exposure. In both species, the 72 h of exposure showed a mean difference of significance at the 0.05 level. Since the crystal proteins bind specifically to certain receptors in the insect’s intestine, certain processes require a longer span of time to exhibit their effects on the mosquito species. Hence, the activity of Bt with respect to span of incubation had a significant effect on A. aegypti and C. quinquefasciatus larvae. Also, the results differed with respect to the species of tested mosquito.

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I. Introduction

Mosquito transmitted diseases have become more of a growing concern over the past decade or so. They are prevalent in more than 100 countries, infecting 300-500 million people and causing about 1 million deaths every year (Zinsser, 1934).

During the 19th century, dengue was considered a benign sporadic disease that caused epidemics at long intervals but in the past five decades, the incidence was reported to have increased 30-folds (Sivanathan, 2006). Transmitted to humans through Aedes species, dengue outbreaks usually occur when mosquito is at its peak. Those infected with dengue can suffer from a spectrum of illnesses ranging from a viral flu to severe and fatal hemorrhagic fever (DHF).

Culex quinquefasciatus was identified as one of the mosquito species that plays a role in the transmission of lymphatic filariasis. Also known as elephantiasis, this mosquito-vectored disease is endemic throughout most of the southern half of the Philippine archipelago (Kron et.al, 2000).

At present, the best control methods for these vector borne diseases are based on vector control (Baird, 2000) primarily accomplished by using chemical insecticide which have been criticized because of the hazards it brought to the environment and its toxicity to non-target organisms, especially humans. Moreover, mosquitoes have already developed resistance to such insecticides.

One alternative measure of control involves the use of entomopathogens that are specific against certain pest species. One is B. thuringiensis (Bt), an ubiquitous, gram-positive, spore-forming bacterium that forms a parasporal crystal during the stationary phase of its growth cycle (Schnepf et al., 1998). Bt produces proteienacious inclusions during sporulation that are toxic to insect larvae upon ingestion. These toxins are highly specific, harmless to humans, vertebrates and plants and are completely biodegradable, leaving no residual toxic products accumulate in the environment (Ibbara et. al., 2003).

A local isolate of B. thuringiensis was identified in this study through its morphological and biochemical characteristics and was tested for toxicity to A. aegypti and C. quinquefasciatus larvae.

II. Materials and Methods

a) Sample Collection

Soil samples were collected from three randomly selected sites at the Vegetable Garden in MSU-IIT, Iligan City. The surface layer of the soil was scraped off with hand shovel and was placed in a sterile plastic container.

b) Isolation of the Bacterial Strain

Isolation of Bt strain was conducted according to the method of Obeidat et. al.(2004).

The suspensions were incubated at 30°C for 24 hours and after, the suspensions were placed in 80°C water bath for 15 minutes. As described by Travers et al., this selection method eliminates most spore-forming bacteria and all non-sporeforming organisms in the soil sample.

Shaeffer-Fulton staining was done to determine the presence of endospores of Bt which differentiates it.
from other Bacillus species. Microscopic observation was performed afterwards.

c) **Cultural Method of Characterization of Bacterial Isolates**

The following differential tests were performed following the identification flow charts on Bergey’s Manual of Determinative Bacteriology: determination of oxygen requirement, starch hydrolysis, catalase test, Voges-Proskauer Test, and test on Triple Sugar Iron agar.

d) **Isolation of Spores and Parasporal Crystals**

A mass production of Bt in nutrient agar plates was done. The plates were incubated for 48 h in 30±2°C. After incubation, 3-ml of sterile distilled water was added to each lawn and were scrapped off from the surface of the agar using a sterile inoculating loop. Using micropipette, the collected lawns were aseptically transferred into 50-ml centrifuge tubes. Sterile distilled water was added to make 10-ml of bacterial suspension and was centrifuged at 5000 rpm for 15 minutes. Pellets (spores and parasporal protein crystals) were washed twice with 10-ml sterile distilled water and centrifuged at 5000 rpm for 5 minutes. The pellets were oven dried at 40-60°C.

e) **Identification of Mosquitoes**

The mosquito larvae samples were collected from Barangay Puga-an and Luinab, Bahayan. Classification of *A. aegypti* and *C. quinquefasciatus* larvae, involves ocular inspection through its significant morphological characteristics (presence of hair in different body parts, structure of air tubes, number of hair in antenna and siphon, and etc.) which are unique among each species.

f) **Larval Bioassay**

The toxicity of the isolate was assayed in triplicate with three different concentrations for *C. quinquefasciatus* (0.5, 1.0 and 3.0%) and two different concentrations for *A. aegypti* (0.5% and 1.0%). Such concentrations were prepared by mixing 25ml of distilled water with 0.125g, 0.25g, 0.75g of the dried *B. thuringiensis* pellets for 0.5, 1.0 and 3.0% concentrations respectively. Fifteen larvae of *C. quinquefasciatus* and twelve larvae of *A. aegypti* for each replicate were placed in a glass container with their respective concentrations of *B. thuringiensis*. The larvae were incubated at 30°C and examined for 72 h in every 24 h interval.

III. **Results and Discussion**

The selection method by Obeidat *et al.* applied on the isolation of bacterial strain eliminated most of the spore-forming bacteria and all non-sporeforming organisms in the soil sample.

The entomopathogenic properties of Bt are due at least in part to the production of δ-endotoxins that make-up the crystalline inclusions characteristic of *B. thuringiensis* strains (Agaisse and Lereclus, 1995).

a) **Analysis for A. aegypti**

Twelve larvae per replicate were observed for mortality rate, treated with Bt with only two different concentrations (0.5% and 1.0%).

Figure 9 and 10 shows the development of adult *A. aegypti*. When exposed to 1.0% Bt spore-crystal concentration, the development slowed down which is not the usual trend for the *A. aegypti* because its pupal stage is short and usually last 1 to 2 days (Lee, 2000). The number of dead larvae also increases with longer length of exposure for both concentrations.

b) **Analysis for C. quinquefasciatus**

Fifteen larvae per replicate were treated with three different concentrations of Bt spore-crystal complex and were observed for mortality rate every 24 h within three days.

Figure 5 shows the effects of the three concentrations on the mortality rate with respect to the span of time when the number of dead larvae was counted. The longer the length of exposure, the more larvae died. Hence, this figure shows that the length of exposure is directly proportional to the number of dead larvae. Larvae pupated more on 1.0% and 0.5% concentrations respectively, while development into adult is more prominent on 0.5% concentration.

c) **Overall Analysis**

The larvicidal effect of *B. thuringiensis* spore-crystal complex was compared for the two mosquito species. The patterns differ among species such that *C. quinquefasciatus* shows no trend since the mortality rate increased from the control to 0.5% concentration yet decreased from 0.5% to 1.0% concentration and increased again on the preceding concentration.

For both *C. quinquefasciatus* and *A. aegypti* larvae, species of mosquito and the incubation period are the factors that posed a significant effect on the mortality rates of the mosquito larvae as shown in Table 7. However, when statistically tested together, incubation and mosquito become insignificant. This implies that these factors had independent effects on the test performed.

Different processes occurred for each tested species due to the difference in the length of their development. The effects of the length of exposure varied with respect to species tested considering that *A. aegypti* has shorter life cycle than *C. quinquefasciatus*. Laboratory studies showed that mosquito larvae require five to ten days for completion. Thus the variation of duration depends on temperature or larval diets (Hawley, 1988).
Bt is more toxic to *C. quinquefasciatus* at higher concentration and longer period of exposure. This entails that the mechanism through which Bt persist its effect on the mortality of the larvae is related to the gradual effect of the bacteria in the insect’s intestine. Longer length of time is required to complete the process of gut integrity disruption and finally to the death of the insect larva from starvation or septicemia.

**IV. SUMMARY AND CONCLUSION**

Results indicate that the activity of *B. thuringiensis* with respect to span of exposure has a significant effect on *A. aegypti* and *C. quinquefasciatus* larvae as well as to the development of the said mosquito species. Also, the results differ with respect to the species of tested mosquito. And so, different effects were exhibited on each tested mosquito, perhaps due to difference in the length of development of each tested species as well as the reported chitinase activity on *A. aegypti*. Furthermore, factors which was not given great emphasis in the study like water preferences of each species and specific age of the larvae, may have contributed to the outcome of the experiment.

**REFERENCES**

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Figure 2: Effects of the different concentrations of B. thuringiensis spore-crystal complex on the mean mortality rate of C. quinquefasciatus larvae with respect to length of exposure.

Table 1: Two-way ANOVA test on the larvicidal activity of B. thuringiensis spore-crystal complex on A. aegypti and C. quinquefasciatus larvae.

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a. R Squared = .639 (Adjusted R Squared = .467)