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INSECTICIDE SUSCEPTIBILITY OF *AEDES AEGYPTI* LARVAE TO *BACILLUS THURINGIENSIS ISRAELENSIS* AND JUVENILE HORMONE IN DENGUE EPIDEMIC AREAS OF SAMUTSONGKHRAM, THAILAND

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ABSTRACT: Dengue hemorrhagic fever (DHF) remains one of Thailand's main public health problems. Samutsongkhram is one of the areas undergoing an epidemic of this disease. To control this disease, public health officers focus on regulating the population of *Aedes aegypti* larvae. The aims of this research were to study the susceptibility of *Ae. aegypti* to *Bacillus thuringiensis israelensis* (Bti) and juvenile hormone, in order to end the spread of this disease in three epidemic DHF outbreak sub-districts of Ladyai, Suanluang and Jompuak. The results revealed that *Ae. aegypti* is susceptible to Bti and juvenile hormone in all areas, with a 100% death rate. Exposure to Bti eradicated the larvae in a very short time of less than 18 hours. In addition, juvenile hormone can completely hinder the growth of *Ae. aegypti* to adult stages. The results demonstrate the effectiveness of these agents for mosquito larvae eradication. Therefore, insecticidal bacterium and juvenile hormone may be an option to reduce the number of DHF patients in Samutsongkhram, Thailand.

Keywords: Insecticide susceptibility, Bacillus thuringiensis israelensis, Juvenile hormone, Aedes aegypti

1. INTRODUCTION

Dengue hemorrhagic fever or DHF is a worldwide public health problem [1], particularly in tropical and sub-tropical areas [2]. This disease is caused by dengue virus, which is carried by *Aedes aegypti* [3]. DHF is also one of Thailand's main health problems [4]. The ratio of infection in Thailand is 219.46 per 100,000 people and the death ratio is at 0.10 (from the report of the Bureau of Vector Borne Diseases, Ministry of Public Health, Thailand), and Samutsongkhram Province has one of highest morbidity rates among DHF outbreak areas [5].

The record of the Samutsongkhram provincial Bureau of Epidemiology in 2015, the patient ratio is 349.7 per 100,000 people. The highest rate of cases were in August and September with 106 and 111 persons, respectively. The sub-districts of Samutsongkhram with the highest disease prevalence are Ladyai, Suanluang, and Jonpluak.

Currently, the Disease Control Department of Thailand specifies three control measures, including physical, biological, and chemical, to regulate the disease-vector mosquito [6]. The best way to prevent and control DHF is to stop the cycle of the *Ae. aegypti* vector. The Public Health Department emphasizes using sand covered by temephos or pesticide spraying to control the mosquito vector. There are reports in many countries including Thailand [7] that *Ae. aegypti* (mosquito larvae) has started to resist the temephos chemical.

Related public health organizations in many countries are interested in replacing temephos with alternative products to control the mosquito vector, such as *Bacillus thuringiensis israelensis* (Bti) and juvenile hormone. Bti is a gram-positive bacterium present in regular soil that can form endospores that produce the insecticidal Delta endotoxin, which is a toxic protein to the gut epithelium of mosquito larvae [8]. An additional agent is juvenile hormone, which can halt mosquito larval and pupal growth [9]. In foreign countries, there is a report that Bti can effectively reduce a number of mosquito larvae in a short period [10] and that juvenile hormone can efficiently eradicate *Ae. aegypti* [11].

In DHF outbreak areas of Samutsongkhram Province, researchers studied the efficacy of Bti and juvenile hormone to eliminate *Ae. aegypti* larvae, in order to find an alternative to temephos chemical usage.

2. MATERIALS AND METHODS

2.1 Mosquito Larva Collection

The study areas in this research are the most infected sub-districts of Samutsongkhram Province (Ladyai sub-district, Muang district; Suanluang sub-

district, Amphawa district; and Jonpluak sub-district, Bangkontee district) (Fig. 1). *Ae. aegypti* larvae were collected from dense household and population areas in each sub-district of DHF outbreak areas by 'Ovitrap' from August to November of 2016. Ten ovitraps (a trap/a house) were set around houses or spaces under people's houses in each sub-district (Figs. 2 and 3). After mosquito trapping for a week, *Ae. aegypti* specimens was collected and sent them to a laboratory in the College of Allied Health Science at Suansunadha University for mosquito species identification and laboratory examinations.

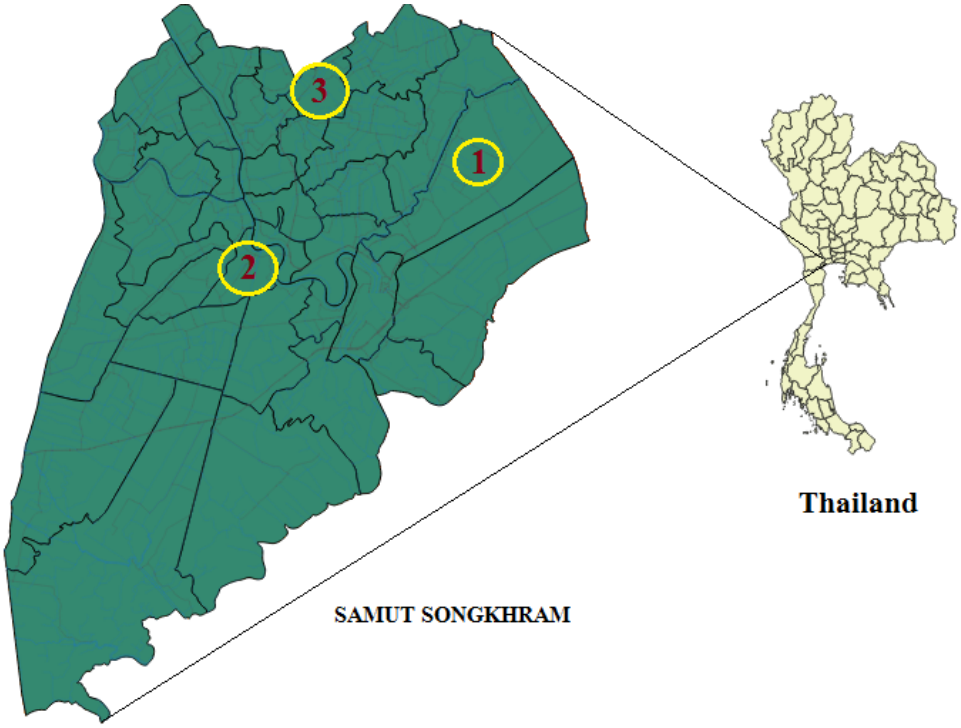

Fig. 1 Map of *Aedes aegypti* Larvae Collection Sites in Samutsongkhram.
1 = Ladyai sub-district, 2 = Suanluang sub-district and 3 = Jonpluak sub-district

Fig. 2 Coconut shells as *Aedes* spp. habitat in the houses that set the ovitrap.

Fig. 3 Big water jar as *Aedes* spp. habitat spread around the houses in Samutsongkhram.

2.2 Examination of Bacillus thuringiensis israelensis and juvenile hormone eradication efficiency

Bacillus thuringiensis of Bacillet 8 WT brand [10,000 ITU/mg (10% W/W)] and juvenile hormone of Sumilarv 0.5 G brand (Pyriproxyfem 0.5% W/W) were used.

First, solution preparations of Bti and juvenile hormone were divided into four concentrations of 10, 1, 0.1, and 0.012 ml/L in water. The serial dilutions were made as per WHO guidelines. Second, a compound with 100 ml of water was placed in each container for testing *Ae. aegypti* larvae and distilled water for controlled *Ae. aegypti*. Each container contained 10 larvae. This method was repeated three times and compared with *Ae. aegypti* Bora Bora strain F1 8 1 (susceptible strain). The susceptible strains were kindly provided by the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University. Deaths associated with Bti were counted every 6 hours for 24 hours, while those associated with juvenile hormone were counted daily until all *Ae. aegypti* were eliminated. In this stage, we recorded the response and number of all mosquito deaths.

2.3 Data Analysis

The deaths of *Ae. aegypti* were counted as a baseline, and then the two types of solutions were added and the average rates of *Ae. aegypti* deaths were determined in each area. Subsequently the death rates were compared between the three areas. Finally, the value on chemical susceptibility of mosquitos was verified under the criteria of the World Health Organization [12] as follows:

Death rate between 98–100% is considered a high rate of chemical susceptibility.

Death rate between 80–97% is a medium rate of chemical susceptibility.

Death rate below 80% is a low rate of chemical susceptibility or is considered as resistant to the chemicals.

The results are considered ineffective if a control group has more than a 20% death rate, and the experiment must be repeated. If the death rate is between 5–20%, the results must be adjusted with Abbott's formula.

3. RESULTS

The average number of larval deaths by *Bacillus thuringiensis var. israelensis* at each time point are shown in Figure 4 for the three sub-districts of Ladyai (Fig. 4a), Suanluang (Fig. 4b) and Jonpluak (Fig. 4c).

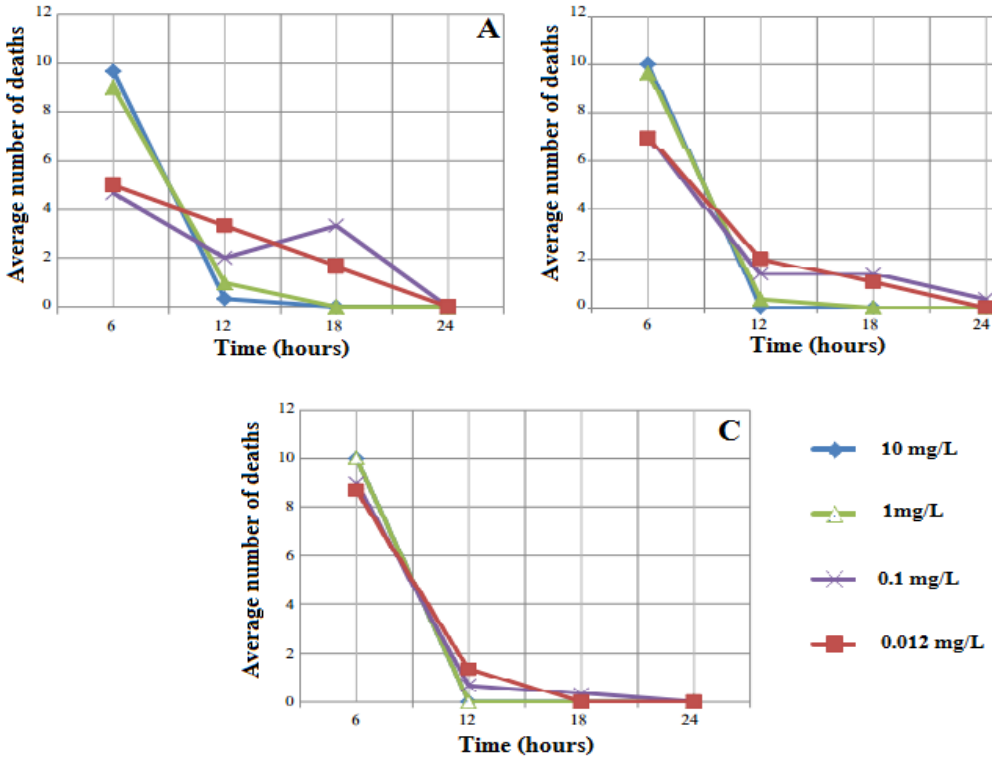

Fig. 4 Average number of larval deaths by *Bacillus thuringiensis var. israelensis* in each period (hours). A = Ladyai sub-district, B = Suanluang sub-district and C = Jonpluak sub-district

The results of susceptibility of larvae to *Bacillus thuringiensis var. israelensis* in the first 6 hours in the Suanluang, Jompluak, and Ladyai sub-districts show that Ladyai has the highest eradication concentration rate at 10 mg/L, followed by 1 mg/L, 0.012 mg/L, and 0.1 mg/L, respectively. The average number of larval deaths were at 9.67, 9, 5 and 4.67 respectively. Suanluang district had the highest eradication concentration rate at 10 mg/L, followed by 1 mg/L, 0.1 mg/L and 0.012 mg/L with average numbers of deaths of 10, 9.67, and 7, respectively. Jompluak sub-district had the highest eradication concentration rate at 10 mg/L and 1 mg/L, followed by 0.1 mg/L and 0.012 mg/L with average numbers of larval deaths of 10, 9, and 8.67, respectively. The mosquito larvae died within 18 hours, as well as the Bora Bora strain, whose death rate was at 100%, where 98–100% is classified as a high rate of Bti susceptibility in all areas.

Aedes mosquito larvae subjected to juvenile hormone were unable to grow into adult stages at every tested concentration, similar to the Bora Bora strain. In the Ladyai and Jompluak sub-districts, the average duration of death was 8 days, while *Ae. aegypti* larvae in the Suanluang sub-district had an average death duration of 7 days. The death rates in all areas were 100% similar to those after Bti exposure, revealing a high rate of susceptibility to juvenile hormone in all areas shown in Figure 5. Dead larvae were not observed in the control groups for the Bti and juvenile hormone treatments.

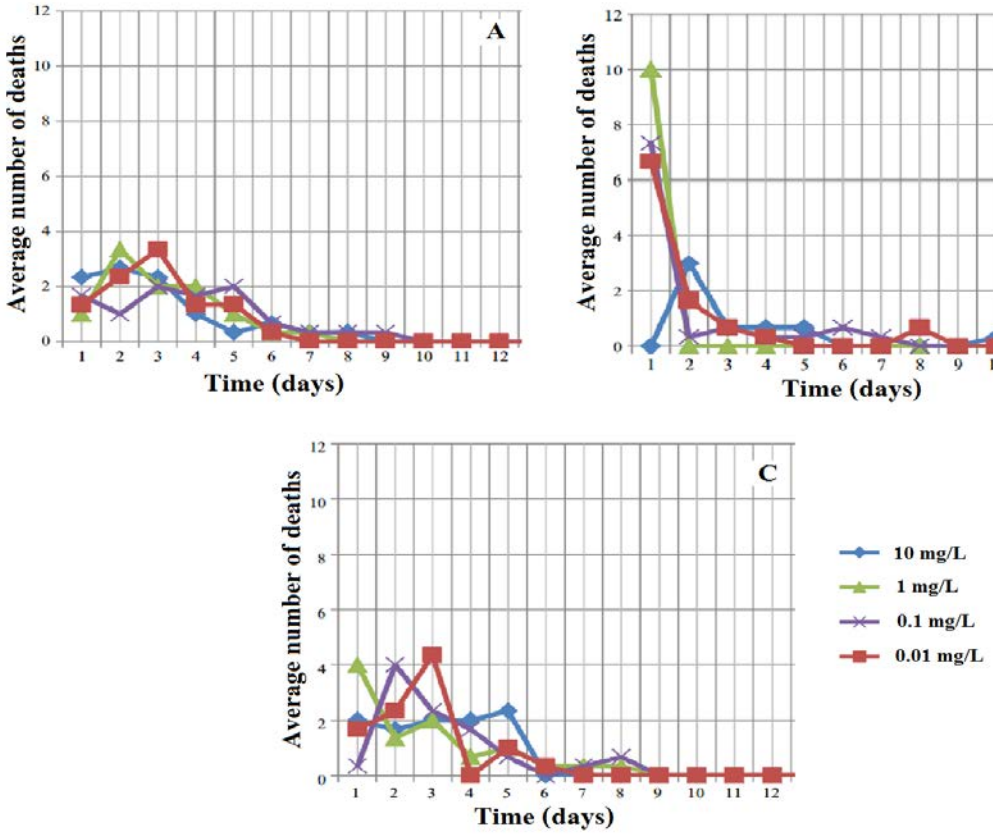

Fig. 5 Average number of larval deaths by Juvenile hormone in each period (days).
A = Ladyai sub-district, B = Suanluang sub-district and C = Jonpluak sub-district

4. DISCUSSION

This experimental research compared the susceptibility of *Ae. aegypti* (mosquito larvae) to *Bacillus thuringiensis var. israelensis* and juvenile hormone in major DHF outbreak areas in Samutsongkhram Province.

Ae. aegypti in three sub-districts of Ladyai, Suanluang and Jonpluak showed a high rate of susceptibility to both Bti and juvenile hormone (100% death rate at all tested concentrations). The death rates of larvae after exposure to either Bti or juvenile hormone in the three areas were similar at 100%. Therefore, both insecticidal substances showed a high effectiveness of larvae eradication in the province, where they have never been used due to the high associated price. The cheaper chemical “temephos” remains the most popular

substance based on prior research indicating a better performance **o**f eradicating *Ae. aegypti* [13].

Our results demonstrate that Bti eradicated the larvae in less than 18 hours (a very short time) at all of the tested concentrations, while juvenile hormone took many days to kill the larvae [14]. The difference is the mechanism of Bti, in which the alkaline salt in larval stomachs is expedited after the larvae swallow the chemical. The larvaes' stomachs are destroyed by the pore-forming toxins, causing paralysis and freezing, which ultimately results in death in a few hours [8]. On the other hand, juvenile will stop the transformation in insect shape, which hinders growth and requires a longer time to execute this creature [11].

5. CONCLUSION

Bacillus thuringiensis var. israelensis (Bti) and juvenile hormone are alternative options to eradicate the larvae of *Ae. aegypti* in DHF outbreak areas of Samutsongkhram Province. These insecticides were effective in eradicating mosquito larvae and can be another option to control house mosquito populations. The only drawback of Bti and juvenile hormone compared to the more popular temephos chemical is a higher price. Nevertheless, Bti and juvenile hormone are appropriate pesticides to be used in areas that the *Ae. aegypti* larvae resist the temephos chemical and may be the leading candidate agent for biological control of mosquitoes in Thailand.

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