

LABORATORY EVALUATION OF *BACILLUS THURINGIENSIS* H-14 AGAINST *Aedes Aegypti* LARVAE IN THE NORTHEAST REGION OF THAILAND

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Abstract. Laboratory bioassays using a preparation of *Bacillus thuringiensis* H-14 (Bt.H-14), namely Skeetal[®] were conducted to determine their effectiveness against late 3rd/early 4th instar larvae of *Aedes aegypti*. The larvae were collected from municipal areas in 7 provinces, namely Burirum, Roi-Et, Khon Kaen, Ubol Ratchatani, Nakorn Phanom, Surin and Nakorn Ratchasima, in the Northeast of Thailand. It was found that for Skeetal, LC₅₀ ranged from 128 to 151 n/l (average 143) and LC₉₀ ranged from 254 to 289 n/l (average 275). The mortality rate of *Ae. aegypti* larvae in the 7 provinces did not differ significantly ($p > 0.05$) at a concentration of 300 n/l. The result of the bioassays show that the preparation of Bt.H-14 is very effective against *Ae. aegypti* larvae in Northeast of Thailand and the mosquito larvae in the various areas were nearly equal in susceptibility to Bt.H-14.

INTRODUCTION

In Thailand malaria, filariasis, dengue hemorrhagic fever and Japanese B encephalitis are important diseases which have mosquitos as vectors (Harinasuta, 1984). One method to control these diseases is the use of chemical insecticides. However, some insecticides are environmentally toxic and harmful to non-target living organisms. An additional problem is the development of resistance to insecticides. Currently, many entomologists have emphasized the importance of developing biological agents as alternatives to chemical insecticides for the control of vectors of human diseases. Many different types of organisms have been evaluated for potential vector control and one of the promising biological agents which has been found is serotype H-14 of *Bacillus thuringiensis* (Bt.H-14), a synonym of *Bacillus thuringiensis* var *israelensis* (Goldberg and Margalit, 1977; de Berjac, 1978). Bt.H-14 has been tested by scientists throughout the world and it was found to be toxic against 72 species of mosquitos (Margalit and Dean, 1985). To date, the organism has been registered for use in the United States and European countries (Service, 1983). Commercial products are now available.

Although bioassays of Bt.H-14 powder against vector species of mosquito have been reported in a number of countries (Margalit and Dean, 1985), very few have been performed in Thailand. Pantuwatana and Youngvanitsed (1984) and Wongsiri and Andre (1984) reported that several species of mosquito in Thailand show susceptibility to Bt.H-14. The objectives of this study were to evaluate the effectiveness of Bt.H-14 against *Aedes aegypti* larvae collected from different provinces of the Northeast Region of Thailand and to compare the mortality rate of the *Ae. aegypti* larvae.

MATERIALS AND METHODS

Collection and rearing of *Ae. aegypti* larvae

Larvae of *Ae. aegypti* were collected from randomly chosen municipalities in each of seven provinces in the Northeast Region of Thailand. The collected larvae were reared to adults and maintained in the laboratory for 1-2 generations. These larvae were kept in a room at 28°C ($\pm 1^\circ\text{C}$) and 80% relative humidity. When the larvae reached late 3rd or early 4th instar, they were ready for testing.

Laboratory testing to determine the lethal concentration

A flowable concentration of Skeetal® containing 600,000 AA ITU/ml was used. The bioassays used in this study were modified from that of de Barjac and Larget (1979). They were carried out in 200 ml beakers containing 150 ml of Bt.H-14 solution in nonchlorinated water in various concentrations, each containing 25 larvae. Three replicates per concentration and three controls were tested. Hence each assay involved 300 larvae of the mosquito exposed to various concentrations of Bt.H-14 and 75 larvae as controls. The mortality data for the mosquito larvae were summed and the lethal concentration at 50 percentile (LC₅₀) and lethal concentration at 90 percentile (LC₉₀) were determined by probit analysis (Finney, 1971).

RESULTS

Ae. aegypti larvae were collected from municipal areas of 7 out of the 17 provinces of the Northeast Region of Thailand. The provinces were Buri Rum, Roi Et, Khon Kaen, Ubon Ratchatani Nakhon Phanom, Surin and Nakhon Ratchasima. The results of laboratory bioassays against larvae of *Ae. aegypti* collected from these areas are presented in Table 1. The results show that LC₅₀ and LC₉₀ against *Ae. aegypti* larvae differed slightly between the 7 provinces. The average

Table 1

Susceptibility of *Aedes aegypti* larvae exposed to Skeetal® continuously for 24 hours

| Locality | Lethal concentration | |
|-------------------|-------------------------|-------------------------|
| | LC ₅₀ (nl/l) | LC ₉₀ (nl/l) |
| Buri Rum | 144 | 270 |
| Roi Et | 149 | 289 |
| Khon Kaen | 128 | 285 |
| Ubon Ratchatani | 140 | 254 |
| Nakhon Phanom | 147 | 264 |
| Surin | 151 | 283 |
| Nakhon Ratchasima | 144 | 281 |
| Average | 143 | 275 |

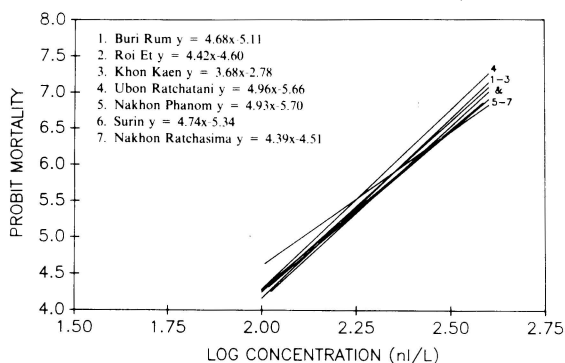


Fig 1—Regression lines of bioassays of *Aedes aegypti* larvae against Skeetal®

LC₅₀ and LC₉₀ to *Ae. aegypti* larvae of all 7 provinces were 143 nl/l (range 128-151) and 275 nl/l (range 254-289), respectively. The regression lines for the bioassays are shown in Fig 1. As is evident from the graphs, the mosquito larvae from the 7 provinces were nearly equally susceptible to Bt.H-14.

The mean percent mortality rates of *Ae. aegypti* larvae collected from the 7 provinces at 300 n/l of Bt.H-14 are shown in Table 2. There was no statistically significant difference between the mean percent mortality rates of the mosquito larvae in the 7 provinces (Student's *t*-test, *p* > 0.05).

DISCUSSION

Bt.H-14 has very high larvicidal activity against several species of mosquitos, especially *Ae. aegypti*.

Table 2

The mean of percent mortality of *Aedes aegypti* larvae in 7 provinces due to Skeetal® at 300 n/l

| Province | Mean mortality ± standard deviation |
|-------------------|--|
| Buri Rum | 89.00 ± 3.31 |
| Roi Et | 89.33 ± 4.98 |
| Khon Kaen | 89.33 ± 3.77 |
| Ubon Ratchatani | 89.00 ± 3.31 |
| Nakhon Phanom | 89.33 ± 4.98 |
| Surin | 88.00 ± 3.26 |
| Nakhon Ratchasima | 88.00 ± 5.65 |

in Thailand (Pantuwatana and Youngvanitsed, 1984; Wongsiri and Andre, 1984; Silapanuntakul *et al*, 1983; Chohanadisai *et al*, 1987; and Voravuthi kunchai *et al*, 1990). The present study also indicates that Bt.H-14, Skeetal[®], is very effective against *Ae. aegypti* larvae. The mosquito larvae collected from the 7 provinces of the Northeast Region of Thailand were nearly equally susceptible to Bt.H-14.

The results of this study will provide basic information for future pilot field experiments on the use of Bt.H-14 for control of *Ae. aegypti* and other mosquitos in Northeast Region of Thailand.

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REFERENCES

- Chowanadisai L, Thanasripukdikul S, Phanthumachinda B. Field trials of granular formulation of *Bacillus thuringiensis* H-14 against *Aedes aegypti* DHF vector of Dengue Hemorrhagic Fever in Thailand. *Commun Dis J* 1987; 13 : 193-200.
- de Barjac H. Une nouvelle varieté de *Bacillus thuringiensis* très toxique pour les moustiques : *B. thuringiensis* var *israelensis* serotype 14. *CR Acad Sci (Paris)*. 1978; 286 : 797-800.
- de Barjac H, Larget I. Proposals for the adoption of a standardized bioassay method for evaluation of insecticidal formulations derived from serotype H.14 of *Bacillus thuringiensis*. WHO document *WHO/VBC/79* 1979; 744.
- Finney DJ. Probit Analysis. 3rd ed. Cambridge : University Printing : House, 1971; pp.50-80.
- Goldberg LJ, Margalit J. Bacterial spores demonstrating rapid larvicidal activity against *Anopheles sergenti*, *Uranotaenia unguiculata*, *Culex univittatus*, *Aedes aegypti* and *Culex pipiens*. *Mosq News* 1977; 37 : 355-8.
- Harinasuta C. Mosquito-borne diseases in Southeast Asia. *Mosq-Borne Dis Bull* 1984; 1 : 1-11.
- Margalit J, Dean D. The story of *Bacillus thuringiensis* var *israelensis* (Bti). *J Am Mosq Control Assoc* 1985; 1 : 1-7.
- Pantuwatana S, Youngvanitsed A. Preliminary evaluation of *Bacillus thuringiensis* serotype H-14 and *Bacillus sphaericus* strain 1593 for toxicity against mosquito larvae in Thailand. *J Sci Soc Thai* 1984; 10 : 101-8.
- Service MW. Biological control of mosquitoes : Has it a future? *Mosq News* 1983; 43 : 113-20.
- Silapanuntakul S, Pantuwatana S, Bhumiratana A, Charoensiri K. The comparative persistence of toxicity of *Bacillus sphaericus* strain 1593 and *Bacillus thuringiensis* serotype H-14 against mosquito larvae in different kinds of environments. *J Invertebr Pathol* 1983; 42 : 387-92.
- Voravuthikunchai S, Wisedrat W, Samasanti W, Suntinanalert P. The efficacy of reference strains of *Bacillus thuringiensis* in controlling mosquito larvae. *Songkhlanakarin J Sci Technol* 1990; 12 : 145-9.
- Wongsiri S, Andre RG. Biological control of mosquitoes in Thailand. *J Sci Soc Thai* 1984; 10 : 73-88.