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# PRELIMINARY EVALUATION OF *BACILLUS THURINGIENSIS* SEROTYPE H-14 AND *BACILLUS SPHAERICUS* STRAIN 1593 FOR TOXICITY AGAINST MOSQUITO LARVAE IN THAILAND*

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**Abstract**

*The larvicidal activity of **Bacillus thuringiensis** serotype H-14 and **Bacillus sphaericus** strain 1593 against several species of laboratory-reared and field-collected mosquito larvae was studied.*

*The larvicidal activity of **B. thuringiensis** (H-14) for several species of mosquito larvae showed 2nd instar larvae of **Aedes aegypti** (Linnaeus) to be the most sensitive with 100% mortality in 20-40 minutes at high doses (10 - 100 mg/liter). The 2nd instar larvae of **Culex quinquefasciatus** (Say), **Ae. albopictus** (Skuse) and a mixed population of **C. mimulus** (Edwards) and **C. vishnui** (Theobald) demonstrated only moderate sensitivity. The 3rd instar larvae of **Anopheles dirus** (Peyton & Harrison), 2nd and 3rd instar larvae of **An. vagus**(Donitz), 3rd instar larvae of **An. maculatus** (Theobald) and **Armigeres subalbatus** (Coquillett), and 2nd instar larvae of **C. tritaeniorhynchus** (Giles) showed relatively low sensitivity. The 3rd instar larvae of **Toxorhynchites splendens** (Wiedemann), and the 4th instar larvae of **Mansonia uniformis** (Theobald) and **M. indiana** (Edwards) were not susceptible.*

*The larvicidal activity of **B. sphaericus** (1593) against several species of mosquito larvae showed **C. quinquefasciatus** to be the most sensitive with 100% mortality in 24 hours ($LC_{50}$ in 2 days, $3.55 \times 10^3$ spores/ml). The 2nd instar larvae of **An. vagus, C. tritaeniorhynchus** and a mixed population of **C. mimulus** and **C. vishnui** demonstrated intermediate sensitivity. The 2nd instar larvae of **Ae. aegypti** and **Ae. albopictus**, the 3rd and 4th instar larvae of **An. dirus, An. minimus** (Theobald), **An. philippinensis** (Ludlow), **An. maculatus** and 4th instar larvae of **An. nivipes** (Theobald) demonstrated less sensitivity ($LC_{50}$ in 2 days, $0.2 - 1.5 \times 10^6$ spores/ml). The 4th instar larvae of **A. subalbatus, M. uniformis** and **M. indiana** were not susceptible.*

## Introduction

Interest in the potential of pathogens for the control of medically important arthropods has been encouraged by the results recently achieved in the development and experimental use of certain spore forming bacteria such as the serotype H-14 of *Bacillus thuringiensis*[1-8] and strains of *B. sphaericus*[9-13]. In addition there is widespread concern about the environmental safety of control programmes and about the development of insecticide resistance[14-16]. With mosquitoes, it has been demonstrated that susceptibility to *B. thuringiensis* (H-14) and *B. sphaericus* varies considerably according to the species tested[1-13]. It is the objective of this study to test the susceptibility of mosquitoes in Thailand against *B. thuringiensis* (H-14) and *B. sphaericus* (1593).

## Materials and Methods

The experimental preparation of *B. thuringiensis* (H-14) used was the Abbott wettable powder (ABG-6108; Lot No. 6404-125) provided by WHO. This preparation had a viable spore count (pour plates incubated for 18 hr at 30 + 1 °C on Trypticase soy agar) of $2.66 \times 10^8$ spores/0.01 mg. *Bacillus sphaericus* (1593) produced by Stauffer Chemical Co. was provided by Dr. S. Singer as a technical powder. This preparation had a viable spore count of $1.12 \times 10^8$ spores/0.01 mg.

The mosquito larvae used in the assays were either from stocks reared in the laboratory of form freshly field-collected larvae. Those from stocks reared in the laboratory were *Ae. aegypti* (had been maintained for 3 years), *C. quinquefasciatus* (had been maintained for 2 years); *An. dirus, An. minimus, An. philippinensis, An. maculatus* and *An. nivipes* (from colonies maintained at Department of Medical Entomology, AFRIMS, for several generations); *M. uniformis* and *M. indiana* (from colonies maintained at Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University). All the rest *(Ae. aegypti, Ae. albopictus, C. quinquefasciatus, C. tritaeniorhynchus, C. mimulus, C. vishnui, An. vagus* and *Armigeres subalbatus)* were those from freshly field-collected larvae which were collected from several provinces (Surat, Songkhla and Yala) as specified in Tables 1 and 2. Larvae from each site were kept separately and transported to a laboratory where they were groups and separated by species. In the case of 2nd instar larvae, some of larvae (about 50%) were randomly selected from the pool and saved for reconfirmed identification by the collaborating medical entomologist (Lt. Col. B. Harrison, AFRIMS). All tests, unless otherwise specified, were conducted at 28 + 1°C in plastic cups containing 10 larvae/cup in a total volume of 100 ml of distilled water/cup. Larval mortality was the criterion used to determine the effects of the various test variables after 24-48 hours of exposure time. If the larvae were not killed in seven days after exposure, they would be considered as not susceptible. All treatments, including the controls, were replicated at least 2 times, and

some were replicated as many as 3 times. Throughout the tests, larvae were fed with ground mouse food daily unless otherwise specified. The results of $LC_{50}$ were estimated by the method of Reed and Muench[17]

Toxicity towards the 4th instar larvae of *Mansonia* spp. was done in plastic cups in the same manner as for the other mosquito larvae except the *Pistia stratiotes* (an aquatic plant) was provided as the host for its larvae and the mosquito larvae were fed daily with rat dung. The $LC_{50}$ value was estimated using the same method.

**Results**

***B. thuringiensis (H-14) toxicity towards mosquito larvae.***

The larvicidal activity of *B. thuringiensis* (H-14) for several species of mosquito is summarized in Table 1. The results showed that the 2nd instar larvae of *Ae. aegypti* which had been colonized in the laboratory for several generations were the most sensitive. There was 100% mortality in 20-40 minutes at the concentrations of 10-100 mg/liter. By contrast, there was only about 30% mortality in 80 min and 28% in 150 min at the same concentrations in the lab-reared larval populations of *C. quinquefasciatus* and *An. dirus,* respectively. The 2nd instar larvae of field-collected *Ae. aegypti, Ae. albopictus,* a mixed population of *C. mimulus* and *C. vishnui,* and of lab-reared *C. quinquefasciatus,* the 3rd instar larvae of lab - reared *An. dirus* and *An. maculatus,* the 2nd and 3rd instar larvae of field-collected *An. vagus,* the 3rd instar larvae of field-collected *Armigeres subalbatus,* and the 2nd instar larvae of field-collected *C. tritaeniorhynchus* was susceptible with varied $LC_{50}$ levels. Whenever the $LC_{50}$ was greater than $10^7$ spores/ml, the suspension was designated as inactive and the test larvae were considered to be not susceptible. The 4th instar larvae of *M. indiana* and *M. uniformis* and the 3rd instar larvae of *T. splendens* were not susceptible under the prevailing lab conditions.

***B. sphaericus (1593)toxicity towards mosquito larvae***

The larvicidal activity of *B. sphaericus* (1593) for several species of mosquito larvae is summarized in Table 2. The results showed that the 2nd instar larvae of field-collected *C. quinquefasciatus* were the most sensitive with 100% mortality in 24 hours at the concentrations of 10-100 mg/liter and with an $LC_{50}$ in 2 days of $3.5 \times 10^3$ spores/ml. The 2nd instar larvae of field-collected *An. vagus,* the 4th instar larvae of field-collected *An. vagus, C. tritaeniorhynchus,* a mixed population of *C. mimulus* and *C. vishnui,* the 2nd instar larvae of field-collected *Ae. aegypti* and *Ae. albopictus,* the 4th instar larvae of field-collected *An. nivipes, the 3rd and 4th* instar larvae of lab-reared *An. dirus, An. minimus, An. philippinensis* and *An. maculatus* were susceptible with varied in $LC_{50}$ levels. The 4th instar larvae of field-collected *Armigeres subalbatus* and the 4th instar larvae of lab-reared *M. uniformis* and *M. indiana* were not susceptible at the given conditions.

**TABLE 1.** Laboratory activity of *Bacillus thuringiensis* (H-14) against mosquito larvae.

| Species | Larval instar | Source of larvae | No. of replicates | $LC_{50}$(organisms/ml) in 48 hrs |
|---|---|---|---|---|
| *Ae. aegypti* | 2nd | Lab colony | 3 | $2.5 \times 10^{2}$[a] |
| | 2nd | Yala, field-collected larvae | 2 | $5.0 \times 10^{4}$ |
| *Ae. albopictus* | 2nd | Songkhla, field-collected larvae | 2 | $5.9 \times 10^{4}$ |
| *C. quinquefasciatus* | 2nd | Lab colony | 3 | $1.0 \times 10^{3}$ |
| *C. tritaeniorhynchus* | 2nd | Yala, field-collected larvae | 2 | $1.0 \times 10^{6}$ |
| Mixed population of *C. mimulus* and *C. vishnui* | 2nd | Yala, field-collected larvae | 2 | $1.0 \times 10^{4}$ |
| *An. dirus* | 3rd | AFRIMS, Lab colony | 3 | $4.7 \times 10^{5}$[a] |
| *An. maculatus* | 3rd | AFRIMS, Lab colony | 3 | $1.9 \times 10^{5}$[a] |
| *An. vagus* | 2nd + 3rd | Yala, field-collected larvae | 2 | $9.5 \times 10^{5}$ |
| *Ar. subalbatus* | 3rd | Yala, field-collected larvae | 2 | $8.5 \times 10^{5}$ |
| *M. uniformis* | 4th | Bangkok, Lab-reared larvae | 3 | not susceptible |
| *M. indiana* | 4th | Bangkok, Lab-reared larvae | 3 | not susceptible |
| *T. splendens* | 3rd | Yala, field-collected larvae | 2 | not susceptible |
| | 3rd | Bangkok, Lab-reared larvae | 3 | not susceptible |

[a]$LC_{50}$ value was obtained in 24 hours.

**TABLE 2.** Laboratory activity of *Bacillus sphaericus* strain 1593 against mosquito larvae.

| Species | Larval instar | Source of larvae | No. of replicates | $LC_{50}$(organisms/ml) in 48 hrs |
|---|---|---|---|---|
| *Ae. aegypti* | 2nd | Surat, field-collected larvae | 2 | $5.6 \times 10^5$ |
| *Ae. albopictus* | 2nd | Songkhla, field-collected larvae | 2 | $4.2 \times 10^5$[a] |
| *C. quinquefasciatus* | 2nd | Yala, field-collected larvae | 2 | $3.5 \times 10^3$ |
| *C. tritaeniorhynchus* | 2nd | Yala, field-collected larvae | 2 | $4.1 \times 10^3$ |
| Mixed population of *C. mimulus* and *C. vishnui* | 2nd | Yala, field-collected larvae | 2 | $2.1 \times 10^4$ |
| *An. vagus* | 2nd | Yala, field-collected larvae | 2 | $4.0 \times 10^3$ |
| *An. dirus* | 3rd + 4th | AFRIMS, Lab colony | 3 | $1.0 \times 10^6$ |
| *An. minimus* | 3rd + 4rd | AFRIMS, Lab colony | 3 | $1.5 \times 10^6$ |
| *An. philippinensis* | 3rd + 4th | AFRIMS, Lab colony | 3 | $1.0 \times 10^6$ |
| *An. maculatus* | 3rd + 4th | AFRIMS, Lab colony | 3 | $1.0 \times 10^6$ |
| *An. nivipes* | 4th | AFRIMS, field-collected larvae | 3 | $2.0 \times 10^5$ |
| *Ar. subalbatus* | 4th | Yala, field-collected larvae | 2 | not susceptible |
| *M. uniformis* | 4th | Bangkok, Lab reared larvae | 3 | not susceptible |
| *M. indiana* | 4th | Bangkok, Lab reared larvae | 3 | not susceptible |

[a]$LC_{50}$ value was obtained in 24 hours.

## Discussion

The susceptibility to *B. thuringiensis* (H-14) and *B. sphaericus* (1593) varied considerably among the mosquito species tested(Tables 1 and 2). *B. thuringiensis* (H-14) was very active against 2nd instar larvae of lab-reared *Ae. aegypti* when compared to 2nd instar larvae of lab-reared *C. quinquefasciatus* and 3rd and 4th instar larvae of lab-reared *An. dirus* tested at the same conditions. It was not active against 4th instar larvae of lab-reared *M. uniformis, M. indiana* and 3rd instar larvae of both lab-reared and field-collected larvae of *T. splendens* at the given conditions. The larvicidal activity of *B. sphaericus* (1593) was better against *Culex* spp. than against *Anopheles* spp. and *Aedes* spp. while it was not active against *Armigeres subalbatus* and *Mansonia* spp. at the given conditions.

Simulated field tests suggest that *B. thuringiensis* (H-14) toxicity in tap water can persist against *Ae. aegypti* for about 2 to 3 months and against *C. quinquefasciatus* for 8 weeks[18]. But the persistence of *B. sphaericus* (1593) toxicity in these conditions persisted in tap water and polluted water against *C. quinquefasciatus* for at least 9 and 6 months, respectively[18]. The persistence of *B. sphaericus* (1593) toxicity against *Ae. aegypti* larvae in similar conditions was about 1 week[18]. Therefore, *B. sphaericus* (1593) is considered to be the most likely good candidate for the control of *Culex* spp. and *Anopheles* spp. and *B. thuringiensis* (H-14) is probably a good candidate for the control of *Aedes* spp. mosquitoes. Again, in planning an approach to the use of microbial control agents, the most significant factors to be considered include production technology, safety, specificity, and the efficacy. Thus, more information is needed before a nationwide programme can be operated especially the cost of application.

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## บทคัดย่อ

การทดสอบคุณสมบัติในการฆ่าลูกน้ำยุงบางชนิดที่เป็นพาหะนำโรคในประเทศไทยของแบคทีเรียชนิด *Bacillus thuringiensis (H-14)* และ *Bacillus sphaericus (1593)* เพื่อประเมินผลการฆ่าลูกน้ำยุงทั้งที่เลี้ยงไว้ในห้องทดลอง และที่เก็บมาจากแหล่งเพาะพันธุ์ในธรรมชาติ ได้กระทำในห้องปฏิบัติการที่มีอุณหภูมิประมาณ 28°C แบคทีเรียชนิด *B. thuringiensis (H-14)* สามารถฆ่าลูกน้ำยุงลายชนิด *Aedes aegypti* และ โดยเฉพาะลูกน้ำยุงลายที่เลี้ยงไว้ในห้องทดลองจะให้ผลดีมาก ลูกน้ำจะถูกฆ่าตายภายในเวลา 20-40 นาที เมื่อใส่ไว้ในน้ำที่มีแบคทีเรียที่ความเข้มข้นสูง ๆ (10-100 มก/ลิตร) ส่วนลูกน้ำยุงชนิดอื่นให้ผลแตกต่างกันไป ขึ้นอยู่กับชนิดและอายุของลูกน้ำยุง แบคทีเรียชนิด *B.thuringiensis (H-14)* มีคุณสมบัติฆ่าลูกน้ำยุงชนิด *Culex quinquefasciatus, Aedes albopictus, Culex mimulus, Culex Vishnui, Anopheles dirus, Anopheles vagus, Anopheles maculatus, Armigeres subalbatus* และ *Culex tritaeniorhyuchus* โดยที่ให้ผลปานกลางและใช้ความเข้มข้นค่อนข้างสูงเมื่อเทียบกับยุงลาย ชนิด *Aedes aegypti* โดยเฉพาะยุงก้นปล่องเกือบทุกชนิดต้องใช้ความเข้มข้นค่อนข้างสูง แบคทีเรีย *B. thuringiensis (H-14)* ไม่มีผลต่อลูกน้ำยุงชนิด *Toxorhynchites splendens, Mansonia unifornis* และ *Mansonia indiana*

สำหรับแบคทีเรียชนิด *Bacillus sphaericus (1953)* นั้นสามารถฆ่าลูกน้ำรำคาญชนิด *Culex quinquefasciatus* ได้ดีมากโดยสามารถฆ่าได้ถึงร้อยละ 100 ภายใน 24 ชม. ในที่ ๆ มีความเข้มข้นสูง สำหรับลูกน้ำยุงชนิดอื่นให้ผลแตกต่างกันไปแล้วแต่ชนิด และอายุของลูกน้ำยุง *B. sphaerius (1953)* มีคุณสมบัติฆ่าลูกน้ำยุงพวก *Culex tritaeniorhyuchus, Culex mimulus, Culex vishnui* และ *Anopheles vagus* ได้ค่อนข้างดีพอ ๆ กับลูกน้ำยุง *Culex quinquefasciates* ส่วนลูกน้ำยุงชนิด *Aedes aegypti, Aedes albopictus, Anopheles dirus, Anopheles minimus, Anopheles nivipes, Anopheles philippinensis, Anopheles maculatus* ให้ผลปานกลาง *B. sphaericus (1593)* ไม่มีผลต่อลูกน้ำยุงชนิด *Armigeres subalbatus, Mansonia unifornis* และ *Mansonia indiana*