IJAMBR 8 (2020) 58-65 ISSN 2053-1818

International Journal of Applied Microbiology and Biotechnology Research
www.bluepenjournals.org/ijambr

doi.org/10.33500/ijambr.2020.08.006

Development of larvicidal *Bacillus thuringiensis var. israelensis* by the Thai NIH and its comparison to *Bacillus sphaericus* and temephos in a selection experiment with the mosquito *Culex quinquefasciatus*

Uruyakorn Chansang*, Nittaya Methawanitpong, Danaporn Saraprug, Porntida Phetsuwan, Nuntaporn Ponsuwan and Pornchai Wiriyasaranont

National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi 11000, Thailand.

Article History
Received 06 September, 2020
Received in revised form 25 September, 2020
Accepted 30 September, 2020

Key words:
***Bacillus thuringiensis* subsp. *israelensis* (Bti),**
***Bacillus sphaericus* (Bsph),**
Temephos,
***Culex quinquefasciatus*,**
Resistance induction.

Article Type:
Full Length Research Article

ABSTRACT

Insecticide resistance poses a serious obstacle to the control of insect vectors. The microorganisms *Bacillus thuringiensis* subsp. *israelensis* (Bti) and *Bacillus sphaericus* (Bsph) have been used as alternative larvicides to a variety of mosquitoes such as *Culex quinquefasciatus* which was reported to be resistant to some groups of insecticides. National Institute of Health (NIH), Thailand has authority and function in the development of knowledge on public health. A Bti was developed in this study and tested following the technical specifications of ISO 17025:2017. *C. quinquefasciatus* Say 1823, a major vector of filariasis in the neighbouring countries has been monitored at the borders of Thailand. The effectiveness and appropriate long-term use of Bti, together with the potential for recovery from insecticide resistance, were tested against the standard susceptible strain of *C. quinquefasciatus* by a selection pressure method. Also, the commercial Bsph and temephos were tested in parallel to determine the suitable time of use. The powdered Bti was produced with a potency of 20,839.98 ITU/mg. The bioassay performed with Bti, Bsph and temephos to standard *C. quinquefasciatus* colonies showed 1.86-, 22.31- and 4.25-fold increases in resistance, respectively, in the 25th generation. The tests were continued without further larvicide exposure to determine the interval over which the resistance decreased. The resistance of the mosquitoes to Bti, Bsph and temephos declined to 0.99, 10.64 and 1.03 in the 31st, 68th and 41st generations, respectively. These studies indicated that the laboratory based-Bti is effective and should be developed as a larvicidal product. *C. quinquefasciatus* developed resistance to Bti slowly, and the induced resistance declined rapidly compared to the resistance to Bsph and temephos.

INTRODUCTION

The National Institute of Health (NIH) is a unit of the Department of Medical Sciences, Ministry of Public Health, Thailand. The institute has authority and functions in parallel with the medical laboratories to meet the requirements of ISO 15189 and the testing and calibration requirements of ISO 17025. Among the functions of the institute, the research and development of laboratory-based knowledge and technologies for public health are categorized as major activities. In addition, the setting of the standards for analyses and laboratory testing services are included in our responsibilities. Insecticide resistance poses a serious obstacle to the effective control of insect vectors, especially in tropical countries. The widespread use of insecticides to control insect pests and disease vectors over the past 50 years has resulted in the emergence of insect resistance to several families of insecticides (WHO, 1992). The microorganism *Bacillus thuringiensis* subsp. *israelensis* (Bti) is increasingly used worldwide for mosquito control and is promising as an alternative and safe larvicide (WHO, 2009; Aksoy et al., 2015; Pruszynski et al., 2017; Amorim et al., 2019). It is a facultative anaerobic, gram-positive bacterium that forms characteristic protein inclusions adjacent to the endospore. It is a naturally occurring soil bacterium with unique toxicity characteristics that can effectively kill mosquito larvae and has low toxicity to nontarget species present in water (Argolo-Filho and Loguercio, 2013). Moreover, it is approved under WHOPES for use in vector control and can be used in drinking- water that will receive little or no further treatment (WHO, 2009). Commercially available Bti strains are sold under a variety of trade names. However, when community vector control is needed for the mosquito control in Thailand, a large amount of insecticides is necessary although source reduction and self-protection are recommended. This includes the use of larvicides applied to the breeding sources of mosquitoes. The commercial Bti sold in Thailand is quite expensive, especially when a large amount is required. Regarding the functions of the Thai NIH, the Biological Control Laboratory has experience in the evaluation of Bti products, meeting the requirements of the ISO 17025:2017 international standard (Certified No. 4016/49). This function applies to products submitted to the Food and Drug Administration for market sales. Among the evaluated Bti products, some were found not to be effective, as they could not meet the evaluation criteria. In response to the public health needs for a mosquito control program and to serve the people by having a good bioinsecticide, a local strain of Bti was developed in a powder form by the Thai NIH. The final formulation of the powdered Bti was bioassayed against a standard strain of *Culex quinquefasciatus*, the nuisance mosquito in Thailand.

C. quinquefasciatus is very abundant and widespread. Because this species of mosquito is the major vector of filariasis in Myanmar, India and China (Simonsen, 2013), it has been monitored at Thai borders as a public health concern (Triteeraprapab, 2000). The Annual Epidemiological Surveillance reports of the Bureau of Epidemiology, DDC Thailand, noted that border areas are at risk of vector-borne diseases, including malaria and filariasis (Annual Epidemiological Surveillance reports, 2015). In addition, high prevalence of *Wuchereria bancrofti* infection was investigated among Myanmar migrants in Thailand (Triteeraprapab, 2001). In 2015, Thailand and neighboring countries joined the ASEAN Economic Community known as AEC 2015. This enhanced the cross-border movement and dramatically increased immigration. Therefore, in this study, this mosquito was selected to test for the effectiveness of the developed Bti. In addition, there have been a variety of reports in which Bti was used against *Aedes aegypti*, the major vector of dengue fever (Amorim, 2019; Ritchie, 2010), while quite a few studies have tested the use of Bti against *C. quinquefasciatus*. The control effectiveness of Bti is typically defined as ITU/mg of product (Rishikesk and Qulennec, 1983). In this study, the *B. thuringiensis* var. *israelensis* standard (Std. 2-11), sub-cultured from international standards (IPS82 for *B. thuringiensis israelensis*), was used for comparison to define the potency of the powdered Bti. Then, to determine the potential for the utilization of this Bti against *C. quinquefasciatus*, resistance induction experiments were carried out both ways to determine the long-term period of use before resistance occurred and the length of the recovery time from resistance. The larvicidal activity was expressed in terms of lethal concentrations (LC50s) according to the bioassay method used. In addition, the larvicides Bsph and temephos, the other mosquito control agents used in the present, were tested in parallel to obtain the appropriate data for these three larvicides to support the mosquito control program. Moreover, there is a plan to develop the Bti larvicide in the form of tablet product for easy use in the management of mosquitos for public health.

MATERIAL AND METHODS

Mosquito strains

The mosquito used in this study was from a susceptible strain of *C. quinquefasciatus*. The colony was established in 1989 and maintained at the NIH, Department of Medical Sciences. Mosquito eggs of the strain were used to establish the new colony used in this study. Larvae were reared in tap water and fed standard amounts of larval food under laboratory conditions at 25±2°C and 75±10% RH, with a light-dark cycle of 12:12 h. The early 4th instar larvae (4-5 days old) of each generation were used for the bioassay tests.

Laboratory Bti production

The strain of Bti evaluated in this study was the local strain collected from Ayutthaya province by Biological Control Section, a unit of the Thai NIH, Department of Medical Sciences. The strain was first isolated from collective soil samples and then purified and identified for the bacterial Bti. This Bti was kept as initial substance and the starters were prepared in nutrient broth (Fluorocult® LMX Broth,

---

*Corresponding author. E-mail: uruyakorn.c@dmsc.moph.go.th. Tel: (662) 951-0000 ext. 99238. Fax: (662) 591-5449.

Merck Germany) shaken for 18 h at a speed of 200 rpm at room temperature. The active growth phase of the bacteria was measured by optical turbidity (OD600 = 1) (Stevenson et al., 2016). Next, the starter was cultured in peptone glucose salt medium (PGSM), which was found to be highly specific for increasing the larvicidal potential of Bti (Chowanadisai and U-mai, 2005). The inoculation medium was shaken for 48 h at a speed of 200 rpm at a temperature of 32°C. Then, the sediment was centrifuged for 15 min at 3,000 rpm at 4°C. The sediment was rinsed with sterile normal saline 2 times before it was dried at -40°C for 24 h. The Bti sediment was crushed into powder and kept in sterile vacuum bottle for the further use.

Potency evaluation of Bti

The developed Bti was bioassayed following the standard protocols described by the World Health Organization (WHO, 1982). The *B. thuringiensis* var. *israelensis* standard (Std. 2-11), subcultured from international standards (IPS82 for *B. thuringiensis israelensis*) and maintained in our laboratory, was used for comparison. The preliminary bioassays were performed to obtain the wide ranges of concentrations caused 10-100% larval mortality in 48 h. Briefly, 25 early 4th instar larvae were transferred to 100 ml of distilled water in 120 ml disposable plastic cups. The cups were treated with 4–5 concentrations of Bti. Each concentration was replicated 4 times in each test. The interval of concentrations was noted and the sets of fine concentrations were prepared and repeated for the same testing method. The median lethal concentrations (LC50) were calculated according to larvicidal activity and were then used for potency calculations (Rishikesk and Qulennec, 1983). The toxicity (ITU/mg) of product tested was determined according to the following formula:

$$\text{ITU/mg of product tested} = \frac{\text{Reference std ITU/mg x LC50 (mg/l) std}}{\text{LC50 (mg/l) product tested}}$$

Selection pressure experiment

The susceptible strain of *C. quinquefasciatus* was subjected to developed Bti and the commercial larvicides Bsph and the sand granule temephos by selection pressure method in the early 4th instar larvae. The new colony was established in the F0 generation, and a total of 2,000 larvae were exposed to each larvicide at the concentration that caused 80-90% mortality. Larval mortality was recorded after 48 h (WHO, 1985) and the LC values of 50% mortalities were calculated using dosage-mortality regression probit analysis. Larvae were considered dead if they could not be induced to move when probed with a needle. Surviving larvae were randomly collected from each generation. They were reared until obtaining adults, to which blood feeding was placed to them to obtain the oviposition. Again, a test was performed to determine the new LC50 to subsequently treated generation. This process was successively extended to F25 generation. The resistance ratio (RR50) was calculated by comparing the LC50 of each filial generation with the LC50 of the susceptible parent generation (F0) as the formula:

$$\text{Resistance ratio} = \frac{\text{LC50 of each filial generation}}{\text{LC50 parent generation (F0)}}$$

After that, the RR50 was monitored after resistance induction for obtaining the decreased level that was similar to that of the F0 generation. The intervals of resistance induction and reduction were noted for each larvicide.

RESULTS

Bti production

The powdered Bti developed in this study has a potency of 20,839.98 ITU/mg compared to *B. thuringiensis israelensis* standard IPS82. According to standard bioassay of the selection pressure method, it was found that this local Ayutthaya Bti strain was successively used during the experiment until to the termination of the tests.

Selection pressure experiment

Three sets of mosquitoes were prepared and used as the parental (F0) generation. Under continuous selection, the mortality results were generally consistent, and the LC50 increased steadily in subsequent generations (Figures 1 and 2). It was found that the three larvicides increased resistance at different times and rates. The RR50 values were 1.86, 22.34 and 4.25 for Bti, Bsph and temephos, respectively, in F25, the last generation in the resistance induction phase. The resistance to Bti increased slowly (Figure 3), while the resistance to Bsph in the F4 generation was 10 times that in the initial colony (Figure 4). For temephos, the mosquitoes showed an intermediate response, with an RR50 between those of Bti and Bsph for the same generation (F25) (Figure 3). After the resistance induction phase was terminated, the Bti-treated mosquitoes had an RR50 (0.99-fold) similar to that of the F1 generation (0.97-fold) when the resistant bioassay tests were continued up to the F35 generation. The decrease in resistance to Bsph was assessed up to the F68 generation, for which the RR50 (10.64-fold) was close to that of the F5 generation (10.50-fold). The selection

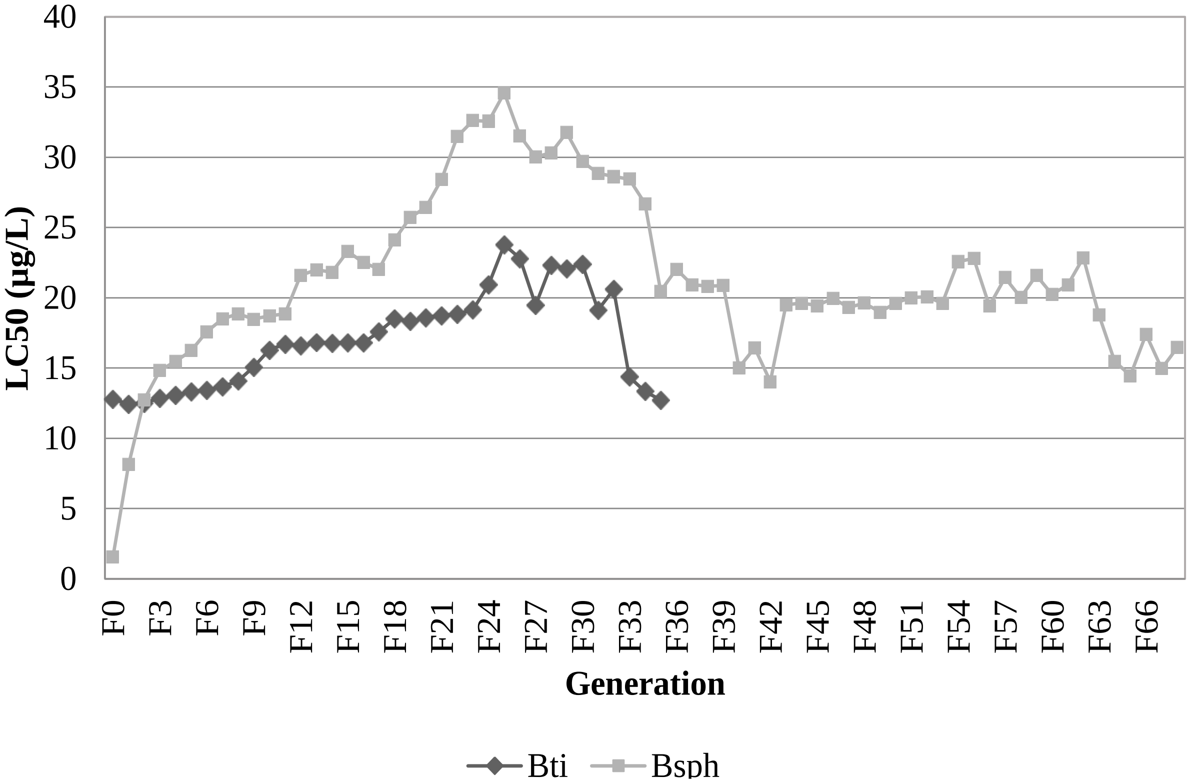

Figure 1. Median lethal concentrations (LC50) of *B. thuringiensis* var. *israelensis* (Bti) and *B. sphaericus* (Bsph) to the standard strain of the mosquito *C. quinquefasciatus* during the induction of resistance (in the F0-F25 generations) and after induction.

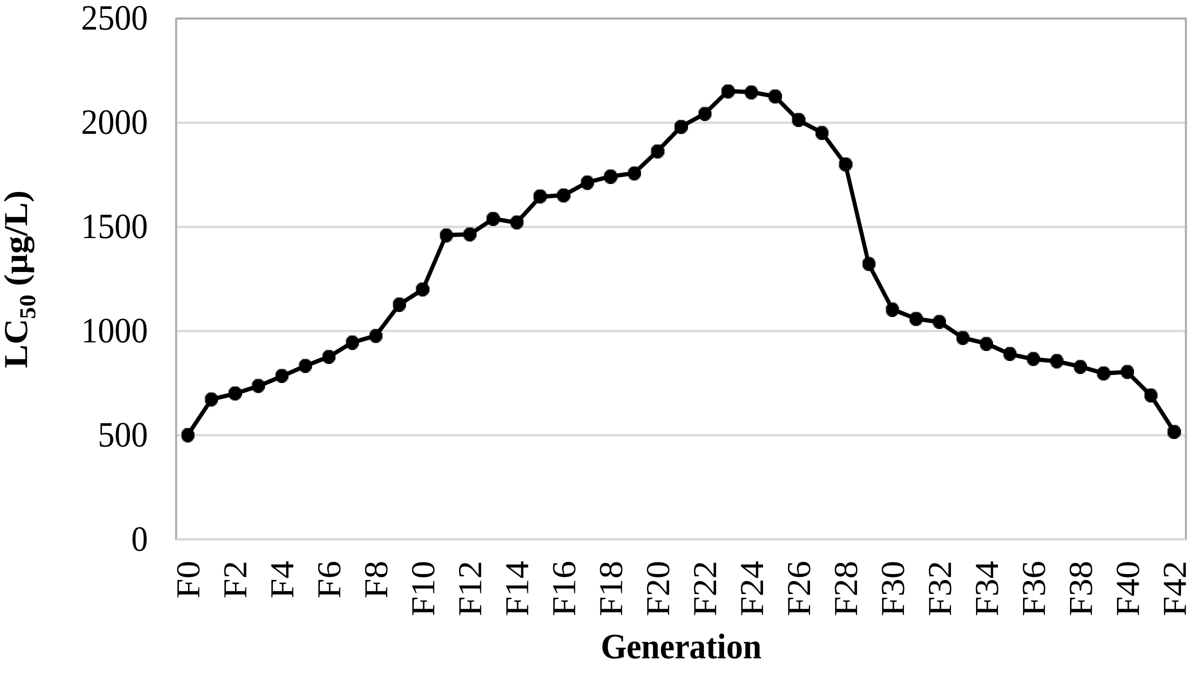

Figure 2. Median lethal concentration (LC50) of temephos to the standard strain of the mosquito *C. quinquefasciatus* during the induction of resistance (in the F0-F25 generations) and after induction.

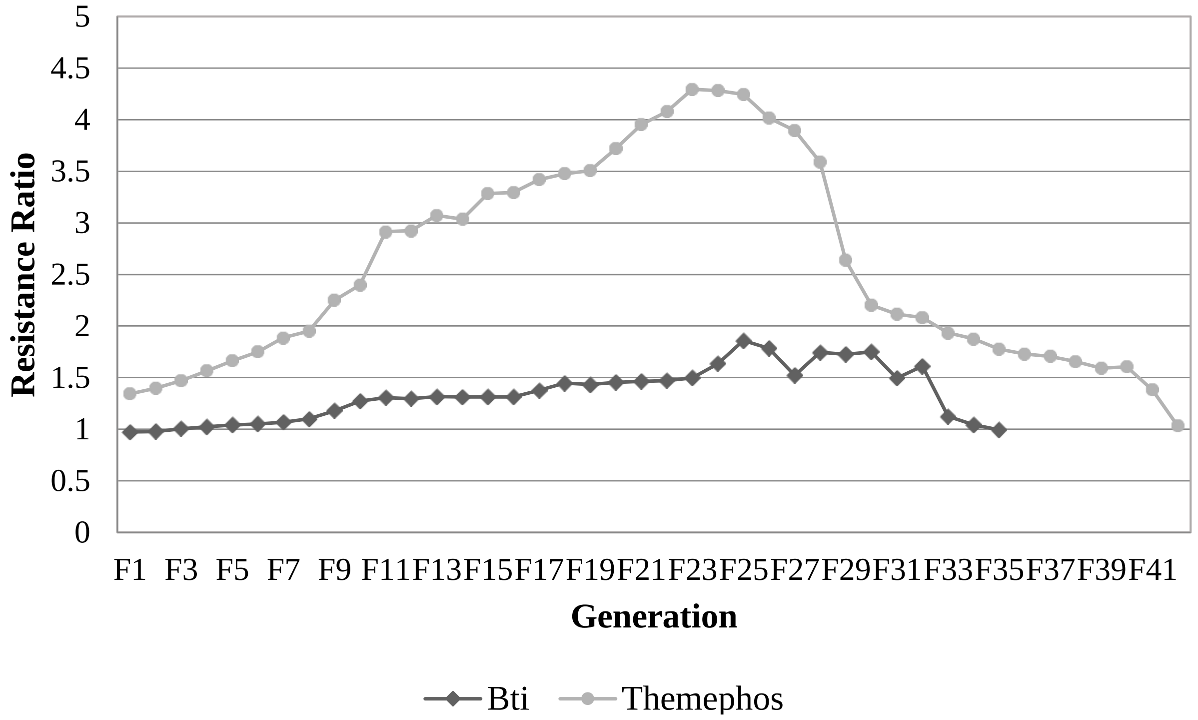

Figure 3. Resistance ratio of the standard strain of the mosquito *C. quinquefasciatus* during the induction of resistance to *B. thuringiensis* var. *israelensis* (Bti) and temephos in the F0-F25 generations and after induction compared to the F0 generation.

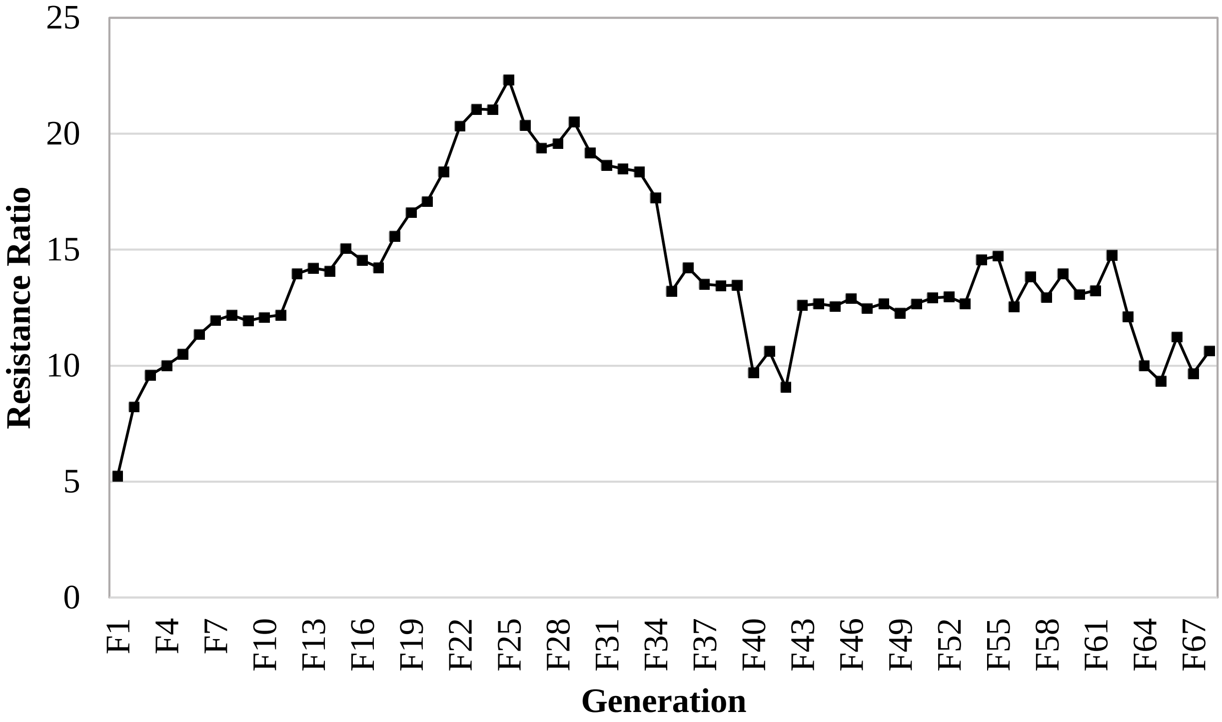

Figure 4. Resistance ratio of the standard strain of the mosquito *C. quinquefasciatus* during the induction of resistance to *B. sphaericus* (Bsph) in the F0-F25 generations and after induction compared to the F0 generation.

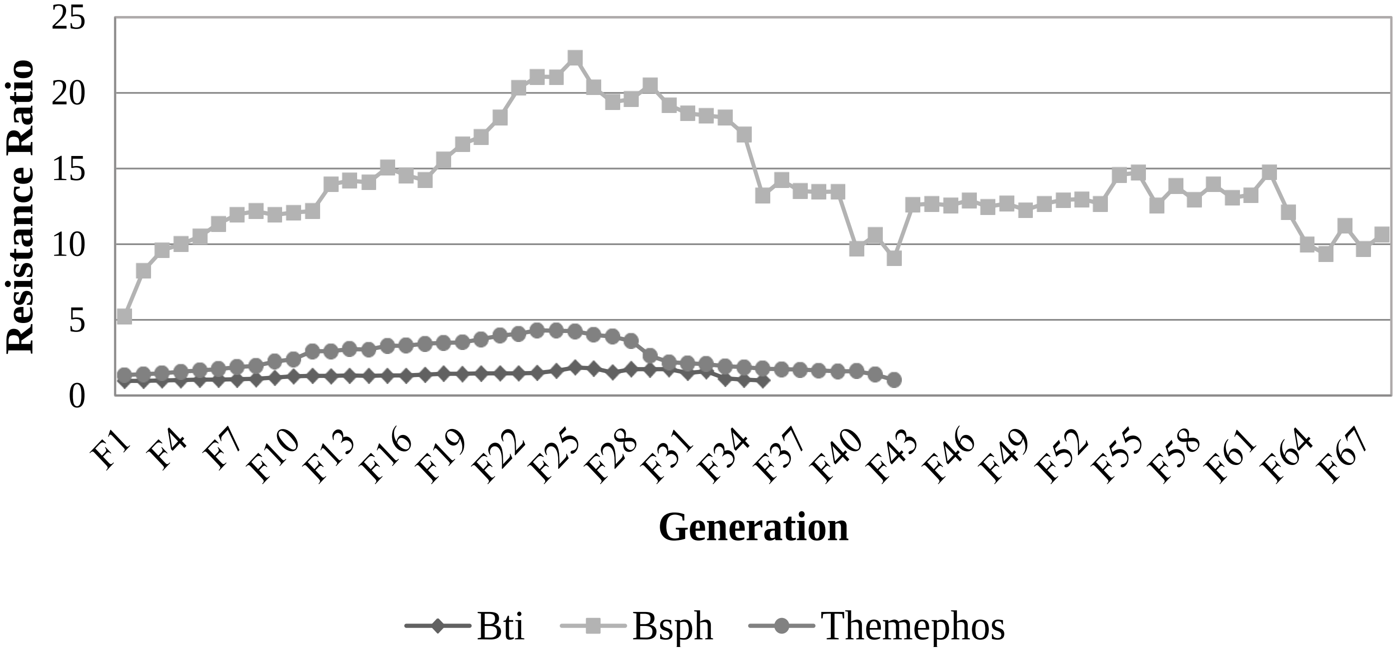

Figure 5. Resistance ratio of the standard strain of the mosquito *C. quinquefasciatus* during the induction of resistance to *B. thuringiensis* var. *israelensis* (Bti), *B. sphaericus* (Bsph) and temephos in the F0-F25 generations and after induction compared to the F0 generation.

experiments with temephos resulted in mosquitoes expressing resistance in a gradually increasing and decreasing pattern. The RR50 of the F41 generation (1.38-fold) was close to that of the F1 generation (1.34-fold). All these data are shown in Figures 3, 4, and 5 for comparison.

The resistant development periods and the length of recovery time from resistance

The resistance development periods of the 3 larvicides were as follows. After 16 months of resistance induction (through generation F25), Bti was still appropriate for mosquito control. After approximately 7 months (generation F35) the susceptibility to Bti was equal to the RR of the parent generation (F0). Bsph was suggested for use but not for the long term, because a 10-fold increase in resistance occurred within 2.5 months, and recovery could not be achieved throughout the study period (up to the F68 generation).

Temephos can be used for mosquito control but should be combined with other control methods for the long-term using. The RR to this larvicide decrease to a level similar to that of the parents within approximately 10 months. These results demonstrated the circulation and combinations of mosquito control agents that could be used to support the mosquito control program.

DISCUSSION

B. thuringiensis var *israelensis* (Bti) is a group of bacteria used as biological control agents for larvae stages of certain dipterans. It has been used for decades to control mosquitoes and black flies (WHO, 2009). In addition, it is generally considered an environmentally safe and effective larvicide. This bacterium has a variety of strains that differ in their Cry and Cyt toxin characteristics, resulting in potential for use against target pests. The insecticidal capacity of this bacterium resides primarily in a diverse group called delta-endotoxins that include Cry and cyt toxins (Xu, 2014). In Thailand, mosquitoes are abundant in both the dry and rainy seasons, and they bite during the day and night. Among these mosquitoes, *A. aegypti* and *C quinquefasciatus* the most abundant and are found everywhere in the country. Mosquito management and vector-borne disease control programs have been listed as priorities due to public health concerns regarding risks and outbreaks. Failures in insecticide usage have occurred in locations worldwide, including Thailand. Resistance to malathion, fenitrothion, and temephos in *A. aegypti* and *C. quinquefasciatus* have been reported in both Bangkok and outlying provinces (Triteeraprapab et al., 2000, Chareonviriyaphap, 2013). *B. thuringiensis* var *israelensis* is an alternative larvicide that has been proven to be effective for the mosquito control. However, the cost of commercial Bti is quite expensive, especially when a large amount is needed. According to the National Institute of Health (NIH), Thailand has authority and functions in the research and development of laboratory knowledge on public health. Larvicidal Bti was developed and tested using effective procedures following the technical operations outlined in the International Organization for Standardization (2017). There have been reports of Bti being produced in various formulations, such as powders, tablets and liquids, for different purposes (that is, research or commercial applications to breeding sites) (Tetreau et al., 2013; El-Bendary, 2016). These products have to be evaluated for potency, measured in international toxic units (ITU/mg), and efficiency against the target mosquito species (Rishikesk and Qulennec, 1983). In this study, Bti was produced as a powder with a potency of 20,839.98 ITU/mg. Then, this powder was tested against *C. quinquefasciatus*, the mosquito for filariasis monitoring at the Thai borders (Triteeraprapab, 2000). The use of Bti in either single or combined formulations to control target mosquito species has been reported during the past two decades. In Kenya, a formulation containing Bti and Bsph was found to significantly reduce the density of Anopheline mosquitoes when it was applied to areas with endemic malaria (Derua et al., 2018). In Florida, Bti was applied against *C. quinquefasciatus* in field plots by using two sprayers. The results indicated that there was no significant difference (P>0.05) in 24 and 48 h larval mortality between the 2 sprayers, with mortality continuously decreasing with increasing distance from the sprayers (Dunford et al., 2014). However, the effectiveness of long-term using of this control agent has not been reported. It has been observed that larvae do not change their behavior to avoid control interventions targeted at their habitats, however under natural selection, larvae can develop resistance to control agents, resulting in limitations of their efficacy. In this study, the long-term effectiveness of the developed Bti and the time to recover from resistance to this larvicide were tested against *C. quinquefasciatus* by the selection pressure method. In addition, the commercial larvicides Bsph and temephos were tested in parallel, and the data were compared to support the mosquito control program. After selection for 25 generations at the LC50 level, the resistance of the mosquitoes to Bit, Bsph and temephos increased by up to 1.86, 22.31 and 4.25 times, respectively. The ability of mosquito larvae to tolerate the toxic compounds of Bti and temephos was examined, and it was found that exposure to these larvicides increased the activity levels of larval detoxifying enzymes (Garcia et al., 2018). For *C. quinquefasciatus,* the induction experiments were studied for resistance to 2.5% spinosad at levels of LC70-90, and resistance was found in early generations (F9) (Xu, 2014). However, the induction of *C. quinquefasciatus* resistance to Bti, Bsph and temephos has not been reported. This is the first study for the appropriate long-term use of these three control agents. Moreover, the interval over which resistance decreased was monitored until the RR were similar to those of the parent generations. Resistance recovery occurred in the 35th and 41st generations for Bti and temephos respectively. Resistance to Bsph also declined gradually after induction but persisted for 10 generations and from the 43rd to the 53rd. While the RR of the 68th generation was 10.64, and then the tests were terminated after over the two years of study. This finding was similar to a report in Thailand that the large-scale use of Bsph in mosquito control programs led to the emergence of resistance in larvae of the *Culex pipiens* complex (Mulla, 2003). These results conformed to the insecticidal characteristics of Bti and Bs. Bti resides delta-endotoxins that include Cry and cyt toxins which display a common three-domain topology (WHO, 2009). While Bsph produces toxin which apparently only binds to a single receptor type on midgut microvilli of mosquito larvae (WHO, 1985). According to a review, it is necessary to increase the development of alternative and innovative strategies for controlling mosquitoes (Achee et al., 2019), and this study provides useful information for the development of an effective bacterial larvicide used for public health. Moreover, the results of this study can be used to develop alternative management strategies, for the control agent selection, and to find practical methods for controlling resistant mosquitoes. Additionally, the Bti larvicide obtained from this study is being developed into the tablet product and test against the field population of *C. quinquefasciatus* for the further use in public health mosquito control program.

ACKNOWLEDGMENTS

The authors are grateful to the National Research Council of Thailand and the NIH, Department of Medical Sciences, for research grants. The authors appreciate the encouragement and constant support of Dr. Ballang Uppapong, Director of the NIH, Department of Medical Sciences, Thailand. We also appreciate the assistance of staff members, Mongkol Riyapan and Atchariya Unat, NIH, Department of Medical Sciences, Thailand.

REFERENCES

Achee N., Grieco J., Vatandoost H., Seixas G., Pinto J., Ching-NG, Martins A. J., Juntarajumnong W., Corbel V., Gouagna C., David J.-P., Logan J. G., Orsborne J., Marois E., Devine G. J. & Vontas J. (2019). Alternative strategies for mosquito-borne arbovirus control. PLOS Neglected Tropical Diseases. 13(3): e0007275. https://doi.org/10.1371/journal.pntd.0006822

Aksoy H. M., Saruhan I., Akca I., Kaya Y., Onder H., Ozturk M. & Aker O. (2015). Isolation and characterization of *Bacillus thuringiensis* isolated from soil and their possible impact on *Culex pipiens* larvae. Egyptian J. Biol. Pest Control. 25(2):439-444.

Amorim Q., da Rocha Bauzer L., Aparecida Braga I. & Lima J. (2019). Evaluation of the persistence of three larvicides used to control *Aedes* *aegypti* in Arapiraca. Northeastern Brazil. J. Am. Mosq. Control Assoc.35(3):192-199.

Annual Epidemiological Surveillance reports 506, DDC Thailand. (2015). Available from URL: http://www.Boedb/surdata/506wk/y56/d76.

Argolo-Filho R. C. & Loguercio L. L. (2013). *Bacillus thuringiensis* is an environment pathogen and host-specificity has developed as an adaptation to Human-generated ecological niches. Insects. 5(1):62-91.

Chareonviriyaphap T., Bangs M. J., Suwonkerd W., Kongmee M., Corbel V. & Ngoen-Klan R. (2013). Review of insecticide resistance and behavioral avoidance of vectors of human diseases in Thailand. Parasit. Vectors. 25:6:280. doi: 10.1186/1756-3305-6-280.

Chowanadisai L. & U-mai N. (2005). Effectiveness of bacterial larvicide products from Thai strain *Bacillus thuringiensis* subsp. *israelensis*. Biotechnology of *Bacillus thuringiensis*. 5:364-382.

Derua Y., Kahindi S., Mosha F., Kweka E., Atieli H., Wang X., Zhou G., Lee M.-C., Githeko A. K. & Yan G. (2018). Microbial larvicides for mosquito control: Impact of long lasting formulations of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* on non-target organisms in western Kenya highlands. Ecol. Evol. 8(15):7563-7573.

Dunford J., Stoops C., Estep A., Britch S., Richardson A., Walker T., Farooq M., Hoel D. F., Platt R. R., Smith V. L., Wirtz R. A. & Kerce J. D. (2014). SR450 and Superhawk XP Applications of *Bacillus thuringiensis israelensis* Against *Culex quinquefasciatus*. J. Am. Mosq. Control Assoc. 30(3):191-198.

El-Bendary M. A. (2006). *Bacillus thuringiensis* and *Bacillus sphaericus* biopesticides production. J. Basic Microbiol. 46(2):158-170.

Garcia A. Z., David M. R., Martins A. J., Freitas R. M., Linss J. G. B., Araujo S. C., Lima J. B. P. & Valle D. (2018). The impact of insecticide applications on the dynamics of resistance: The case of four *Aedes aegypti* populations from different Brazilian regions. PLoS Negl. Trop. Dis. 12(2): e0006227. doi: 10.1371.

International Organization for Standardization (ISO/IEC 17025:2017) (2017). General requirements for the competence of testing and calibration laboratories. 3rd ed. Geneva. International Organization for Standardization (ISO).

Mulla M. S., Thavara U., Tawatsin A., Chomposri J., Su T. (2003). Emergence of resistance and resistance management in field populations of tropical *Culex quinquefasciatus* to the microbial control agent *Bacillus sphaericus*. J. Am. Mosq. Control Assoc. 19:39-46.

Pruszynski C. A., Hribar L. J., Mickle R. & Leal A. L. (2017). A large scale biorational approach using *Bacillus thuringiensis israeliensis* (Strain AM65-52) for managing *Aedes aegypti* populations to prevent dengue, chikungunya and zika transmission. PLoS ONE. 12(2): e0170079. doi:10.1371/journal.pone.0170079.

Rishikesk N. & Qulennec G. (1983). Introduction to a standardized method for the evaluation of the potency of *Bacillus thuringiensis* serotype H-14 based products. Bulletin of the World Health Organization. 61:93-97.

Ritchie S., Rapley L. & Benjamin S. (2010). *Bacillus thuringiensis* var. *israelensis* (Bti) provides residual control of *Aedes aegypti* in small containers. Am. J. Trop. Med. Hyg. 82(6):1053-1059.

Simonsen P. & Mwakitalu M. (2012). Urban lymphatic filariasis. Parasitol. Res. 112(1):35-44.

Stevenson K., McVey A., Clark I., Swain P. & Pilizota T. (2016). General calibration of microbial growth in microplate readers. Sci. Report. 6:38828. doi: 10.1038/srep38828.

Tetreau G., Patil C., Chandor-Proust A., Salunke B., Patil S. & Després L. (2013). Production of the bioinsecticide *Bacillus thuringiensis* subsp. *israelensis* with deltamethrin increases toxicity towards mosquito larvae. Appl. Microbiol. 57(2):151-156.

Triteeraprapab S., Kanjanopas K., Suwannadabba S., Sangprakarn S., Poovorawan Y. & Scott A. (2000). Transmission of the nocturnal periodic strain of *Wuchereria bancrofti* by *Culex quinquefasciatus*: establishing the potential for urban filariasis in Thailand. Epidemiol. Infect. 125(1):207-212.

Triteeraprapab S., Nuchprayoon I., Porksakorn C., Poovorawan Y. & Scott A. L. (2001). High prevalence of *Wuchereria bancrofti* infection among Myanmar migrants in Thailand. Ann. Trop. Med. Parasitol. 95(5):535-538.

World Health Organization (1982). Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides. Retrieved June 22, 2020. Available online at https://apps.who.int/iris/handle/10665/69615.

World Health Organization (1985). Bioassay method for the titration of *Bacillus sphaericus* preparations with RB standard. Informal consultations on the development of *Bacillus sphaericus* as a microbial larvicide. Geneva: World Health Organization. Available online at https://apps.who.int/iris/handle/10665/60326.

World Health Organization (1992). Vector resistance to pesticides. Fifteenth Report of the WHO Committee on Vector Biology and Control. 35:143-148.

World Health Organization (2009). *Bacillus thuringiensis israelensis* (Bti) in drinking-water; Background document for development of WHO Guidelines for Drinking-water Quality. Retrieved June 10, 2020. Available online at https://www.who.int/water_sanitation_health/gdwqrevision/RevisedFourthEditionBacillusthuringiensis_Bti_July272009_2.pdf.

Xu C., Wang B. C., Yu Z. & Sun M. (2014). Structural insights into *Bacillus thuringiensis* Cry, Cyt and Parasporin Toxins. Toxins. 6(9):2732-2770.