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SHORT COMMUNICATION

EVALUATING THE EFFICACY OF COMMERCIAL *Bacillus thuringiensis israelensis* FOR *Aedes albopictus* LARVAL CONTROL IN VARIOUS LAND USE

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ABSTRACT

A commercial bacterial product of *Bacillus thuringiensis israelensis* (Bti); Mosquito BTI from USA was tested for its efficacy in exterminating the local populations of *Aedes albopictus* larvae. Larval surveys were carried out to obtain field populations of *Ae. albopictus* from various land use namely dengue-risk and dengue-free housing localities as well as agrarian localities like oil palm plantations, rubber estates and paddy fields. A 24-hour larval bioassay using Mosquito BTI was performed on each *Ae. albopictus* population. Only a maximum of 2.00% mortality was observed by the end of the first four hours of exposure while a maximum of 25.00% mortality was displayed at 24 hours of exposure. Results showed the potential use of Bti in reducing local *Ae. albopictus* larval populations but more than 24 hours may be required to achieve complete mortalities among these populations upon exposure to Bti. Some limitations of this study were also discussed.

Keywords: *Bacillus thuringiensis israelensis*, Bti, *Aedes albopictus*, mosquito larvae.

ABSTRAK

Satu produk komersial bakteria; *Bacillus thuringiensis israelensis* (Bti); Mosquito BTI dari USA telah diuji untuk menentukan keberkesanannya dalam menghapuskan populasi tempatan larva *Aedes albopictus*. Kajian taburan larva telah dijalankan untuk mendapatkan populasi lapangan *Ae. albopictus* dari pelbagai kawasan kegunaan tanah seperti kawasan perumahan dengan kes denggi dan bebas denggi serta kawasan pertanian seperti ladang kelapa sawit, ladang getah dan sawah padi. Bioasai larva selama 24 jam telah dilaksanakan menggunakan Mosquito BTI ke atas setiap populasi *Ae. albopictus*. Hanya kematian maksimum 2.00% dapat dilihat pada penghujung empat jam pertama pendedahan, manakala kematian maksimum 25.00% dapat dilihat pada akhir 24 jam pendedahan. Keputusan ini menunjukkan potensi penggunaan Bti dalam mengurangkan populasi tempatan larva *Ae. albopictus* namun lebih dari 24 jam mungkin diperlukan untuk mencapai kematian sepenuhnya dalam kalangan populasi ini selepas pendedahan kepada Bti. Beberapa kekangan dalam kajian ini juga turut dibincangkan.

Kata kunci: *Bacillus thuringiensis israelensis*, Bti, *Aedes albopictus*, larva nyamuk.

Dengue is a mosquito-borne infectious disease transmitted by *Aedes aegypti* and *Aedes albopictus* (Ferreira et al. 2023). An estimated 400 million dengue cases are occurring annually throughout the world (Zaidi et al. 2022). Dengue continues as among the major public health concern in Malaysia due to its high morbidity (Sakinah et al. 2021). The development of dengue vaccine to protect the communities from this infection is still at an early stage. Therefore, vector control continues as the hallmark of the dengue control strategies. Chemical control using insecticides has become the most commonly selected method in controlling mosquito vectors, but continuous use of insecticides has steered to the emergence of insecticide resistance among these mosquito populations. The occurrence of insecticide resistance among dengue vectors has been reported in many countries including Malaysia (Asgarian et al. 2023; Elia-Amira et al. 2018; Rajendran et al. 2021; Rasli et al. 2021; Wan-Norafikah et al. 2010). The development of mosquito resistance against insecticides reduces the efficacy of vector control activities conducted. Hence, there is an urgency to introduce an alternative to overcome dengue spread.

Bacillus thuringiensis israelensis (Bti) is a gram-positive bacteria that is capable in producing toxin crystal proteins (Alam et al. 2022). Bti toxin is environmentally safe and has minimal effects on non-target organisms (Odom et al. 2022). The efficacies of Bti products are varied as Bti toxin crystals are highly selective to only certain mosquito species and few dipteran families (Carvalho et al. 2021). As for now, not many Bti-related studies that were carried out upon *Ae. albopictus*. Bti has been proven effective against *Ae. albopictus* populations from certain countries like Brazil, Switzerland, Italy, China, Greece and Cameroon (Balaska et al. 2020; Suter et al. 2017; Su et al. 2019; Yougang et al. 2020). In Malaysia, Bti application has been previously reported at a locality each in Sarawak and Selangor (Bohari et al. 2020; Zaki et al. 2020). Henceforth, this study was aimed to determine the efficacy of the commercial Bti product; Mosquito BTI in controlling *Ae. albopictus* larval populations from various types of land use.

Human dwellings within housing and agrarian localities were included in this study. The housing localities encompassed the dengue-free and dengue-risk housing localities, whereas, the agrarian localities comprised of the oil palm plantations, rubber estates and paddy fields (Table 1). All dengue-free housing localities were free from any reported dengue cases for the past five years while there were reported dengue cases within five recent years in dengue-risk housing localities chosen for this study as disclosed by the Ministry of Health Malaysia. Oil palm plantations, rubber estates and paddy fields were chosen to represent the agrarian localities following the recognition on them as the primary Malaysian industrial crop (Department of Agriculture Peninsular Malaysia 2015). There was no vector control activity that has been carried out in these agrarian localities since no mosquito-borne infection has ever been reported from these localities. Three study localities represented each type of housing and agrarian locality. Therefore, a total of fifteen field populations were involved in this study. Conversely, the reference strain was represented by the laboratory strain that has been cultured in the laboratory for nearly ninety generations without any insecticide selection.

Table 1. Description of study localities

State	District	Study Localities	Coordinates	Elevation (m) (Above Sea Level)
Housing locality: Dengue-free housing localities				
Selangor	Shah Alam	Alam Nusantara, Setia Alam **(Selangor DF)**	03˚06.692’N, 101˚28.134’E	34
Kedah	Padang Serai	Taman Serai Wangi, Mukim Kulim **(Kedah DF)**	05˚31.301’N, 100˚32.673’E	3
Pahang	Temerloh	Taman Seberang Temerloh **(Pahang DF)**	03˚26.985’N, 102˚26.743’E	19
Housing locality: Dengue-risk housing localities				
Johor	Kota Tinggi	Felda Air Tawar 2 **(Johor DR)**	01˚40.552’N, 104˚01.340’E	5
Selangor	Shah Alam	Kg. Padang Jawa, Seksyen 17 **(Selangor DR)**	03˚03.000’N, 101˚29.200’E	1
Federal Territory of Kuala Lumpur	Cheras	Kg. Cheras Baru **(Kuala Lumpur DR)**	03˚06.630’N, 101˚45.101’E	89
Agrarian locality: Oil palm plantations				
Johor	Kota Tinggi	University of Malaya Oil Palm Research Plantation, Jementah **(Johor OP)**	02˚01.727’N, 103˚51.924’E	28
Selangor	Klang	Jalan Paip Kiri, Meru **(Selangor OP)**	03˚09.201’N, 101˚27.535’E	5
Pahang	Temerloh	Taman Paya Pulai **(Pahang OP)**	03˚27.642’N, 102˚28.098’E	42
Agrarian locality: Paddy fields				
Selangor	Kuala Selangor	Parit 3, Ban 3, Tanjung Karang **(Selangor PD)**	03˚29.770’N, 101˚09.288’E	-25
Kedah	Kulim	Kg. Terat Batu, Mukim Sidam Kanan **(Kedah PD)**	05˚32.741’N, 100˚32.350’E	9
Negeri Sembilan	Kuala Pilah	Kg. Padang Lebar Terachi, Tanjong Ipoh **(Negeri Sembilan PD)**	02˚44.520’N, 102˚07.787’E	81
Agrarian locality: Rubber estates				
Selangor	Sungai Buloh	Sungai Pelong **(Selangor RU)**	03˚12.549’N, 101˚32.436’E	39
Pahang	Temerloh	Taman Jaya 8 **(Pahang RU)**	03˚27.423’N, 102˚27.638’E	43

Johor	Kota Tinggi	Malaysian Rubber Board, Desaru **(Johor RU)**	01˚33.844'N, 104˚14.267'E	23

Kg. = Kampung

The sampling of *Aedes* larvae was performed via a larval survey at each study locality. All possible natural and man-made mosquito breeding habitats were inspected for any existence of mosquito larvae. All mosquito larvae found in these breeding spots were collected and brought to the Laboratory Animal Care Unit (LACU), Faculty of Medicine, Universiti Teknologi MARA (UiTM). These mosquito larvae were fed with ox liver powder and half-cooked ox liver chunks and further raised till adulthood. The morphological identification was made on all emerged adult mosquitoes by referring to the taxonomic keys (Jeffery et al. 2012). Only *Ae. albopictus* adult mosquitoes (F0) were blood-fed in mosquito cages to produce the eggs of successive generation (F1). Hatched eggs (F1) were fed with similar larval diet and raised until third instar of larvae which then be used for the larval bioassays of this research work.

An American commercial product of *Bacillus thuringiensis israelensis* (Bti) bacteria (Plus Evergroup Sdn Bhd); Mosquito BTI was utilized in this study. This product is offered for consumer buying in the Malaysian market. The mixture of Mosquito BTI was prepared at 0.02 g/L as recommended by the manufacturer. The larval bioassay using Mosquito BTI was performed according to the guidelines by World Health Organization (WHO) (2016) with minor modifications. A total of 0.02 g/L of Mosquito BTI mixture was prepared in a jar with dechlorinated water and left for four hours before the starting of the larval bioassay. This Mosquito BTI mixture was then divided into four test cups with 200 ml per replicate. Twenty-five (25) late third instar larvae were introduced into each test cup and two control cups. The control cups only contained 200 ml of dechlorinated water. The larval bioassay using Mosquito BTI was performed on the reference strain and all field populations. Larval mortality was recorded for each replicate at every ten minutes for the first four hours and at 24 hours of exposure.

The results obtained from the preliminary larval bioassay using the reference strain and the larval bioassay using the field populations of *Ae. albopictus* larvae were subjected to the same statistical analysis. The mortality percentage and the lethal time for 50% of the population (LT_{50}) were attained using the SPSS statistical analysis software while the resistance ratio (RR) value for every population was calculated as below:

$$\text{Resistance Ratio (RR)} = \frac{\text{LT50 of field population}}{\text{LT50 of reference strain}}$$

RR value of <5 demonstrated the susceptibility of the field population. RR value of $5 \leq RR \leq 10$ revealed a moderate resistance while RR value of >10 suggested a high resistance of the field population (WHO 2016).

In the larval bioassay, all *Ae. albopictus* larval populations from housing and agrarian localities were exposed to Mosquito BTI for 24 hours. During the first four hours of exposure, only the reference strain as well as the field populations of Pahang RU, Selangor DF, Johor DR and Kuala Lumpur DR demonstrated mortalities between 1.00% and 2.00% (Table 2). There was no mortality recorded for the rest of *Ae. albopictus* larval populations. Due to this very low and zero mortality during the first four hours of exposure, the lethal time value for 50% of the population (LT_{50}) and also the resistance ratio (RR) for each population tested were unable to be calculated.

However, after 24 hours of exposure period, *Ae. albopictus* larval populations from all localities showed mortalities between 1.00% and 25.00%. *Aedes albopictus* larvae from Pahang OP, Kedah PD and Pahang DF displayed the lowest mortality percentage while the highest mortality percentage was exhibited by *Ae. albopictus* larvae from Kedah DF. These bioassay results were also grouped according to the types of localities in which *Ae. albopictus* larval populations from dengue-risk housing localities showed the lowest mortality percentage (3.67%±0.33) while the highest mortality percentage was observed among *Ae. albopictus* larval populations from dengue-free housing localities (11.33%±7.13).

In this study, the Mosquito BTI mixture was prepared at four hours before the bioassays. This is following the manufacturer's claim that Mosquito BTI will be effective to kill mosquito larvae within four to 24 hours. Our observation showed that from four to eight hours after the Mosquito BTI mixture preparation, very low mortalities were recorded. Furthermore, at 24 hours of exposure time, less than 30% of the larvae were dead. These findings indicate that more than 24 hours is needed to reach the high or complete mortalities of *Ae. albopictus* larvae for all populations tested. A significant difference ($P \leq 0.05$) was displayed in the mortality percentage between all field populations with the reference strain at the first four hours of exposure time but not at 24 hours of exposure period.

There was also a limitation of this study. As recommended by the manufacturer, the Mosquito BTI mixture was prepared at 0.02 g/L in a plastic jug at four hours before the conduct of larval bioassays. However, during the transfer of every 200 ml of Mosquito BTI mixture into the replicate cup, only the Mosquito BTI mixture was transferred into it, leaving the particle of Mosquito BTI in the preparation jug. The Mosquito BTI particle was very hard and unable to be shattered. This limitation could jeopardize the end results of this study as more than four hours may be needed for the Bti to be fully released from the particles. Higher mortality percentages may be achieved if the Mosquito BTI particles could be maintained in each replicate cup. Therefore, some modifications on the larval bioassays could be done by preparing each replicate in a bigger container so that the Mosquito BTI particle could be remained in each replicate of 1 L and thus, increase the number of dead larvae. Furthermore, another study could be carried out involving a longer time of exposure and observation in order to verify the manufacturer's claim that Mosquito BTI will be effective for up to fourteen days.

Table 2. Means Percent mortality (SE)of *Aedes albopictus* larvae from different types of localities against commercial *Bacillus thuringiensis israelensis* bacteria; Mosquito BTI at 4 hours and 24 hours of the exposure time

Types of Localities	**Study Localities**	**Means Percent Mortality At 4 Hours of The Exposure Time (%)**		**Means Percent Mortality At 24 Hours of The Exposure Time (%)**	
		By Locality	**By Types Of Localities**	**By Locality**	**By Types Of Localities**
Reference	Laboratory	2.00±2.00	2.00±2.00	8.00±5.42	8.00±5.42
Dengue-free housing locality	Selangor DF	1.00±1.00	0.33±0.33	8.00±4.32	11.33±7.13
	Kedah DF	0.00±0.00		25.00±5.26	
	Pahang DF	0.00±0.00		1.00±1.00	
Dengue-risk housing locality	Johor DR	1.00±1.00	0.6±0.33	3.00±1.91	3.67±0.33
	Selangor DR	0.00±0.00		4.00±1.63	
	Kuala Lumpur DR	1.00±1.00		4.00±0.00	
Agrarian locality: Oil palm plantation	Johor OP	0.00±0.00	0.00±0.00	13.00±3.42	7.00±3.46
	Selangor OP	0.00±0.00		7.00±1.00	
	Pahang OP	0.00±0.00		1.00±1.00	
Agrarian locality: Paddy field	Selangor PD	0.00±0.00	0.00±0.00	10.00±5.29	6.67±2.85
	Kedah PD	0.00±0.00		1.00±1.00	
	Negeri Sembilan PD	0.00±0.00		9.00±5.26	
Agrarian locality: Rubber estate	Selangor RU	0.00±0.00	0.33±0.33	4.00±1.63	4.67±0.67
	Pahang RU	1.00±1.00		6.00±3.83	
	Johor RU	0.00±0.00		4.00±2.31	
One way ANOVA		F=7.767 df=15 P=0.706	F=3.750 df=5 P=0.036	F=3.242 df=15 P=0.001	F=0.496 df=5 P=0.773

Percent mortality at 4 hours or 24 hours of the exposure time (%) = Mean of mortality larvae+Standard Error (S.E.)

The Bti application for the control of mosquito larvae has started as early as 1980s (Derua et al. 2022). However, the use of Bti in controlling *Ae. albopictus* larvae is still underreported. Complete mortalities have been exhibited among field populations of *Ae. albopictus* larvae in foreign countries like Brazil, Switzerland, Italy, China, Greece and Cameroon upon exposure to Bti (Balaska et al. 2020; Suter et al. 2017; Su et al. 2019; Yougang et al. 2020). On the other hand, Bti has been tested in the field operations in Sibu, Sarawak and Selangor, Malaysia which displayed significant decline of *Aedes* indices (Bohari et al. 2020; Zaki et al. 2020).

In summary, Mosquito BTI has the potential to be utilized by the residents at the local housing and agrarian localities selected for this study. Bti could be implemented as a domestic biological tool in controlling or avoiding the breeding of *Ae. albopictus* larvae in natural and man-made containers available within the premise compounds. However, more than 24 hours are needed by the Mosquito BTI to become significantly effective and the Mosquito BTI particles are suggested to be maintained in each breeding container so that the effectiveness of the Mosquito BTI could be maximized. Further studies to understand and rectify these two issues will help to highlight the beneficial use of Bti as an alternative mosquito larval control tool for the consumers in Malaysia.

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AUTHORS DECLARATIONS

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Declarations

No ethical issue is required for this research.

Data Availability Statement

Not applicable.

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