Tropical Biomedicine 27(3): 493–503 (2010)

Susceptibility of field-collected *Aedes aegypti* (L.) (Diptera: Culicidae) to *Bacillus thuringiensis israelensis* and temephos

Loke, S.R.[1,2*], Andy-Tan, W.A.[1,2], Benjamin, S.[3], Lee, H.L.[2] and Sofian-Azirun, M.[1]
[1]Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia
[2]Medical Entomology Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia
[3]Valent BioSciences Corporation, Malaysia
Corresponding author email: joserong@yahoo.com
Received 13 March 2010; received in revised form 8 July 2010; accepted 19 July 2010

Abstract. The susceptibility status of field-collected *Aedes aegypti* (L.) from a dengue endemic area to *Bacillus thuringiensis israelensis* (Bti) and temephos was determined. Since August 2007, biweekly ovitrap surveillance (OS) was conducted for 12 mo in 2 sites, A & B, in Shah Alam, Selangor. Site A was treated with a Bti formulation, VectoBac® WG at 500 g/ha, from December 2007 – June 2008 while Site B was subjected to routine dengue vector control activities conducted by the local municipality. *Aedes aegypti* larvae collected from OS in both sites were bred until F3 and evaluated for their susceptibility. The larvae were pooled according to 3 time periods, which corresponded to Bti treatment phases in site A: August – November 2007 (Bti pre-treatment phase); December 2007 – June 2008 (Bti treatment phase); and July – September 2008 (Bti post-treatment phase). Larvae were bioassayed against Bti or temephos in accordance with WHO standard methods. Larvae collected from Site A was resistant to temephos, while incipient temephos resistant was detected in Site B throughout the study using WHO diagnostic dosage of 0.02 mg/L. The LC_{50} of temephos ranged between 0.007040 – 0.03799 mg/L throughout the year in both sites. Resistance ratios (LC_{50}) indicated that temephos resistance increased with time, from 1.2 – 6.7 folds. The LC_{50} of *Ae. aegypti* larvae to Bti ranged between 0.08890 – 0.1814 mg/L throughout the year in both sites, showing uniform susceptibility of field larvae to Bti, in spite of Site A receiving 18 Bti treatments over a period of 7 mo. No cross-resistance of *Ae. aegypti* larvae from temephos to Bti was detected.

INTRODUCTION

Dengue fever (DF) and dengue haemorrhagic fever (DHF) are the most significant vector-borne viral diseases globally. The causative dengue virus, a flavivirus, is principally transmitted by the *Aedes* spp. mosquitoes. The virus has four antigenically similar but immunologically distinct serotypes. In the absence of an effective vaccine, vector control is the only method to prevent transmission of this viral disease. Unfortunately, insecticide resistance has continued to spread and affect disease control programmes in many countries (WHO, 1980).

The current strategy of the Vector Borne Disease Control Programme (VBDCP) in Malaysia against dengue is source reduction, larviciding and adulticiding in outbreak sites. Fogging with insecticides is conducted within a radius of 200 – 400 m of a reported dengue case, and repeat fogging is conducted within 7 – 10 d after the first fogging (Lee *et al.*, 2008). Adulticides that are commonly used in mosquito control programme are pyrethroids, while temephos is

recommended as the larvicide (Ang & Satwant, 2001; Lee *et al.*, 2008). In spite of fogging in the infected area and the latest guidelines on treatment, dengue remains a major public health problem. The lack of community awareness and information on the field dengue vector susceptibility status to the commonly used insecticides have impeded the success of dengue vector control programme. Therefore, examination on susceptibility status of field populations of *Aedes* spp. larvae and adults from the dengue endemic areas to insecticides that are commonly used in mosquito control is important.

The principal larvicide used in the control of dengue vectors is temephos (Abate®). In Malaysia, it has been used since the first DF outbreak in 1973. It is added in potable containers as 1% (w/w) sand granules to control breeding in stagnant waters (Cheong, 1978). This formulation is available as 100 gram sachets for consumer purchase and it is also distributed by the local authorities to residents from dengue infected premises. The 30 years of temephos use warrants an investigation into the susceptibility status of the dengue vector larvae to be constantly carried out.

Bacillus thuringiensis israelensis (Bti) was used to replace temephos in dengue epidemic countries with temephos resistance. Bti is the predominant biological control agent applied in the mosquito control programme in the United States, Asia and Europe (Becker, 2004). The effectiveness of its larvicidal activity has been well-documented since its introduction more than 20 years ago and the development of resistance to Bti in the field population to all mosquito species has not been reported (Lacey, 2007). In this study, the state of Selangor was chosen as the study site due to continuous high incidences of dengue reported in the recent years. In 2007, 15,871 of 48,846 total dengue cases were reported in this state; 21,262 of 49,335 and 18,676 of 41,486 dengue cases were reported respectively in 2008 and 2009 (Ministry of Health, Malaysia, 2009; 2010). The state capital of Selangor, Shah Alam, was identified by the local authority as one of the areas in Selangor with high number of dengue cases. In the early 2009, 14 dengue hot spots were identified in Shah Alam (Salina, 2009). Temephos was the only larvicide used by the residents in this area. During the one-year ovitrap surveillance, Bti treatment (500g/ha) was conducted simultaneously for 7 mo from December 2007 – June 2008 in this area. The objective of this study was to monitor both temephos and Bti susceptibility in the field dengue vector population for a year.

MATERIALS AND METHODS

Study Period

August 2007 – September 2008

Study Site: Section 17, Shah Alam, approximately 50 km from Kuala Lumpur City Centre. Two designated sites were chosen in this area: Site A (8 ha) with 300 houses; and Site B (10 ha) with 400 houses. Both of the residential areas had rows of double storey terrace houses or clusters of 4 houses. The distance between 2 rows of houses was approximately 10 m. The front yard designed as a garden (approximately 18 m^2) was common in most of the houses. Artificial ponds and aquaria were observed in several houses at both sites at the front yard. Back yard space is limited, thus vegetation was hardly found. Discarded garbage was thrown at the back lane. Covered drums for water storage purpose were also found at the back lane. The local authority at Site B carried out adulticiding when dengue cases were reported during the study period. Bti treatment (500 g/ha) was conducted simultaneously for 7 mo from December 2007 – June 2008 in Site A. The Bti treatment was carried out at biweekly basis in the Bti-treatment phase. After 7 times of biweekly Bti treatments, adjustment was made from biweekly to weekly Bti-treatment for 7 Bti treatments. After that, biweekly Bti treatment was re-implemented for 4 times until the Bti-treatment phase was completed.

Ovitrap Surveillance (OS)

The gravid mosquito adult population was monitored by using ovitraps as described by Lee (1992). The ovitrap consisted of a 300 mL plastic container with straight, slightly tapered sides. The container exterior was coated with a layer of black oil paint. Each ovitrap was filled with approximately 200 mL seasoned tap water and a hardboard paddle (10.0 x 2.5 x 0.3 cm) was placed in the water with the rough surface upwards. The paddle served as an oviposition substrate for the female mosquitoes.

A total of 60 ovitraps were placed indoors and outdoors in 30 randomly selected houses in each site. OS was conducted twice a month, the first and third week of the month. All ovitraps were collected after five days and brought to the Medical Entomology Unit, Institute for Medical Research (IMR).

In the laboratory, the ovitrap contents including the paddles were transferred into respective plastic containers filled with seasoned fresh water. Sufficient quantity of water was then added to ensure the paddle was fully submerged. A piece of half-cooked beef liver was added into each container as larval food. The containers were covered to prevent wild mosquitoes from ovipositing and were left in the laboratory for 7 days. The hatched larvae were identified and counted on the 3rd day post collection. Only mosquito larvae of 3rd instar and beyond were picked and accounted for during the count. Larval counting was subsequently carried out every alternate day until day 7 post collection, thus providing sufficient time for all eggs to hatch. Only *Aedes aegypti* mosquitoes were kept for further analysis. The larvae were pooled and bred until F3 and evaluated for temephos and Bti susceptibility in accordance to three time frames intervals which corresponded to the pre-treatment of Bti (August – November 2007), treatment of Bti (December 2007 – June 2008) and post-treatment application of Bti (July – September 2008).

Larval Bioassays

Temephos

This test was conducted according to WHO (1981) larval susceptibility bioassay. Disposable paper cups of 300 mL capacity were used in the bioassays. Tests were run at 6 series concentrations of 3 replicates. Stock solution of 5000 mg/L temephos was prepared in ethanol and further diluted to 50 mg/L. One hundred mL of seasoned tap water was added into each test cups. The cups were left for 30 min after pipetting the required volume of insecticide into them. After that, twenty-five 3rd instar larvae were introduced into each cup and 150 mL water was further added to make up the final volume to 250 mL. Larval mortality was recorded after 24 hours post exposure. Control cups received only 1 mL ethanol. To determine temephos resistance of field larvae, the diagnostic dosage (DD) was used. According to WHO standard (WHO, 1981), the temephos DD for *Ae. aegypti* is 0.02 mg/L. The technical grade (94.5%) of temephos used in the study was provided by BASF Co., Malaysia.

Bacillus thuringiensis israelensis(*Bti*)

Bti susceptibility testing was conducted in accordance to an Institute for Medical Research (IMR) protocol [(IMR/IDRC/ENTO/SOP/01, 2002, unpublished)]. Each test consisted of 6 different concentrations of three replicates. A commercial formulation, VectoBac® WG (Lot 155-563-PG) manufactured by Valent BioSciences Corporation, USA was used. Stock solution of Bti was prepared in distilled water. Primary stock prepared was 100000 mg/L and a serial dilution was prepared to make up a final stock of 100 mg/L. Assays were conducted in disposable paper cups which held 200 mL test volume. Batches of 25 late 3rd instar larvae were introduced in 50 mL distilled water. Bti dilutions were prepared by dispensing the required volume from the working concentrations followed by addition of the remaining water volume to make the total volume of 150 mL. Larval

mortality was recorded after 24 hours post exposure. Control cups were not treated with Bti.

Bioassay of Bti and temephos against laboratory strain of *Aedes aegypti*

The lab strain of *Ae. aegypti* obtained from insectary of IMR, has been established for more than a thousand generation. This lab strain was used as a reference because it is not exposed to any insecticide since it was established. F1018 and F1020 generations were used for assays conducted on different months. The procedure of susceptibility testing of Bti and temephos against laboratory strain of *Ae. aegypti* was similar to the field strain *Ae. aegypti* susceptibility testing mentioned above. Each bioassay series involved 6 concentrations of 3 replicates with 25 larvae in each cup. A range of concentrations inducing mortality between 10 – 90% was used to determine the LC (lethal concentration) values.

Data Analysis

Mortality was recorded after 24 h exposure and subjected to probit analysis using the statistics software SPSS (version 11.5). Only concentrations causing between 10 – 90% mortality were included in the analysis. Based on the computed LC_{50} & LC_{90} values, the resistance ratios, RR_{50} & RR_{90} of temephos and Bti were determined. The resistance ratio (RR) was determined by comparison with the LC_{50}/LC_{90} values of field *Ae. aegypti* and the susceptible laboratory strain *Ae. aegypti*. Mean of the LC_{50} and LC_{90} values for laboratory strain obtained from the 2 generations mentioned above was calculated and used in the RR formulation.

Determination of RR for Bti:

$$RR_{50/90} = \frac{LC_{50/90} \text{ field } \textit{Ae. aegypti}}{LC_{50/90} \text{ laboratory strain } \textit{Ae. aegypti}}$$

Determination of RR for temephos:

$$RR_{50/90} = \frac{LC_{50/90} \text{ field } \textit{Ae. aegypti}}{LC_{50/90} \text{ laboratory strain } \textit{Ae. aegypti}}$$

Temephos resistance/susceptibility status of mosquito was evaluated according to WHO interpretation (WHO, 1998): larval mortality > 98% = temephos susceptible; mortality < 80% = temephos resistance; and mortality in the range of 80% - 98% defines possibility of resistance that needs further confirmation.

RESULTS

The Bti susceptibility of the field *Ae. aegypti* was determined over a year and categorized into three time frames: first 3 mo (August – November 07); subsequent 7 mo (December 07 – June 08); and final 3 mo (July – September 08). This time frame was used for the purpose of correlating the susceptibility trend to Bti after introduction of Bti treatments in site A.

Temephos susceptibility trend was also determined to the above mentioned time frames where the larvae were pooled within each time frame. This was done due to insufficient field population, because in Site A dengue vector population was very much reduced due to Bti treatments and also in Site B due to adulticiding conducted by health authorities for 2 mo (May – June 08) because of dengue outbreaks.

Bti susceptibility

Susceptibility of field *Ae. aegpti* larvae to Bti is shown in Table 1. Both generations of the lab strain *Ae. aegypti* from IMR were highly susceptible to Bti with mean LC_{50} of 0.04002 mg/L. Field larvae from Site A (Bti treated site) were susceptible to Bti at pre-treatment phase, with LC_{50} of 0.1226 mg/L (RR_{50} 3.1 folds) followed by 0.1156 mg/L with (RR_{50} 2.9 folds) at Bti-treatment phase. There was a slight decrease in susceptibility of field larvae in Site A with LC_{50} of 0.1814 mg/L (RR_{50} 4.5 folds) at post-treatment phase. In general, field larvae from Site A were susceptible to Bti throughout the three phases. From August – November 07, LC_{50} for Site B was 0.09221 mg/L (RR_{50} 2.3 folds). For the following months, from December 07 – June 08 and July – September 08, respectively, the LC_{50}

Table 1. Susceptibility of laboratory-bred and field *Ae. aegypti* to Bti

	LC_{50} (mg/L) (95% C.L.)	RR_{50}	LC_{90} (mg/L) (95% C.L.)	RR_{90}
Lab Strain				
F1018	0.04088 (0.03786–0.04622)	–	0.06274 (0.05333–0.08469)	–
F1020	0.03916 (0.03558–0.04247)	–	0.1150 (0.09987–0.1418)	–
Mean ± S.E.	0.04002 ± 0.00		0.08887 ± 0.03	
August – November 07				
Site A (Bti Pre-treatment Phase)	0.1226 (0.1142–0.1323)	3.1	0.2531 (0.2221–0.3008)	2.9
Site B	0.09221 (0.07774–0.1208)	2.3	0.1798 (0.1323–0.4251)	2.0
December 07 – June 08				
Site A (Bti Treatment Phase)	0.1156 (0.1042–0.1284)	2.9	0.2519 (0.2150–0.3134)	2.8
Site B	0.1329 (0.1008–0.1683)	3.3	0.3091 (0.2221–0.8361)	3.5
July – September 08				
Site A (Bti Post-treatment Phase)	0.1814 (0.1665–0.1965)	4.5	0.3527 (0.3060–0.3983)	4.0
Site B	0.08890 (0.08318–0.09512)	2.2	0.1946 (0.1732–0.2255)	2.2

was 0.1329 mg/L (RR_{50} 3.3 folds) and 0.08890 mg/L (RR_{50} 2.2 folds). Overall, field larvae from Site B were susceptible to Bti in the three time frames.

Temephos susceptibility

Both lab strains of *Ae. aegypti* from IMR were highly susceptible to temephos with mean LC_{50} of 0.005690 mg/L (Table 2). Susceptibility to temephos from August – November 07 was at LC_{50} of 0.007910 mg/L at Site A (RR_{50} 1.4 folds) followed by 0.02088 mg/L (RR_{50} of 3.7 folds) from December 07 – June 08 and LC_{50} of 0.03799 mg/L (RR_{50} 6.7 folds) from July – September 08. At Site B, LC_{50} was 0.007040 mg/L (RR_{50} 1.2 folds) from August – November 07, followed by 0.01022 mg/L (RR_{50} 1.8 folds) and 0.01485 mg/L (RR_{50} 2.6 folds), from December 07 – June 08 and July – September 08, respectively. Decreased susceptibility of field *Ae. aegypti* to temephos was observed in both sites. The field larvae showed variations in susceptibility when subjected to diagnostic dosage (Table 3). According to WHO interpretation (WHO, 1998), incipient resistance was observed in Site A (88%) from August – November 07 and resistance was accounted for the subsequent months

Table 2. Susceptibility of lab-bred and field *Ae. aegypti* to temephos

	LC_{50} (mg/L) (95% C.L.)	RR_{50}	LC_{90} (mg/L) (95% C.L.)	RR_{90}
Lab Strain				
F1018	0.006600 (0.006310–0.006890)	–	0.008130 (0.007730–0.008700)	–
F1020	0.004780 (0.003600–0.005380)	–	0.009040 (0.007920–0.01254)	–
Mean ± S.E	0.005690 ± 0.00		0.008585 ± 0.00	
August – November 07				
Site A	0.007910 (0.006110–0.009740)	1.4	0.02471 (0.01798–0.04591)	2.9
Site B	0.007040 (0.005650–0.008130)	1.2	0.01552 (0.01271–0.02309)	1.8
December 07 – June 08				
Site A	0.02088 (0.01930–0.02265)	3.7	0.03924 (0.03467–0.04633)	4.6
Site B	0.01022 (0.009130–0.01120)	1.8	0.01668 (0.01501–0.01932)	1.9
July – September 08				
Site A	0.03799 (0.02801–0.04792)	6.7	0.07058 (0.05404–0.15804)	8.2
Site B	0.01485 (0.01075–0.02016)	2.6	0.02791 (0.02043–0.1092)	3.3

Table 3. Resistance status of field *Ae. aegypti* to temephos diagnostic dosage (0.02 mg/L)

	% Mortality	
	Site A	Site B
August – November 07	88	100
December 07 – June 08	44	91
July – September 08	11	67
Control (IMR susceptible strain F 1018 & 1020)	100	

(44% and 11%). However, in Site B, the population was susceptible to temephos from August – November 07 (100%), and showed mortality levels indicating insipient resistance and resistant from December 07 – June 08 and July – September 08 respectively (91% and 67%). Complete mortality was observed for lab strain *Ae. aegypti*.

DISCUSSION

The development of insecticide resistance remains the main obstacle in the mosquito control programme. In this study, Bti treatment was intensively applied for 7 mo in Section 17, Shah Alam. Prior to this, Bti was never used in vector control in this area. According to the local authority, sand granule temephos was the only larvicide used in the area and distributed to the residents to be applied in their premises.

In general, field mosquitoes were highly susceptible to Bti throughout the three time frames. Application of Bti did not alter the Bti susceptibility of field *Ae. aegypti* larvae at the treatment and post-treatment phases as the RR_{50} remained at 2.9 – 4.5 folds in Bti treated site (Site A). The result was similar to the study in Alabama and Florida. Liu *et al*. (2004) indicated that *Culex quinquefasciatus* Say larvae were highly susceptible to Bti although the larvae had been extensively exposed to Bti. In Germany, after 10 years of Bti application in mosquito control programme by the German Mosquito Control Association (KABS) to suppress *Aedes vexans*, the population in the Bti treated area remained susceptible to Bti when compared to the untreated area (Becker and Ludwig, 1993). In 2008, Lee *et al.* conducted space spray application in a suburban residential area and in a temporary settlement site in the state of Selangor, Malaysia. The wide area spray application gave good significant reduction in both *Ae. aegypti* and *Aedes albopictus* populations. In Cambodia, Bti was applied into portable cement water jars holding rain or river or well waters. Water dispersible granule and tablet formulations of Bti were introduced into the jars and the efficacy and persistence in the treated jars were observed until 12 wk post-treatment in the treated jars. Large scale commune studies in Cambodia for 2 consecutive years showed that a single application of Bti in all 'in use' containers controlled the adult *Ae aegypti* population for 10 - 12 wk post treatment (Setha *et al*., 2007). In Columbia, Armengol *et al.* (2006) also showed that a tablet formulation of Bti had reduced pupae formation by more than 80% in the artificial container for 12 wk. In Nuevo León State, Mexico, the dengue control programme had applied VectoBac® 12AS, a Bti liquid formulation into the 200-gal metal water drums. Larval indices were reduced to zero in the 2-week treatment period (Ponce *et al*., 2002). In India, Sharma *et al*. (2008) had evaluated the persistence of Bti in a heavy breeding site of *Anopheles* sp. and *Culex* sp. by applying Bti into different habitats. Application of Bti at 0.5 ml/m^2 had significantly reduced the density of anophelines in clear water for 2 wk; and double the dosage at 1.0 ml/m^2 managed to control breeding of *Cx. quiquefasciatus* in polluted waters for 2 wk intervals.

When the field larvae were tested against temephos, the results indicated that some degree of resistance had developed in field strain *Ae. aegypti*. RR_{50} increased 1.8 folds in Site A, as well as in Site B where RR_{50} has increased to 1.4 folds from July – September 08 when compared to December 07 – June 08. Decreased susceptibility of field *Ae. aegypti* to temephos was observed in both Site A and Site B. This was evaluated by conducting bioassays at the temephos diagnostic dosage. The populations in Site A and Site B were subjected to temephos diagnostic dosage, 0.02 mg/L. Survival of any *Ae. aegypti* larvae from exposure to 0.02 mg/L would indicate the possibility of resistance among population tested. Results in Table 3 showed 88% mortality from August – November 07, 44% mortality from December 07 – June 08 and subsequently 11% mortality from July – September 08 in Site A, confirming the level of resistance

among populations of *Ae. aegypti*. Similar trend of susceptibility reduction was observed in Site B, with 100% mortality from August – November 07, 91% mortality from December 07 – June 08 and 67% mortality from July – September 08. Resistance appeared to exist in the population since pre-treatment in Site A. WHO (1998) has categorized 80 – 97% mortality in a susceptibility testing as the possibility of resistance that needs to be confirmed. The results in the diagnostic dosage tested in the subsequent phases have provided evidence of resistance development in Site A. Although temephos resistance was detected in both sites, however, susceptibility to Bti was not affected. In a cross-resistance study conducted by WHO (1979), temephos-selected *Cx. quinquefasciatus* larvae were susceptible to Bti, again indicating chemical resistant population remained susceptible to Bti.

Resistance of field mosquitoes in the study area was unknown. Inheritance of resistance could be the reason for the occurrence of chemical resistance (Coker, 1958). The possibility of long-term application of temephos in mosquito control contributing to chemical resistance was considered. In the first evaluation of temephos susceptibility to field strain *Ae. aegypti* collected from Kuala Lumpur in 1984, the results showed that while the field larvae exhibited tolerance to temephos, resistance was not detected (Lee *et al.*, 1984). The re-evaluation of the susceptibility of *Ae. aegypti* to temephos at various dosages was conducted by Lee & Lime (1989) who showed that field larvae from Jinjang, Kuala Lumpur, the location where first dengue outbreak occurred in 1973-74, had low levels of resistance to temephos. However, temephos was still effective under operational dosage of 1 mg/L. Similar finding was observed by Chen *et al.* (2005) who reported that although the field *Ae. aegypti* exhibited resistance to the diagnostic dosage (0.012 mg/L), complete mortality to operational dosage at 1 mg/L was observed. In the international realm, the first nationally integrated programme to monitor resistance status was the Brazilian Health Foundation (FUNASA) in 1999, where field-collected larvae of *Ae. aegypti* exhibited resistance to temephos in most of the municipalities in the state of Rio de Janeiro and Espírito Santo. These 2 states were intensively exposed to temephos, fenitrothion and malathion since 1986 in the Brazilian Dengue Control Programme (Lima *et al.*, 2003). Another study conducted in both the 2 states in 2001, indicated the populations had resistance to temephos compared to those of 2 years earlier (Braga *et al.*, 2004). Similar findings were obtained in the study conducted in the Caribbean area and in neighboring countries where temephos was used for *Ae. aegypti* control for more than 10 years in the regions (Georghiou *et al.*, 1987; Rawlins & Joseph, 1995). Temephos resistance also was reported in other Latin America countries. The highest resistance ratio (RR_{50}) was found in Havana City with 100 folds higher than susceptible strain, followed by Costa Rica (80 folds) and Jamaica (50 folds), (Rodríguez *et al.*, 2007). In Thailand, resistance to temephos was detected in high dengue endemic districts where temephos was applied as the larvicide in vector control programme (Jirakanjanakit *et al.*, 2007). *Aedes* mosquitoes collected in 5 subdistricts in Thailand exhibited different degrees of resistance to temephos (Ponlawat *et al.*, 2005). In Argentina, field larvae exhibited incipient resistance to temephos three years later with resistance ratio increased from 1.30 folds in the year 2004 to 3.10 folds in 2007 (Seccacini *et al.*, 2008).

In conclusion, the data obtained provided information on the susceptibility status of *Ae. aegypti* in Shah Alam. Temephos resistant was detected in Site A, while incipient resistant was detected in Site B. Microbial insecticides may be important for mosquito control especially in situations when local strains are resistant to other chemical insecticides. Therefore, it is important to alternate temephos with other larvicides, such as

Bacillus thuringiensis israelensis so as to maximize the outcome of dengue control programme.

Acknowledgements. The authors thank the Director-General of Health, Malaysia for permission to publish. They are also grateful to Dr. Nazni W.A. for helpful discussion. Assistance in the field rendered by the staff of Unit of Medical Entomology, Infectious Disease Research Centre, IMR is gratefully acknowledged. This project was supported by a grant from the National Institutes of Health, Ministry of Health (No. JPP-05-006). Works mentioned in this paper partially fulfilled the University of Malaya M.Sc. requirement of the first author.

REFERENCES

Ang, K.T. & Satwant, S. (2001). Epidemiology and new initiatives in the prevention and control of dengue in Malaysia. *Dengue Bulletin* **25**: 7 – 14.

Armengol, G., Hernandez, J., Velez, J.G. & Orduz, S. (2006). Long-lasting effects of a *Bacillus thuringiensis* Serovar *israelensis* experimental tablet formulation for *Aedes aegypti* (Diptera: Culicidae) control. *Journal* of *Economic Entomolgy* **99**: 1590–1595.

Becker, N. (2004). Microbial control of mosquito vectors. 1st Asean Congress of Parasitology and Tropical Medicine & 40th Annual Scientific Seminar of MSPTM. 23–25 March 2004. In: Symposium 10: *Bti/Bsp*, pg. 80.

Becker, N. & Ludwig, M. (1993). Investigation on possible resistance in *Aedes vexans* field populations after a 10-year application of *Bacillus thuringiensis israelensis. Journal of the American Mosquito Control Association* **9**: 221–224.

Braga, I.A., Lima, J.B.P., Soares, S.S. & Valle, D. (2004). *Aedes aaegypti* resistance to temephos during 2001 in several municipalities in the State of Rio de Janeiro, Sergipe, and Alagoas, Brazil. *Memórias do Instituto Oswaldo Cruz* **99:** 199–203.

Chen, C.D., Nazni, W.A., Lee, H.L. & Sofian-Azirun, M. (2005). Weekly variation on susceptibility status of *Aedes* mosquitoes against temephos in Selangor, Malaysia. *Tropical Biomedicine* **22**: 195–206.

Cheong, W.H. (1978). The present status of dengue fever/dengue haemorrhagic fever and its control in West Malaysia. *Asian Journal of Infectious Diseases* **2**: 136–138.

Coker, W.Z. (1958). The inheritance of DDT resistance in *Aedes aegypti*. *Annals of Tropical Medicine and Parasitology* **52**: 443–455.

Georghiou, G.P., Wirth, M., Tran, H., Saume, F. & Knudsen, A.B. (1987). Potential for organophosphate resistance in *Aedes aegypti* (Diptera:Culicidae) in the Caribbean area and neighboring countries. *Journal of Medical Entomology* **24**: 290-294.

IMR/IDRC/ENTO/SOP/01. (2002). Standard operating procedure. Laboratory evaluation of microbial agent. Unpublished technical report, 6 pages.

Jirakanjanakit, N., Saengtharatip, S., Rongnoparut, P., Duchon, S., Bellec, C. & Yoksan, S. (2007). Trend of temephos resistance in *Aedes (Stegomyia)* mosquitoes in Thainland during 2003-2005. *Environmental Entomology* **36**: 506–511.

Lacey, L.A. (2007). *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. *The American Mosquito Control Association Bulletin* No. 7. **23**: 133–163.

Lee, H.L. (1992). *Aedes* ovitrap and larval survey in several suburban communities in Selangor, Malaysia. *Mosquito-Borne Diseases Bulletin* **9**: 9–15.

Lee, H.L., Lee, T.W., Law, F.M. & Cheong, W.H. (1984). Preliminary studies on the susceptibility of field collected *Aedes (Stegomyia) aegypti* (Linneaus) to Abate® (temephos) in Kuala Lumpur. *Tropical Biomedicine* **1**: 37–40.

Lee, H.L. & Lime, W. (1989). A re-evaluation of the susceptibility of field-collected *Aedes (Stegomyia) aegypti* (Linnaeus) larvae to temephos in Malaysia. *Mosquito-Borne Diseases Bulletin* **6**: 91–95.

Lee, H.L., Chen, C.D., Masri, M.S., Chiang, Y.F., Chooi, K.M. & Benjamin, S. (2008). Impact of larviciding with a *Bacillus thuringiensis israelensis* formulation, VectoBac WG®, on the dengue mosquito vectors in a dengue endemic site in the state of Selangor, Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* **39**: 601–609.

Lima, J.B.P., Pereira Da Cunha, M., Silva-Jr, R.C.S., Galardo, A.K.R., Soares, S.S., Braga, I.A., Ramos, R.P. & Valle, D. (2003). Resistance of *Aedes aegypti* to organophosphates in several municipalities in the state of Rio de Janeiro and Espírito Santo, Brazil. *American Journal of Tropical Medicine and Hygiene* **68**: 329–333.

Liu, H., Cupp, E.W., Micher, K.M., Guo, A. & Liu, N. (2004). Insecticide resistance and cross-resistance in Alabama and Florida strains of *Culex quinquefasciatus*. *Journal of Medical Entomology* **41**: 408–413.

Ministry of Health, Malaysia. Director General of Health Malaysia. Kenyataan akhbar ketua pengarah kesihatan Malaysia. Penduduk leka-denggi menular. 30 January 2009. Available at: http://moh.gov.my/MohPortal/DownloadServlet?id=2453&type=2. 10 October 2009.

Ministry of Health, Malaysia. Director General of Health Malaysia. Kenyataan akhbar ketua pengarah kesihatan Malaysia. Situasi semasa demam denggi dan chikungunya di Malaysia. Bagi minggu epid 52/2009 (27 Dis. 2009 hingga 2 Jan. 2009). 5 January 2010. Available at: http://www.infosihat.gov.my/menuutama/Press_Denggi_Chiku/KPK_Minggu_52_2009.pdf. 11 Jan 2010.

Ponce, G.G., Flores, A.E., Badii, M.H., Rodríguez-Tovar, M.L. & Fernández-Salas, I. (2002). Laboratory evaluation of VectoBac® AS against *Aedes aegypti* in Monterrey, Nuevo León, Mexico. *Journal of the American Mosquito Control Association* **18**: 341–343.

Ponlawat, A., Scott, J.G. & Harrington, L.C. (2005). Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thainland. *Journal of Medical Entomology* **42**: 821–825.

Rawlins, S.C. & Joseph Ou, H.W. (1995). Resistance in some Caribbean populations of *Ae. aegypti* to several insecticides. *Journal of the American Mosquito Control Association* **11**: 59–65.

Rodríguez, M.M., Bisset, J.A. & Fernández, D. (2007). Levels of insecticide resistance and resistance mechanism in *Aedes aegypti* from some Latin American countries. *Journal of the American Mosquito Control Association* **23**: 420–429.

Salina, K. (2009). 14 dengue hot spots identified in Shah Alam. *The Star*, 3 February. Available at: www.starproperty.my/PropertyGuide/HomeProtection/115/0/0

Seccacini, E., Lucia, A., Zebra, E., Licastro, S. & Masuh, H. (2008). *Aedes aegypti* resistance to temephos in Argentina. *Journal of the American Mosquito Control Association* **24**: 608–609.

Setha, T., Chantha, N. & Socheat, D. (2007). Efficacy of *Bacillus thuringiensis israelensis*, VectoBac® WG and DT, formulations against dengue mosquito vectors in cement potable water jars in Cambodia. *Southeast Asian Journal of Tropical Medicine and Public Health* **38**: 261–268.

Sharma, S.K., Upadhyay, A.K., Haque, M.A., Raghavendra, K. & Dash, A.P. (2008). Field evaluation of previously untested strain of biolarvicide (*Bacillus thuringiensis israelensis* H14) for mosquito control in an urban area of Orissa, India. *Journal of the American Mosquito Control Association* **24**: 410–414.

WHO. (1979). Data sheet on the biological control agent, *Bacillus thuringiensis* serotype H-14 (de Barjac 1978). WHO/VBC/79.750 Rev. 1 VBC/BCDS/79.01.

WHO. (1980). Resistance of vectors of disease to pesticides. Fifth report of the WHO Expert Committee on Vector Biology and Control. WHO Technical Report Series 655.

WHO. (1981). Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC/81.807.

WHO. (1998). Test procedures for insecticide resistance monitoring in Malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. Report of the WHO informal consultation. 28-30 September 1998, WHO, Geneva, Switzerland. WHO/CDS/CPC/MAL/98.12.