IMPACT OF LARVICIDING WITH A *BACILLUS THURINGIENSIS ISRAELENSIS* FORMULATION, VECTOBAC WG®, ON DENGUE MOSQUITO VECTORS IN A DENGUE ENDEMIC SITE IN SELANGOR STATE, MALAYSIA

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Abstract. The field bioefficacy of a wettable granule (WG) formulation of *Bacillus thuringiensis israelensis* (Bti), VectoBac WG (Bti strain AM65-52) against dengue vectors, *Aedes aegypti* and *Ae albopictus*; was evaluated in a suburban residential area (TST) and in a temporary settlement site (KB) in the state of Selangor, Malaysia. Pre-control ovitrap surveillance of the trial sites indicated a high population of both types of *Aedes* mosquitoes. The populations were monitored continuously by weekly ovitrapping. Bti was sprayed biweekly at a dosage of 500 g/ha by using a mist-blower. The spray application was targeted into outdoor larval habitats. If required, Bti formulation was also applied directly into indoor water-holding containers at 8 g/1,000 l. Based on ovitrap surveillance, a significant reduction in *Aedes* populations was evident 4 weeks after initiating the first Bti treatment. The ovitrap index (OI) and the larvae density decreased drastically in both trial sites. In TST, the indoor OI was significantly reduced from 57.50±7.50% to 19.13±5.49% ($p<0.05$), while the outdoor OI decreased from 38.89±11.11% to 15.36±5.93%. In KB, similarly, the OI was significantly reduced by more than half, from 66.66±6.67% to 30.26±2.99% ($p< 0.05$). In all cases, the reduction in OI was paralleled by reduction in larval density.

INTRODUCTION

Dengue is the most serious mosquito-vector borne disease in Malaysia. Since the first nation-wide dengue outbreak in 1973, the incidence of this disease has increased many fold over the years and in 1996 the number of dengue cases exceeded 10,000 for the first time. For the year 2005, between 1 January and 9 April, 12,813 Malaysians were hospitalized with dengue fever or dengue hemorrhagic fever (DHF). The disease also claimed 41 lives (New Sunday Times, April 2005). During this period, the Selangor State recorded the highest number of dengue and DHF cases (5,028 cases with 17 deaths) followed by Kuala Lumpur, the capital city, with 2,269 cases and 9 deaths. Both *Aedes aegypti* and *Ae. albopictus* were incriminated as vectors of dengue.

The Vector Borne Disease Control Program (VBDCP) in Malaysia focuses on source reduction and adulticiding by fogging with chemical insecticides, such as malathion and pyrethroids, in affected areas. Fogging is conducted within a radius of 200 meters of suspected dengue cases, and repeat fogging is conducted within 7-10 days after the first fogging. The public is expected to larvicide with temephos (ABATE SG®) in water receptacles

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that are harboring *Aedes* immatures or in potential larval habitats.

Despite these measures, dengue continues to occur. This may be attributed to the fact that adulticiding is only conducted after the introduction of the virus in the human population; and the effect of adulticiding is transient without any residual activity. Thus, mosquito vector control conducted after the onset of dengue has not prevented dengue outbreaks. Effective mosquito vector control prevents the emergence of adult mosquitoes and subsequent introduction of dengue virus into the human population. To achieve these objectives, intensive larviciding is the most effective means of controlling and eliminating *Aedes* vectors. Temephos sand granules have been used to reduce *Aedes* breeding in containers since 1974. Recent studies have, however, indicated the presence of *Aedes* larval resistance to temephos (Chen *et al*, 2005). An alternative larvicide that is as safe and effective as temephos is *Bacillus thuringiensis israelensis* (*Bti*) which is a microbial agent known to be highly and specifically effective against mosquito larvae and devoid of undesirable side-effects. The present study was undertaken to determine the impact of larviciding with a Bti formulation VectoBac WG (Bti strain AM65-52) on the adult mosquito vector population in a dengue endemic site in Selangor State, Malaysia. VectoBac WG has been successfully evaluated by the WHO Pesticide Evaluation Scheme (WHOPES) for mosquito larviciding (WHO, 2007).

MATERIALS AND METHODS

Study Site

The Selangor State Vector Borne Disease Control Program presented a list of 55 localities and a weekly report of dengue/DHF cases in 2004. The locality, Taman Samudera (TS), was chosen as the study site based on the criterion that it had weekly reported cases in the first 22 weeks of the year, with a total of 23 serologically confirmed dengue cases.

Taman Samudera (Fig 1) is a suburban residential area located 16 km southeast of Kuala Lumpur. It is divided into TS Utara (North), TS Selatan (South), TS Timur (East) and TS Barat (West), covering an area of about 100 ha. TS Utara (TSU), TS Selatan (TSS) and TS Timur (TST) have double storey residential terrace houses; and TS Barat (TSB) is a commercial block with factories and shops. TSS and TST harbored 84% of the reported dengue/DHF cases. Thus, ovitraps were placed to collate baseline data regarding mosquito vectors in TSS and TST. The ovitraps were placed in 10% of the total number of residential houses at each site, *ie* 20 houses each in TSS and TST. Ovitraps were placed indoors and outdoors at the selected houses.

A temporary settlement area of 4 ha, adjacent to TST was also selected for the trial. The temporary settlement area, Kg. Banjar (KB), is at one of the entry points to TST. This settlement area is not indicated on the local map. It has about 200 wooden or wooden-cement houses, haphazardly constructed on the site. Discarded garbage was strewn throughout the site; the area had poor drainage. Vegetation was observed, and thirty ovitraps were placed at this site.

The owners' consent was obtained to place ovitraps in their residences. For all surveillance, including pre- and post-treatment surveillance, the ovitraps were placed in the same houses, unless the residents were not in the house at the time of placing the ovitraps. The ovitraps were then placed in neighboring houses.

Ovitrap surveillance

The ovitrap used in this study has been described by Lee (1992). It is a 300 ml plastic container with straight, slightly tapered sides. The opening measures 7.8 cm in diameter, the base diameter is 6.5 cm and the container is

Note:

Within the solid boundary is the VectoBac WG treated site, Taman Samudera Timur and Majlis Perbandaran Selayang (Kg. Banjar)

Within the dashed boundary is the untreated site, Taman Samudera Selatan

(Source: RIMMAN Street Directory–Kuala Lumpur and Klang Valley. Published by RIMMAN International Sdn Bhd and UBD, 2004, Map 131)

Fig 1–Street map of the study site : Taman Samudera, Selangor.

9.0 cm in height. The outer wall of the container is coated with a layer of black glossy oil paint. An oviposition paddle (measuring 10 cm x 2.5 cm x 0.3 cm) made from dark brown colored hardboard was placed in each ovitrap container. Tap water was added to a level of 5.5 cm in each container. After each surveillance, the ovitrap containers and paddles were washed with hot water to destroy any remaining unhatched eggs. At each site, 6 pretreatment ovitrap surveillance studies were conducted from August to September 2004; followed by 6 concurrent ovitrap surveillance studies during VectoBac WG treatment (November 2004- February 2005) and 2 post-treatment surveys were carried out (February to March 2005).

The ovitraps were placed indoors and outdoors in randomly selected houses at each study site. In this study, "indoors" refers to the interior of the house within its 4 walls, while "outdoors" refers to outside the house but confined to the immediate vicinity of the house. All ovitraps were collected 5 days after placement and were replaced with fresh ovitraps with paddles. Ovitraps that were lost/damaged were replaced at each visit.

Identification of larvae

The collected ovitraps were brought back to the laboratory and the contents (including the paddle) were transferred into plastic containers filled with fresh tap water. The ovitrap contents were kept for 9 days, providing sufficient time for all eggs to hatch. A small piece of fresh beef liver was introduced as larval food. The hatched larvae were subsequently counted and identified at the 3rd instar stage.

Data analysis

The total number of ovitraps with *Aedes* sp larvae (positive ovitrap) and total number of *Aedes* sp larvae found in all collected ovitraps with each survey were recorded. The ovitrap index and larval density were determined where: Ovitrap Index (OI) is the total number of positive ovitraps / total number of collected ovitraps x 100; Larval Density (LD) is the total number of larvae / total number of collected ovitraps. All data were statistically analyzed using a *t*-test at a significance level of $p = 0.05$.

VectoBac WG treatments

Bti treatment was targeted to all identified potential and actual outdoor larval habitats, via direct or spray application, providing complete coverage of all larval habitats including vegetation, such as leaf axils, tree holes, bushes, etc. For direct application, VectoBac WG (8 g/1,000l) was introduced into all water holding containers (≥50 liters in volume). The containers were treated only once during this study period.

For spray application, VectoBac WG (500 g/ha) was mixed onsite into the required amount of water and was discharged with a motorized backpack mist blower (Stihl SR420). The flow rate of the nozzle and the swath of the spray were pre-calibrated. A pre-treatment exercise was conducted to determine the required amount of water to disperse the Bti into all larval habitats. In KB, the temporary settlement area was strewn with litter and vegetation, the entire site of 4 ha was spray treated. A factory dealing with automobile batteries adjacent to KB was not treated as we did not have access to the premises. In TST the principal outdoor larval habitat was the concrete perimeter drains, running through each lane in the study site and the sparse vegetation. The concrete drains holding stagnant clear water and the vegetation (2 ha in size in an area of 14 ha) were spray treated. The spray treatments in KB and TST were conducted every 2 weeks, beginning 24 November, 2004, with the last spray on 2 February, 2005. Spraying was conducted between 07:30-10:30 by a 4-man team.

Taman Samudra Selatan (TSS), which is comprised of two storey houses similar to TST, is separated from the treated area by a two lane road, was not sprayed, but was used as an untreated control.

RESULTS

The efficacy of VectoBac WG treatment evaluated by ovitrap index and larval density is described in Table 1. The dengue vector population changes are shown in Fig 2. The pre-treatment ovitrap surveillance (25 August to 22 September 2004) found large *Ae. aegypti* and *Ae. albopictus* populations in both TST and KB, with *Ae. aegypti* predominating. In TST, indoor and outdoor surveillance found mean ovitrap indexes (OI) for both *Ae. aegypti* and *Ae. albopictus* of 36.45 ± 4.14% and 43.05 ± 6.16%, respectively (Tables 1a and 1b), showing the presence of large *Aedes* populations in this area. In KB, the mean OI was 52.96 ± 5.04% (Table 1c). The predominance of *Ae. aegypti* was confirmed by larval density. In TST, the indoor *Ae. aegypti* larval density was 9.59 ± 2.67 and 0.19 ± 0.14 for *Ae. albopictus*. A similar result was found outdoors (8.89 ± 2.01 *vs* 1.77 ± 0.62). In KB, *Ae. aegypti* predominant (8.88 ± 2.17 larvae per ovitrap *vs* 3.08 ± 0.67 for *Ae. albopictus*) (Table 1).

VectoBac WG treatments were not immediately implemented after pre-treatment surveillance due to a logistical problem. The treatments were initiated on 24 November 2004, 2 months after the pre-treatment surveillance, thus the actual *Aedes* sp mosquito density at the study site on initiation of Bti treatment was not known. Ovitrap surveillance conducted with the first 2 Bti treatments (24 November and 8 December) indicated the *Aedes* sp mosquito density then was greater than the

Table 1
Impact of larviciding with *Bacillus thuringiensis israelensis* formulation (VectoBac WG) on *Aedes aegypti* and *Aedes albopictus* populations. Mean ovitrap index and larval density per surveillance ± SE.

Table 1a : Taman Samudra Timur (TST) Indoors

Study period	Ovitrap surveillance	Ovitrap index (%)	Larval density	
			Ae. aegypti	*Ae. albopictus*
Pre-treatment (25 Aug - 22 Sep 2004)	*n* = 6	36.45±4.14 [a]	9.59±2.67 [b]	0.19±0.14 [b]
Bti treatments (24 Nov - 17 Dec 2004)	*n* = 2	57.50±7.50 [a]	26.97±1.47 [a]	0.30±0.30 [b]
Bti treatments (27 Dec - 14 Feb 2005)	*n* = 4	19.13±5.49 [a]	4.27±2.43 [ab]	0 [b]

[a] : significantly different at p = 0.05; [b] : not significantly different at p>0.05

Table 1b. Taman Samudra Timur (TST), Outdoors

Study period	Ovitrap surveillance	Ovitrap index (%)	Larval density	
			Ae. aegypti	*Ae. albopictus*
Pre-treatment (25 Aug - 22 Sep 2004)	*n* = 6	43.05±6.16 [ab]	8.89±2.01 [b]	1.77±0.62 [b]
Bti treatments (24 Nov - 17 Dec 2004)	*n* = 2	38.89±11.11 [b]	8.90±4.30 [b]	2.14±0.64[ab]
Bti treatments (27 Dec - 14 Feb 2005)	*n* = 4	15.36±5.93 [ab]	3.19±1.22 [b]	0.22±0.21[ab]

[a] : significantly different at p = 0.05; [b] : not significantly different at p>0.05

Table 1c. Kampong Banjar (KB)

Study period	Ovitrap surveillance	Ovitrap index (%)	Larval density	
			Ae. aegypti	*Ae. albopictus*
Pre-treatment (25 Aug - 22 Sep 2004)	*n* = 6	52.96±5.04 [ab]	8.88±2.17 [ab]	3.08±0.67 [ab]
Bti treatments (24 Nov - 17 Dec 2004)	*n* = 2	66.66±6.67 [ab]	26.46±3.34 [a]	2.85±1.88[b]
Bti treatments (27 Dec - 14 Feb 2005)	*n* = 4	30.26±2.99 [a]	5.94±1.53 [ab]	0.44±0.44 [ab]

[a] : significantly different at p = 0.05; [b] : not significantly different at p>0.05

density at the time of the pre-treatment surveillance. The efficacy of Bti treatment was measured in relation to the ovitrap surveillance data collected concurrently with the first 2 Bti treatments.

The OI and larval density decreased dramatically at both trial sites 4 weeks after initiating Bti treatment. In TST, the indoor OI was significantly lower (57.50 ± 7.50% to 19.13 ± 5.49%) ($p<0.05$), while the outdoor OI also decreased (38.89 ± 11.11% to 15.36 ± 5.93%) (Tables 1a and 1b). In KB, the OI decreased significantly (66.66 ± 6.67% to 30.26 ± 2.99%) ($p< 0.05$). In all cases, the reduction in OI was paralleled by a reduction in both *Ae. aegypti* and *Ae. albopictus* larval density. At both treated sites, *Ae albopictus* was well suppressed (Fig 2).

The untreated control, TSS, initially showed a large *Aedes* population both indoors and outdoors (Fig 3). The ovitrap index reached a maximum of 70.59% indoors and 63.16% outdoors. However, on the 6th ovitrapping, the local health staff conducted extensive space spraying of chemical adulticide for 5 weeks due to an outbreak of dengue in the area. The *Aedes* sp population then declined.

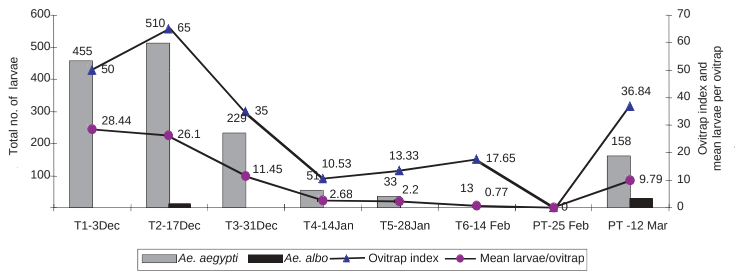

Fig 2a: Taman Samudera Timur (TST) Indoors.

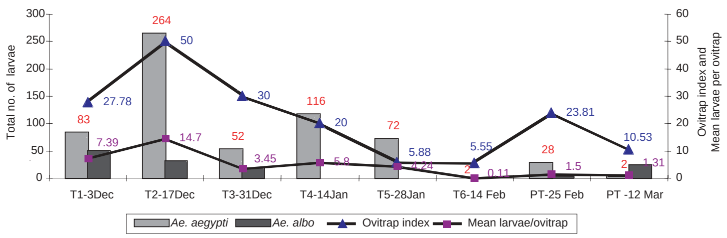

Fig 2b: Taman Samudera Timur (TST) Outdoors.

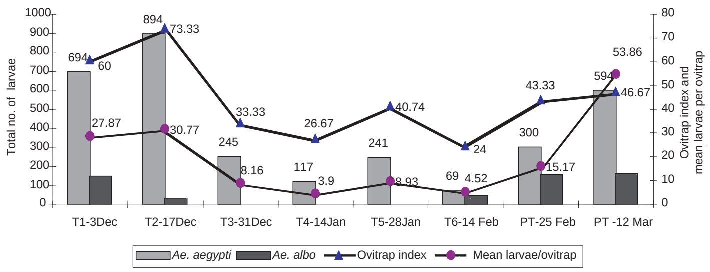

Fig 2c: Kampong Banjar (KB).

Fig 2–Trend of *Aedes aegypti* and *Aedes albopictus* populations treated with VectoBac WG as measured with ovitrap surveillance conducted on alternate weeks with treatment (T) and post-treatment (PT).

DISCUSSION

In this study, Bti treatment was applied to all identified potential and actual outdoor larval habitats via direct or spray application to provide a complete coverage of all larval habitats. In TST, the urban residential area, there were fewer water containers, and in Kg. Banjar, a temporary settlement area, had few water holding containers since this area had piped water. Thus, the larvicide treatment was focused on an area-wide cold spray application of the VectoBac WG formulation of Bti, which successfully reduced the field mosquito adult populations of *Ae. aegypti* and *Ae. albopictus*. This indicates it is possible to control *Aedes* vectors by larviciding with a biocide.

The larviciding technique used in this study differed from the conventional method

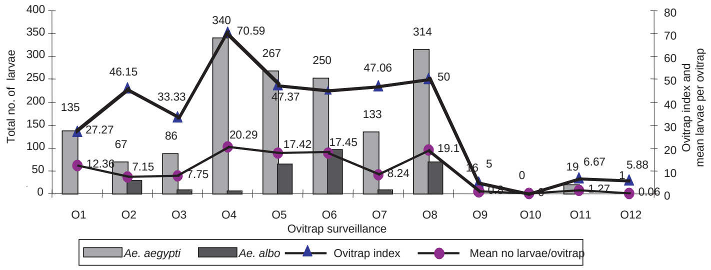

Fig 3a. Indoor larval population of *Aedes aegypti* and *Aedes albopictus* at the untreated site, Taman Samudra Selatan.

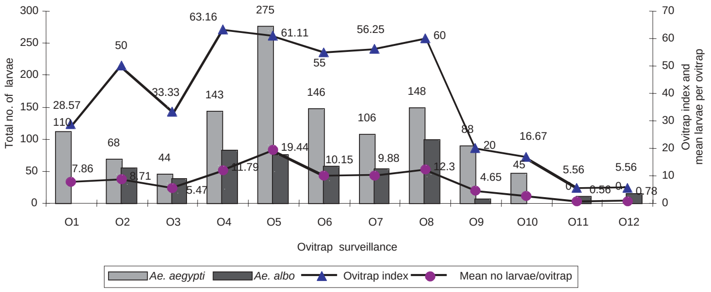

Fig 3b. Outdoor larval population of *Aedes aegypti* and *Aedes albopictus* at the untreated site, Taman Samudra Selatan.

Fig 3–Larval population of *Aedes aegypti* and *Aedes albopictus* at the untreated site, Taman Samudra Selatan.

of larvicide application in that mass application of Bti was through the use of a motorized back pack sprayer, Stihl SR 420, which has an engine displacement of 56.5 cc, a power output of 3.5 hp, and a standard mist blower nozzle that is able to deliver the Bti spray mixture to target larval habitats (horizontal distance of 12- 30 m; vertical height of 11.5 m). The covered concrete perimeter drains in TST and the dense vegetation in KB were uniformly covered with Stihl SR 420. This may account for the effectiveness of Bti, since application of Bti could ensure complete coverage of all larval habitats, actual and potential. Conventional manually applied larvicide could not accomplish this due to the nature of the application method and equipment. Therefore, wide area spraying of Bti with the appropriate equipment is recommended for routine use in dengue control programs.

It is essential to treat all larval habitats within the flight range of *Aedes* sp mosquitoes at the targeted treatment site. The OI of KB was significantly reduced from an average of 67% to an average of 31%, but a further reduction could have been achieved if the factory dealing with automobile batteries adjacent to this site had been treated. The OI threshold for a possible dengue outbreak has been calculated to be about 30% (Kamilan, 2001).

The concrete perimeter drains with stagnant clear water are a principal larval habitat for *Aedes* sp mosquitoes. The water from TST drains was tested for oviposition in the insectarium and was found to support the colonization of *Ae. aegypti* from eggs to adulthood. In a no choice test, there was no significant difference in the numbers of eggs, larvae, pupae and adults colonized from drain water and seasoned tap water ($p > 0.05$), indicating that *Ae. aegypti* can oviposit their eggs on a substrate which is readily available. In a choice test, the number of eggs laid by *Ae. aegypti* in drain water (1,630.67 ± 204.26) was significantly more than that in seasoned tap water (221.33 ± 53.18) ($p < 0.05$). The number of eggs was 6 fold higher in drain water compared to seasoned tap water. Furthermore, the oviposition activity index (OAI) was 0.71, indicating that the drain water was more attractive compared to the seasoned tap water as an oviposition substrate. This study also showed the pH and BOD values for both drain water and seasoned tap water were not significantly different from each other ($p > 0.05$), indicating that water from the drain did not contain organic content, *ie* the water was clean and clear. Significant water conductivity

($p < 0.05$) and the presence of bacteria could have contributed to the site selection for egg laying for *Ae. aegypti*. The drain water successfully supported the colonization of immature mosquitoes, with the emergence of 824.33 ± 13.96 adult mosquitoes. The ratio of male to female mosquitoes was 1:1. This study concluded that a concrete drainage system with clear stagnant water provides a suitable habitat for breeding of the dengue vector, *Ae. aegypti* (Chen *et al*, personal communication). In this study, the entire perimeter drain was well treated with the Bti spray mixture.

It was observed the *Aedes* population did not decrease during the first 2 treatments of 4 weeks. The population declined abruptly only after the second treatment primarily due to the presence of adults which continued to survive the earlier treatments as Bti which is a larvicide. After 2 treatments (4 weeks), existing adults would have died and without replacement from the larvae which were eliminated by Bti treatments, the *Aedes* population began to decline until almost being decimated.

The application frequency of every 2 weeks was effective. Similar results were also observed in trials conducted at Brunei Shell Petroleum (BSP). BSP conducted Bti spraying once every 2 weeks being applied with a Stihl SR 380 into dense vegetation harboring *Ae. albopictus* larvae. The target *Ae. albopictus* adult population was significantly reduced from an ovitrap index of 47.13 ± 7.48 to 15.4 ± 3.36 ($p< 0.05$) (Brunei Shell Petroleum, 2004). Hence, with actual use of a Bti formulation biweekly, optimal dengue vector control may be achieved.

The original intention of using TSS as a control (untreated) site did not materialize due to a dengue outbreak and the subsequent adulticiding activities of the health staff in TSS. Nevertheless, even in the absence of a useful untreated site, the impact of Bti on the *Aedes* population was still evidenced by the increase in the natural population after Bti application was stopped, indicating that the suppression of the *Aedes* population was due to Bti and not the natural fluctuation of the wild mosquito population.

Larviciding is an important component in dengue control due to the presence of transovarial dengue virus in larvae of Malaysian *Aedes* mosquitoes (Lee *et al*, 1997; Rohani *et al*, 1997). Infected larvae were shown to be the reservoir host of the dengue virus during inter-epidemic periods. More recently, Lee and Rohani (2005) found the early presence of infected larvae is related to subsequent dengue outbreaks.

The application of larvicides for dengue control is still following the traditional mode of direct application via traditional sprayers. Larvicides, such as temephos and anti-malaria oil are applied using a knapsack sprayer directly into larva breeding areas. Such a method, though effective, is slow, time-consuming, labor-intensive and at times provides poor coverage. Space spraying of a larvicide such as the Bti used in this study was found to be superior to traditional methods of application in terms of effectiveness, coverage and labor.

Another important observation was application of Bti did not inflict any undesirable effects on non-target organisms, such as fish, poultry, pet birds, which were present, especially in the surroundings of KB. All animal feed containers holding water were treated with Bti. No reports of death of these organisms during Bti treatment came to us during or after the trial period. This reiterates the environmental biosafety of Bti which has been reported by many workers from different parts of the world.

In conclusion, a wide application of VectoBac WG at target larval habitats is able to provide control of dengue mosquito vectors.

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REFERENCES

Brunei Shell Petroleum. Getting rid of a nuisance the biological way. Shell Health Digest, 2004.

Chen CD, Nazni WA Lee HL, Sofian Azirun M. Susceptibility of *Aedes aegypti* and *Aedes albopictus* to temephos in four study sites in Kuala Lumpur City Centre and Selangor State, Malaysia. *Trop Biomed* 2005; 22: 207-16.

Kamilan D. Preliminary study on the use of ovitrap and sequential sampling techniques for the surveillance of dengue vectors in Terengganu state. *Vector J* 2001; 7: 53-8.

Lee HL. *Aedes* ovitrap and larval survey in several suburban communities in Selangor, Malaysia. *Mosq Borne Dis Bull* 1992; 9: 1-7.

Lee HL, Mustafakamal Ibrahim, Rohani A. Does transovarian transmission of dengue virus occur in Malaysian *Aedes aegypti* and *Aedes albopictus* ? *Southeast Asian J Trop Med Public Health* 1997; 28: 230-2.

Lee HL, Rohani A. Transovarian transmission of dengue virus in *Aedes* vectors in relation to dengue outbreak in an urban area in Malaysia. *Dengue Bull* 2005; 29: 1-6.

Rohani A, Lee HL, Asmaliza SI, Zainah S. Detection of dengue virus from field *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) adults and larvae. *Southeast Asian J Trop Med Public Health* 1997; 28: 138-42.

WHO. WHO specifications and evaluations for public health pesticides. *Bacillus thuringgiensis* subspecies *israelensis* strain AM65-52. [Cited 2007 Dec 10]. Available at: URL: http://www.who.int/whopes/quality/Bti_eval_spec_Jun_07.pdf