Effect of chemical fogging on immature *Aedes* mosquitoes in natural field conditions

K B Chua, I L Chua, I E Chua, K H Chua

ABSTRACT

Introduction: Dengue and dengue haemorrhagic fever are common and serious arboviral diseases endemic in a number of countries situated in both the tropical and subtropical belts.

Methods: A prospective study was carried out to examine the environmental factors influencing the ovipositing behaviour of gravid female *Aedes* mosquitoes in a typical urbanised residential environment in Malaysia. This study reports the effect of the usual ultra-low volume fogging of insecticides carried out by public health officers on the collection of immature *Aedes* mosquitoes using ovitraps.

Results: Throughout the study, no dead immature *Aedes* mosquitoes was noted in any of the ovitraps set up in all of the fogging and immediate post-fogging periods. The mean number of days of ovitrapping for immediate pre-fogging, fogging and post-fogging periods were 10.3, 10.1 and 10.4 days, respectively. There was no statistically significant difference in the mean duration of ovitrapping cycle among the immediate pre-fogging, fogging and immediate post-fogging periods. The total number of immature *Aedes* mosquitoes collected in the immediate post-fogging periods was more than the immediate pre-fogging periods, and both were more than the fogging periods. However, there was no statistically significant difference in the total number of immature *Aedes* mosquitoes collected at various periods. It was not unusual to find dead insects, spiders and even small animals collected in ovitraps or environment in the fogged locality within 48 hours of chemical fogging.

Conclusion: In this study, the usual chemical fogging in natural environment was ineffective in breaking the reproductive lifecycle by eliminating gravid female *Aedes* mosquitoes.

Keywords: *Aedes* mosquitoes, chemical fogging, dengue fever, mosquitoes, ovitraps

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INTRODUCTION

Dengue fever (DF) and dengue haemorrhagic fever (DHF) are serious diseases that are endemic in a number of countries situated in both the tropical and subtropical belts. Globally, it is the most common and widespread anthropod-borne arboviral infection today. The geographical spread, incidence and severity of DF and DHF are increasing in the Americas, Southeast Asia, the Eastern Mediterranean, and the Western Pacific. Some 2.5 to 3 billion people live in areas where dengue viruses can be transmitted. It is estimated that each year, there are about 20 million infections with 240,000 cases of DHF and at least 12,000 to 15,000 deaths(1). The incidence rate of clinically-diagnosed DF and DHF reported in Malaysia is showing an upward trend, from 8.5 cases per 100,000 population in 1988 to 123.4 cases per 100,000 population in 1998. There are more cases of DF than DHF with a ratio of 16-25:1 over the last five years. The case fatality rate for DHF remains high, ranging from 5% to 6% per annum for both children and adults(2).

Dengue fever was reported in 1902 in Penang and has become a major public health problem in Malaysia, particularly with the appearance of the first DHF outbreak, also in Penang, in 1962(3). Both diseases are caused by any of the four serotypes of dengue viruses which are transmitted by mosquitoes, *Aedes aegypti* and *Aedes albopictus*, easily found in Malaysia. These mosquitoes breed in artificial and natural containers in and around houses and in construction sites. Rapid industrial and economic development over the last two decades have brought about massive infrastructure changes and created conducive man-made environments for breeding of *Aedes* mosquitoes. These were reflected by the finding of most cases of both DF and DHF among the urban population (70-80%) with the highest incidence in the working and school-going age groups, which correlate with the relatively high *Aedes* index in construction sites, factories and schools(4-6).

To date, there is still no effective vaccine available to control the occurrence and periodic

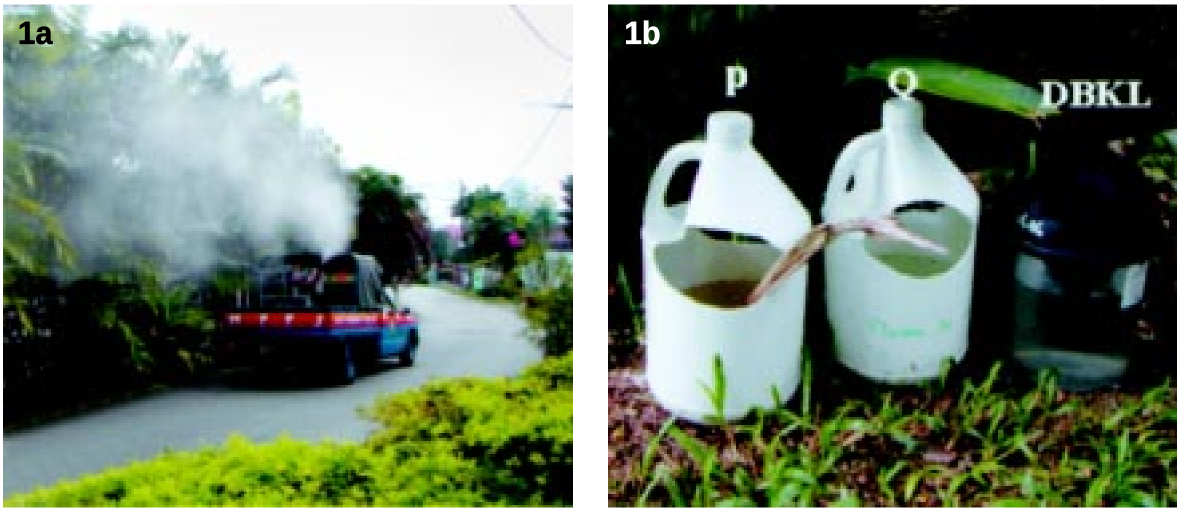

Fig. 1 Composite photograph shows (a) the process of ultra-low volume fogging of insecticide using vehicle-mounted equipment, and (b) types of ovitraps used in ovitrapping to study possible effects of chemical fogging in an urban residential environment.

recurrent outbreaks of DF and DHF(7). Thus, to reduce the incidence of the disease and control of periodic outbreaks, the authorities and public still depend very much on the conventional measures of dengue vector controls, such as public health education and public involvement, environmental management, biological and chemical control of both the adult and immature *Aedes* mosquitoes. Chemicals (insecticides) have been used to control *Aedes aegypti* since the turn of century. Current methods for applying insecticides (e.g. organophosphate group, pyrethroid group, temephos) include larvicide applications, perifocal treatment and space spraying(7). The effectiveness of these insecticides have been well-documented in studies carried out in the laboratories and under controlled field conditions(8-12). This study reports the effect of ultra-low volume fogging of chemical insecticides on the immature *Aedes* mosquitoes in a typical urban residential environment in natural field conditions.

METHODS

This is an on-going project to study the oviposit behavior of *Aedes* mosquitoes (*A. aegypti* and *A. albopictus*) in natural field conditions starting on March 1, 2002. The study was carried out in an established residential housing estate in the city of Petaling Jaya, peninsular Malaysia (GPS co-ordinate: 03^{0} 05.649N, 101^{0} 37.045E; elevation: 62M). The ovitrapping cycles in which fogging took place was taken as the fogging periods. The cycles of ovitrapping prior to and after each fogging period were taken as immediate pre-fogging periods and post-fogging periods, respectively.

Conventional space spraying, or commonly called ultra-low volume (ULV) fogging, was adopted to deliver the required chemical insecticides into the environment in the studied area. Field workers employed by the Majlis Perbandaran Petaling Jaya (MPPJ, Petaling Jaya City Council) were responsible to carry out the job. Vehicle-mounted generators and equipment (Fig. 1a) were used to aerosolise and deliver the chemical insecticides. The insecticides used for fogging throughout the study period was Aqua-Resigen (S-bioallethrin 0.14% w/w, permethrin 10.11% w/w, piperonyl butoxide 9.96% w/w, inactive base 79.79% w/w) and was delivered into the field at a concentration of 1 in 10 dilution in accordance with the recommendation of the Vector Control Unit, Vector-borne Disease Section, Ministry of Health.

Three permanent sites within the garden were chosen for setting up the ovitraps. Each site contained three types of ovitraps: (i) a preliminary identified most-favoured type of ovitrap (Q); (ii) a similar ovitrap as the former but contains a leaflet of dried *Ptychosterma macathurii* palm leaves (P); and (iii) an ovitrap introduced by Kuala Lumpur Municipal Council (DBKL) (Fig. 1b). The clean ovitraps were each filled with 1.5 litres of clear rainwater and placed in shaded areas within a house garden at various distances from the road. Sites A, B, and C were located 4, 7 and 15 metres, respectively, from the edge of the road in front of the house.

Examination of ovitraps for the presence of mosquito larvae was carried out daily. The end-point for harvesting the immature mosquitoes (larvae and pupae) from all ovitraps was taken whenever an early mosquito pupa was noted in any of the containers. The larvae in each trapping container were respectively transferred into a smaller transparent plastic container fitted with a perforated screw-cap for ventilation and enumerated accordingly. The larvae in the holding transparent containers were fed with fish pellet (Azoo Co, Malaysia) until mature adult mosquitoes emerged. Preliminary identification for the species of the collected larvae was made

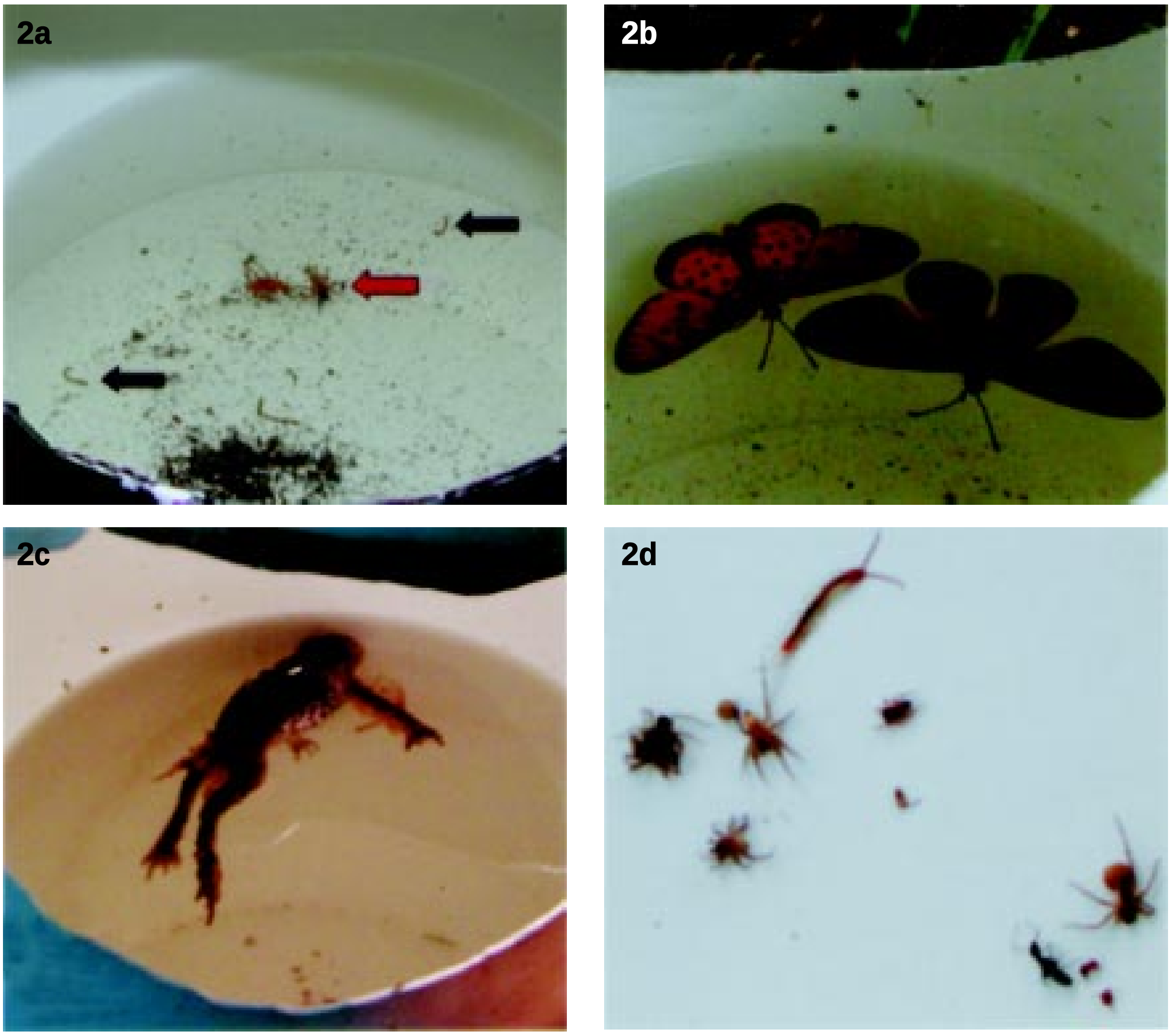

Fig. 2 (a) Photograph shows a dead spider and an unidentified insect (red arrow). Black arrows indicate live *Aedes* mosquito larvae. Photographs show (b) a pair of dead butterflies and (c) a dead frog collected in the ovitraps within 48 hours post-fogging. (d) Photograph shows dead ants, spiders and a baby centipede found in the garden within 48 hours post-fogging.

based on the morphological characteristics of the larvae. Final identification for the species of mosquitoes collected was based on morphological characteristics of the adult mosquitoes. Though both *A. aegypti* and *A. albopictus* were collected in this study, no attempt was made to count them separately. They were all counted together as *Aedes* species.

After each cycle of ovitrapping, the containers were thoroughly washed and scrubbed with a mild detergent, especially the inner surface, to ensure that no residual eggs which adhered to the surface, were carried forward to the next cycle of study. The containers were then rinsed with clean rain or tap water, and subsequently refilled with rainwater before being placed back in the respective locations but at a different orientation to reduce possible bias due to relative position of the containers with each other. In each cycle, the number of immature mosquitoes collected from three different types of containers in each location were pooled together and enumerated as the total of immature mosquitoes collected for that specific location during analysis.

The derived data was tabulated in appropriate worksheets using the Microsoft Excel program and evaluated by the paired t-test (Wilcoxon signed ranks test) using the Statistical Package for Social Sciences (SPSS) (Chicago, IL, USA) and Epi Info 6 (Center for Disease Control and Prevention, Atlanta, USA) free computer programme for any statistical significant association. A p-value of 0.05 or less was taken as the level of significant association for each ordinal variable with the relevant adjusting variables. In the analysis, the immature mosquitoes included larvae of all stages of development and pupae.

RESULTS

This component of the study extended from March 1, 2002 to August 31, 2004. During this whole study period, seven ULV foggings of chemical insecticides were carried out by field workers of the MPPJ in the studied area. The date and time of each

Table I. Number of days taken for the first *Aedes* pupa to appear in any of the ovitraps starting from the day of setting up the ovitraps in relationship to chemical fogging carried out in natural field conditions.

Date (time)	Immediate pre-fogging period			Fogging period			Immediate post-fogging period		
of fogging	Date Set	Date collect	Duration	Date set	Date collect	Duration	Date set	Date collect	Duration
27/10/2002 (1830hr)	12/10/02	23/10/02	**11**	27/10/02	7/11/02	**10**	9/11/02	19/11/02	**10**
30/12/2002 (1945hr)	14/12/02	22/12/02	**8**	23/12/02	1/01/03	**9**	2/01/03	11/01/03	**9**
25/01/2003 (1855hr)	12/01/03	22/01/03	**10**	25/01/03	4/02/03	**10**	8/02/03	19/02/03	**11**
26/03/2003 (1850hr)	5/03/03	16/03/03	**11**	17/03/03	29/03/03	**12**	2/04/03	11/04/03	**9**
20/08/2003 (1845hr)	4/08/03	15/08/03	**11**	17/08/03	28/08/03	**11**	29/08/03	10/09/03	**12**
22/04/2004 (0800hr)	3/04/04	13/04/04	**10**	15/04/04	25/04/04	**10**	26/04/04	7/05/04	**11**
11/08/2004 (1835hr)	23/07/04	3/08/04	**11**	10/08/04	19/08/04	**9**	21/08/04	1/09/04	**11**
Total			**72**			**71**			**73**
Average duration			**10.3**			**10.1**			**10.4**

Date set: The date of setting up the ovitraps for that study cycle.

Date collect: The date of harvesting all immature Aedes mosquitoes whenever a first pupa was noted in any of the ovitraps for that study cycle.

Duration: Number of days.

Table II. Combined number of immature *Aedes* mosquitoes collected in three different types of ovitraps placed at three different locations in relationship to chemical fogging carried out in natural field conditions.

Combined number of immature *Aedes* mosquitoes collected in three different types of ovitraps at locations A, B, C												
Study	Immediate pre-fogging period				Fogging period				Immediate post-fogging period			
number	A	B	C	Total	A	B	C	Total	A	B	C	Total
1	60	39	15	**114**	29	41	10	**80**	52	38	65	**155**
2	47	40	80	**167**	62	64	55	**181**	45	40	65	**150**
3	67	11	0	**78**	39	0	17	**56**	87	26	18	**131**
4	87	31	43	**161**	60	30	64	**154**	102	48	41	**191**
5	9	85	116	**210**	31	0	15	**46**	18	37	115	**170**
6	49	43	13	**105**	81	81	38	**200**	49	33	35	**117**
7	64	39	25	**128**	88	51	63	**202**	61	84	56	**201**
Total	319	249	267	**963**	302	216	199	**919**	353	222	339	**1,115**
Mean				**138**				**131**				**159**

respective ULV fogging was recorded (Table I). On all occasions of ULV fogging, no dead immature *Aedes* mosquitoes were noted in any of the ovitraps set up as a consequence of chemical fogging in all of the fogging and the immediate post-fogging periods.

The duration, expressed as the number of days, recorded for each cycle of ovitrapping with respect to the respective period of immediate pre-fogging, fogging, and immediate post-fogging periods, are shown in Table I. The mean number of days of ovitrapping for immediate pre-fogging, fogging and post-fogging periods were 10.3, 10.1 and 10.4 days, respectively. There was no statistically-significant difference in the mean duration of ovitrapping cycle between the immediate pre-fogging and fogging periods ($Z = 0.378$, $p = 0.705$). There was also no significant difference in the mean duration of ovitrapping cycle between fogging and post-fogging periods ($Z = 0.687$, $p = 0.492$). In comparison with the mean duration of ovitrapping cycles between the pre-fogging and post-fogging periods, there was also no statistical significant difference ($Z = 0.333$, $p = 0.739$).

The number of pooled immature *Aedes* mosquitoes collected in ovitraps placed at three different locations (A, B and C) in the garden for each cycle of ovitrapping during the immediate pre-fogging, fogging and post-fogging period are shown in Table II. The total number of pooled immature *Aedes* mosquitoes collected in the immediate pre-fogging, fogging, and immediate post-fogging periods were 963, 919, and 1115, respectively, with a mean of 138, 131, and 159, respectively. There was no statistically-significant difference in the total number of immature *Aedes* mosquitoes collected between the immediate pre-fogging and fogging periods ($Z = 0.169$, $p = 0.866$); fogging and immediate post-fogging periods ($Z = 0.847$, $p = 0.397$); and immediate pre-fogging and immediate post-fogging periods ($Z = 1.352$, $p = 0.176$).

The results of analysis of subset data also did not show any statistically-significant difference of the quantity of immature *Aedes* mosquitoes collected in ovitraps placed at various distances from the source of ULV chemical fogging. The respective statistical values in comparison with immediate pre-fogging and fogging periods, fogging and immediate post-fogging periods, and immediate pre-fogging and immediate post-fogging periods were ($Z = 0.169$, $p = 0.866$), ($Z = 0.338$, $p = 0.735$), and ($Z = 0.943$, $p = 0.345$) for site A; ($Z = 0.507$, $p = 0.612$), ($Z = 0.507$, $p = 0.612$), and ($Z = 0.314$, $p = 0.753$) for site B; and ($Z = 0.254$, $p = 0.799$), ($Z = 0.676$, $p = 0.499$), and ($Z = 1.352$, $p = 0.176$) for site C, respectively.

DISCUSSION

Controlling DF and its severe forms, DHF and dengue shock syndrome, and vector mosquitoes are urgent public health problems in dengue endemic countries. In dengue control, early case detection in sentinel health facilities, early warning system of impending outbreaks, and prompt counter-measures for vector control, have been considered essential and are still very much in practice in most parts of the tropical and subtropical regions where dengue is endemic[7]. A widely-favoured approach, particularly during epidemics or impending epidemics, is to spray ULV aerosols of chemical insecticides from equipment-mounted road vehicles to kill the adult mosquitoes. Previously, this approach had been widely accepted as highly effective to eliminate adult *Aedes* mosquitoes in the outbreak area and have some limited effect on their immature forms to abort an impending epidemic or achieve good rapid control of an epidemic.

Recently, there is much controversy on the effectiveness of ULV fogging of insecticides on *Aedes* to control epidemics[13]. A previous study in Thailand showed that although two treatments of malathion by ULV fogging three days apart gave a 99% reduction of adult mosquitoes, their numbers returned to pre-treatment level within two weeks[14]. Several recent studies in the Americas indicated a much lower impact, with their numbers returning to the pre-treatment baseline within just a few days[15,16]. A mathematical model by Newton and Reiter indicates that ULV has minimal impact on disease incidence even when multiple applications are made, although the peak of the epidemic may be delayed[17].

In this study, ULV chemical fogging of insecticides did not increase the duration of ovitrapping cycles in both fogging and immediate post-fogging periods. It reflected the failure of chemical fogging on *Aedes* mosquitoes to complete their lifecycle of reproduction in natural field conditions. It also indirectly indicated that chemical fogging did not eliminate or reduce the number of gravid female *Aedes* mosquitoes in the fogged locality to prolong the duration of ovitrapping cycles significantly. This study showed that the total number of immature *Aedes* mosquitoes collected during the fogging period was only marginally reduced when compared with the pre-fogging period, but the difference was not statistically significant. Interestingly, though it was not statistically significant in this relatively small study, the quantity of immature *Aedes* mosquitoes collected in the immediate post-fogging period was more than that in the immediate pre-fogging period. The possibility of this rebound phenomenon needs further study as it may have future implications on the continual use of chemical insecticides to control vector mosquitoes in the environment, especially the effect on the natural predators of *Aedes* mosquitoes.

In this prospective study, it was not rare to find dead insects and spiders and even small animals such as snails and frogs (Fig. 2) in ovitraps placed in the garden meant for the mosquitoes within 48 hours of post-fogging. In addition, dead insects such as ants and spiders (Fig. 2) could also be found on careful search of the garden, especially at the inter-phase between the garden wall and lawn, within 48 hours after chemical fogging. Whether the destruction of spiders and frogs, natural predators of mosquitoes, by chemical fogging of insecticides meant for the *Aedes* mosquitoes contributed to the rebound increase in the number of immature *Aedes* mosquitoes in this study needs further verification.

Thus, more studies are urgently required to investigate the impact of chemical fogging on the environment, especially the effect on predators of mosquitoes, as well as the questionable effectiveness of these chemical insecticides on the control of *Aedes* mosquitoes in the field. Though the findings of properly-designed field studies have wide implications in the control of dengue outbreaks, which are precipitated by a build-up of *Aedes* mosquitoes, this study encountered a couple of shortcomings. Besides a relatively small sample size in this study, the authors did not have any control on the frequency, type and pattern of chemical fogging.

The national guideline of carrying out perifocal ULV fogging of insecticides in response to a reported case of DF in a locality is in line with recommended World Health Organisation guidelines for chemical control to suppress a dengue epidemic or to suppress an imminent outbreak. The perifocal ULV fogging schedule was to fog the locality sequentially twice at seven days apart. In this study, observation showed that no such sequential ULV fogging was carried out. Thus, this study was based on only one fogging and not two or more, as should normally be done in an outbreak situation. A possible reason for this single fogging was probably that the initial fogging was carried out based on the clinical report of a case of DF but was subsequently confirmed not to be a dengue case.

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REFERENCES

1. Mosquito-borne hemorrhagic fevers of South-East Asia and the Western Pacific. Bull World Health Organization 1966; 35:17-33.
2. Ministry of Health Malaysia. Annual Reports of Vector-borne Disease Control Programme, 1988-2001.
3. Rudnick A, Tan EE, Lucas JK, et al. Mosquito-borne haemorrhagic fever in Malaya. Br Med J 1965; 5445:1269-72.
4. Gubler DJ, Meltzer M. Impact of dengue/dengue hemorrhagic fever on the developing world. Adv Virus Res 1999; 53:35-70.
5. Monath TP, Heinz FX. Flaviviruses. In: Fields BN, Knipe DM, Howley PM, et al, eds. Fields Virology. Vol 1. 2nd ed. New York: Raven Press, 1990; 763-814.
6. Wallis RC. A study of oviposition activity of mosquitoes. Am J Hyg 1954; 60:135-68.
7. World Health Organization. Dengue haemorrhagic fever: diagnosis, treatment and control. Handbook of the World Health Organization. Geneva, 2000: 1-84.
8. Yap HH. Vector control in Malaysia: present status and future prospects. J Malaysian Soc Hlth 1984; 4:7-12.
9. Yap HH, Chong ASC, Adanan CR, et al. Field efficacy of a pyrethroid microemulsion (Pesguard PS 102) as ULV ground aerosols against three urban mosquito species. Trop Biomed 1996; 13:59-63.
10. Yap HH, Chong ASC, Adanan CR, et al. ULV field studies of combined spraying of Aqua Resigen and Vectobac AS12 against *Aedes aegypti, Aedes albopictus* and *Culex quinquefasciatus*. Trop Biomed 1997; 14:57-63.
11. Yap HH, Khoo TC, Tan HT, et al. Comparative adulticidal and larvicidal effects of thermal fogging formulations of Resigen and malathion against *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* (Say) in urban areas, Malaysia. Trop Biomed 1988; 5:125-30.
12. Yap HH, Lee YW, Chong ASC, et al. Bioefficacy of pyrethroid formulation (Pesguard PS 102) in thermal fogging application against urban mosquito species of public health importance. Trop Biomed 1999; 16:17-22.
13. Gratz NG. Emergency control of *Aedes aegypti* as a disease vector in urban areas. J Am Mosq Control Assoc 1991; 7:353-65.
14. Pant CP, Mount GA, Jatanasen S, et al. Ultra-low-volume ground aerosols of technical malathion for the control of *Aedes aegypti* L. Bull World Health Organ 1971; 45:805-17.
15. Chadee DD. An evaluation of malathion ULV spraying against caged and natural populations of Aedes aegypti in Trinidad, West Indies. Cah ORSTOM Ser Entomol Med Parasitol 1985; 23:71-4.
16. Focks DA, Kloter KO, Carmichael GT. The impact of sequential ultra-low volume ground aerosol applications of malathion on the population dynamics of *Aedes aegypti* (L.). Am J Trop Med Hyg 1987; 36:639-47.
17. Newton EAC, Reiter P. A model of the transmission of dengue fever with an evaluation of the impact of ultra-low volume (ULV) insecticide applications on dengue epidemics. Am J Trop Med Hyg 1992; 47:709-20.