INSECTICIDE SUSCEPTIBILITY STATUS OF FIELD-COLLECTED *AEDES* (*STEGOMYIA*) *AEGYPTI* (L.) AT A DENGUE ENDEMIC SITE IN SHAH ALAM, SELANGOR, MALAYSIA

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Abstract. Biweekly ovitrap surveillance (OS) was conducted for a year (August 2007 - September 2008) at two different dengue endemic sites in Shah Alam, Selangor, Malaysia, 50 km from Kuala Lumpur. *Aedes aegypti* collected from these 2 locations were raised to the F3 stage and subjected to a WHO standard bioassay method to determine lethal time (LT) against pyrethroids (permethrin 0.75%, cyfluthrin 0.15%), organophosphates (malathion 5.0%, fenitrothion 1.0%), carbamates (propoxur 0.1%, bendiocarb 0.1%) and organochlorine (DDT 4.0%). Insecticide susceptibilities were analyzed for one year. *Aedes aegypti* were resistant to DDT with a mortality range of 0 - 13.3% throughout the year at both sites. Susceptibilities to pyrethroids and carbamates varied throughout the year. In contrast, susceptibilities to pyrethroids and carbamates varied throughout the year: resistant to propoxur, bendiocarb and permethrin with mortality of < 80% in most months; but, showed incipient resistant to cyfluthrin in most months. Mosquitoes were consistently susceptible to malathion and fenitrothion, with complete mortality during most months. They were especially susceptible to malathion with LT_{50} values of 21.32 - 36.37 minutes, suggesting effectiveness of malathion for control of dengue.

Keywords: *Aedes aegypti*, ovitrap surveillance, WHO standard bioassay, susceptibility

INTRODUCTION

Dengue and dengue hemorrhagic fever (DF/DHF) are a major public health problem in Malaysia and have been since the first outbreak in 1973. In the absence of a dengue vaccine, control of dengue vectors, *Aedes aegypti* (L.) and *Aedes albopictus* Skuse, with chemical insecticides is the only means of combating DF/DHF. However, insecticide resistance has hampered the effectiveness of the vector control program.

Development of insecticide resistance among insects generally occurs due to selection of individuals that can survive a lethal dosage of insecticide. When an

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insecticide is used repeatedly, it induces resistance by a process of selection, which kills individuals with a susceptible gene and selects resistant genotypes (Brown, 1986). Devonshire and Field (1991) suggested insecticide resistance may be due to gene amplification.

Reviewing the historical background of insecticides used for dengue control programs, DDT was introduced for *Aedes aegypti* control via residual spraying in 1954 by Reid. Until the major outbreak of dengue fever and dengue hemorrhagic fever in 1973, bioresmethrin was used in thermal and cold fogging. However, because fogging this chemical was expensive, and not effective against larvae, bioresmethrin was soon replaced by other chemicals (Cheong, 1986). Malathion was recommended for the control of *Ae. aegypti* in 1986 by the Ministry of Health (MOH) (Vythilingam *et al*, 1992) and in 1996 was replaced by pyrethroids (PY), such as permethrin (Ang and Singh, 2001).

In the past several decades, studies on the susceptibility of mosquitoes in Malaysia to various insecticides have been conducted. In 1958, Coker reported DDT resistance in *Ae. aegypti* and suggested DDT resistance in this strain was monofactorial inheritance, *ie* the resistance was associated with a single gene inheritance. When DDT was replaced by organophosphates (OPs), resistance to organophosphorous compounds, such as malathion, fenitrothion and temephos, has been found among *Culex quinquefasciatus* and *Ae. aegypti* (Lee *et al*, 1984; Lee and Lime, 1989; Nazni *et al*, 1998, 2005). In 1996, pyrethroids (PY) were introduced in the vector control program, with resulting PY-resistance among *Cx. quinquefasciatus* (Nazni *et al*, 2005). In spite of information regarding insecticide susceptibility among mosquitoes in the field, we lack information about the resistance of *Ae. aegypti* in dengue endemic areas.

Our study site, Shah Alam (the capital of the State of Selangor), had 14 dengue hotspots in 2009 and was identified as an area with large number of dengue cases in the State of Selangor, Malaysia (Salina, 2009). Selangor State reported 18,676 cases of dengue in 2009 (MOH, Malaysia, 2010a). Until 3 July 2010, a total of 24,240 dengue cases with 81 deaths had been reported nationwide for 2010, compared to 25,234 cases with 62 deaths during the corresponding period in 2009. Although there was a 4% decrease in dengue cases, there was a 31% increase in fatalities. Selangor reported the largest number of cases (10,699) among the states of Malaysia (MOH, Malaysia, 2010b). Two localities named Site A and Site B were selected for ovitrap surveillance during August 2007 - September 2008. *Ae. aegypti* collected from these two localities were tested against various classes of insecticides. Because fogging with insecticides is the major method of controlling dengue vectors, it is necessary to ensure these insecticides are still effective. Hence, the susceptibility of *Ae. aegypti* was monitored continuously for 1 year in this study.

MATERIALS AND METHODS

Study site

This study was carried out during August 2007 - September 2008 in section 17, Shah Alam, approximately 50 km from Kuala Lumpur City, Malaysia. Two sites were chosen: Site A (8 ha) with 300 houses and Site B (10 ha) with 400 houses. Both were residential areas with rows of two storey terrace houses or clusters of 4 houses. The distance between 2 rows of houses was approximately 10 m. Heavy

vegetation was observed at both study sites. Site A was treated with *Bacillus thuringiensis israelensis* (Bti) at 500 g/ha for 7 months from December 2007 - June 2008. Site B was treated with chemical adulticides by the local authority when dengue cases were reported during the study period.

Ovitrap surveillance (OS) and adult mosquito colonization

Sixty ovitraps were placed indoors and outdoors in 30 randomly selected houses at each site. Modified ovitraps were used for surveillance as described by Lee (1992). OS was conducted twice a month, the first and third weeks of the month. The traps were left out for 5 days then collected and brought to the laboratory.

In the laboratory, the ovitrap contents, including oviposition paddles, were transferred into respective plastic containers filled with fresh water. A sufficient quantity of water was then added to ensure the paddle was fully submerged. A piece of half-cooked beef liver was added to each container as larval food and L3/L4 larvae were identified and counted. Larval counting was carried out every alternate day until day 7 post-collection. All containers were covered to prevent wild mosquitoes from ovipositing. Only *Ae. aegypti* mosquitoes were kept for further testing. The larvae were pooled and bred until F3. Non-blood fed adult female mosquitoes aged 2 - 5 days were selected for adult bioassay. WHO insecticide monitoring test kits were comprised of WHO test tubes and WHO impregnated paper. In this study, the mosquitoes were tested against 4% DDT, 1% fenitrothion, 5% malathion, 0.1% propoxur, 0.1% bendiocarb, 0.15% cyfluthrin and 0.75% permethrin for 1 hour per WHO instructions.

Bioassay procedure

The adult bioassay was conducted according to WHO standard procedure (WHO, 1981a, b) for determining the susceptibility status. The non-blood fed adult female mosquitoes aged 2-5 days were kept in clean paper cups covered with a fine mesh. Each cup contained 15 healthy mosquitoes. The mosquitoes were exposed to the insecticide impregnated paper in the WHO test kits. Each insecticide was tested using three replicates and three controls. During exposure, the knock down number was recorded every five minutes for 1 hour. For pyrethroids, exposure tubes were held horizontally and the knockdown rate was recorded every minute for 1 hour. After 1 hour, mosquitoes were transferred to clean paper cups, fed with 10% sugar solution and mortality was recorded 24 hours post-exposure.

Data analysis

Susceptibility test data were analyzed according to WHO criteria (Davidson and Zahar, 1973; WHO, 1998): 98-100% mortality indicated susceptibility; 80-98% mortality indicated possibility of resistance that needed further confirmation, and <80% mortality indicated resistance.

The results were then evaluated by probit analysis (SPSS version 11.5; SPSS, Chicago, IL) to compute the LT_{50} values. Susceptibilities of field-collected *Ae. aegypti* mosquitoes were tested by month. The resistance ratio (RR) was calculated by dividing the LT_{50} of the field strain by the LT_{50} of the susceptible strain maintained at the Medical Entomology Unit:

$$\text{Resistance Ratio (RR)} = \frac{LT_{50} \text{ of field strain}}{LT_{50} \text{ of laboratory strain (susceptible)}}$$

Table 1
Susceptibility of *Ae. aegypti* to 0.75% permethrin.

	0.75% Permethrin (1 hour exposure time)			
	LT_{50} (min) 95% (CL)	Regression line	RR_{50}	24 h post-exposure mortality (%)
Susceptible strain	13.95 (13.28 - 14.57)	Y = 5.06x - 5.79	-	100
Field strain				
Site A				
Sep 2007	179.63 (116.72 - 556.85)	Y = 2.58x - 5.81	12.9	31.1
Oct 2007	37.84 (36.74 - 38.98)	Y = 4.68x - 7.38	2.7	80.0
Nov 2007	29.72 (28.40 - 31.00)	Y = 3.01x - 4.43	2.1	75.6
Dec 2007	45.27 (43.60 - 47.19)	Y = 3.89x - 6.44	3.2	77.8
Jan 2008	52.06 (48.64 - 56.56)	Y = 3.83x - 6.58	3.7	77.8
Feb 2008	152.36 (105.61 - 384.21)	Y = 2.76x - 6.02	10.9	54.5
Apr 2008	129.36 (103.45 - 188.92)	Y = 3.09x - 6.52	9.3	55.6
Jul 2008	*	(-)	-	20.0
Sep 2008	377.36 (173.29 -5,681.43)	Y = 1.80x - 4.64	27.1	8.9
Site B				
Sep 2007	253.66 (150.10 - 858.71)	Y = 1.79x - 4.30	18.2	42.2
Nov 2007	417.47 (-)	Y = 1.86x - 4.88	29.9	64.4
Dec 2007	41.52 (40.32 - 42.72)	Y = 8.88x - 4.36	3.0	42.2
Jan 2008	66.22 (60.46 - 76.02)	Y = 3.71x - 6.76	4.7	36.7
May 2008	515.81 (196.28 - 37,291.38)	Y = 1.64x - 4.46	37.0	6.7
Jun 2008	* (-)	-	-	11.1
Jul 2008	* (-)	-	-	11.1
Aug 2008	* (-)	-	-	4.4
Sep 2008	440.43 (173.40 - 53,118.80)	Y = 1.60x - 4.23	31.6	6.7

*mortality < 10% in 1 hour exposure period

RESULTS

Pyrethroids susceptibilities

Mosquitoes from Site A were resistant to permethrin throughout the year with ≤ 80% mortality during all months (Table 1). A trend of decreasing susceptibility to permethrin was seen over time, beginning from October 2007 and continuing to September 2008 with resistance ratios (RR) ranging between 2.1-27.1 fold. Resistance to permethrin increased 10 times by September 2008. A similar trend of decreasing susceptibility to permethrin over time was observed at Site B where mortality rates were 4.4-64.4%. The RR values (3.0-37.0) showed *Ae. aegypti* was highly resistant. The resistance increased at least 10 times by May 2008.

Susceptibility to cyfluthrin fluctuated throughout the year at Site A (Table 2), with the RR ranging between 1.6-7.1 and 45-97.8% mortality rates. The field populations had resistance. Similar findings were seen at site B. Complete mortality by 24 hours exposure was seen in May 2008. The RR ranged from 2.0-5.2 throughout the year.

Table 2
Susceptibility of *Ae. aegypti* to 0.15% cyfluthrin.

	0.15% Cyfluthrin (1 hour of exposure time)			
	LT_{50} (min) 95% (CL)	Regression line	RR_{50}	24 h post-exposure mortality (%)
Susceptible strain	9.54 (8.88 - 10.16)	Y = 10.95x - 10.73	-	100
Field strain				
Site A				
Sep 2007	49.20 (47.46 - 51.24)	Y = 4.55x - 7.70	5.2	77.8
Oct 2007	15.05 (13.82 - 16.19)	Y = 3.86x - 4.54	1.6	93.3
Nov 2007	22.62 (21.79 - 23.41)	Y = 6.04x - 8.18	2.4	95.6
Dec 2007	30.38 (29.26 - 31.52)	Y = 3.57x - 5.29	3.2	88.9
Jan 2008	17.91 (16.96 - 18.82)	Y = 3.78x - 4.74	1.9	97.8
Feb 2008	36.73 (35.73 - 37.76)	Y = 5.06x - 7.92	3.8	84.4
Apr 2008	40.32 (39.12 - 41.58)	Y = 4.65x - 7.47	4.2	84.1
Jul 2008	67.61 (61.85 - 76.08)	Y = 3.60x - 6.60	7.1	45.0
Sep 2008	31.34 (30.21 - 32.48)	Y = 3.66x - 5.47	3.3	97.8
Site B				
Sep 2007	31.2 (30.36 - 32.04)	Y = 6.01x - 8.98	3.3	97.6
Nov 2007	27.56 (26.30 - 28.74)	Y = 5.98x - 8.61	2.9	93.3
Dec 2007	19.44 (18.03 - 20.76)	Y = 2.83x - 3.64	2.0	90.9
Jan 2008	30.38 (29.26 - 31.52)	Y = 3.57x - 5.29	3.2	88.6
May 2008	25.39 (24.46 - 26.31)	Y = 3.96x - 5.57	2.7	100
Jun 2008	37.36 (35.89 - 38.94)	Y = 3.21x - 5.05	3.9	57.8
Jul 2008	37.37 (36.25 - 38.52)	Y = 4.52x - 7.11	3.9	62.8
Aug 2008	33.81 (32.81 - 34.82)	Y = 4.63x - 7.08	3.5	91.1
Sep 2008	49.23 (46.76 - 52.25)	Y = 3.09x - 5.23	5.2	45.5

Organophosphates susceptibilities

Field mosquitoes caught at site A had 100% mortality to malathion during all months except April 2008 (93.3%) with a RR ranging from 1.2 to 2.0. At Site B, complete mortality was observed throughout the year with the RR ranging from 1.4 to 1.9 (Table 3). Low mortality at 1 hour post-exposure with fenitrothion revealed mosquito populations at both Sites A and B were not as susceptible to malathion. However, complete mortality was seen by 24 hours to fenitrothion (Table 4).

Carbamates susceptibilities

The mosquitoes at Site A had 31.8-95.3% mortality with propoxur and RR of 1.0-2.3 throughout the year. At Site B, the mosquitoes showed varying resistance to propoxur throughout the year with mortality rates ranging between 13.6-68.9% and RR of 0.9-3.2 (Table 5).

Similar susceptibilities to bendiocarb were observed at Site A (Table 6). The mortality rates were 33.3-100% and the RR ranged between 0.9-2.0. A low RR did not confer higher mortality since RR values in

Table 3
Susceptibility of *Ae. aegypti* to 5.0% malathion.

	5.0% Malathion (1 hour of exposure time)			
	LT_{50} (min) 95% (CL)	Regression line	RR_{50}	24 h post-exposure mortality (%)
Susceptible strain	18.20 (17.72 - 18.66)	Y = 10.45x - 13.17	-	100
Field strain				
Site A				
Sep 2007	23.93 (23.44 - 24.42)	Y = 12.39x - 17.09	1.3	100
Oct 2007	21.32 (20.82 - 21.80)	Y = 11.03x - 14.65	1.2	100
Nov 2007	28.82 (28.21 - 29.43)	Y = 9.57x - 13.97	1.6	100
Dec 2007	33.8 (32.76 - 34.79)	Y = 10.34x - 15.82	1.9	100
Jan 2008	25.51 (22.59 - 28.72)	Y = 8.57x - 12.05	1.4	100
Feb 2008	32.99 (32.49 - 33.52)	Y = 13.31x - 20.20	1.8	100
Apr 2008	31.93 (31.32 - 32.53)	Y = 10.03x - 15.09	1.8	93.3
Jul 2008	30.44 (29.94 - 30.93)	Y = 15.90x - 23.59	1.7	100
Sep 2008	36.37 (35.62 - 37.10)	Y = 8.36x - 13.04	2.0	100
Site B				
Sep 2007	27.65 (26.95 - 28.33)	Y = 8.61x - 12.42	1.5	100
Nov 2007	34.97 (34.41 - 35.52)	Y = 13.24x - 20.44	1.9	100
Dec 2007	29.99 (26.91 - 33.43)	Y = 8.89x - 13.13	1.6	100
Jan 2008	25.58 (-)	Y = 6.43x - 9.06	1.4	100
May 2008	26.12 (25.61 - 26.63)	Y = 11.72x - 16.61	1.4	100
Jun 2008	33.76 (33.14 - 34.37)	Y = 10.49x - 16.03	1.9	100
Jul 2008	24.86 (24.16 - 25.59)	Y = 11.00x - 15.35	1.4	100
Aug 2008	33.94 (33.12 - 34.76)	Y = 12.63x - 19.33	1.9	100
Sep 2008	26.58 (26.00 - 27.18)	Y = 10.61x - 15.11	1.5	100

Table 4
Susceptibility of *Ae. aegypti* to 1.0% fenitrothion.

	1.0% Fenitrothion (1 hour of exposure time)	
	24 hour post-exposure mortality (%)	
Susceptible strain	100	100
Field strain	Site A	Site B
Sep 2007	100	100
Oct 2007	100	100
Nov 2007	100	100
Dec 2007	100	100
Jan 2008	100	100
Feb 2008	100	100
Apr 2008	100	100
Jul 2008	100	100
Sep 2008	100	98

Table 5
Susceptibility of *Ae. aegypti* to 0.1% propoxur.

	0.1% Propoxur (1 hour of exposure time)			
	LT_{50} (min) 95% (CL)	Regression line	RR_{50}	24 h post-exposure mortality (%)
Susceptible strain	46.24 (43.63 - 49.38)	Y = 5.62x - 9.36	-	100
Field strain				
Site A				
Sep 2007	49.18 (46.18 - 53.18)	Y = 5.36x - 9.06	1.1	57.8
Oct 2007	55.14 (50.82 - 62.21)	Y = 4.70x - 8.18	1.2	64.4
Nov 2007	51.89 (49.31 - 54.39)	Y = 5.82x - 9.98	1.1	95.3
Dec 2007	64.22 (58.02 - 77.68)	Y = 5.32x - 9.61	1.4	47.7
Jan 2008	103.6 (91.20 - 129.22)	Y = 3.68x - 7.42	2.2	43.2
Feb 2008	47.72 (45.29 - 50.66)	Y = 6.49x - 10.89	1.0	86.7
Apr 2008	91.3 (83.62 - 103.11)	Y = 4.10x - 8.03	2.0	70.2
Jul 2008		ND		
Sep 2008	106.11 (75.93 - 1,101.99)	Y = 4.45x - 9.01	2.3	31.8
Site B				
Sep 2007	58.3 (54.30 - 65.10)	Y = 6.38x - 11.27	1.3	68.9
Nov 2007	69.15 (58.93 - 108.66)	Y = 5.63x - 10.35	1.5	55.6
Dec 2007	85.94 (69.82 - 184.58)	Y = 5.63x - 10.88	1.9	50.0
Jan 2008	64.05 (57.33 - 76.72)	Y = 4.32x - 7.80	1.4	48.9
May 2008	75.19 (65.59 - 106.41)	Y = 6.47x - 12.13	1.6	18.8
Jun 2008	73.48 (64.38 - 142.84)	Y = 8.72x - 16.28	1.6	28.9
Jul 2008	43.39 (41.12 - 45.87)	Y = 6.18x - 10.11	0.9	64.4
Aug 2008	74.06 (63.27 - 99.13)	Y = 3.67x - 6.87	1.6	35.6
Sep 2008	149.17 (87.79 - 41,683.96)	Y = 3.17x - 6.89	3.2	13.6

December 2007 and April 2008 (0.9 fold) did not show complete mortality 24 hours post-exposure (December 2007, 86.7%; April 2008, 95.1%). At Site B, resistance to bendiocarb remained stable with mortality that ranged 53.3-68.9%, except in July (44.4%) and September 2008 (26.7%). Generally, the mosquito population at Site B was resistant to bendiocarb.

DDT susceptibility

Aedes aegypti at both Sites A and B were highly resistant to DDT. Mortality was hardly seen after 1 hour of exposure and 24 hours post-exposure. No mortality was observed during most months, except in September (2.2%) and December 2007 (13.3%) at Site A. A similar pattern was seen at Site B with no mortality during 4 months (November 2007, April 2008, July 2008, and September 2008); relatively low mortality was seen during the rest of the test period (Table 7).

DISCUSSION

Field collected *Ae. aegypti* from Shah Alam exhibited varying levels of resistance to PYs (permethrin, cyfluthrin), CARBs (propoxur, bendiocarb) and DDT.

Table 6
Susceptibility of *Ae. aegypti* from Site A to 0.1% bendiocarb.

	1.0% Bendiocarb (1 hour of exposure time)			
	LT_{50} (min) 95% (CL)	Regression line	RR_{50}	24 h post-exposure mortality (%)
Susceptible strain	39.25 (37.47 - 41.01)	Y = 7.97x -12.71	-	100
Field strain				
Site A				
Sep 2007	37.48 (35.89 - 39.15)	Y = 9.45x - 14.87	1.0	100
Oct 2007	47.29 (44.82 - 50.29)	Y = 6.29x - 10.53	1.2	82.2
Nov 2007	44.11 (41.10 - 46.80)	Y = 5.07x - 8.33	1.1	93.2
Dec 2007	36.28 (34.37 - 38.07)	Y = 7.34x - 11.45	0.9	86.7
Jan 2008	77.56 (65.23 - 131.93)	Y = 4.79x - 9.05	2.0	33.3
Feb 2008	45.44 (42.87 - 48.52)	Y = 5.50x - 9.11	1.2	73.3
Apr 2008	35.86 (32.86 - 38.83)	Y = 4.18x - 6.49	0.9	95.1
Jul 2008		ND		
Sep 2008	76.14 (65.24 - 135.89)	Y = 6.60x - 12.41	1.9	52.3
Site B				
Sep 2007	83.03 (68.36 - 128.41)	Y = 3.77x - 7.24	2.1	54.3
Nov 2007	49.02 (46.69 - 51.87)	Y = 7.23x - 12.22	1.2	68.9
Dec 2007	107.89 (96.11 - 136.74)	Y = 5.82x - 11.83	2.7	53.3
Jan 2008	49.1 (46.83 - 51.86)	Y = 7.48x 12.66	1.3	62.2
May 2008	67.71 (61.07 - 81.17)	Y = 5.89x - 10.77	1.7	64.4
Jun 2008	47.98 (45.35 - 51.26)	Y = 5.97x - 10.03	1.2	66.7
Jul 2008	67.24 (60.82 - 83.03)	Y = 6.65x -12.15	1.7	44.4
Aug 2008	54.51 (50.04 - 62.70)	Y = 4.34x - 7.54	1.4	68.9
Sep 2008	131.7 (86.78 - 771.92)	Y = 3.27x - 6.94	3.4	26.7

ND, not done

Table 7
Susceptibility of *Ae. aegypti* from Site A to 4.0% DDT.

	4.0% DDT (1 hour of exposure time)	
	24 h post-exposure mortality (%)	
Susceptible strain	35.6	35.6
Field strain	Site A	Site B
Sep 2007	2.2	8.9
Oct 2007	0	0
Nov 2007	0	13.3
Dec 2007	13.3	2.2
Jan 2008	0	4.4
Feb 2008	0	2.2
Apr 2008	0	0
Jul 2008	0	0
Sep 2008	0	0

Table 8
Dengue cases and history of insecticide application in Shah Alam, Selangor, 2008.

No.	Month	No. cases		Date	Insecticide	Thermal/ULV	Site
		DF	DHF				
1	January	1	0				
2	February	0	0	22/02/2008	Resigen	Thermal	A
3	March	0	0				
4	April	0	0				
5	May	3	0	09/05/2008	Resigen	Thermal	B
				09/05/2008	Resigen	Thermal	B
				16/05/2008	Resigen	Thermal	B
6	June	6	0	04/06/2008	Resigen	Thermal	B
				04/06/2008	Resigen	Thermal	B
				05/06/2008	Resigen	Thermal	B
				10/06/2008	Resigen	Thermal	B
				10/06/2008	Resigen	Thermal	B
				17/06/2008	Resigen	Thermal	B
				24/06/2008	Resigen	Thermal	B
				24/06/2008	Resigen	Thermal	B
				25/06/2008	Resigen	Thermal	B
				30/06/2008	Resigen	Thermal	B
7	July	3	0	09/07/2008	Mospray	Thermal	B
				10/07/2008	Mospray	Thermal & ULV	B
				21/07/2008	Mospray	Thermal	B
				25/07/2008	Mospray	Thermal	B
				25/07/2008	Mospray	ULV	B
8	August	2	0	26/08/2008	Mospray	Thermal & ULV	B
9	September	1	0	02/09/2008	Mospray	Thermal & ULV	B

Data source: Shah Alam City Council

They were susceptible to OPs (malathion, fenitrothion). Permethrin resistance was observed at both Sites A and B. Site B had a higher resistance level to permethrin with higher RR values and lower mortality 24 hours post-exposure. Mortality with permethrin was extremely low (<10%) from May 2008 onwards at Site B. Shah Alam Municipality used fogging at Site B when a dengue case was reported (Table 7). Twenty thermal foggings conducted druing May- September 2008 at Site B; the insecticide used during the operation was permethrin. At Site A, thermal fogging was conducted in February 2008. Continual use of insecticides to control dengue vectors has created selection pressure resulting in the emergence of resistant populations. In Thailand, deltamethrin and permethrin have been the most common insecticides used for vector control. In a resistance studies conducted during 2003-2005 permethrin resistance was detected at all study sites in Thailand (Ponlawat *et al*, 2005; Jirakanjanakit *et al*, 2007). Most mosquito populations showed resistance

to deltamethrin and permethrin. Thus, it is not surprising that permethrin resistance occurred at our study sites in Shah Alam due to the intensive exposure of mosquitoes to permethrin fogging operations during dengue outbreaks.

Permethrin resistance has been documented to develop faster than with other insecticides. Nazni *et al* (1998) in Malaysia reported the RR of permethrin was 12 fold that of malathion after 9 generations for permethrin and 8 generations for malathion among field-collected *Culex quinquefasciatus* Say mosquites. These results are similar to studies conducted by Hidayati *et al* (2005) who reported permethrin resistance developed at a faster rate than malathion and temephos resistance. The resistance developed faster in field strain than in laboratory-permethrin-selected mosquitoes (Wan-Norafikah *et al*, 2008). Resistance to insecticides can eventually extend to other compounds that share a similar mode of action. Our results show incipient resistance and resistance to cyfluthrin were detected in *Ae. aegypti* even though this compound was not used in the vector control program. Development of resistance to cyfluthrin in this context may be explained by cross-resistance among PYs. In California, a permethrin selected strain of *Cx. quinquefasciatus* showed a relatively broad spectrum of resistance to related PYs (WHO, 1980). Somboon *et al* (2003) found *Ae. aegypti* from northern Thailand was resistant to the two commonly used insecticides for dengue control: permethrin and deltamethrin. They also had resistance to another PY insecticide, etofenprox, although this insecticide was not widely used for dengue control in Thailand.

PYs resistance was associated with cross-resistance to DDT, since both PYs and DDT act on the nervous system (Hemingway and Ranson, 2000). Detoxifying enzyme, oxidase and monooxgenase are associated with cross-resistance between DDT and PYs (Brogdon and McAllister, 1998). In our study, DDT resistance was observed throughout the year at Sites A and B suggesting cross-resistance between PYs and DDT in these areas. Our results agree with that of Lee *et al* (1998), where adult *Ae. albopictus* Skuse collected from Kuala Lumpur were resistant to permethrin and DDT. Cross-resistance between DDT and PYs has been reported in numerous locations throughout the world. In Colombia, application of lambda-cyhalothrin for malaria control in recent years has resulted in resistance to this insecticide. DDT resistance was detected in the same population. Historically, DDT was used for malaria control in Colombia before it was replaced by lambda-cyhalothrin; DDT resistance was sustained in the population for 17 years after the termination of DDT usage (Fonseca-González, *et al*, 2009). Adult mosquitoes collected from eight countries in Latin America exhibited DDT resistance; most had developed resistance or incipient resistance to PYs (Rodríguez *et al*, 2007). DDT was exclusively used for dengue control in the past in Thailand before it was replaced by PYs. DDT resistance is widely spread throughout Thailand and confers cross-resistance to other insecticides (Paeporn *et al*, 2005; Jirakanjanakit *et al*, 2007). A different finding with DDT resistance was seen in Vietnam where *Ae. aegypti* was found resistant to DDT, but susceptible to PYs (Huong *et al*, 2004). Casimiro *et al* (2006) reported *Anopheles funestus* from southern Mozambique were susceptible to DDT but resistant to PYs, suggesting the a kdr-type target site resistance mechanism was not present in these populations.

Aedes aegypti from Sites A and B

were susceptible to OPs, malathion and fenitrothion with 100% mortality during most months of the year. CARBs resistance (propoxur and bendiocarb) was seen in *Ae. aegypti* populations. Malathion was replaced with pyrethroid formulations in 1996 in the vector control program in Malaysia (Ang and Singh, 2001); malathion resistance among *Ae. aegypti* is rarely reported in this country. A review by Brown in 1986 showed malathion resistance was detected in *Ae. albopictus* mosquitoes in Malaysia. Nazni *et al* (2005) reported field collected *Cx. quinquefasciatus* from Kuala Lumpur had high levels of resistance to malathion and fenitrothion. In Thailand, *Ae. aegypti* was susceptible to OP compounds (Somboon *et al,* 2003; Paeporn *et al,* 2005; Ponlawat *et al,* 2005; Sathantriphop *et al,* 2006). The tested populations of *Ae. aegypti* were resistant to PYs, but still susceptible to malathion and fenitrothion. These results are similar to our findings which showed resistance to permethrin, but susceptibility to malathion and fenitrothion. In Sri Lanka, malathion was applied heavily with indoor spraying in the malaria and filariasis control program. Widespread use of malathion has resulted in resistance to malathion in *Culex* and *Anopheles* mosquitoes in these areas. However, the dengue vectors, *Ae. aegypti* and *Ae. albopictus* were found to be susceptible to malathion in spite of intensive use of malathion in malaria and filariasis epidemic areas (Karunaratne and Hemingway, 2001). Similar conditions also occurred in Cuba, Venezuela, Costa Rica and Jamaica, where *Ae. aegypti* was susceptible to malathion regardless of heavy application of this insecticide in the *Ae. aegypti* control program in those countries (Coto *et al,* 2000).

Propoxur and bendiocarb resistance were observed at Sites A and B during most months of the year. This is the first report of CARB-resistance of *Ae. aegypti* in Malaysia. In Townsville, Australia, *Ae. aegypti* was found to be resistant to bendiocarb, although bendiocarb was never used in the Townsville vector control program. Propoxur and bendiocarb have never been introduced in any vector control program in Malaysia, yet resistance was detected suggesting these findings are related to cross-resistance (Canyon and Hii, 1999). In a nationwide insecticide resistance evaluation conducted by Jirakanjanakit *et al* (2007), only one strain in the whole nation, from Mae Wong, Nakhon Sawan Province, was resistant to propoxur. Liu *et al* (2004a) reported absence or very low levels of resistance to propoxur in field collected *Ae. albopictus* from Alabama and Florida. However, Liu *et al* (2004b) found cross-resistance between propoxur and OPs in *Cx. quinquefasciatus* mosquitoes from the two locations mentioned above. *Cx. pipens,* from filariasis endemic areas of Egypt, was found to be resistant to all four classes of insecticides, including propoxur and bendiocarb (Zayed *et al,* 2006). Low-level resistance to CARBs was reported in Mozambique due to bendiocarb use in the malaria control program in some localities (Casimiro *et al,* 2006).

Bioassay data shows temporal variations in susceptibility to all insecticides were seen. A similar finding of variations in insecticide susceptibility was also reported by Lee *et al* (1998) after 10 weeks of insecticide susceptibility monitoring. The bioassay data provide useful baseline information about susceptibility of field collected mosquitoes to commonly used insecticides in vector control programs. OP compounds, such as malathion, had the greatest toxicity against *Ae. aegypti* among the four classes of insecticides tested in a dengue endemic area, Shah

Alam, suggesting the urgent need to revert to malathion in the dengue control program. The presence of resistance of *Ae. aegypti* in a dengue endemic area to multiple insecticides reveals current vector control strategies need to be reviewed.

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REFERENCES

Ang KT, Singh S. Epidemiology and new initiatives in the preventive and control of dengue in Malaysia. *Dengue Bull* 2001; 25: 7-14.

Brogdon WG, McAllister J. Insecticide resistance and vector control. *Emerg Infect Dis* 1998; 4: 605-13.

Brown AWA. Insecticides resistance in mosquitoes: A pragmatic review. *J Am Mosq Control Assoc* 1986; 2: 123-40.

Canyon DV, Hii JKL. Insecticide susceptibility status of *Aedes aegypti* (Diptera: Culicidae) from Townsville. *Aust J Entomol* 1999; 38: 40-3.

Casimiro S. Coleman M, Mohloai P. Hemingway J, Sharp B. Insecticide resistance in *Anopheles funestus* (Diptera: Culicidae) from Mozambique. *J Med Entomol* 2006; 43: 267-75.

Cheong WH. The vectors of dengue and dengue haemorrhagic fevers in Malaysia. In: Rudnick A, Lim TW, eds. Dengue fever studies in Malaysia. *Inst Med Res Malaysia Bull* 1986; 23: 155-67.

Coker WZ. The inheritance of DDT resistance in *Aedes aegypti*. *Ann Trop Med Parasitol* 1958; 52: 443-55.

Coto MMR, Lazcano JAB, Fernández DM, Soca A. Malathion resistance in *Aedes aegypti* and *Culex quinquefasciatus* after its use in *Aedes aegypti* control programs. *J Am Mosq Control Assoc* 2000; 16: 324-30.

Davidson G, Zahar AR. The practical implications of resistance of malaria vectors to insecticides. *Bull World Health Organ* 1973; 49: 475-83.

Devonshire AL, Field LM. Gene amplification and insecticide resistance. *Annu Rev Entomol* 1991; 36: 1-23.

Fonseca-González I, Quiñones ML, McAllister J, Brogdon WG. Mixed-function oxidases and esterases associated with cross-resistance between DDT and lambda-cyhalothrin in *Anopheles darlingi* Root 1926 populations from Colombia. *Mem Inst Oswaldo Cruz* 2009; 104: 18-26.

Hemingway J, Ranson H. Insecticide resistance in insect vectors of human disease. *Annu Rev Entomol* 2000; 45: 371-91.

Hidayati H, Sofian-Azirun M, Nazni WA, Lee HL. Insecticide resistance development in *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) larvae against malathion, permethrin and temephos. *Trop Biomed* 2005; 22: 45-52.

Huong VD, Ngoc NTB, Hien DT, Lien NTB. Susceptibility of *Aedes aegypti* to insecticides in Viet Nam. *Dengue Bull* 2004; 28: 179-83.

Jirakanjanakit N, Rongnoparut P, Saengtharatip S, *et al*. Insecticide susceptible/resistance status in *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) in Thailand during 2003-2005. *J Econ Entomol* 2007; 100: 545-50.

Karunaratne SHPP, Hemingway J. Malathion resistance and prevalence of the malathion carboxylesterase mechanism in populations of mosquito vectors of disease in

Sri Lanka. *Bull World Health Organ* 2001; 79: 1060-4.

Lee HL. *Aedes* ovitrap and larval survey in several suburban communities in Selangor, Malaysia. *Mosq Borne Dis Bull* 1992; 9: 9-15.

Lee HL, Lime W. A re-evaluation of the susceptibility of field-collected *Aedes* (Stegomyia) *aegypti* (Linnaeus) larvae to temephos in Malaysia. *Mosq Borne Dis Bull* 1989; 6: 91-5.

Lee HL, Asikin N, Nazni WA, Sallehuddin S. Temporal variations of insecticide susceptibility status of field-collected *Aedes albopictus* (Skuse) in Malaysia. *Trop Biomed* 1998; 15: 43-50.

Lee HL, Lee TW, Law FM, Cheong WH. Preliminary studies on the susceptibility of field-collected *Aedes* (Stegomyia) *aegypti* (Linnaeus) to Abate® (temephos) in Kuala Lumpur. *Trop Biomed* 1984; 1: 37-40.

Liu H, Cupp EW, Guo A, Liu N. Insecticide resistance in Alabama and Florida mosquito strains of *Aedes albopictus*. *J Med Entomol* 2004a; 41: 946-52.

Liu H, Cupp EW, Micher KM, Guo A, Liu N. Insecticide resistance and cross-resistance in Alabama and Florida strains of *Culex quinquefasciatus*. *J Med Entomol* 2004b; 41: 408-13.

Ministry of Health (MOH), Malaysia. Director General of Health Malaysia. Press statement by Director General of Health, Malaysia. Current situation of dengue fever and chikungunya in Malaysia for epidemiology week 52/2009 (27 Dec 2009 to 2 Jan 2009) (In Bahasa Malaysia). Kuala Lumpur: MOH, 2010a. [Cited 2010 Feb 10]. Available from: URL: http://www.infosihat.gov.my/menuutama/Press_Denggi_Chiku/KPK_Minggu_52_2009.pdf 11 Jan 2010

Ministry of Health (MOH), Malaysia. Director General of Health Malaysia. Press statement by Director General of Health, Malaysia. Current situation of dengue fever and chikungunya in Malaysia for epidemiology week 26/2010 (27 Jun 2010 to 3 July 2010) (In Bahasa Malaysia). Kuala Lumpur: MOH, 2010b. [Cited 2010 Jul 16]. Available from: URL: http://www.moh.gov.my/press_releases/38

Nazni WA, Lee HL, Azahari AH. Adult and larval insecticide susceptibility status of *Culex quinquefasciatus* (Say) mosquitoes in Kuala Lumpur Malaysia. *Trop Biomed* 2005; 22: 63-8.

Nazni WA, Lee HL, Sa'diyah I. Rate of resistance development in wild *Culex quinquefasciatus* (Say) selected by malathion and permethrin. *Southeast Asian J Trop Med Public Health* 1998; 29: 849-55.

Paeporn P, Supaphathom K, Sathantriphop S, Mukkhun P, Sangkitporn S. Insecticide susceptibility of *Aedes aegypti* in Tsunami-affected areas in Thailand. *Dengue Bull* 2005; 29: 210-3.

Ponlawat A, Scott JG, Harrington LC. Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand. *J Med Entomol* 2005; 42: 821-5.

Rodríguez MM, Bisset JA, Fernández D. Levels of insecticide resistance and resistance mechanisms in *Aedes aegypti* from some Latin American countries. *J Am Mosq Control Assoc* 2007; 23: 420-9.

Salina K. 14 dengue hot spots identified in Shah Alam. *The Star* 2009 Feb 3. [Cited 2011 Apr 20]. Available from: URL: http://www.starproperty.my/PropertyGuide/HomeProtection/115/0/0

Sathantriphop S, Paeporn P, Supaphathom K. Detection of insecticides resistance status in *Culex quinquefasciatus* and *Aedes aegypti* to four major groups of insecticides. *Trop Biomed* 2006; 23: 97-101.

Somboon P, Prapanthadara L, Suwonkerd W. Insecticide susceptibility tests of *Anopheles minimus* s.l., *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus* in northern Thailand. *Southeast Asian J Trop Med Public Health* 2003; 34: 87-93.

Vythilingam I, Chiang GL, Lee HL, Singh KI. Bionomics of important mosquito vectors in Malaysia. *Southeast Asian J Trop Med Public Health* 1992; 23: 587-603.

Wan-Norafikah O, Nazni WA, Lee HL, *et al*. Detection of permethrin resistance in *Aedes albopictus* Skuse, collected from Titiwangsa Zone, Kuala Lumpur, Malaysia. Proceeding of the 3rd ASEAN Congress of Tropical Medicine and Parasitology, 2008: 69-77.

World Health Organization (WHO). Present status of research on resistance of vectors to pesticides. *VBC/EC/80.19*.1980

World Health Organization (WHO). Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphates and carbamate insectcicides establishment of the base-line. *WHO/VBC/81.805.* 1981a.

World Health Organization (WHO). Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphates and carbamate insectcicides - diagnostic test. *WHO/VBC/81.806*. 1981b.

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

Zayed ABB, Szumlas DE, Hanafi HA, *et al*. Use of bioassay and microplate assay to detect and measure insecticide resistance in field populations of *Culex pipiens* from filariasis endemic areas of Egypt. *J Am Mosq Control Assoc* 2006; 22: 473-82.