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Weekly variation on susceptibility status of *Aedes* mosquitoes against temephos in Selangor, Malaysia

Chen, C.D.[1], Nazni, W.A.[1], Lee, H.L.[1] and Sofian-Azirun, M.[2]
[1]Medical Entomology Division, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia.
[2]Institute Science Biology, Faculty of Science, University Malaya, 50603 Kuala Lumpur, Malaysia.

Abstract. Larvae of *Aedes aegypti* and *Aedes albopictus* obtained from 6 consecutive ovitrap surveillance (OS) in Taman Samudera and Kg. Banjar were evaluated for their susceptibility to temephos. Larval bioassays were carried out in accordance with WHO standard methods, with diagnostic dosage (0.012 mg/L) and operational dosage (1 mg/L) of temephos respectively. *Aedes aegypti* and *Ae. albopictus* obtained from six OS in Taman Samudera showed resistance to diagnostic dosage of temephos with percentage mortality between 5.3 to 72.0 and 9.3 to 56.0, respectively, while *Ae. aegypti* and *Ae. albopictus* obtained from Kg. Banjar showed resistance to temephos with percentage mortality between 16.0 to 72.0 and 0 to 50.6, respectively. Only two strains of *Ae. aegypti* from Kg. Banjar were susceptible to temephos with 93.3% (OS 2) and 100% (OS 3) mortality. The 50% mortality at lethal time (LT_{50}) for all strains of *Ae. aegypti* and *Ae. albopictus* tested against operational dosage of temephos showed range between 36.07 to 75.69 minutes and 58.65 to 112.50 minutes, respectively, and complete mortality was achieved after 24 hours. Our results indicated that there is weekly variations of the resistance status for *Ae. aegypti* and *Ae. albopictus*. *Aedes* susceptibility to temephos is changing from time to time in these two study sites. It is essential to continue monitoring the resistance of this vector to insecticides in order to ensure the efficiency of program aimed at vector control and protection of human health.

INTRODUCTION

Dengue fever (DF) and dengue haemorrhagic fever (DHF) are most important vector-borne diseases in tropical, subtropical and temperate regions of the world (Gubler *et al.*, 1998). Millions of people are infected by DF and DHF annually (Jacobs, 2000). *Aedes aegypti* and *Aedes albopictus* plays a crucial role in the transmission of these infections (Rebecca, 1987; Lam, 1993; Lee & Inder, 1993; Nogueira *et al.*, 1999).

Malaysia has suffered from epidemics of DF and DHF since the first major national DF and DHF outbreak occurred in 1973 during which 969 cases and case fatality ratio of 5.6/100 were reported by Ministry of Health (Lee, 1994a). Currently, a dengue vaccine is not available and the only method of controlling or preventing DF and DHF is to combat the vector mosquitoes. Thus, insecticide resistance represents a threat for efficacy of vector control. The use of chemical agents is one of the most important methods of controlling vectors of medical importance. Larvaciding is the first step in chemical mosquito control, since the mosquitoes are killed at the breeding site, prior to dispersing and infesting a community. Since early 1970, WHO has recommended temephos (0,0,0'0'-tetramethyl-0,0'-thiodiphenylene phosphorothiorate) or Abate® for control of *Aedes* mosquitoes (WHO, 1985) and it has been used extensively in the past 30 years for control of *Ae. aegypti* and *Ae. albopictus* in Malaysia.

For many years, resistance was "detected" in an insect population only when it had evolved to the point where it

had no obvious impact on a control program. Today the early detection and monitoring of resistance is recognized as a vital part of resistance management. Resistance management is a relatively new area of research that is directed at developing insecticide use strategies that minimize the rate of evolution of resistance (Ferrari, 1996). Thus, this study was conducted to monitor the acute toxicity of temephos against *Ae. aegypti* and *Ae. albopictus* in two dengue endemic sites.

MATERIALS AND METHODS

Mosquito strains

Six continuous ovitrap surveillance was conducted in Taman Samudera (Gombak, Selangor) and Kampung Banjar (Gombak Selangor). Ovitraps as described by Lee (1992a) was used in this study. The ovitrap consists of 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 9.0 cm in height. The outer wall of the container is coated with a layer of black oil paint. An oviposition paddle made from hardboard (10 cm x 2.5 cm x 0.3 cm) was placed diagonally into each ovitrap. Each ovitrap was filled with tap water to a level of 5.5 cm. Ovitraps were placed indoors and outdoors. In this study, "indoor" is referred to the interior of the house, while "outdoor" is referred to outside of the house but confined to the immediate vicinity of the house (Lee, 1992b). All the ovitraps were collected after 5 days and replaced with fresh ovitraps and paddles. The hatched larvae were subsequently identified at 3rd instar. All strains of larvae were colonized until 1st generation (F1) and late 3rd or early 4th instar larvae were used for bioassay.

Insecticide

For larval bioassay testing, diagnostic dosage, 0.012 mg/L of temephos (WHO, 1992) was prepared from technical grade of temephos with 95.6% wt/wt, while for operational dosage, 1 mg/L of temephos was prepared from 1.1% a.i. sand granule formulation of Abate® temephos.

Bioassay against larvae

Larval bioassay was performed by using the WHO method (WHO, 1981). The temephos test concentration (diagnostic dosage and operational dosage) were prepared by pipetting the appropriate standard insecticide solution into 300 ml drinking paper cups filled with 200 ml distilled water and 25 late 3rd or early 4th instar larvae were added. Any larvae showing abnormalities were discarded. The water was then topped up to 250 ml using distilled water. The cups were held at room temperature of 28°C and 70% relative humidity. Larval mortality was recorded every 10 minutes until 120 minutes (2 hours) and after 24 hours. At least 3 replicates of each concentration were conducted. The control (untreated) consisted of 1 ml of ethanol added to the distilled water.

Data analysis

The test results obtained from bioassay were pooled and analysed using Probit Analysis Program of Raymond (1985) to obtain the lethal time values. The resistance ratio (RR) was determined as follow:

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

Values of RR greater than 1 is indicative of resistance and values less than or equal to 1 are considered susceptible.

RESULTS

Diagnostic Dosage, 0.012 mg/L temephos

Figure 1 and 2 showed the percent mortality of *Aedes* sp. from Taman Samudera and Kg. Banjar to 0.012 mg/L on exposure for 24 hours. In all the 6-times ovitrap surveillance (OS) of field *Ae. aegypti* and *Ae. albopictus* larvae, the percentage mortality ranges from 5.3% to

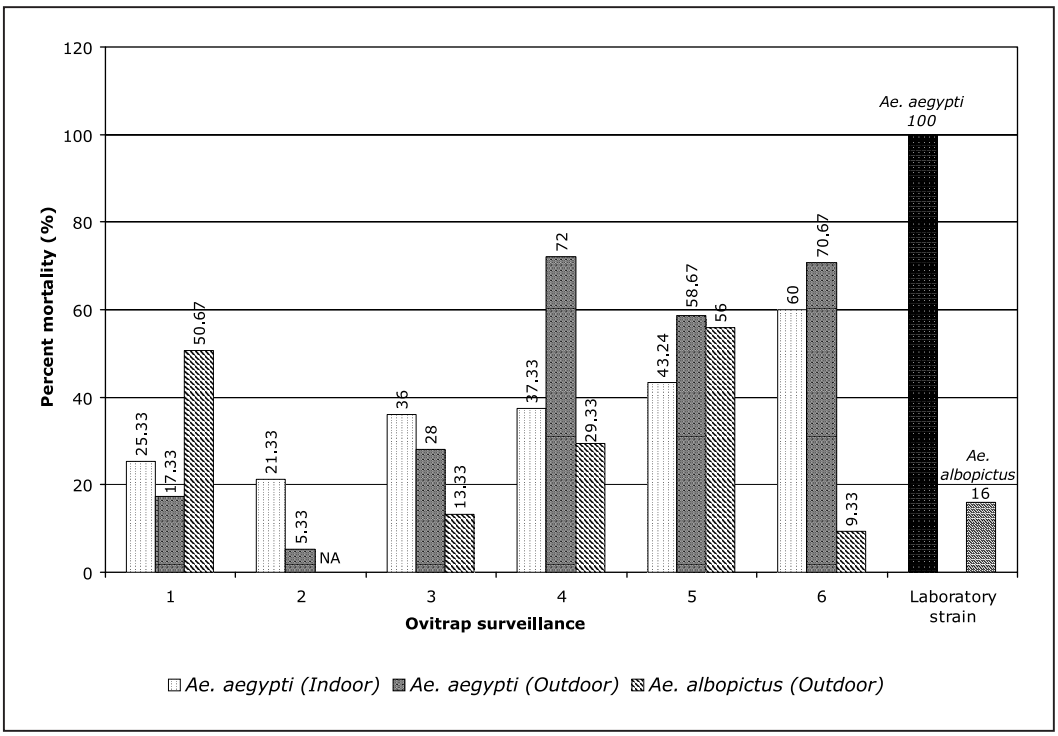

Figure 1. Percent mortality of *Aedes* sp. from Taman Samudera, Selangor to 0.012 mg/L on exposure for 24 hours.

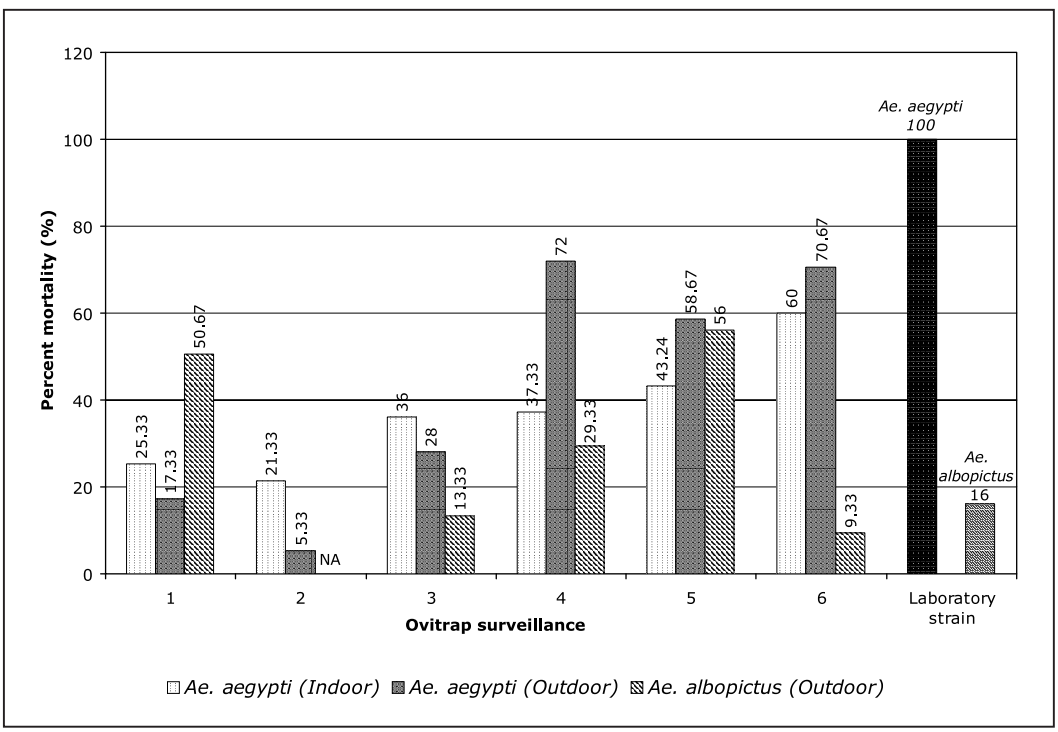

Figure 2. Percent mortality of *Aedes* sp. from Kg. Banjar, Selangor to 0.012 mg/L on exposure for 24 hours.

72.00% and 9.33% to 56.00% respectively in Taman Samudera and 0% to 50.67% mortality in *Ae. albopictus* in Kg. Banjar. Only 4 strains of *Ae. aegypti* showed resistance to temephos with mortality less than 80%.

Laboratory strain of *Ae. aegypti* showed complete mortality after 24 hours test, while laboratory strain of *Ae. albopictus* showed high resistance to temephos with only 16.0% mortality. The reason of low percentage mortality of laboratory strain *Ae. alobopictus* is because it is newly colonized and has been maintained for 8 generations only, while *Ae. aegypti* (F952) has been colonized for the past 30 years.

There was no mortality observed within 2 hours for bioassay with 0.012 mg/L temephos in all the field strains *Ae. albopictus*.

Operational Dosage, 1 mg/L temephos

Table 1 shows the LT_{50}, regression line and resistance ratio of *Ae. aegypti* and *Ae. albopictus* larvae obtained from Taman Samudera, Selangor tested against operational dosage of temephos. In the bioassays with 1 mg/L of temephos, there were no significant difference between LT_{50} of indoor and outdoor *Ae. aegypti* obtained from Taman Samudera ($p > 0.05$), which LT_{50} ranged from 44.10 minutes (OS 6) to 75.69 minutes (OS 3), and resistance ratio ranged from 1.04 to 1.79. However, LT_{50} of outdoor *Ae. albopictus* were significantly higher than both indoor and outdoor *Ae. aegypti* ($p < 0.05$), with range from 58.65 minutes (OS 1) to 93.61 minutes (OS 4). Resistance ratio of *Ae. albopictus* obtained from outdoor Taman Samudera were 1.75 (OS 5) to 1.20 (OS 4) folds than laboratory strain. However, our study found that there were 2 field strains of *Ae. albopictus* obtained from outdoor Taman Samudera were more susceptible than laboratory strain with resistance ratio 0.75 (OS 5) and 0.85 (OS 1). The LT_{50} of *Ae. albopictus* was about 1.24 to 2.12 folds higher than *Ae. aegypti* in Taman Samudera.

The LT_{50}, regression line and resistance ratio for the operational dosage of temephos of *Ae. aegypti* and *Ae. albopictus* larvae obtained from Kg. Banjar, are presented in Table 2. In comparison with laboratory strain, only 3 strains of *Ae. aegypti* from Kg. Banjar showed potential of resistance development, showing LT_{50} value ranging from 43.51 minutes (OS 4) to 55.68 minutes (OS 6), and resistance ratio was in the range of 1.03 to 1.32. Two field strains of *Ae. aegypti* were more susceptible than laboratory strain, that is *Ae. aegypti* obtained from OS 2 (LT_{50} = 36.07 minutes, resistance ratio = 0.85) and OS 3 (LT_{50} = 37.01 minutes, resistance ratio = 0.87). All strains of *Ae. albopictus* from Kg. Banjar were resistant in comparison to laboratory strain, with LT_{50} ranging from 82.78 minutes (OS 3) to 112.50 minutes (OS 6), and resistance ratio ranged from 1.06 to 1.45. This indicated that potential of resistance development in *Ae. albopictus* obtained from this site. *Ae. albopictus* obtained from Kg. Banjar was also significantly resistant compared to *Ae. aegypti* ($p < 0.05$), ranging from 1.49 to 3.12 folds.

Based on the LT_{50} value, the results in Figure 3 and 4 showed that all strain of *Ae. albopictus* from Taman Samudera and Kg. Banjar need more time to achieve 50% mortality compared to *Ae. aegypti* against 1 mg/L, the operational dosage of temephos. Beside this, complete mortality was observed in all strains of *Ae. aegypti* exposed to temephos within 120 minutes (Figure 3, 4, 5, 6 and 7), while this was not observed in *Ae. albopictus*. Thus, this indicated *Ae. albopictus* was more resistant to temephos compared to *Ae. aegypti* for the strains obtained from these 2 study sites.

However, in the 24 hours test with 1 mg/L temephos, complete mortality was observed in all strains of *Ae. aegypti* and *Ae. albopictus*.

Table 1. LT_{50}, regression line and resistance ratio of *Ae. aegypti* and *Ae. albopictus* collected from Taman Samudera, Selangor tested against 1 mg/L temephos

Ovitrap Surveillance	Ae. aegypti (Indoor)			Ae. aegypti *(Outdoor)*			Ae. albopictus(Outdoor)		
	LT_{50} (C.L.)	Regression Line	Resistance Ratio	LT_{50} (C.L.)	Regression Line	Resistance Ratio	LT_{50} (C.L.)	Regression Line	Resistance Ratio
1	46.78 (45.14 – 48.34)	Y = 13.79x – 155.91	1.11	54.42 (52.87 – 55.97)	Y = 15.79x – 180.26	1.29	65.81 (62.82 – 68.53)	Y = 7.37x – 82.07	0.85
2	54.65 (53.11 – 56.18)	Y = 16.07x – 183.55	1.29	55.98 (54.46 – 57.46)	Y = 17.09x – 195.82	1.32		NA	
3	75.69 (73.33 – 77.98)	Y = 9.95x – 113.20	1.79	53.31 (50.90 – 55.23)	Y = 12.76x – 144.68	1.26	82.92 (80.24 – 85.53)	Y = 9.29x – 105.75	1.07
4	47.67 (45.93 – 49.29)	Y = 12.68x – 143.10	1.13	46.34 (44.92 – 47.71)	Y = 16.69x – 189.62	1.10	93.61 (90.02 – 97.69)	Y = 6.71x – 75.33	1.20
5	44.88 (43.47 – 46.22)	Y = 16.92x – 192.10	1.06	48.09 (46.07 – 50.06)	Y = 8.69x – 95.37	1.14	58.65 (54.83 – 62.33)	Y = 3.84x – 40.19	0.75
6	62.96 (60.70 – 65.27)	Y = 8.93x – 100.40	1.49	44.10 (42.61 – 45.55)	Y = 14.25x – 166.00	1.04	83.08 (80.33 – 85.90)	Y = 8.13x – 91.88	1.07
Mean ± SE for LT_{50}	55.44 ± 4.88	–	1.31	50.37 ± 1.98	–	1.19	76.81 ± 6.36	–	0.99
Laboratory Strain	42.31 (41.33 – 43.28)	Y = 15.51x – 175.29	–	42.31 (41.33 – 43.28)	Y = 15.51x – 175.29	–	77.81 (74.76 – 80.80)	Y = 6.96x – 77.75	–

C.L. = Conference Limit
NA = Not Available

Table 2. LT_{50}, regression line and resistance ratio of *Ae. aegypti* and *Ae. albopictus* collected from Kg. Banjar, Selangor tested against 1 mg/L temephos

Ovitrap Surveillance	*Ae. aegypti*			*Ae. albopictus*		
	LT_{50} (C.L.)	Regression Line	Resistance Ratio	LT_{50} (C.L.)	Regression Line	Resistance Ratio
1	41.25 (39.70 – 42.77)	Y = 12.13x – 135.89	1.00	101.34 (98.39 – 104.68)	Y = 10.26x – 118.20	1.30
2	36.07 (34.77 – 37.35)	Y = 14.19x – 167.37	0.85	89.82 (86.92 – 92.82)	Y = 8.63x 98.13	1.15
3	37.01 (35.68 – 38.32)	Y = 14.63x – 164.28	0.87	82.78 (79.87 – 85.79)	Y = 7.43x – 83.56	1.06
4	43.51 (40.10 – 46.13)	Y = 7.18x – 78.59	1.03	86.77 (83.42 – 90.32)	Y = 6.61x – 73.91	1.12
5	45.19 (43.16 – 46.98)	Y = 11.21x – 125.67	1.07	100.61 (96.54 – 105.56)	Y = 6.77x – 76.29	1.29
6	55.68 (53.65 – 57.67)	Y = 10.15x – 114.21	1.32	112.50 (108.32 – 118.22)	Y = 9.31x – 107.19	1.45
Mean ± SE for LT_{50}	43.12 ± 2.90	–	1.02	95.64 ± 4.54	–	1.23
Laboratory Strain	42.31 (41.33 – 43.28)	Y = 15.51x – 175.29	–	77.81 (74.76 – 80.80)	Y = 6.96x – 77.75	–

C.L. = Conference Limit

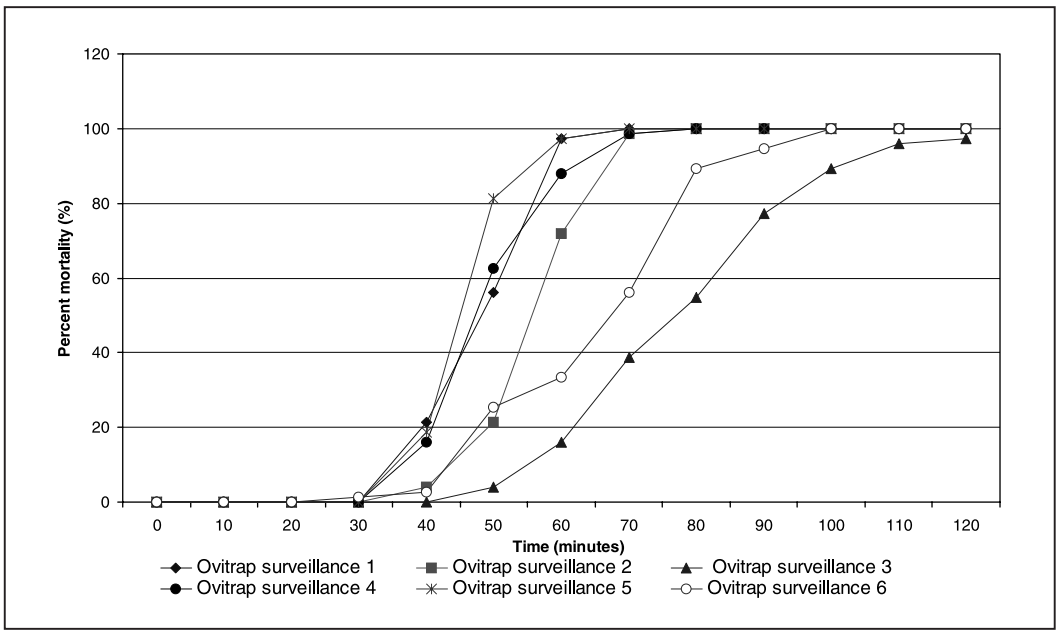

Figure 3. Percent mortality of *Ae. aegypti* (indoor) from Taman Samudera, Selangor exposed to 1 mg/L temephos for 120 minutes.

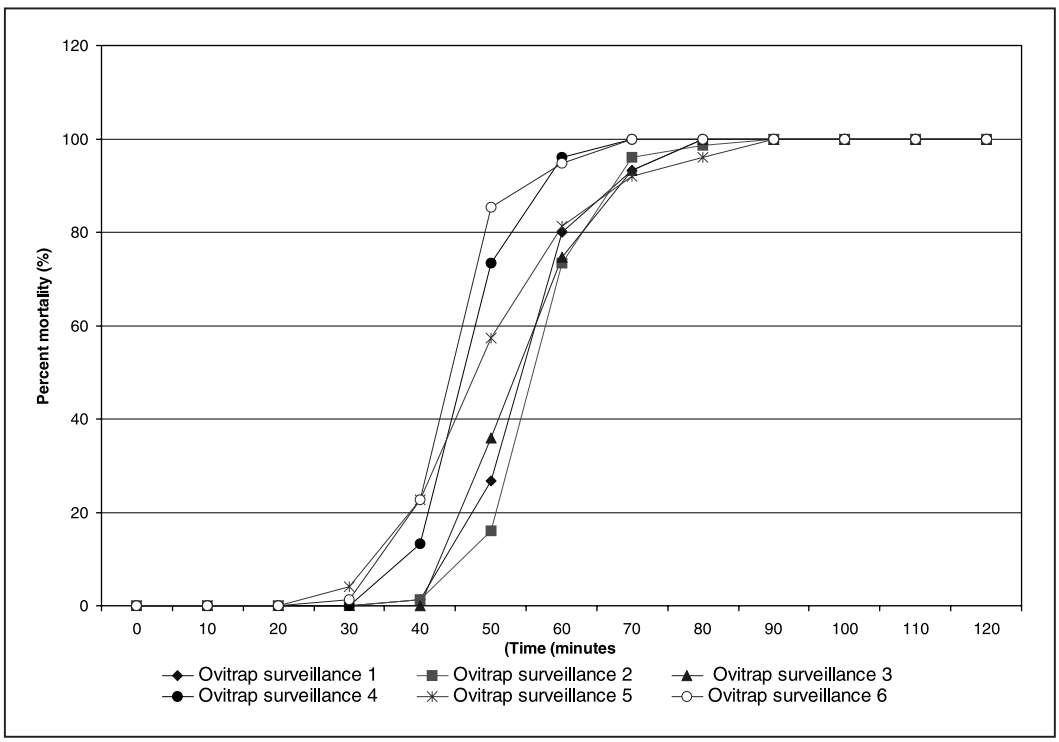

Figure 4. Percent mortality of *Ae. aegypti* (outdoor) from Taman Samudera, Selangor exposed to 1 mg/L temephos for 120 minutes.

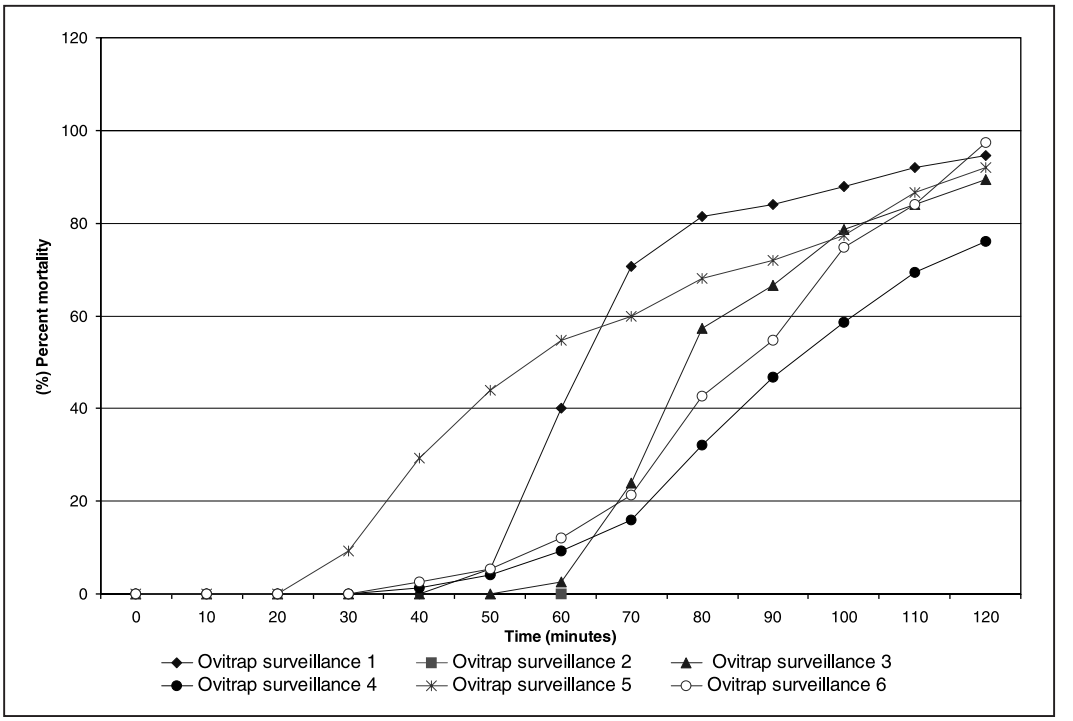

Figure 5. Percent mortality of *Ae. albopictus* (outdoor) from Taman Samudera, Selangor exposed to 1 mg/L temephos for 120 minutes.

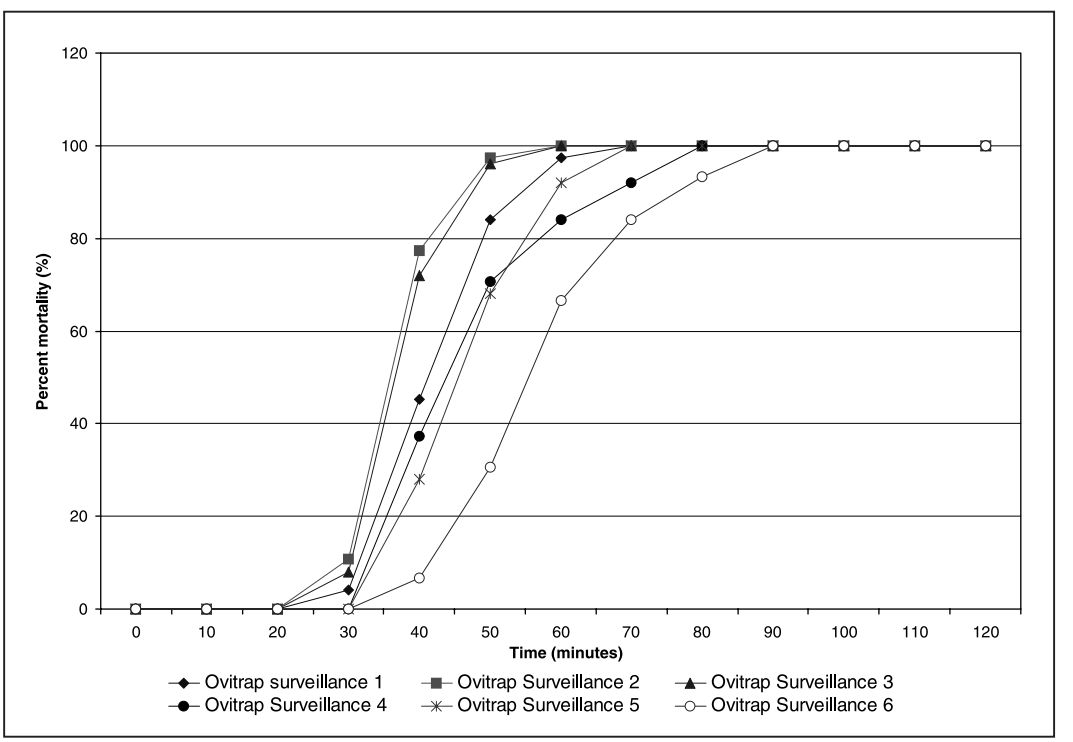

Figure 6. Percent mortality of *Ae. aegypti* from Kg. Banjar, Selangor exposed to 1 mg/L temephos for 120 minutes.

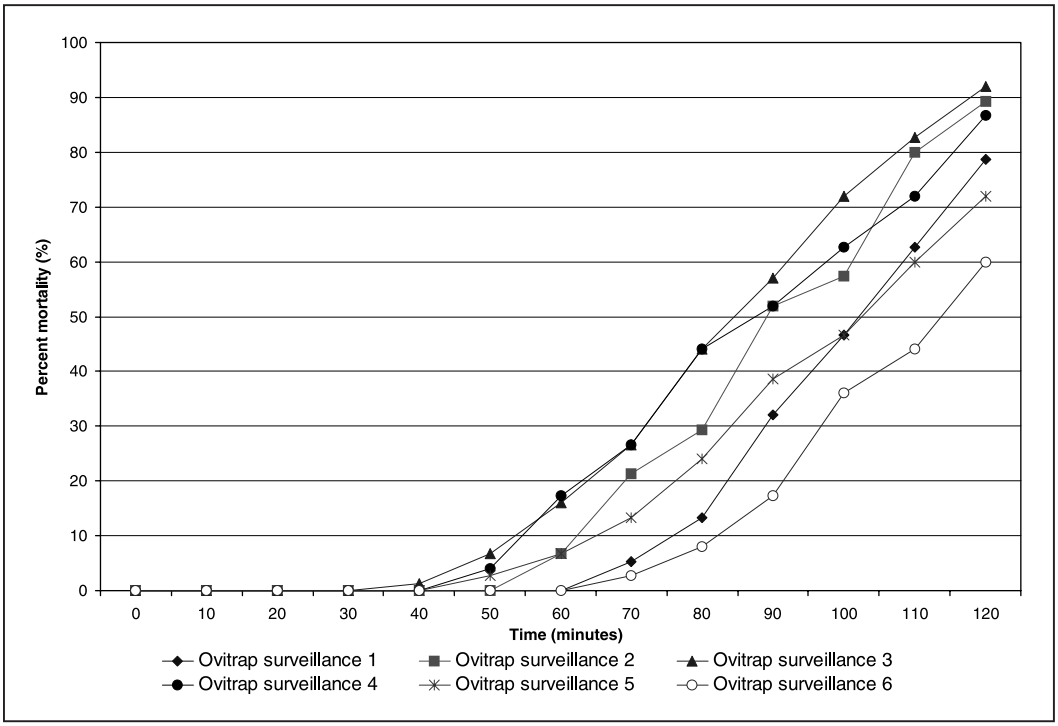

Figure 7. Percent mortality of *Ae. albopictus* from Kg. Banjar, Selangor exposed to 1 mg/L temephos for 120 minutes.

DISCUSSION

The simplest method for detecting resistance against insecticide is by using the diagnostic dosage test. The diagnostic dosage is a predetermined insecticide dose that is known to be lethal to a high proportion of susceptible individuals and tolerant to a high proportion of resistant individuals (Ferrari, 1996). In cases where control failure is evident in the field, the diagnostic dosage test can be used to confirm resistance.

Bioassay results obtained from 6 consecutive ovitrap surveillance (OS), with 0.012 mg/L temephos indicated weekly variations of the resistance status for *Ae. aegypti* and *Ae. albopictus* in Taman Samudera and Kg. Banjar. Similar variations in the resistant gene frequencies of Malaysian field *Culex quinquefasciatus* adults had also been reported by Lee & Tadano (1994). This time-dependent variations were supposedly due to the presence of vast gene pool in the field mosquito populations which continuously provide genetic variability to population at any particular point of time (Lee *et al.*, 1998).

Lima *et al.* (2003) used 0.012 mg/L temephos against adult *Ae. aegypti* and found that monitoring temephos adult bioassay for 120 minutes indicated 100% mortality was not achieved in any tested population.

Only one strain of *Ae. aegypti* from OS 3 in Kg. Banjar was susceptible to 0.012 mg/L temephos with 100% mortality, and one strain of *Ae. aegypti* from OS 2 showed possible resistance developed to temephos with 93.33% mortality. On the other hand, 24 hours test against 6 strains of *Ae. albopictus* and 4 strains of *Ae. aegypti* showed resistance to 0.012 mg/L temephos, which were less than 80%. Thus, this indicates the presence of resistance gene in the field population.

The development of resistance to temephos in *Ae. aegypti* larvae has been reported in Malaysia (Lee & Lime, 1989),

Thailand (Chareonviriyaphap *et al*., 1999; Paeporn *et al*., 2003), Cambodia (Polson *et al*., 2001), Vanuzuella (Mazzarri & Georghiou, 1995) and Brazil (Macoris *et al*., 2003); while development of the resistance to temephos in *Ae. albopictus* has also been reported in Malaysia (Lee *et al*., 1998; Nazni *et al*., 2000) and Thailand (Ponlawat *et al*., 2005). However, Luna *et al*. (2004) and Dalla *et al*. (1994) reported that *Ae. aegypti* obtained from Curitiba, Brazil in 2003 and *Ae. albopictus* obtained from Veneto, Italy were highly susceptible to temephos.

Resistance could be attributed to the continual selection pressure from control activities instituted by vector control programs to suppress the population of *Ae. aegypti* and *Ae. albopictus* by fogging as has been reported by Lee *et al*. (1996).

In our study, laboratory strain of *Ae. albopictus* was showing low mortality against diagnostic dosage of temephos, this may due to this strain was newly colonized and has been maintained for 8 generations only in the laboratory.

Strain resistant to a given chemical may be due to the result of genetic, operational or biological factors. According to de Carvalho *et al*. (2004), genetic and biological factors are characteristic of different populations and include the frequency and the dominant character of resistance genes, isolation, endogamy and the population's reproductive potential. On the other hand, Brogdon & McAllister (1998) reported that operational factors are due to the use of insecticides and may appear as a result of selection pressure.

Resistant genes are rare and after prolonged periods of selection, as individuals carrying susceptible alleles die (Brown, 1986). The degree of dominance of the resistant gene influences the growth of the insect populations under selective pressure. When the resistant gene is recessive, the growth of insect is slower; when it is dominant, the growth of insect is become faster (Georghiou & Taylor, 1977).

Lee (1994b) reported that sequentially applied higher dosages of insecticides are required to select against the resistant gene. There is therefore a need to redefine the diagnostic dosage of temephos against *Aedes* mosquitoes, as it appeared that resistance in the field populations are rather widespread in this mosquito. However, the results obtained from bioassay with 1 mg/L temephos tested against both *Ae. aegypti* and *Ae. albopictus*, complete mortality was achieved with 24 hours test. This again indicates that temephos is still a very effective larvicide against both *Ae. aegypti* and *Ae. albopictus*.

In Kg. Banjar (settlement area), there were numerous containers for water storage. All these containers should be treated with 1 mg/L of temephos (operational dosage) to avoid the breeding of *Aedes* larvae. Lee & Winita (1993) found that earthen jars treated with 1 mg/L of temephos were effective in causing complete larval mortality of *Ae. albopictus* up to 91 days post-treatment.

This study also implicated that larval *Ae. albopictus* were less susceptible than *Ae. aegypti* to temephos. This was similar as reported by Romi *et al*. (2003).

Ae. aegypti and *Ae. albopictus* susceptibility to temephos is changing from time to time. It is essential to continue monitoring the resistance status of dengue vector to insecticides in order to ensure the efficiency of the programs for vectors control and the protection of human health. Another possible alternative is the use of biological insecticide *Bacillus thuringensis israelensis* (*Bti*) as resistant to *Bti* had not been reported in any regions till today.

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