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Susceptibility of *Aedes aegypti* and *Aedes albopictus* to temephos in four study sites in Kuala Lumpur City Center and Selangor State, Malaysia

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Abstract. Larvae obtained from Taman Samudera (Gombak, Selangor), Kampung Banjar (Gombak, Selangor), Taman Lembah Maju (Cheras, Kuala Lumpur) and Kampung Baru (City centre, Kuala Lumpur) were bioassayed with diagnostic dosage (0.012 mg/L) and operational dosage (1 mg/L) of temephos. All strains of *Aedes aegypti* and *Aedes albopictus* showed percentage mortality in the range of 16.00 to 59.05 and 6.4 to 59.50 respectively, after 24 hours. LT_{50} values for the 6 strains of *Ae. aegypti* and *Ae. albopictus* were between 41.25 to 54.42 minutes and 52.67 to 141.76 minutes respectively, and the resistance ratio for both *Aedes* species were in the range of 0.68 to 1.82 when tested with operational dosage, 1 mg/L temephos. These results indicate that *Aedes* mosquitoes have developed some degree of resistance. However, complete mortality for all strains were achieved after 24 hours when tested against 1 mg/L temephos.

INTRODUCTION

Aedes aegypti (Linnaeus) and *Aedes albopictus* Skuse have been incriminated in the transmission of classical dengue fever (DF) and dengue haemorrhagic fever (DHF) in many urban areas of South-east Asia (Smith, 1956; Hammon, 1966; Rudnick, 1967). As in other South East Asia countries, *Ae. aegypti* and *Ae. albopictus* are widely distributed throughout Malaysia and have been incriminated as vectors of dengue (Lo & Narimah, 1984; Yap, 1984; Chan & Counsilman, 1985; Lee & Cheong, 1987; Rebecca, 1987; Lam, 1993; Lee & Inder, 1993). Their control is important in diseases prevention. Although chemical insecticides were successful in controlling mosquitoes in the past decades, the development of resistance has hampered their use.

Temephos or 0,0,0'0'-tetramethyl-0,0'-thiodiphenylene phosphorothiorate is an organophosphorus (OP) compound with very low mammalian toxicity and is not harmful to human when used at operational dosages (Laws *et al*, 1967). Temephos has been in use for control of mosquito larvae (*Ae. aegypti*, *Culex* spp and *Anopheles* spp) in portable water since the early 1970s. It has been useful in the control of dengue and dengue haemorrhagic fever, malaria and filariasis (WHO, 1991). In Malaysia, temephos (Abate®) is recommended as a larvicide by Ministry of Health and widely used since 1973.

Since this insecticide has been in use for a very long time, the susceptibility status of this insecticide is now being questioned by many researchers and the public. The objective of this study was to determine the susceptibility status of the mosquito population of *Ae. aegypti* and *Ae. albopictus* obtained from the dengue prone areas.

MATERIALS AND METHODS

Mosquito strains

The field strains were collected by using ovitraps from Taman Samudera (Gombak, Selangor), Kampung Banjar (Gombak, Selangor), Taman Lembah Maju (Cheras, Kuala Lumpur) and Kampung Baru (City center, Kuala Lumpur). These sites were selected since a large number of dengue cases have been reported from there.

Ovitrap surveillance

Ovitraps as described by Lee (1992a) was used in this surveillance. 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 indoor and outdoor. In this study, "indoor" is referred to the interior of the house, while "outdoor" referred to outside of the house but confined to the immediate vicinity of the house (Lee, 1992b). All ovitraps were collected after 5 days. 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 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 procedures recommended by WHO (1981) were used. Bioassay was conducted in 300 ml disposable paper cup. Twenty-five late 3rd or early 4th instar larvae were exposed to temephos in 250 ml distilled water. The cups were held at room temperature of 28°C and 70% relative humidity. At least 3 replicates of each dosage of temephos were conducted. The control (untreated) consisted of 1 ml of ethanol added to the distilled water. The larval mortality was recorded every 10 minutes until 120 minutes (2 hours). The larval mortality was also recorded 24 hours after exposure.

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 shows the percentage mortality of *Ae. aegypti* and *Ae. albopictus* from 4 study sites against 0.012 mg/L temephos (WHO diagnostic dosage) after exposure for 24 hours. The larvae of laboratory strain *Ae. aegypti* (F952), which has been colonized in the Medical Entomology Unit, Institute for Medical Research, showed 100% mortality to the diagnostic dosage of temephos (0.012 mg/L). All field strains of *Ae. aegypti* were resistant to temephos showing percentage mortality in the range of 16.00 to 59.02 after 24 hours. *Ae. aegypti* obtained from Taman Lembah Maju showed highest mortality among all the field strains, while *Ae. aegypti* obtained from Kg. Banjar was the most resistant strain with the lowest percentage mortality.

On the other hand, laboratory strain *Ae. albopictus* (F8) from Medical Entomology Unit, Institute for Medical

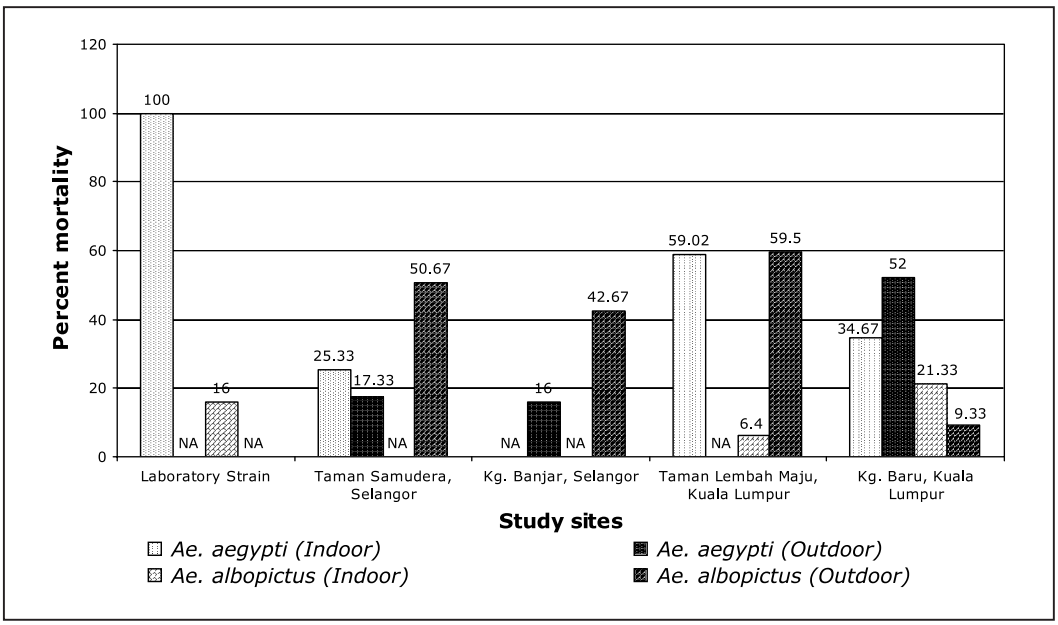

Figure 1. Percent mortality of *Aedes* sp. from 4 study sites to 0.012 mg/L temephos on exposure for 24 hours.

Research was highly resistant, showing percentage mortality of 16.00 when tested against diagnostic dosage of temephos. Outdoor and indoor *Ae. albopictus* obtained from Taman Lembah Maju indicated the highest and lowest percentage mortality among all field strains of *Ae. albopictus*, with 59.50 and 6.40 respectively, after 24 hours.

There was zero mortality within 2 hours exposure period to diagnostic dosage of temephos for both *Ae. aegypti* and *Ae. albopictus*.

Operational Dosage, 1 mg/L temephos

The LT_{50} of various strains of *Ae. aegypti* and *Ae. albopictus* from the 4 study sites exposed to 1 mg/L operational dosage of temephos is shown in Figure 2. The LT_{50} values are defined as the lethal time required to kill 50% of the test mosquitoes.

For *Ae. aegypti*, the LT_{50} values of the 6 strains (indoor and outdoor) were between 41.25 to 54.42 minutes. Most of the field strains required a longer time to be killed than the laboratory strain (42.31 minutes) when tested with operational dosage of temephos, except strains from Kg. Banjar and Kg. Baru showing 41.25 and 41.90 minutes respectively. However, complete mortality was observed within 2 hours for all the 6 strains of *Ae. aegypti* (Figure 3 to 6).

For *Ae. albopictus*, the LT_{50} values for the 6 strains were between 52.67 to 141.76 minutes. Strains from Taman Samudera and Taman Lembah Maju outdoor showed 65.81 and 52.67 minutes respectively, showing a shorter time to be killed incomparison to the laboratory strain showing 77.81 minutes. Only strain from Taman Lembah Maju outdoor showed complete mortality within 2 hours when tested against temephos at operational dosage (Figure 5). This indicated that *Ae. albopictus* from this site was the most susceptible strain among the 6 strains collected from the 4 study sites.

In comparison between species within the same study site, our study indicated that *Ae. aegypti* was more susceptible to temephos compared to *Ae. albopictus* as shown in Figure 2.

Table 1 shows the regression line and the resistance ratio (RR) of *Ae. aegypti* and *Ae. albopictus* from the 4 study sites against operational dosage of temephos. The RR for the 6 strains of *Ae. aegypti* and

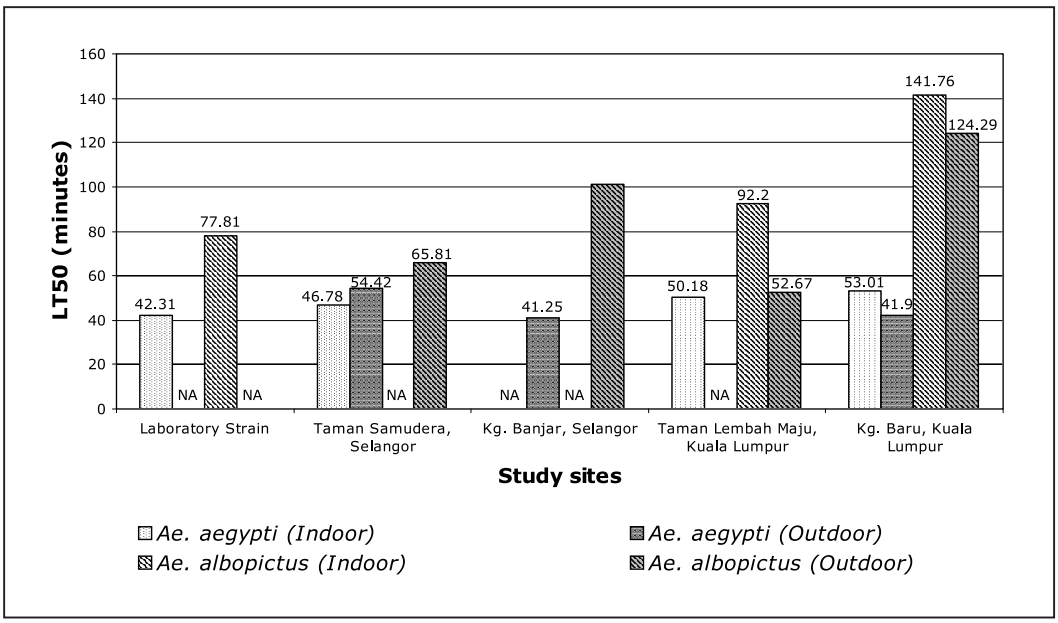

Figure 2. LT_{50} of *Aedes* sp. from 4 study sites exposed to 1 mg/L temephos.

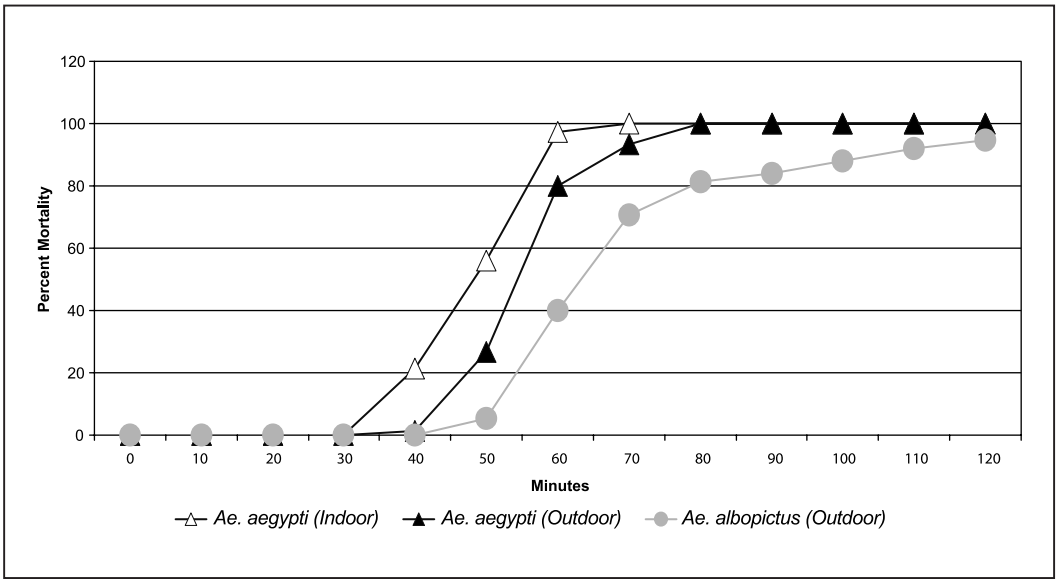

Figure 3. Percent mortality of *Aedes* sp. (Taman Samudera, Selangor) exposed to 1 mg/L temephos for 120 minutes.

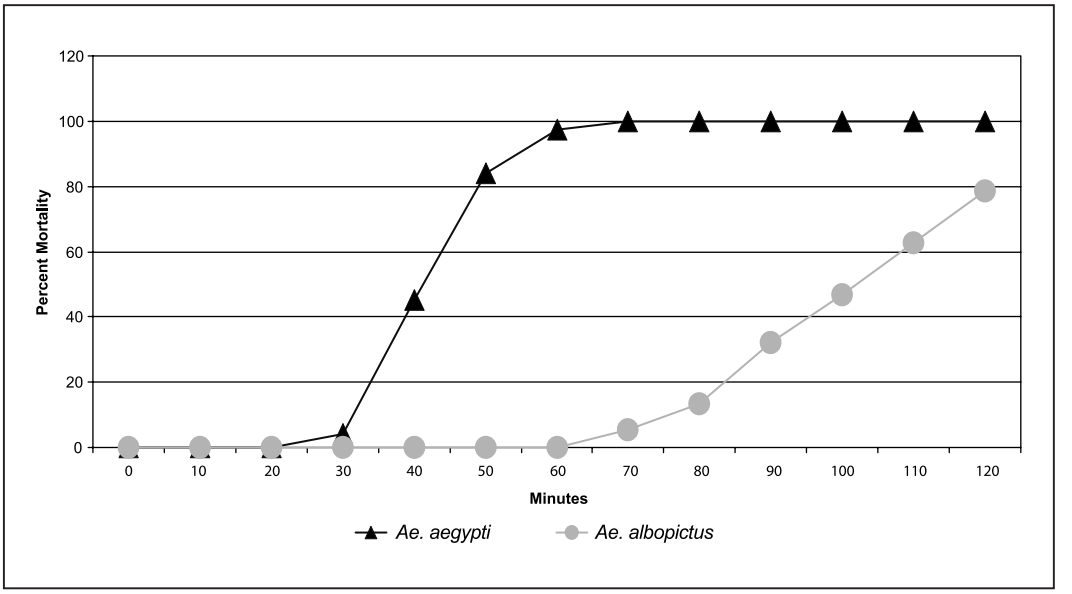

Figure 4. Percent mortality of *Aedes* sp. (Kg. Banjar, Selangor) exposed to 1 mg/L temephos for 120 minutes.

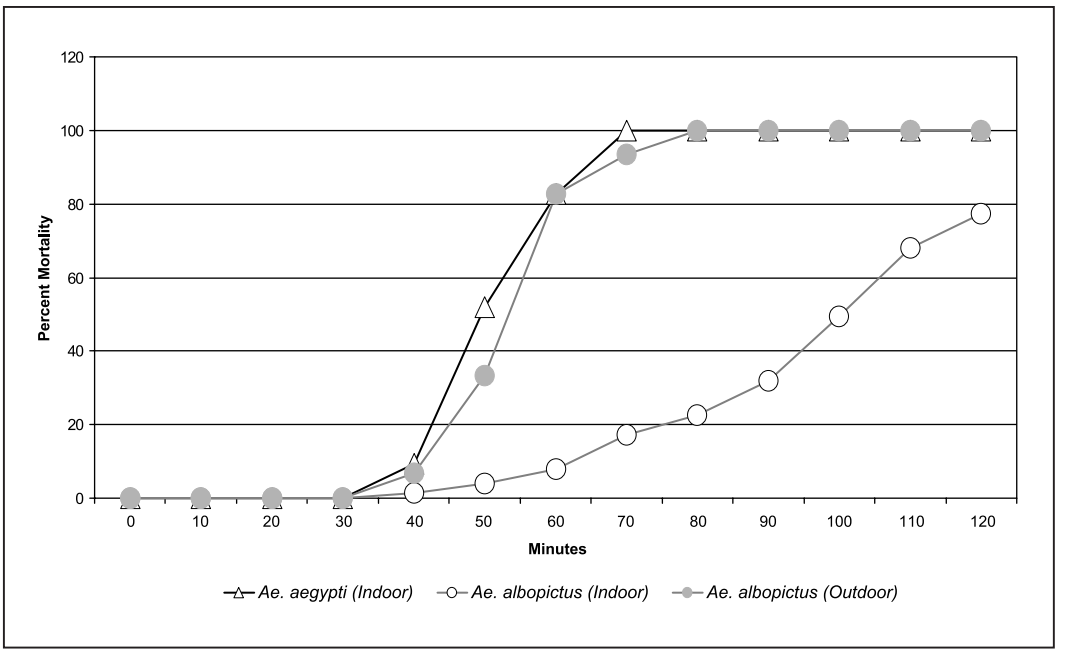

Figure 5. Percent mortality of *Aedes* sp. (Taman Lembah Maju, Kuala Lumpur) exposed to 1 mg/L temephos for 120 minutes.

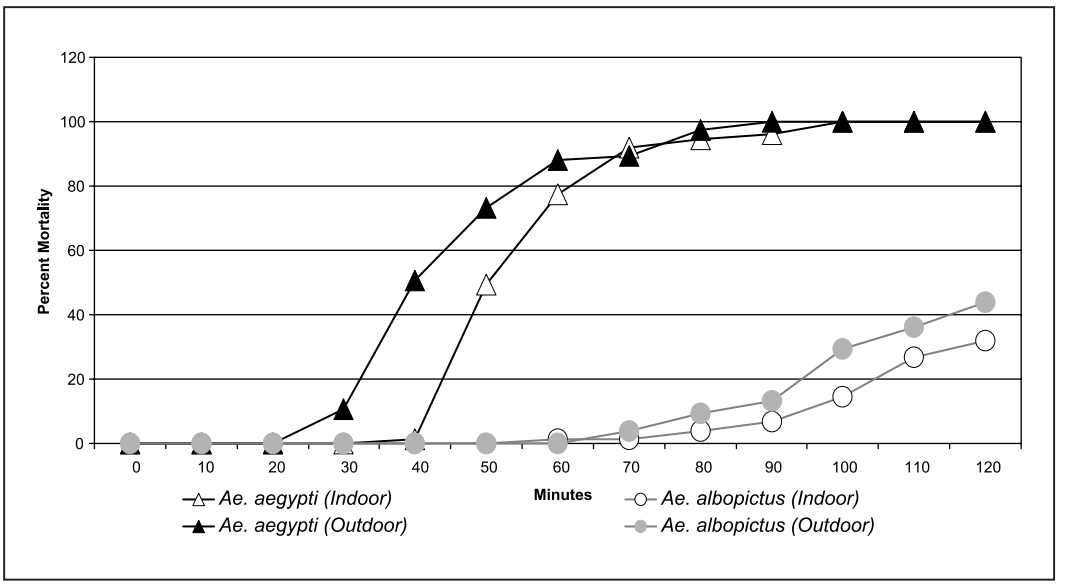

Figure 6. Percent mortality of *Aedes* sp. (Kg. Baru, Kuala Lumpur) exposed to 1 mg/L temephos for 120 minutes.

Ae. albopictus range between 0.97 to 1.29, and 0.68 to 1.82 respectively. Hence indicating a possibility of resistance development in the future. However, complete mortality for all strains were achieved after 24 hours.

DISCUSSION

The percentage mortality of all strains of *Ae. aegypti* and *Ae. albopictus* to the diagnostic dosage of temephos ranged from 6.40 to 59.50%, indicating that the mosquitoes have developed resistance. *Ae. aegypti* has been reported to show resistance to the diagnostic dosage of temephos (0.012 mg/L) in many countries (Brown, 1986; Rawlins, 1998; Campos & Andrade, 2001; de Carvalho *et al.*, 2004).

The use of temephos at 0.012 mg/L as the diagnostic dosage, was recommended by WHO (1992). However, variations in the absolute values of the diagnostic dosage have been used by laboratories around the world in the resistance monitoring program and sometimes, even among different tests performed by the same laboratory in different years. For instance, the laboratory located in Sao Paulo used 0.008 mg/L as the diagnostic dosage during 1999 monitoring and 0.012 mg/L for 2002/2003 assays (Braga *et al.*, 2004). Beside this, many of the researchers used 0.02 mg/L of temephos as diagnostic dosage (WHO, 1981), such as Polson *et al.* (2001) from Cambodia, Lee (1991) from Malaysia, Liew *et al.* (1994) from Singapore, and Paeporn *et al.* (2004) from Thailand. The researchers reported that temephos resistance in *Aedes* mosquitoes has been detected.

This study indicated that in some study sites, *Ae. aegypti* were more resistant than *Ae. albopictus* strains to 0.012 mg/L temephos. This could be due to the fact that this species prefers resting indoor, and this likely to be exposed to household insecticides while *Ae. albopictus*, prefers to rest outdoor in the vegetation. The frequencies of *Ae. aegypti* being in contact with the household insecticides in indoor is greater. Moreover the indoor environment has a smaller surface area and is enclosed. It is true that fogging activity is conducted during dengue cases but this is not done consistently. Hence the selection pressure has not been built up

Table 1. LT_{50}, regression line and resistance ratio of *Ae. aegypti* collected from 4 study sites tested against 1 mg/L temephos

Strain		*Ae. aegypti*			*Ae. albopictus*		
		LT_{50} (C.L.)	Regression line	Resistance ratio	LT_{50} (C.L.)	Regression line	Resistance ratio
Laboratory strain		42.31 (41.33 – 43.28)	Y = 15.51x – 175.29	–	77.81 (74.76 – 80.80)	y = 6.96x – 77.75	–
Taman Samudera	Indoor	46.78 (45.14 – 48.34)	Y = 13.79x – 155.91	1.11		NA	
	Outdoor	54.42 (52.87 – 55.97)	y = 15.79x – 180.26	1.29	65.81 (62.82 – 68.53)	y = 7.3x – 82.07	0.85
Kg. Banjar	Indoor		NA			NA	
	Outdoor	41.25 (39.70 – 42.77)	y = 12.13x – 135.89	0.97	101.34 (98.39 – 104.68)	y = 10.26x – 118.20	1.30
Taman Lembah Maju	Indoor	50.18 (48.45 – 51.97)	y = 12.81x – 144.89	1.19	98.20 (94.27 – 102.85)	y = 6.66x – 74.89	1.26
	Outdoor		NA		52.67 (50.99 – 54.34)	y = 13.27x – 150.54	0.68
Kg. Baru	Indoor	53.01 (51.03 – 54.89)	y = 10.37x – 116.56	1.25	141.76 (129.39 – 166.47)	y = 6.93x – 79.19	1.82
	Outdoor	41.90 (39.60 – 44.03)	y = 6.91x – 75.34	0.99	124.29 (116.69 – 137.39)	y = 7.01x – 79.80	1.60

C.L. = Confidence Limit. NA = Not Available

yet in *Ae. albopictus*. Moreover in outdoor site, it is an open space and the chances of *Ae. albopictus* not being hit by the insecticide droplets could be greater.

In Malaysia, the recommended dosage of Abate® sand granules applied to domestic stored water is 1g/10L water which is equivalent to 1 mg/L of active ingredient. This is about 83 folds higher than the WHO diagnostic dosage (0.012 mg/L). If applied accordingly, it is still highly effective for *Aedes* control (Lee & Lime, 1989).

The results of the bioassay indicated that some degree of resistance to operational dosage, 1 mg/L temephos existed in field strains of *Ae. aegypti* and *Ae. albopictus*, with a range of resistance ratio from 1.11 to 1.82. Thus, this implicates the emergence of resistance to temephos for both *Ae. aegypti* and *Ae. albopictus*. Although resistance was detected in this study, complete mortality was achieved for all strains within 24 hours. Thus, indicating Abate® is still effective under operational field conditions.

It is important to note that temephos may become completely ineffective at a dosage of 1 mg/L due to the emergence of higher levels of temephos resistance among the *Aedes* larva, as reported by Georghiou *et al.* (1987). The presence of resistance in the natural population may probably be due to the fact that temephos has been used for controlling both *Ae. aegypti* and *Ae. albopictus* since the DF and DHF outbreak in 1973 in Malaysia (Cheong, 1978). Moreover, a high level of resistance to temephos may also be due to the frequencies of fogging for mosquito control, as dengue cases have always been reported in all these study sites. Beside that, Lee *et al.* (1998) reported factor such as migration may also influence the susceptible individuals in the field population.

How rapidly an insecticide becomes ineffective will depend on the selection pressure for resistance, which is determined by how long and how often the insecticide is being used, how many breeding sites are treated and the dosage used (Hudson, 1983). Hudson (1983) also reported that in focal treatment (adding insecticide directly to the breeding ground), the development of resistance may be delayed by using high dose to kill the heterozygous resistant larvae as well as the susceptible larvae.

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