

Research Note

Laboratory bioefficacy of nine commercial formulations of temephos against larvae of *Aedes aegypti* (L.), *Aedes albopictus* Skuse and *Culex quinquefasciatus* Say

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Abstract. The bioefficacy of nine commercial formulations of temephos against *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* larvae were evaluated in the laboratory. WHO larval bioassay with operational dosage of temephos at 1 mg/L was performed. The larval mortality was recorded every 5 minutes until complete mortality was achieved. All formulations of temephos exhibited various toxicity level against *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus*. Generally, larvae of *Cx. quinquefasciatus* was susceptible to all formulations of temephos, followed by *Ae. aegypti* and *Ae. albopictus*.

Temephos or 0,0,0'0'-tetramethyl-0,0'-thiodiphenylene phosphorothiorate is one of a few registered organophosphates used to control mosquito larvae and is the only organophosphate exhibiting appreciable larvicidal activity. Temephos (Abate®) was first registered in 1965 as an emulsifiable concentrate for the control of mosquito larvae. Since then, several granular formulations have been developed (Health Council of the Netherlands, 2003).

Temephos is used to control mosquito, midge, gnat, punkie and sandfly larvae in non-potable water (stagnant, saline, brackish and temporary water bodies), waters that are high in organic contents, highly polluted water, including moist areas, woodland pools, shallow ponds, edges of lakes, swamps, marshes, tidal waters, intertidal zones, catch basins and tyre piles. It is also

used widely in portable water due to its low mammalian toxicity. The annual usage of temephos per year is approximately 25,000 to 40,000 pounds of active ingredient (Environmental Protection Agency, 2001)

The different formulations of temephos were developed to accommodate various conditions and equipment used in mosquito abatement programmes. Emulsifiable concentrates are designed for use in spray applications when there is unobstructed access to the breeding site. In cases where vegetation around the breeding area are dense, granular formulations were used to penetrate the target sites (Health Council of the Netherlands, 2003).

The use of temephos is one of the most important chemical methods of controlling vectors of medical importance. Larviciding is the first step in mosquito control, since

mosquitoes are killed at the breeding site, prior to dispersing and infesting a community. Temephos is very low in mammalian toxicity and is not harmful to human when used at operational dosages (Laws *et al.*, 1967). Temephos has been used for the control of mosquito larvae (*Aedes aegypti*, *Culex* spp. and *Anopheles* spp.) in drinking water since the early 1970s. It has been found useful in the control of dengue and dengue haemorrhagic fever, malaria and filariasis vectors (WHO, 1991).

The objective of this study is to evaluate the bioefficacy of nine commercial formulations of temephos against *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* larvae in the laboratory.

The susceptible strain of *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* obtained from insectarium of Medical Entomology Unit, Institute for Medical Research, Malaysia were used.

The nine commercial formulations of temephos used were: Abate[®] 1.1G (Malaysia), Creek[®] 1.0G (Malaysia), Abate[®] 1% SG (Thailand), Vactor[®] 1G (Thailand), Saixaco[®] 1G (Thailand), Lavifor[®] SG (Thailand), Chemfleetsanabate[®] (Thailand), SaiGPO-2[®] (Thailand) and Azai-SS[®] (Thailand). The dilutions were prepared as recommended by the manufacturer to produce the final total release dosage of 1 mg active ingredient per liter (1 mg a.i./L).

Larval bioassay was performed in accordance to the guidelines by WHO (1981). Bioassay was conducted in 300 ml disposable paper cups. 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±2°C and 70% relative humidity. The larval mortality was recorded every 5 minutes until complete mortality was achieved. At least 3 replicates of each dosage of temephos were conducted. The control (untreated) was distilled water. The test results obtained from bioassay were pooled and analysed using probit analysis of Raymond (1985) to obtain the lethal time values.

Table 1 showed the LT₅₀ and regression line of *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* exposed to nine

formulations of temephos. All formulations of temephos exhibited various toxicity level against *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus*. Abate[®] 1% SG (Thailand) exhibited higher toxicity against larvae of *Ae. aegypti* and *Ae. albopictus*, with LT₅₀ at 23.11 minutes and 59.69 minutes, respectively. On the other hand, Abate[®] 1.1% G (Malaysia) exhibited high toxicity against larvae of *Cx. quinquefasciatus*, with LT₅₀ at 17.74 minutes. However, low toxicity was exhibited by Azai-SS[®] against larvae of *Ae. aegypti* (LT₅₀ at 121.32 minutes), *Ae. albopictus* (81.34% larvae mortality after 24 hours post treatment) and *Cx. quinquefasciatus* (LT₅₀ at 68.61 minutes), in comparison to other commercial formulations of temephos. This study indicated that *Cx. quinquefasciatus* was highly susceptible to temephos, in comparison to *Ae. aegypti* and *Ae. albopictus*.

In Malaysia, the study on the susceptibility status of field collected *Ae. aegypti* larvae was conducted by Lee *et al.* since 1984. Their results indicated that the LC₅₀ values of field collected *Ae. aegypti* were about 2–3 folds higher than laboratory strain, showing some degree of tolerance to temephos (Abate[®]). However, LC_{99,9} values of all studied areas were much lower than diagnostic dosage. Thus, no significant resistance to temephos was detected.

A follow-up study was conducted by Lee & Lime (1989). Again, the LC₅₀ values of field collected *Ae. aegypti* larvae were higher than the laboratory strain, while the LC_{99,9} values of larvae collected from some areas were higher than the diagnostic dosage, indicating the emergence of very low resistance of these strains. However, there was no indication that temephos was ineffective at operational dosage.

Lee (1991) conducted a study on temephos susceptibility in *Ae. aegypti* larvae collected from Selangor, Perlis and Terengganu and, showed that the field strain *Ae. aegypti* was less susceptible to temephos relative to the laboratory strain with resistance ratio of 1.20 – 1.33.

A study on the susceptibility status of Singapore *Aedes* vectors against temephos

Table 1. Susceptibility of *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* larvae tested against 9 commercial formulations of temephos at a dosage of 1 mg/L

Temephos formulations	<i>Ae. aegypti</i> LT ₅₀ (95% C.L.)	<i>Ae. albopictus</i> LT ₅₀ (95% C.L.)	<i>Cx. quinquefasciatus</i> LT ₅₀ (95% C.L.)
Abate® 1.1G (Malaysia)	50.26 (48.77 – 51.71)	65.61 (62.04 – 69.06)	17.74 (16.66 – 18.82)
Creek® 1.0G (Malaysia)	74.04 (72.28 – 75.78)	81.35 (77.49 – 85.18)	29.32 (28.07 – 30.42)
Abate® 1% SG (Thailand)	23.11 (21.93 – 24.32)	59.69 (57.15 – 62.00)	22.25 (19.07 – 25.64)
Vactor® 1G (Thailand)	50.00 (46.65 – 53.55)	84.14 (81.81 – 86.51)	27.37 (26.11 – 28.43)
Chemfleetsandabate® (Thailand)	94.19 (92.07 – 96.26)	287.78 (262.13 – 311.71)	42.19 (39.57 – 44.38)
SaiGPO-2® (Thailand)	97.42 (95.30 – 99.50)	344.55 (326.54 – 366.26)	49.87 (48.67 – 51.01)
Saixaco® 1G (Thailand)	57.21 (55.07 – 59.25)	64.00% mortality within 8 hours exposure 89.33% mortality after 24 hours exposure	59.70 (56.64 – 62.48)
Lavifor® SG (Thailand)	58.14 (55.89 – 60.28)	77.34% mortality within 8 hours exposure 100% mortality after 24 hours exposure	65.87 (61.77 – 70.21)
Azai-SS® (Thailand)	121.32 (94.61 – 155.55)	6.67% mortality within 8 hours exposure 81.34% mortality after 24 hours exposure	68.61 (49.76 – 94.59)

C.L. = Confidence Limit

was conducted by Liew *et al.* (1994). They compared the resistance ratio of the larval population collected in 1993 to data from 1979, indicated a 3.5 fold increase in LD₅₀'s for both *Ae. aegypti* and *Ae. albopictus* and the LD₉₀ values had increased proportionately. *Ae. albopictus* was slightly more resistant than *Ae. aegypti*.

The susceptibility status in field-collected (Kuala Lumpur) *Ae. albopictus* larvae was reported by Lee *et al.* (1998). In this 10 weeks study, larvae of *Ae. albopictus* were highly susceptible to temephos,

although increased tolerance to temephos was detected once in the first week with resistance ratio 0.067 to 4.52.

Study conducted by Chen *et al.* (2005) shows some degree of *Aedes* resistance against operational dosage of temephos, indicating the emergence of temephos resistance in *Aedes* populations obtained from the field. Their study also showed that *Ae. albopictus* was more resistant towards temephos, in comparison to *Ae. aegypti*.

Generally, the recommended field dosage of 1.0 mg/L is still effective in

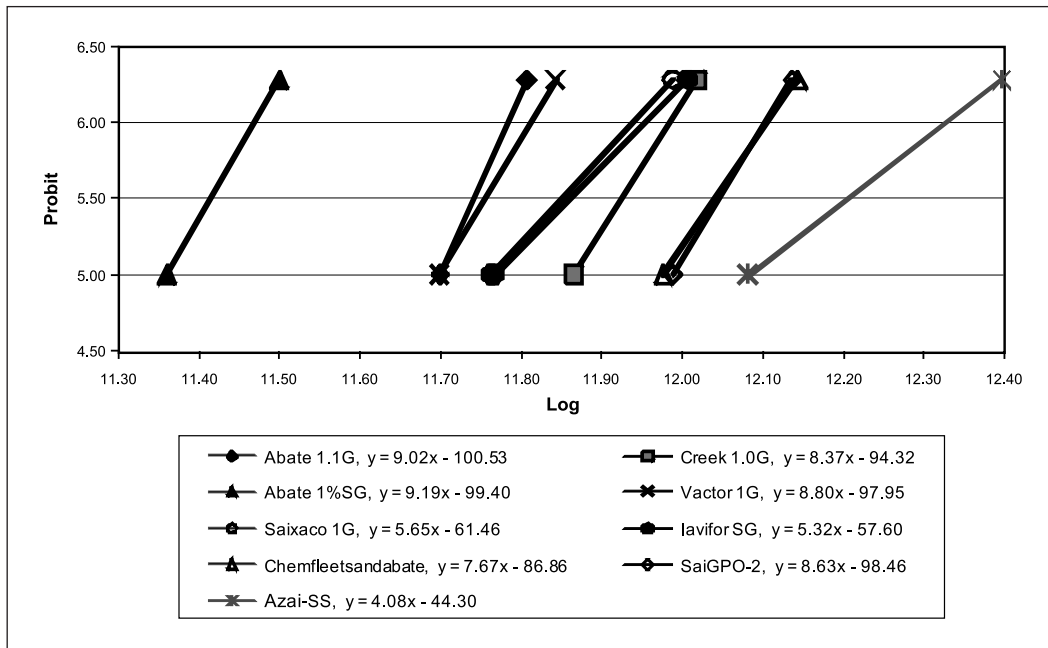


Figure 1. Regression lines of *Ae. aegypti* larvae tested against 9 formulations of 1 mg/L temephos.

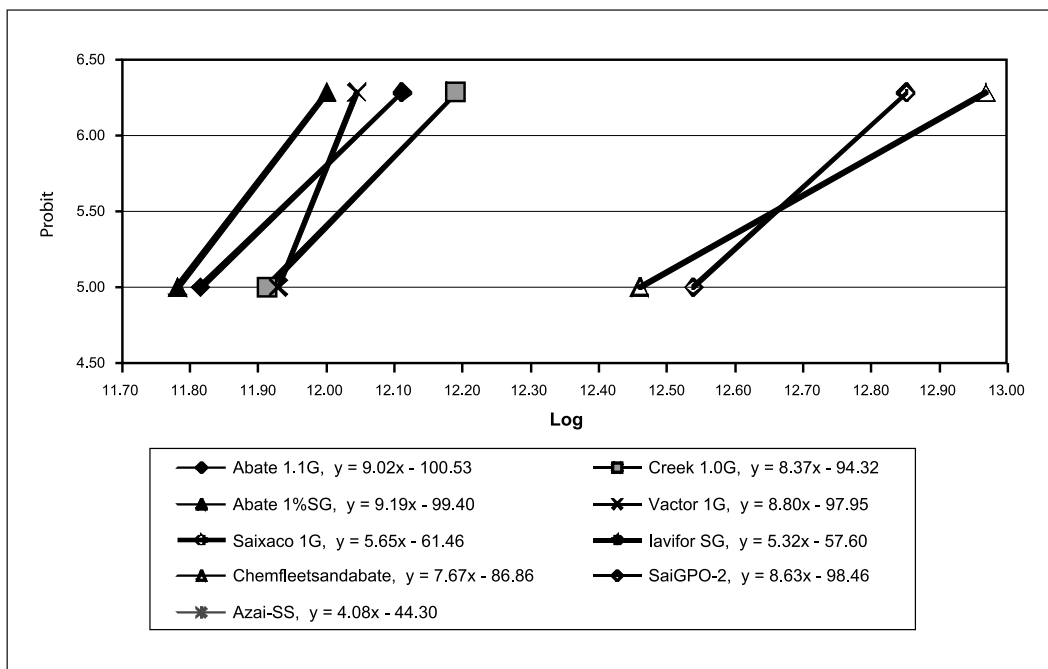


Figure 2. Regression lines of *Ae. albopictus* larvae tested against 9 formulations of 1 mg/L temephos. No regression lines were obtained from *Ae. albopictus* larvae tested against Saixaco® 1G, Lavifor® SG and Azai-SS®.

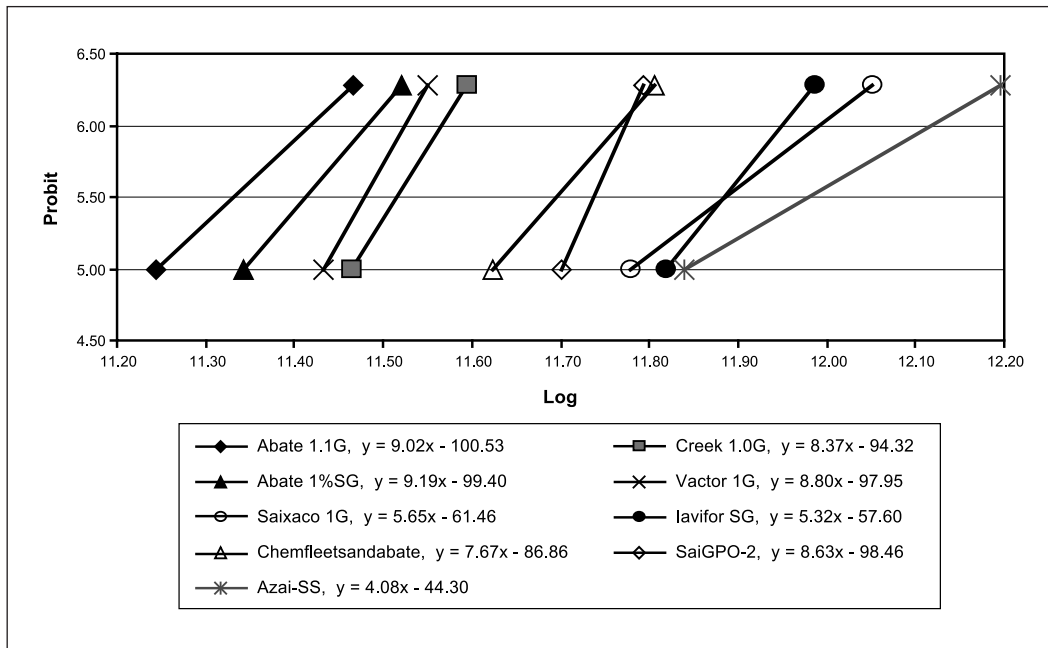


Figure 3. Regression lines of *Cx. quinquefasciatus* larvae tested against 9 formulations of 1 mg/L temephos.

controlling the vectors, but many reports on the decreasing susceptibility of *Aedes* larvae against temephos were reported within the previous 2 decades. Thus, this study suggested that standardization on manufacture procedure of insecticides is needed. Besides, the stability of insecticides and the duration of their effectiveness are also determined by the storage temperature and moisture / humidity (Fishel, 2002). Protection from temperature extremes is important because either freezing or excess heat can shorten the shelf life of insecticides and may reduce their effectiveness. In addition, the insecticides containers should not be stored where they are exposed to direct sunlight, to avoid overheating and degradation of insecticides (Robertson & Pope, 2005; Ogg *et al.*, 2007). Fishel (2001) reported that granule formulations are not affected by low temperature, while moisture is the greatest factor affecting their storage, as it can cause caking that may reduce the effectiveness of the insecticides.

The use of same insecticide exhibiting different level of toxicity may cause early emergence of insecticide resistance and finally failure in controlling the vectors. The field evaluation of temephos should be conducted from time to time, to ensure the efficacy and residual activity of temephos formulations applied in this region are still effective in suppressing the vector populations.

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