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SCIENTIFIC NOTE

SUSCEPTIBILITY OF ADULT FIELD STRAINS OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN SINGAPORE TO PIRIMIPHOS-METHYL AND PERMETHRIN

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ABSTRACT. The susceptibilities of field strains (F1) of *Aedes aegypti* and *Ae. albopictus* adult females to pirimiphos-methyl and permethrin were investigated and compared with a susceptible laboratory strain (S) by using a filter paper (12 × 15 cm) impregnation method. The resistance ratio between the 50% lethal concentration values (RR_{50}) of the F1 and the S strains shows that the RR_{50} of F1 *Ae. aegypti* for pirimiphos-methyl was 1.5 and the RR_{50} of F1 *Ae. aegypti* for permethrin was 12.9. The RR_{50} of F1 *Ae. albopictus* for pirimiphos-methyl was 1.4, and that for permethrin was 1.8. This indicates that the field strain *Ae. aegypti,* but not *Ae. albopictus,* has developed resistance to permethrin. However, both species are still susceptible to pirimiphos-methyl. Therefore, control with pirimiphos-methyl will still be effective.

KEY WORDS *Aedes aegypti, Aedes albopictus,* pirimiphos-methyl, permethrin, insecticide resistance

Aedes aegypti (L.) and *Ae. albopictus* (Skuse) are the primary and secondary vectors, respectively, of dengue fever and dengue hemorrhagic fever in Singapore. The control strategy is one that integrates source reduction, public education, law enforcement, and insecticide treatment (Tan 1997). Insecticide treatment is not used routinely; it is used only during outbreaks where fast elimination of infected adult mosquitoes is needed to curtail disease transmission. Excessive use of insecticides may result in the development of resistance in the vectors, thereby resulting in ineffective fogging.

Resistance in *Ae. aegypti* and *Ae. albopictus* has been reported in many countries within the Asia Pacific region, such as Malaysia, Thailand, Vietnam, Japan, and China, and in the South American countries, such as Venezuela and the Caribbean Islands (WHO 1986, 1993; Wu et al. 1992; Mazzarri and Georghiou 1995; Rawlins and Joseph 1995). In Singapore, Ong et al. (1980) reported that field *Ae. aegypti* were resistant to DDT, dieldrin, and propoxur, whereas field *Ae. albopictus* was resistant to fenitrothion, DDT, and dieldrin.

During the 1970s, malathion was used in Singapore to control adult *Aedes* mosquitoes. The *Ae. aegypti* and *Ae. albopictus* recorded 100% mortalities for malathion at a dosage of 5.0% (Ong et al. 1980). However, the use of malathion was discontinued because residents detested the smell of the insecticide. Hence, bioresmethrin, a synthetic pyrethroid insecticide, replaced malathion. In the 1980s, bioresmethrin was replaced by permethrin (Reslin® 50E [17% w/v permethrin]). In 1991, when permethrin resistance was detected in field *Ae. aegypti* (Vector Control and Research Department [VCRD], unpublished data), the organophosphorous insecticide pirimiphos-methyl was chosen to replace permethrin. Pirimiphos-methyl has been used in the *Aedes* control program for fogging for approximately 9 years now. This study was done to assess the susceptibility of *Ae. aegypti* and *Ae. albopictus* to pirimiphos-methyl and permethrin in Singapore.

Field strains of *Ae. aegypti* and *Ae. albopictus* larvae were collected during our daily survey (over a period of 6 months) of breeding sites at various areas of Singapore. They were reared to adults and bloodfed for egg-laying. The first generation adults (F1) were used for the tests. Long-established laboratory strains (S) of *Ae. aegypti* and *Ae. albopictus* of Singapore origin (since 1994) were used as susceptible reference strains.

Batches of approximately 1,000 F1 eggs on filter paper from each colony of *Ae. aegypti* and *Ae. albopictus* were placed in plastic trays containing aged tap water. Small pieces of boiled ox liver and yeast granules were given to the larvae as food. Adults were fed with a 10% sugar solution. The laboratory conditions were kept at 25.8 ± 1.40°C and 79.5 ± 5.20% relative humidity.

Pirimiphos-methyl (92% w/w; Zeneca Agrochemicals, Maldstone, United Kingdom) and permethrin (97.21% w/w; Hoechst Schering AgrEvo GmbH, Environmental Health, D-65926 Frankfurt, Germany) diluted in acetone were used to prepare a series of concentrations. The concentrations (% w/w) of pirimiphous-methyl for F1 *Ae. aegypti* were 0.028, 0.041, 0.052, 0.061, and 0.092, and those for S *Ae. aegypti* were 0.012, 0.018, 0.037, 0.045, and 0.061. Series of concentrations (% w/w) of pirimiphos-methyl for F1 and S *Ae. albopictus* were prepared at 0.008, 0.009, 0.012, 0.015, and

Table 1. Susceptibility of adult *Aedes aegypti* and *Aedes albopictus* field and susceptible laboratory strains to pirimiphous-methyl, 1998.[1]

Species	Strain	No. adults tested	LC_{50} (95% CL)	LC_{95} (95% CL)	RR_{50}[2]	RR_{95}[3]
Aedes aegypti	S-Lab	1800	0.0320 (0.0308–0.0331)	0.0587 (0.0558–0.0623)	1.5	1.4
	Field	1800	0.0478 (0.0466–0.0491)	0.0819 (0.0780–0.0867)		
Aedes albopictus	S-Lab	2250	0.0098 (0.0089–0.0107)	0.0138 (0.0115–0.0166)	1.4	1.6
	Field	1350	0.0137 (0.0133–0.0141)	0.0219 (0.0208–0.0233)		

[1] LC, lethal concentration; RR, resistance ratio; CL, confidence limit; S-Lab, susceptible laboratory strain.
[2] RR_{50} was calculated by dividing the field-strain LC_{50} with susceptible strain LC_{50}.
[3] RR_{95} was calculated by dividing the field-strain LC_{95} with susceptible strain LC_{95}.

0.021 and 0.005, 0.008, 0.009, 0.012, and 0.015, respectively. For permethrin, the series of concentrations prepared for F1 and S *Ae. aegypti* were 1.940, 3.240, 4.860, 6.940, and 9.270 and 0.050, 0.100, 0.500, 1.000, and 1.500 respectively; those for F1 and S *Ae. albopictus* were 0.050, 0.080, 0.120, 0.250, and 0.500 and 0.025, 0.036, 0.050, 0.071, and 0.125, respectively. Exactly 3 ml of each concentration was introduced onto a stainless steel tray (19.8 × 13.3 cm). A piece of filter paper (Whatman #1, 15 × 12 cm) was placed on it immediately to absorb the solution. The filter paper was dried for 1 h and sealed in aluminium foil. The control papers were coated only with acetone. All papers were used within 3 days of their preparation.

The test procedures were modified from WHO standard methods (WHO 1981). Sugar-fed females 3–7 days old were used for the tests. Twenty-five female mosquitoes were introduced into each test kit lined internally with paper impregnated with 1 concentration of insecticide. The females were exposed to the insecticide for 1 h and then transferred to holding kits with clean filter paper covering the internal walls. Their mortality was observed at 24 h. Small cotton pads soaked in 10% sugar solution were placed on top of each test kit as food. A series of 5 concentrations and a control were used in the tests for each strain of mosquitoes. There were 3 replicates for each concentration. Three or more tests were carried out for each strain of mosquitoes.

Mortality data were analyzed by using the probit analysis program by Michel Raymond (1987), based on Finney (1952). Resistance ratios (RR), which are the ratios of the 50% lethal concentration (LC_{50}) values of the field strains to the LC_{50} values of the respective susceptible strains, were determined on the basis of the laboratory-susceptible strains of each species for each insecticide.

The values of LC_{50}, LC_{95}, and RR_{50} for pirimiphos-methyl in *Ae. aegypti* and *Ae. albopictus* adults are presented in Table 1. The RR_{50}s of F1 *Ae. aegypti* and *Ae. albopictus* to pirimiphos-methyl were 1.5 and 1.4, respectively. The small ratio indicates that *Ae. aegypti* and *Ae. albopictus* are still susceptible to pirimiphos-methyl. This finding further confirmed that Actellic® EC_{50} (pirimiphos-methyl) in *Aedes* control program—used since November 1992—is still effective in Singapore.

The susceptibility statuses of field *Ae. aegypti* and *Ae. albopictus* to permethrin are presented in Table 2. The results show that the RR_{50}s of *Ae. aegypti* and *Ae. albopictus* are 12.9 and 1.8, respectively. Similarly, the RR_{95} for *Ae. aegypti* is also higher than *Ae. albopictus,* i.e., 8.9 and 2.6, respectively. The high resistance ratio of field *Ae. aegypti* indicates that the field *Ae. aegypti* population still shows a relatively high resistance level for permethrin.

In 1991, Reslin 50E (permethrin) was found to be less effective in controlling *Ae. aegypti* field populations by use of thermal fogging (VCRD, unpublished data). The World Health Organization (WHO) bioassay using 0.25% w/v permethrin-impregnated papers (supplied by WHO) was performed on *Ae. aegypti* to determine their susceptibility. The percentage mortality of *Ae. aegypti* adults exposed to the papers for an hour was only 0.5% (VCRD, unpublished data). The current susceptibility test of *Ae. aegypti* to permethrin also showed a high resistance ratio, with an RR_{50} of 12.9

Table 2. Susceptibility of adult *Aedes aegypti* and *Aedes albopictus* field and susceptible laboratory strains to permethrin, 1999.[1]

Species	Strain	No. adults tested	LC_{50} (95% CL)	LC_{95} (95% CL)	RR_{50}[2]	RR_{95}[3]
Aedes aegypti	S-lab	1800	0.2876 (0.19308–0.4282)	1.6587 (0.8628–3.2086)	12.9	8.9
	Field	1800	3.7171 (3.1141–4.4213)	14.7556 (9.859–22.645)		
Aedes albopictus	S-Lab	1350	0.0686 (0.0651–0.0725)	0.1753 (0.1563–0.2016)	1.8	2.6
	Field	1350	0.1219 (0.11356–0.13084)	0.4632 (0.4022–0.5487)		

[1] LC, lethal concentration; RR, resistance ratio; CL, confidence limit; S-Lab, susceptible laboratory strain.
[2] RR_{50} was calculated by dividing the field-strain LC_{50} with susceptible strain LC_{50}.
[3] RR_{95} was calculated by dividing the field-strain LC_{95} with susceptible strain LC_{95}.

(Table 2). This indicates that the resistance of the F1 *Ae. aegypti* population in Singapore to permethrin still persists even though they had not been exposed to this insecticide for 9 years. The decrease in sensitivity of field *Ae. aegypti* to permethrin is likely to be the result of the species' long-term exposure to this insecticide in the 1980s.

For the field strain of *Ae. albopictus,* the resistance ratios (RR_{50} and RR_{95}) are small, although there is a significant difference to the susceptible laboratory strain in a parallelism test ($P \leq 0.05$). There might be low selection pressure for *Ae. albopictus* to the insecticide because this species is often found outdoors, whereas the *Aedes* adult control measure in the 1980s that used fogging machines and permethrin was targeted at the indoor *Ae. aegypti.*

Pirimiphos-methyl has been used in adult *Aedes* control for the last 9 years. Current susceptibility tests of both field *Ae. aegypti* and *Ae. albopictus* to pirimiphos-methyl show that there is no evidence of resistance to this insecticide. However, as permethrin possesses less of a hazard to humans than organophosphates, its reintroduction into the mosquito control program in Singapore will be appreciated. Therefore, the effectiveness of permethrin to field *Aedes* mosquitoes will still be monitored closely, with a view to revert back to its use at an appropriate time.

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