

CONTROL OF MOSQUITO VECTORS OF TROPICAL INFECTIOUS DISEASES: (2) PYRETHROID SUSCEPTIBILITY OF *Aedes aegypti* (L.) COLLECTED FROM DIFFERENT SITES IN THAILAND

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Abstract. Four strains (SS, BS, A and B) of *Aedes aegypti* collected from different sites in Bangkok and at different times were examined for their pyrethroid susceptibility. Mosquito coils containing *dl, d*-T80-allethrin, *d, d*-T-prallethrin and methoxymethyl-tetrafluorobenzyl tetramethyl-cyclopropanecarboxylate (K-3050) with or without a synergist were tested by the 25 m³ semi-field test method. One strain (SS) was the most susceptible with KT_{50} values of about <30 minutes for all mosquito coils, while the other three strains (BS, A and B) were found to be around 10 to 20 times more tolerant to pyrethroids than the SS strain. A similar tendency for the pyrethroid susceptibility of the four strains was obtained with tests by topical application method. In field efficacy tests, mosquito coils with *d, d*-T-prallethrin 0.20% plus N-(2-ethylhexyl)bicyclo-[2,2,1]-hept-5-ene-2,3-dicarboximide as a synergist exhibited a repellent effect of about 85%, while those with K-3050 0.10% plus the synergist exhibited a greater repellent effect of about 90%. In contrast, the repellent effect of commercial *dl, d*-T80-allethrin 0.20% coils was as low as about 50%. The *d, d*-T-prallethrin and K-3050 coils with the synergist were confirmed to be highly effective in repelling *Ae. aegypti*.

INTRODUCTION

Dengue fever, an infectious disease transmitted by mosquitoes, is prevalent in South-east Asia, India, Africa, and the Americas, and is expanding worldwide. As a control measure, it is essential to prevent mosquito bites since the development of an effective vaccine against dengue is still in the future. According to one study (Eshita, 1997), *Ae. aegypti*, the main vector for dengue, comprises more than 90% of the total number of mosquito species

collected from houses in northeast Thailand. Given these findings, mosquito coils containing several pyrethroids as active ingredients are an important way to control *Ae. aegypti* mosquitoes, which are active in the daytime.

We reported the efficacy of mosquito coils containing several pyrethroids and a synergist against three tropical mosquito species in a previous paper (Katsuda *et al*, 2008). In this paper, the pyrethroid susceptibilities of four *Ae. aegypti* strains collected from different sites in Bangkok were reported based on the KT_{50} values of pyrethroid mosquito coils in the 25 m³ test room and the LD_{50} values of the three pyrethroids used in the tested mosquito coils by topical application. The field efficacy test results in districts A are also presented in this paper.

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MATERIALS AND METHODS

Tested mosquito coils

All the tested mosquito coils used in this study were prepared using pyrethrum powder as a combustible, and *Machilus thunbergii* powder (Tabu powder) and starch as binders, by the conventional techniques. Each mosquito coil weighed about 13 g and had a burning time of about 7 hours. The three pyrethroids and their doses in the mosquito coil formulations were as follows: 1). *dl, d*-T80-allevethrin, 0.50% weight by weight (w/w); 2). *d, d*-T-prallethrin (Katsuda, 1971, 1977), 0.10 and 0.20% (w/w); and 3). methoxymethyltetrafluorobenzyl tetramethylcyclopropanecarboxylate (K-3050; Katsuda *et al*, 2001), 0.05 and 0.10% (w/w).

In addition to each one containing a pyrethroid, a synergist, S:N-(2-ethylhexyl)bicyclo [2,2,1]hept-5-ene-2,3-dicarboximide, was also added at a ratio of 2 times the active ingredient for testing.

Commercially available *dl, d*-T80-allevethrin 0.20% mosquito coils purchased in Bangkok were also tested in this study for comparative purposes.

The LC₅₀ values in rats for the acute inhalation toxicity of mosquito coils containing *d, d*-T-prallethrin 0.20% plus S or K-3050 0.10% plus S were both above >5,000 mg/m³, similar to *dl, d*-T80-allevethrin coils in commercial use worldwide.

Test mosquitoes

The four *Aedes aegypti* strains used in this study were BS strain (Thung Khru District, Bangkok, 2005), SS strain (Pom Prap Sattru Phai District, Bangkok, 1977), A strain (Thung Khru District, Bangkok, 2007), and B strain (Tha Phra, Bangkok Yai District, Bangkok, 2007).

All mosquito strains were reared in the insectarium of the Department of Medical Entomology, Faculty of Tropical Medicine,

Mahidol University.

Semi-field test (25 m³) of the mosquito coils

For this study, the semi-field test method was adopted to evaluate the test for good reproductivity and reliability. The tests were conducted in a test room (25 m³) in the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University in Bangkok, at an average daily temperature of 25-27°C and a relative humidity of 70-80%.

White paper was laid on the floor of the test room (25 m³). One hundred female mosquitoes (3-6 days old, sucrose-fed) were released into the room and a test mosquito coil was lit in the center of the floor. Mosquitoes found on the floor of the room (knocked down mosquitoes) were counted from the beginning of the experiment. After 120 minutes of exposure, all mosquitoes found on the floor were transferred to a clean container with absorbent cotton soaked in a 3% sugar solution, and kept at 26±2°C for 24 hours to observe for mortality. The process was replicated 3 to 5 times per formulation. KT₅₀ values were determined by time and percent knocked down, using the Bliss's probit method (Bliss, 1937).

Topical application test

Mosquitoes were anesthetized at low temperature. An acetone solution of 0.25 µl containing different doses of active ingredients was topically applied to the dorsal surface of the abdomen of the insects. The mosquitoes were maintained at 26±2°C for 24 hours and observed for mortality.

LD₅₀ values were determined based on the dosage, and mortality was calculated according to Bliss's probit method (Bliss, 1935).

Field efficacy test

Field efficacy tests were conducted in District A in Bangkok where dengue was prevalent for the previous 2 years. The trial was conducted from July 17 to August 24, 2007.

Landing counts were carried out on at 4

human subjects at 4 plots (a control plot and 3 coil-used plots) in one week, and repeated for 4 weeks using 4 houses.

For each plot, each human subject was seated in the house for 4 hours from 9:00 AM to 01:00 PM, and all landing *Ae. aegypti* mosquitoes on the human subject during that time were collected.

The first of 4 consecutive test days per week was allotted to the control plot, the second day to *d, d*-T-prallethrin 0.20% plus S coil plot, the third day to the K-3050 0.10% plus S coil plot and the fourth day to the commercial *dl, d*-T80-allethrin 0.20% coil plot. After completing this set of 4 test plots per week per house, the 4 human subjects rotated houses to compensate for the variation in attractiveness to mosquitoes. Thus each field efficacy test covered a period of 4 weeks, making a total number of 64 test plots.

To evaluate the mosquito coils, the mean number of collected mosquitoes per one house on one human subject was calculated. Then the repellent effects for the test plots against the control plot were determined according to the following formula:

$$\text{Repellent effect (\%)} = \frac{M_1 - M_2}{M_1} \times 100$$

where M_1 = Mean no. of collected mosquitoes from the control plot,

M_2 = Mean no. of collected mosquitoes from the test plot.

RESULTS

Semi-field test of the mosquito coils

Table 1 shows the bioefficacy of mosquito coils against *Ae. aegypti* strains in the semi-field tests.

Mosquitoes were exposed to volatilized pyrethroids for 120 minutes and KT_{50} values for >120 minutes were considered as a presumed value. Therefore, mosquito coils with

KT_{50} values <100 minutes were defined as "effective".

Based on the KT_{50} values for the mosquito coils, the SS strain was the most susceptible among these *Ae. aegypti* strains as the KT_{50} values for all the mosquito coils were about <30 minutes with a rapid knock down effect. The BS strain was found to be around 10 to 20 times more tolerant to pyrethroids than the SS strain.

The test results showed that the KT_{50} values for mosquito coils containing *d, d*-T-prallethrin 0.20% plus S and K-3050 0.10% plus S against the A and B strains were close to those against the BS strain, confirming the high practicability of these mosquito coils.

However, the bioefficacy of the *dl, d*-T80-allethrin 0.20% coils commercially available in Bangkok was extremely low against the A and B strains and its KT_{50} values were not determined since the percentage of knocked down insects even after 120 minutes' exposure was 0%.

Topical application test

Table 2 shows test results of the three pyrethroids against four strains of *Ae. aegypti* strains by topical application.

The pyrethroid susceptibility of the SS strain was the greatest based on the LD_{50} values obtained. BS, A and B strains showed almost the same levels of susceptibility, being around 10 to 20 times more tolerant to pyrethroids than the SS strain. This finding was consistent with the test results of the mosquito coils in the 25 m³ test room semi-field tests.

Field efficacy test

Table 3 shows the mean numbers of collected (landing) *Ae. aegypti* mosquitoes per house / per human subject in the A district tests, and Fig 1 is a graph of their variations.

The number of collected mosquitoes for the control plot varied the greatest, and the descending order of the bioefficacy of the coils

Table 1
Bioefficacy of mosquito coils against *Ae. aegypti* strains in the 25 m³ semi-field tests.

Tested mosquito coil (w/w %)	Strains of <i>Ae. aegypti</i>							
	SS		BS		A		B	
	KT ₅₀ (min)	KT ₅₀ (min)	Ratio (BS/SS)	KT ₅₀ (min)	Ratio (A/SS)	KT ₅₀ (min)	Ratio (B/SS)	
<i>dl, d</i> -T80-Allethrin	0.20 ^a	-	ND ^c	ND	ND			
	0.50	12.9	170	13.1	-	-		
<i>d, d</i> -T-Prallethrin	0.10	20.7	171	8.3	-	-		
	0.10+ S ^b	11.9	120	11.8	-	-		
	0.20	6.1	130	21.4	-	-		
K-3050	0.20+ S	6.0	85	14.1	102	17.0	98 16.3	
	0.05	31.9	353	11.1	-	-		
	0.05+ S	13.6	184	13.5	-	-		
	0.10	8.6	117	13.6	-	-		
	0.10+ S	7.8	67	8.6	66	8.5	59 7.6	

^aA product commercially available in Bangkok.

^bN-(2-ethylhexyl)bicyclo[2,2,1]hept-5-ene-2,3-dicarboxyimide; amount = 2 times the active ingredient.

^cNot determined.

Table 2
LD₅₀ values of several pyrethroids against *Ae. aegypti* strains by topical application method.

Pyrethroid	Strains of <i>Ae. aegypti</i>							
	SS		BS		A		B	
	LD ₅₀ (µg/insect)	LD ₅₀ (µg/insect)	Ratio (BS/SS)	LD ₅₀ (µg/insect)	Ratio (A/SS)	LD ₅₀ (µg/insect)	Ratio (B/SS)	
<i>dl, d</i> -T80-Allethrin	0.0092	0.0637	6.9	0.0702	7.6	0.0925	10.1	
<i>d, d</i> -T-Prallethrin	0.0011	0.0181	16.5	0.0169	15.4	0.0192	17.5	
K-3050	0.0013	0.0196	15.1	0.0154	11.8	0.0305	23.5	

Table 3
Reduction in the number of collected mosquitoes by use of mosquito coils in field tests conducted in District A.

Active ingredients in tested mosquito coil repellent effect (w/w %)	Mean no. of collected mosquitoes (mosquitoes/house/person)	Repellency %
<i>dl, d</i> -T80-Allethrin	0.20 ^a	49
<i>d, d</i> -T-Prallethrin	0.20+ S ^b	83
K-3050	0.10+ S	87
Control	-	-

^aA product commercially available in Bangkok.

^bN-(2-ethylhexyl)bicyclo[2,2,1]hept-5-ene-2,3-dicarboxyimide; amount = 2 times the active ingredient.

are: commercial *dl, d*-T80-allethrin 0.20% coil, the *d, d*-T-prallethrin 0.20% plus S coil and the K-3050 0.10% plus S coil plots. This indicates that the lower the KT_{50} values of mosquito coils in 25 m³ semi-field tests, the smaller the variation in field efficacy test data. Compared with the mean number of 16.8 mosquitoes/house/person for the control plot, the mean number for the *dl, d*-T80-allethrin coil plot was 8.6, half that of the control. The mean numbers decreased to as low as 2.8 and 2.2 for the *d, d*-T-prallethrin and K-3050 coil plots, respectively.

There were also significant differences between the control and the three test plots as well as between the *dl, d*-T80-allethrin coil and the other two coils tested ($p < 0.05$) indicating the excellent repellent effects of *d, d*-T-prallethrin and K-3050 mosquito coils against *Ae. aegypti*.

The mosquito repellent effects for the test plots versus the control plot are also shown in Table 3.

The *d, d*-T-prallethrin coils exhibited a great repellent effect, of about 85%, and the K-3050 coils an even greater higher repellent effect of about 90%, while the repellent effect of the conventionally used commercial *dl, d*-

T80-allethrin 0.2% coils was as low as about 50%.

DISCUSSION

In regard to the pyrethroid susceptibilities of the four strains of mosquitoes collected from different sites in Bangkok, all BS, A and B strains were found to be more tolerant to the pyrethroids compared with the SS strain. These mosquito strains have possibly developed pyrethroid resistance since the strain was collected in 1977.

In the 25 m³ semi-field tests, mosquito coils were evaluated in terms of KT_{50} values for their bioefficacy in repelling mosquitoes or suppressing blood-sucking by mosquitoes.

The lethal doses per insect were determined from the LD_{50} values 24 hours post-treatment with topical application. These LD_{50} values were necessary to determine the pyrethroid resistance. Both test results were consistent in spite of their different test objectives.

Proper evaluation may be difficult in this kind of field efficacy test for biting insects, since data reproductivity may be poor due to differences in test environments such as house design, the characteristics of the test rooms, population density of mosquitoes and placement of mosquito coils. However, data from the field and semi-field testing showed that the *d, d*-T-prallethrin and K-3050 mosquito coils used for this study were especially effective against field *Ae. aegypti* mosquitoes and may contribute greatly to the reduction of biting by *Ae. aegypti* in dengue prevalent areas.

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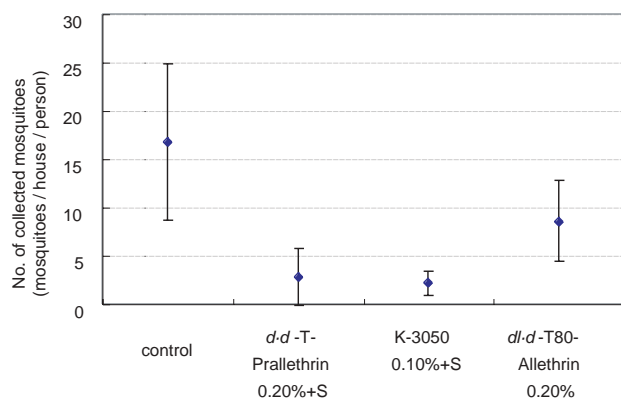


Fig 1—Number of collected mosquitoes by using mosquito coils in the A district field tests.

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