CONTROL OF MOSQUITO VECTORS OF TROPICAL INFECTIOUS DISEASES: (3) SUSCEPTIBILITY OF *AEDES AEGYPTI* TO PYRETHROID AND MOSQUITO COILS

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Abstract. We collected 11 groups of *Aedes aegypti* mosquitoes from various locations of Thailand. After rearing in the laboratory, the colonies were tested for KT_{50} values to *dl,d-T80*-allethrin 0.5% mosquito coils in a 25 m^3 room semi-field test and KD_{50} and LD_{50} values to *dl,d-T80*-allethrin by a topical application method. Two groups of mosquitoes were susceptible to allethrin similar to a SS (known allethrin sensitive) group, and other 9 groups showed various levels of lower susceptibility to allethrin; of these 6 had susceptibilities similar to a BS (known allethrin resistant) group with extremely low susceptibility, while the remaining 3 groups had susceptibilities to allethrin between the SS and BS groups. The KD_{50} values with the topical application were found to correlate highly with the KT_{50} values in the 25 m^3 room semi-field test, providing a useful test method for insect susceptibility evaluation. The allethrin mosquito coils, even at higher concentrations, had no activity against the 6 decreased susceptibility groups, similar to the BS group. With the 25 m^3 room semi-field test, mosquito coils with *d,d-T*-prallethrin at concentrations of 0.1 to 0.15% plus a synergist and those with methoxymethyl-tetrafluorobenzyl tetramethylcyclopropane carboxylate (K-3050) at a concentration of 0.1% plus a synergist were found to be highly effective against these mosquito groups. These two pyrethroids had smaller KD_{50} and LD_{50} values for topical application, and were more effective than *dl,d-T80*-allethrin, having the potential to control *Ae. aegypti* mosquitoes with low allethrin susceptibility.

INTRODUCTION

Dengue fever, an infectious disease transmitted by mosquitoes, is prevalent in Southeast Asia, India, Africa, and America. Since an effective vaccine against dengue is still in the future and treatment is not presently available, it is essential to protect humans from mosquito bites. There are two main methods for the control of vectors, namely larviciding and adulticides. After the Second World War, the WHO took a leading role in tackling mosquito vectors of tropical diseases, using residual spraying and larviciding with DDT, organophosphates and pyrethroids. Although vector-borne diseases were reduced or eliminated in many areas of the world, some vast regions still have a

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high disease prevalence due to the development of resistance to insecticides. Recently the world took another look at the importance of treatment with adulticides. Since the main vector for dengue, *Ae. aegypti* mosquitoes, is active and sucks blood during the daytime, mosquito coils are one tool used to repel or prevent their blood sucking.

We previously reported the bioefficacy of mosquito coils containing several pyrethroids and a synergist against *Ae. aegypti*, *Culex pipiens quinquefasciatus* and *Anopheles dirus* as well as the mechanism of action of the synergist (Katsuda *et al*, 2008a). In a second paper (Katsuda *et al*, 2008b), we demonstrated the repellency or blood-sucking suppressing effect of mosquito coils in human subjects under field efficacy conditions was highly correlated with KT_{50} values on 25 m^3 room semi-field tests and the latter method could be adopted to evaluate the repellency of mosquito coils. We also found the presence of a high allethrin-susceptible *Ae. aegypti* strain (SS colony) and a low susceptible one (BS colony) in Thailand, and reported the allethrin susceptibilities of 2 mosquito groups collected in Bangkok having a history of dengue fever (Thung Kru and Tha Phra districts) were similar to those of the BS group. Against the *Ae. aegypti* BS group with low allethrin susceptibility, current allethrin mosquito coils were found to give unsatisfactory insecticidal efficacy even when the allethrin concentrations were raised.

Thanispong *et al* (2008) reported 9 strains of *Ae. aegypti* mosquitoes from different localities in Thailand were subjected to susceptibility tests by being exposed to insecticide-treated papers and *Ae. aegypti* from different localities were strongly resistant to DDT and permethrin.

This paper deals with the pyrethroid susceptibilities of 11 *Ae. aegypti* groups collected from various districts having a history of dengue fever. After being reared in the laboratory, these groups were tested to determine their KT_{50} values with *dl,d-T80*-allethrin 0.5% mosquito coils in a 25 m^3 room semi-field test and their KD_{50} and LD_{50} values with *dl,d-T80*-allethrin using a topical application method. Several pyrethroids with higher efficacy than *dl,d-T80*-allethrin were evaluated by the 25 m^3 room semi-field test and topical application method as well. The test results are reported here.

MATERIALS AND METHODS

Test mosquito coils

All the test mosquito coils used in this study were prepared using plant powders as combustibles, *Machilus thunbergii* powder (Tabu powder) and starch as binders. Each mosquito coil weighed about 13 g and had a burning time of about 7 hours. The three synthetic pyrethroids and their concentrations in the mosquito coil formulations were as follows: 1). *dl,d*-T80-allethrin, 0.50% (w/w); 2).*d,d*-T-prallethrin (Katsuda, 1971, 1977), 0.10% and 0.15% (w/w); and 3). Methoxymethyl-tetrafluorobenzyl tetramethylcyclopropanecarboxylate (Katsuda *et al*, 2001), 0.10% (w/w).

In products 1). and 2). a synergist-S; N-(2-ethylhexyl)bicyclo[2,2,1]- hept-5-ene-2,3-dicarboxyimide- was added to each pyrethroid test mosquito coil at 2 times the active ingredient.

Test mosquitoes

The locations for the *Ae. aegypti* groups used in this study are shown in Fig 1. These include the BS group (Thung Kru District, Bangkok, 2005), the SS group (Pom Prap Sattru Phai District, Bangkok, 1977), and the 11 field groups(labeled A-K) collected from locations having an increased frequency of dengue fever cases in the East, West, South, North, Northeast and central parts of Thai-

land. Of these, the A group (Thung Kru District, Bangkok, 2007) and B group (Tha Phra District, 2007) collected in the suburbs of Bangkok have had their susceptibilities studied previously (Katsuda *et al*, 2008b).

All collected mosquito groups were reared for several generations in the insectorium of the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University after having been collected from the field.

Test methods

Semi-field test (25 m^3 room). For this study, the semi-field test method (25 m^3 room) described in our previous papers (Katsuda *et al*, 2008a,b) was used. The tests were run with an average daily temperature of 25-27ºC and a relative humidity of 70-80%. White paper was laid on the floor of the room. 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. Knocked-down mosquitoes were counted 12 times at intervals of 5-20 minutes for 120 minutes from the beginning of the experiment. After 120 minutes exposure, all test mosquitoes were transferred, by use of a sucking apparatus, to a clean container with absorbent cotton soaked with 3% sugar solution, and kept at 26±2ºC for 24 hours to observe for mortality. The experiment was replicated 2-3 times per formulation. KT_{50} values were determined for time and knockdown percentage data according to the Bliss' probit method (Bliss, 1937).

Topical application method. The pyrethroid was diluted with acetone to make test solutions containing various concentrations of the active ingredient. Female mosquitoes (3-6 days old) were anesthetized under low temperature, and 0.25 µl acetone solution was topically applied to the dorsal thorax of each mosquito. The mosquitoes were observed for knockdown after 30 minutes and mortality after being held for 24 hours 26±2ºC. The KD_{50} and LD_{50} values were determined from the obtained data regarding dosage and reaction percentage according to Bliss' probit method (Bliss, 1935).

RESULTS

Allethrin susceptibilities of *Ae. aegypti*: Semi-field tests (25 m^3 room) with mosquito coils

Table 1 shows the KT_{50} values with *dl,d-T80*-allethrin 0.5% mosquito coils in a 25 m^3 room semi-field test against *Ae. aegypti* mosquitoes.

The mosquitoes were exposed to volatilized pyrethroids for 120 minutes. For test plots where the knockdown percentage failed to reach 50% after 120 minutes, the KT_{50} values were shown as >120 minutes.

As reported previously (Katsuda *et al*, 2008b), the KT_{50} value for the SS group was 13 minutes. The BS group had a more than 10 times lower susceptibility, with a KT_{50} value of >120 minutes.

We regarded *Ae. aegypti* mosquitoes with KT_{50} values below 60 minutes as susceptible (Group I) and those with KT_{50} values more than 60 minutes as less susceptible, then classified those with KT_{50} values of 60 - 120 minutes into Group II and those greater than 120 minutes into Group III.

Of the 11 groups (A through K), F and I were classified as being in Group I, their KT_{50} values were 13 minutes and 35 minutes, respectively, and were regarded to be similar to the SS group. The J, H and D groups had susceptibilities between the SS and BS groups with their KT_{50} values of 76 minutes, 96 minutes and 99 minutes, respectively, and were placed in Group II.

The mosquitoes from 6 other groups had knocked-down percentages below 50 % even after 120 minutes. With uncalculated

Table 1
Allethrin susceptibilities of *Ae. aegypti* colonies in 25 m^3 room semi-field tests using a *dl*, *d*-T80-allethrin 0.5% mosquito coil.

Sensitivity group[a]	Mosquito group	KT_{50}: minutes		Ratio[b]
		Range	Mean value (minutes)	
	SS		13	1
	BS		> 120	13
I	F	< 60 minutes	13	1
	I		35	3
	J		76	6
II	H	60 - 120	96	7
	D		99	8
	E			
	G		KT_{50}:	
III	A	> 120	Not calculated	>> 10
	K			
	B			
	C			

[a]Sensitivity groups are classified based on KT_{50} values obtained in a 25 m^3 room semi-field test with a *dl*, *d*-T80-allethrin 0.5% mosquito coil as follows: I, below 60 minutes; II, 60 - 120 minutes; III, more than 120 minutes.
[b]Ratios are given against the SS group as 1.

KT_{50} values, these colonies were found to be more than 10 times less susceptible than the SS colony.

Allethrin susceptibilities of *Ae. aegypti*: Topical application method

Table 2 shows the KD_{50} and LD_{50} values with *dl,d-T80*-allethrin topical application against 11 *Ae. aegypti* groups collected from various districts.

The ratios to the SS colony KD_{50} values with topical application and those with corresponding LD_{50} values showed only slight differences from each other. The former ratios were similar to the KT_{50} values for *dl,d-T80*-allethrin 0.5% mosquito coils in the 25 m^3 room semi-field test as described in Table 1. This high correlation between the KT_{50} values in the 25 m^3 room semi-field test and the KD_{50} values 30 minutes after treatment by topical application proved to be useful as this provides an index for insect susceptibility evaluation.

Efficacy of mosquito coils against *Ae. aegypti* colonies with low allethrin susceptibility: Semi-field tests (25 m^3 room) with mosquito coils

Table 3 shows the test results of mosquito coils containing *d,d-T*-prallethrin or pyrethroid of K-3050, both with higher efficacy than *dl,d-T80*-allethrin, in a combination with a synergist S: N-(2-ethylhexyl) bicyclo[2,2,1]hept-5-ene- 2,3-dicarboxyimide, with the 25 m^3 room semi-field test.

Against *Ae. aegypti* mosquitoes in Groups II and III, *d,d-T*-prallethrin 0.1-0.15% and K-3050 0.10% mosquito coils were found to have high efficacy.

Table 4 shows the classification of the 11 *Ae. aegypti* mosquito groups based on the KT_{50} values with *dl,d-T80*-allethrin 0.5%

Table 2
Allethrin susceptibilities of *Ae. aegypti* mosquitoes as determined by topical application using *dl, d*-T80-allethrin.

Sensitivity group[a]	Mosquito group	KD_{50} (30 minutes)		LD_{50} (24 hours)	
		µg/insect	Ratio[b]	µg/insect	Ratio[b]
	SS	0.0038	1	0.0092	1
	BS	0.0360	9	0.0637	7
I	F	0.0060	2	0.0190	2
	I	0.0219	6	0.0706	8
	J	0.0201	5	0.0446	5
II	H	0.0282	7	0.0693	8
	D	0.0218	6	0.0497	5
	E	0.0312	8	0.0366	4
	G	0.0488	13	0.0625	7
III	A	0.0533	14	0.0702	8
	K	0.0561	15	0.0788	9
	B	0.0616	16	0.0925	10
	C	0.0743	20	0.0842	9

[a]Groups are classified based on KT_{50} values obtained in a 25 m^3 room semi-field test with a *dl, d*-T80-allethrin 0.5% mosquito coil as follows: I, below 60 minutes; II, 60-120 minutes; III, more than 120 minutes.
[b]Ratios are given against the SS group as 1

Table 3
Pyrethroid susceptibilities of *Ae. aegypti* colonies in a 25 m^3 room semi-field test by using mosquito coils with each pyrethroid.

Sensitivity group	Mosquito group	KT_{50} (minutes)			
		dl,d-T80-allethrin	*d,d*-T-Prallethrin		K-3050
		0.5%	0.10%+S	0.15%+S	0.10%+S
	SS	13	12	-	8
	BS	> 120	120	100	67
I	F	13	11	-	-
II	D	99	87	-	88
III	E	> 120	60	44	77
	G	> 120	81	-	-

Synergist S, N-(2-ethylhexyl)bicyclo[2,2,1]hept-5-ene-2,3-dicarboxyimide

mosquito coils and effective pyrethroids and their concentrations in mosquito coils for each group.

Against *Ae. aegypti* colonies in Group I, commercial allethrin 0.2 to 0.3% mosquito coils available in Thailand had good efficacy. For mosquitoes in Group II, *dl,d-T80*-allethrin mosquito coils needed to have higher

Table 4
Activity of mosquito coils against *Ae. aegypti* mosquitoes with low pyrethroid susceptibility.

Sensitivity group	Mosquito group	Active ingredient and concentration of coil				
		dl,d-T80-Allethrin		*d,d*-T-Prallethrin		K-3050
		0.2-0.3%	≥0.5%	0.10%+S	0.15%+S	0.10%+S
I	F, I	○	–	–	–	–
II	J, H, D	x	○	○	○	○
III	E, G, A K, B, C	x	x	△~○	○	○

○, Effective; △, moderately effective; x, not effective; –, not tested

Table 5
Pyrethroid susceptibilities of *Ae.aegypti* mosquitoes in a topical application test using *d,d*-T-Prallethrin and K-3050.

Sensitivity group	Mosquito group	*d,d*-T-Prallethrin				K-3050			
		KD_{50}(30 min)		LD_{50} (24 hrs)		KD_{50} (30 min)		LD_{50} (24 hrs)	
		µg/insect	Ratio[a]	µg/insect	Ratio[a]	µg/insect	Ratio[a]	µg/insect	Ratio[a]
	SS	0.0006		0.0011	1	0.0005	1	0.0013	1
	BS	0.0090	15	0.0181	17	0.0074	15	0.0196	15
I	F	0.0016	3	0.0028	3	0.0007	1	0.0019	2
II	D	0.0026	4	0.0124	11	0.0058	12	0.0361	28
III	E	0.0058	10	0.0078	7	0.0062	12	0.0112	9
	G	0.0138	23	0.0215	20	0.0063	13	0.0212	16

[a]Ratios are given against the SS colony as 1

concentrations of at least 0.5%. *d,d-T*-prallethrin 0.1% or K-3050 0.1% mosquito coils were found to have good efficacy. Against mosquitoes in Group III, *d,d-T*-prallethrin 0.1 to 0.15% or K-3050 0.1% mosquito coils were highly effective.

Efficacy of mosquito coils against *Ae. aegypti* colonies with low allethrin susceptibility: Topical application method

Table 5 shows the results of *d,d-T*-prallethrin and K-3050 against one or several *Ae. aegypti* mosquito groups.

Both *d,d-T*-prallethrin and K-3050 were highly effective with several times lower KD_{50} and LD_{50} values than those for *dl,d-T80*-allethrin (Table 2) in all cases, confirming their practicability for possible control of *Aedes aegypti* mosquitoes with low allethrin susceptibility.

DISCUSSION

To study the pyrethroid susceptibilities of *Ae. aegypti* mosquitoes, 11 groups of mosquitoes were collected from various loca-

Ae. aegypti group	Locations and year of collection
SS	Pom Prap Sattru Phai, BKK (1977)
BS	Thung Khru, BKK(2005)
A	Thung Khru, BKK(2007)
B	Tha Phra, BKK(2007)
C	Uttaradit (2008)
D	Chiang Rai (2008)
E	Surat Thani (2008)
F	Kanchanaburi(2008)
G	Nakhon Sawan(2008)
H	Chanthaburi(2008)
I	Chon Buri (2008)
J	Songkhla (2008)
K	Phang-Nga (2008)

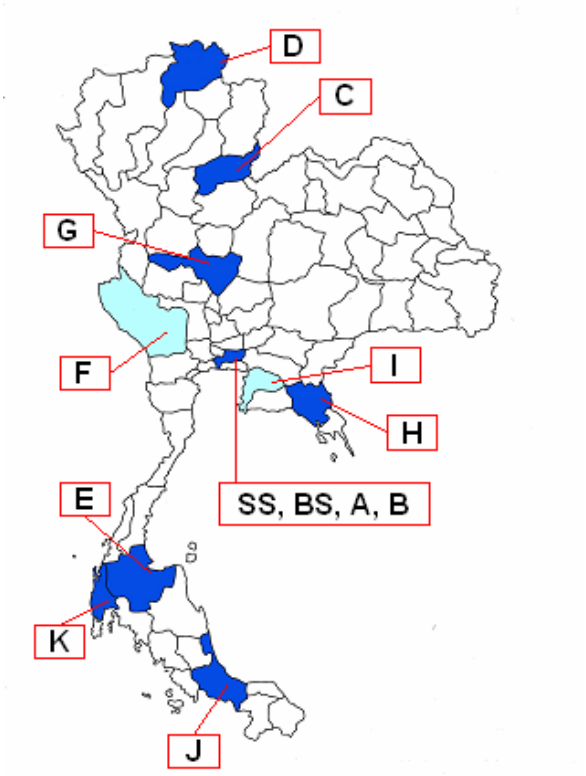

Fig 1–*Ae. aegypti* colonies collected from various districts.

tions in Thailand having a history of dengue fever. *Ae. aegypti* mosquitoes with low allethrin susceptibility are widely distributed in Thailand. They have probably developed resistance to chemicals over the past 30 years since the SS mosquito group was first collected. One reason for resistance is probably the tremendous use of organochlorine insecticides in the past may have generated *kdr* mechanisms in mosquito species to pyrethroids.

Thanispong *et al* (2008) reported that 9 strains of *Ae. aegypti* mosquitoes from different locations in Thailand showed strong resistance to DDT and permethrin when tested by exposure to papers treated with insecticides. In the present study the pyrethroid susceptibilities of *Ae. aegypti* mosquitoes from 11 locations were assessed by the practical 25 m^3 room semi-field test with *dl,d-T80*-allethrin 0.5% mosquito coils and topical application method using *dl,d-T80*-allethrin with which the KD_{50} and LD_{50} values per mosquito for each group were determined as an absolute evaluation index. It is apparent from both studies *Ae. aegypti* colonies with low allethrin susceptibility are widely distributed in Thailand, although their test results cannot be compared directly due to different testing methods.

To manage dengue outbreaks, the best method is to prevent mosquito bites of *Ae. aegypti*, a daytime biting mosquito, that is; to protect the host from mosquito bites using mosquito coils. In our earlier paper (Katsuda *et al*, 2008b), mosquito coils containing *d,d*-T-prallethrin plus S and K-3050 plus S were found to be highly effective

against BS group mosquitoes. Although the BS colony with low allethrin susceptibility shows cross resistance to all pyrethroids other than allethrin, the *d,d*-T-prallethrin and K-3050 mosquito coils were found to be extremely effective against all 11 *Ae. aegypti* field colonies.

According to our recent studies, mosquito coils containing natural pyrethrins were effective against less susceptible *Ae. aegypti* mosquitoes as well (unpublished). A synthetic pyrethroid, *d,d*-T-prallethrin is similar to natural pyrethrins in chemical structure and is composed of only carbon, hydrogen and oxygen atoms. This makes us consider *d,d*-T-Prallethrin to be preferable to K-3050 having fluorine atoms in its chemical structure from mammalian safety view points. *d,d*-T-Prallethrin mosquito coils with efficacy and safety can contribute greatly to the management of dengue fever. Further studies regarding their practical use will be carried out.

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