CONTROL OF MOSQUITO VECTORS OF TROPICAL INFECTIOUS DISEASES: (1) BIOEFFICACY OF MOSQUITO COILS CONTAINING SEVERAL PYRETHROIDS AND A SYNERGIST

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Abstract. The bioefficacy of mosquito coils containing several pyrethroids were tested in a 25 m^3 room against *Culex pipiens quinquefasciatus*, *Aedes aegypti* and *Anopheles dirus.* The test results were compared with tests against *Culex pipiens pallens* in Japan. Based on the KT_{50} values (the 50% knockdown time) of mosquito coils containing *dl, d*-T80-allethrin, *d*, *d*-T-prallethrin and methoxymethyl-tetrafluorobenzyl tetramethyl-cyclopropanecarboxylate (K-3050) at doses of 0.05-0.5% (w/w) with or without a synergist, the pyrethroid susceptibility of the four mosquito species was as follows: *Cx. p. quinquefasciatus* was several times more tolerant to pyrethroids than *Cx. p. pallens, Ae. aegypti* was a further several times more tolerant than *Cx. p. quinquefasciatus*, and *An. dirus* was more susceptible than *Cx. p. pallens* (KT_{50} value: about half of *Cx. p. pallens*). The order of their susceptibilities is common for pyrethroids. Mosquito coils containing *d, d*-T-prallethrin and K-3050 at doses of 0.05-0.2% (w/w) and N-(2-ethylhexyl)bicycle-[2,2,1]-hept-5-ene-2,3-dicarboxyimide as a synergist at a ratio of 2 times the active ingredient were highly effective against *Ae. aegypti*, the most important mosquito vector for dengue fever.

INTRODUCTION

Mosquito-borne infectious disease is one of the most important public health issues to address in the 21st Century. The development of vaccines for malaria and dengue fever is widely considered to be a time-consuming process and technically difficult. About 500 million persons suffer from malaria each year with more than one million deaths (WHO, 2000). One of the most seriously affected areas is sub-Saharan Africa. Dengue, which was predominantly a Southeast Asian disease, is now prevalent in India, Africa, and the Americas, expanding worldwide. As a preventive measure, it is essential to prevent mosquito bites.

Although epidemic Japanese encephalitis transmitted by *Culex tritaeniorhynchus* has not been reported in Japan since 1992, measures against this disease must be prepared for the possible northern extension of mosquito habitats due to global warming. Attention should also be given to the potential spread of West Nile infection to Japan.

After the Second World War, the WHO took a leading role in tackling mosquito vectors of tropical diseases, using methods such as residual spraying and larviciding with DDT, organophosphates and pyrethroids. Despite

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these efforts, the results remain unsatisfactory.

The mosquito coil, one of the most common household insecticide formulations for the control of adult mosquitoes, has incorporated pyrethroids and natural pyrethrins as active ingredients due to their rapid effects on insects using minimal dosages with low risk to mammals. The history of mosquito coils dates back to 1890 when stick-type mosquito coils were first commercialized in Japan. Then the shape was improved to a spiral type with a burning time of about 7 hours in 1895. Mosquito coils of this type have been widely used, and still top the list of annual worldwide use of the four major types of household insecticide formulations of mosquito coils, aerosols, vaporizing mats and liquid vaporizers.

Until now efficacy data of various pyrethroids against *Cx. p. pallens* have been obtained from laboratory tests using mosquito coils. However, few test reports against mosquito vectors of tropical infectious diseases are available. For the purpose of controlling mosquito vectors of tropical infectious diseases, we started studies in 2005 by preparing mosquito coils containing various pyrethroids.

Based on test results with *Cx. p. pallens*, efficacy tests of mosquito coils containing several pyrethroids with or without a synergist in a typical room (25 m^3) against tropical mosquito species were designed for *Cx. p. quinquefasciatus*, *Ae. aegypti* and *An. dirus*.

This paper reports efficacy results of *dl, d*-T80-allethrin and two other pyrethroids against three tropical mosquito species. The mechanisms of the synergistic effects in the mosquito coil are also discussed in this paper.

MATERIALS AND METHODS

Test mosquito coils

All the test mosquito coils used in this study were prepared using pyrethrum powder as a combustible, and *Machilus thunbergii* powder (Tabu powder) and starch as binders, according to the conventional procedures. Each mosquito coil weighed about 13 g and had a burning time of about 7 hours. The three synthetic pyrethroids and their doses in the mosquito coil formulations were as follows:

1). *dl, d*-T80-allethrin, 0.27 and 0.50% weight per weight (w/w); 2). *d, d*-T-prallethrin (Katsuda,1971,1977), 0.10, 0.15 and 0.20% (w/w); and 3). Methoxymethyl-tetrafluorobenzyl tetramethylcyclopropane-carboxylate (K-3050; Katsuda *et al*,2001), 0.05, 0.10 and 0.15% (w/w).

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

Test mosquitoes

The four species of mosquitoes used in this study were *Culex pipiens pallens* (Kincho colony, Toyonaka, Osaka, 1965), *Culex pipiens quinquefasciatus* (Mae Sot District, Tak Province, Thailand, 2006), *Aedes aegypti* (Thung Kru District, Bangkok, 2005), and *Anopheles dirus* (Chon Buri Province, Thailand, 1997).

All mosquito species were reared for several generations in the insectorium of the Research and Development Laboratory of Dainihon Jochugiku Co, Ltd and the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University.

Bioefficacy test method

The conventional test methods such as the cylinder method, open cylinder method, glass box method and Peet-Grady method generally yielded test conditions with a much higher aerial concentration of test pyrethroids due to their small test space compared with a typical room (25 m^3), causing great data deviation from the actual states. For mosquito coil formulations whose efficacy depends on the aerial pyrethroid concentration, the typi-

cal room method (25 m^3) was adopted as it gives good reproductivity and reliability in data.

The studies with *Cx. p. pallens* were conducted in a typical room (25 m^3) in the Research and Development Laboratory of Dainihon Jochugiku Co, Ltd at an average daily temperature of 24-27ºC and a relative humidity of 50-80%. The other three tropical mosquito species were tested in the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University at an average daily temperature of 25-27ºC and a relative humidity of 70-80%.

White paper was laid on the floor of the room. Next 100 female mosquitoes (3-6 day-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 from the beginning of the experiment. After 120 minutes' exposure, all test mosquitoes were transferred 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 process was replicated 3-5 times per formulation. KT_{50} values were determined for time and knockdown percentage data according to the Bliss' probit method (Bliss,1937).

Verification test of the synergistic effect of the mosquito coil

To verify the mechanism of the efficacy-promoting effect, the following four test plots were set to determine the bioefficacy in a typical room (25 m^3) using *Cx. p. pallens* : (1) one coil containing *dl, d*-T80-allethrin (0.27%) alone; (2) one coil containing *dl, d*-T80-allethrin (0.27%) plus synergist S (0.54%); (3) one coil containing synergist S (0.54%) alone; and (4) two coils of (1) and (3) were burnt in a room at the same time to compare the efficacy of this plot with that of mosquito coil (2).

Gas chromatographic examination of synergists

The following synergist samples were subjected to GC analysis by a Shimadzu GC apparatus equipped with a flame ionization detector and a glass column (3 mm i.d. x 1 m) packed with 5% silicone SE-30 on Chromosorb WAW-DMCS (80-100 mesh) at a column temperature of 185ºC: (1) synergist S; (2) N-(2-ethylhexyl)-1-isopropyl-4-methyl-bicyclo[2,2,2]oct-5-ene-2,3-dicarboxyimide (Sy-500); and (3) piperonyl butoxide (PBO; Wachs,1947).

The peaks corresponding to each sample on the gas chromatogram were checked for retention time compared with the three pyrethroids as described in the "Test mosquito coils" section.

Measurements of the volatilization rates of pyrethroids

To an outlet on the top of a glass bell with an inner volume of about 1.5 liters, two glass tubes for trapping (about 25 mm i.d.x10 cm) packed with about 6 g of silica gel were connected in series, and linked to a suction pump. A two gram sample of a test mosquito coil set was placed in the center of the floor covered with the bell. The coil was lit and trapping of volatilized substances was initiated by sucking the air at a rate of 8 liter/minute. After the coil burned out, all volatilized substances trapped in the tubes and attached to the inside of the bell were washed out and subjected to GC analysis to determine the amount of recovered pyrethroid. The volatilization rate of the pyrethroid was calculated from the data obtained and the theoretical amount of the pyrethroid in 2 g of the test mosquito coil.

RESULTS

Bioefficacy tests of mosquito coils against 4 mosquito species

Table 1 shows the bioefficacy of mosquito coils containing each pyrethroid alone and those containing each pyrethroid plus a synergist.

In the current test, mosquitoes were ex-

posed to volatilized pyrethroids for 120 minutes, and KT_{50} values for >120 minutes were calculated 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 containing *dl, d*-T80-allethrin, *d*, *d*-T-prallethrin and K-3050 against the four mosquito species, *Cx. p. quinquefasciatus* was several times more tolerant to pyrethroids than *Cx. p. pallens; Ae. aegypti* was a further several times more tolerant than *Cx. p. quinquefasciatus*, and *An. dirus* was more susceptible than *Cx. p. pallens* (the KT_{50} value was about half of that for *Cx. p. pallens*).

All mosquito coils containing the three pyrethroids plus the synergist showed greater speed and killing than those without the synergist. The promoting effect of synergist (S) was observed at lower concentrations approximating typical use so that evaluating such synergistic effects at higher concentrations was inappropriate.

The ratios for the efficacy-promoting effect of S in *dl, d*-T80-allethrin 0.27% mosquito coils were 1.5 against *Cx. p. pallens* and 4.8 against *Cx. p. quinquefasciatus,* respectively based on KT_{50} values. Against *Cx. p. quinquefasciatus*, effective mosquito coils needed *dl, d*-T80-allethrin 0.27% plus S or *dl, d*-T80-allethrin >0.5% if there was no S. In contrast, *dl, d*-T80-allethrin mosquito coils showed low efficacy against *Ae. aegypti* even at a high concentration of 0.5%.

The addition of S to *d, d*-T-prallethrin mosquito coils more than doubled the efficacy against *Cx. p. quinquefasciatus,* showing the use of mosquito coils with *d*, *d*-T-prallethrin 0.10% plus S to be promising. Against *Ae. aegypti,* mosquito coils with *d, d*-T-prallethrin 0.20% plus S were effective with ratios for the efficacy-promoting effect of S at around 1.5.

Table 1
Bioefficacy of mosquito coils containing pyrethroid alone and containing pyrethroid and a synergist.

Test mosquito coil	(w/w %)	*Cx. p. pallens*		*Cx. p. quinquefasciatus*		*Ae. aegypti*		*An. dirus*	
		KT_{50} (min)	Mortality (%)	KT_{50} (min)	Mortality (%)	KT_{50} (min)	Mortality (%)	KT_{50} (min)	Mortality (%)
dl, d-T80-Allethrin	0.27	28.3	20	196	4	361	15	-	-
	0.27+ S[a]	18.6	27	41	35	174	21	11.1	100
	0.50	20.8	28	72	55	170	29	8.0	100
d, d-T-Prallethrin	0.10	20.6	36	108	25	171	22	8.3	91
	0.10+ S	14.5	51	55	30	120	24	8.0	91
	0.15	14.0	39	100	47	140	25	-	-
	0.15+ S	11.4	53	42	55	100	30	-	-
	0.20	13.1	67	63	50	130	28	-	-
	0.20+ S	10.3	92	24	71	85	30	8.1	100
K-3050	0.05	25.6	25	64	19	353	11	14.6	98
	0.05+ S	15.8	42	53	24	184	16	13.9	100
	0.10	13.9	43	33	27	117	32	-	-
	0.10+ S	10.8	90	18	73	67	39	8.7	100
	0.15	10.5	98	20	43	60	37	-	-
	0.15+ S	10.2	100	15	51	47	43	-	-

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

Table 2
Bioefficacy test results of test plots (1)~(4).

Tested mosquito coils	*Cx. p. pallens*	
	KT_{50} (min)	Mortality (%)
(1) *dl, d*-T80-Allethrin (0.27%)	28.3	20
(2) *dl, d*-T80-Allethrin (0.27%) + S (0.54%)	18.6	27
(3) S (0.54%)	No knocked-down mosquitoes	0
(4) Simultaneous burning of (1) and (3)	19.7	25

The efficacy-promoting effect ratios for S in K-3050 mosquito coils were 1.0-1.8 against both *Cx. p. pallens* and *Cx. p. quinquefasciatus*, and mosquito coils of K-3050 0.05% alone were found effective against both species. Against *Ae. aegypti*, mosquito coils of K-3050 0.10% plus S gave good results while those without the synergist had relatively lower efficacy.

Efficacy-promoting effect of synergists in the mosquito coil

Insect physiological aspects of efficacy-promoting effect. Table 2 shows the bioefficacy test results of test plots (1)~(4). As shown in Table 2, mosquito coil (3) (synergist S alone) had no efficacy. In test plot (4) where two coils of (1) and (3) were burnt at the same time, the efficacy obtained showed an apparent synergistic effect compared with mosquito coil (1) (pyrethroid alone), confirming that insect physiological mechanism is the main factor for the effect of the synergist in the mosquito coil.

Physical aspects of efficacy-promoting effect. According to the above test, the efficacy of the test plot (4) was slightly inferior to that of mosquito coil (2) (pyrethroid plus S). Presuming the physical aspects associated with the promotion of pyrethroid volatilization in the mosquito coil might also contribute to the effect of the synergist in addition to the insect physiologic mechanisms, we conducted tests as follows.

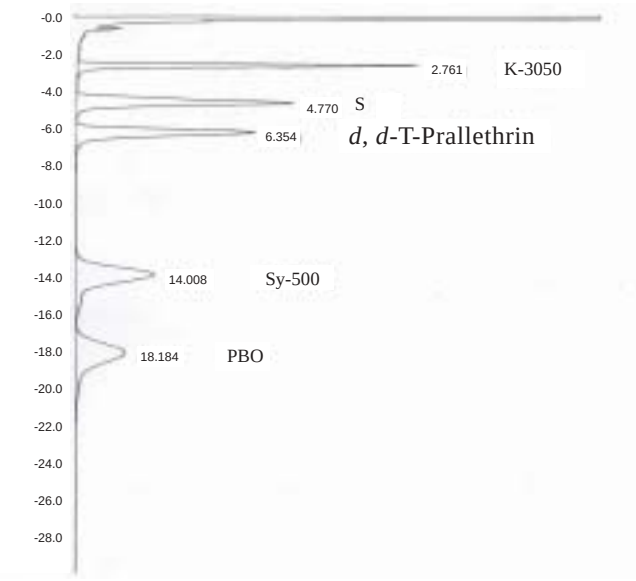

Fig 1–Gas chromatograms[a] of some synergist samples and the three pyrethroids.

[a]GC analysis was carried out according to conditions described in the Materials and Methods section. The peak of *dl, d*-T80-allethrin was omitted as the peaks of *dl, d*-T80-allethrin and *d, d*-T-prallethrin newly overlapped each other.

Gas chromatograms of some synergist samples and the three pyrethroids are shown in Fig 1. As indicated in Fig 1, the peak of S having a synergistic effect was close to those for *dl, d*-T80-allethrin, *d, d*-T-prallethrin and K-3050. On the other hand, N-(2-ethylhexyl)-1-isopropyl-4-methylbicyclo [2,2,2]oct-5-ene-

2,3-dicarboxyimide (Sy-500) and piperonyl butoxide (PBO) whose peaks were far from those of the three tested pyrethroids gave no synergistic effect when used in combustion-accompanying mosquito coil formulations.

Measurements showed that the pyrethroid volatilization rate in the mosquito coil varied with the pyrethroid tested. Increases in the volatilization rate by adding S were about 8%, 19% and 5% for *dl, d*-T80-allethrin, *d, d*-T-prallethrin and K-3050 mosquito coils, respectively.

DISCUSSION

After examining several pyrethroid mosquito coils against *Cx. p. quinquefasciatus*, *Ae. aegypti,* and *An. dirus*, *dl, d*-T80-allethrin, a conventional pyrethroid, was found to be effective against *Cx. p. quinquefasciatus* and *An. dirus* by the addition of a synergist or at a high concentration (0.5%) that is 2 times the conventional dosage, but exhibited very low efficacy against *Ae. aegypti* even at high concentrations.

Conversely, *d, d*-T-prallethrin and K-3050 were more effective against *Ae. aegypti*, and can be used to develop highly effective mosquito coils with high efficacy against *Ae. aegypti* with the addition of an efficacy-promoting agent, such as a synergist.

Although mortality after 24 hours was <100% in most tests, other tests with *Cx. p. pallens* that had not died after 24 hours confirmed that they lost a great deal of their blood-sucking capacity.

It was also confirmed that the typical room method (25 m^3) gives good reproductivity and reliability in data for tests of mosquito coil formulations. The relationship between the KT_{50} values obtained in the 25 m^3 tests and their practical effectiveness will be examined in future research.

The verification test results (Table 2) showed that the main factor for the effect of the synergist in the mosquito coil is related to the physiological mechanism of the insect.

Yamamoto (1973) reviewed the mechanism of synergists based on the insect physiology, given that synergists increase the insecticidal efficacy of pyrethroids by acting as an inhibitor of mixed function oxidase (MFO), thereby preventing the oxidative metabolism of pyrethroids, or by promoting the penetration of pyrethroid into the insect's exoskeleton and enabling the pyrethroids to reach action sites in the nervous system.

In studies of the synergist-formulated mosquito coils in the 1950s, Katsuda *et al* (1971) prepared mosquito coils containing natural pyrethrins as an active ingredient and piperonyl butoxide at various concentrations, conducted efficacy tests against *Cx. p. pallens* and *Musca domestica* using the cylinder method, and reported (Nagasawa *et al*,1952) that no synergistic action was observed in the tests.

Our recent studies that the synergist S exhibits a high synergistic effect in the mosquito coil formulation and its mechanism is based on both the physiological aspects of the insect and physical ones associated with the mosquito coil formulation.

Considering a report by Eshita (1997) that *Ae. aegypti*, the most important mosquito vector for dengue comprises more than 90% of the species and total number of mosquitoes collected from houses in northeast Thailand, the mosquito coils we have developed are highly useful against this mosquito.

In conclusion, the bioefficacy test results in this study suggested that mosquito coils containing *d, d*-T-prallethrin or K-3050 plus a synergist (S) are effective against *Cx. p. quinquefasciatus, Ae. aegypti,* and *An. dirus*.

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