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Control of DF/DHF Vector, *Aedes* Mosquito, with Insecticides

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Abstract: *Aedes aegypti*, the principal vector of DF/DHF, mainly breeds in household water jars. The breeding sites are definite and, thus, the larviciding will become the most effective measures as well as source reduction. However, the following facts make it difficult. 1) Water in the jars is used and refilled. The replenishment of water leads to dilution of larvicides. 2) Many small breeding sites are overlooked, such as water at the bottom of household plant pots.

To solve these problems, two new larval control methods were examined. The first was the slow release formulation containing the insect growth regulator, pyriproxyfen, which highly inhibited adult emergence of mosquitoes. The formulation kept concentration enough to inhibit adult emergence, even after replacing water in the container. The second was the utilization of blood-fed females as a vehicle of pyriproxyfen to small larval habitats. When the black-color adult resting traps treated with pyriproxyfen were kept inside a house, it was confirmed that the mosquitoes contacted with pyriproxyfen and carried it to small containers with water.

Permethrin-incorporated bednet was introduced as a tool for preventing of further virus dispersion from patients by mosquito bitings. Momentary contact of the females with the netting resulted in high mortality. However, early diagnosis method of patients will be essential. For a self-protection, inhibitory effect of a mosquito coil on host-seeking behaviour of *Aedes aegypti* was demonstrated.

Key words: Insecticide, *Aedes aegypti*, Vector control

INTRODUCTION

The space sprays of adulticides during an epidemic period and the applications of larvicides to breeding sites have been employed as measures of vector control with insecticides for many years (Gratz, 1993). These could be mainly executed by a municiparity in co-operation with communities. If the coverage area and timing for space spray is correctly determined, effective control of epidemic can be expected. In addition, administrative demonstration effect to the communities might be also expected, because of noisy sound caused by spray machine and rapid reduction in mosquito density. Since the main larval habitats of *Aedes aegypti* are definite place such as household water jars, the

larviciding is the most effective measure of vector control. However, many small and inconspicuos breeding sites such as water at the bottom of household flower pots should be also covered with larvicides. Use of mosquito coils and mats is very familliar to Asian peopole for a self protection, but improvement of economical conditions in community is essential to make use possible.

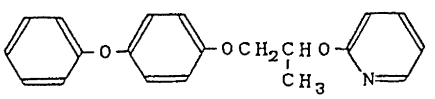
In this paper, a slow release formulation containing an insect growth regulator (IGR) and an utilization of adults of *Aedes aegypti* as a vehicle of IGR were presented as a new approach to vector control. An insecticide-incorporated bednet for patients and inhibitory effect of a mosquito coil on host-seeking behaviour of the females were also presented.

Slow release formulation containing IGR, pyriproxyfen, for control of larvae

The chemical structure of pyriproxyfen, which is a juvenile hormone mimic and inhibits adult emergence of mosquitoes, is shown in Fig. 1 with the inhibitory activity of adult emergence against mature larvae of *Anopheles*, *Culex* and *Aedes* mosquitoes (Hatakoshi *et al*, 1987). The IC50 value, which is the concentration to be required for 50% inhibition of the emergence, of pyriproxyfen is 0.023 ppb against *Aedes aegypti*, while that of temephos, which is an organophosphorus insecticide, is 4.5 ppb. Thus, it is evident that pyriproxyfen has about 200 times higher activity than temephos.

Fig. 2 shows the concept of the slow release formulation containing pyriproxyfen. The formulation was made of a synthetic polymer incorporated with pyriproxyfen at 5% by a new technology. Even though all of water in a jar is replaced after the treatment, the active ingredient is continuously released from the formulation and its concentration in the

Pyriproxyfen (Insect growth regulator)



Larvicides	50 % Inhibition of Adult Emergence (ppb)		
	Cx. pipiens	An. stephensi	Ae. aegypti
Pyriproxyfen	0.0046 (369)	0.043 (63)	0.023 (196)
Methoprene	0.013 (131)	0.54 (5)	0.77 (6)
Temephos	1.7 (1)	2.7 (1)	4.5 (1)

Fig. 1. The chemical structure of the new insect growth regulator, pyriproxyfen, and the inhibitory activity of adult emergence of mosquitoes

refilled water will reach to effective level to inhibit adult emergence. Additional advantages are as follows; 1) Since the active ingredient has no odor, the treated water do not smell odor, 2) The shape of the formulation is slender strip type to be cut with a scissor for use and this easiness of use will make community participation possible, 3) Since materials except for the active ingredient is not dissolved in water, the treated water will keep transparent, 4) Momentary recognition of the formulation in a jar will make confirmation of treatment easy.

The laboratory experiment under the simulated condition assuming replenishment of water in a jar was conducted (Fig. 3). Sixty mg of the formulation was cut with a scissor and put into water of 25 litres in a container. When all amount of the active ingredient was dissolved at once in the water, its concentration could be calculated to be 0.2 ppm. The water of small volume was sampled from the container to be inoculated with last instar larvae of *Aedes aegypti* after 7 days. The remaining water was discarded, followed by refilling fresh water of 25 litres. The same procedure was repeated at 7 days interval. As shown in Fig. 3, the adult emergence from the larvae inoculated in the sampled water was highly inhibited at every sampling times. It was evident that pyriproxyfen was continuouly released from the formulation into the refilled water. Practical assessment of the formulation against *Aedes aegypti* is under field trial at Bangkok.

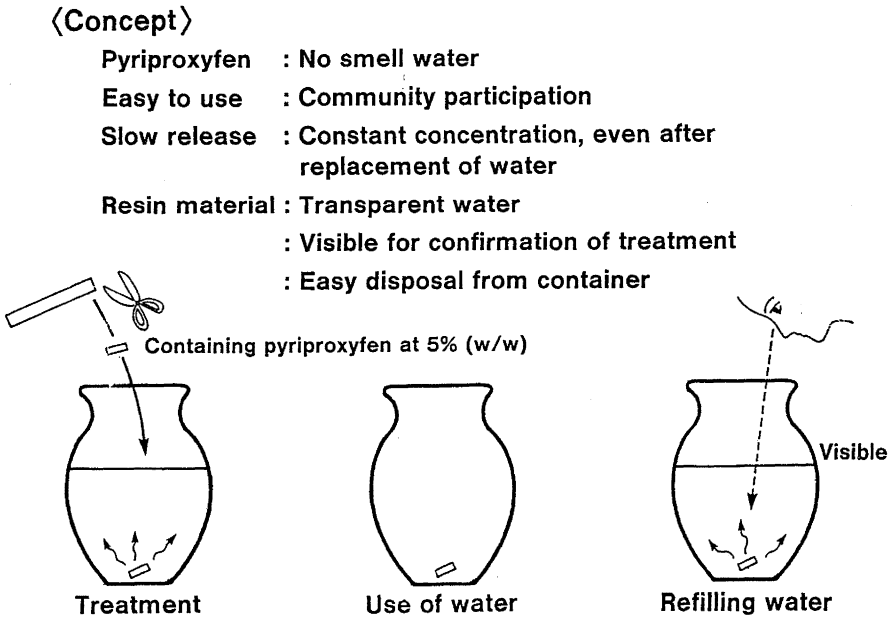


Fig. 2. The concept of the slow release formulation (streched micro porous resin molding) containing pyriproxyfen for larval control

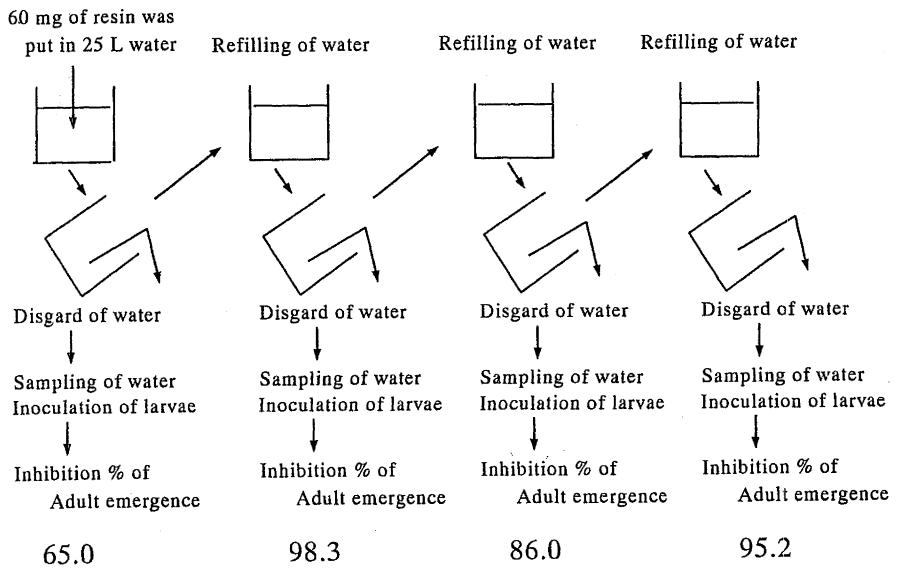


Fig. 3. Inhibitory activity of adult emergence of the new formulation containing pyriproxyfen against *Aedes aegypti* under the simulated condition assuming replenishment of water in a jar

Utilization of adults of *Aedes aegypti* as a vehicle of pyriproxyfen

The main resting place of *Aedes aegypti* is well known as dark place inside houses. When a black color resting trap treated with pyriproxyfen is placed in a house, the adults will rest and contact with pyriproxyfen on the trap. Among them, blood-fed females are expected to transfer the chemical from the body surface to small larval habitats, when they lay eggs there (Fig. 4).

In a laboratory, we could observe that the adult emergence from the larvae kept in a container with water in a cage was highly inhibited, when a blood-fed female, which had contacted with pyriproxyfen at 1 g/m^2 for 30 min, was liberated into the cage. It was obvious that pyriproxyfen was transfered from body surface of the female to the water, when the female laid eggs (Itoh *et al.*, 1993). Then, transmissibility of pyriproxyfen was assessed in a field at Bangkok. Black nylon netting was treated with pyriproxyfen at 1.5 g/m^2 and hold inside a black color bamboo basket. These adult resting traps and ovipositional cups with water for confirmation of the transmissibility were arranged in a house (Fig. 5). Brown color paper with rough surface was lined inside the cups for ovipositional place. The cups were kept in the house for 4 days and brought back to the laboratory of Mahidol University near the experimental site to be inoculated with the last instar larvae of *Aedes aegypti*. Table 1 shows the number of eggs laid and inhibition % of adult emergence from larvae in each cup. The adult emergence from some cups was highly inhibited. For instance, the number of eggs laid was 52 and the inhibition % of adult emergence was 72 in the cup No. 8 on 2nd 4 day. Even though no evidence of

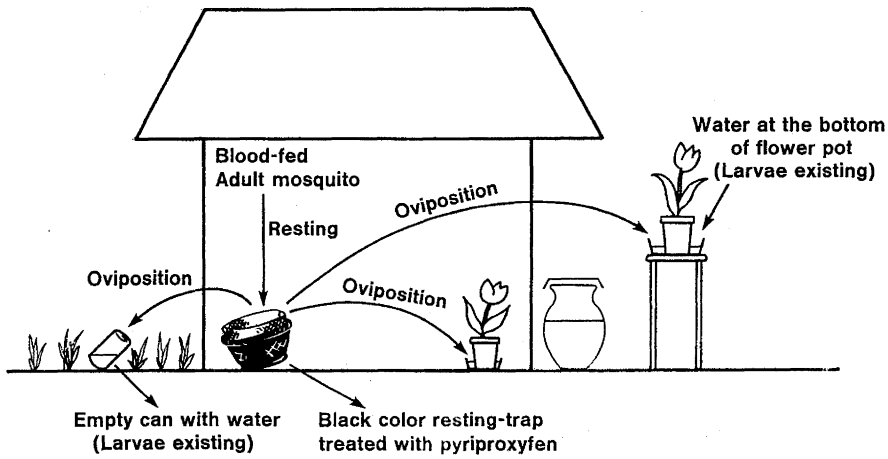


Fig. 4. Utilization of adults of *Aedes aegypti* as a vehicle of pyriproxyfen for small and inconspicuous larval habitats

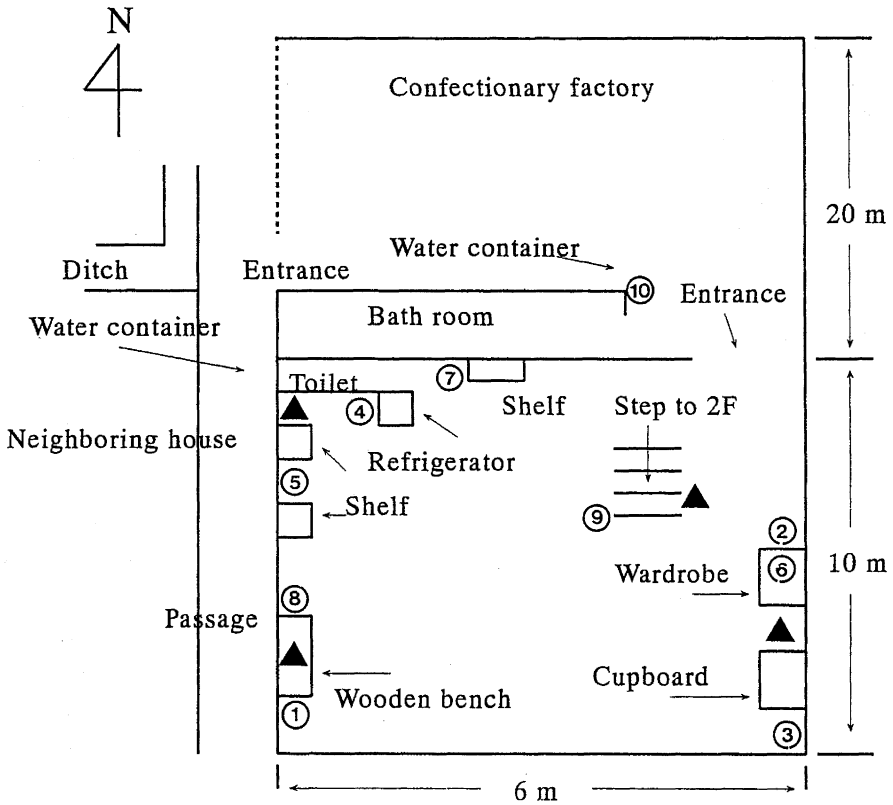


Fig. 5. Diagram of the experimental house in Bangkok (Black triangle: Resting trap, White circle: Oviposition cup)

Table 1. Inhibition of adult emergence from larvae inoculated into cup-water which was kept inside the house for 4 days

Observation items	Cup No.									
	1	2	3	4	5	6	7	8	9	10
	1st 4 days									
No. of eggs laid	42	0	0	31	23	0	41	0	0	0
Inhibition %	62	0	14	0	0	0	57	6	0	0
	2nd 4 days									
No. of eggs laid	0	0	0	79	18	32	11	52	4	7
Inhibition %	16	100	44	2	0	0	2	72	37	0
	3rd 4 days									
No. of eggs laid	23	0	36	21	12	63	9	0	0	0
Inhibition %	5	5	11	0	0	5	5	30	5	5
	4th 4 days									
No. of eggs laid	0	0	0	15	29	39	1	0	0	0
Inhibition %	84	92	7	0	36	3	19	76	84	3

oviposition was observed, the adult emergence was also highly inhibited. For instance, the number of egg was 0 and the inhibition % was 100 in the cup No. 2 on 2nd 4 day. The latter result suggests possibility that any adults played as the vehicle of the chemical to the ovipositional cups. In fact, dead males could be observed in some cups. Further large field trial of pyriproxyfen-treated resting trap become interested.

Permethrin-incorporated bednet for patients

The concept of a new approach with the bednet is prevention of further virus dispersion from patients by mosquito bitings (Fig. 6). However, establishment of an early diagnosis of patients is essential for this approach. The netting was made of a synthetic polymer incorporated with permethrin at 2%. The mesh size of the netting was wide to provide good air-ventilation, and was adopted from the following reason. When the size was less than width of wing expance of flying mosquitoes, the mosquitoes rested on the netting before passing through, thus allowing them time to pick up a lethal amount of permethrin (Itoh *et al.* 1986).

Fig. 7 shows short contact test results. Females of *Aedes aegypti* was confined on the netting for 3 and 7 min, and transfered in a cup with cotton soaked with sugar solution for observation of mortality after 24 hrs. Mortality was 100% in both exposure times of 3 and 7 min. If an early diagnosis become possible, this bednet will be a promissing tool for prevention of virus dispersion.

Concept : Prenvention of virus dispersion from patients
: Ready use
: Good air-ventilation

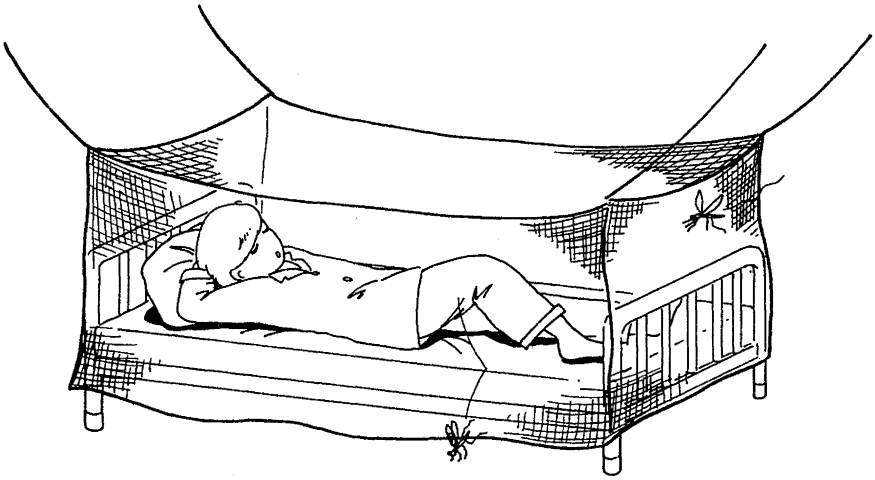
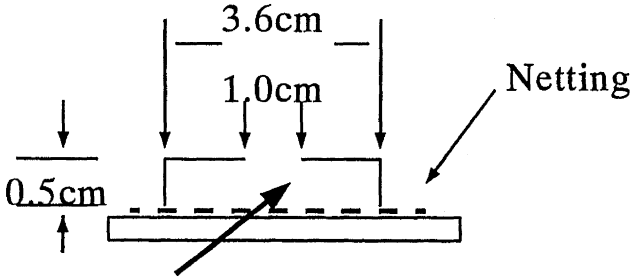


Fig. 6. Permethrin-incorporated bednet for patients



Feamles were confined for 3 or 7 min.

Experimental results

Exposure time	Mortality % after 24 hr.
3 min	100
7 min	100

Fig. 7. Efficacy of permethrin-incorporated netting against females of *Aedes aegypti*

Effect of mosquito coil on host-seeking behaviour of mosquitoes.

A mosquito coil is an effective measure for self-protection from mosquito bitings. Mosquito behaviour on biting cycle is shown in Fig. 8 (Chadwick, 1975). Biting behaviour starts from host-seeking, followed by landing, palpation, probing and sucking-blood. When a mosquito coil disturbes host-seeking, mosquitoes can not bite.

A mosquito coil was ignited in 28 m^3 chamber, of which air was ventilated 5 times per hour. A volunteer sat down in the chamber and one female of *Aedes aegypti* was released into the chamber. The length of time until the female landed on the volunteer was recorded for 3 min. When the blank coil without active ingredient was ignited, the female could land on the volunteer after 1.2 min (Fig. 9 : Teshima, 1992). When BPMC, which is a carbamate insecticide, coil was ignited, the female could land after 1.5 min. However, *d*-allethrin, which is a pyrethroid insecticide, coil at 0.2% was ignited, no female could arrive at the host within 3 min. Thus, *d*-allethrin as an active ingredient of coil formulation is desireble in comparison with BPMC. Improvement of economical condi-

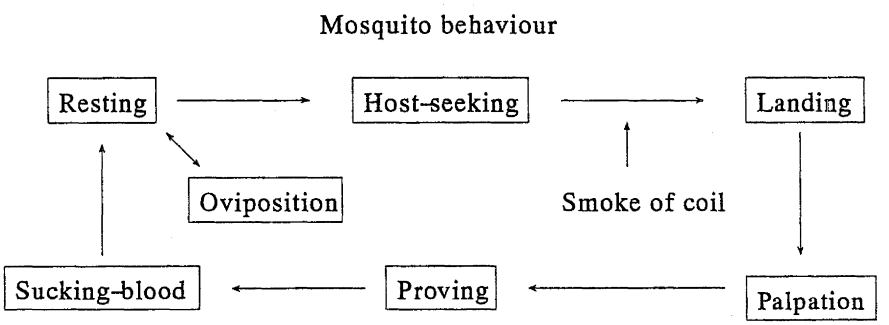


Fig. 8. Biting behaviour of mosquitoes

Active ingedient	Conc. (%)	Landing (%) on man	Time required to land on man (1 — 2 min)	KT50 (min)
d-allethrin	0.1	61	1.4	55
	0.2	0	No mosquito land	39
	0.3	0	No mosquito land	17
BPMC	1.5	89	1.5	>60
Blank coil	-	90	1.2	>60
Untreated	-	100	0.9	>60

Fig. 9. Inhibitory effect of mosquito coil on host-seeking behaviour of *Aedes aegypti* (KT50 means time to be required for 50% knocked-down of mosquito)

tions in community will be necessary for wide spread use of mosquito coil.

Conclusion

Vector control strategy with insecticides should be concentrated to both the space spray of adulticides and larvicidings to the main breeding sites. These should be executed by municiparity. The new formulation of pyriproxyfen make community participation possible, due to easiness of treatment. Pyriproxyfen-treated resting trap and permethrin -incorporated bednets can be performed as supplementary measures in co-operation with community. As a self-protection, use of a mosquito coil will become more important with improvement of economical conditions in the community.

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