Utilization of Bloodfed Females of *Aedes aegypti* as a Vehicle for the Transfer of the Insect Growth Regulator, Pyriproxyfen, to Larval Habitats

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Abstract: Bloodfed females of *Ae. aegypti* were exposed to a surface treated with pyriproxyfen at 1.0 g/㎡ for 30 min and then allowed to lay eggs in cups of water containing 4th instar larvae in a cage. Adult emergence from the immatures was highly inhibited, and transmission of pyriproxyfen from the females to the water was revealed. The transfer of the chemicals to the water decreased with time before the blood meal. Chemical analysis for pyriproxyfen on the exoskeleton of treated females demonstrated the rapid disappearence of the compound. Pyriproxyfen obviously affected egg maturation of females treated before blood meals, as the number of eggs deposited decreased concurrently with the number of days before the blood meals. Utilization of adults of *Ae. aegypti* as a vehicle of pyriproxyfen was examined at a house in Thailand. The black−colour nettings treated with the chemical at 1.5 g/㎡ and ovitraps containing water were arranged inside the house. The ovitraps were collected after 4 days to count the number of eggs deposited. The 4th instar larvae of *Ae. aegypti* were innoculated in the water in the trap. Adult emergence from the larvae was highly inhibited at certain ovitraps. This experimental result suggests that the adults which inhabited in the house came to contact with the treated nettings and carried pyriproxyfen to the water of ovitraps.

Key words: Insect growth regulator, *Aedes aegypti*, Vector control

INTRODUCTION

Schlein and Pener (1990) monitored *Culex pipiens* feeding on sugar solution sprayed onto plants. They then added spores of *Bacillus sphaericus* to the spray solution. As a result, the *B. sphaericus*−carrier mosquitoes caused larval mortality in breeding sites 60−100 m from the treated area. Their experiments suggested the utilization of blood−fed female *Ae. aegypti* as a vehicle for transfer of larvicides to the small and inconspicuous larval habitats such as water at the bottom of household plant pots. When the female lays eggs in or near the water, the larvicide on their bodies may be released into the water to kill the exising larvae. The insect growth regulator, pyriproxyfen, was tested as a larvicide as it was not lethal to adult mosquitoes and had a high activity of adult emergence inhibition (Kawada *et al.*, 1988). The above hypothesis was supported under both laboratory and field conditions.

Materials and Methods

Technical grade of pyriproxyfen (4−phenoxyphenyl (RS)−2−(2−pyridyloxy) propyl ether, purity 95.2%, Sumitomo Chemical Co. Ltd, Osaka) was diluted with a 50:1 mixture of acetone and rape seed oil, and applied onto a film of polyethylene terephthalate at 1.0 g active ingredient per ㎡. The film was used to line the inside wall of a WHO susceptibility test kit tube. Twenty females of *Ae. aegypti* were confined to the test kit for 30min. They had been given blood meal of a chicken 3 days before the treatment. One, 3 and 5 treated females were released into a mosquito cage. A plastic cup lined with filter paper, containing 20 last instar larvae in 100ml of water, was placed inside the cage. The females were allowed to lay eggs on the filter paper for 1 night. The filter paper was removed the next day. The larvae were fed daily and reared until the adult emerged.

The influence of the timing of treatment with pyriproxyfen to females was examinend for the inhibitory effect on adult emergence. The females were treated with pyriproxyfen 4 days before, on the day of, and one and 3 days after feeding on chicken blood. Five females were released into a cage, in which a cup with larvae in 100ml of water was placed. The females were allowed to lay eggs for 1 night, and the number of eggs laid was counted on the next day. The larvae were reared for observation of adult emergence.

Chemical analysis of pyriproxyfen on the body of the females after treatment was done. Fifty femlaes were treated with pyriproxyfen for 30 min as mentioned above, and kept in a cage with a cotton ball soaked with 5% sugar solution. Out of the 50 females, 10 were randomly sampled for chemical analysis of pyriproxyfen on the day of the treatment and 1, 2, 5 and 7 days after the treatment. Pyriproxyfen was extracted from the mosquitoes with 2ml of hexane solution containing an appropriate amount of fenpropathrin as an internal standard for 1hr. The quantity of the active ingredient was determined using HPLC.

Transfer of pyrirpoxyfen from *Ae. aegypti*, which inhabits in a house, to water was assessed in Bangkok. Experimental site was a house located at Wat Makok district of Bangkok. Black nylon netting was treated with acetone solution of pyripoxyfen to provide dosage at 1.5 g active ingredient per ㎡. The netting was hold inside of a black color bamboo basket to make an adult resting traps. Four resting traps were arranged in the room of the house. Ten ovitraps, which were lined with brown color paper for ovipositional site, were also arranged in the same room (Fig. 1). After 4 days of the arrangement, the ovitraps were collected to be inoculated with last instar larvae of *Ae. aegypti* at Mahidol University. By observation of adult emergence from larvae, transfer of pyriproxyfen to water was determined.

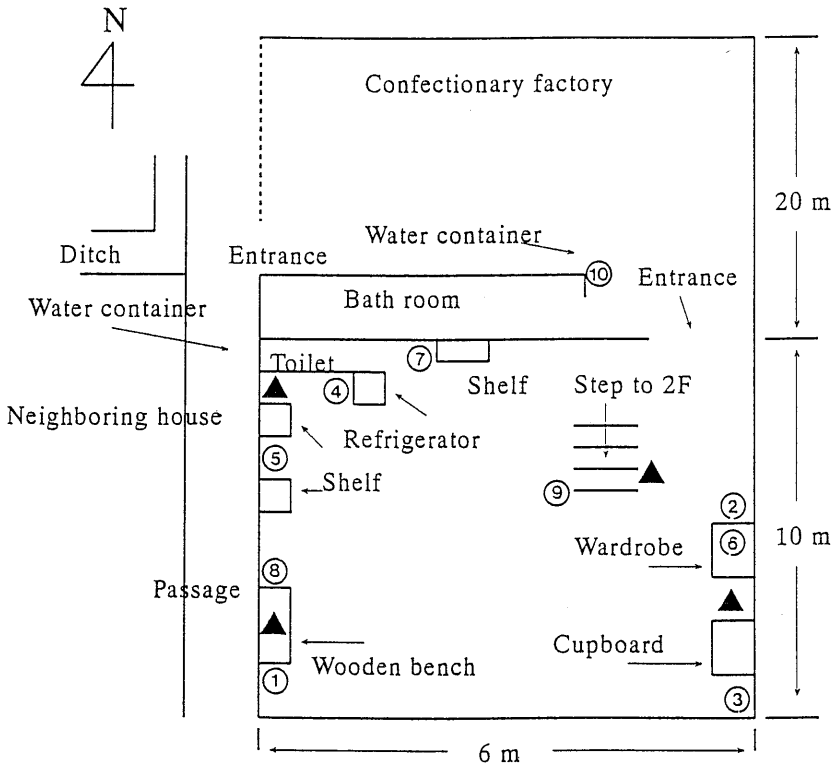

Fig. 1. Arrangement of adult resting traps treated with pyriproxyfen (black triangle) and ovitraps (white circle with number) in a experimental house.

Results and Discussion

Table 1 shows inhibitory activity of three larvicides on adult emergence of *Culex, Anopheles* and *Aedes* mosquitoes. The IC_{50} value indicates the concentration to be required for 50% inhibition of adult emergence from larval stage. The organophosphorus larvicide, abate, inhibited 50% adult emergence of *Ae. aegypti* at 4.5 ppb. Pyriproxyfen inhibited 50% adult emergence of *Ae. aegypti* at 0.023 ppb and its activity was 200 times higher than that of abate.

Table 1. Inhibitory activity of adult emergence from last instar larvae

Chemicals	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)
Abate	1.7(1)	2.7(1)	4.5(1)

* Relative activity to Abate

When 5 untreated females were released in a cage, 95% of larvae could normally emerge (Fig. 2). However, adult emergence rates from larvae were 6.7, 10.0 and 0% in the cases of 1, 3 and 5 treated females per cage. It was evident that pyriproxyfen−treated females affected adult emergence from larvae. Vapour of pyriproxyfen from the treated females did not seem to affect the adult emergence, because normal adult emergence of larvae in the cup covered with netting was observed in preliminary experiment.

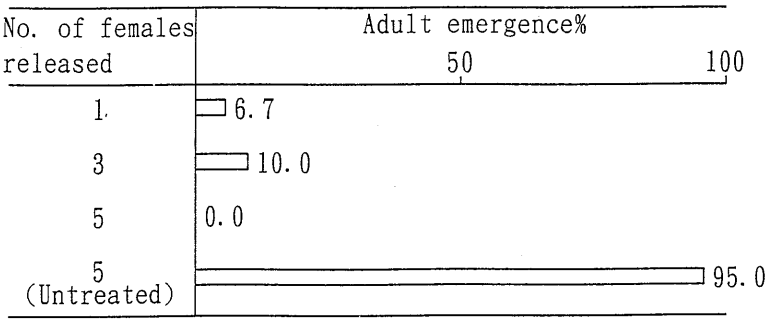

Fig. 2. Adult emergence inhibition by pyriproxyfen−treated bloodfed female *Ae. aegypti.*

The effect on larvae in water when pyriproxyfen was treated before or after blood meals is shown in Fig. 3. In the case of the untreated females, the number of eggs laid in a cup was 402 and adult emergence rate of larvae was 96.7%. When the females were treated 4 days before blood meals, the number of eggs laid was 17 and adult emergence rate was 76.7%. On the contrary, when the females were treated 3 days after blood meals, the number of eggs laid was 193 and adult emergence rate was 5.0%. A reduction in emergence rates was directly related to the time of feeding, whereas the number of eggs laid by the treated females increased with days after blood meals. Judson and Lumen (1976) reported

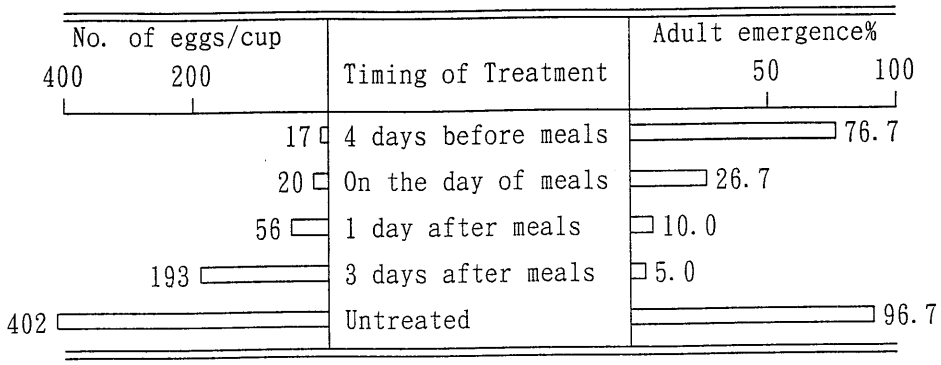

Fig. 3. Adult emergence inhibition by female *Ae. aegypti* treated with pyriproxyfen before and after blood meals.

the effect of synthetic JH analogues on egg development in *Ae. aegypti*. The treatment before or up to 24hr after blood meals inhibited egg maturation, but not after 24hr of blood meals. In Fig. 3, the small number of eggs observed by the treatment before blood meals might be due to inhibition of egg maturation of the females. Then, there is a possibility that higher adult emergence rate of larvae was due to fewer frequency of landing on water by the females treated before blood meals. Because they might have a weaker desire to oviposit. Two kinds of effects of pyriproxyfen may be expected. When bloodfed females are exposed to pyriproxyfen, they carry enough pyriproxyfen to the larval water to inhibit adult emergence. When unfed females are exposed to pyriproxyfen, the number of eggs laid after blood meals decreases.

Table 2 shows persistence of pyriproxyfen picked up by *Ae. aegypti* from the treated film. When the females were confined in the test kit lined with pyriproxyfen−treated film for 30 min, 1.49μg of the chemical was picked up by one female. If all the chemical was solubilized in 500ml, the concentration can be calculated as 2.98 ppb. This concentration was quite enough to inhibit adult emergence from *Aedes* larvae, because the IC_{50} value of pyriproxyfen against the larave was 0.023 ppb as shown in Table 1. However, pyriproxyfen was not detected 7 days after treatment and this rapid disappearance might allow high adult emergence of larave when the chemical was treated to the females before blood meals as shown in Fig. 3.

Table 2. Persistence of pyriproxyfen picked up by *Ae. aegypti* females from pyriproxyfen-treated film.

Days after treatment	Pyriproxyfen (μg) detected per 10 females	Remaining %
0	14.9	100.0
1	8.6	57.9
2	4.9	32.7
5	0.5	3.1
7	Not detected	0.0

Table 3 shows adult emergence of larvae inoculated into the ovitraps which were kept for 4 days in the room with the adult resting trap treated with pyrirpoxyfen. Adult emergence from larave in certain traps was highly inhibited. The number of eggs laid was 52 and inhibition % of adult emergence was 72 in the trap No. 8 on 2nd 4 day. Even though no evidence of oviposition was observed, adult emergence was also highly inhibited. The number of eggs was 0 and inhibition % of adult emergence was 100 in the trap No. 2 on 2nd 4 day. These results suggest possibility that any adults will act as a vehicle of pyriproxyfen to the water. In fact, dead males could be observed in a certain traps.

In conclusion, the utilization of blood fed female *Ae. aegypti* as a vehicle for transfer of pyriproxyfen to small and inconspicuous larval habitats was suggested under laboratory

conditions. Pyripoxyfen obviously affected egg maturation of females treated before blood meals. When the adult resting traps treated with pyrirproxyfen were arranged in a house, both males and females which inhabit in the house might be expected to become a vehicle of pyrirpxyfen to water.

Table 3. Adult emergence from larvae inoculated into ovitraps which were kept inside a 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

REFERENCE

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