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UTILIZATION OF BLOODFED FEMALES OF *AEDES AEGYPTI* AS A VEHICLE FOR THE TRANSFER OF THE INSECT GROWTH REGULATOR PYRIPROXYFEN TO LARVAL HABITATS[1]

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ABSTRACT. Bloodfed female *Aedes aegypti* were exposed to a surface treated with pyriproxyfen at 1.0 g/m² for 30 min and then allowed to lay eggs in cups of water containing 4th-instar larvae. Adult emergence from the immatures was highly inhibited, and transmission of pyriproxyfen from the females to the water was revealed. The transfer of the chemical to the water decreased with time before the blood meal. Chemical analysis for pyriproxyfen on the exoskeleton of treated females demonstrated the rapid disappearance 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.

INTRODUCTION

Aedes aegypti (Linn.) is the main vector of dengue and dengue hemorrhagic fever in Thailand. Household water jars around houses are the main larval breeding sites. There are also many other small breeding sites such as water at the bottom of household plant pots. Reduction of the number of the artificial breeding sites is difficult, but important.

Schlein and Pener (1990) monitored *Culex pipiens* Linn. feeding on sugar solution sprayed onto plants. They then added spores of *Bacillus sphaericus* Neidi to the spray solution. The *B. sphaericus*-carrier mosquitoes caused larval mortality in breeding sites 60–100 m from the treated area. Their experiments suggested the utilization of bloodfed female *Ae. aegypti* as a vehicle for a larvicide for small and inconspicuous larval habitats. When the females lay eggs in or near the water, the larvicide on their bodies may be released into the water to kill existing larvae. The insect growth regulator pyriproxyfen was tested as a larvicide as it was not lethal to adult mosquitoes and had a high emergence inhibition (Kawada et al. 1988). In this paper, the above hypothesis was supported under laboratory conditions.

MATERIALS AND METHODS

Test chemical: We tested pyriproxyfen (4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether, purity 95.2%, Sumitomo Chemical Co. Ltd., Osaka). An emulsifiable concentrate was formulated containing the active ingredient, an emulsifier (Sorpol SM200, Thoho Chemical Co. Ltd., Tokyo), and xylene, in a ratio of 5:10:85 w/w.

Mosquitoes: Three different colonies of *Ae. aegypti* were used. The first colony was collected as immature larvae at the Wat Makok district of Bangkok, Thailand, on July 8, 1992, and was designated as WC (wild-caught) larvae. The 2nd colony originated from a laboratory colony reared for many years at the Department of Medical Entomology at Mahidol University in Bangkok, and was designated as ML. The 3rd colony originally came from larvae collected in Bangkok in 1975 and reared for successive generations at 25°C and 16L–8D photoperiod conditions, and was designated as SL. All of the females used were 10–12 days old.

Determination of susceptibility of WC and ML colony larvae: The emulsifiable concentrate of pyriproxyfen was diluted with distilled water to provide prescribed concentrations and 1 ml of the solution was added to 99 ml of distilled water in a 150-ml plastic cup in which 20 of the WC 4th-instar larvae were introduced. The larvae were fed daily with a small amount of larval food (50:50 mixture of powdered rat diet [CE-2, Nihon Clea Co. Ltd., Tokyo] and dried yeast [Ebios, Asahi Beer Co. Ltd., Tokyo]). They were reared at 26 ± 1°C and were observed until the adults emerged. The experiments were replicated 3

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Table 1. Inhibitory effect of pyriproxyfen on adult emergence of *Aedes aegypti* larvae.

Concentration of pyriproxyfen (ppb)	Percentage of adult emergence[1]	Percentage of emergence inhibition[2]	IC_{50} (ppb)
	WC larvae		
10.0	0.0	100.0	
1.0	6.7	83.3	
0.1	8.3	79.3	0.056
0.01	35.0	12.5	
0.001	48.3	—	
Untreated	40.0		
	ML larvae		
10.0	0.0	100.0	
1.0	1.7	98.2	
0.1	10.0	89.7	0.011
0.01	50.0	48.3	
0.001	86.7	10.3	
Untreated	96.7		

[1] No. of emerged adults/no. of larvae used × 100.
[2] 100 − (% of adult emergence from treated water/% of adult emergence from untreated water) × 100. The larvae that died in the treated cups were almost in the pupal stage.

Table 2. Adult emergence inhibition by pyriproxyfen-treated bloodfed female *Aedes aegypti*.

No. females released in cage	Replicate no.	No. dead females	Oviposition evidence	No. newly emerged adults[1]	Mean % adult emergence
1	1	0	0	0	
	2	0	0	4	6.7
	3	0	0	0	
2	1	1	+	5	
	2	0	+	0	10.0
	3	0	+	1	
5	1	1	+	0	
	2	2	+	0	0.0
	3	1	+	0	
5	1	0	+	17	
Untreated	2	0	+	20	95.0
	3	0	+	20	

[1] The larvae that died in the treated cups were almost in the pupal stage.

times. The percentage of emergence was calculated and then the percentage of emergence inhibition was obtained according to the following formula: percentage of inhibition = 100 − (% of emergence from treated water/% of emergence from untreated water) × 100. The inhibition concentration value (IC_{50}), which is the concentration required for 50% emergence inhibition, was calculated by Bliss's probit analysis (Bliss 1934). Susceptibility of the 4th-instar larvae of the ML colony was compared with that of the WC larvae. These laboratory experiments were carried out at Mahidol University.

Transfer of pyriproxyfen from female mosquitoes to water: Technical grade pyriproxyfen 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 m^2. The film was then used to line the inside wall of a WHO susceptibility test kit tube (4 cm diam × 12 cm high) and held in place by a wire ring. Twenty females of the SL colony were confined to the test kit for 30 min at 25°C. They had been given a chicken blood meal 3 days before the treatment. One, 3, and 5 treated females were released into a mosquito cage (20 × 20 × 30 cm). A plastic container (7 cm top diam, 5 cm bottom diam × 10 cm high) lined with filter paper containing 20 4th-instar larvae (SL colony) in 100 ml of distilled water was placed inside the cage. The females were allowed to lay eggs overnight on the filter paper. The filter paper was removed the next day for observation of egg deposition. Mortality of adult mosquitoes liberated into the cage was also observed. Larvae were fed daily and reared at 25°C until adults emerged.

The influence of the time of treatment of females with pyriproxyfen was examined for an inhibitory effect on adult emergence. SL colony females were treated with pyriproxyfen 1 and 4 days before, and 1 and 3 days after feeding on chicken blood. Five females were released into a mosquito cage in which a container with 100 ml of water and larvae was placed. The females were allowed to lay eggs overnight, and the number of eggs laid and adult mortality was observed the next day. The larvae in the container were reared at 25°C for observation of adult emergence.

Chemical analysis of pyriproxyfen on the body of female mosquitoes after treatment: Fifty females from the SL colony were treated with pyriproxyfen for 30 min, and kept in a cage with a cotton ball soaked with 5% sugar solution. Ten of these females 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 for 1 h with 2 ml of hexane solution containing an appropriate amount of fenpropathrin ((RS)-α-cyano-3-phenoxybenzyl-2,2,3,3-tetramethyl-cyclopropanecarboxylate) as an in-

Table 3. Adult emergence inhibition by pyriproxyfen-treated female *Aedes aegypti* before and after the blood meals.

Time of treatment	Replicate no.	No. dead females	Total no. eggs laid in one night	Total no. newly emerged females[1]	Mean % adult emergence
4 days before meals	1	1	0	12	
	2	0	0	17	76.7
	3	0	50	17	
On the day of meals	1	0	7	7	
	2	0	45	9	26.7
	3	0	9	0	
One day after meals	1	0	5	5	
	2	0	126	1	10.0
	3	0	36	0	
3 days after meals	1	0	81	3	
	2	1	212	0	5.0
	3	1	286	0	
Untreated	1	0	415	20	
	2	0	401	20	96.7
	3	0	390	18	

[1] The larvae that died in the treated cups were almost in the pupal stage.

ternal standard. The quantity of active ingredient was determined using a Hitachi L-4000 HPLC apparatus equipped with a Sumipax ODS A-212 column in the conditions of 230 nm wave length of detector and acetonitrile/water (75:25 v/v) liquid phase at 1 ml/min flow rate.

RESULTS AND DISCUSSION

The IC_{50} values of pyriproxyfen were calculated to be 0.056 and 0.011 ppb against the WC and ML colony larvae, respectively, as shown in Table 1. The difference in susceptibility between the 2 colonies was about 5 times. Adult emergence rate of the WC larvae in untreated water was 40.0%, and that of the ML colony was 96.7%. The comparatively lower emergence rate for the WC larvae might have been due to a parasitic infection or possibly the difference in environmental conditions between the field-collected and the laboratory-maintained larvae. Hatakoshi et al. (1987) reported an IC_{50} value of 0.023 ppb for pyriproxyfen on larvae of the SL colony; the difference in susceptibility between the WC and SL colony larvae is only 2.5 times and thus we decided to use the SL colony for larval control in Bangkok.

Table 2 shows the emergence inhibition effects on larvae from pyriproxyfen carried by treated bloodfed females. Adult emergence rates from the larvae were 6.7, 10.0, and 0% in the cases of 1, 2, and 5 treated females/cage. Adult emergence was 95.0% from the cage with the 5 untreated females. It was evident that pyriproxyfen-treated females affected adult emergence from larvae. Even though no evidence of oviposition was observed in the case of one treated female/cage, the emergence rate was quite low. This suggests that pyriproxyfen was carried to the water surface or to the wetted filter paper by the treated female. In the latter case, pyriproxyfen may have migrated from the filter paper to the water, and further observations should be made on the inhibitory effect of pyriproxyfen without filter paper. The possibility that the vapor of pyriproxyfen from the treated females affected adult emergence seems highly unlikely. Our preliminary experiment indicated normal adult emergence from larvae in a cup covered with netting to prevent the treated females from making contact with the inside of the cup.

The effect on larvae in water when pyriproxyfen was applied before or after blood meals is shown in Table 3. Adult emergence rates were 76.7, 26.7, 10.0, and 5.0% with treatment 4 days before, on the day of, 1 day after, and 3 days after a blood meal. A reduction in emergence rates was directly related to the time of feeding, whereas the number of eggs laid by treated females increased with days after blood meals. There are 2 possibilities for the increase in adult emergence with treatment 4 days before blood meals. Pyriproxyfen is a juvenile hormone (JH) mimic that suppresses egg development and maturation of adult mosquitoes. Judson and de Lumen (1976) reported the effect of 2 synthetic

JH analogues on egg development in *Ae. aegypti*. Treatment before or up to 24 h after blood meals inhibited egg maturation, but not after 24 h. We also observed that the number of eggs laid by treated females decreased when treatment was made before blood meals (Table 3). The frequency of landing on the water by prebloodfeeding treated females was lower than for postbloodfeeding females. Perhaps this was due to the prebloodfeeding females having fewer mature eggs and a weaker desire to oviposit. The lower landing frequency of treated females may result in smaller releases of pyriproxyfen into the water. The other possibility is that the amount of pyriproxyfen on the surface of the cuticle of the females may decrease with time, due to decomposition or penetration into the body through the cuticle. This would lead to smaller amounts of pyriproxyfen entering the water. Table 4 shows the persistence of pyriproxyfen on the body of the females after treatment. Pyriproxyfen was not detectable after day 7. There was a reduction in the number of eggs laid by treated females. Pyriproxyfen evidently affected egg maturation of females treated before blood meals. Thus, two kinds of effects of pyriproxyfen may be expected: 1) When unfed females are exposed to pyriproxyfen, the number of eggs laid after blood meals decreases, and 2) when bloodfed females are exposed to pyriproxyfen, they carry sufficient pyriproxyfen to the larval water to inhibit adult emergence.

When females were confined in the test kit lined with pyriproxyfen-treated film for 30 min, 1.49 μg of pyriproxyfen was picked up by one female (Table 4). If all the pyriproxyfen was solubilized in 500 ml of water, the concentration would be 2.08 ppb. This concentration was quite enough to inhibit adult emergence from *Aedes* larvae, as the IC_{50} value of pyriproxyfen against 3 colonies was 0.011–0.056 ppb (Table 1). However, rapid disappearance of pyriproxyfen from the body surfaces of females, as shown in Table 4, suggests the possibility for transfer of the compound to the water. Pyriproxyfen or its metabolite might be excreted through malpighian tubules back into the water, as it is not probable that pyriproxyfen, which is insoluble in water, is removed from the waxy layer of the cuticle and dissolved in water. This possibility should be clarified by further study.

Our laboratory experiments may support the possibility of utilizing bloodfed female mosquitoes of *Aedes aegypti* as a vehicle for pyriproxyfen for the control of mosquitoes in small and inconspicuous larval habitats.

Table 4. Persistence of pyriproxyfen picked up by *Aedes aegypti* females from pyriproxyfen-treated film.

Days after larvicide treatment	Pyriproxyfen (μg) detected per 10 females				
	Replicate no.				
	1	2	3	Mean	Percentage remaining
0	13.7	12.7	18.8	14.9	100.0
1	7.0	8.4	10.4	8.6	57.9
2	4.9	4.7	5.0	4.9	32.7
5	0.6	0.4	0.5	0.5	3.1
7	ND[1]	ND	ND	ND	0.0

[1] ND = not detected.

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