Effectiveness of pyriproxyfen-controlled release block against larvae of *Aedes* (*Stegomyia*) *aegypti* in Kuala Lumpur, Malaysia

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Abstract

This study was conducted to evaluate the effect of a commercially available pyriproxyfen, an insect growth regulator (IGR) on the larvae of a dengue vector, *Ae. aegypti*. The study site was the surrounding area of the Medical Entomology Unit, Institute for Medical Research (IMR), Kuala Lumpur (N03°10.167′, E101°41.919′). Pyriproxyfen-controlled release blocks with dosages of 10% w/w and 20% w/w were used to treat a set of earthen jars placed outdoors. Untreated jars were also set up as controls. Fifty laboratory-bred 2nd instar larvae were introduced into each earthen jar and observed daily. The number of adults that emerged was recorded and the larval mortality was calculated. The indicators of effectiveness of IGR for these studies were: (i) duration of effectiveness, and (ii) percentage of emergence inhibition (EI). There was a significant difference in the number of emerged adults obtained from the untreated and treated earthen jars up to 25 weeks ($p<0.05$). The duration of effectiveness of pyriproxyfen caused 80% emergence inhibition in earthen jars treated with 10% w/w and 20% w/w pyriproxyfen up to 22 and 25 weeks, respectively. Pyriproxyfen-controlled release block is an effective method of controlling mosquito larvae for several months. The method of application of the block is simple and straightforward and can therefore be used easily.

Keywords: *Aedes aegypti*; Pyriproxyfen; Controlled release block; Duration of effectiveness; Emergence inhibition.

Introduction

Aedes aegypti is a principle vector of dengue in many parts of the world. It is one of the major domestic groups of mosquitoes that are pests of man as well as a vector of disease. In many areas of the world, this species commands considerable attention in term of its management and control[1]. The number of options available for mosquito control at the present time are also limited. The control of this mosquito is still dependent on the use of chemical insecticides.

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Although insecticides are invaluable in preventing and controlling damage to agricultural products and to the health of man and animals, they are not without side-effects on the environment and its biota[2]. There is a critical need to find and develop new agents and products for the control of this and other important species of mosquitoes. Insect growth regulators (IGRs) are now increasingly used to control *Aedes* and other mosquito larvae. These compounds have unique modes of action, and are often selective and do not persist in the environment. Such attributes are desirable when dealing with the problem of pest resurgence, secondary pest outbreaks and insecticides resistance[3].

Pyriproxyfen, 2-[1-methyl-2-(4-phenoxyphenoxy) ethoxyl] pyridine, is a new generation of IGR. It is a juvenile hormone analogue and a relatively stable aromatic compound. It functions as an insecticide by overloading the hormonal system of the target insect, ultimately affecting its egg production, brood care and other social interactions, and inhibiting its growth[4]. Pyriproxyfen works well against public health insects like houseflies and mosquitoes[5]. Pyriproxyfen is reported to exhibit 95% inhibition of the emergence of mosquito larvae and its effects on mosquito larvae having lasted for two months after application[6]. Although the treated mosquito larvae continue to pupate, however, their emergence is inhibited by the action of pyriproxyfen[7].

The controlled release block used in this study was impregnated with 10% w/w and 20% w/w a.i. (active ingredient) of pyriproxyfen granules. Pyriproxyfen-controlled release block is claimed to be an easy method applicable in areas such as drains, ponds, lakes, etc., where mosquitoes breed.

This study was conducted to evaluate the commercially available pyriproxyfen-controlled release block used for the control of *Ae. aegypti* larvae in earthen jars.

Materials and methods

Test site

The study was conducted in the area surrounding the Medical Entomology Unit, Institute for Medical Research (IMR), Jalan Pahang, Kuala Lumpur (N03°10.167', E101°41.919').

Insect growth regulator

A formulation of insect growth regulator, pyriproxyfen-controlled release block, was used in this study. Two concentrations of controlled release block were provided, each containing pyriproxyfen at 10% w/w a.i. and 20% w/w a.i. The formulation was provided by Zero-Moz Japan Sdn. Bhd.

Test containers

Earthen jars were used as mosquito breeding containers in this study. Earthen jars each with an opening of 52 cm in diameter, base diameter of 35 cm and 47 cm in height were prepared and placed outdoors. Three replicates of each were used in each research arm of the study (Table 1). Each earthen jar held 60 L tap water. Before initiating the study, all containers were washed with tap water and tested for the presence of contaminant, such as insecticides, by introduction of 50 *Ae. aegypti* 2nd instar larvae. The larvae were observed until complete emergence as adult.

Table 1: Set-up of earthen jars for testing

Earthen jar	Chemical (active ingredient)	Number of replicates
Untreated	None	3
Treated (with controlled release block)	Granular pyriproxyfen 10% w/w	3
	Granular pyriproxyfen 20% w/w	3

Trial procedures

Each pyriproxyfen-controlled release block was placed into earthen jar (3 replicates) and labelled. Three earthen jars without pyriproxyfen-controlled release block served as untreated control. In each test, 50 laboratory-bred 2nd instar larvae were introduced into each earthen jar and observed daily. Pupae were collected, recorded and transferred into paper cups covered with net. The total number of adults emerged was recorded and the larvae mortality rates were calculated. A total of 50% of water (30 L) was removed and added into the earthen jars every alternate day. The same procedure was repeated by adding fresh batch of larvae (50 larvae) into each earthen jar weekly.

Data analysis

The indicators of effectiveness of pyriproxyfen-controlled release block for these studies were:

(1) duration of effectiveness of each dosage, and

(2) percentage of emergence inhibition (EI) =

$$\frac{\text{Number of larvae introduced} - \text{Number of adult emerged}}{\text{Number of larvae introduced}} \times 100\%$$

A cut-off point of EI ≥ 80% was considered to be effective.

If percentage of untreated EI was > 5%, the percentage of treated EI was corrected by Abbott's formula:

$$\frac{\%\ \text{treated EI} - \%\ \text{untreated EI}}{100 - \%\ \text{untreated EI}} \times 100\%$$

Results and discussion

Table 2 shows the number of pupae, adult emergence and emergence inhibition obtained from earthen jars treated with 10% w/w and 20% w/w pyriproxyfen-controlled release block that were impregnated with 10% w/w and 20% w/w pyriproxyfen granules. The result showed a significant difference on the number of pupae collected from all treated (10% w/w and 20% w/w) and untreated earthen jars ($p<0.05$). However, there was no significant difference on the number of pupae collected from the 6th week onwards ($p>0.05$), indicating that pyriproxyfen exhibited low larvicidal activity against *Ae. aegypti*. This is similarly reported by Lee et al.[8] in which studies were carried out on the bio-efficacy and duration of the effectiveness of pyriproxyfen (Sumilarv 0.5%) as direct applications for the control of larvae of *Ae. aegypti* and *Ae. albopictus*. At 79.5 mg/L and 159.0 mg/L, pyriproxyfen showed low larvicidal activity but provided very effective control of adult emergence against larvae of *Ae. aegypti* and *Ae. albopictus*[8].

A significant difference in the number of adult emergence was observed in both the

Table 2: Number of pupae, emergence of adult and emergence inhibition obtained from earthen jars treated with 10% w/w and 20% w/w pyriproxyfen-controlled release block

Week	Test period	Mean ± SE of collected pupae				Mean ± SE adult emerged			
		Untreated	Treated		One way ANOVA	Untreated	Treated		One way ANOVA
			10% w/w	20% w/w			10% w/w	20% w/w	
1	5 Feb – 11 Feb	48.00 ± 1.00	15.67 ± 10.73	37.33 ± 3.28	F = 6.42 p = 0.032	48.00 ± 1.00	0.00 ± 0.00	0.00 ± 0.00	F = 2304.00 p = 0.000
2	12 Feb – 18 Feb	43.18 ± 3.18	21.67 ± 11.79	9.33 ± 1.20	F = 5.85 p = 0.039	43.18 ± 3.18	0.00 ± 0.00	0.00 ± 0.00	F = 184.38 p = 0.000
3	19 Feb – 25 Feb	40.67 ± 2.03	2.00 ± 0.58	1.33 ± 0.33	F = 333.26 p = 0.000	40.67 ± 2.03	0.00 ± 0.00	0.00 ± 0.00	F = 401.38 p = 0.000
4	26 Feb – 4 Mar	39.00 ± 2.65	10.33 ± 5.90	4.33 ± 2.03	F = 22.41 p = 0.002	39.00 ± 2.65	0.00 ± 0.00	0.00 ± 0.00	F = 216.59 p = 0.000
5	5 Mar – 11 Mar	20.50 ± 7.50	0.33 ± 0.33	0.00 ± 0.00	F = 7.34 p = 0.024	20.50 ± 7.50	0.00 ± 0.00	0.00 ± 0.00	F = 7.47 p = 0.024
6	12 Mar – 18 Mar	45.33 ± 1.76	39.33 ± 1.33	36.33 ± 4.10	F = 2.91 p = 0.131	45.33 ± 1.76	0.00 ± 0.00	0.00 ± 0.00	F = 663.36 p = 0.000
7	19 Mar – 25 Mar	41.47 ± 0.88	35.33 ± 2.85	33.67 ± 1.86	F = 8.44 p = 0.018	41.33 ± 1.20	0.00 ± 0.00	0.00 ± 0.00	F = 1186.23 p = 0.000
8	26 Mar – 1 Apr	30.33 ± 14.40	0.67 ± 0.33	0.67 ± 0.67	F = 4.23 p = 0.134	44.00 ± 5.00	0.00 ± 0.00	0.00 ± 0.00	F = 77.44 p = 0.003
9	2 Apr – 8 Apr	49.67 ± 0.33	47.00 ± 3.00	40.00 ± 2.65	F = 4.64 p = 0.061	49.00 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	F = 7137.34 p = 0.000
10	9 Apr – 15 Apr	49.33 ± 0.67	42.33 ± 3.38	25.00 ± 6.56	F = 8.57 p = 0.017	48.67 ± 0.88	0.00 ± 0.00	0.00 ± 0.00	F = 3058.84 p = 0.000
11	16 Apr – 22 Apr	47.33 ± 2.19	46.33 ± 2.73	43.33 ± 3.38	F = 0.55 p = 0.604	46.33 ± 2.33	0.00 ± 0.00	0.00 ± 0.00	F = 395.38 p = 0.000
12	23 Apr – 29 Apr	44.00 ± 2.08	36.33 ± 2.40	41.67 ± 2.03	F = 3.26 p = 0.110	40.00 ± 2.52	0.00 ± 0.00	0.00 ± 0.00	F = 251.95 p = 0.000
13	30 Apr – 6 May	42.00 ± 2.08	38.67 ± 2.40	40.67 ± 3.18	F = 0.42 p = 0.677	40.00 ± 1.53	0.00 ± 0.00	0.00 ± 0.00	F = 683.50 p = 0.000

Week	Test period	Mean ± SE of collected pupae				Mean ± SE adult emerged			
		Untreated	Treated		One way ANOVA	Untreated	Treated		One way ANOVA
			10% w/w	20% w/w			10% w/w	20% w/w	
14	7 May – 13 May	43.33 ± 2.73	38.00 ± 3.21	34.00 ± 4.73	F = 1.64 p = 0.271	41.67 ± 2.40	0.00 ± 0.00	0.00 ± 0.00	F = 301.46 p = 0.000
15	14 May – 20 May	41.67 ± 2.91	39.67 ± 2.96	35.33 ± 3.76	F = 1.00 p = 0.420	38.33 ± 1.67	0.00 ± 0.00	0.00 ± 0.00	F = 526.80 p = 0.000
16	21 May – 27 May	44.33 ± 3.48	37.67 ± 2.33	33.67 ± 6.33	F = 1.51 p = 0.294	43.67 ± 3.76	0.00 ± 0.00	0.00 ± 0.00	F = 134.89 p = 0.000
17	28 May – 3 June	46.00 ± 2.65	35.33 ± 4.91	33.33 ± 5.04	F = 2.46 p = 0.166	41.67 ± 1.67	0.00 ± 0.00	0.00 ± 0.00	F = 622.61 p = 0.000
18	4 June – 10 June	41.33 ± 1.86	34.67 ± 3.84	33.00 ± 3.21	F = 2.04 p = 0.210	39.67 ± 2.19	0.00 ± 0.00	0.00 ± 0.00	F = 328.12 p = 0.000
19	11 June – 17 June	37.67 ± 2.19	36.00 ± 1.53	36.00 ± 3.61	F = 0.14 p = 0.874	36.67 ± 1.76	0.00 ± 0.00	0.00 ± 0.00	F = 1302.32 p = 0.000
20	18 June – 24 June	36.67 ± 5.78	42.00 ± 2.08	40.67 ± 2.60	F = 0.52 p = 0.620	30.00 ± 8.33	1.67 ± 0.08	0.00 ± 0.00	F = 12.29 p = 0.008
21	25 June – 1 July	Not available							
22	2 July – 8 July	44.00 ± 1.15	37.67 ± 1.45	26.33 ± 10.68	F = 2.05 p = 0.210	44.00 ± 1.15	3.00 ± 0.58	0.00 ± 0.00	F = 1092.89 p = 0.000
23	9 July – 15 July	46.50 ± 1.50	27.00 ± 5.51	16.33 ± 1.86	F = 13.21 p = 0.010	45.50 ± 2.50	12.33 ± 3.53	0.33 ± 0.33	F = 71.58 p = 0.000
24	16 July – 23 July	Not available							
25	24 July – 30 July	48.00 ± 1.00	35.00 ± 14.50	41.67 ± 4.91	F = 0.35 p = 0.719	47.67 ± 0.33	31.67 ± 12.86	9.33 ± 6.98	F = 5.19 p = 0.049
26	31 July – 6 Aug	36.67 ± 1.20	37.67 ± 2.85	34.33 ± 5.17	F = 0.24 p = 0.792	33.00 ± 3.06	33.67 ± 2.40	30.67 ± 5.36	F = 0.17 p = 0.848
27	7 Aug – 13 Aug	34.33 ± 12.20	46.00 ± 1.53	43.67 ± 2.33	F = 0.73 p = 0.520	31.33 ± 11.17	42.00 ± 0.58	19.33 ± 9.33	F = 1.82 p = 0.241

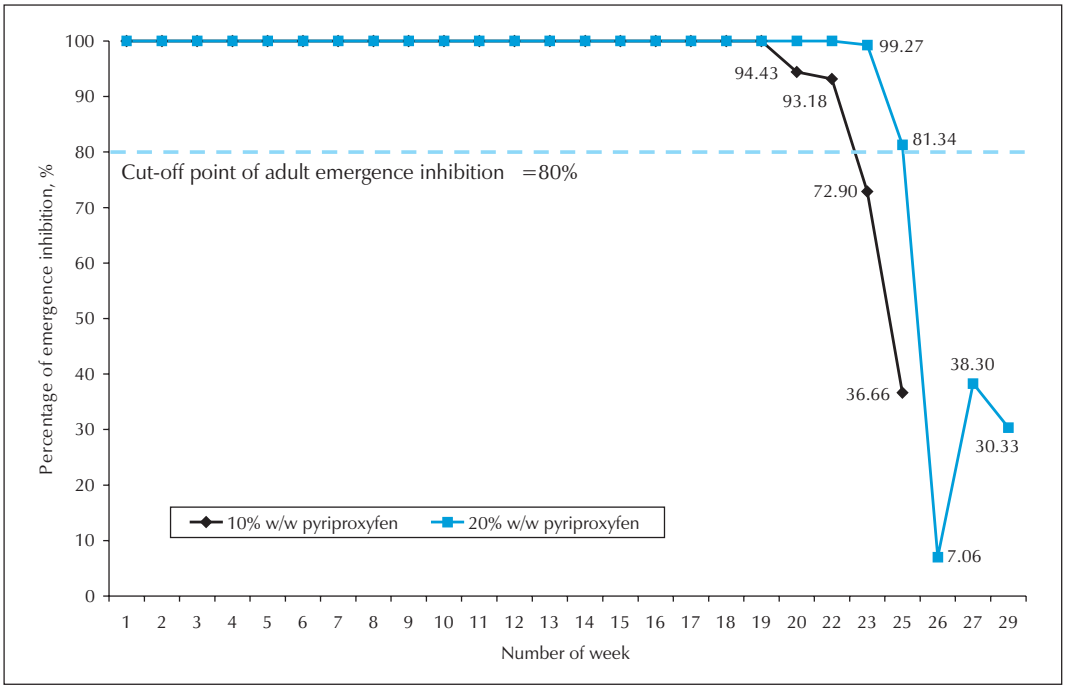
SE = standard error; $p>0.05$ = no significant difference; $p<0.05$ = significant difference; $p<0.01$ = highly significant difference

treated and untreated earthen jars up to 25 weeks ($p<0.05$). The result indicated that in the untreated jar, not all the pupae successfully emerged as adults throughout the trial period. In the earthen jars treated with 10% w/w and 20% w/w pyriproxyfen granules, although some larvae pupated successfully, none of these pupae could emerge as adults up to 19 and 22 weeks, respectively. This finding was similar to that reported by Sihuincha et al.[7], where larvae continued to pupate but failed to emerge. After this, both earthen jars (treated with 10% and 20% pyriproxyfen granules) exhibited e"80% emergence inhibition for another 3 weeks, indicating that both 10% and 20% pyriproxyfen were able to inhibit the emergence of adult *Ae. aegypti* for 22 weeks (5 months) and 25 weeks (6 months), respectively (Figure).

In Cambodia, the inhibition of adult emergence of *Ae. aegypti* in simulated domestic water storage containers by using controlled-release formulation of pyriproxyfen showed that at target dosages of 18, 27 and 36 µg/L of a.i., inhibition of adult emergence remained above 95% for at least two months. After three months at 18 µg/L a.i., the residual efficacy was significantly lower than for the higher dosages ($p<0.05$)[9]. At the higher dosages, inhibition of adult emergence was > or = 87% for six months[9].

The persistence and efficacy of pyriproxyfen were evaluated in two final concentrations of 0.01 and 0.05 mg/L against *Ae. aegypti* larvae in laboratory conditions using three types of containers, i.e. cement box, glass bottle and plastic bucket, in the University of

Figure: Percentage emergence inhibition of *Ae. aegypti* exposed to 10% w/w and 20% w/w pyriproxyfen-controlled release block in earthen jars



Federal de Minas Gerais. The study indicated that a persistency of 45 and 90 days by using 0.01 and 0.05 mg/L final concentrations of pyriproxyfen respectively was observed[10].

In another study, pyriproxyfen was tested against *Ae. aegypti* at 0.01 and 0.02 mg of active ingredient (a.i.) per litre of water in 60 litre earthen jars. Both concentrations provided 100% control for four months. Moreover, in field trial condition, pyriproxyfen at a dosage of 0.02 mg a.i. per litre provided 100% control for 10 weeks against *Ae. albopictus*[11].

In Japan, blood-fed female *Ae. aegypti* were exposed to a surface treated with pyriproxyfen at 1.0 g/m^2. The results showed that transmission of pyriproxyfen from females to the water was revealed[12]. Pyriproxyfen affected the egg maturation of females treated before blood meals, as the number of eggs deposited decreased concurrently with the number of days before the blood meal[12].

The use of pyriproxyfen-treated oviposition containers to achieve horizontal transfer of pyriproxyfen to mosquito oviposition sites also can be a field management technique based on mosquito biology and behaviour. A study conducted in the North Carolina State University showed that horizontal transfer of pyriproxyfen by *Ae. albopictus* from a container with a treated ovistrip (0.3 or 0.4 mg/cm^2) to an untreated microcosm resulted in 14% – 38% inhibition[13].

Besides controlling *Aedes*, many researchers have also reported that pyriproxyfen was able to control *Culex* mosquitoes in Israel[14], Egypt[15], Florida[16] and Bangladesh[17]; and *Anopheles* mosquitoes in Sri Lanka[18] and Solomon Island[19].

The current study showed that pyriproxyfen-controlled release block is an effective method of controlling mosquito larvae for several months. The method of application of the block is simple and straightforward, and can therefore be used easily. This method can be applied in areas such as drains, ponds and lakes where mosquitoes breed and in which a long-term control is desired.

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