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**SIMULATED FIELD EFFECTIVENESS OF
ZERO-MOZ CONTROLLED RELEASE BLOCK®
AGAINST LARVAE OF *Anopheles maculatus* Theobald**

by

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INTRODUCTION

In Malaysia, there are about 431 species of mosquitoes belonging to 20 genera (Abu & Salmah, 1990). Mosquitoes are important vectors of several tropical diseases, such as malaria, dengue, Japanese encephalitis and so on. They also are nuisance pest in many countries.

Anopheles mosquitoes are the notorious vectors of disease to man (Service, 1980). *Anopheles* mosquitoes can transmit the malaria parasite, *Plasmodium* species. *Anopheles* mosquitoes are active between sunrise and sunset. *Anopheles malculatus* is the most important vector in hills and mountains that have been cleared of forest. The larvae are found in clean and slow flowing water which is exposed to direct sunlight (Sulaiman, 2000).

The use of chemical agents is one of the most important methods of controlling these vectors. Among the chemicals, insect growth regulators have become an important tool for the control of mosquitoes. A variety of insect growth regulators (IGRs) have exhibited excellent activity in the laboratory and field against a wide range of stagnant and floodwater mosquitoes (Mulla *et al.*, 1986).

Pyriproxyfen 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 egg production, brood care and other social interactions, and inhibiting growth (Glancey *et al.*, 1990). Pyriproxyfen works well against public health insects like houseflies and mosquitoes (The



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British Crop Protection Council, 1991). Pyriproxyfen is reported to have 95% inhibition of emergence for mosquito larvae and its effects on mosquito larvae lasted for two months after application (Miyamoto *et al.*, 1993).

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 drain, ponds, lakes etc where mosquito breeds.

OBJECTIVE

This study was conducted to evaluate the commercially available pyriproxyfen controlled release block for its impacts on the control of *An. maculatus* larvae in earthen jars.



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MATERIALS AND METHODS

Test site

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

Test Insect

Larvae of lab-bred *An. maculates* were used in the test. This colony is maintained in the lab for > 10 years and not exposed to any control agents.

Insecticide

A formulation of insect growth regulator, ZERO-MOZ Controlled Release Block® was used in this study. Two concentrations of ZERO-MOZ Controlled Release Block® was provided each containing granular pyriproxyfen 10% w/w a.i. and 20% w/w a.i., respectively.

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 outdoor. Three replicates of each were used in each research arm of the study (Table 1). Each earthen



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jar held 60 L tap water. Before initiating the study, all containers were washed with tap water and tested for the presence of contaminant by introduction of 25 *Anopheles maculatus* 2nd instar larvae. The larvae were observed until complete emergence as adult.

Table 1. Setup of earthen jars for testing

Earthen jar	Chemical (active ingredient)	Number of replicates
Untreated	None	3
Treated	Granular pyriproxyfen 10% w/w	3
(with ZERO-MOZ Controlled Release Block®)	Granular pyriproxyfen 20% w/w	3

Trial procedures

Each ZERO-MOZ Controlled Release Block® was placed into earthen jar (3 replicates) and labeled. Three earthen jars served as untreated control without ZERO-MOZ Controlled Release Block®. A total of 25 laboratory-bred 2nd instar larvae were introduced into each earthen jar. All earthen jars were observed daily. Pupae were collected, recorded and transferred into a paper cup 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 (25 larvae) into each earthen jar weekly.


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Data analysis

The indicators of effectiveness of ZERO-MOZ Controlled Release Block® for these studies were:

- a) residual activities of each dosage, and
- b) 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 \geq 80% is considered to be effective.

If percentage of untreated EI is $> 5\%$, the percentage of treated EI should be corrected by Abbott's formula:

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



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Figure 1. ZERO-MOZ Controlled Release Block® was provided containing pyriproxyfen granules



Figure 2. Earthen jar treated with 10% w/w ZERO-MOZ Controlled Release Block®



Figure 3. Earthen jar treated with 20% w/w ZERO-MOZ Controlled Release Block®

A handwritten signature in black ink, likely belonging to Chen Chee Dhang.

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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 ZERO-MOZ Controlled Release Block® that are impregnated with 10% w/w and 20% w/w pyriproxyfen granules. The result shows the significant level of the number of pupae collected from all treated (10% w/w and 20% w/w) and untreated earthen jars were inconsistent when analyzed with one way ANOVA, indicating that pyriproxyfen shows low larvicidal activity against *An. maculatus*.

A significant difference on number of adult emergence was observed in both treated and untreated earthen jars up to 45 weeks ($p < 0.05$). The result indicates 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 throughout the study period, indicating 100% emergence inhibition was achieved up to 45 weeks.

This study is still in progress to determine the 80% emergence inhibition rates of the earthen jars treated with 10% w/w and 20% w/w pyriproxyfen granules.

Yapabandara & Curtis (2004) reported that single treatment of pyriproxyfen effectively inhibited the emergence of adult mosquitoes in the riverbed pools for a period of 190 days. The treatment caused significant reduction of the adult populations of *An. culicifacies* (78%) and *An. subpictus* (72%). Similarly,



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incidence of malaria was reduced in the treatment villages by about 70% (95% confidence interval 58-78%) compared with the controls.

Yapabandara & Curtis (2002) also reported pyriproxyfen at dosages of 0.01 and 0.1mg/l were tested in the laboratory and complete inhibition of emergence was found at both concentrations.

Okazawa *et al.* (1991) reported that adult emergence of *An. punctulatus* was inhibited completely for 2 months at a dosage of 0.1 mg/L, for one month at 0.05 mg/L and 0.01 mg/L, and for 20 days at 0.02 mg/L. Death of test insects were observed at the pupal stage and at adult emergence. The mortality rate at adult emergence increased with the duration of larval rearing and with the elapse of time after application.

Besides controlling *Anopheles* mosquitoes, many researchers also reported that pyriproxyfen was able to control *Culex* mosquitoes in Israel (Schwartz *et al.*, 2003), Egypt (El-Shazly & Refaie, 2002), Florida (Nayar *et al.*, 2002) and Bangladesh (Ali *et al.*, 1999); and *Aedes* mosquitoes in Malaysia (Lee *et al.*, 2005; Vythilingam *et al.*, 2005), Cambodia (Chang *et al.*, 2006) and Brazil (Resende & Gama, 2006).

This study shows that ZERO-MOZ 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 is applicable in areas such as drain, ponds, lakes where mosquito breeds and in which long term control is desired.



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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 \pm SE of collected pupae				Mean \pm SE adult emerged				Emergence inhibition (%)	
		Untreated	Treated		One way ANOVA	Untreated	Treated		One way ANOVA	Treated	
			10% w/w	20% w/w			10% w/w	20% w/w		10% w/w	20% w/w
1	6 Aug - 12 Aug	21.00 \pm 1.52	15.67 \pm 1.45	10.67 \pm 0.67	F = 16.47 P = 0.004	21.00 \pm 1.52	0.00 \pm 0.00	0.00 \pm 0.00	F = 190 P = 0.000	100	100
3	20 Aug - 26 Aug	20.00 \pm 1.73	16.67 \pm 2.60	14.67 \pm 0.67	F = 2.13 P = 0.200	20.00 \pm 1.73	0.00 \pm 0.00	0.00 \pm 0.00	F = 133.65 P = 0.000	100	100
5	3 Sep - 9 Sep	24.33 \pm 2.03	19.33 \pm 0.88	9.33 \pm 0.88	F = 30.87 P = 0.000	24.33 \pm 2.03	0.00 \pm 0.00	0.00 \pm 0.00	F = 143.65 P = 0.000	100	100
7	17 Sep - 23 Sep	21.67 \pm 1.20	15.67 \pm 1.76	16.67 \pm 1.45	F = 4.67 P = 0.060	21.67 \pm 1.20	0.00 \pm 0.00	0.00 \pm 0.00	F = 106.48 P = 0.000	100	100
9	1 Oct - 7 Oct	18.33 \pm 2.03	13.00 \pm 2.00	9.67 \pm 2.33	F = 4.22 P = 0.072	18.33 \pm 2.03	0.00 \pm 0.00	0.00 \pm 0.00	F = 81.53 P = 0.000	100	100
11	15 Oct - 21 Oct	24.67 \pm 0.33	18.00 \pm 2.08	15.33 \pm 1.20	F = 11.82 P = 0.008	24.67 \pm 0.33	0.00 \pm 0.00	0.00 \pm 0.00	F = 5588.70 P = 0.000	100	100
13	29 Oct - 4 Nov	21.00 \pm 1.53	16.67 \pm 0.88	17.33 \pm 1.20	F = 3.58 P = 0.095	21.00 \pm 1.53	0.00 \pm 0.00	0.00 \pm 0.00	F = 188.39 P = 0.000	100	100
15	12 Nov - 18 Nov	23.33 \pm 0.33	16.00 \pm 1.15	16.00 \pm 2.51	F = 6.95 P = 0.027	23.33 \pm 0.33	0.00 \pm 0.00	0.00 \pm 0.00	F = 4998.06 P = 0.000	100	100
17	26 Nov - 2 Dec	21.33 \pm 1.86	15.33 \pm 1.20	16.33 \pm 0.88	F = 5.46 P = 0.045	21.33 \pm 1.86	0.00 \pm 0.00	0.0 \pm 0.00	F = 131.51 P = 0.000	100	100

SE = standard error

p > 0.05 = no significant different

p < 0.05 = significant different

p < 0.01 = highly significant different

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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 (cont')

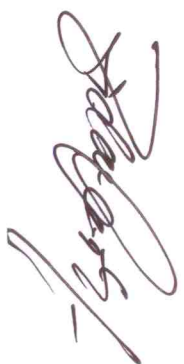
Week	Test period	Mean \pm SE of collected pupae				Mean \pm SE adult emerged				Emergence inhibition (%)	
		Untreated	Treated		One way ANOVA	Untreated	Treated		One way ANOVA	Treated	
			10% w/w	20% w/w			10% w/w	20% w/w		10% w/w	20% w/w
19	10 Dec - 16 Dec	23.33 \pm 0.33	14.33 \pm 1.45	14.00 \pm 1.53	F = 18.47 P = 0.003	23.33 \pm 0.33	0.00 \pm 0.00	0.00 \pm 0.00	F = 4998.06 P = 0.000	100	100
21	24 Dec - 30 Dec	23.33 \pm 0.33	17.33 \pm 0.88	16.67 \pm 0.33	F = 40.71 P = 0.000	23.33 \pm 0.33	0.00 \pm 0.00	0.00 \pm 0.00	F = 4998.06 P = 0.000	100	100
23	7 Jan - 13 Jan	22.00 \pm 1.73	14.67 \pm 0.67	16.67 \pm 1.20	F = 8.82 P = 0.016	22.00 \pm 1.73	0.00 \pm 0.00	0.00 \pm 0.00	F = 161.72 P = 0.000	100	100
25	21 Jan - 27 Jan	21.00 \pm 1.16	16.67 \pm 1.20	15.67 \pm 1.45	F = 4.93 P = 0.054	21.00 \pm 1.16	0.00 \pm 0.00	0.00 \pm 0.00	F = 323.73 P = 0.000	100	100
27	4 Feb - 10 Feb	20.67 \pm 1.76	17.33 \pm 0.33	16.33 \pm 1.33	F = 3.11 P = 0.118	20.67 \pm 1.76	0.00 \pm 0.00	0.00 \pm 0.00	F = 137.93 P = 0.000	100	100
29	18 Feb - 24 Feb	22.67 \pm 1.45	16.67 \pm 1.20	16.67 \pm 0.88	F = 8.34 P = 0.019	22.67 \pm 1.45	0.00 \pm 0.00	0.00 \pm 0.00	F = 244.44 P = 0.000	100	100
31	3 Mar - 9 Mar	23.67 \pm 0.33	17.33 \pm 1.20	16.67 \pm 1.45	F = 12.27 P = 0.008	23.67 \pm 0.33	0.00 \pm 0.00	0.00 \pm 0.00	F = 5144.80 P = 0.000	100	100
33	17 Mar - 23 Mar	20.33 \pm 1.45	17.00 \pm 1.15	17.33 \pm 1.67	F = 1.63 P = 0.273	20.33 \pm 1.45	0.00 \pm 0.00	0.00 \pm 0.00	F = 196.58 P = 0.000	100	100
35	31 Mar - 6 Apr	22.00 \pm 1.00	15.67 \pm 0.88	18.33 \pm 1.45	F = 7.82 P = 0.021	22.00 \pm 1.00	0.00 \pm 0.00	1.0 \pm 0.00	F = 484.00 P = 0.000	100	100

SE = standard error

p > 0.05 = no significant different

p < 0.05 = significant different

p < 0.01 = highly significant different



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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 (cont')

Week	Test period	Mean \pm SE of collected pupae				Mean \pm SE adult emerged				Emergence inhibition (%)	
		Untreated	Treated		One way ANOVA	Untreated	Treated		One way ANOVA	Treated	
			10% w/w	20% w/w			10% w/w	20% w/w		10% w/w	20% w/w
37	14 Apr - 20 Apr	21.67 \pm 2.03	17.67 \pm 2.03	15.67 \pm 1.20	F = 2.89 P = 0.132	21.67 \pm 2.03	0.00 \pm 0.00	0.00 \pm 0.00	F = 113.95 P = 0.000	100	100
39	28 Apr - 4 May	22.67 \pm 0.88	16.67 \pm 2.60	17.67 \pm 1.20	F = 4.89 P = 0.055	22.67 \pm 0.88	0.00 \pm 0.00	0.00 \pm 0.00	F = 663.65 P = 0.000	100	100
41	12 May - 18 May	24.00 \pm 0.58	16.67 \pm 2.03	10.67 \pm 2.96	F = 10.11 P = 0.012	24.00 \pm 0.58	0.00 \pm 0.00	0.00 \pm 0.00	F = 1712.25 P = 0.000	100	100
43	26 May - 1 June	20.67 \pm 1.20	16.33 \pm 2.33	23.00 \pm 17.00	F = 0.12 P = 0.892	20.67 \pm 1.20	0.00 \pm 0.00	0.00 \pm 0.00	F = 296.70 P = 0.000	100	100
45	9 June - 15 June	18.67 \pm 1.76	15.00 \pm 1.15	23.00 \pm 16.00	F = 0.18 P = 0.836	18.67 \pm 1.76	0.00 \pm 0.00	0.00 \pm 0.00	F = 112.53 P = 0.000	100	100
SE = standard error		p > 0.05 = no significant different				p < 0.05 = significant different				p < 0.01 = highly significant different	


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CONCLUSION

ZERO-MOZ Controlled Release Block® containing 10% w/w and 20% w/w pyriproxyfen granules is able to completely inhibit the emergence of adult *An. maculatus* for 45 weeks (10 months).



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DISCLAIMER STATEMENT

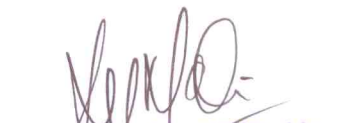
This proposed test is merely to assess the effectiveness of ZERO-MOZ Controlled Release Block® for mosquito control in response to request by the supplier. It does not constitute an endorsement of the products. All expenses of the test will be fully borne by the supplier. The supplier is fully responsible to supply the products. The Institute for Medical Research (IMR) cannot be held responsible for the loss, damage or otherwise of products after taken reasonable measures to prevent and/or reduce such occurrence. All data collected belong to IMR and such data shall not be published, mentioned, broadcasted or otherwise without prior written permission from the Director of IMR.

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