ABSTRACT. The insect growth regulator pyriproxyfen was tested against Aedes aegypti at 0.01 and 0.02 mg of active ingredient (AI) per liter of water in 60-liter earthen jars. Both concentrations provided 100% control for 4 months. In additional experiments where 10 liters of water were replaced fortnightly, 100% control was still obtained over 4 months with 0.02 mg AI/liter and greater than 93–100% control was obtained over 4 months with 0.01 mg AI/liter. In less-controlled field-trial conditions, pyriproxyfen at a dosage of 0.02 mg AI/liter provided 100% control for 10 wk against Aedes albopictus even though water was replaced either daily or weekly. Although the activity of pyriproxyfen declines after 10 wk, those tests in the plastic tubs showed much higher levels of sustained residual activity compared to those in the earthen jars. Pyriproxyfen did not have an impact on nontarget organisms.

KEY WORDS Aedes aegypti, Aedes albopictus, pyriproxyfen, earthen jars, plastic tubs

INTRODUCTION

Currently, dengue fever and dengue hemorrhagic fever are considered to be the most important arboviral diseases of humans in terms of their public health impact (Gubler 1989). Over the past years, disease incidence and distribution have steadily increased with more than 2–3 billion people at risk of infection, and an estimated 20 million dengue cases annually (WHO 1997). Container-breeding Aedes aegypti (Linnaeus) and A. albopictus (Skuse) serve as the primary and secondary vectors, respectively. Both these species breed in water-storage jars in Malaysia.

At present, only space spraying provides immediate removal of infected mosquitoes. Long-term dengue control measures include the use of chemicals as larvicide, personal protection, health education, and source reduction. Insecticide use still remains a major component of any control strategy, especially during an outbreak. Chemicals frequently used are those belonging to the organophosphate and pyrethroid classes of insecticide (WHO 1997).

With the current trends in dengue incidence worldwide and without an effective vaccine or treatment, it is expected that the widespread use of insecticides will continue. This practice will likely lead to the selection of resistant strains among exposed vector populations and render current insecticides less effective, leading to a need for the identification of replacement control strategies.

In Malaysia, temephos (Abate® 1% sand granules) has been the larvicide of choice for the control of immature Aedes aegypti; however, the susceptibility of this mosquito to temephos is decreasing (Lee and Lime 1989). Although Bacillus thuringiensis var. israelensis (Bti) can be used to prevent these vectors from breeding, the bacteria cannot self-replicate and as such the residual activity is inadequate. Controlled semifield studies show that Bti can be effective for about 7–12 wk (Mulla et al. 2004, Vilarrinos and Monnerat 2004) in undisturbed conditions. Hence, it is essential to evaluate alternative insecticides that could be used in the event of resistance.

Insect growth regulators (IGRs), in general, appear to be selective for mosquitoes and at practical rates have no apparent ill effect on prevailing nontarget organisms (Mulla et al. 1986, Schaefer et al. 1988). Most IGRs are known to have low mammalian toxicity and a good margin of safety to fish and wildlife. Assessment of the activity of pyriproxyfen against Anopheles species in their natural environment in the Solomon Islands showed high levels of adult emergence inhibition (Suzuki et al. 1989, Okawaza et al. 1991).

This study was designed to evaluate the residual effectiveness of pyriproxyfen against dengue vectors over time under both laboratory and field conditions found in Malaysia.

MATERIALS AND METHODS

Laboratory trial: Laboratory-bred 3rd-instar Aedes aegypti were used for the study. Sumilarv 0.5G® (manufactured by Sumitomo Chemical Company, Japan) was the chosen formulation, with a content of 0.5% (w/w) pyriproxyfen. The 2 dosages tested were 0.01 mg active ingredient (AI) per liter and 0.02 mg AI/liter. Sixty-liter earthen jars with glazed interior walls were used for the experiment. Five replicates were used for each dosage. The jars were filled with 50 liters of water. Five of the jars were treated with 0.1 g of pyriproxyfen 0.5G and another 5 were treated with 0.2 g. Two jars were left untreated as controls. One hundred rd-instar Aedes aegypti were introduced in each jar in the form of a small piece of liver was added for the larvae. The jars were kept in the laboratory for any larval, pupal, or adult development during a cumulative period of 2 wk. The same observations were conducted at fortnightly intervals. The percentage of adults emerged was calculated. At fortnightly intervals, 10 Ae. aegypti were introduced into each jar, and the same observations were conducted. The residual activity of each dosage was tested in the same conditions found in field experiments. The percentage emergence of adults was considered to be the most important parameter of any control strategy, and as such the residual activity is inadequate.

Field trial: Field trial involved the use of earthen jars with 60 liters of water. These were arranged 2 rows of tap water was added in each jar. The jars were then filled with 50 liters of water. Five of the jars were treated with 0.1 g of pyriproxyfen 0.5G and another 5 were treated with 0.2 g. One hundred rd-instar Aedes aegypti were introduced in each jar.
Wg.

I.

Percentage emergence inhibition of *Ae. aegypti* after application of pyriproxyfen at 0.01 and 0.02 mg of active ingredient per liter in water in earthen jars.

*Ae. aegypti* were introduced into each of the jars. A small piece of liver was added to each jar as food for the larvae. The jars were examined every 3 days for any larval, pupal, or adult mortality and the cumulative data were recorded at the termination of the test when adult emergence was completed in control jars and no living larvae or pupae remained. The percentage of adult emergence was then calculated. At fortnightly intervals a fresh batch of larval *Ae. aegypti* was introduced into the jars and the same observations were continued to determine residual activity of each dosage.

**Simulated field trial:** The simulated field trial was conducted in the same type of jars. The same procedure was carried out except that 10 liters (20%) of water was replaced from the jars fortnightly and 10 liters of fresh water added. One hundred 3rd-instar *Ae. aegypti* were introduced into the jars and the same observations were continued to determine residual activity of each dosage.

**Field trial:** Field trials were conducted in 60-liter earthen jars, with glazed interior walls and 60-liter plastic tubs. These containers were maintained outdoors under a plastic tarpaulin to protect them from rain, direct sunlight, and falling debris. A total of 16 containers (8 earthen jars and 8 plastic tubs) were arranged in 2 rows side by side. Fifty liters of tap water was added to all containers and 0.2 g of pyriproxyfen 0.5G was added to 6 of the earthen jars and 6 of the plastic tubs to give a final concentration of 0.02 mg Al/liter. The remaining 4 containers served as controls. All containers remained uncovered to allow oviposition of wild mosquitoes.

From 3 of the earthen jars and plastic tubs, 10 liters (20%) of water was removed daily and replaced with the same amount of fresh water; whereas from the other 6 containers, 25 liters (50%) of water was removed weekly and replaced with the same amount of fresh water. The same procedure was also carried out for the control containers. All containers were observed daily for the presence of mosquito larvae and pupae. All pupae were collected and maintained in plastic cups covered with plastic netting. Survival in both the treated and control containers was determined by counting the number of imagoes that had successfully emerged from the pupal exuviae.

The efficacy of the formulation through time was assessed as % EI in treatment containers adjusted for any pupal mortalities in corresponding controls with the formula of Mulla et al. (1974).

Percent emergence was assessed as:

\[
\text{% emergence} = \frac{\text{number of images successfully emerged}}{\text{total number of pupae}} \times 100.
\]

**RESULTS**

In the laboratory trial 100% El was obtained for 4 months at both dosage rates, as shown in Fig. 1.
During the 5th and 6th months, the higher dosage (0.02 mg AI/liter) still gave 83% and 44% EI, whereas the lower dosage (0.01 mg AI/liter) fell to zero.

In the simulated field trial where water was replaced fortnightly, 100% EI was obtained over 4 months in jars with the higher dosage, as shown in Fig. 2. In the 5th and 6th months, the EI was 20% and 12%, respectively. In jars with the lower dosage, 100% EI was obtained for 2 months, as shown in Fig. 2, with a respectable 99.6% and 92.8% control maintained through the 3rd and 4th months, respectively.

In the difficult field-trial conditions, 100% EI was obtained for 10 wk in earthen jars where water was replaced daily (Fig. 3). In week 11 the EI was 60% and by week 15 almost all larvae emerged as adults. However, in plastic tubes, 100% control was obtained until week 13 and subsequently at week 15 the EI was more than 90% (Fig. 3).

In the trial where water was replaced weekly, 100% EI was obtained in the earthen jars until week 10 and by week 15 the EI was 14% (Fig. 4). However, in the plastic tubs 100% EI was obtained until week 11 and subsequently at week 15 the EI was 86.7% (Fig. 4).

In all control containers the pupae collected emerged successfully. The larvae found in this experiment were *Ae. albopictus*. The activity profile of pyriproxyfen in both earthen jars and plastic tubs was similar up to the 10th week after treatment, but thereafter, the activity of pyriproxyfen in the plastic tubs showed much higher levels of sustained residual activity compared to those of the earthen jars and this was significant (at $P < 0.05$). However, no significant differences ($P > 0.05$) were observed in the EI between the daily and weekly replenishing of water within the same type of containers at the 15th week after treatment. Emergence inhibition was achieved either in the pupal stage whereby the imago failed to emerge or when adults could not completely detach from their pupal exuviae.

It was observed in this study that pyriproxyfen appears to be highly effective against *Ae. albopictus*. During the 11th week the same aquatic stage in both the control and treated earthen jars and the treated earthen and plastic tubs, and in 2 plastic tubs pyriproxyfen against *Ae. albopictus* was observed.

In the laboratory at the 11th week temperature in the aqeus. During the 11th week the same aquatic stage in both the control and treated earthen jars and the treated earthen and plastic tubs, and in 2 plastic tubs pyriproxyfen against *Ae. albopictus* was observed.

**D**
was 14% (Fig. 4). A 92% EI was obtained at week 15 the EI
appears to be highly specific against mosquito lar-
avae. During the 11th week of the study, water bugs (order Hemiptera, family Corixidae) were observed in both the control earthen jars. The same bugs were also observed in 1 of the treated earthen jar at the 12th week after treatment. The following week the same aquatic bugs were observed in 3 of the treated earthen jars, 1 of the control plastic tubes, and in 2 plastic tubes where the activity of pyriproxyfen against larval *Ae. albopictus* remained high. No apparent effects on the aquatic bugs were observed.

**DISCUSSION**

In the laboratory study it was observed that pyriproxyfen was highly effective against *Ae. aegypti* for 4 months (16 wk) even with replacement of water, whereas in the field trial pyriproxyfen was effective against *Ae. albopictus* for 10 wk with dail-

general, temperature may have a direct profound effect on IGRs, because Schaefer et al. (1988) found pyriproxyfen to be stable in water, but its stability is lower at higher temperature. Similarly, the activity of S-methoprene, another type of IGR, was greatly affected by increasing temperatures (Glare and O’Callaghan 1999). Studies by WHO (2001) and Nayar et al. (2002) with the same formulation of pyriproxyfen also reported a complete EI against both *Ae. aegypti* and *Ae. albopictus* for 6 wk in plastic tubs placed outdoors.

From this study it is evident that pyriproxyfen appears to be highly effective against container-breeding *Aedes* sp. In addition, the results concur with those from studies conducted by Schaefer et al. (1988), whereby pyriproxyfen was observed to have no apparent effect on aquaticcoleopterans, whereas in our study no effect was noted on aquatic hemipterans.

Furthermore, in addition to including mortality in the pupal stage, incomplete emergence has been observed. This could be due to morphological aberrations that lead to failure of the imago to suc-

Fig. 4. Percentage emergence inhibition of *Aedes albopictus* after application of pyriproxyfen at 0.02 mg of active ingredient per liter and 50% of water replacement weekly.
Pyriproxyfen is not only effective against Ae. aegypti but also against other species of mosquitoes. In field studies in Sri Lanka it was shown that only 2 applications per year of this IGR were required compared to 12 applications per year of temephos or oil (Yapabandara and Curtis 2002). Before this, in small-scale field trials, it has been shown that with pyriproxyfen at a target dosage of 0.01 mg Al/l the vector density of Anoph eles culicifacies Gi les and An. subpictus Grassi and also the prevalence of malaria declined significantly in the treated area compared to the control area (Yapabandara et al. 2001).

Even Culex quinquefasciatus Say, which is resistant to most insecticides, has shown good susceptibility to pyriproxyfen. In a study conducted in Dar es Salaam, it was found that at a dosage of 0.1 mg Al/l, the emergence inhibition of Cx. quinquefasciatus was effective for 4 wk during the rainy season and 11 wk during the dry season (Chavase et al. 1995). In Florida, both laboratory and field trials using pyriproxyfen against Ae. albopictus and Cx. quinquefasciatus showed 80–100% EI for 6 wk (Nayar et al. 2002).

Pyriproxyfen has shown long-term effectiveness against Ae. aegypti and Ae. albopictus. With pyriproxyfen, mortality can occur in larvae soon after application, but with time, mortality is more often observed during adult emergence. Adult emergence was completely inhibited for 4 months even with removal and addition of water. Because the acute toxicity (oral median lethal dose [LD50] in rats of >5,000 mg/kg and dermal LD50 in rats of >2,000 mg/kg) is very low and skin sensitization is negative (in guinea pigs), pyriproxyfen seems to have good potential for use in vector control. For treatment of drinking water, pyriproxyfen at a dosage not exceeding 0.01 mg Al/liter may be used (Nayar et al. 2002). Pyriproxyfen provides a good alternative when larvae development reach to currently used insecticides.

ACKNOWLEDGMENTS

We thank Dr. Ng. Kok Han, Director, Institute for Medical Research for permission to publish this paper, Sumatomo Chemical Enviro-Agro Asia Pacific Sdn. Bhd, for the supply of Sumilarv 0.5G, and staff of Entomology Unit IMR for their help. BML and RH were supported by Malaysian Technical Cooperation Program and Asian Development Bank, respectively, for the duration of this project.

REFERENCES CITED


