Tropical Biomedicine 32(4): 684–692 (2015)

Evaluation of insect growth regulators, temephos and *Bacillus thuringiensis israelensis* against *Aedes aegypti* (L) in plastic containers

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Received 24 May 2014; received in revised form 24 October 2014; accepted 20 April 2015

Abstract. The residual activities of 5 insect growth regulators (IGRs) were studied and compared to operational dosage of temephos (1 mg/L) and *Bacillus thuringiensis israelensis* (Bti) (0.008 mg/L). The IGRs, temephos and Bti were applied into plastic containers containing 5 litres of water. Thirty *Aedes aegypti* larvae were added into each container weekly. Efficacy of these IGRs were evaluated for effective duration of each dosage and the percentage of emergence inhibition (EI). An end-point of EI/mortality $\geq$ 50% was considered to be effective. Pyriproxyfen possessed the longest residual activity in both indoor (43 weeks) and outdoor (26 weeks) conditions, followed by temephos (26 weeks in indoor and 16 weeks in outdoor). Although the residual activity of Bti in indoor lasted 8 weeks which was longer than cyromazine and diflubenzuron; however, it was least effective in outdoor which only lasted 2 weeks. This study revealed that pyriproxyfen possessed good residual effect among test IGRs compared to temephos and Bti. The use of IGRs can be an alternative long-term control measure in stagnant water body.

INTRODUCTION

Currently, dengue is considered as the most important arboviral disease in term of its public health importance in tropical, subtropical and temperate regions of the world (Gubler, 1989; Gubler *et al.*, 1998). Dengue is endemic in Malaysia since the first documented case in 1902, while the first major national dengue outbreak occurred in 1973 (Skae, 1902; Lee, 1994). *Aedes aegypti* (L.) has been incriminated as the primary vector in the transmission of dengue fever (DF) and dengue haemorrhagic fever (DHF) (Chen *et al.*, 2005).

Without an effective dengue vaccine and specific treatment, the use of chemical agents is one of the most important methods of controlling dengue vector. The control approaches used by the Vector Borne Disease Control Program (VBDCP) in Malaysia are fogging with chemical insecticides and source reductions in affected areas (Lee *et al.*, 2008). Larviciding using temephos is recommended by WHO since early 1970 for the control of container-breeding *Aedes* mosquitoes (WHO, 1985). In Malaysia, temephos (Abate® 1% sand granules) was widely used by the public to control the immature of *Ae. aegypti* for the last 3 decades. However, several studies in Malaysia had shown that the susceptibility of *Aedes* larvae to temephos is decreasing due to the development of resistance (Lee & Lime, 1989; Chen *et al.*, 2005). Another larvicide, *Bacillus thuringiensis* var. *israelensis* (Bti) is a microbial control agent known for the efficacy and selectivity against mosquito larvae. Although *Bacillus thuringiensis* var. *israelensis* (Bti) can be used as an alternative

control agent, the bacteria cannot self-replicate and thus the residual efficacy is reduced (Vythilingam *et al*., 2005).

Insect growth regulators (IGRs) interfere development of immature insects including mosquito larvae and have no apparent ill effect on non-target organisms including mammals (Mulla *et al.*, 1986; Mulla, 1995). Insect growth regulators are now increasingly used to control *Aedes* and other mosquito larvae. Most IGRs are being developed to satisfy all the factors that enable larviciding more desirable when dealing with problem of pest/disease outbreaks. Through hormonal imbalance and inhibition of chitin formation caused by IGRs, these chemicals suppress insect embryogenesis, metamorphosis, and adult emergence (Mulla, 1995). In past decade, Lam (1990), Mulla (1995), Seccacini *et al*. (2008) and Chen *et al*. (2008) have reported studies on laboratory evaluation and field efficacy of a number of IGRs against mosquito larvae.

This study was designed to evaluate the residual effectiveness of five IGRs, namely pyriproxyfen, methoprene, diflubenzuron, cyromazine and novaluron in comparison to temephos and Bti.

MATERIALS AND METHODS

Ethical Notes

This research was regulated by the Medical Review and Ethics Committee (MREC), the Ministry of Health, Malaysia. No specific permits were required for this study, which did not involve endangered or protected species.

Test container

Plastic containers with an opening of 22.0cm in diameter, base diameter of 19.5cm and 21.7cm in height were used. Five replicates were used for each chemical. Before initiating the study, all containers were washed with tap water and tested for the presence of any larvicidal contaminant by introducing 30 lab-bred early 3rd instar *Ae. aegypti* larvae. The larvae were observed daily until complete emergence as adults.

Test insecticides

Five insect growth regulators (IGRs) used in this study were pyriproxyfen 0.5% w/w GR (granules), methoprene 1.3% w/w GR (granules), diflubenzuron 25% w/w WP (wettable powder), cyromzine 75% w/w WP (wettable powder) and novaluron 10% w/w EC (emulsifiable concentrate). Bti wettable granule (VectorBac WG, recommended dosage = 8g/1000L) and temephos sand granule (Abate 1.1G recommended dosage = 1 mg/L) were also tested in this study.

Test insect

Laboratory reared 3rd instar *Ae. aegypti* were used in the test. The colony was maintained in the laboratory for more than 30 years and not exposed to any control agents.

Trial Procedure

The trial procedure was modified according to the protocol used by Chen *et al*. (2008). The applied concentration of IGR was 10 times of 90% emergence inhibition (EI_{90}) (Table 1). The EI_{90} of each IGR against laboratory strain of *Ae. aegypti* was obtained by using standard larval bioassay procedures recommended by WHO (1981). Bti (VectorBac WG) and temephos (Abate®) were also tested for comparison purpose. Five containers holding 5 litres of water were set up in indoor (laboratory condition) and outdoor (simulated field condition) under the eave for each chemical. Five containers without chemicals served as untreated control. In this study, "indoor" is referred to the interior of the house while "outdoor" is outside the house but confined to the immediate vicinity of the house (Lee, 1992). In each arm of study, 30 laboratory 3rd instar larvae were introduced into each plastic container and mortality of larvae, pupae and adults was monitored daily. A small piece of liver was added to each container as larvae food. In both experiments, the containers were covered with net to prevent oviposition of wild mosquitoes and to prevent emerged adults from escaping from the containers. The live larvae and pupae were collected, recorded and transferred into paper cups covered with net for observation until all

Table 1. Concentration of EI_{90} of each IGRs against laboratory strain of *Ae. aegypti* and test concentration (10 x EI_{90}) used in this study

Insect growth regulator	EI_{90} (mg/L) against *Aedes aegypti* (95% C.L.)	10 X EI_{90} (mg/L) used in this study
Pyriproxyfen	0.076 (0.051–0.144)	0.761
Methoprene	0.025 (0.011–0.129)	0.245
Diflubenzuron	0.011 (0.004–0.103)	0.111
Cyromazine	0.664 (0.458–1.139)	6.636
Novaluron	0.003 (0.001–0.014)	0.033

C.L. = Confidence Limit

Table 2. Residual activity of 5 IGRs, temephos and Bti against *Ae. aegypti* larvae in plastic containers placed in indoor and outdoor

Insecticide	Number of Weeks			
	Indoor		Outdoor	
	100% EI	≥ 50% EI	100% EI	≥ 50% EI
Diflubenzuron	6	11	4	6
Cyromazine	7	12	6	8
Novaluron	15	23	9	13
Pyriproxyfen	28	43	15	26
Methoprene	12	21	9	15
Temephos	22	26	12	16
Bti	8	14	1	2

EI = Emergence Inhibition
Bti = *Bacillus thuringiensis israelensis*

individuals died or emerged as adults. A 50% of the total volume of water was removed and replenished weekly. The same procedure was repeated by adding fresh batch of larvae (30 larvae) into each container weekly.

Statistical Analysis

Statistical software (SPSS v11.5) was used to analysis the data. The indicators of effectiveness of tested chemicals for these studies were:

i. duration of effectiveness of tested chemical, and

ii. percentage of emergence inhibition (EI) =

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

An end-point of emergence inhibition (EI) or mortality ≥ 50% was considered effective. If percentage of untreated EI was > 5% the percentage of treated EI was corrected by Abbott's formula:

$$\frac{\text{\% treated EI} - \text{\% control EI}}{100 - \text{\% control EI}} \text{ X } 100\%$$

RESULTS

Figure 1 shows the weekly EI of *Ae. aegypti* in indoor plastic containers treated with 5 IGRs, temephos and Bti. Complete emergence inhibition/mortality of *Ae. aegypti* larvae was found in pyriproxyfen treated containers for 28 weeks, followed by temephos (22 weeks), novaluron (15 weeks),

methoprene (12 weeks), Bti (8 weeks), cyromazine (7 weeks) and diflubenzuron (6 weeks). By using 50% emergence inhibition as the indicator of residual efficacy, pyriproxyfen exhibited longest residual effect lasted for 43 weeks before declining to 50% EI and lower on week 44. The residual activity of larvicides against *Ae. aegypti* in containers placed indoor in descending order was: pyriproxyfen > temephos > novaluron > methoprene > Bti > cyromazine > diflubenzuron with 50% EI at 43 weeks, 26 weeks, 23 weeks, 21 weeks, 14 weeks, 12 weeks, and 11 weeks, respectively.

Figure 2 shows the weekly EI of *Ae. aegypti* in plastic containers treated with 5 IGRs, temephos and Bti under outdoor condition. The plastic containers placed outdoor treated with pyriproxyfen induced complete inhibition for 15 weeks, followed by temephos (12 weeks). Both novaluron and methoprene showed complete inhibition for 9 weeks, while cyromazine, diflubenzuron and Bti showed complete inhibition for 6 weeks, 4 weeks and 1 week, respectively. The residual activity of pyriproxyfen against *Ae. aegypti* under outdoor condition exhibited up to 26 weeks ≥ 50% of emergence inhibition. The residual efficacy of containers treated with pyriproxyfen was the longest while the shortest was treated by Bti with 2 weeks of residual effect. The residual activity of larvicides against *Ae. aegypti* in containers placed outdoor in descending order was: pyriproxyfen > temephos > methoprene > novaluron > cyromazine > diflubenzuron > Bti with 50% EI at 26 weeks, 16 weeks, 15 weeks, 13 weeks, 8 weeks, 6 weeks and 2 weeks, respectively. In all untreated containers, all the pupae emerged successfully.

DISCUSSION

Our results showed that pyriproxyfen was the most effective IGR in terms of duration with complete inhibition and residual activity throughout the experiment under indoor and outdoor conditions. In indoor conditions,

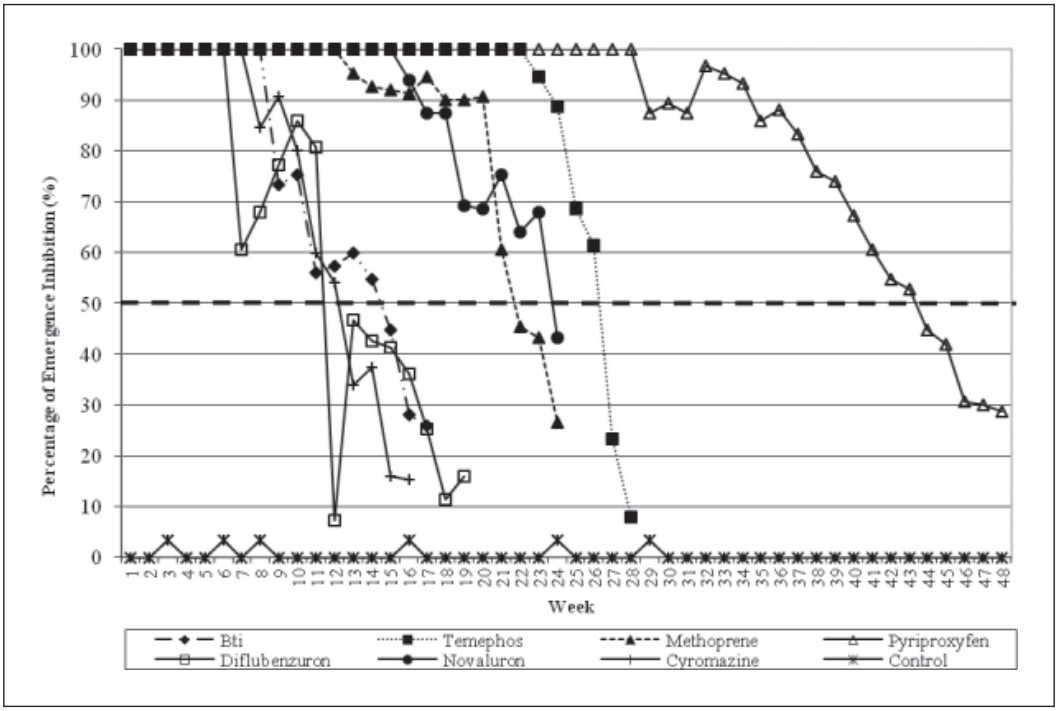

Figure 1. Bioefficacy of insect growth regulators, temephos and Bti against *Ae. aegypti* in plastic containers under indoor condition. Dotted line indicated the residual efficacy at cut-off point of ≥50% EI

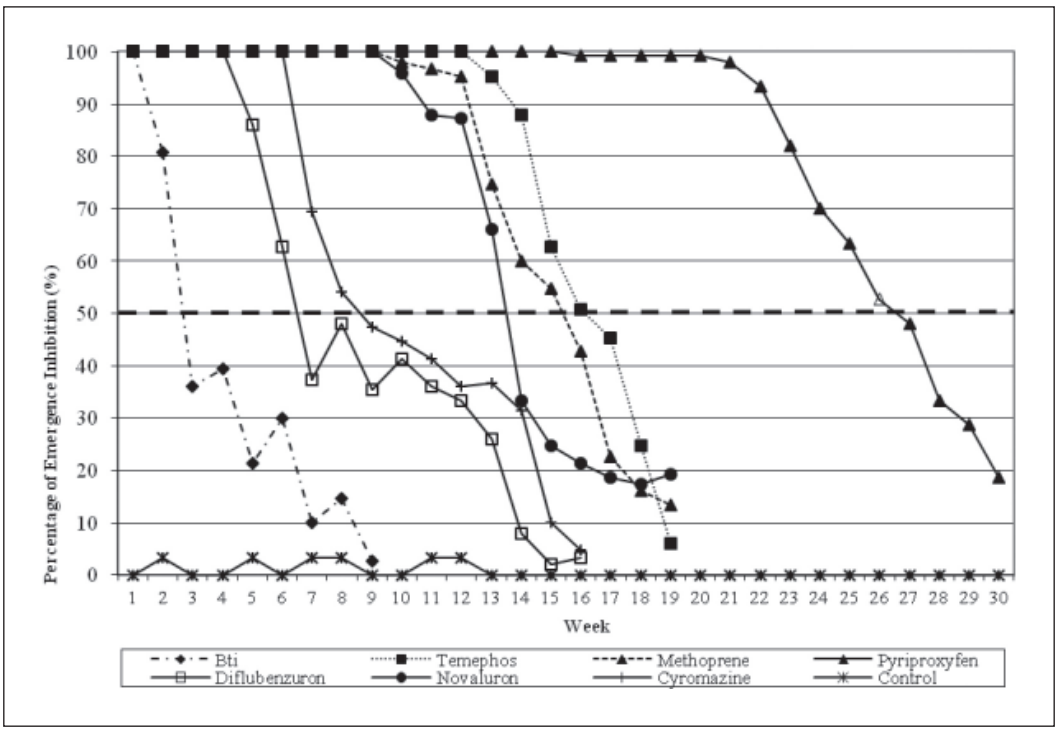

Figure 2. Bioefficacy of insect growth regulators, temephos and Bti against *Ae. aegypti* in plastic containers under outdoor condition. Dotted line indicated the residual efficacy cut-off point at ≥50% EI

treatment with pyriproxyfen showed 28 weeks of complete inhibition and residual activity up to 43 weeks. Vythilingam *et al.* (2005) reported that 0.01 and 0.02 mg/L pyriproxyfen were highly effective against *Ae. aegypti* for 16 weeks with replacement of water under laboratory trial and simulated field trial. Seccacini *et al.* (2008) also reported that the 0.1 mg/L granular sand formulations of pyriproxyfen remained active for over 4 months (>16 weeks). Studies by WHO (2001) and Nayar *et al.* (2002) also reported complete EI against *Ae. aegypti* for 6 weeks in plastic tubs placed outdoor.

The outdoor containers treated with diflubenzuron showed complete inhibition for 4 weeks, similar to that reported by Chen *et al.* (2008). Lam (1990) reported that the duration of effectiveness after application of wettable formulation of diflubenzuron (Dimilin® WP-25) in septic tanks to control *Ae. albopictus* breedings was up to 8 weeks. Seccacini *et al.* (2008) reported that in a simulated field study, the 0.1mg/L granular formulation of diflubenzuron was able to control *Ae. aegypti* up to 4 months (≈16 weeks). Unlike our results, Thavara *et al.* (2007) reported that the efficacy of the 0.02 mg/L of tablet and granular formulations lasted for 21 and 22 weeks post-treatment, respectively. Under the conditions where half of the water in treated jar was removed and refilled, tablet and granular formulation achieved 96–100% EI up to 21 weeks post-treatment (Thavara *et al.*). Cetin *et al.* (2006) conducted a study on diflubenzuron (25% wettable powder and 4% granular formulation) against *Culex pipens*. Their results indicated that both formulations tested at 0.01, 0.02 and 0.03 mg a.i./L were able to achieve 100% adult inhibition up to 4 weeks post treatment.

The residual efficacy of methoprene, novaluron and cyromazine were shorter than pyriproxyfen and temephos but exhibited longer residual activity than diflubenzuron and Bti. Nayar *et al.* (2002) reported that the residual activity of 0.02 and 0.05 mg/L of

methorpene was less effective compared to same concentration of pyriproxyfen with EI 22.3–93.7% during 6 weeks of observation. An experiment conducted by Mulla *et al.* (2003) in Thailand under field condition showed that EC10 of novaluron (0.05 – 1 mg/L) exhibited 86 – 96% of EI for about 190 days (≈27 weeks), while 0.001 – 0.02 mg/L achieved 80 – 100% of EI for 2 months (≈8 weeks). Because of the scarcity of data on residual activity of cyromazine against *Ae. aegypti*, the result obtained in this study was useful for consideration of future field evaluation. Recently, Suman *et al.* (2013) have shown that pyriproxyfen has the tendency to adsorb the various substrates that might be the possible explanation for extending the efficacy via retaining more compound even after changing the water for several weeks.

In the present study, temephos showed second longest residual activity in both indoor and outdoor conditions. Temephos is an organophosphorus compound with very low mammalian toxicity and has been used for the control of *Aedes* larvae in potable water since the early 1970s (Chen & Lee, 2006). Chen & Lee (2006) reported that the residual effect of 1 mg a.i./L. temephos in earthen jar lasted 15 weeks under laboratory condition. Mulla *et al.* (2004) reported that glazed clay water storage jars treated with temephos sand granules (1%) and temephos zeolite granules (1%) yielded almost 100% mortality for more than 6 months (≈24 weeks) and Thavara *et al.* (2004) also reported that a single application of temephos zeolite granules at 1 mg a.i./L provided high and satisfactory control period of at least 3 months (≈12 weeks) in water storage containers in field under normal water use practices.

Plastic containers treated with Bti exhibited 14 weeks of residual larvicidal activity in indoor but only 2 weeks in outdoor. Lee & Zairi (2005) reported that more than 80% reduction of mosquitoes was recorded in earthen jars treated with Bti up to 40 days, while Lima *et al.* (2005) reported larvae mortality of 70% or more attained for 2 – 5 weeks in containers treated with Bti. The field efficacy of Bti reported by Lee & Cheong (1987) was up to 6 weeks. Chen *et al.* (2009) also reported that 80% larvae mortality was obtained in earthen jars without plants up to 10 weeks while earthen jars with aquatic plants achieved more than 50% mortality up to 7 weeks. According to Becker *et al.* (2010), although $Cobalt^{60}$ source is well suited for Bti product sterilization without significantly reducing their toxicity, exposure to strong sunlight appear to reduce the larvicidal effect of Bti. Becker *et al.* (1992) also reported that the LC_{90} value at sunny sites (LC_{90}= 0.235 ± 0.036 ppm) was 4 time higher than in shaded conditions (LC_{90} = 0.054 ± 0.008 ppm) in which the third-instar *Culex pipiens* was treated with Bti powder at the same time and under identical conditions with temperature of 25 ± 1°C.

In general, the residual activity in outdoor conditions was reduced compared to indoor because insecticides in outdoor containers were degraded by sunlight and heat as the stability of insecticides are affected by direct sunlight and temperature. Robertson & Pope (2005) and Ong *et al.* (2007) reported that freezing and excess heat can shorten the shelf life of insecticides and direct sunlight also will degrade the insecticides. Ho *et al.* (1990) conducted an experiment by exposing IGRs to ultraviolet irradiation or heat management (45°C – 60°C) and showed that diflubenzuron and flufenoxuron were very stable but the other tested IGRs were not which included methoprene. However, the degradation rate of the insecticide by sunlight and heat in this trial was not studied.

In addition to possessing good effectiveness, formulation is another factor affecting the residual activity. Seccacini *et al.* (2008) reported that the emulsifiable concentrate formulations (EC) of diflubenzuron diminished the concentration of the compound ingested by larvae due to instability in water and low aqueous solubility, on the other hand, the EC pyriproxyfen was 5 times more active than the technical grade. Emulsifiable concentrates (EC) are liquid formulations in which the active ingredient has been dissolved in oil or solvents that can be mixed with water or oil for spraying purpose. Wettable powders (WP) are dry powdered pesticides formulations contain wetting and

dispersing agents which suitable for some active ingredients which cannot be formulated into EC. Chen *et al.* (2008) reported that the diflubenzuron WP mixed well in water and did not produce turbidity which was similar to our observation. In this study, sand granule (GR) formulation of insecticides performed better than EC and wettable powder (WP). The sand granule formulation was designed to sink to the bottom of the water body to release the active ingredient slowly so that the concentration is maintained in treated water body. Thavara *et al.* (2007) showed that residue efficacy of the granular formulation of diflubenzuron was up to 22 weeks post-treatment, indicating this formulation provides significantly long residual activity.

In terms of user preference, direct application method is simple and can be easily applied in areas such as drains and ponds and in places where long-term control is desired. The IGRs do not smell or produce turbidity in treated water like temephos. Moreover, pyriproxyfen, methoprene, diflubenzuron and novaluron have been accepted by WHO for application in drinking water (WHO, 2008). The IGRs induce late mortality after treatment and this is a desirable feature of a control agent since mosquito larvae and other vectors are important food source for aquatic animals (Mulla, 1995). However, the treated larvae will still be present and alive until late mortality occurs due to the mode of action of IGR, and this may discourage the use of these insecticides in some countries. In countries like Malaysia, the presence of *Aedes* larvae is ground for the enforcement officers to take legal action against the house-owners in spite of the application of IGR (Plan of action Ministry of Health Malaysia). Thus, the user and the enforcer should be educated on the use of IGR.

In conclusion, pyriproxyfen has shown long-term effectiveness against immature stages of *Ae. aegypti* compared with other IGRs and larvicides. It appears to be one of the best alternatives to conventional chemical insecticides such as temephos where *Aedes* larvae had been shown to develop resistance.

Acknowledgements. The authors thank the University of Malaya for funding this study (Project No. RP003B/13SUS). This study is part of the M.Sc. thesis of the first author, University of Malaya, Kuala Lumpur, Malaysia.

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