FIELD EFFECTIVENESS OF AUTO-DISSEMINATION TRAP WITH PYRIPROXIFEN AGAINST CONTAINER-BREEDING *AEDES* IN HIGH-RISE CONDOMINIA

W.A.Nazni[1*], G.N.Teoh [1], N Oreenaiza[1], H. Farah[1], O. Suhana[1], A. Chandru[1], M. Khairul Asuad[1], A. Norazizah[1], M. Azman[1], O.Topek, Y. Liang[3], D. Greenhalgh[3], Rosemary Lees[4] and H.L. Lee [2]

[1] *Medical Entomology Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia*

[2] *Vector Borne Disease Control Branch, Disease Control Division, Ministry of Health, Putrajaya, Malaysia.*

[3] *Department: Mathematics and Statistics, Address: Livingstone Tower, 26 Richmond Street, Glasgow, Scotland, UK, G1 1XH*

[4] *LITE (Liverpool Insect Testing Establishment), Vector Biology Department, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA.*

*Corresponding: *Nazni WA, Medical Entomology Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia,* Email: nazni@imr.gov.my

Abstract

The continued outbreaks of dengue in endemic areas, unabated despite the use of conventional vector control methods, necessitate the development of new control tools, as existing dengue mosquito control technology is effective only to a limited extent. An insect growth regulator-treated auto-dissemination trap has been developed against *Aedes* mosquitoes, whereby a female mosquito ovipositing in the treated trap contaminates her body with the IGR and transfers it to other containers as she continues to skip oviposit. We evaluated the bioefficacy of auto-dissemination traps treated with 0.004% pyriproxyfen (PPF), an IGR, for 44 weeks in a 3-block condominium complex in Sri Subang, Selangor, Malaysia from February to the end of December 2014. Four treated traps were installed on each floor and the traps were replenished with PPF solution biweekly. Standard ovitrapping was conducted to monitor the *Aedes* population density and so assess the efficacy of the auto-dissemination traps. Dengue epidemiological monitoring was also conducted by national health authorities. Oviposition in the auto-dissemination traps increased over the study period, indicating that gravid female *Aedes* mosquitoes were attracted to oviposit in these traps. However, not a single live larva was observed in any auto-dissemination trap, indicating that complete larval mortality was induced by PPF. Following introduction of an additional 8 treated traps on every floor from the 16th week of treatment onwards a reduction in ovitrap index (OI) was measured, from 90% to 33% by the 20th week of treatment. Correspondingly, the number of dengue cases reported reduced from 53 cases in 2013 to 13 cases in 2014 was significant. Although *Aedes* populations fluctuated over the course of the study period, these results suggest that the auto-dissemination trap could be a promising dengue control tool. Future research should be directed to determine the optimal concentration of PPF and the optimal number of PPF traps to deploy to ensure maximum control of dengue.

Keywords : Auto-dissemination, Pyriproxyfen, Ovitrap index, *Ae.aegypti,* Dengue

INTRODUCTION

Aedes aegypti (Linnaeus, 1762) is not indigenous to Malaysia. It is believed to originate from tropical Africa and to have been introduced to Malaysia via India at the turn of the 20^{th} century (Smith, 1956). On the other hand, *Aedes albopictus* (Skuse, 1894) is native to Southeast Asia, including Malaysia. Both species are prolific breeders in artificial containers and are vectors of human arboviruses, such as dengue, Zika and chikungunya, with *Ae. aegypti* being the primary vector for dengue (Rudnick, 1986). In recent years, dengue outbreaks have attained epidemic proportions, causing a significant public health impact through high levels of morbidity and mortality. Presently, dengue is reported in over 100 endemic countries in the world, with up to 500,000 cases of dengue haemorrhagic fever and 22,000 deaths reported annually (WHO, 2016). Dengue is the only serious arbovirus in Malaysia with 101,357 dengue cases and 237 deaths reported in 2016 (MOH, 2017). The resurgence of dengue over the years makes the control of *Ae. aegypti* extremely urgent, especially in the absence of specific antiviral treatment, effective tetravalent vaccine, prophylaxis, or therapeutic agents. To date, vector control is the key means of combating this arboviral threat and the only tool available for interruption of dengue transmission.

The concept of auto-dissemination of a control agent combines a 'pull' strategy of attracting wild gravid females to deposit their eggs and a 'push' in the dispersal or transfer of the control agent (microbes or chemicals), which are innocuous to the adult mosquito, to other target habitats. This method exploits the skip oviposition behaviour of the *Aedes* mosquitoes (Davis et al., 2016). This strategy has been pioneered using microbes which can multiply themselves in containers, such as entomopathogenic fungi and baculoviruses (Soper, 1978; Yu, 1997; Klein, 1999), and nematode auto-dissemination has even been considered (Lacey et al., 1993). Recently proposed approaches exploit *Edhazardia aedis* (Microsporidia) and the entomopathogenic fungus *Metarhizium anisopliae* as agents for controlling *Ae. aegypti*. This innovative concept was first proposed by Itoh et al., (1994), who conclusively demonstrated that gravid female *Ae. aegypti* contaminated with the insect growth regulator pyriproxyfen (PPF) were able to transfer lethal concentrations to larval habitats. The effect was enhanced by the skip oviposition behaviour of gravid *Ae. aegypti* females, which distribute their eggs throughout multiple containers (Mogi and Mokry, 1980). Besides using auto-dissemination trap for *Aedes* control, study has also been demonstrated in laboratory and semi-field using another specific IGR, novaluron and K+ channel modulators against *Anopheles quadrimaculatus* (Daniel et al., 2018). This strategy could potentially reduce the malaria burden in malaria endemic countries.

In the present study, we used a simple auto-dissemination device, consisting of a modified ovitrap containing pyriproxyfen, to contaminate gravid female mosquitoes during oviposition, allowing them to disseminate the chemical to other *Aedes* oviposition sites. The objective of this study was to evaluate the impact of auto-dissemination trap in reducing the natural *Aedes* population leading to reduction of dengue cases in a urbanised high-rise codominia.

MATERIALS AND METHODS

Trial site

The experimental field site is located in an area consisting of 3 highly urbanized residential condominia known as Ridzuan Court Sunway, Selangor (Figure 1). The built-up area of the condominia is 3.0 hectares and consists of 3 blocks, namely Block A (27 levels), Block B (27 levels) and Block C (26 levels), totalling 847 units, and a 5-level building with a car park, convenience stalls and food stalls (not included in the test site). Ridzuan Court is a highly urbanised, densely populated area in the Klang Valley, which accounts for about 60% of the total dengue cases in Malaysia.

The 3 blocks are high rise condominia with GPS coordinates of N3 04.767 E101 36.369 (Block A), N3 04.700 E101 36.395 (Block B), and N3 04.793 E101 36.362 (Block C). Each block has 10 units of houses on each floor, and each floor is approximately 7,500 sq ft. The corridors in Block A and Block B are 88 m long compared to 96 m in Block C. Blocks A and B each have an area of 3,168 m^2 and Block C has an area of 3,456 m^2. The condominia and associated swimming pool are surrounded by green shrubs and cultivated trees.

A dengue hotspot in Malaysia is defined as a location whereby at least two dengue cases occur within a week and further cases then continue to occur within a one month period. This area has been a declared dengue hotspot since week 52, 2013. These condominia were selected as the trial site since it had proven very difficult to manage dengue transmission in these blocks using conventional methods; it was therefore used to evaluate the effectiveness of a novel auto-dissemination trap in the interruption of dengue transmission.

Preparation of test chemical

The chemical used was a juvenile hormone analogue, pyriproxyfen [(4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propylether] used at a concentration of 0.004% w/v pyriproxyfen (Sumilarv 0.5G®, Sumitomo Chemical Corporation, Osaka, Japan) throughout the study, and prepared by dissolving 800 g of Sumilarv 0.5G® in 87.5 L of filtered and seasoned water and leaving it to stand for a minimum of 48 hours. The solution was then stirred for 4 hours using a high speed stirrer and filtered before adding into 12.5 L of hay infusion. Polyhexamethylene 0.004% w/v was added as a preservative and the prepared solution was stored away from direct sunlight in a cool dry place before use.

Auto-dissemination trap device

The auto-dissemination trap used to topically contaminate gravid mosquitoes seeking an oviposition site, was black in colour, as preferred by gravid *Aedes* (Beckel, 1955). The device, 13.0 cm high, 11.0 cm wide at the bottom and 14.8 cm wide at the top, was filled with water to within 2.5 cm of the brim (Figure 2). It could remain stable and upright due to its vase-like design, i.e. being slim at the bottom and broader at the top. Occasionally during the study the condominia experienced strong wind (authors' observations) but no effect on the devices was observed.

The ~600 ml water used to fill the device contained pyriproxyfen (PPF) (0.004%) and a food substrate which functioned as a lure to attract the gravid females. Three lateral holes, each with a diameter of 2.0 cm, were drilled to enable mosquitoes to gain entry into the device and become contaminated with pyriproxifen as they oviposited. Two pieces of tissue paper (Scott, Kimberly-Clark, 9 cm by 6 cm) were attached to the inside of the device, on opposite walls, to serve as an oviposition substrate.

Environmental parameters

Throughout the study period, temperature and relative humidity were recorded weekly, on each occasion when auto-dissemination traps were refilled and papers with eggs collected, using a thermal hygrometer (Model: EL-USB-2).

Pre-treatment *Aedes* population baseline study in study site

Ovitrap surveillance was conducted to determine the baseline *Aedes* population density in these trial sites prior to conducting the auto-dissemination experiment, using standard ovitraps (Fay and Perry, 1965; Fay and Eliason, 1966). This technique was used because the ovitraps were previously found to be sensitive and efficient in measuring the population of *Aedes*. The ovitrap consists of the same container as the auto-dissemination device but filled with untreated water and a paddle made from hard cardboard (3 cm x 10 cm) introduced to serve as a resting site for the gravid females to oviposit. Four ovitraps were placed on each floor during the pre-treatment period, five meters apart along the corridors outside the apartment units, a total of 356 ovitraps across the 3 blocks. The ovitraps were placed in protected and low-traffic areas that provided shade, as favoured by container-dwelling species in oviposition site selection (Vezzani, 2009), and collected weekly. The number of positive ovitraps and larval density per ovitrap was recorded, and larvae were identified by species to determine the ratio of *Ae. aegypti* to *Ae. albopictus*. These data gave an indication of the size of the *Aedes* population. The baseline survey was carried out continuously for 4 weeks (week 47 to week 51 of 2013) and again for a week (week 6-7 of 2014) before the deployment of the auto-dissemination traps. All deployed ovitraps were collected before the intervention started.

Placement of auto-dissemination traps

The effectiveness of utilising *Aedes* adult females as a vehicle for the transfer of pyriproxyfen to larval habitats was evaluated under field conditions for a total of 11 months (44 weeks) from February 2014 to the end of December 2014. A total of 356 pyriproxyfen-treated devices were placed in the same locations as in the baseline data collection, and monitored twice weekly in the same way. During the first week of the study, the auto-dissemination traps were observed for the presence of live larvae. Because of the complete absence of live larvae after the first week of setting the auto-dissemination traps, it was decided that it was not necessary to continue weekly collection of auto-dissemination egg papers. This decision was confirmed by the complete absence of live larvae in the auto-dissemination traps throughout the study. Hence, from then on the auto-dissemination trap papers were collected every 2 weeks and replaced with new ones, and the auto-dissemination traps were refilled with 0.004% pyriproxyfen solution up to the 250 ml mark. The oviposition papers were labelled with block, placement site and date, and air dried and stored at room temperature (27-28°C) prior to manual counting of eggs. The eggs were allowed to hatch in plastic containers (16 cm length x 10.5 cm width x 4 cm height) containing 200 ml of seasoned tap water. These containers were checked for larvae 1 - 2 times per week for 2 weeks post-hatching. The mean numbers of eggs collected every other week from each block were used to assess the efficacy of the auto-dissemination trap using the standard ovitrap index and the *Aedes* egg index over the whole period of intervention.

On week 22 of 2014 (the 15th treatment week), an additional 8 auto-dissemination traps per floor were placed in Block B to evaluate the impact of additional auto-dissemination traps on the mosquito population. The traps were placed along the corridors, in the waiting area near to the lifts and also at the emergency staircase of each level. The total number of auto-dissemination traps in the 3 blocks was increased to 552.

Post-intervention procedure

Standard ovitraps were placed monthly amidst the auto-dissemination traps at a distance of 0.5 m from the waiting area of the lifts on every floor of the 3 blocks, a total of 90 ovitraps, for a period of 7 days. The placement of standard ovitraps was to assess the effectiveness of the auto-dissemination trap since the absence of eggs or presence of deformed immatures in the standard ovitraps would indicate transfer of pyriproxifen by female mosquitoes into subsequent ovitraps. The data collected from data logger showed that there was minimal variation in relative humidity and temperature. If an ovitrap index was lower than the initial ovitrap index prior to intervention using auto-dissemination traps then this could indicate adult population suppression. The larval density and the species of *Aedes* were recorded using both the eggs that hatched and the larvae in the ovitraps.

This surveillance was performed continuously for 11 months. Ovitraps were collected after seven days and immediately transported to the Institute for Medical Research (IMR) laboratory. The contents of each ovitrap together with the paddle were poured individually into labelled plastic containers (15 cm x 7 cm x 8.5 cm) and covered. All larvae were counted and identified under compound microscopes (NIKON ECLIPSE E200, Japan) at the third or fourth instar using taxonomy keys prepared by the IMR.

Dengue epidemiology

Pre- and post-intervention dengue epidemiological data for 2013-2015 were obtained from the Disease Control Division, Ministry of Health via its eDengue database, which is updated daily by epidemiological reports uploaded by local health departments. As dengue is a notifiable disease in Malaysia, epidemiological data are routinely screened and verified by dedicated public health specialists before being loaded into the eDengue database. This method is chosen because the eDengue database is reliable and rigorously tested source of data. Epidemiological data were used to determine the impact of the auto-dissemination traps on dengue transmission.

Data Analysis

Entomological data for the pre- and post-treatment periods were recorded using Excel Microsoft Office and presented as mean ± standard error. All data were analysed using SPSS version 17.0, and statistical significance was determined at $p<0.05$. The data were tested for normality and variance homogeneity using Komolgorov-Smirnnov and Levene's tests. Abnormally distributed data were arcsine log transformed to stabilize the variance. Independent sample t-test (parametric) or Mann-Whitney U-test (non-parametric) and one-way analysis of variance (ANOVA) or Kruskal-Wallis tests were applied to determine the significant differences. A correlation test was performed using the Pearson correlation.

RESULTS

Data on temperature and relative humidity

Malaysia lies on the tropical belt and throughout the year rain is prevalent which affects the temperature and relative humidity at the study site, the Ridzuan Condominia site, The maximum and minimum temperatures observed during the 11 month study period were 32.15°C ± 0.03°C and 27.04°C ± 0.39°C, respectively, while the maximum and minimum values for relative humidity were 82.0% ± 2.35 and 60.40% ± 0.51.

Abundance of *Aedes* population in study site

Baseline data collection before the intervention revealed that two species of mosquito co-existed in this trial site, with *Ae. aegypti* being the dominant mosquito (96.22%) over *Ae albopictus* (3.78%) in the 3 blocks (A, B and C) as shown in Figure 3, Ovitrap surveillance showed that *Ae. aegypti* was ovipositing on every floor from the ground floor to the 27th floor in all the 3 blocks. The ovitrap index (OI) for *Ae. aegypti* in Block A, Block B and Block C was 64.57% ± 7.06, 44.52% ± 2.81 and 25.73% ± 2.07, respectively. In comparison, *Ae. albopictus* breeding was less intense, with the OI in block A to C being 2.54% ± 1.32, 2.66% ± 2.06 and 0.50% ± 0.31, respectively. The OI of *Ae. aegypti* was 15-39 fold higher compared with *Ae. albopictus* in this trial site during the pre-trial surveillance.

There was equal distribution of *Ae. aegypti* larvae in ovitraps by floor: there was no difference in numbers of larvae between lower (Floors 1-10), middle (Floors 11-20) and higher (Floors 21-28) (F=1.546, df=2, p=0.219). This data did not correspond to the results of a previous study conducted in a high rise building in Singapore (Lee et al., 2013), which found that *Ae. aegypti* prefer to breed within 6–8 floors of ground level. In our study, *Ae. albopictus* did not show a clear preference for breeding. In Block B, *Ae. albopictus* were found breeding both on the lower floors (1- 6) and on the higher levels (22-27).

Intervention using auto-dissemination traps with 0.004% pyriproxyfen

A total of 552 auto-dissemination traps were deployed in the 3 blocks during the intervention phase. It was noteworthy that *Aedes* females had oviposit into most of these traps. During our intervention period of 11 months, no larvae were found surviving in any auto-dissemination trap, although eggs were present and notably some had hatched, as observed under the dissecting microscope, no larvae survived to L2 when the egg papers from the field were submerged in seasoned tap water for further hatching. The absence of pupal exuviae in the auto-dissemination traps or plastic containers in the lab also indicated that none of the larvae emerged into adults. Therefore, 100% inhibition of pupal and adult emergence was observed in all the containers containing egg papers treated with PPF throughout the study period. The mean number of eggs based on two week collection for Block A, B and C were recorded (Figure 4).

The mean ovitrap indices for *Ae. aegypti* and *Ae. albopictus* before intervention for all blocks combined were 44.79% ± 11.35 and 1.90% ± 1.34, and after intervention were 53.43% ± 9.11 and 10.59% ± 4.07, respectively. Intervention using 4 auto-dissemination traps on every floor of all 3 blocks continuously for sixteen weeks (26 March 2014 to 11 June 2014) failed to impact the ovitrap index, showing that deployment of auto-dissemination traps at this density did not control the *Ae. aegypti* population over this period. Instead, a trend of increasing numbers of eggs laid in the auto-dissemination traps was indicative of an increasing *Aedes* mosquito population (Figure 4). The solution in the auto-dissemination trap contained hay infusion to enhance the trap's attractiveness to gravid females seeking to oviposit. Studies have also demonstrated that water infusion with leaf matter were effective in attracting *Ae. albopictus* females (Ponnusamy et al., 2010; Gaugler et al., 2012). In the surveillance program, we followed normal practice and did not use hay infusion in the standard ovitraps as our objective was to monitor the effect of the auto-dissemination traps and thus it was the relative abundance before, during and after intervention that was of interest.

In order to magnify the effect of auto-dissemination, the number of auto-dissemination traps was increased, with an additional 8 auto-dissemination traps deployed on every floor from the 1st to the 27th floor in Block B because the ovitrap index was consistently higher in Block B than in Blocks A or C. Thus, a total of 324 traps were deployed in Block B from the initial 116 traps, a 2.8 fold increase, which resulted in a reduction in *Aedes* egg index (the percentage of auto-dissemination systems testing positive with *Aedes* eggs within 2 weeks) from 5.69 to 3.44 (a 39.54% reduction) within 10 weeks post-application. Within the same period, the *Aedes* egg index increased from 5.64 to 6.09 (7.90% increase) in Block A and reduced from 5.19 to 4.07 (22.16% reduction), in Block C (Figure 5).

Table 1: Number of eggs laid in Block B increased from 116 to 324 after deployment of auto- dissemination traps (Independent sample T-test).

Block	*Aedes* egg index (%)	*Aedes* egg index (%) with additional traps in Block B	*P*-value
A	5.64	6.09	0.459
B	5.69	3.44	0.000
C	5.19	4.07	0.473

The *Aedes* egg index was significantly lower in Block B after deployment of the extra pyriproxyfen treated auto-dissemination traps compared to before (*p*=0.000) but there was no significant difference in either Block A (*p*=0.459) or C (*p*=0.473) after intervention. The mean number of *Aedes* eggs laid per paper (Figure 5) was significantly reduced after the increased intervention of 8 additional traps per floor in Block B (Independent Sample T-test (*F*=1.089, *df*=16, *p*=0.000, Table 1). The regression or trend line in Figure 4 demonstrates a slight but significant decrease in *Aedes* egg index (paired t-test, *p*=0.000, R^2=0.11). There was also a significant reduction in the mean number of eggs per paper in Block B compared to Block A and Block C (*p*=0.000, R^2=0.118, Figure 5). On the other hand, even though the *Aedes* egg index was reduced, no effect of pyriproxyfen transferred by mosquitoes into the standard ovitraps was observed in Block A, Block B or Block C because there was no larval mortality nor any deformities observed in these standard ovitraps. This strongly suggests that a higher concentration of pyriproxyfen is required to induce the auto-dissemination effect in these traps via the skip oviposition behaviour. Despite this, after the intervention was increased with extra auto-dissemination traps, there was a reduction in the ovitrap index by 18.5%, 56.67% and 6.67% in Block A, B and C, respectively, compared to the previous month's OI.

During the overall field assessment period of 11 months, a total of 272,666 eggs were laid in the auto-dissemination traps by *Aedes* mosquitoes; all of this large number of eggs were non-viable due to the pyriproxyfen treatment in the auto-dissemination traps.

A significant correlation (r=0.795, *p*=0.001) was found between OI and number of larvae per trap in all 3 blocks (Figure 6) showing that OI was also indicative of larval density in the study area.

None of the *Aedes* OI values in the 3 blocks showed an obvious decrease during the study period (Figure 7). The trend in OI was similar when all *Aedes* data was pooled and when *Ae. aegypti* data was separated (Figure 8), with an increase in OI measured during the intervention.

The OI pre-intervention (baseline) and OI during the study as shown in Figure 8 indicated prior to introduction of intervention, the ovitraps index was consistent, however upon introduction of intervention the ovitraps index fluctuated. It is interesting to note the plateau in the trend line in the pre-intervention (R^2=2×10^{-5}) compared to during intervention trend line (R^2=0.41), evidence of an impact. The exponential increase in OI could be due to the migration of mosquitoes from the neighbourhood localities (WHO, 2003)

Another interesting phenomenon observed was the influence of auto-dissemination trap deployment on the *Ae. albopictus* population (Figure 9). There was a significant increase in the number of *Ae. albopictus* in the standard ovitraps after the *Ae. aegypti* population was reduced. Prior to the introduction of the auto-dissemination trap the ovitrap index for *Ae. albopictus* was in the range of 0.00-4.05% ± 3.40 for Block A, B and C. However during the intervention period the ovitrap index was in the range of 3.33% ± 1.93 to 19.28% ± 8.14 for Blocks A, B and C. Figure 9 depicts the declining ovitrap index pre-treatment (R^2=0.95) compared to the during intervention trend line (R^2=0.59), indicating a strong correlation over time. This could be due to the reduced inter-specific competition and *Ae. albopictus* subsequently capitalising on the reduced competition from *Ae. aegypti* to fill in the niche left by *Ae. aegypti*. This highlights the necessity of operationally deploying auto-dissemination traps both indoors and outdoors to control both *Aedes* species. This study was conducted using the design of comparing pre- and post- intervention data as opposed to using a control site as discussed in (Erlanger et al., 2008).

The auto-dissemination traps appeared to suppress indigenous dengue cases and dengue transmission (Figures 10 and 11). Fifty three (53) confirmed dengue cases were reported in 2013 prior to the trial, and 13 cases at the end of the trial in 2014 (p=0.006, Mann Whitney U-test). The correlation between the average OI across the blocks and total dengue cases in the next two weeks although positive was weak (R^2=0.069, p=0.46). The trial site is considered as a dengue hotspot and is exposed to fogging conducted by the local health authorities when there is a notified case. The trial shows a reduction in the number of dengue cases when the auto-dissemination traps were used. The regression or trend line in Figure 10 showed that there was a significant decrease in dengue incidence rate during the auto-dissemination trap intervention. Dengue cases in 2015 then increased drastically after the removal of the auto-dissemination trap. For example, by week 40 during the treatment year there had been 10 cases, which significantly increased to 40 cases in 2015 during the same epidemiology week EW 8 to EW 50 (p=0.005, Mann Whitney U-test). The regression or trend line in Figure 11 shows a significant increase in dengue incidence during the post-intervention phase when the auto-dissemination traps were removed. Even though the ovitrap indices (Figure 7) were inconclusive, the number of eggs per paper decreased throughout the study (Figure 5) possibly indicating suppression of the *Aedes* spp. population. It is plausible that the reduction in dengue transmission could be attributed to a decrease in the number of adult *Aedes*.

Discussion

Various methods are in use for vector control depending on the ecosystem and climatic conditions of the endemic area. Although chemical, biological and environmental management techniques are still widely used, the battle against the *Aedes* mosquitoes has been going on for the better part of the last century with limited success in terms of sustainable control. *Aedes* species are unmanageable using conventional practices, largely due to limited long-term sustainability of the control measures themselves, a result of the cryptic larval habitats preferred by the *Aedes* species which are difficult to reach by traditional methods of insecticide application such as space spraying.

The aim of our work was to assess the feasibility of this novel approach to control dengue in high rise buildings in urbanised areas, which in many parts of the world are faced with frequent dengue outbreaks. In addition to investigation on the impact on OI due to deployment of auto-dissemination traps on dengue transmission in the Ridzuan condominia, based on dengue epidemiology data obtained from the eDengue database for nationwide dengue management, Ministry of Health, Malaysia.

The concept of "auto-dissemination" strategy may be incorporated with other innovative tools for dengue vector control to help overcome the development of resistance in dengue vectors, primarily *Ae. aegypti* and *Ae. albopictus*, which has been reported for the past several decades. The oviposition behaviour of these vectors, which typically scatter the eggs from a single gonotrophic cycle among multiple potential breeding sites, facilitates control strategies which exploit auto-dissemination (Caputo et al., 2012; Davis et al., 2016). This approach may also overcome the major constraint encountered in vector control by larviciding via chemical or biological insecticides in targeting the hard to reach cryptic larval habitats such as hidden man-made containers. *Aedes* mosquitoes are attracted towards darker, shadier areas, making this artificial PPF treatment device attractive for them to rest and lay eggs. In Thailand, a similar PPF-treated device was developed and evaluated in outdoor tunnels and field trials, and was found to significantly reduce the adult catch via BG-Sentinel traps post-treatment. A reduction in egg production by females exposed to PPF was also observed (Ponlawat et al., 2013). Auto-dissemination under lab, semi-field and field trials has been studied in several countries (Nayar et al., 2002; Sihuincha et al., 2005; Ponlawat et al., 2013, Ohba et al., 2013). In a public cemetery in Peru auto-dissemination traps showed 42–98% *Aedes aegypti* inhibition of emergence (Devine et al., 2009). In another study in Italy, modified sticky trap coated with 5% pyriproxyfen powder provided 40 – 70 % mortality (Caputo et al., 2012). Dual treatment stations using pyriproxyfen powder and oil boosted auto-dissemination efficacy by improving toxicant attachment and retention on contaminated females (Wang et al., 2013). Our study have demonstrated the approach's feasibility in a real - time Malaysian setting.

One vital finding of this project was that the deployment of an auto-dissemination trap was apparently able to reduce dengue transmission. During the study, auto-dissemination traps were continuously present at the trial site and thus removed 272,666 eggs that had been laid and this number of adult emergence had been removed from the condominia.. This could be the reason for the decrease in dengue transmission

Ovitrap surveillance has remained one of the most commonly employed methodologies in entomologic surveillance globally (Manica et al., 2017), since its development by Fay and Eliason (1966). Reiter and Nathan (2001) reported that ovitraps are useful in assessing the impact of vector control measures targeting the breeding and dispersal of local *Ae. aegypti* populations. Ovitraps can also be used to investigate breeding populations and species composition in locations where control measures are being evaluated. Ovitrapping may be inappropriate as a proxy indicator for adult density in the study sites as observed in our study, though it remains the only practical surveillance tool available. The OI fluctuated throughout the study duration in every block; it was not necessarily true that if there was a high number of *Ae. aegypti* larvae in the container one week, the number would also be high in the following week, indicating a fluctuating population. Migration of *Aedes* mosquitoes into the study site, via human residents using the elevators at the condominia could be one explanatory factor, for example.

Reduced fecundity of female *Aedes* mosquitoes treated with pyriproxyfen was reported by Ponlawat *et al.* (2013) and Ohba *et al.* (2013). We achieved a similar result only after the additional 8 pyriproxyfen-treated traps per floor deployed in Block B. We considered from the initial results that a higher dose, either by increasing the concentration of pyriproxyfen in each trap or by increasing the number of traps in the study site, was needed to impact the number of surviving offspring of adult mosquitoes. The latter was chosen during this study since this was expected to increase the contact frequency of the *Aedes* mosquitoes with these traps. Mosquitoes are known to groom themselves (Walker and Archer, 1988), and during this process contaminated mosquitoes could easily remove the biocide formulation from their body. To ensure that the auto-dissemination phenomenon will occur in operational programmes, the concentration should also be increased to a level where the mosquito adults can pick up a sufficient concentration to transfer a lethal dose to subsequent breeding containers. The concentration as indicated by other studies carried out in field ranged from 0.5% - 20% (Suman et al., 2004; Isik et al., 2017; Devine et al., 2017). However in our study the concentration was 0.004% which was 125 - 5000 folds lower. This may be the reason why no auto-dissemination effect was observed in the current field study. In our laboratory studies we noticed ovitraps without pyriproxyfen was contaminated by *Aedes aegypti* when placed in the same cage (unpublished data).

The concentration of 0.004% pyriproxyfen in the auto- dissemination trap was developed by a local company. The concentration was chosen based on laboratory bioefficacy study. Moreover pyriproxyfen was used in close small volume container without any running water. Therefore a lower concentration was required for these trials in the field.

The most encouraging result from our study was that not a single auto-dissemination trap from 552 traps deployed in Block A, B and C contained any pupal exuviae, despite the frequent presence of eggs in traps. Pyriproxyfen had been known to show ovicidal activity (Suman et al., 2013) besides having pupicidal action, pyriproxyfen also sterilizes adult females which decreases spermatogenesis in male *Anopheles balabacensis*. (Iwanaga and Kanda, 1988) and ceased egg diapause early in *Ae. albopictus* (Suman et al., 2015). This suggests an added value that the auto-dissemination traps can contribute to integrated vector management programmes, overcoming the limitations of the larviciding approach when there are cryptic and hard-to-find containers in dengue endemic areas. Kawada et al. (1993) stated that PPF did not impair adult activity, and its effectiveness against *Ae. aegypti* larvae was proven at extraordinarily low concentrations. Moreover, auto- dissemination stations with new designs and other compounds (Mark et al., 2016) as well as new formulations (e.g., IGRs in combination with bacterial toxins of spinosad or fungi *B. bassiana*) may further enhance effectiveness of auto-dissemination traps (Nicole et al., 2019).

This study is an operational deployment of auto-dissemination trap showing reduction of dengue transmission in a real life situation. The number of dengue cases was reduced from 53 cases in 2013 to 13 cases in 2014. This decrease has been shown to be statistically significant and is even more convincing if put in the context of the overall increase in total number of dengue cases in Malaysia, which increased from 43,346 in 2013 to 108,698 in 2014. However, the number of dengue cases increased from 13 cases in 2014 to 57 cases in 2015, post-deployment. This 338% increase is much higher than the proportional increase in the dengue cases in Malaysia overall of 11% from 108,698 in 2014 to 120,863 in 2015, suggesting that whilst the traps were present dengue transmission was reduced.

Although the outcomes of this trial were promising, the approach should be used synergistically with other control tools such as residual spraying to maximise the potential control of dengue transmission. The shortcomings of this tool are the requirement of regular servicing of the traps and the associated costs, though based on the rate of evaporation from this trap we suggests that bimonthly servicing should be sufficient to implement this trap operationally. Given the failure of any eggs laid in these traps during the experiment to develop past first instar larvae there is no danger of creating breeding sites. Public engagement activities should be conducted prior to the application of the auto-dissemination trap. Community participation is crucial to ensure the approach's sustainability and the effective use of auto-dissemination traps to control dengue.

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