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

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Abstract. Continued outbreaks of dengue in endemic areas, unabated despite use of conventional vector control methods, necessitate development of new control tools as existing dengue mosquito control technologies are effective only to a limited extent. An insect growth regulator (IGR)-treated auto-dissemination trap was developed against *Aedes* spp, in which a female mosquito ovipositing in the treated trap is contaminated with an IGR [0.004% pyriproxyfen (PPF)] and transfers the IGR to other containers as the mosquito continues to oviposit. Four PPF auto-dissemination traps were placed on each floor of a 3-block condominium complex in Sri Subang, Selangor, Malaysia for 44 weeks (February to December 2014). Traps were replenished with PPF solution biweekly. Dengue epidemiological monitoring was also conducted by national health authorities. Oviposition in auto-dissemination traps increased over the study period, indicating an attraction for gravid female *Aedes* spp. No single live larva was observed in any auto-dissemination trap, indicating complete larval mortality induced by PPF. Following introduction of eight additional treated traps on every floor from week 16 onwards, a reduction in ovitrap index from 90 to 33% by week 20 was observed. Correspondingly, number of reported dengue cases was reduced from 53 in 2013 to 13 cases in 2014 (p-value = 0.006). Although *Aedes* spp populations fluctuated over the course of the study period, the results suggest auto-dissemination traps as a promising dengue control tool. Future research should be directed to determine the optimal PPF concentration and number of PPF treated auto-dissemination traps required to be deployed for ensuring maximum control of dengue.

Keywords: *Aedes* species, auto-dissemination trap, dengue, insect growth regulator, pyriproxyfen

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INTRODUCTION

Aedes aegypti (Linnaeus, 1762) is not indigenous to Malaysia and is believed to originate from tropical Africa and to have been introduced to Malaysia via India at the turn of the 20th 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 chikungunya, dengue and Zika, 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. The global incidence of dengue has grown dramatically in recent decades. About half of the world's population is now at risk. There are an estimated 100-400 million infections each year. The case mortality rate of severe dengue is below 1% with good health care (WHO, 2020). Dengue is the only predominant arbovirus-borne disease in Malaysia, and up to the 52nd epidemiological week in 2019, 130,101 cases of dengue were reported with 182 deaths (CodeBlue, 2020).

Resurgence of dengue over the past years (Phillips, 2008) makes control of *Ae. aegypti* extremely urgent, especially in the absence of any 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 eggs and a 'push' in the dispersal or transfer of a control agent (microbes or chemicals), which are innocuous to the adult mosquitoes, to other target habitats. This method exploits the 'skip' oviposition behavior of *Aedes* mosquitoes, in which the female will oviposit in multiple sites within a single gonotrophic cycle (Davis *et al*, 2016). This strategy was pioneered using microbes that can multiply in containers, such as entomopathogenic fungi and baculoviruses (Soper, 1978; Yu and Brown, 1997; Klein and Lacey, 1999), and nematode auto-dissemination also has been considered (Lacey *et al*, 1993). Recently proposed approaches exploit *Edhazardia aedis* (Microsporidia) and entomopathogenic fungus *Metarhizium anisopliae* as agents for controlling *Ae. aegypti* (Buckner *et al* 2017). This innovative concept was first proposed by Itoh *et al* (1994) who conclusively demonstrated gravid female *Ae. aegypti* contaminated with insect growth regulator pyriproxyfen (PPF) were able to transfer lethal concentrations to larval habitats. The effect was enhanced by the skip oviposition behavior of gravid *Ae. aegypti* females that distribute eggs throughout multiple containers (Mogi and Mokry, 1980). In addition, laboratory and semi-field tests demonstrated the application of specific IGRs, novaluron and K+ channel modulators, in auto-dissemination traps against *Anopheles quadrimaculatus* (Swale *et al*, 2018).

Here, a simple auto-dissemination device, consisting of a modified ovitrap containing pyriproxyfen to contaminate gravid female mosquitoes during oviposition, thereby allowing them to disseminate the chemical to other *Aedes* oviposition sites, was evaluated in urban high-rise condominiums for its efficacy in reducing natural *Aedes* spp population and dengue cases.

MATERIALS AND METHODS

Trial site

The trial site, an urban area of 3.0

hectares in Ridzuan Court Sunway, Selangor, Malaysia, situated three residential condominiums, namely, Block A (27 levels), Block B (27 levels) and Block C (26 levels), with a total of 847 units, and a 5-level building with a car park, convenience stalls and food stalls (not included in the test site). The condominiums and associated swimming pool are surrounded by green shrubs and cultivated trees. Ridzuan Court is located in a densely populated area in the Klang Valley, which accounts for about 60% of the total dengue cases in Malaysia.

A dengue hotspot in Malaysia is defined as a location where at least two dengue cases occur within a week and further cases then continue to occur within one-month period. This area has been a declared dengue hotspot since week 52 of the year 2013. These condominiums were selected as the trial site as it had proven very difficult to manage dengue transmission in this area using conventional methods (Tee *et al*, 2019).

Test chemical preparation

A juvenile hormone analogue, 0.004% (w/v) pyriproxyfen [(4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propylether] (PPF) (Sumilarv 0.5G®; Sumitomo Chemical Corp, Osaka, Japan) was prepared by dissolving 800 g of Sumilarv 0.5G® in 87.5 liters of filtered water allowed to remain at ambient temperature for a minimum of 48 hours, then solution was stirred for 4 hours using a high speed stirrer filtered by high power centrifuge (OKATZ Power Tools, Selangor, Malaysia.) before adding to 12.5 liters of hay infusion (One Team Networks Sdn Bhd, Selangor, Malaysia). Polyhexamethylene (0.004% w/v) (Nissho Manufacturing Co Ltd, Gifu, Japan) was added as a preservative and solution (seasoned water) was stored in a cool dry place shielded from direct sunlight until used.

Auto-dissemination trap construction

A black auto-dissemination trap (13.0 cm (height) cm x 11.0 cm (bottom width) x 14.8 cm (top width), fitted with a removable cover, to topically contaminate gravid mosquitoes seeking an oviposition site, as preferred by gravid *Aedes* (Beckel, 1955) was filled with 600 ml of 0.004% (w/v) PPF solution (Fig 1). Three holes, 2.0 cm in diameter, were drilled on the side to enable mosquito's entry into the trap for oviposition. Two pieces of tissue paper (6 cm x 9 cm) (Scott Kimberly-Clark Products (M) Sdn Bhd, Johor, Malaysia) were attached to the inside of the trap, on opposite walls, to serve as oviposition sites.

Environmental parameters determination

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

Pre-treatment baseline *Aedes* spp. population data collection

Baseline *Aedes spp.* population density at test sites prior to conducting auto-dissemination trial was conducted using standard ovitraps consisting 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 (Fay and Perry, 1965; Fay and Eliason, 1966). Four ovitraps were placed on each condominium floor, five meters apart, along corridors outside the apartment units (a total 356 ovitraps in the three condominiums) in protected

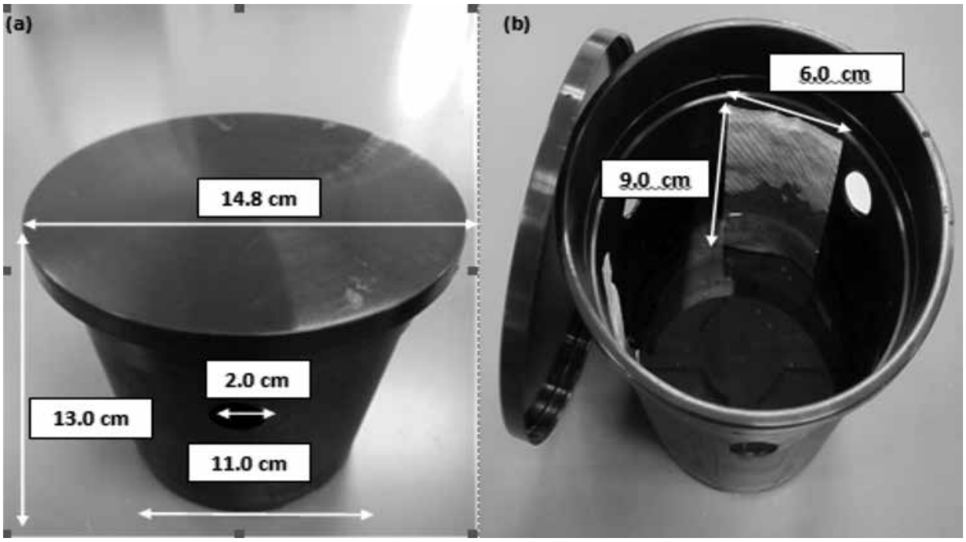

Fig 1-Auto-dissemination trap

(a) External view; (b) Internal view. The trap is filled with 600 ml of 0.004% (w/v) pyriproxyfen solution (juvenile hormone analogue) and lined with tissue papers on two sides. Three holes (2.0 cm in diameter) are drilled into the side to allow ingress and egress of gravid female mosquitoes.

and low-traffic areas that provided shade, as favored by container-dwelling species in oviposition site selection (Vezzani, 2009), and collected weekly. The number of positive ovitraps, number of larvae per ovitrap and *Ae. aegypti*: *Ae. albopictus* ratio were recorded. 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 deployment of auto-dissemination traps (all standard ovitraps being removed prior to trial).

Auto-dissemination trap placement

Auto-dissemination trap trial was conducted for a period of 44 weeks, from February 2014 to December 2014. PPF-treated auto-dissemination traps (n = 356) were placed in the same locations as in the baseline data collection trail and monitored twice weekly as described above. During week 1 of the study, auto-dissemination traps were observed for the presence of live larvae, and, owing to absence of live larvae after week 1, this was discontinued (confirmed by absence of live larvae in the auto-dissemination traps throughout the study). Auto-dissemination trap papers were collected every two weeks and replaced with new papers, and auto-dissemination traps were refilled with 0.004% (w/v) PPF solution up to the 250 ml mark. Oviposition papers were labelled (placement site and date), air dried and stored at room temperature (27-28°C) prior to manual counting of eggs, which 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. Containers were checked for larvae 1-2 times per week for 2 weeks post-hatching. Average number of eggs collected every other week from each condominium was used to assess efficacy of auto-dissemination trap using a standard ovitrap index (OI), the percent positive ovitraps with *Ae. aegypti* and *Ae. albopictus* against total number of ovitraps recovered per surveillance trip (Lee, 1992), and *Aedes* spp egg index, percent auto-dissemination system positive with *Aedes* eggs within a 2- week period (Buckner *et al*, 2017), during the whole trial period.

On week 22 of 2014 (15^{th} treatment week), an additional eight auto-dissemination traps per floor were placed in Block B (making a total of 552 traps) to evaluate the impact of additional auto-dissemination traps on mosquito population. The additional traps were placed along corridors, in waiting areas near lifts and at emergency staircase of each floor.

Intervention procedures

In order to assess the effectiveness of auto-dissemination traps, standard ovitraps were placed monthly among the auto-dissemination traps at a distance of 0.5 m from the waiting area of lifts on every floor of the three condominiums (a total of 90 standard ovitraps) for a period of seven days. Absence of eggs or presence of deformed immatures in standard ovitraps indicates transfer of PPF by female mosquitoes into subsequent ovitraps. Larvae numbers and species of *Aedes* were recorded using both eggs that hatched and larvae in ovitraps. Ovitraps were collected after seven days and immediately transported to the Institute for Medical Research (IMR) laboratory, Kuala Lumpur. Content of each ovitrap together with that on paddle were placed individually into labelled covered plastic containers (15 cm x 7 cm x 8.5 cm). All larvae were counted and identified under a compound microscope (Nikon Eclipse E200; Nikon Imaging Japan, Tokyo, Japan) at the third or fourth instar using in-house taxonomy keys.

Dengue epidemiology

Pre- and post-intervention dengue epidemiological data for 2013-2015 were obtained from the Disease Control Division, Ministry of Health, Malaysia via an eDengue database, updated daily by epidemiological reports from local health departments (Ministry of Health, 2017). As dengue is a notifiable disease in Malaysia, epidemiological data are routinely screened and verified by dedicated public health specialists before being loaded into eDengue database. This method was chosen because the eDengue database is a reliable and rigorously tested source of data. Epidemiological data were used to determine the impact of auto-dissemination traps on dengue transmission.

Entomological surveillance

The entomological surveillance was conducted at the study sites by the Medical Entomology Team from Medical Entomology Unit, Institute for Medical Research (IMR), Kuala Lumpur, Malaysia. The larvae and pupae collected in the ovitraps after each surveillance period were transferred to Medical Entomology Laboratory and analysed by the Medical Entomology staff. Each and every larva and pupa from ovitrap containers was identified and recorded carefully by species and localities of each trap. The larvae species were identified and the ovitrap index (OI) was calculated. The *Aedes* eggs yield from the auto-dissemination traps placed at the study sites were also removed and counted on bi-weekly basis. The entomological indices from this study consisted of:

i. Ovitrap Index (OI): Number of positive ovitraps against the total number of ovitraps collected in percentage for each surveillance trip.

ii. *Aedes* Egg Index: Number of traps positive with *Aedes* eggs against total number of auto-dissemination traps collected in percentage for each surveillance trip.

Data analysis

Entomological data in pre- and post-treatment periods were recorded using Excel Microsoft Office and presented as mean ± standard error of mean (SEM) and analyzed using the Statistical Package for the Social Sciences (SPSS) version 17.0 (IBM, Chicago, IL), and statistical significance is accepted at *p*-value <0.050. Data were tested for normality and variance homogeneity using Komolgorov-Smirnnov and Levene's tests. Abnormally distributed data were arcsine log transformed to normalize the variation. 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 significance of differences. Correlation test was performed using a Pearson correlation method.

RESULTS

Temperature and relative humidity

Malaysia lies on the tropical belt and rain is abundant throughout the year. The maximum and minimum temperature observed during the 44 weeks study period were 32.15 ± 0.03 °C and 27.04 ± 0.39 °C, respectively, and maximum and minimum relative humidity observed were 82 ± 2 and 60.4 ± 0.51 percent, respectively.

Aedes spp population at study site prior to intervention trial

Baseline data collected prior to intervention revealed existence of two *Aedes* spp, *Ae. aegypti* (96.22%) and *Ae albopictus* (3.78%) in the three condominiums (Blocks A, B and C) (Fig 2). From ovitrap surveillance data, *Ae. aegypti* oviposited from ground to 27th floor in all the three blocks. OIs for *Ae. aegypti* in Blocks A, B and C were 65 ± 7, 44 ± 3 and 26 ± 2 percent, respectively, and those of *Ae. albopictus* 2 ± 1, 3 ± 2 and 0.5 ± 0.3 percent, respectively; OI of *Ae. aegypti* was 15-39-fold higher than that of *Ae. albopictus*. There was equal distribution of *Ae. aegypti* larvae in ovitraps on each floor. Similar data were obtained with *Ae. albopictus*.

Intervention using 0.004% (w/v) PPF auto-dissemination traps

Females *Aedes* oviposited in 100 % of the 0.004% (w/v) PPF auto-dissemination traps (*n* = 552), but during the 44 weeks of the study period, no surviving larvae were found in any auto-dissemination trap, although eggs were present and, notably, some had hatched as observed under a dissecting microscope but no larvae survived to L2 stage when eggs collected on filter papers were submerged in seasoned tap water for further hatching. Absence of pupal exuviae in auto-dissemination traps or plastic containers in the laboratory indicated that no larva emerged into adults, *ie* 100% inhibition of pupal and adult emergence from paper-deposited eggs.

OIs (mean ± SEM) of *Ae. aegypti* and *Ae. albopictus* before intervention for all three condominiums blocks were 45 ± 11 and 2 ± 1 percent respectively, and after intervention using four auto-dissemination traps on every floor continuously for sixteen weeks (26 March 2014 to 11 June 2014), OIs were 53 ± 9 and 11 ± 4 percent, respectively (Fig. 4). A lesser post-intervention OI indicates

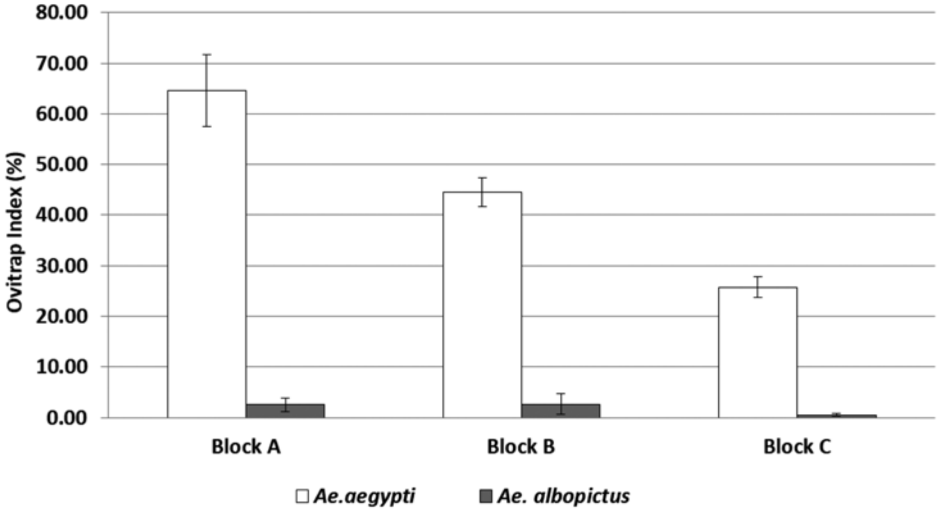

Fig 2-Baseline mean ovitrap index of *Aedes aegypti* and *Ae. albopictus* from week 48 of 2013 to week 6 of 2014

Mosquito traps ($n = 4$) as described in legend to Fig 1 but without pyriproxyfen and fitted with paddle made from hard cardboard (3 cm x 10 cm) to serve as a resting site for gravid females to oviposit, were placed on each floor, five meters apart along corridors outside apartment units, of three test condominiums (making a total of 356 ovitraps). Traps were inspected every two weeks and solution and paddle replaced. Results are shown as mean ± standard error of mean (SEM).

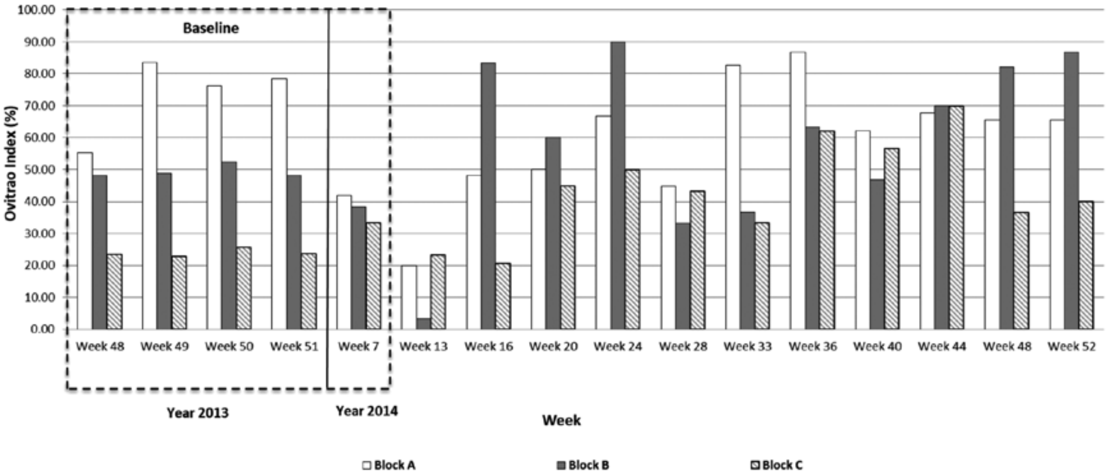

Fig 3-Ovitrap index during an intervention trial of 0.004% (w/v) pyriproxifen auto-dissemination traps placed in Ridzuan condominium complex, Bandar Sunway, Selangor, Malaysia during 2014

Auto-dissemination traps ($n = 356$) were placed in locations and monitored as described in legend to Fig 2 except cardboard paddle was replaced with three pieces of tissue paper. From week 48 of 2013 to week 7 of 2014, baseline data were collected as described in legend to Fig 2.

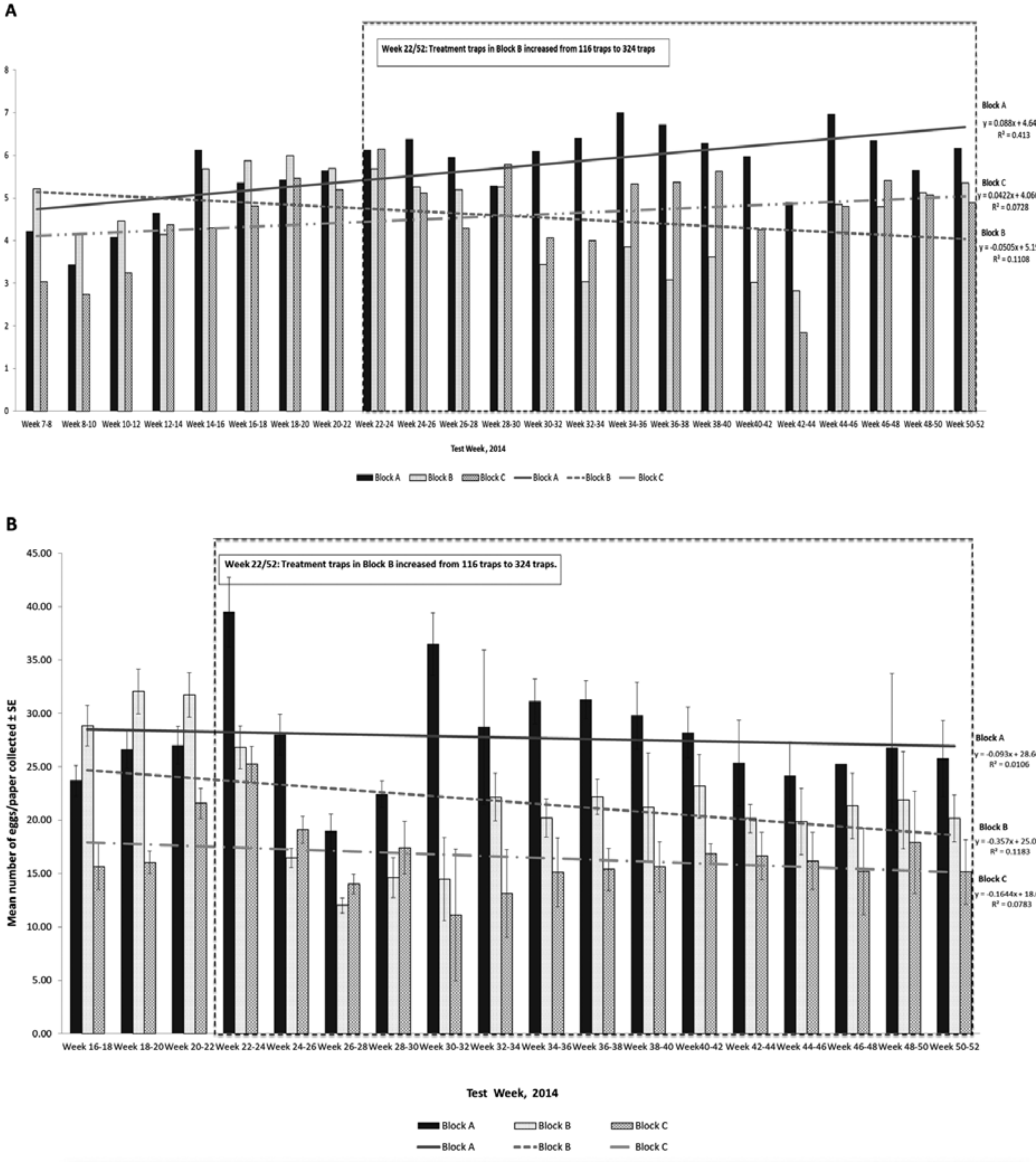

Fig 4-*Aedes* spp egg index (percent trap positive with *Aedes* eggs within a 2-week period) (A) and mean number of eggs per collection paper ± standard error of mean (SEM) (B) during an intervention trial of 0.004% (w/v) pyriproxifen auto-dissemination traps placed in Ridzuan condominium complex, Bandar Sunway, Selangor, Malaysia during 2014

Auto-dissemination traps (n = 356) were placed in locations and monitored as described in legend to Fig 2. Linear graph depicts linear least square fit of data.

adult population suppression. Thus, intervention in all three condominiums under these test conditions failed to impact OI values of *Ae. aegypti* population over this period although there is a significant increase in OI values for *Ae. albopictus*.

In order to amplify the effect of auto-dissemination traps, the numbers of these traps were increased by deploying eight more traps from 1st to the 27th floor in Block B as OI of this Block was higher than that of Block A or C. The 2.8-fold increase in number of auto-dissemination traps resulted in a reduction in *Aedes* spp egg index from 5.69 to 3.44 (39.5% reduction) within 10 weeks after implementation (Fig 4). Within the same period, *Aedes* spp egg index increased from 5.64 to 6.09 (7.9% increase) in Block A and reduced from 5.19 to 4.07 (22.1% reduction) in Block C. During the 44-week intervention period a total of 272,666 eggs were laid in the auto-dissemination traps by *Aedes* spp, all non-viable due to presence of 0.004% (w/v) PPF solution.

On the other hand, although *Aedes* spp egg index was reduced (in Blocks B and C), no effects of pyriproxyfen transferred by mosquitoes into the supplemented standard ovitraps were observed in all three condominiums as evidenced by absence of larval mortality or deformities in these standard ovitraps. This suggests a higher concentration of pyriproxyfen is required to induce the auto-dissemination effect in these traps via skip oviposition behavior. However, after extra auto-dissemination traps were introduced in Block B, mean OI value decreased 18.5, 56.7 and 6.7% in Blocks A, B and C, respectively compared to mean OI value of the previous months (data not shown).

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 auto-dissemination trap trial was implemented to help reduce dengue cases in the test area. Fifty-three confirmed dengue cases were reported in 2013 prior to the trial, and 13 cases at the end of the trial in 2014 (p-value = 0.006, Mann Whitney U-test). Correlation between average OI of the three condominiums and total dengue cases in the following two weeks, although positive, was weak (Fig 5). In 2015, dengue cases increased drastically after cessation of the auto-dissemination trap trial (Fig 6).

DISCUSSION

Various methods are in use for vector control depending on ecosystems and climatic conditions of the endemic area. Although chemical, biological and environmental management techniques are still widely used, the battle against *Aedes* mosquitoes has been going on for the better part of the last century with limited success in terms of sustainable control (Halstead, 2000). *Aedes* spp are unmanageable using conventional practices, largely due to limited long-term sustainability of control measures, a result of cryptic larval habitats preferred by *Aedes* mosquitoes that are difficult to reach by traditional methods of insecticide application such as space spraying.

The strategy of an auto-dissemination system can be incorporated into other innovative tools for dengue vector control to help overcome the development of insecticide resistance in dengue vectors, primarily *Ae. aegypti* and *Ae. albopictus*, reported for the past several decades (Ishak *et al*, 2015). The oviposition behavior

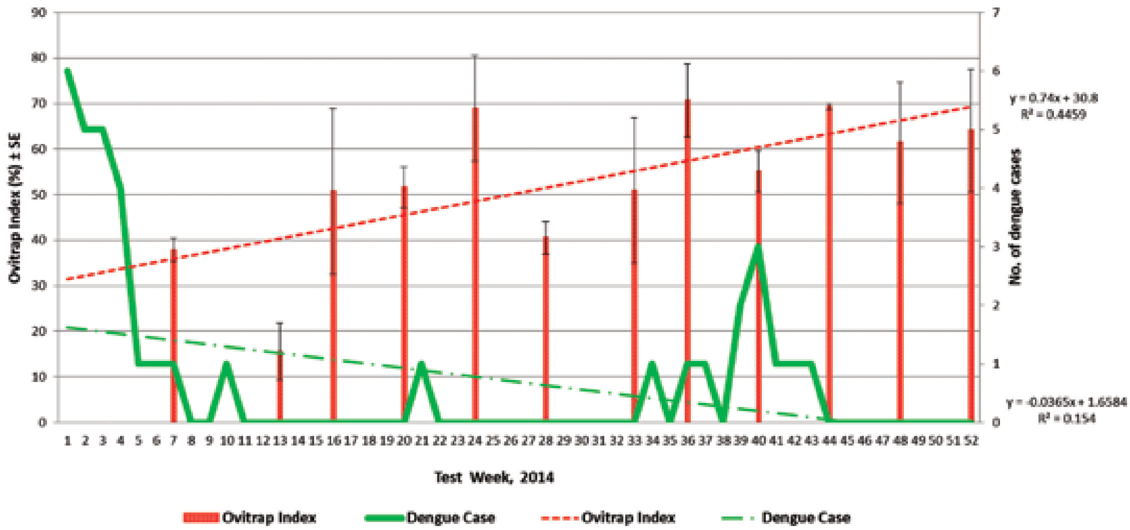

Fig 5-Ovitrap index and dengue cases in Ridzuan condominium complex, Bandar Sunway, Selangor, Malaysia throughout the 2014 study period

Auto-dissemination traps containing 0.004% (w/v) pyriproxifen were distributed in the condominiums and ovitrap index [% ± standard error (SE)] determined as described in legend to Fig 2. Linear graph depicts least square fit of data. Dengue data were obtained the Disease Control Division, Ministry of Health, Malaysia via eDengue database.

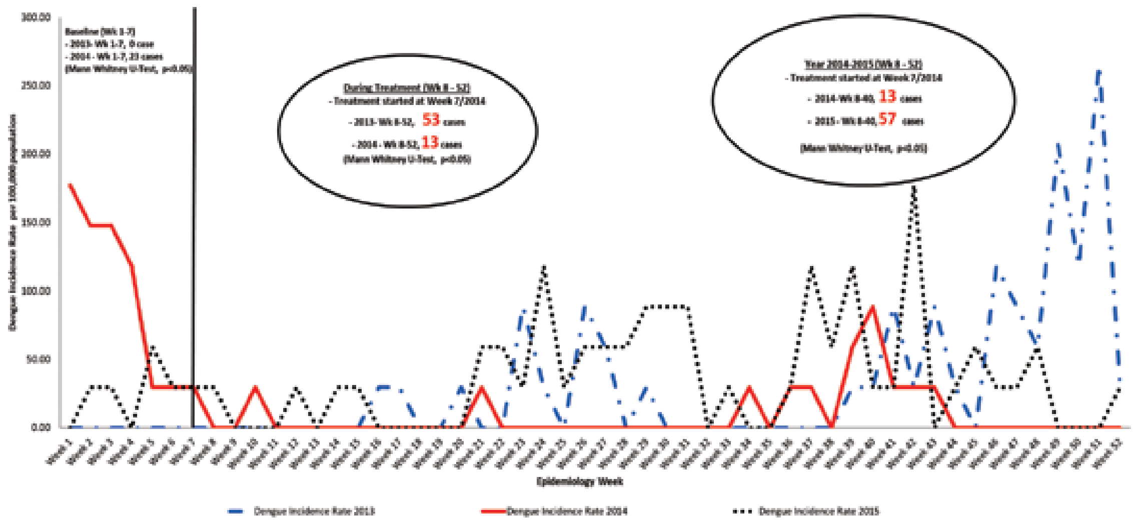

Fig 6-Dengue incidence rate in in Ridzuan condominium complex, Bandar Sunway, Selangor, Malaysia from 2013 -2015

X-axis indicates week in 2014 during which 0.004 % (w/v) pyriproxyfen auto-dissemination traps were deployed. Linear graph depicts least square fit of data. Dengue data were obtained the Disease Control Division, Ministry of Health, Malaysia via eDengue database.

of these vectors, typically scattering eggs from a single gonotrophic cycle among multiple 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 chemical or biological insecticides in targeting cryptic larval habitats such as hidden man-made containers. *Aedes* mosquitoes are attracted towards darker, shadier areas, making this auto-disseminating PPF treatment device attractive for egg deposition. In Thailand, a similar PPF-treated device evaluated in outdoor tunnels and field trials showed significantly reduced adult catch using BG-Sentinel traps post-treatment, together with a reduction in egg production by females exposed to PPF (Ponlawat *et al*, 2013). In a public cemetery in Peru introduction of auto-dissemination traps resulted in 42-98% inhibition of *Ae. aegypti* emergence (Devine *et al*, 2009). In Italy, a sticky trap coated with 5% PPF powder produced 40-70% mortality (Caputo *et al*, 2012). Dual treatment using PPF powder and oil raised auto-dissemination efficacy by improving toxicant attachment and retention on contaminated females (Wang *et al*, 2014).

The present study demonstrates the feasibility of this approach in a condominium setting in an urban region of Malaysia. One vital finding is deployment of an auto-dissemination trap was apparently able to reduce dengue transmission. During the study period, auto-dissemination traps continuously present at the trial site removed 272,666 eggs that would otherwise have developed into potential dengue virus-carrying adult mosquitoes.

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 ovitraps usefulness in assessing impact of vector control measures targeting 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. However, ovitrap index may be inappropriate as a proxy indicator for adult density in study sites as observed in our study, though it remains the only practical surveillance tool available. OI fluctuated throughout the study duration in every condominium but 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. No differences in larva numbers between lower (floors 1-10), middle (floors 11-20) and higher (floors 21-28) ($F = 1.546$, $df = 2$, p-value = 0.219) were observed in the preset study, but Lee *et al* (2013) found *Ae. aegypti* prefer to breed within 6-8 floors from ground level. Introduction of *Aedes* mosquitoes into the study sites via human residents using condominium elevators might provide an explanation.

The solution employed in an auto-dissemination trap contained hay infusion to enhance the trap's attractiveness to gravid females seeking to oviposit. Water infused with leaf matter is effective in attracting *Ae. albopictus* females (Ponnusamy *et al*, 2010; Gaugler *et al*, 2012). In the pre-intervention surveillance period, hay infusion water was not used in the standard ovitraps as the study objective was to monitor the effect of auto-dissemination traps and thus it was the relative abundance before, during and after intervention that was of interest.

Another interesting phenomenon observed in the present study was the influence of auto-dissemination trap deployment on *Ae. albopictus* population, showing a significant increase in the number of *Ae. albopictus* in the standard ovitraps after *Ae. aegypti* population was reduced. This could be due to reduced inter-specific competition and *Ae. albopictus* subsequently capitalizing on the compromised competition from *Ae. aegypti* to fill the niche. This highlights the necessity of operationally deploying auto-dissemination traps both indoors and outdoors to control both *Aedes* spp. The present study used a comparison of pre- and post- intervention data as opposed to using a control site as previously reported (Erlanger *et al*, 2008).

Reduced fecundity of female *Aedes* mosquitoes treated with PPF was reported by Ohba *et al* (2013) and Ponlawat *et al* (2013). Similar results were obtained only after additional PPF-treated traps per floor deployed in Block B. Although a higher dose of PPF in the traps would have achieved the same effect, the course of action taken was expected to increase contact frequency of *Aedes* mosquitoes with these traps. Mosquitoes groom themselves (Walker and Archer, 1988) and during this process contaminated mosquitoes can easily remove biocide from their body. In order to ensure auto-dissemination phenomenon will occur in the intervention strategy, biocide concentration should also be increased to a level that 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*, 2014; Devine *et al*, 2009; Isik *et al*, 2017). In the present study PPF concentration was 125-5,000 folds lower, accounting for the absence of auto-dissemination effect. The PPF concentration used in the auto-dissemination trap was based on a laboratory bioefficacy study, in which PPF was placed in a small volume closed container accounting for the low concentration required.

The most encouraging result from the present study was that not a single auto-dissemination trap from 552 traps deployed in the three test condominiums contained any pupal exuviae, despite the presence of eggs in traps. PPF has ovicidal activity (Suman *et al*, 2013) in addition to being a pupicide, and sterilizes adult females thereby decreasing spermatogenesis in male *Anopheles balabacensis*. (Iwanaga and Kanda, 1988) and causes early cessation of egg diapause in *Ae. albopictus* (Suman *et al*, 2015). These properties indicate an added value that PPF auto-dissemination traps can contribute to integrated vector management programs, overcoming limitations of the larvicide approach when there are cryptic and hard-to-find containers in dengue endemic areas. Kawada *et al* (1993) noted PPF does not impair adult activity and its effective against *Ae. aegypti* larvae is at extremely low concentrations. Auto-dissemination traps of new designs and using other compounds (Kartzinel *et al*, 2016) as well as new formulations (*eg* IGRs in combination with bacterial toxins of Spinosad or fungus *Beauveria (bassiana*) may further enhance effectiveness of auto-dissemination traps (Achee *et al*, 2019).

The present study was an operational deployment of auto-dissemination traps to show whether such a strategy could reduce dengue transmission in a real-life situation. The number of dengue cases was reduced in 2014 (deployment period) from that in 2013 (pre-deployment). Although the decrease was small it is 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 (Woon *et al*, 2016). The number of dengue cases in the test area increased 338% in 2015 following cessation of deployment of auto-dissemination traps, much higher than the overall increase (11%) in the dengue cases in Malaysia from 2014 to 2015 (The Star, 2016) highlighting the role of auto-dissemination traps in dengue control. Even though OI values were inconclusive, the number of eggs per paper in the traps decreased throughout the test period indicating suppression of *Aedes* spp population. It is plausible to conclude that the reduction in dengue transmission could be attributed (in part) to a decrease in the number of adult *Aedes* mosquitoes.

The shortcomings of the auto-dissemination strategy are requirement of regular servicing of the traps and associated costs, but based on the rate of evaporation from the traps bimonthly servicing should be sufficient. Given the failure of any eggs laid in the traps to develop past first instar larva stage there is no danger of creating breeding sites

In conclusion, the outcomes of this trial were promising but the strategy should be used in conjunction with other control tools, such as residual spraying, to maximize control of dengue transmission. Public engagement should be conducted prior to application of the auto-dissemination traps. Community participation is crucial to ensure the strategy sustainability and effective use of auto-dissemination traps to control dengue in the community.

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