# Monitoring *Aedes* Population using Ovitrap Index and Larval Abundance in an Urban University Residence

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### ABSTRAK

Ovitrap autosidal berperanan penting dalam pemantauan dan kawalan vektor denggi, Aedes aegypti and Aedes albopictus. Beberapa jenis ovitrap ini telah digunakan di Malaysia termasuklah Mosquito Larvae Trapping Device (MLTD) yang diperkenalkan oleh Dewan Bandaraya Kuala Lumpur. Kajian ini bertujuan untuk menilai kepadatan larva Aedes dan pembolehubah meteorologi setempat di sebuah penempatan pelajar di Cheras, Kuala Lumpur. MLTD (n=30) dengan umpan larutan baja NPK (5:5:5) organik telah digunakan. Pengumpulan larva dan penyelenggaraan ovitrap telah dilakukan setiap minggu selama lapan minggu. Pemeliharaan larva dan penentuan spesies telah dijalankan di makmal. Daripada sejumlah 2,152 larva Aedes, 85% merupakan Ae. albopictus manakala selebihnya Ae. aegypti. Ae. albopictus lebih banyak dikumpulkan di luar berbanding di dalam bangunan (purata larva per ovitrap, 9.28 lawan 6.08). Sebaliknya, Ae. aegypti lebih banyak dikumpulkan di dalam berbanding di luar bangunan (1.72 lawan 0.86). Ovitrap indeks yang paling tinggi adalah semasa minggu kelima (90%) dan keenam (93%). Minggu pertama menunjukkan ovitrap indeks yang paling rendah (30%). Analisis mendapati korelasi positif antara kepadatan larva Aedes dan suhu maksimum (r=0.830, p=0.011), manakala kelembapan relatif minimum menunjukkan korelasi negatif (r=-0.778, p=0.023). Pemantauan rutin bagi peringkat pramatang vektor denggi boleh memberikan maklumat mengenai kepadatan dan naik turun populasi nyamuk setempat. Apabila digandingkan dengan faktor meteorologi sejajar, maklumat ini dapat memberi panduan bagi perlaksanaan kawalan vektor sebagai sebahagian strategi pencegahan denggi.

Kata kunci: Aedes, cuaca, denggi, indeks, kelembapan, larva, surveilans, suhu

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## ABSTRACT

Autocidal ovitraps have played a significant role in surveillance and control of dengue vectors, Aedes aegypti and Aedes albopictus. Malaysia has witnessed the deployment of several types of autocidal ovitraps, which includes the Mosquito Larvae Trapping Device (MLTD) introduced by Kuala Lumpur City Hall. This study aimed to assess Aedes larval abundance and local meteorological variables in a university residence, in Cheras, Kuala Lumpur. MLTD (n=30) baited with organic NPK fertiliser (5:5:5) solutions were deployed. Larvae collection and trap servicing were performed weekly for a duration of eight weeks. Rearing and species identification for larvae were conducted in the laboratory. Out of 2,152 Aedes larvae, 85% of them were Ae. albopictus whilst the remaining were Ae. aegypti. Outdoor collection of Ae. albopictus surpassed its indoor collection (mean larvae per trap of 9.28 versus 6.08). Conversely, an indoor collection of Ae. aegypti was greater than its outdoor collection (mean larvae per trap of 1.72 versus 0.86). The highest ovitrap indices were observed in Week 5 and 6 which were 90% and 93%, respectively. Week 1 had the lowest ovitrap index, 30%. Our analyses revealed a positive correlation between Aedes larval abundance and maximum temperature (r=0.830, p=0.011) whereas minimum relative humidity was shown to have a negative correlation (r=-0.778, p=0.023), with the larval abundance. Routine monitoring of dengue vectors at its immature stages can provide information on the density and fluctuation of the local mosquito population. Coupled with concurrent meteorological variables, it can guide vector control operations as part of dengue prevention strategies.

Keywords: Aedes, dengue, humidity, index, larva, surveillance, temperature, weather

### **INTRODUCTION**

Dengue is a mosquito-borne disease known to be associated with a spectrum of clinical presentations, including febrile acute illness, haemorrhagic neurological and manifestations, and dengue eye disease such as maculopathy (Umi Kalthum & Wong 2012). Malaysia is one of the countries in South-east Asia dealing with endemic dengue infection every year over the past few decades. The year 2019 recorded a higher number

of dengue cases than the previous year. The current total cumulative number of dengue cases from all states in Malaysia reached 130,101 including 182 deaths. Selangor contributed to the vast majority of dengue cases along the 2019 period (72,543). In Kuala Lumpur and Putrajaya alone, the number of cases reported was up to 15,424 (Ministry of Health Malaysia 2020). This clearly shows that dengue infection is highly prevalent in urban areas with high population density.

Dengue virus is transmitted

primarily by *Aedes* mosquito, namely Aedes aegypti (Linnaeus) and Aedes albopictus (Skuse). These species are found in Malaysia at a great extent, leading to the commencement of various local studies to investigate their abundance. A recent outdoor ovitrap survey in Kuala Lumpur revealed a mosquito population dominated by Ae. albopictus (Ahmad-Azri et al. 2019). A similar finding was reported by ovitrap collections in the city fringe of Seremban, Malaysia (Sahani et al. 2012). Other studies conducted in different states also reported that the two species were found to share breeding sites in both indoor and outdoor settings (Norzahira et al. 2011: Wan-Norafikah et al. 2012) and dominant species changes according to the area (Wan-Norafikah et al. 2012). Meteorological factors, such as relative humidity and temperature of the environment, also played a huge role as factors affecting the abundance and development of Aedes mosquitoes (Yusof et al. 2018; Reinhold et al. 2018). Having a clear picture and better understanding of the nature of these vectors, interventions can be tailored accordingly in order to halt their abundance from spreading exponentially hence interfering with the transmission of dengue viruses.

Dengue vector surveillance is one of the necessary measures taken by Malaysia's local health authority in response to dengue cases. The larval survey that is being implemented in Malaysia has its drawbacks (Shah & Sani 2011; Shah et al. 2012) which can be mitigated with ovitrap surveillance. Kuala Lumpur City Hall introduced an autocidal ovitrap called Mosquito Larval Trapping Devices (MLTD). MLTD added with *Bacillus thuringiensis israelensis* (*Bti*) were used for surveillance and control of dengue vectors (Azil et al. 2011). In general, leaf infusion from local plants, either dried (e.g. hay) or fresh, have been used to increase collection of oviposition traps. Besides these, animal food pellet and organic NPK fertiliser were used for the same purpose (Ahmad-Azri et al. 2019).

To the best of our knowledge, there is no study reporting the relationship between MLTD ovitrap index and meteorological variables in an urban university residence, Malaysia. In addition, NPK fertiliser solution had never been added to MLTD ovitrap. Hence, the objective of this study was to investigate the abundance of immature *Aedes* spp. and meteorological factors that are associated with its fluctuation in a student residence in Cheras using MLTD baited with NPK fertiliser solution.

# MATERIALS AND METHODS

# Study Site

This study was conducted in a student residence in Cheras. Kuala Lumpur with a size of approximately 15,010.65 m<sup>2</sup> (161,573 ft<sup>2</sup>). There were six blocks of four-storey buildings interconnected by covered walkways. The area is surrounded with tall trees, low-lying shrubs and planted with landscape plants. The residence is well managed, appeared clean with a good drainage system. Past dengue

cases involving students residing in the residence had been reported (personal communications with the students, March 2019) and the surrounding areas were known as hotspots for dengue cases (Ministry of Health Malaysia 2020).

# **Ovitrap Surveillance**

Organic NPK fertiliser [nitrogen (N), phosphorus (P) and potassium (K), ratio 5:5:5] was utilised as an oviposition attractant, for the eight weeks of study following its proven effectiveness in previous studies (Ahmad-Azri et al. 2019; Anderson & Davies 2014). A total of 30 MLTD were used for this study. MLTD is known to be an autocidal ovitrap. The ovitrap is made up of cylinder-shaped plastic container measuring 24 x 13.5 cm with a lid, funnel, and black jacket. It can contain 1.6 L of water and weighs about 200 g each (Ahmad-Azri et al. 2019). These MLTD ovitraps were used to collect the larvae with 15 MLTD placed inside and outside of the student residential buildings, respectively. Ovitrap locations were determined partly based on the findings of our pilot study which provided us with information that the ovitraps had successfully attracted some mosquitoes and were in suitable locations. This minimises trap failure caused by animals or vandalism. The ovitraps also were placed in covered area, away from direct sunlight, rain and strong winds.

Organic NPK fertiliser solution with a concentration ratio of 5:5:5 was added up to the cut-off limit of the device. Weekly samplings were done, whereby the ovitraps were collected from the designated area every seven days for a total duration of eight weeks. The samples were then brought to the Entomology Laboratory, Department of Parasitology and Medical Entomology, Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM) for larvae preservation before species identification process.

# Identification of Larvae

The contents of each ovitrap were first transferred to different plastic containers with labels. The larvae were fed on fish food and reared until the fourth instar. Next, the larvae were examined using stereo and light microscopes in the laboratory for species identification. The number of larvae collected in each positive MLTD ovitraps and the identified species was recorded.

# Meteorological Data Collection

Two data loggers were installed at key locations, both indoor and outdoor, to collect the data for temperature (°C) and relative humidity (%). The data was retrieved at the end of each collection week and the data loggers were set again for the upcoming week. Meteorological data collected during this study were relative humidity, as well as minimum and maximum temperature.

# **Ethical Consideration**

The study was approved by UKM Ethics Committee (IRB Ref no: UKM.

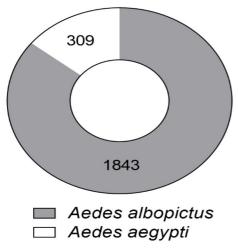


Figure 1: Total number of *Aedes* larvae collected from eight weeks of surveillance in the study site

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#### **Data Analysis**

The data obtained from this study was analysed as follows: (i) Ovitrap Index (OI), also known as the MLTD index, the percentage of positive ovitrap (for *Aedes* spp.) over the total number of ovitrap deployed at the sites; (ii) Mean number of larvae per ovitrap, the total number of larvae against the total number of ovitrap recovered.

The significant distribution of the mean number of both *Aedes* larvae species per ovitraps was calculated using Mann-Whitney test. Spearman correlation was also performed to find the correlation between the mean number of larvae and meteorological variables. The data analysis was performed using SPSS (version 22) (IBM Corp., Armonk, NY). The level of statistical significance was determined at p≤0.05.

#### RESULTS

A total of 2,152 *Aedes* larvae were collected from both indoor and outdoor settings throughout the study period. Figure 1 shows the total

Table 1: Comparison of mean number of larvae per ovitrap between *Ae. aegypti* and *Ae. albopictus* from indoor and outdoor settings and overall ovitrap index for eight weeks of continuous surveillance

Collection week	Ovitrap index (%)	Mean number <u>+</u> SE larvae per ovitrap				
		Ae. aegypti		Ae. albopictus		
		Indoor	Outdoor	Indoor	Outdoor	
Week 1	30.00	$0.00 \pm 0.00$	$0.00 \pm 0.00$	$0.53 \pm 0.32$	0.73 <u>+</u> 0.35	
Week 2	70.00	0.47 <u>+</u> 0.33	1.80 <u>+</u> 1.06	1.60 <u>+</u> 0.91	12.00 <u>+</u> 3.52	
Week 3	66.67	1.23 ± 0.78	1.77 <u>+</u> 0.98	1.31 ± 0.87	14.00 <u>+</u> 5.21	
Week 4	73.33	1.27 <u>+</u> 0.61	1.00 <u>+</u> 0.41	5.33 <u>+</u> 2.91	8.53 <u>+</u> 1.62	
Week 5	90.00	4.00 ± 1.85	0.73 ± 0.53	11.00 <u>+</u> 3.26	12.33 <u>+</u> 3.20	
Week 6	93.33	2.80 ± 1.65	0.80 <u>+</u> 0.36	$9.00 \pm 2.40$	15.93 <u>+</u> 5.49	
Week 7	66.67	0.67 <u>+</u> 0.25	4.53 <u>+</u> 1.49	0.27 <u>+</u> 0.21	4.13 <u>+</u> 2.24	
Week 8	73.33	2.67 <u>+</u> 1.28	0.67 <u>+</u> 0.37	14.87 <u>+</u> 5.16	8.40 ± 4.03	
Overall mean	70.41 <u>+</u> 6.80	1.72 <u>+</u> 0.38	0.86 <u>+</u> 0.20	6.08 <u>+</u> 1.00	9.28 <u>+</u> 1.28	
SE = standard e	rror					

Collection —	Ovitrap index (%)		Mean number $\pm$ SE larvae per ovitrap		
week	Indoor	Outdoor	Indoor	Outdoor	Mann- Whitney U
Week 1	20.00	40.00	0.53 <u>+</u> 0.32	0.73 <u>+</u> 0.35	U = 5787.50 z = -2.67 p = 0.008
Week 2	53.33	86.67	2.07 <u>+</u> 0.94	13.80 <u>+</u> 3.59	
Week 3	46.67	86.67	2.54 <u>+</u> 1.49	15.77 <u>+</u> 0.62	
Week 4	53.33	93.33	6.60 <u>+</u> 3.11	9.53 <u>+</u> 1.73	
Week 5	80.00	100.00	$15.00 \pm 4.50$	13.07 <u>+</u> 3.44	
Week 6	86.67	100.00	11.80 <u>+</u> 3.50	16.73 ± 5.54	
Week 7	80.00	53.33	5.20 <u>+</u> 1.59	4.40 <u>+</u> 2.29	
Week 8	73.33	73.33	17.53 <u>+</u> 5.84	9.07 <u>+</u> 4.10	

Table 2: Ovitrap index and mean number of *Aedes* larvae per ovitrap with respect to the location (indoor and outdoor) from eight weeks of continuous surveillance

number of collected larvae segregated into species. The percentage of *Ae. albopictus* larvae (85%) collected throughout the eight weeks of study was higher than *Ae. aegypti*, to almost

six folds. Table 1 shows the weekly abundance of Ae. aegypti and Ae. albopictus retrieved from both indoor and outdoor ovitraps with overall OI ranges from 30.00-93.33%. During Week 1, the OI was 30.00% despite none of Ae. aegypti being collected. This was due to the presence of Ae. albopictus larvae which contributed the number of positive of ovitraps. For the indoor setting, the mean number of Ae. aegypti per ovitrap ranged from 0.00+0.00 to 4.00+1.85; while it ranged from 0.00+0.00 to 4.53+1.49 for ovitraps retrieved from the outdoor setting. Nevertheless, the mean number of Ae. albopictus per ovitrap from indoor ovitraps ranged from 0.27+0.21 to 14.87+5.16 while outdoor ovitraps yield ranged from 0.73+0.35 to 15.93+5.49. The mean numbers

of Ae. aegypti larvae gathered from indoor ovitraps were moderately high at Week 3 to Week 6, ranging from 1.23±0.78 to 4.00±1.85 and also at Week 8 (2.67±1.28). Ae. albopictus were constantly higher in the outdoor settings compared to indoors, except for Week 8 in which the indoor collection of Ae. albopictus was higher compared to outdoor collection (14.87+5.16 versus 8.40+4.03). Overall, aegypti were predominantly Ae. found in indoor settings (1.72±0.38) rather than outdoor (0.86+0.20). In contrast, the overall mean number of Ae. albopictus larvae was higher in outdoor than indoor with mean of 9.28±1.28 and 6.08±1.00, respectively.

The OI and mean number of *Aedes* larvae from indoor and outdoor settings were shown in Table 2. Indoor OI ranged from 20.00% to 86.67%; while outdoor OI ranged from 40.00-100.00%. The ovitraps retrieved from outdoor settings were all positive for harbouring *Aedes* larvae for two consecutive weeks, at Week 5 and

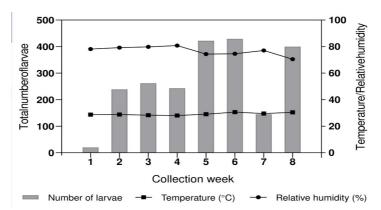


Figure 2(a): Relation between *Aedes* larval abundance with average temperature and average relative humidity

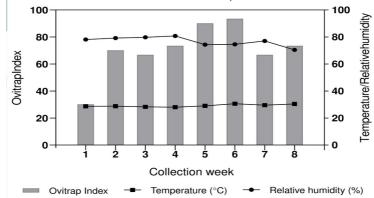


Figure 2(b): Relation between overall ovitrap index with average temperature and average relative humidity

6. The mean number of larvae per ovitraps from indoor settings ranged from 0.53+0.32 to 17.53+5.84; while at its lowest, the outdoor mean number of larvae per ovitraps was 0.73±0.35 and 16.73±5.54 at its peak at Week 6 of study. A Mann-Whitney test indicated that the distribution of mean number of both Aedes larvae per ovitraps were significantly different between indoor (mean rank = 108.73, n = 120 and outdoor (mean rank = 132.27, n = 120) settings, U =5787.50, z = 2.67 (corrected for ties), two-tailed. Minimump=0.008, maximum indoor temperature and relative humidity ranged from 25.5-33°C and 47.5-88.5%, respectively, whereas minimum-maximum outdoor temperature and relative humidity ranged from 23-37°C and 47.5-93.5%, respectively.

To examine the fluctuation trend of entomological variables regardless of *Aedes* species, we present here Figure 2a & 2b. Week 1 demonstrated the lowest larval abundance and ovitrap index. These parameters rose steadily in the next three weeks (Weeks 2, 3 and 4) when the average temperature was approximately 28°C and the average relative humidity reached

Meteorological variables	r	P value	
Maximum temperature	0.830	0.011	
Minimum temperature	0.204	0.629	
Maximum relative humidity	-0.299	0.471	
Minimum relative humidity	-0.778	0.023	

Table 3: Correlation of meteorological variables with larval abundance

80.8% by Week 4. A sharp increase in larval abundance was observed in the following two weeks (Weeks 5 and 6) when the humidity drops to 74% and temperature ranges from 29.1-30.6°C; OI was highest during these weeks. Both parameters decreased in Week 7 before increasing again in Week 8.

To demonstrate the relationship of average temperature and average relative humidity on the abundance of Aedes larvae, non-parametric correlation test (Spearman Correlation) was performed because the data obtained were not normally distributed. manifested Larval abundance moderate correlation on weekly relative average temperature and humidity. It was shown that larval abundance was directly correlated with maximum temperature (r=0.830; p=0.011) and inversely correlated with minimum relative humidity (r=-0.778; p=0.023), with both of these correlations being statistically significant. Correlation coefficients for minimum and maximum temperature and relative humidity with larval abundance were stated in Table 3.

Correlation coefficient (*r*) between average temperature and relative humidity was -0.857 with a p-value of 0.007. This shows that these two variables are inversely correlated meaning relative humidity decreases when temperature increases. Correlation between OI and number of larvae demonstrated a high correlation coefficient, r of 0.880 with a p-value of 0.004. This suggests that the two parameters can be used interchangeably to monitor changes in mosquito population density.

### DISCUSSION

Based on our study, the overall predominant site of breeding of Aedes spp. was outdoors compared to indoors. This is largely due to the outdoor preference of Ae. albopictus, the dominant species, for breeding. Our findings were parallel with a similar urban study conducted in Keramat, Kuala Lumpur and Shah Alam, Selangor in which indoor and outdoor ovitrap collections revealed that Ae. aegypti was more frequently found indoors, whereas Ae. albopictus bred more in outdoor ovitraps, with the latter being the dominant species in both areas (Noor-Afizah et al. 2018). Similar findings were also reported by Wan-Norafikah et al. (2009) and Rozilawati et al. (2015) in their studies conducted in Kuala Lumpur. Ae. aegypti are predominantly higher in indoor settings as they preferred to breed in a place with high human density due to its anthropophilic

nature, whereas *Ae. albopictus* prefer to rest and breed outdoors where vegetation and natural water-holding containers are available.

addition, outdoor In extreme temperatures, up to 36-37°C as recorded by our data loggers might not be favourable for Ae. aegypti to thrive. For instance, adult survivorship of field strains was greatly affected when the temperature reaches 36°C (Marinho et al. 2016) and egg production and hatching significantly reduced at 35°C when compared to 30°C (Costa et al. 2010). According to Marinho et al. (2016), who conducted a study on three different populations of Ae. aegypti in Brazil, the largest number of eggs per female was observed at 28°C and the lowest was at 36°C. Furthermore, embryonic development took longer at 36°C as compared to 28°C and 33°C for two out of three populations. It was found that the extreme temperature of 39°C suppressed the embryonic development and survival of larval stages (Marinho et al. 2016). Our indoor temperature ranged from 25.5-33°C, supporting the optimal development of the species.

On the other hand, a wider range of temperature and relative humidity is more accommodating to *Ae. albopictus.* The species has been shown to have high endurance to weather anomalies enabling it to survive in temperate, subtropical and tropical countries (Reinhold et al. 2018). Rozilawati et al. (2016) discovered that immature stages of *Ae. albopictus* successfully developed into adults at 35°C with survival rates of 68% and 86% for Kuala Lumpur and Selangor field strains, respectively. At 40°C, these strains managed to develop until L3 stage. However, tolerance to weather and climate might be different with geographical regions as mosquito strains of different genetic compositions exist.

The abundance of immature Aedes spp. larvae showed a consistent increase with maximum temperature. This is supported by the studies done by Rohani et al. (2011) and Madi et al. (2012). These studies, including have measured ambient ours, air temperatures instead of water temperature of larval habitats. Changes of temperature in these habitats directly affect the larval and pupal development, while air temperature determines the development of eggs and adult phase. Measuring water temperature in breeding containers can help to better understand the role of fluctuating temperature on immature stages development (Waldock et al. 2013). Unlike temperature, our study showed an increase in relative humidity will decrease the abundance of immature Aedes spp. or vice versa (i.e. a significant negative correlation), which is in line with studies by Wan-Norafikah et al. (2009). In contrast, Rohani et al. (2011) and Madi et al. (2012) reported a consistent increase in the larval abundance with increasing maximum relative humidity. The discrepancy between our results and others' may be due to the duration of the ovitrap collections in which a clearer picture can be obtained when data from one year or more is examined. Relative humidity influences longevity, fecundity, oviposition and

larvae survival of Aedes (Costa et al. 2010). At high humidity, mosquitoes generally live longer and produce more eggs. Relative humidity also directly affects the evaporation rates of vector breeding sites. The effect of the same temperature but dissimilar relative humidity on mosquito development was previously investigated (Costa et al. 2010). In their laboratory study, they found out that lower humidity (60% versus 80%) at the same temperature cause less egg hatching, and oviposition inhibited for some Ae. aegvpti females, which indicates that humidity does play a role in determining the magnitude of temperature effect on mosquito's life cycle. However, in realworld situations, this effect might be occurring subtler in the environment due to the constant fluctuations of relative humidity in a tropical climate, especially outdoors which can range from 47.5-93.5%, as occurred in our study.

This study had incorporated the use of NPK fertiliser as an attractant for female Aedes mosquitoes. Bacterial growth in fertiliser-baited ovitraps contributes to the availability of food for the larvae. Previous study by Darriet et al. (2010) stated that NPK fertiliser is a potential alternative of attractant for Aedes mosquitoes. NPK fertiliser is commercially available and easier to prepare as compared to hay infusion. Besides, it is suitable to be used in ovitrap that is placed indoors as it does not bring any foul or unpleasant smell, unlike the hay or leaf infusions. According to Margues et al. (2013), ammonia (NH<sub>2</sub>) volatiles released from water were able to attract the female

Aedes mosquitoes to oviposit. In Malaysia, Ahmad-Azri et al. (2019) has demonstrated the effectiveness of NPK fertiliser solution as an oviposition attractant for Ae. albopictus and Ae. aegypti. It was reported in another study conducted in Timor Leste that NPK fertilisers are found to be attractive to gravid Ae. albopictus and may be useful in the control and monitoring program (Anderson & David 2014). In addition, Darriet et al. (2010) has shown that Ae. aegypti was also attracted to the solution. Study to compare its attractancy between the two mosquito species has yet to be conducted, but evidence stated here shows its potential as an oviposition attractant for both mosquito species.

8-week ovitrap collection An limit us from acquiring more data to associate entomological and weather variables. This is unavoidable to due to some technical and manpower constraints. An extended period of ovitrap surveillance would be more meaningful as it could strengthen the predictive capacity of the data which would bear a more conclusive relationship between entomological meteorological variables. and Nonetheless, short-term surveillance entomological indices using as demonstrated in our study can be utilised to rapidly assess the status of vector population density in an area. This can trigger vector control actions whenever deemed necessary, especially in a large dengue outbreak where swift vector control need to be taken.

### CONCLUSION

Based on this study, the total number of Aedes species collected throughout the study proved that Ae. albopictus was the dominant species in the area, especially outdoors, as compared to Ae. aegypti, which was more frequently found in the indoor area. A targeted control method based on the distribution of each respective species can be formulated and used together with current interventions. Furthermore, the study showed that larval abundance is directly correlated with maximum temperature and inversely correlated with minimum relative humidity. Thus, a predictive model of Aedes spp. abundance can be developed with the integration of these parameters provided by data from previous and extensive future studies. This study also had utilised MLTD with NPK fertiliser solution as an attractant for the surveillance of immature Aedes abundance, which has been proven to be effective in monitoring Aedes larval abundance.

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