UNIVERSITI PUTRA MALAYSIA

FIELD EFFICACY OF INTELLIGENT MOSQUITO SYSTEM (I.M.O.S) WITH XMOS MINI AEROSOL AGAINST Aedes IN 17TH COLLEGE, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

FIELD EFFICACY OF INTELLIGENT MOSQUITO SYSTEM (I.M.O.S) WITH XMOS MINI AEROSOL AGAINST *Aedes* IN 17TH COLLEGE, UNIVERSITI PUTRA MALAYSIA

Siti Najiha Binti Abu Bakar, Banulata Gopalsamy & Latifah Binti Saiful Yazan

Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, UPM

Introduction: Dengue fever is an endemic disease that critically affects subtropical and tropical regions of the world. Female *Aedes* mosquitoes are the only known vector that can transmit dengue virus (DENV) and cause dengue fever (DF). Recent DF cases have been reported in Malaysia with highest cumulative cases in Selangor state. Despite the ongoing efforts from the Ministry of Health in controlling and preventing the transmission of dengue virus, the cases continue to rise due to uncontrolled factors. Xmos mini aerosol is an intervention that is able to kill adult mosquitoes. It contains 0.76% (w/w) metofluthrin as the main ingredient along with nano tech slow release technology. **Objective:** This study determined the efficacy of I.M.O.S (Intelligent Mosquito House) by using Xmos mini aerosol in reducing *Aedes* mosquito population in 17th College, Universiti Putra Malaysia (UPM). **Methodology**: Prior to the experiment, the *Aedes* mosquito population was determined in all blocks of the 17th College, UPM. Ovitraps were set up in each floor of the block for seven days to collect the mosquitoes. The eggs were counted and houses with high mosquito population were identified to test the I.M.O.S intervention. Another block with similar mosquito population was chosen as control. I.M.O.S. was installed above the entrance door of the hall and two rooms and was set to automatically spray at 6.30 am and 4.00 pm every day. No intervention was used in the control house. The efficacy test was conducted for three months. On day 30,60 and 90, *Aedes* mosquitoes (obtained from Sumitomo Chemical) were placed in cages with 20 mosquitoes in each cage and was hung at a distance of 10 m from I.M.O.S. The mosquitoes were exposed for two hours and the mortality caused by I.M.O.S were recorded throughout the 24 hours exposure. Following three months of I.M.O.S installation, the units were removed. The number of mosquito eggs population was calculated. Percentage of adult knockdown and percentage of adult mortality were determined by using two-way ANOVA and unpaired T-test, respectively. **Result:** The mean number of *Aedes* mosquito eggs and ovitrap showed no significant difference ($p>0.05$) between control and treatment blocks. On the other hand, we found significant differences ($p<0.05$) in the percentage of knockdown of adult *Aedes* mosquitoes (10, 20, 30, 60 and 120 minutes after exposure) and the mortality of adult *Aedes* mosquitoes after 24 hours exposure. **Conclusion:** Nano and slow release technology of I.M.O.S proved that this type of intervention can kills adult *Aedes* mosquitoes. Thus, it is a potential intervention for vector control and management.

Keywords: Aedes mosquito; aerosol; dengue virus; dengue fever; ovitrap; intelligent mosquito system.

ABSTRAK

KEBERKESANAN LAPANGAN *INTELLIGENT MOSQUITO SYSTEM* (I.M.O.S) TERHADAP POPULASI Aedes DI KOLEJ TUJUH BELAS, UNIVERSITI PUTRA MALAYSIA

Siti Najiha Binti Abu Bakar, Banulata Gopalsamy & Latifah Binti Saiful Yazan

Jabatan Sains Bioperubatan, Fakulti Perubatan dan Sains Kesihatan, UPM

Pengenalan: Demam denggi merupakan penyakit endemik yang sangat mempengaruhi secara kritikal di rantau subtropika dan tropikal. Nyamuk *Aedes* betina adalah satu-satunya vektor yang diketahui mampu menyebarkan virus denggi (DENV) dan menyebabkan demam denggi. Kes demam denggi baru-baru ini yang dilaporkan di Malaysia dengan kes kumulatif tertinggi adalah di negeri Selangor. Walaupun terdapat usaha murni yang telah dilaksanakan oleh Kementerian Kesihatan dalam menangani dan mencegah penularan virus denggi, namun kes-kes tersebut terus meningkat akibat daripada faktor yang tidak dikekang. Xmos aerosol mini ialah sebuah intervensi yang mampu membunuh nyamuk dewasa. Ia mengandungi 0.76% (w/w) metofluthrin sebagai bahan utama dan juga dilengkapi dengan teknologi nano pelepasan laun. **Objektif:** Kajian ini menentukan keberkesanan IMOS (*Intelligent Mosquito House*) dengan menggunakan Xmos aerosol mini dalam pengurangan populasi nyamuk *Aedes* di Kolej Tujuh Belas, Universiti Putra Malaysia (UPM). **Metodologi:** Populasi nyamuk *Aedes* ditentukan di semua blok Kolej Tujuh Belas, UPM sebelum memulakan eksperimen. Ovitrap telah dipasangkan di setiap tingkat blok selama tujuh hari untuk tujuan pengumpulan nyamuk. Telur nyamuk dihitung dan rumah dengan populasi nyamuk yang tinggi telah dikenal pasti untuk menguji intervensi IMOS. Blok yang berlainan dengan populasi nyamuk yang persis telah dipilih sebagai kontrol. IMOS telah dipasang di pintu masuk rumah dan dua buah bilik dan telah ditetapkan untuk semburan pada 6.30 pagi dan 4.00 petang setiap hari secara automatik. Tiada intervensi digunakan di dalam rumah kontrol. Ujian keberkesanan telah dijalankan selama tiga bulan. Pada hari ke-30, 60 dan 90, 20 ekor nyamuk *Aedes* (diperolehi daripada Sumitomo Chemical) telah diletakkan di dalam sangkar dengan di setiap sangkar dan telah digantung pada jarak 10 meter dari IMOS. Nyamuk telah didedahkan selama dua jam dan mortaliti yang disebabkan oleh IMOS telah direkodkan sepanjang pendedahan 24 jam. Unit telah dikeluarkan setelah pemasangan I.M.O.S. selama tiga bulan. Bilangan populasi telur nyamuk telah dihitung. Peratus pehempasan nyamuk dewasa dan peratus mortaliti nyamuk dewasa telah ditentukan dengan menggunakan ANOVA dua hala dan ujian-T tak bersandar. **Keputusan:** Bilangan min telur nyamuk *Aedes* dan ovitrap menunjukkan tiada perbezaan signifikan ($p > 0.05$) di antara kontrol dan blok yang dirawat. Sebaliknya, kami mendapati perbezaan signifikan ($p < 0.05$) dalam peratusan pehempasan nyamuk *Aedes* dewasa (10, 20, 30, 60 dan 120 minit selepas pendedahan) dan nyamuk *Aedes* dewasa selepas pendedahan selama 24 jam. **Kesimpulan:** Teknologi nano pelepasan laun IMOS telah membuktikan bahawa jenis intervensi ini boleh membunuh nyamuk *Aedes* dewasa.

Oleh itu, ini merupakan intervensi yang berpotensi untuk kawalan dan pengurusan vektor.

Kata kunci: Nyamuk Aedes; aerosol; virus denggi; demam denggi; ovitrap; *Intelligent Mosquito System*.

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LIST OF ABBREVIATIONS

DENV	Dengue virus
DF	Dengue fever
DHF	Dengue haemorrhagic fever
DSS	Dengue shock syndrome
OI	Ovitrap Index
WHO	World Health Organization
I.M.O.S	Intelligent Mosquito System
NS	Non-structural

CHAPTER 1

INTRODUCTION

1.1 Background

Dengue fever is an endemic disease that critically affects the subtropical and tropical regions of the world. Female *Aedes* mosquitoes are the only known vector that can transmit dengue virus (DENV) and cause dengue fever (DF). DENV is transmitted and spread specifically by *Aedes aegypti* and *Aedes albopictus.* DENV is a single stranded RNA virus of Flaviviridae family. The flavivirus circulates in the blood of infected person for 2 – 3 days and develops symptoms such as sudden onset fever, severe flu, severe headache, nausea, vomiting, swollen glands, retro orbital pain, muscle and joint pains (Ratini,2019). People with a second or subsequent dengue infection as well as those with weakened immune systems are at higher risk for developing severe form of viral illness which are dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Ratini, 2019).

DENV1, DENV 2, DENV3 and DENV4 are four different serotypes of the virus. All serotypes can infect people and cause DF, DHF and DSS. Recovery from one infection

provides lifelong immunity to that specific serotype. However, it gives only a short time of immune-cross reaction to another serotype. This statement is supported by (WH0, 2019).

The disease today is widespread in over 100 countries. These include Western Pacific, South East Asia, Eastern Mediterranean, America and Africa. However, the most affected countries are South East Asia and Western Pacific. According to the research that was done before 1970, there were only total of 9 countries that was affected with dengue epidemics. Dengue cases in Malaysia has risen since the first major epidemic in 1973 (Wallace et al., 1980). In Malaysia, iDenggi (2020) reported 57,920 dengue cases from 29 December 2019 until 9 July 2020.To add, the cumulative number of death due to dengue infection from January until July were 94 deaths (iDengue, 2020). It clearly showed that demographic and social developments such as population development, urbanization and modern transportation significantly affected the increase in number of dengue cases. Higher infectious incidence due to different virus serotypes raised the rate of genetic modification in viruses. As a result, it will increase the chances of DENV genotypes with higher severity of the DF (Gubler, 2002).

Due to the failure of vector management, the increasing intensity of dengue has given more opportunity to scientist to develop the dengue vaccines, hence making it effective tetravalent dengue vaccine for global public health. For several decades, the dengue vaccine development has been in progress but the complex pathology of the illness, the importance to control four virus serotypes and low in investment by vaccine developers have inhibited the process (Guzman et al., 2015). Good intervention

practices should be adhered to as we wait for the new tools of vaccines, antiviral drugs and improved diagnostic to be found (Guzman et al., 2015). Hence, there is a need for implementation of a new intervention to reduce the *Aedes* mosquito population. According to Packierisamy et al., (2004) as observed in Malaysia, dengue vector activities are intensely dependent on human resource. However, current government-funded integrated dengue vector control is not fully effective to prevent dengue outbreaks in Malaysia. Hence, they suggested on further work to evaluate the impact of using insecticide as one of the solutions to reduce dengue transmission (Packierisamy et al., 2004).

Intelligent mosquito system (I.M.O.S) with Xmos mini aerosol introduced by One Team Network Sdn. Bhd. is one of the alternatives to combat this issue. Xmos mini aerosol contains 76 % of Metofluthrin as active ingredient and is highly effective to kill adult mosquitoes. Metofluthrin has higher vapor pressure and deliver macro-particles into air that lasts for 8 hours (Personal communication). Matsuo et al., (2005) explained that metofluthrin shows high effectiveness of knockdown especially towards mosquitoes as well as other insects. Moreover, this ingredient has low toxicity towards mammal and high volatility (Matsuo et., 2005).

1.2 Objectives

1.2.1 General objective

To determine the efficacy of I.M.O.S by using Xmos mini aerosol in reducing *Aedes* mosquito population in 17th College, Universiti Putra Malaysia (UPM).

1.2.2 Specific objectives

1. To determine the effectiveness of I.M.O.S against adult *Aedes* mosquitoes.
2. To compare the knockdown of adult *Aedes* mosquitoes in treatment and control area.

1.3 Hypothesis

The use of I.M.O.S will effectively reduce the *Aedes* mosquito population and kill the adult *Aedes* mosquitoes in 17th College, UPM.

CHAPTER 2

LITERATURE REVIEW

2.1 Dengue fever

Dengue fever (DF) is becoming a global threat due to its highly endemic infectious disease in many tropical and subtropical regions. The virus responsible for causing dengue fever is known as dengue virus (DENV). There are four types of DENV serotypes which are DENV1, DENV2, DENV3 and DENV4 (Khetarpal and Khanna, 2016). DENV serotype distribution in Malaysia has been internally inconsistent. For example, both DENV 1 and DENV 2 dominated other serotypes during the 1996–1998 of dengue outbreaks. Somehow, DENV 3 is commonly circulated while DENV 4 is a rare serotype (Suppiah et al., 2018). These viruses can be transmitted through female *Aedes* mosquito, specifically *A. albopictus* and *A. aegypti* from an infected person. Dengue infection results in different degrees of symptoms depending on the types of DENV starting from moderate asymptomatic dengue fever (DF) to severe haemorrhagic syndrome (DHF) and dengue shock symptoms (DSS). In some cases, it could lead to death. When a person is infected by DENV, they will experience a fever which is self-limiting that typically last for 5-7 days. Dengue fever's clinical features vary by the patient's age (Khetarpal and Khanna, 2016). According to the World Health Organization (WHO), common symptoms during febrile phase including headache, muscle and joint pain, nausea, vomiting, swollen glands and rash. Besides that, they

may experience severe DF 3 – 7 days after illness onset. Severe form of dengue may occur due to plasma leaking, accumulation of fluid, severe bleeding and organ failure (WHO, 2020). In addition, the possibility of death due to severe DF is high. During critical phase, there are warning symptoms which it will manifest severe abdominal pain, persistent vomiting, bleeding gums, tachycardia, presence of blood in vomit and fatigue. Careful monitoring over the next 24 hours to 48 hours is necessary if patient develop these symptoms during critical phase to prevent complications and mortality risk (WHO, 2020). A person can develop a lifelong immunity against DENV serotype after full recovery of infection. However, second infection may increase the risk of severe dengue if infected by other serotypes. Furthermore, after recovery, cross immunity to other DENV serotype is partially developed but for only a short term (WHO, 2020).

2.2 Dengue virus and serotypes

Dengue infection is caused by arbovirus, which belongs to *Flaviviridae* family consisting of four distinct serotypes including DENV 1, 2, 3 and 4 (Chew et al., 2016). This virus comes from *Flavivirus* genus and they have different antigenicity (Uno and Ross, 2018). Generally, it is a single stranded RNA virus that encoded three structural proteins which are protein C, membrane protein M and envelope protein E that forms the virus particle. On the other hand, it also has seven non-structural proteins including NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 (Cheah et., 2014). Such NS proteins are essential for viral genome replication and assembly. Mathew et al., (1998) stated that serotype-specific epitope on structural proteins is less prevalent compared to

recognition of serotype-cross-reactive epitopes on NS3 and NS1.2a. The structure of the virus is enveloped and roughly spherical, approximately 11,000 kb in length. It includes a type I cap at the 5′ end and lacks a 3′ poly(A) tail (Uno and Ross, 2018). RNA genome undergo translation when it reaches the cytoplasm, and it will eventually translate into single polyprotein which will divided into 10 individual viral protein (Pu et al., 2017).

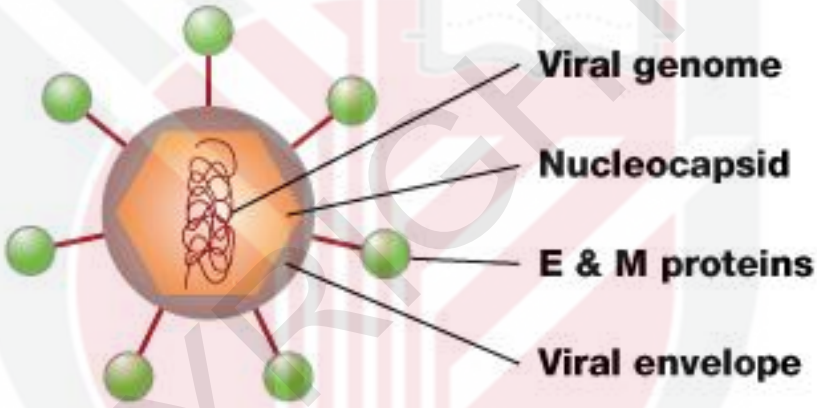

Figure 1 : Dengue virus structure

Source: https://www.nature.com/scitable/topicpage/dengue-viruses-22400925

(Nature Education, 2014)

A surveillance meta-analysis by Guo et al., (2017) showed serotypes data between 1990 to 2015 indicating DENV-2 was the highest monoinfection, followed by DENV-1, DENV-3 and DENV-4. After 2010, DENV-1 and DENV-2 are the most widespread serotype in American and African areas whereas in European areas, DENV-1 is the most common. In southeast Asia region, all four dengue serotypes are circulating

consistently (Guo et al., 2017). DENV-2 and DENV-3 are recognized as 'Asian' serotypes since they are often associated with secondary dengue infection (WHO, 2020).

2.3 Prevalence of dengue fever

In 2012, WHO announced that about 50–100 million new dengue infections occur annually worldwide with a 30-fold rise in global incidence over the last 50 years (Guo et al., 2012 as cited in WHO, 2012). Recent estimates in South East Asia also show a continued increase in the incidence of dengue and has the highest number of outbreaks compared to the Western Pacific and American regions (Chew at al., 2016; Guo et al., 2017). Chew et al., (2016) stated that the incidence of dengue increased with age in Malaysia's urban and rural areas, where the cases reached 90 percent of those aged 70 years and above (Chew et al., 2016). In Malaysia, iDenggi (2020) reported 57,920 of dengue cases starting from 29 December 2019 until 9 July 2020. The cumulative number of death due to dengue infection from January until July 2020 were 94 deaths (iDengue, 2020).

2.4 Pathogenesis of dengue fever

As for now, the exact pathogenesis of DF is not well understood (Cheah et al., 2014). In order to understand the possible function of pathways in dengue pathogenesis and immunity, further work is needed in research. Generally, for certain circumstances,

severe form of dengue can develop after primary infection if a person is infected by DENV (Katzelnick et al, 2018). Simmons et al. (2015) showed that Langerhans cells that is located at the dendritic cells of the skin possess the highest viral growth titers. This statement support that the dermis is an important site of virus infection (Simmons et al., 2015). According to Mongkolsapaya et al. (2013), the result indicates that T-cell activation is the main cause of DENV. To add, the severity of the disease is related to the magnitude of T-cell responses as well as tumour necrosis factor-α (Mongkolsapaya et al., 2013). DENV attaches itself to a target cell in the first exposure to the mosquito bite by forming highly sulphated glycosaminoglycan heparin sulfate, which eventually promotes the DENV penetration through a high affinity receptor. On the contrary, antibody-dependent enhancement (ADE) takes place in the secondary infection, where DENV enters via the Fcγ- receptors and facilitates the development of complexes with the antibodies. After that, a complex is formed during the combination of virus and specific antibody involving mononuclear cells, B-lymphocytes, dendritic cells via a FcR mediated endocytosis (Cheah et al., 2014).

2.5 *Aedes* Mosquito

Generally, *Aedes aegypti, Aedes albopictus*, and *Aedes polynesiensis* are known as the most common vectors for dengue transmission (Mcfee et al., 2018). When mosquitoes bite a person infected with the virus, they become infected. Infected mosquitoes can then spread the virus by bites to others (CDC, 2020) These mosquitoes rest in dark, cold and indoor places. For instance, the mosquito rests in bathrooms, closets, under beds, and behind curtains. In addition, the mosquito prefers to bite during predawn and

dusk hour despite the fact that they can bite at any time of the day (Mcfee et al., 2018). Many of the natural vertical transmission studies of DENV in Asia indicate that *A. aegypti* and *A. albopictus* are abundant in India and Thailand (Lima et al., 2018). In Malaysia, only two mosquitoes are commonly related to DF which are *A. aegypti* and *A. albopictus* (CDC, 2020). Depending on the habitat, precipitation, temperature and other factors, mosquitoes can live for two to four weeks. In fact, only female mosquitoes feed on blood in order to produce eggs (CDC, 2020).

According to Sprenger and Wuithiranyagool (1986), *A. albopictus* also known as the Asian tiger mosquito is first recorded in 1985 at Texas, United States (Sprenger and Wuithiranyagool, 1986). The bold black scales and silver white scales on the palpus and tarsi make adult *A. albopictus* clearly recognizable. The scutum can be recognize by the white stripe at the head's dorsal surface and running down to the thorax. Male *A. albopictus* has feathery plumous and their proboscis is slightly different than female due to nectar feeding. Commonly, males are 20 percent smaller than females despite they having similar morphology. It has white and black bands on their legs on each of the tarsal segment (University of Florida, 2004). *A. albopictus* is active during day-time and known as outdoor feeder. Despite the fact that they rest indoors, they favour to feed on human (Boyer et al., 2018). Generally, *A. aegypti* breeds in artificial containers such as water jars, discarded containers, drums, tires and tin cans. Primarily, *A. aegypti* is classified as anthropophilic because they are attracted to human blood for feeding. They rest indoor and also known as day biting mosquito (Boyer et al., 2018). According to Alikhan et al., (2014), the mosquitoes has been identified to have silvery white scales on the vertex of the head and lyre-shaped pattern at scutum with white stripes at the first to forth segments of their legs.

a) *Aedes albopictus* b) *Aedes aegypti*

Figure 2: *Aedes* mosquitoes that cause dengue fever.

Source a) : http://entnemdept.ufl.edu/creatures/aquatic/asian_tiger.htm (University of Florida, 2004)

Source b) : https://www.cdc.gov/zika/vector/range.html (CDC, 2020)

2.5.1 Life cycle of *Aedes* mosquito

Basically, the life cycle *of A. aegypti* and *A. albopictus* is same and consist of four stages; eggs, larva, pupa and adult stages. For an egg to mature into an adult, it takes around 7- 10 days.

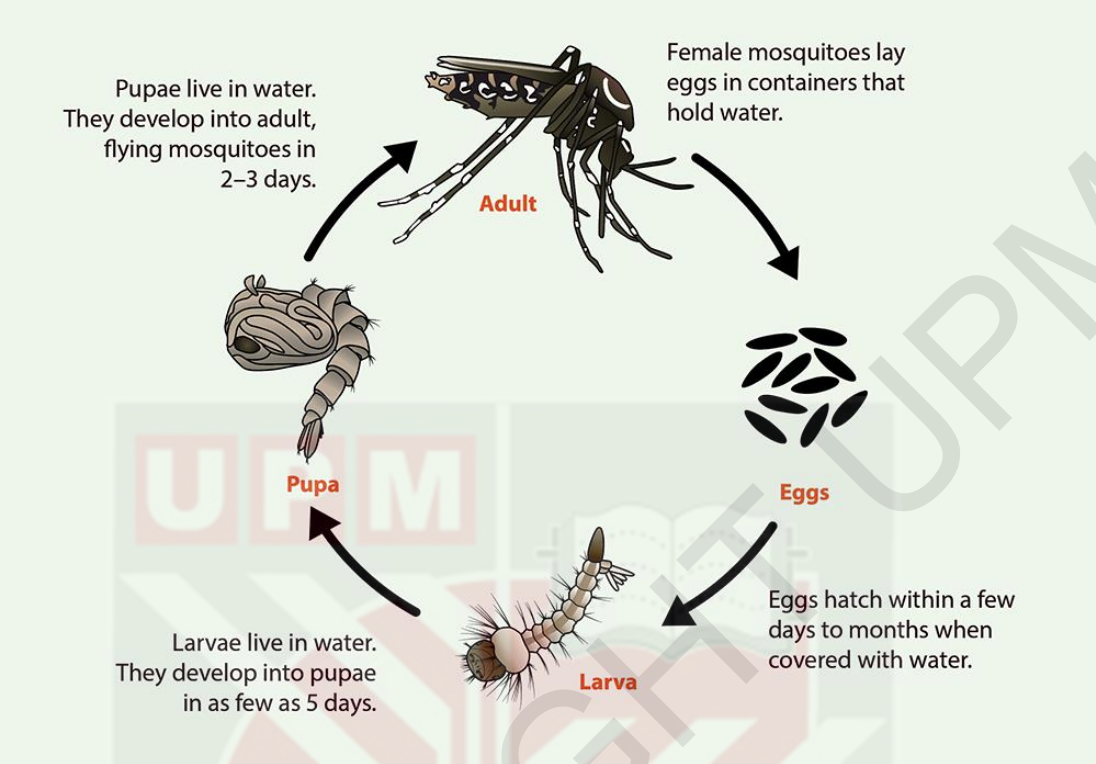

Figure 3: Life cycle of *Aedes* mosquito

Source : https://www.cdc.gov/mosquitoes/about/life-cycles/aedes.html (CDC, 2020)

Commonly, female mosquitoes lay eggs on the interior walls of any type of artificial containers, even with absence of water, the eggs still can survive for more than 8 months without water. *Aedes* species only require a small amount of water to oviposit. (CDC, 2020). Later, a larva, also known as 'wigglers', start to hatch when water covers the eggs. Larva live in aquatic environment as well as pupae. After that, the pupa will break their skin off and develops into an adult mosquito. Within two to three days, an adult mosquito will emerge. In order to produce eggs, adult female mosquitoes feed on human and animals blood. Female mosquitoes look for water sources to oviposit

after feeding. In fact, *A. aegypti* prefer to rests near humans and also indoor in order to feed on human blood. In contrast, *A. albopictus* rest in, near homes and also thickets (CDC, 2020).

2.6 Dengue control and management

Mosquito populations around the world are widespread and abundant despite decades of vector control programs (Marcondes and Ximenes, 2016). However, different types of interventions, resources, period of study will affect the effectiveness of any vector control program which may explain in part of varying degree of success between studies. On the other hand, due to poor reporting of the study design, observational methodologies, heterogeneity and indirect results, the quality of data seemed to be low to very low (Bouzid et al., 2016).

Generally, the most common vector management is categorised as physical control (physical actions in eliminating mosquitoes), biological control (biological agent is used), chemical control (chemical agent used) and integrated vector (combination of more than two interventions) or also known as Integrated Vector Management (Bouzid et al., 2016). As practiced in Malaysia, dengue vector control activities are strongly dependent on human resources. In fact, a strong and large human workforce is needed to carry out the numerous district-level activities of dengue vector management, surveillance and prevention (Packierisamy et al., 2004). In addition, trained healthcare that performs inspections, fogging and larvaciding activities is one of the pillar of the

national control activities in preventing the rise of dengue cases. Frontliner including medical doctors and entomologist are needed to provide technical support in this issue. As a result, human resources represent the most important community to combat against *Aedes* mosquitoes (Packierisamy et al., 2015). Fitzpatrick et al., (2017) reported six countries with middle-income economies and high number of dengue cases accounts for an estimated 15% of the global dengue burden (Fitzpatrick et al., 2017)

2.6.1 Physical control

Physical controls consist of regular cleaning of mosquito breeding sites which is artificial containers, cover of the containers and ovitraps (Lima, Goulart and Neto, 2015). The first was routine washing of containers using a home-crafted paste created from detergent and bleach. In one study,1784 houses were selected in Honduras and from the first test, it indicates the formula did not have the desired impact at the specified dosage where 5 tablespoons of sugar and one tablespoon of detergent was used. Later, a new formula was created by using 1:2 bags of chlorine bleach along with 1:2 bags of detergent. As a result, there were significant reduction in the number of pupae and larvae (Fernandez et al., 2018).

Besides, there is another intervention in controlling the life cycle of mosquito by collecting the eggs in the ovitraps as well as covers of the container. Cheng, Barnett

and Goodwin (1982) proved that by using this method, the entomological incides in intervention areas should decrease (Cheng, Barnett and Goodwin, 1982)

In controlling dengue vector by using physical control, sticky traps and NS 1 antigen test kit of dengue can be used as a screening device. A constructive approach would be more effective for system controls than existing reactive approaches. This preliminary test revealed that the sticky trap is an inexpensive and effective form of catching *Aedes* mosquito. The NS1 antigen detection kit is a basic device which can be used by public health staff to show the existence of an infected mosquito and thus prevention action should be taken before an outbreak occurs (Lau et al., 2015). Sticky traps may be an effective option for tracking and managing dengue vector spread and intensity (Roslan et al., 2017)

2.6.2 Biological control

Biological controls tend to reduce entomological indices better than chemical controls, whereas educational campaigns can reduce breeding environment. In five population trials in Vietnam, Copepods (*Mesocyclops spp.*) were successful vector control, which includes long-term larval and adult *Aedes* controls (Lazora et al., 2015). According to Han and colleagues study, they measured the impact of larvivorous fish by using single and multiple species in the water storage containers. The usage of larvivorous fish appears to be identified as a possible WHO dengue vector management tool, and the ability of fish for dengue prevention or management is likely to vary from that of malaria. It is mostly because *Aedes* vectors, unlike other malaria vectors, primarily

thrive in isolated man-made containers usually varying in size from 0.5 L soda bottles discarded to 400 L domestic water tanks. Han et al., (2015) reported a significant reduction in larva incides by using larvivorous fish as a standalone treatment. The findings from the three efficacy trials showed that larvivorous fish has the ability to contribute successfully to the management of immature stages of dengue vector (Han et al., 2015). Larvivorous fish eat rapidly and can kill all immature stages from tanks within 24 hours. Nevertheless, these findings suggest nothing more than the capacity of fish to eradicate larvae from the container in which they reside and any actual dengue-endemic situation will need large coverage levels of several effective containers in order to have some effect on vector species and on dengue transmission (Han et al., 2015).The results indicate that the use of larvivorous fish, whether employed as a single agent or in conjunction with other interventions, may substantially minimize immature vector stage infestations. Nonetheless, there is no proof to prove the effectiveness of larvivorous fish in the population as a single agent (Han et al, 2015).

Bacillus thuringiensis israelensis (Bti), a bacterium that develops toxic proteins that result in high mortality between larvae after ingestion, was used as a dengue prevention measure in Boyce et al., (2013). Although Bti may be successful in decreasing the amount of immature *Aedes* in handled samples, very little evidence exists that dengue morbidity can be decreased when utilizing Bti alone. As for now, there is currently inadequate data to support the fact Bti as a single agent for long term dengue vector management and DF prevention. The data provided from the effectiveness studies shows that in a number of breeding sites, Bti may be successful in regulating the immature stages of dengue vector mosquitoes. The killing impact is simple, usually

removing all immature stages within 24 hours of treated containers, with a residual effect lasting from two to four weeks (Boyce et al, 2013).

2.6.3 Chemical control

According to Bouzid et al., (2016), they listed insecticide spraying, insecticide treated curtains, nets and screens and larvicide application as chemical controls (Bouzid et al., 2016). Meta-analysis of nine pre-post insecticide spray and aerosol experiments by Das and colleagues indicated a statistically significant 10 percent drop in House Index (Das et al., 2014). George et al., (2015) reviewed the potency of temephos in water tanks. By using temephos as an individual intervention, it recorded a decrease in the immature stages post-intervention opposed to their respective control group. It was found that the treated sources were larvae-free for a variable period of time depending on the application season, number of applications, temephos dose, control technique and method of operation (George et al., 2015)

Wilson and colleagues reported that materials treated with insecticide could minimize the spread of disease but recorded low *A. aegypti* mortality rates demonstrated substantial resistance to insecticides, which significantly reduces the efficacy of this form of control measure (Wilson et al., 2014).

2.6.4 Integrated vector management

Integrated vector management relates to the combined usage of two or more control interventions. This method of management is preferred because it is considered to be more reliable, reflected in the amount of systemic reviews that are important. Higher quality studies of the effect of vector control strategies on human occurrence is needed because integrated vector controls may not always have showed an increase in the efficiency (Bouzid et al, 2016) For an efficient management system, the World Health Organization (WHO) suggested Integrated Vector Management (IVM). Plus, IVM strategies aims to increase disease-vector management performance, cost-effectiveness, ecological soundness and sustainability. Furthermore, it is described as "a logical decision-making method for optimally using vector control tools. (Lima,Goulart and Neto, 2015 ; WHO, 2012).

Commonly, in IVM, the combination of larvivorous fish with other biological control measures and temephos larvacide in water storage with other control measures were used (Han et al., 2015; Bouzid et.al 2016). A study conducted by George et al., (2015) showed a significant reduction in entomological indices by using temephos along with other chemical vector. However, the efficacy of temephos depended on various variables, including distribution efficiency, water turnover rate, water content, organic debris, temperature and sunlight exposure (George et al., 2015).

2.6.4.1 AedesTech Mosquito Home System

Figure 4: AedesTech Mosquito Home System

AedesTech Mosquito Home System (AMHS) is a commercial and autocidal ovitrap which is used to reduce the *Aedes* population, as it uses the principle of 'lure and kill'. This commercial traps are manufactured by One Team Network Sdn. Bhd. In addition, AMHS will attract adult female *Aedes* mosquito to oviposit inside the ovitraps by using pyriproxyfen (PPF) as attractant (Latifah et al., 2020). This attractant will prevent immature stages of *Aedes* emerges into an adult mosquito. Basically, PPF is used as insecticide and a hormone analogue that will interrupt the life cycle of *Aedes* mosquito, thus prevent the transmission of DENV (Hustedt et al., 2020).

2.7 Vaccine for dengue virus

The development of dengue vaccines has been a challenging task due to the presence of four antigenically distinct DENV serotypes in which each serotype is able to induce cross reactivity and disease-enhancing antibody reaction to the other three serotypes. Over the last decades, the development of dengue vaccine has progressed despite the current challenges to produce effective dengue vaccine (Khetarpal and Khanna, 2016). There are some dengue such as multivalent attenuated, chimeric, DNA and inactivated vaccines have been developed. Primarily, these vaccines work by enhancing immune responses to DENV envelopes (E) and non-structural-1 proteins (NS1) (Liu et al., 2016).

Of these vaccines, live attenuated vaccine is produced and licensed by Sanofi Pasteur in late 2015. It is known as the world's first dengue vaccine, CYD-TVD or Dengvaxia. The candidate for the Sanofi Pasteur tetravalent dengue vaccine is composed of 4 recombinant live attenuated vaccines based on the backbone of a yellow fever vaccine. Additionally, each expressing the prM and envelope genes of one of the four DENV serotypes (Guy, Saville and Lang, 2010). On the other hand, this vaccine only provides partial protection against DENV2 due to the unexplained incidence of nine years old children that was hospitalized for severe dengue (Liu et al., 2016).

An effective and specific treatment or vaccine against dengue fever can be developed if there is better understanding in the molecular mechanism of DENV pathogenesis (Chen et al., 2018). Toresi et al., (2017) suggested that long-term protective immune responses and high-titer neutralizing antibody responses to all four DENV serotypes need to be induced simultaneously for a safe and effective vaccine. To add, following DENV infection and DENV vaccine administration, it would be a major step forward if we could understand the ways DENV can modulate immunological responses (Toresi et al., 2017).

CHAPTER 3

METHODOLOGY

3.1 Study area

This research was carried out at the 17th College of Universiti Putra Malaysia. This college is located in Serdang, Selangor. It consists of four residential blocks including Block A, B, C and D. Seventeen College accommodation design is ' Apartment Style ' with four rooms per house and t wo students per room. Blocks A, B and C are accommodated by female students while Block D is for male students. There are four wings with five levels in each of the block. Lake can be found about 50 meters from the entrance of the college. All blocks were used for the pre-treatment. During the intervention, Block B was used as treatment block whereas Block C was the control block.

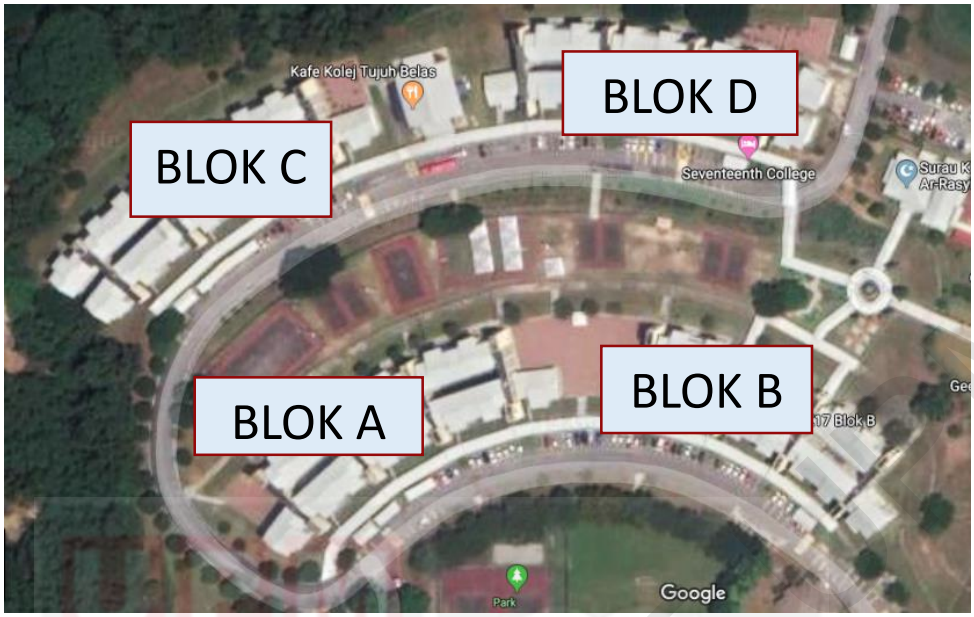

Figure 5: Aerial view of 17th College, UPM

Figure 6: Map view of 17th College, UPM

3.2 Materials

3.2.1 Ovitrap

Ovitrap was sponsored by One Team Networks Sdn Bhd**.** A total of 160 of ovitraps with QR code were installed along the front and back staircase of each level of Block A, B, C and D for the pre-treatment. Non-woven tissue (17.5 cm x 7.3 cm) was placed in each ovitrap for six days to allow the mosquitoes to oviposit. All the tissues were collected, and the eggs deposited on the tissues were counted to access the mosquito population for every block. This procedure was carried out every month.

3.2.2. Temperature and Humidity device

Three sets of humidity and temperature data logger of Temperature Technology of Australia were provided by One Team Networks Sdn Bhd. The device was placed in the living room, room 1 and room 2 in the selected houses for 24 hours for temperature and humidity recording.

Figure 7: Humidity and temperature device

3.2.3 Female non-gravid *Aedes aegypti*

A total of 1,200 females non-gravid *Aedes aegypti* were supplied by Sumitomo Chemical Enviro- Agro Asia Pacific Sdn. Bhd located in Senawang, Negeri Sembilan.

3.2.4 Intelligent Mosquito System (I.M.O.S) with Xmos mini aerosol

Figure 8: I.M.O.S with Xmos mini aerosol

I.M.O.S with Xmos mini aerosol was kindly sponsored by One Team Networks Sdn Bhd. A total of fifteen I.M.O.S and 45 cans of Xmos mini aerosol were used in five selected houses during the intervention. Each house contains three I.M.O.S where it was installed at three different areas which are hall, room 1 and room 2. Before the trial, all the Xmos mini aerosol were pre-weighed and the weight loss was recorded after assessment. A new Xmos mini aerosol was replaced after 45 or 69 days and the whole efficacy study was assessed for 60 days.

3.3 Methodology

3.3.1 Pre-treatment assessment

During the pre-treatment, 160 ovitraps were installed at the front and back staircase of each levels. The ovitraps were attached to the stairs by using cable tie (8 inches). The mosquito population was assessed by counting the eggs of mosquito on the substrate inside the ovitrap after six days. Two blocks with highest population were chosen for the treatment. The mean number of eggs and ovitrap index (OI) were calculated by using the following formulas.

$$\text{Mean number of eggs} = \frac{Total\ number\ of\ eggs\ in\ ovitraps}{Total\ number\ ovitraps\ examined} \times 100$$

$$\text{Ovitrap index, OI} = \frac{No.\ of\ positive\ ovitrap\ (with\ eggs)}{No.\ of\ ovitrap\ deployed} \times 100\%$$

Figure 9 : Ovitrap located at the staircase

3.3.2 Intervention

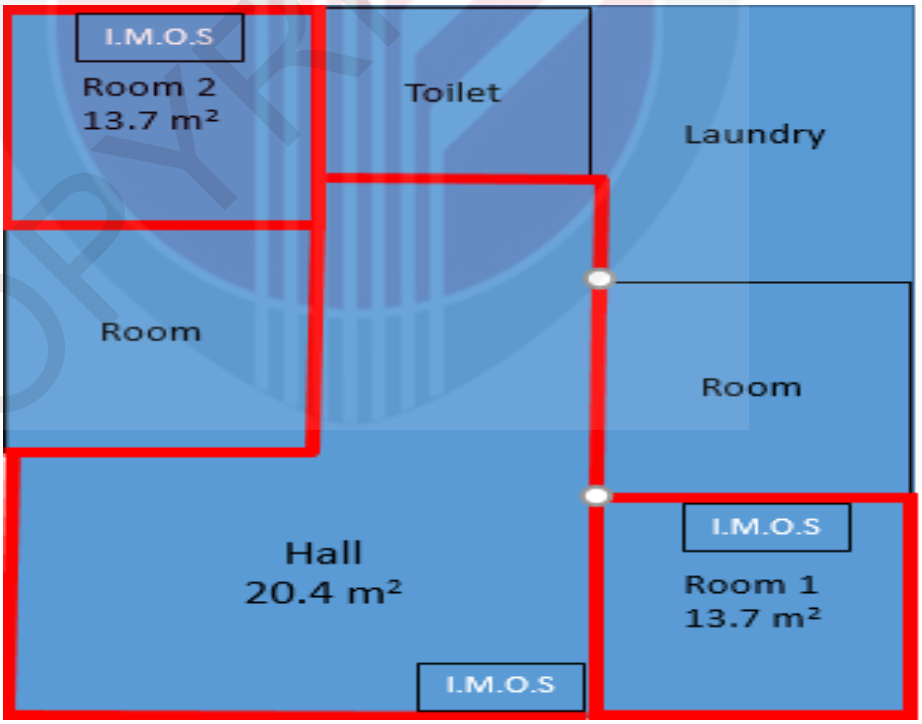

Figure 10: The size of hall, room 1 and room 2 at 17th College, UPM

Figure 10 shows the size of hall, room 1 and room 2 of all houses in 17th College. The size for hall is 20.4 m^2 while 13.7 m^2 for room 1 and room 2. For the intervention study, Block B was chosen as treatment block while Block C was the control block. A total of fifteen I.M.O.S with Xmos mini aerosol were placed inside the five selected houses in Block B. There was no installment of I.M.O.S used in the control houses. In each of the treated house, three I.M.O.S with Xmos mini aerosol was installed separately in the hall, room 1 and room 2. Xmos mini aerosol was set to spray at two time points, 6.30 am and 4.00 pm. At each spraying time 6 sprays were released in room 1 and 2 while 9 sprays were released in the hall.

Figure 11: Net where the female *Aedes* mosquitoes were placed.

3.3.4 *Aedes* mosquito eggs counting

All the substrates from the ovitrap were air-dried at room temperature. The substrates were placed under magnifying glass to manually calculate the number of mosquito eggs.3.3.5 Adult efficacy study

Twenty *Aedes* adult female mosquitoes (non-gravid and non-blood fed) provided by Sumitomo Chemical Enviro-agro Asia Pacific Sdn. Bhd. were placed in the net (Figure 5). The nets were hung 10 feet away from I.M.O.S in the five treatment houses and control houses. Starting from 4:30 pm to 6:30 pm, the knockdown efficacy was calculated by measuring the number of dead mosquitoes in the net. Then, all nets weremoved to the laboratory. The percentage of mortality after 24 hours of exposure was then calculated. This adult efficacy study was conducted thrice at day 30, 60 and 90 (every 30 days) during the intervention phase.

$$Percentage\ of\ knockdown\ efficacy: \frac{Number\ of\ mosquito\ knockdown}{Total\ number\ of\ mosquito\ in\ net} x100$$

3.3.6 Data analysis

Data were presented as mean ± SE. Data were analyzed by using Statistical Package for Social Science (SPSS) Version 15. Two-way ANOVA was used to compare means on knockdown of adult *Aedes* mosquitoes between control and treatment blocks at different time points. Percentage of adult knockdown between room and hall and the percentage of adult Aedes mosquitoes after 24 hours were calculated by using unpaired T- test. A value was considered significant at $p \leq 0.05$.

CHAPTER 4

RESULTS

4.1 *Aedes* mosquito population

Figure 12 shows the mean number of *Aedes* mosquito eggs collected in 17th College during pre-treatment, intervention 1 and intervention 2 between control and treatment blocks. The population was high and not significantly different ($p < 0.05$) with each other during the pre-treatment. During day 30 and 60 indicated as intervention 1 and 2, respectively, the mosquito population was not significant different ($p<0.05$) between the control and treatment blocks.

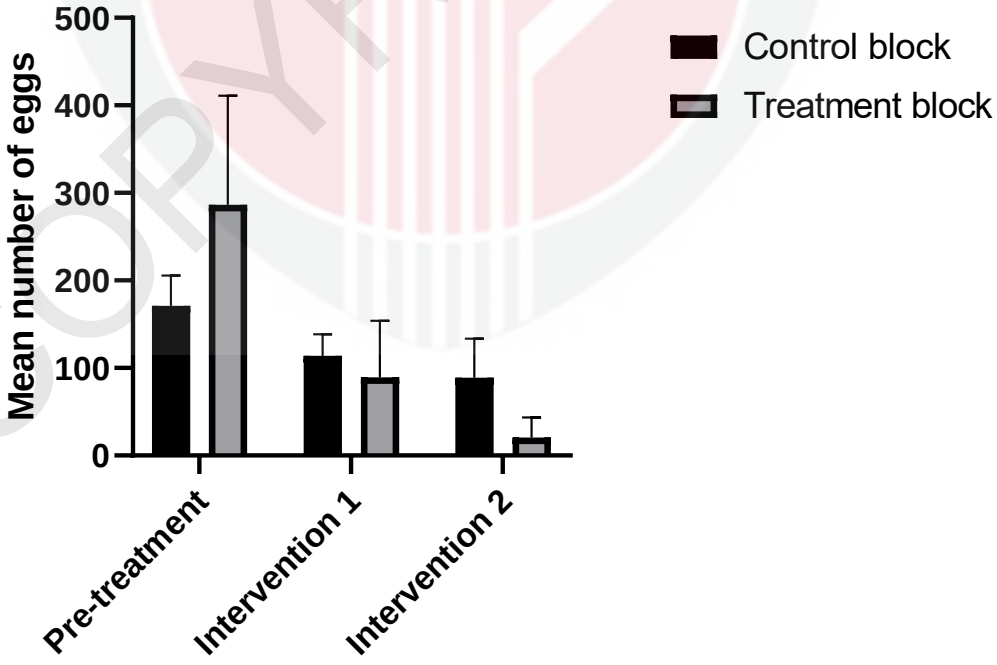

Figure 12: Mean number of eggs during pre-treatment, intervention 1 and intervention 2 between control and treatment blocks

Figure 13 shows the appearance of *Aedes* mosquitoes' eggs on tissue sample collected from an ovitrap in 17th College, UPM. The eggs were black in colour and laid singly.

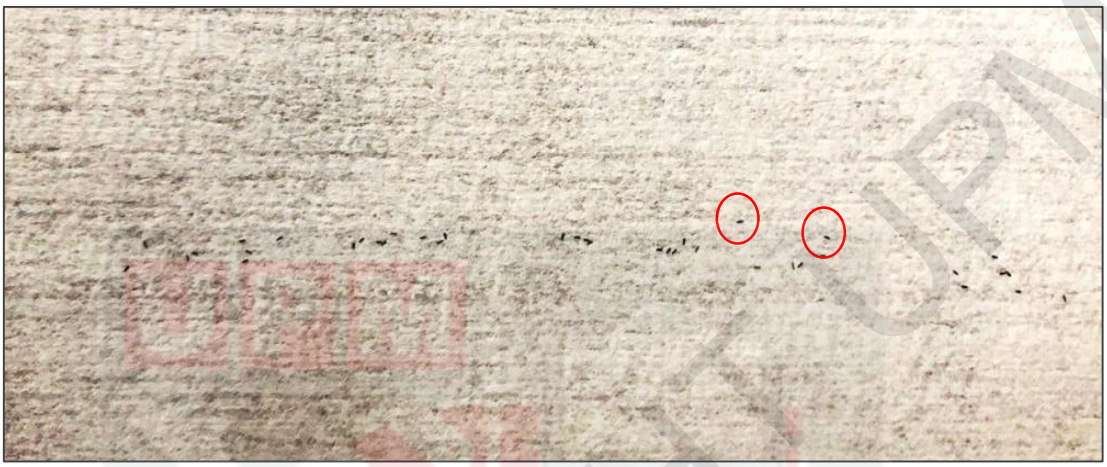

Figure 13: Eggs of *Aedes* mosquitoes (red circles) attached on the tissue collected from ovitrap as observed from naked eyes

4.2 Ovitrap Index (OI)

Figure 14 depicts the Ovitrap Index (OI) in 17th College, UPM. The OI shows similar pattern with the mean number of eggs indicating that there was no significant difference ($p>0.05$) between control and treatment blocks during pre-treatment, intervention 1 and intervention 2.

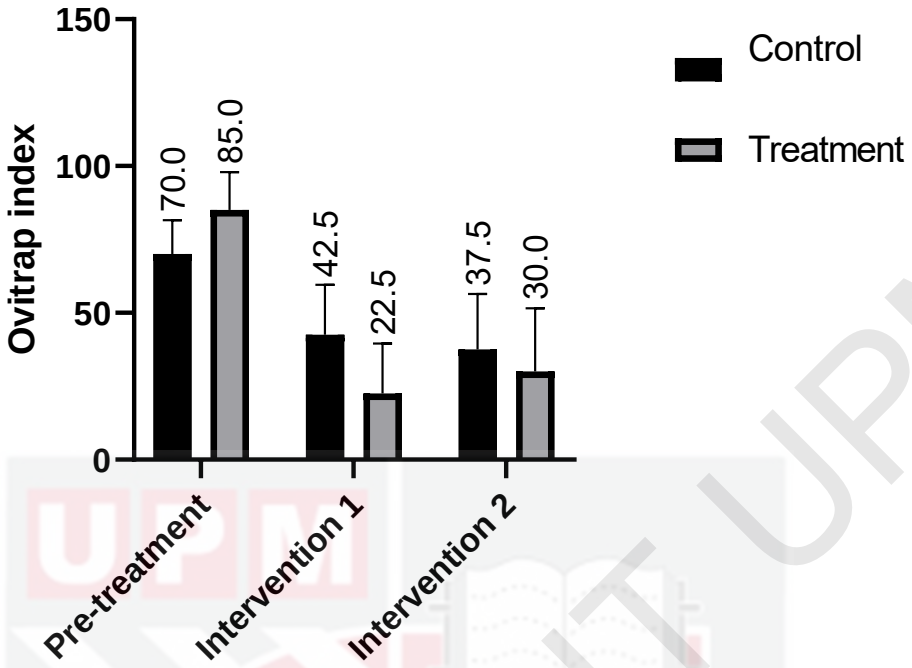

Figure 14: Ovitrap index (OI) during pre-treatment, intervention 1 and intervention 2 between control and treatment blocks

4.3 Knockdown of Adult *Aedes* mosquitoes

The knockdown percentage of adult *Aedes* mosquitoes in the treatment block was significantly higher ($p<0.05$) than the control block at all time points (10, 20, 30, 60 and 120 minutes (Figure 15).

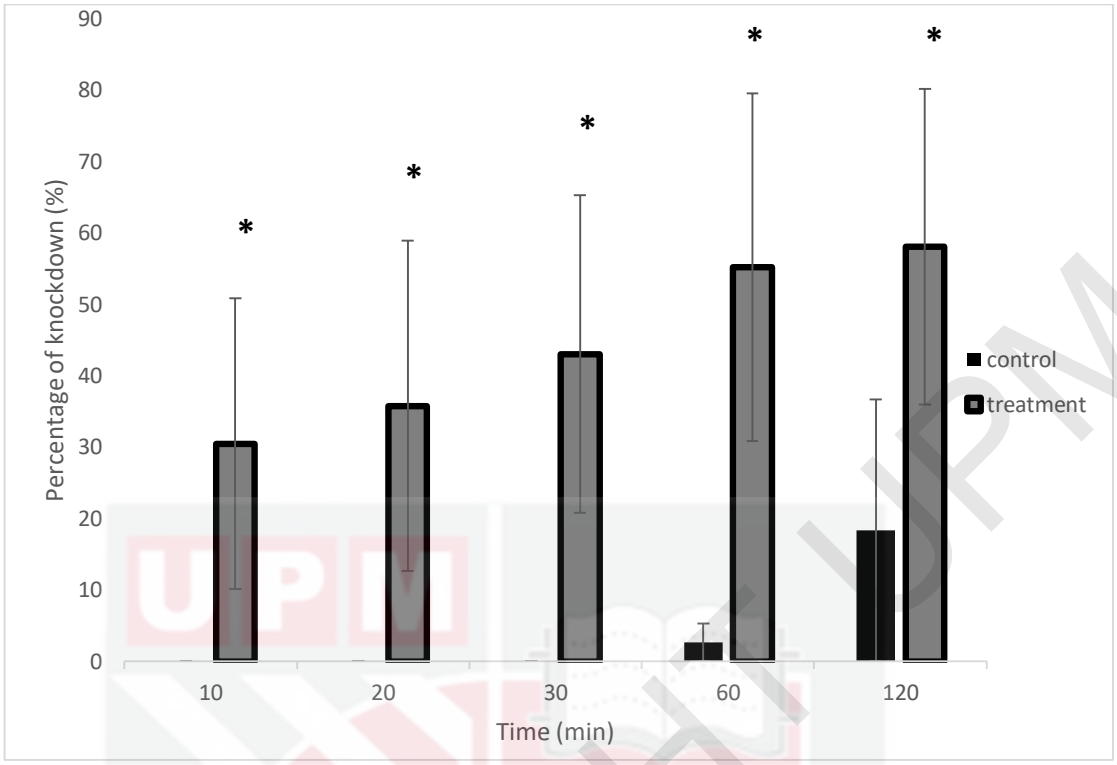

Figure 15: Percentage of knockdown at (10, 20, 30, 60 and 120 min) between control and treatment blocks. * significantly different at $p<0.05$

The percentage of knockdown of adult *Aedes* mosquitoes between room and hall at the treated areas in 17th College, UPM showed no significant difference ($p>0.05$) (Figure 16).

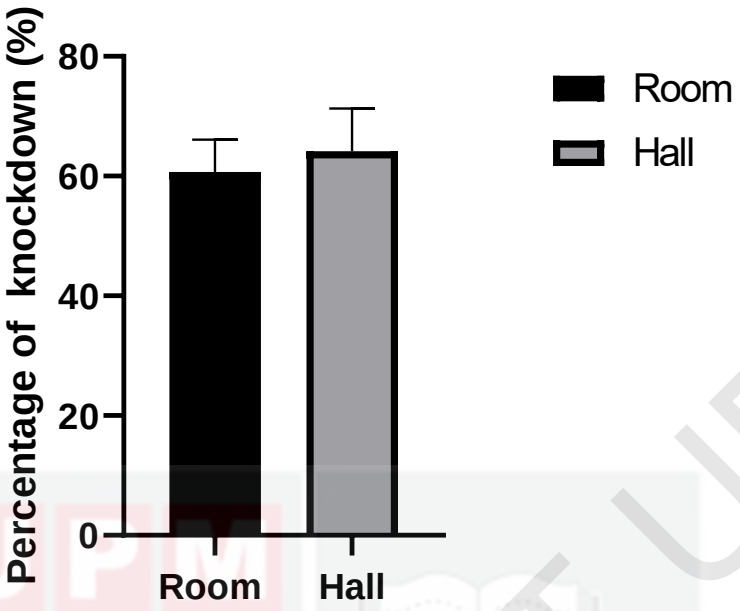

Figure 16: Percentage of knockdown of adult *Aedes* mosquitoes

Figure 17 presents the mortality of adult *Aedes* mosquitoes after 24 hours exposure. There was a significant difference ($p>0.05$) between control and treatment block. The treatment employed significantly increase the percentage of mortality of adult *Aedes* mosquitoes after 24 hours of exposure as represented in Figure 16.

Figure 17: Percentage of mortality between control and treatment blocks after 24 hours of exposure. * significantly different at $p<0.05$

4.4 Mean temperature and relative humidity

Table 1 shows the highest and lowest temperature and relative humidity measured at control and treatment blocks. It shows that the mean temperature was 29ºC and the relative humidity was 74 % throughout the study.

	Highest	**Lowest**	**Mean**
Temperature (ºC)	30.7	27.3	29
Relative Humidity (%)	80.2	67.3	74

Table 1: Mean temperature and humidity measured at control and treatment blocks

CHAPTER 5

DISCUSSION

According to iDenggi (2020), dengue cases in Malaysia remains high with 66, 379 cases and 109 deaths reported from December 2019 until August 2020. Selangor is the popular hotspot for dengue cases since it reported the highest number of cases among all states in Malaysia (iDenggi, 2020). Despite the intensive and extensive efforts by health agencies and government-funded integrated vector control, all the vector control activities and prevention are not fully effective in reducing dengue outbreaks. Therefore, there is a need for new intervention and a further study of insecticide as one of the solutions to reduce dengue cases (Packierisamy et al., 2004).

We determined the mosquito population by calculating the mean number of eggs and ovitrap index (OI). The eggs were black and laid singly on the substrate indicated characteristic of *Aedes aegypti* (Rueda, 2014). During the pre-treatment, the mosquito population was high. This could be due to artificial containers that hold clear water indoor and outdoor of 17th College and enhance the hotspot of *Aedes aegypti* to oviposit (Rozilawati et al., 2007). Moreover, the presence of lakes in front of the 17th College could be the reason of high mosquito population. However, the use of I.M.O.S was not significantly reducing the mosquito population, indicating that the treatment has no effect on the *Aedes*'s eggs.

We need to consider and evaluate how long the efficacy of the treatment formulation lasts in the air when exposed against *Aedes* mosquitoes. *Ae.aegypti* shows an ability to escape from an open windows and doors (Moore et al., 2007). If this escape occurs, it will cause female *Aedes* mosquitoes to be able to oviposit as well as increases the eggs population. Throughout this study, we used commercial ovitraps to collect the eggs. During the USA *Ae.aegypti* eradication program that was held three years ago, it was the beginning of the use of ovitrap (Fay & Eliason, 1966 ; Fay & Perry, 1965; Jakob & Bevier, 1969). Ovitrap is now a popular *Aedes* surveillance strategy in several areas, including South and North America, Asia and Europe, due to its sensitive means to detect mosquito and inexpensive (ECDC, 2012; Mogi et al., 1988; Reiter, Amador & Colon, 1991). Generally, ovitraps is used to enhance and attract female *Ae. aegypti* to oviposit inside the container (Fay and Perry, 1965).

I.M.O.S with Xmos mini aerosol is a chemical vector control that is able to kill adult mosquitoes where it contains 76% of metofluthrin as active ingredient. Previous study by Kawada et al., (2004) and Ujihara et al., (2004) reported multilayer paper strips impregnated with metofluthrin gives positive result as repellent against mosquitoes whether in field application or laboratory (Kawada et al. 2004, Ujihara et al. 2004). According to Tamara et al., (2017), metofluthrin has the ability to reduce the biting activity of *Ae aegypti*. When the mosquitoes fly in the zone of metofluthrin, they will experience confusion and this lead to knockdown (Tamara et al., 2017). In this study, it was discovered that metofluthrin was able to cause knockdown at (10, 20, 30, 60 and 120 min) and high mortality after 24 hours of exposure towards adult *Aed*es mosquitoes. Metofluthrin is one efficient method to prevent biting by *Ae aegypti* due

to its special and successful mechanism to disturb the biting activity (Tamara et al., 2017).

Metofluthrin is highly lethal to *Ae. aegypti* in the confined space of an apartment at Queensland, Australia (Rapley et al., 2009). Shono (2004) reported that the use of metofluthrin coils effectively cause reduction in landing counts of *Ae.aegypti* (Shono, 2004). One Team Network Sdn Bhd claimed metofluthrin has high vapor pressure and deliver macro-particles into air and provides up to eight hours of protection. This statement can be supported by Kawada et al., (2006) where metofluthrin has greater vapor pressure than d-allethrin and permethrin. Due to its characteristic, metofluthrin can vaporize at normal temperature while other phyrethroid are not able to vaporize due to their inability to evaporate effectively and require heating source for evaporation (Kawada et al., 2006). In this study, we tested the efficacy of I.M.O.S at two different areas in the treated houses including hall (20.4 $m^{2)}$ and room (13. 7 m^2). We discovered that the use of I.M.O.S was highly effective to cause mortality against *Aedes* mosquitoes despite the difference size between hall and room.

According to One Team Network Sdn Bhd, they reported that I.M.O.S will cause mortality even with four sprays in room with 30 m^2. They recommended additional sprays if the room area is more than 30 m^2 (personal communication). Throughout our study, I.M.O.S was set to automatically release 6 sprays per session . Moreover, our hall and room area are slightly smaller from the area mentioned by this company. This indicate the mortality cause by I.M.O.S was not affected by hall and room size. Jonathon et al., (2017) found that metofluthrin that was installed in the rooms within

1 m showed 90 percent of reduction in *Ae. aegypti* biting activity (Darbro et al., 2017). During the intervention study, we placed *Ae. Aegypti* 10 m from the I.M.O.S and it causes 67 % of death at treatment area. Metofluthrin could be used to protect from mosquito biting, as it demonstrates good knockdown performance against mosquitoes and safety profile toward mammals. Metofluthrin is ideal to be used in numerous current source products such as coil of mosquito, as well as in modern products such as ventilator vaporizers and paper strips (Ujihara et al., 2014).

Throughout our study, we placed the temperature and humidity device in the treated and control houses. The mean temperature was 29 ^{o}C and the mean humidity was 74 RH. *Ae. aegypti* females were activable to travel between 15 ° C and 32 ° C, whereas flight was feasible at high temperatures at 10 ° C and 35 ° (Rowley and Graham, 1968). *Ae. aegypti* was most aggressive at a temperature of 28 ^{o}C, which was found optimum for the genus and this range of optimal temperatures showed the mosquitoes suit perfectly with our measured temperature range (Rowley and Graham, 1968; Connors, 1924). Furthermore, metofluthrin also displays strong vapor activity at room temperature (Ujihara et al., 2004). Rowley and Graham (1968) stated optimal values have been assumed for all vital mosquito activities was around 27 ° C and 80 percent RH. Relative humidity had little impact towards virgin female *Ae. aegypti* as well as their flight activity is still within 30 to 90 percent (Rowley and Graham, 1968). Our measured temperature and humidity of this study coincides with optimum survival for mosquito activities.

CHAPTER 6

CONCLUSION

6.1 Conclusion

Intelligent mosquito home system (I.M.O.S) with Xmos mini aerosol was effective in killing adult *Aedes* mosquitoes in 17th College, Universiti Putra Malaysia. However, I.M.O.S was not successful in reducing the *Aedes* mosquito population in 17th College.

6.2 Recommendations for further study

This study can be conducted in more populated area to see the effectiveness of I.M.O.S at a larger scale. Moreover, it also can be conducted towards residents of different socio-economic and education background to see the consistency of the outcomes.

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APPENDICES

Pre-treatment data by using ovitrap

Table 2: Number of egg of *Aedes* mosquito during pre-treatment study

Block	Wing	Level	Pre-treatment				
			No. of eggs			Total	Grand Total
			Front	Back	Total		
C	1	G	28	21	49	186	660
		1	4	0	4		
		2	29	0	29		
		3	8	18	26		
		4	7	71	78		
	2	G	32	38	70	204	
		1	8	0	8		
		2	0	32	32		
		3	13	25	38		
		4	33	23	56		
	3	G	26	31	57	131	
		1	28	27	55		
		2	1	0	1		
		3	1	0	1		
		4	4	13	17		
	4	G	37	25	62	139	
		1	22	0	22		
		2	12	9	21		
		3	0	34	34		
		4	0	0	0		

Block	Wing	Level	Pre-treatment				
			No. of eggs			Total	Grand Total
			Front	Back	Total		
B	1	1	25	66	91	181	1092
		2	0	3	3		
		3	23	0	23		
		4	63	1	64		
		5	0	0	0		
	2	1	25	21	46	150	
		2	7	0	7		
		3	11	29	40		
		4	6	0	6		
		5	50	1	51		
	3	1	107	104	211	409	
		2	0	28	28		
		3	26	0	26		
		4	5	50	55		
		5	7	82	89		
	4	1	33	12	45	352	
		2	39	30	69		
		3	91	16	107		
		4	15	50	65		
		5	0	66	66		

Intervention 1 data by ovitrap

Table 3: Number of egg of *Aedes* mosquito during Intervention 1 study

Block	Wing	Level	Intervention 1				
			No. of eggs			Total	Grand Total
			Front	Back	Total		
C	1	G	0	0	0	81	386
		1	0	18	18		
		2	22	5	27		
		3	22	0	22		
		4	14	0	14		
	2	G	27	25	45	105	
		1	17	0	0		
		2	0	23	0		
		3	0	9	0		
		4	0	4	0		
	3	G	79	0	79	93	
		1	0	0	0		
		2	0	0	0		
		3	0	0	0		
		4	14	0	14		
	4	G	43	55	78	107	
		1	0	2	0		
		2	0	0	0		
		3	7	0	60		
		4	0	0	11		

Block	Wing	Level	Intervention 1				
			No. of eggs			Total	Grand Total
			Front	Back	Total		
B	1	G	106	11	117	155	358
		1	0	0	0		
		2	0	0	0		
		3	0	0	0		
		4	0	27	0		
	2	G	33	30	63	114	
		1	0	19	19		
		2	0	5	5		
		3	0	0	0		
		4	0	0	0		
	3	G	0	0	0	0	
		1	0	0	0		
		2	0	0	0		
		3	0	0	0		
		4	0	0	0		
	4	G	0	60	0	127	
		1	1	0	1		
		2	0	0	0		
		3	12	0	12		
		4	1	0	67		

Intervention 2 data by ovitrap

Table 4: Number of egg of *Aedes* mosquito during Intervention 2 study

Block	Wing	Level	Intervention 2				
			No. of eggs			Total	Grand Total
			Front	Back	Total		
C	1	G	24	14	38	70	356
		1	0	4	4		
		2	0	24	24		
		3	0	4	4		
		4	0	0	0		
	2	G	45	0	45	45	
		1	0	0	0		
		2	0	0	0		
		3	0	0	0		
		4	0	0	0		
	3	G	15	0	15	92	
		1	0	5	5		
		2	0	7	7		
		3	0	32	32		
		4	0	33	33		
	4	G	73	5	78	149	
		1	0	0	0		
		2	0	0	0		
		3	60	0	60		
		4	11	0	11		

Block	Wing	Level	Intervention 2				
			No. of eggs			Total	Grand Total
			Front	Back	Total		
B	1	G	13	0	13	54	82
		1	0	1	1		
		2	5	2	7		
		3	22	11	33		
		4	0	0	0		
	2	G	0	0	0	1	
		1	0	0	0		
		2	0	0	0		
		3	1	0	1		
		4	0	0	0		
	3	G	0	0	0	13	
		1	0	0	0		
		2	1	0	1		
		3	0	0	0		
		4	0	12	12		
	4	G	0	0	0	14	
		1	1	0	1		
		2	0	0	0		

Table 5: Percentage of knockdown during Trial 1

Hostel	Location / Time (min)	Percentage of Adult Knockdown					Percentage of adult mortality
		10	20	30	60	120	24 hours
B-G-10	Room 1	5	5	10	15	40	50
	Room 2	35	40	95	100	100	100
	Living Room	40	45	100	100	100	100
B-1-6	Room 1	5	5	5	5	5	15
	Room 2	0	0	0	0	5	15
	Living Room	5	5	5	25	25	35
B-2-8	Room 1	25	25	30	30	35	55
	Room 2	20	20	30	75	100	100
	Living Room	35	35	35	100	100	100
B-3-4	Room 1	0	10	10	10	30	40
	Room 2	5	5	5	10	10	25
	Living Room	0	5	5	10	15	35
B-4-5	Room 1	50	75	75	80	80	100
	Room 2	30	30	40	40	45	65
	Living Room	20	20	20	50	50	70
C-G-12	Room 1	0	0	0	0	10	15
	Room 2	0	0	0	5	20	25
	Living room	0	0	0	20	25	25
Mean ± SE	Room 1	17	24	26	28	38	52
	Room 2	18	19	34	45	54	61
	Living Room	20	22	33	57	58	68

Table 6: Percentage of knockdown during Trial 2

Hostel	Location /Time (min)	Percentage of Adult Knockdown					Percentage of adult mortality
		10	20	30	60	120	24 hours
B-G-10	Room 1	60	70	90	90	90	100
	Room 2	55	100	100	100	100	100
	Living Room	40	90	100	100	100	100
B-1-6	Room 1	15	20	20	60	100	100
	Room 2	20	25	30	60	100	100
	Living Room	0	20	25	40	100	100
B-2-8	Room 1	55	90	90	90	100	100
	Room 2	75	100	100	100	100	100
	Living Room	45	100	100	100	100	100
B-3-4	Room 1	100	100	100	100	100	100
	Room 2	100	100	100	100	100	100
	Living Room	100	100	100	100	100	100
B-4-5	Room 1	100	100	100	100	100	100
	Room 1	100	100	100	100	100	100
	Living Room	100	100	100	100	100	100
C-G-12	Room 1	0	0	0	0	0	5
	Room 2	0	0	0	0	0	5
	Living room	0	0	0	0	0	0
Mean ± SE	Room 1	66	76	80	88	98	100
	Room 2	70	85	86	92	100	100
	Living Room	57	82	85	88	100	100

Table 7: Two-way ANOVA for Ovitrap Index (OI)

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Control block - Treatment block								
Pre-treatment	70.00	85.00	-15.00	11.93	4	4	1.257	18.00
Intervention 1	42.50	22.50	20.00	11.93	4	4	1.676	18.00
Intervention 2	37.50	30.00	7.500	11.93	4	4	0.6286	18.00

Table 8: Two-way ANOVA for percentage of knockdown between control and treatment

Mean			Standard Error		
Mins	control	treatment	Mins	control	treatment
10	0	30.5033	10	0.00000	20.32593
20	0.0000	35.7767	20	0.00000	23.13869
30	0.0000	43.0567	30	0.00000	22.24350
60	2.6667	55.2200	60	2.66667	24.33233
120	18.3333	58.1100	120	18.33333	22.09122

Table 9: Unpaired T test for percentage of mortality between control and treatment

Unpaired t test	
P value	0.0383
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=3.042, df=4

Table 10: Unpaired T test for percentage of knockdown between room and hall

Unpaired t test	
P value	0.7050
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.3895, df=10