Efficacies of the *Aedes* Tech Mosquito Home System (A-MHS) and Insecticidal Mosquito spray (X'MOS) at Universiti Malaysia Terengganu

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Introduction

A-MHS is an innovation that incorporated a common mosquito ovitrap with growth regulator (IGR), pyriproxyfen. This technology is purposely targeting container breeding mosquitoes such as *Aedes* at immature stages in which it will inhibit the growth and develpopment of mosquito larvae. The insect growth regulator is a group of insecticides that inhibit and retard the growth of insect juveniles including *Aedes* mosquitoes' larvae (Mulla, 1995). The exposure of a minimum concentration of pyriproxyfen on field collected *Aedes* mosquitoes has resulted on their reproductions status where the egg laying, egg hatching and adult emergence processes were significantly interrupted (Yadav et al., 2019).

Pyriproxyfen has been used in pest insects' control including mosquitoes and flies (Geden & Devine (2012). In USA, pyriproxyfen is used mainly for mosquito control (Suman et al., 2018) The exposure of pyriproxyfen that act as juvenile hormone analog at an early stage of ovarian development of *Ae. albopictus* were more efficiently effect their embryonic development.

Auto-dissemination is a process of insect management that offers benefit to the economics (save product and labour) the chemicals' contaminated insects will transfer the lethal concentration to other insects via oviposition, mating, aggregation or any other behaviours (Gaugler et al., 2012). Auto-dissemination methods have been studied for more than a decade as an important use in mosquito vector control programs that targeting container breeding mosquitoes such as *Ae. aegypti* and *Ae. albopictus*. Where "attract and kill" system is used to create lethal ovitrap (Swale et al., 2018).

X'MOS is an insecticide aerosol that convenient to kill adult mosquitoes with metofluthrin as its active ingredient. Metofluthrin is a volatile synthetic pyrethroid that used as an insect repellent. The use of metofluthrin has been proven to knockdown adult mosquitoes. The objectives of this study are to determine the efficacies of the *Aedes* Tech Mosquito Home System (A-MHS) and insecticidal mosquito spray X'MOS against *Aedes* mosquitoes and to prove pyriproxyfen auto dissemination occurred when using A-MHS.

Methodology

Study site and *Aedes* population

UMT student housing consisted of 8 buildings with each has 4 to 5 levels (Figure 1). For preliminary study, MH0 (A-MHS with water only) will be distributed in all eight buildings (5 MH0/ floor) to gauge the *Aedes* population in the area. The MH0 servicing (changing water and replacing oviposition substrates) will be conducted weekly for four weeks. Data that will be obtained are number of eggs and egg's hatching rates for each building and floor.

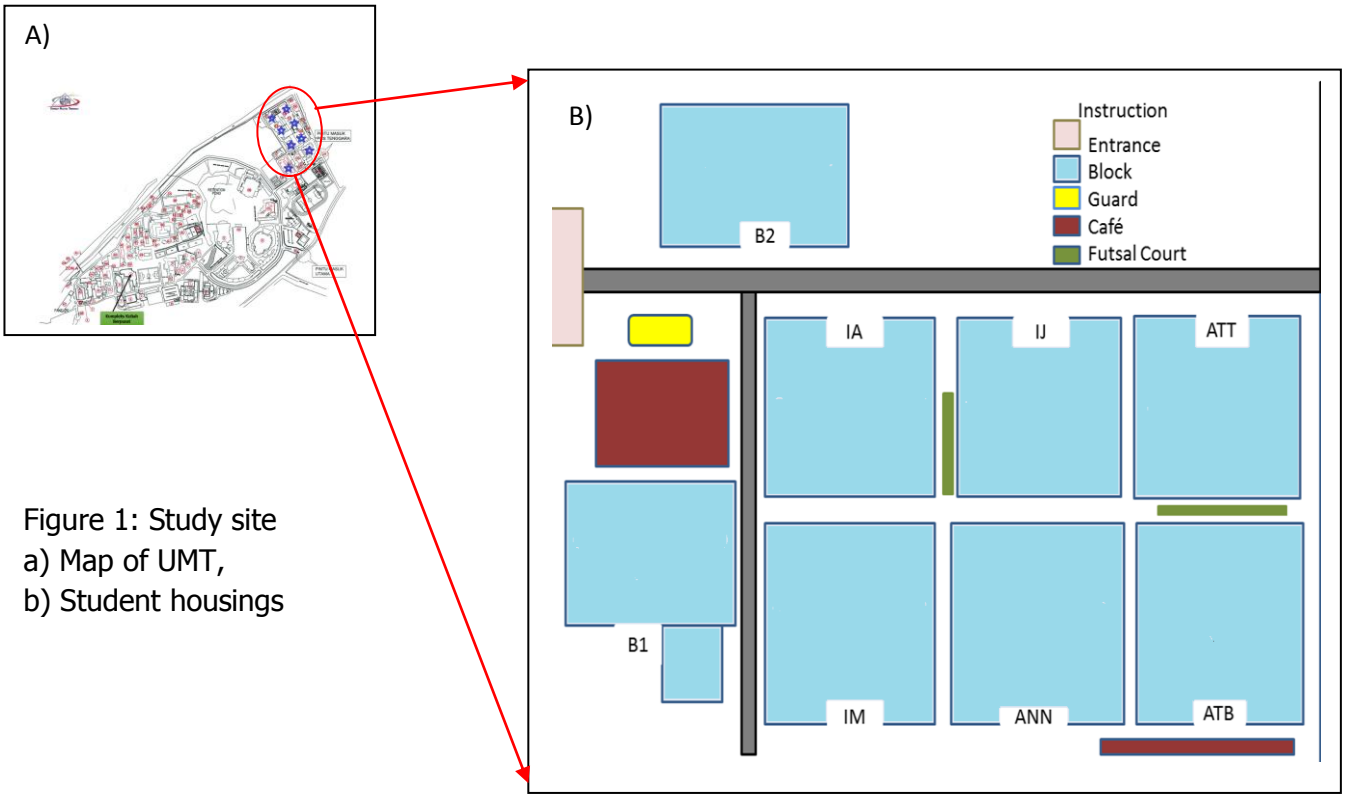

Figure 1: Study site
a) Map of UMT,
b) Student housings

In week 8, each building will be assigned with one of the following treatments.

Treatment		Building involved	Duration	Detail of treatment	Aim
1.	**Control** MH0 –A-MHS with water (without insecticide-pyriproxyfen)	2 Buildings	8 weeks	- Five MH0 will be placed in each floor - Oviposition substrates will be collected weekly for 8 weeks	Serve as control to be compared with other treatments.
2.	**MH1 only** MH0 –A-MHS with water MH1 – A-MHS containing insecticide pyriproxyfen	2 Buildings	8 weeks	- Five mosquito home (2 MH0 and 3 MH1) will be distributed at each floor - Oviposition substrates will be collected weekly for 8 weeks - Eggs will be counted - Hatching rate will be determine - Water sample from MH0 will be collected and tested for the presence of insecticide by using Liquid chromatography–mass spectrometry (LC-MS)	To determine the efficacy of MH -in attracting *Aedes* oviposition. -prevent larva development - to prove pyriproxyfen auto-dissemination occurred.
3.	**XMOS only**	2 Buildings	6 weeks	Apply XMOS following product instruction. • Xmos were sprayed once a day at 1800h inside buildings along the corridor of selected student housings with 1m interval for each spray. Determine re-occurrence of household insect pests **MH0 will be placed in the hallway/ building throughout experiment period to determine whether XMOS application will effect *Aedes* population in the building.**	To determine the efficacy of XMOS -Against household pest -effect XMOS on immature/ breeding site present in the treated area
4.	**MH1 + XMOS combination**	2 Buildings	6 weeks	Treatment 2 + Treatment 3	To determine the efficacy of combination treatment (MH and XMOS) in reducing *Aedes* population in student housing area.

After 8 weeks of the above treatments, observation on *Aedes* population in student housing area will be continued by placing MH0 in each building as post-treatment observation.

Laboratory test: Auto-dissemination pyriproxifen

Methodology

Auto-dissemination test was conducted in Makmal Penyelidikan Vektor, UMT. Four oviposition cups (8cm height x 4cm diameter) were arranged at each corner, inside of an insect cage (30cm x 30cm x 30cm)(Figure 2) . A strip (20cmx7.5cm) made up from disposable microfiber wipes was placed in each cup. Three cups were half-filled with dechlorinated tap water and another one cup was half-filled with pyriproxyfen solution (One Team Network). Adult *Ae. albopictus* mosquitoes used in this study were from eggs collected from the field.

Twenty five mated females *Ae. albopictus* (3 to 4 days old) were released into the insect cage. The females were allowed to blood fed on laboratory rat restrained in wire mesh daily for 15 minutes until all mosquitoes died. A cotton ball soaked in 10% sucrose solution was continuously provided in the cage as adult food. The cotton ball was changed every two days to avoid fungal growth. A total of five similar set up was prepared as replicates. For control group, another five cages each have four cups half-filled with dechlorinated tap water were prepared.

Each cup was taken out of the cage to check for the presence of eggs on oviposition strip and larva in the water daily. Eggs on the paper strips were counted under dissection microscope. Larvae developed in the water were counted. No larva food provided throughout the experiment. Different hand gloves were used in handling different cup to prevent contamination of pyriproxyfen due human error.

All the eggs laid from each cup were immersed in water in different container to hatch. Data on hatching eggs, immature survival and adult emergence were recorded.

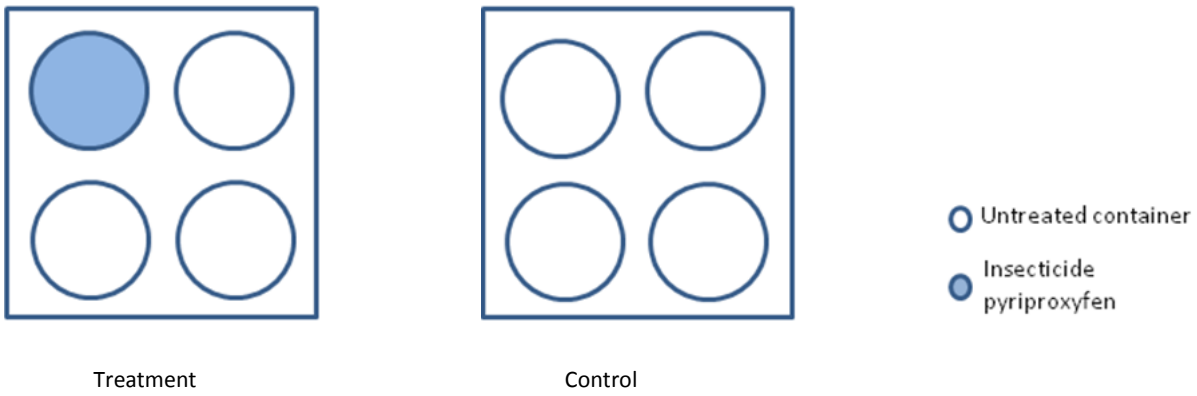

Figure 2: Arrangement cups in treatment and control cages for auto-dissemination test.

Results

Efficacies of A-MHS and XMOS in the field

Result showed that egg population of *Aedes* in all buildings fluctuated during 20 weeks observation. Accept for B1 building which consistently showed low population throughout study period, while at other sites ovitraps showed high positive ovitrap (Figure 3) and mean egg count (Figure 4) during preliminary and decreased during treatment and post treatment periods.

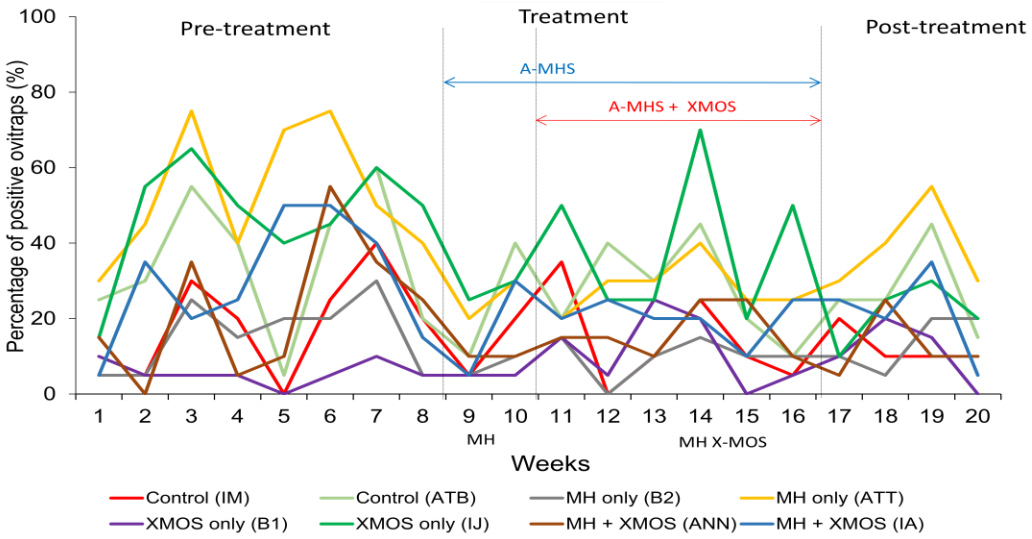

Figure 3: Percentage of positive ovitraps in UMT student housings

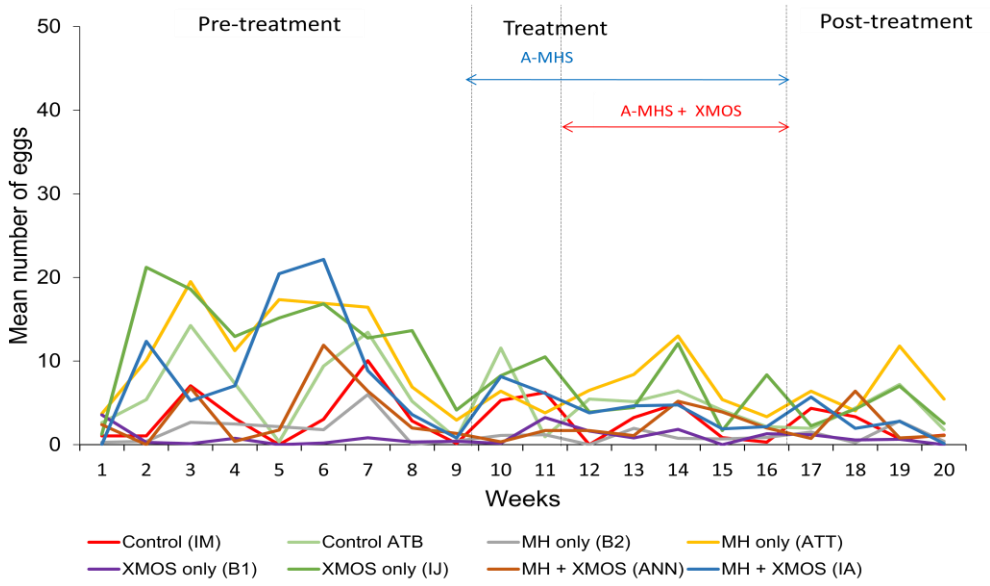

Figure 4: Mean number of eggs in UMT student housings

Approximately 50 to 90% of eggs collected during preliminary observation successfully hatched when immersed in water (Figure 5). None of the eggs collected from MH and MH +XMOS hatch during treatment periods (week 8-16). While eggs collected from building treated with XMOS only (week 11- 16) showed hatch rate of 45 to 90 %.

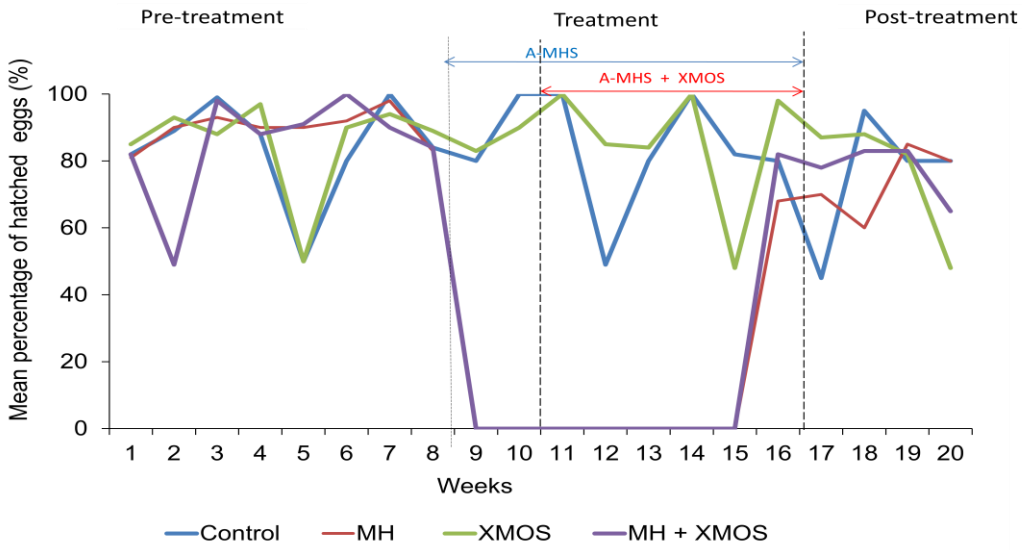

Figure 5: Mean percentage of hatched eggs in UMT student housings.

All eggs collected from treated (A-MHS) and untreated ovitraps not hatch for two buildings (Building B2 and ATT) during eight weeks of treatment. Similar (Table 1) results were observed in buildings (ANN and IA) treated with A-MHS together with XMOS spray. The eggs collected during eight weeks treatment period did not hatch.

Compared to buildings (BI and IJ) received XMOS sprays only for six weeks, eggs collected from ovitraps showed 70 to 100% hatch. While eggs from Control buildings (IM and ATB) also showed 70 to 100% successful hatch.

Table 1: Total number of eggs collected from student housings and percentage of hatch (%) during treatment weeks (8 weeks)

		Treated ovitrap		Untreated ovitrap	
Treatment (Building)	Level	Total egg	% hatch	Total egg	% hatch
MH only (B2)	1	28	0	0	0
	2	0	0	0	0
	3	1	0	38	0
	4	37	0	11	0
	5	39	0	0	0
MH only (ATT)	1	139	0	152	0
	2	380	0	23	0
	3	0	0	102	0
	4	55	0	121	0
MH + XMOS (ANN)	1	191	0	17	0
	2	53	0	40	0
	3	0	0	0	0
	4	19	0	26	0
MH + XMOS (IA)	1	106	0	262	0
	2	47	0	161	0
	3	16	0	24	0
	4	32	0	0	0
XMOS only (B1)	1			81	0
	2			92	0
	3			0	0
	4			45	0
	5			8	0
XMOS only (IJ)	1			265	0
	2			395	0
	3			254	0
	4			152	0
Control (IM)	1			129	0
	2			229	0
	3			14	0
	4			37	0
Control (ATB)	1			276	0
	2			239	0
	3			195	0
	4			75	0

Beside *Aedes* mosquitoes (*Aedes albopictus* and *Aedes aegypti*), other insects such as ants, beetle, flies and coakroach were also presence in the XMOS treated buildings (ANN, IA, BI, IJ). Ants from species *Solenopsis geminata* and *Monomorium pharaonis* were observed unaffected by the XMOS sprays. The ants were observed foraged on food baits placed in the buildings. Flies (*Drosophila* sp.), cockroach (*Blattella germanica* and *Periplaneta americana*) and crazy ant, *Paratrechina longicornis* were found affected by the XMOS. The insects were observed presence during pre- and post treatment periods but absence during treatment period. The list of insects observed during study period is presented in Table 2.

Table 2: Insect observed in the study sites during six weeks XMOS treatment

Insect/ Period	**Presence of insect**			**Status by XMOS**
	Pre	**Treatment**	**Post**	
1. Fire ant *Solenopsis geminata*	+	+	+	Unaffected
2. Longhorn crazy ant *Paratrechina longicornis*	+	-	+	Affected
3. Pharaoh ant *Monomorium pharaonis*	+	+	+	Unaffected
4. African black beetle *Heteronychus arator*	+	-	+	Unaffected
5. Cockroach *Blattella germanica*	+	-	+	Affected
6. Cockroach *Periplaneta americana*	+	-	+	Affected
7. Fly *Drosophila* sp.	+	-	+	Affected

Auto dissemination test in laboratory

Female *Aedes albopictus* survival decreased with age for both treatment (Figure 6A) and control (Figure 6B) groups. The oldest ages observed were 23 and 22 days for treatment and control groups respectively. Females started to lay eggs at the age of seven days old (mean=2 eggs) for treatment group. The last batch of eggs was laid at the age of 22 days old (mean=3 eggs) and the highest number of eggs was laid at the age of 13 days (mean=60.6 eggs). While for control group, the earliest presence of eggs was at 6 days old (mean=43 eggs) and the last eggs was laid on day 21 days old. The highest number was mean= 122 eggs lay at day 14. Average lifespan of *Aedes albopictus* female was 10.31 and 10.96 days for treatment and control groups respectively (Figure 7).

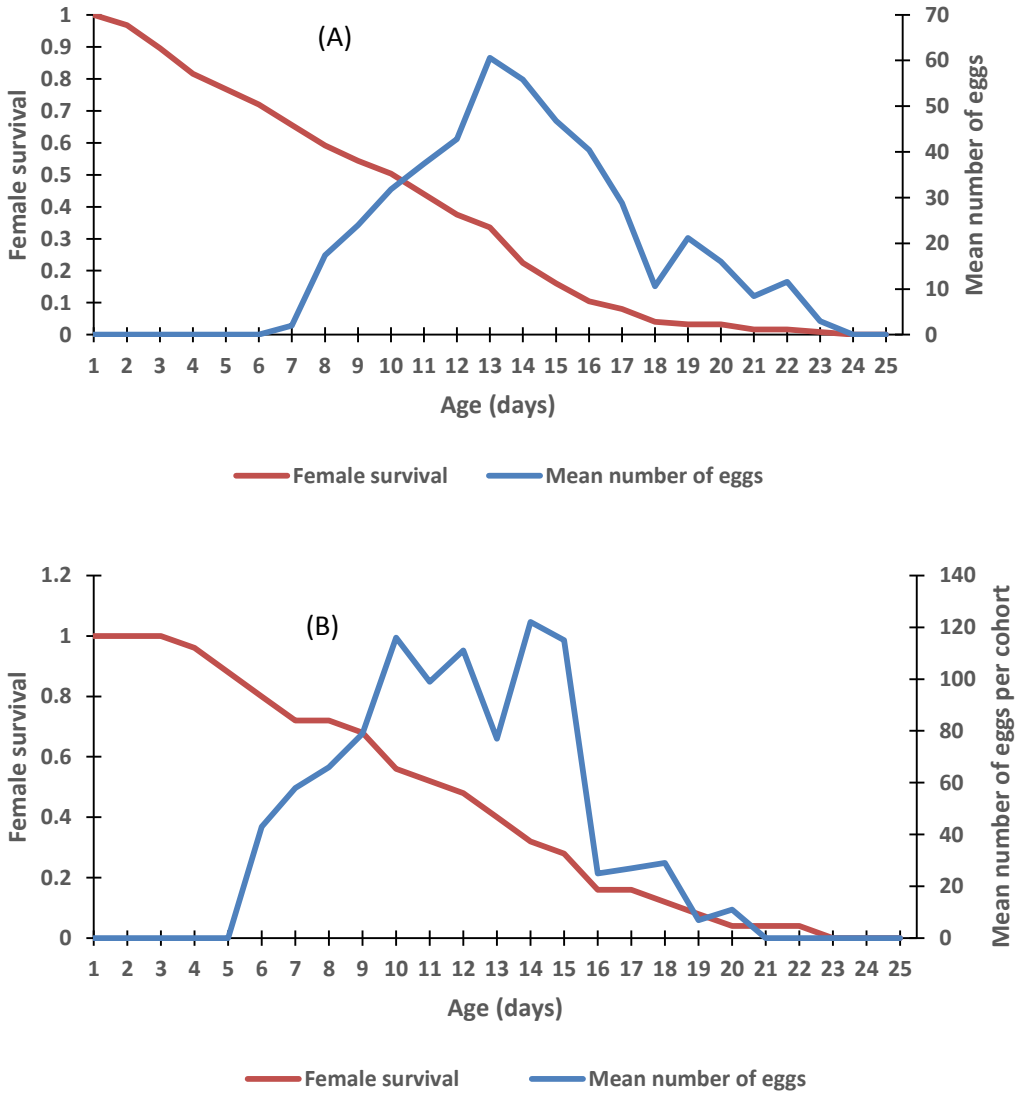

Figure 6: Survival and eggs produced by *Aedes albopictus* cohort for (A) treatment and (B) control groups during auto dissemination test.

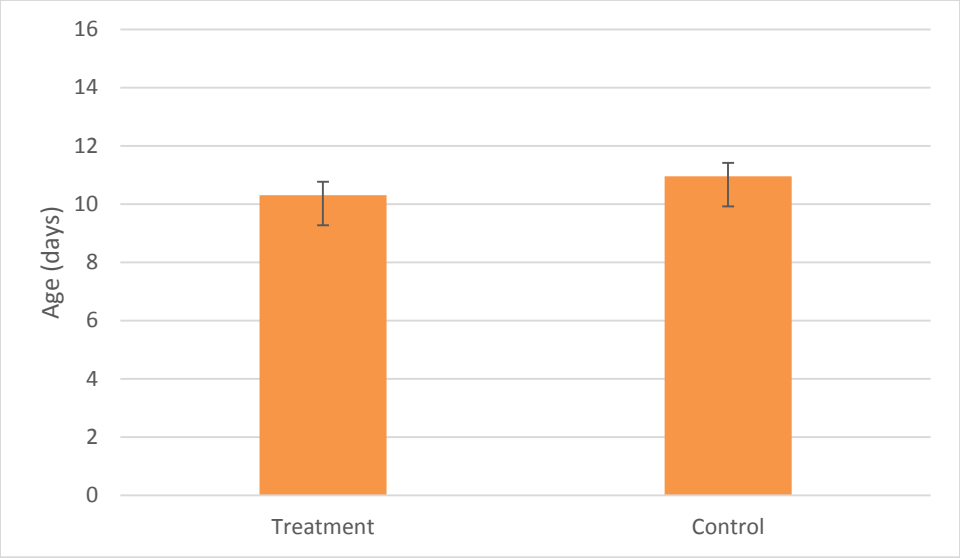

Figure 7: Average life span of *Aedes albopictus female* in auto dissemination test

It was found that in treatment group, *Aedes albopictus* laid eggs in all cups (treated and untreated). Percentages of hatching (%) ranged between 56.4 to 58.6. Low hatching of 1% was observed in cup containing pyriproxyfen solution. The oldest immature stage observed to survive after eggs hatched was 2nd stage larvae.

For control group, 47 to 50% off egg hatch was observed. The larvae successfully reach late stage (4th stage larva and pupa) and eventually one adult emerged (Table 3).

Table 3: Percentage of hatching (%) from different cages.

Cage	Containers	Mean egg	Mean % of hatching	Oldest stage survived	Adult emergence
Treatment	C1(water)	107.8	58.00	2nd	0
	C2(water)	119.2	56.40	2nd	0
	C3(water	89.6	58.60	2nd	0
	C4(Pyriproxyfen)	137.6	1.00	2nd	0
Control	C1	219	50	4th	0
	C2	207	50	Pupa	0
	C3	278	43	Pupa	1 (male, *Ae. Albopictus*)
	C4	250	47	4th	0

Conclusion

From the present study, A-MHS utilization successfully reduced *Aedes* population in student housings. Looking at the hatching percentage data, probable auto dissemination occurred since no egg hatching was observed for the eggs from both treated untreated ovitraps. In laboratory, eggs from untreated cups in treatment group hatched but none successfully emerged into adults proven that female *Aedes albopictus* probably transferred small dose of pyriproxyfen to untreated cups. During oviposition adult females transfer lethal concentrations of an insect growth regulator (IGR) to the oviposition site and ensued with the reduction of mosquito population through larval control (Swale et al., 2018).

X'MOS aerosol (Metofluthrin 0.76%) also reduced the number of eggs collected in treated buildings probably by killing and repelling gravid female from entering the buildings. Other insects affected by XMOS were Cockroaches (*Blattella germanica* and *Periplaneta Americana*) fly, Drosophilla sp., crazy ant (*Paratrechina longicornis*). Ritchie & Divine (2013) reported that female *Ae. aegypti* placed in metofluthrin treated room were affected and those female adults were knocked down after 15-30 minutes after exposure. This proves that the metofluthrin based formulation used for X'MOS spray helps to reduce the population of targeted insects.

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