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16 May 2013

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Dear Sir

I am enclosing the interim test report for the research of laboratory and small scale field trial of mosquito home formulation (ASC 0.04% w/w and BACM 0.04% w/w) against dengue vector *Aedes aegypti* in a tropical environment. (One Tean Network 001/2013)

With all good wishes.

Yours sincerely

Dr Zairi Jaal
Professor
& Coordinator

Encs

STRICTLY CONFIDENTIAL

001/2013

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INTERIM TEST REPORT

TITLE

LABORATORY & SMALL SCALE FIELD TRIAL EVALUATION OF MOSQUITO HOME FORMULATIONS (ASC 0.0400% w/w AND BACM 0.0400% w/w) AGAINST DENGUE VECTOR *Aedes aegypti* IN A TROPICAL ENVIRONMENT

TESTING FACILITIES

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DATE SAMPLE/S RECEIVED	**: 05-FEB-2013**
DATE TEST STARTED	**: 13-FEB-2013**
DATE TEST COMPLETED	**: 18-APR 2013**
DATE OF THE TEST REPORT	**: 25-APR-2013**

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1.0 TITLE

Laboratory & field evaluation of **Mosquito Home** formulations (ASC 0.0400% w/w and BACM 0.0400% w/w) against dengue vector *Aedes aegypti & Aedes albopictus* in a tropical environment.

2.0 OBJECTIVES

1. To conduct laboratory evaluations on **Mosquito Home** with the anti-mosquito formula (ASC 0.0400% w/w and BACM 0.0400% w/w) in terms of egg laying & hatching activity against dengue vector *Aedes aegypti & Aedes albopictus.*
2. To conduct a field trial evaluations on **Mosquito Home** formulations (ASC 0.0400% w/w and BACM 0.0400% w/w) in terms of transferring effect & population reduction against dengue vector *Aedes albopictus.*

3.0 RESEARCH PERSONNEL

Professor Dr Zairi Jaal (Principal Investigator)
Mr Adanan Che Rus (Research Scientist, Vector Control Research Unit)
Supporting staff of VCRU, USM to assist

4.0 MATERIALS AND METHODS

4.1 TEST INSECT

Adult *Aedes aegypti* from well establish colony at Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia was used in this study.

4.2 TEST MATERIALS

4.2.1 Code ASC 0.0400% w/w Insect Growth Regulator (IGR)
4.2.2 Code BACM 0.0400% w/w IGR + 0.0400% w/w Anti-microbial agent

4.3 TEST METHOD

4.3.1 Egg laying Test:

Batches of 20 blood feed adult mosquitoes were released into the cage (30 cm x 30 cm x 30 cm). A cone shape wetted filter paper, soaked in five concentration dilutions of each anti larvae formulations (0.0040% w/w, 0.0020% w/w , 0.0008% w/w & 0.0004% w/w) was introduced into the cage for collecting *Aedes aegypti* eggs. The mosquitoes were allowed to lay their eggs up to 24 hours. Three replicates were set up and an equal number of controls were set up simultaneously with filtered tap water. The test containers were held at 25–28 °C, humidity 60-80% and preferably a photoperiod of 12 h light followed by 12 h dark (12L:12D). After 24 h exposure, the numbers of eggs were recorded.

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Table 1: Dilution rate for the medium in the testing from ASC 0.0400% w/w and BACM 0.0400% w/w

From Formulation	Dilution rate	Final concentration on the oviposition medium (%w/w)
ASC 0.0400% w/w and BACM 0.0400% w/w	1ml:99ml	0.0004
	2ml:98ml	0.0008
	5ml:95ml	0.0020
	10ml:90ml	0.0040
Control	Control	0.0000

4.3.2 Egg Hatching, Larva Survival & Adult Emergence Test

All the eggs collected in the egg laying test were hatch in their respective medium (soaked in five dilutions of each anti larvae formulations (ASC and BACM). The number of eggs hatching were counted up to 72 hours based on the number of larvae emerged. And the larvae were maintained in the rearing tray until they turn into pupae and then monitor the adult emergence. Batches of 20 third or fourth instar larvae were transferred by means of strainers, screen loops or droppers to small disposable test cups, each containing 100ml of water with different concentration of anti mosquito formulations. Small, unhealthy or injured larvae were removed and replaced. The depth of the water in the cups should remain between 5 cm and 10 cm; deeper levels may cause undue mortality.

The appropriate volume of dilution was added (as recommended by manufacturer) to 100 ml water in the cups to obtain the desired target dosage, Four replicates were set up and an equal number of controls were set up simultaneously with filtered tap water, to which 1 ml alcohol (or the solvent used in the formulation) was added. Each test was conducted three times on different days. For pro-longed exposures, larval food was added to each test cup. The test containers are held at 25–28 °C and preferably a photoperiod of 12 h light followed by 12 h dark (12L:12D). After 24 h exposure, larval mortality was recorded. For slow-acting insecticides, 48 h reading was required. Moribund larvae were counted and added to dead larvae for calculating percentage mortality. Dead larvae were those that cannot be induced to move when they were probed with a needle in the siphon or the cervical region. Moribund larvae were those incapable of rising to the surface or not showing the characteristic diving reaction when the water was disturbed.

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If more than 10% of the control larvae pupate in the course of the experiment, the test was discarded and repeated. If the control mortality was between 5% and 20%, the mortalities of treated groups should be corrected according to Abbott's formula:

$$Mortality\ (\%) = \frac{X - Y}{X} 100\ ,$$

where X = percentage survival in the untreated control and Y = percentage survival in the treated sample.

4.3.3 Laboratory Study - Carry Over Factor

Batches of 20 blood feed adult mosquitoes were released into the cage (30 cm x 30 cm x 30 cm). A cone shape wetted filter paper (soaked in five dilution of each anti larvae product (ASC 0.0400% w/w and BACM 0.0400% w/w) was introduced into the cage for collecting *Aedes aegypti* eggs. Another cone shape filter paper soaked in only filtered water (water volume 200ml) also introduce into the cage. The mosquitoes were allowed to lay their eggs up to 24 hours. Three replicates were set up and an equal number of controls were set up simultaneously with filtered tap water. The test containers were held at 25–28 °C, humidity 60-80% and preferably a photoperiod of 12 h light followed by 12 h dark (12L:12D). After 24 h exposure, the number of egg was recorded.

All the eggs collected in the first part of the test (filter paper soaked in anti mosquito formulation) and only filtered water were hatched in their respective medium (ASC and BACM in 0.0040, 0.0020, 0.0008, 0.0004% w/w respectively). The number of eggs hatched were counted up to 72 hours based on the number of larvae emerged. Batches of 20 late second instars larvae were transferred by means of strainers, screen loops or droppers to small disposable test cups, each containing 100ml of water with different concentration of anti mosquito formulations. Small, unhealthy or injured larvae were removed and replaced. The depth of the water in the cups was remaining between 5 cm and 10 cm; deeper levels may cause undue mortality. And the larvae were maintained in the rearing tray until they turn into pupae and the monitor the adult emergence.

From all the laboratory evaluation and results, one of the suitable formulation to proceed for the small scale field trial study within the USM's campus.

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4.3.4 Small Scale Field Trial Study - Carry over Factor

In the small scale field trial, the **Mosquito Home** was placed in one location at Universiti Sains Malaysia Campus, Penang, known as "Durian Valley". The **Mosquito Home** traps were set in the above mentioned location.

Ovitraps were set up with distance of five meter apart between each other in four directions, namely North, South, East and West. There were five ovitraps in each of the respective direction.

Pre-treatment trial period of two weeks, with standard ovitraps (without any treatment in the water) were set up on the respective places to monitor the population baseline of the mosquitoes at the study location. After the first two weeks pre-treatment period, the **Mosquito Home** station was set up at the centre point of the above mentioned 20 ovitraps setting in the four directions. The mosquito population at the study site was monitor further for another three weeks to evaluate the transferring effect in the field conditions. All data collections were done weekly.

Another location approximately one kilometer away from the above mentioned Durian Valley study site was selected near to "Desasiswa Gemilang" and the setting of the ovitraps were the same with the four directions for mosquito population fluctuation comparison as the control reference. However, some of the direction cannot reach the distance of 25 meters.

4.4 DATA ANALYSIS

Data from all replicates were pooled for analysis. Number of eggs laid was calculated from total data. Bioassays were repeated at least three times, using new solutions or suspensions and different batches of adult each time.

Standard deviation of the means of number of egg laid/adult emergence were calculated and recorded.

A test series was valid if the relative standard deviation is less than 25%.

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5.0 RESULT

Table 2 show that eggs has been laid for the all treatment (the water has been treated with the ASC 0.0400% w/w and BACM 0.0400% w/w) larvae control solution at five different concentrations. The mean number of *Aedes aegypti* egg laid in the trial was normal for all the four concentration when compare to control. The adult female mortality exceeded 70% after oviposition for all the lower dilutions (0.0020% w/w, 0.0008% w/w and 0.0004% w/w). For the highest concentrated tested at 0.0040% w/w (40ppm) of both formulations of ASC and BACM, all the adult female mosquitoes (100%) died after laying eggs on the filter papers. This demonstrated that the formulation has no repellency effect against female *Aedes aegypti* mosquito to be attracted to them to lay their eggs there and get themselves kill without any difficulty.

Table 3 show the number of eggs hatched for all treatments (the water has been treated with the ASC and BACM anti larvae solution with 0.0040%w/w, 0.0020% w/w, 0.0008% w/w and 0.0004% w/w dilutions). Almost all of *Aedes aegypti* eggs were hatched in all 4 concentrations when compared to control. However, there were no single adult emergence was recorded for all concentrations tested. From the observation, all the larvae grew normally and became pupae but all of them failed to emerge into an adult mosquito. This showed clearly that the formulations are highly effective to block all the adult mosquito emergence and kill all of them at pupa stage even with the lowest concentration of 0.0004% w/w (4.0 ppm) .

Table 4 showed that the mosquitoes can lay eggs on both oviposition medium prepared (0.0004% w/w & 0.0008% w/w) . The number of eggs was less in the normal oviposition medium. All the eggs in the normal oviposition medium was deposited 12 hour after mosquitoes released. This showed that the mosquito laid their eggs in the Mosquito Home Station first prior to the normal ovi-trap. Almost all the eggs were hatched in both mediums. But in the anti mosquito larvae solution no adult emergence was recorded (pupae cannot emerge to become an adult). All larvae in normal medium and control hatch and emerged into adult mosquitoes. It showed that the carry over factor was not effective when the secondary breeding container's water volume is too much (100ml), as well as the Mosquito Home Station's concentration of 4ppm (0.0004% w/w) & 8ppm (0.0008% w/w) respectively are too low.

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Table 5 showed that **Mosquito Home** station with the ASC 0.0020% w/w (20 ppm) could potentially reduce the mosquito population significantly, base on Ovitrap indeks (OI) :

After one week, the OI reduction is up to 40%. After two weeks, the OI reduction is up to 75% and after three weeks, the OI reduction is up to 80%.

Table 6 it showed that the carry over factor effect with the ASC 0.0020% w/w (20 ppm) could go up to the distance of 25 meters from the Mosquito Home station on all the directions of North, East, West & South.

6.0 CONCLUSION

Anti mosquito formulation ASC and BACM could kill up to 100% of the adult mosquito that come to lay their eggs in the medium containing both formulations as low as 0.0004% w/w (4 ppm). These two formulations also inhibited the adult emergence of 100% at their lowest concentration of 4 ppm. This low and effective dosage is always contains within the station without being releases into the environment nor into the air, this would be very useful for eco-friendly mosquito control.

The egg laying female attracted to the Mosquito Home Station, they pick up the mosquito control solution from the station, and could contaminates the nearby breeding sites, thus killing the larva there and reduces the population within a few weeks time. When set up Mosquito Home station in the field, the ASC 0.0020% w/w (20 ppm) could delivered up to 80% population reduction, based on ovi-trap indexes and effective for up to 20 meter radius.

The above mentioned demonstrated the positive effect of carry over factor from in the small scale field study with the 20 ppm dosage, this also explains that why those observation were not effective from the laboratory studies where they were tested with too low concentration of 4 ppm & 8 ppm respectively.

This Mosquito Home Station concept is potentially a revolutionary tool to control *Aedes spp,* which they have a unique egg-laying characteristic of distributes their eggs over as many breeding containers as they could possibly find to maximize their offspring's survival chances, the Mosquito Home Station is making use of these egg-laying female mosquito behavior as the mechanical vector to bring the formulation to cross contaminate to other hidden breeding sites that nobody could find, killing their own offspring as well as other mosquito during their aquatic stage before they become adult.

25th April 2013

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Appendixes :

Table 2: Mean number of eggs laid (20 female) for *Aedes aegypti* on the oviposition medium mix with the anti larvae formulation (ASC 0.0400% w/w and BACM 0.0400% w/w) and mean number of adult mosquitoes dead after laying eggs.

Formulation	Diluted Concentration (w/w %)	Mean No of egg ± SD	Mean No of Adult mosquito dead ± SD
ASC 0.0400 % w/w	0.0004	**1935±7.64**	**14.67±0.33**
	0.0008	**1894±3.99**	**15.33±0.33**
	0.0020	**1833±4.67**	**17.00±0.57**
	0.0040	**1944±3.99**	**20.00±0.00**
	0.0000	**1904±3.84**	**0.00±0.00**
BACM 0.0400 % w/w	0.0004	**1838±5.24**	**15.33±0.33**
	0.0008	**1899±29.74**	**16.33±0.33**
	0.0020	**1827±4.33**	**16.67±0.33**
	0.0040	**1911±5.51**	**18.00±0.58**
	0.0000	**1897±8.14**	**0.00±0.00**

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Table 3: Mean number of eggs hatched (filter paper) for *Aedes aegypti* on the medium mix with the anti larvae product (ASC 0.0400% w/w and BACM 0.0400% w/w) and mean number of adult mosquitoes emergence (20 larvae per replicate).

Formulation	Diluted Concentration (%)	Mean No of egg ± SD	Mean No of Adult mosquito emergence ± SD
ASC 0.0400 % w/w	0.0004	**1935±7.64**	**0.00±0.00**
	0.0008	**1894±3.99**	**0.00±0.00**
	0.0020	**1833±4.67**	**0.00±0.00**
	0.0040	**1944±3.99**	**0.00±0.00**
	0.0000	**1904±3.84**	**100.00±0.00**
BACM 0.0400 % w/w	0.0004	**1838±5.24**	**0.00±0.00**
	0.0008	**1899±29.74**	**0.00±0.00**
	0.0020	**1827±4.33**	**0.00±0.00**
	0.0040	**1911±5.51**	**0.00±0.00**
	0.0000	**1897±8.14**	**98.99±0.58**

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Table 4: Mean number of eggs laid (20 female) for *Aedes aegypti* on the oviposition medium mix with the anti larvae formulations, both ASC & BACM at 0.0004% w/w (4 ppm) & 0.0008%w/w (8 ppm) and normal medium, Mean number of eggs hatched (filter paper) and mean number of adult mosquitoes emergence (20 larvae per replicate).

Formulation	Concentration Dilution (% w/w)	Mean No of egg ± SD	Mean No of eggs hatching ± SD	Mean % of Adult emergence ± SD
ASC 0.0400% w/w	0.0004 (a)	**1233.67±6.12**	**1232.33±5.61**	**0.00±0.00**
	Water (a)	**256.00±18.33**	**250.00±15.09**	**100.00±0.00**
	0.0008 (b)	**1502.67±9.53**	**1500.33±9.59**	**0.00±0.00**
	Water (b)	**274.67±3.18**	**271.67±2.03**	**100.00±0.00**
	0.0000	**1666.33±15.30**	**1666.00±15.31**	**100.00±0.00**
BACM 0.0400% w/w	0.0004 (a)	**1704.00±9.64**	**1699.67±6.06**	**0.00±0.00**
	Water (a)	**268.33±4.91**	**265.33±2.96**	**100.00±0.00**
	0.0008 (b)	**1614.67±15.19**	**1613.67±14.19**	**0.00±0.00**
	Water (b)	**275.33±7.53**	**273.67±6.94**	**100.00±0.00**
	0.0000	**1727.33±9.59**	**1724.33±9.21**	**100.00±0.00**

Only two concentrations were selected for this trial base on the result in egg laying testing.

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Table 5: Percentage of ovitrap index in Durian Valley and Desasiswa Gemilang before and after setting **Mosquito Home** Station. Only formulation ASC 0.04% w/w was used for this study. The concentration used was 0.0020% w/w (20 ppm).

	Direction of Ovitrap from the Mosquito Home Station				
	East	**West**	**North**	**South**	**Mean OI**
1st Week	100	100	80	100	95
2nd Week	100	100	100	100	100
3rd Week	60	60	60	60	60
4th Week	20	20	20	40	25
5 Week	20	20	0	20	20
	Direction of Ovitrap in the Control				
	East	**West**	**North**	**South**	**Mean OI**
1st Week	100	80	80	100	90
2nd Week	100	100	100	100	100
3rd Week	80	100	100	100	95
4th Week	100	80	100	100	95
5 Week	100	100	100	100	100

Colum highlighted with green was pretreatment week

The Colum Highlighted with red was treatment week (with Mosquito Home Trap

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Table 6: Percentage of ovitrap index in Durian Valley before and after setting **Mosquito Home** Station base on distance on each direction

	Distance From Mosquito House Trap					
East	**5m**	**10m**	**15m**	**20m**	**25m**	**TOTAL OI**
1st Week	20	20	20	20	20	100
2nd Week	20	20	20	20	20	100
3rd Week	0	20	20	20	0	60
4th Week	0	0	0	0	20	20
5 Week	0	0	0	0	20	20
West	**5m**	**10m**	**15m**	**20m**	**25m**	
1st Week	20	20	20	20	20	100
2nd Week	20	20	20	20	20	100
3rd Week	20	20	0	20	0	60
4th Week	0	0	20	0	0	20
5 Week	0	0	0	0	20	20
North	**5m**	**10m**	**15m**	**20m**	**25m**	
1st Week	20	20	20	20	0	80
2nd Week	20	20	20	20	20	100
3rd Week	20	20	20	0	0	60
4th Week	20	20	0	0	0	40
5 Week	0	0	0	0	0	0
South	**5m**	**10m**	**15m**	**20m**	**25m**	
1st Week	20	20	20	20	20	100
2nd Week	20	20	20	20	20	100
3rd Week	20	20	0	0	20	60
4th Week	0	0	0	0	20	20
5 Week	0	0	0	20	20	40

Colum highlighted with green was pretreatment week

The Colum Highlighted with red was treatment week (with Mosquito House Trap

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