



## FINAL REPORT

### TITLE

**PRELIMINARY LABORATORY BIO-EFFICACY EVALUATION OF DRBEDBUGS AND INVISIBLE DRMOS NATURAL BAD BUGS REPELLENT AGAINST FLYING INSECT (*Aedes Aegypti*) USING MICE REPELLENT TEST AND CRAWLING INSECTS (GERMAN COCKROACHES *Blattella Germanica*) USING HARBOURAGE CHOICE TEST METHOD IN VCRU, USM, PENANG, MALAYSIA.**

DRMOS LABORATORY

Household & Public Health, R&D Division


Shah Alam / Malaysia

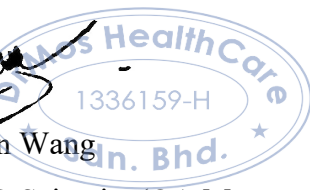
Date: 26 Oct 2021

Prepared By :

Name

Designation

  
: Dr Lee Yean Wang  
: Chief R & D Scientist (QA Manager)



DATE SAMPLE/S RECEIVED: 1 September 2022

DATE TEST STARTED: 5 October 2022

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## 1.0 TITLE

Preliminary laboratory Bio-efficacy evaluation of DrBedBugs and Invisible DrMos Natural Bad Bugs Repellent against flying insect (*Aedes aegypti*) using mice repellent test and crawling insects (German cockroaches *Blattella germanica*) using harbourage choice test method in VCRU, USM, Penang, Malaysia.

## 2.0 OBJECTIVE

To evaluate the bio-efficacy of DrBedBugs and Invisible DrMos Natural Bad Bugs Repellent against flying insect (*Aedes aegypti*) using mice repellent test and crawling insects (German cockroaches *Blattella germanica*) using harbourage choice test method in VCRU, USM, Penang, Malaysia.

## 3.0 RESEARCH PERSONNEL

Dr Lee Yean Wang (Principal Investigator)

Lee Jie Xi (Research Assistant)

Lee Jie Yu (Research Assistant)

Technical Staff of DrMos Laboratory to assist.

## 4.0 MATERIALS AND METHODS

### 4.1 Test Samples

A minimum of 1 liter of DrBedBugs and Invisible DrMos Natural Bad Bugs Repellent were provided by the company, DrMos HealthCare Sdn Bhd. The samples tested are as followed:

<b>Samples</b>	<b>active ingredient</b>
DrBedBugs Natural Insects Repellent	Melaleuca (Aus), Lavender (France), Eucalyptus (Aus), Peppermint (UK), Rosemary (Spain), Invisible Technology Ingredients from USA & Germany, Langkawi Blue Water
Invisible DrMos Natural Bad Bugs Repellent	Lemongrass (Thailand), Eucalyptus (Aus), Peppermint (UK), Rosemary (Spain), Invisible Technology Ingredients from USA & Germany, Langkawi Blue Water

### **Mosquitoes Repellent Test using Wired trapped mice (Mice Repellent Test)**

This test is conducted in a Glass Chamber measuring 70cm x 70cm x 70cm (Figure 1). A total of 20 laboratory-cultured, sucrose-fed adult female mosquitoes (*Aedes aegypti*) aged 2-5 days are released into the chamber.

DrBedBugs and Invisible DrMos Natural Bad Bugs Repellent is mosquito inhibitor and repellent, as such, during the evaluation, a “wired trapped mice” (figure 2) treated with DrBedBugs and Invisible DrMos Natural Bad Bugs Repellent at the dose of 2 ml / mice was placed in the chamber to count the mosquito bite/landing before treatment (pre-treatment) and post-treatment for an exposure time period of 5 minutes. The post-treatment assessment were conducted at 30, 60, 120, 180 and 240 minutes. A “mosquito bite” test using “wired trapped mice” control was carried out where the mice does not spray or use any household product.



Figure 1: Glass Chamber



Figure 2: Wired Trapped Mice

The calculations of percentage of reduction have taken into consideration natural biting behaviour of untreated (control) mice at pretreatment and post-treatment based on the following formula:

$$\% \text{ Reduction} = 100 - \frac{(C1 \times T2)}{(T1 \times C2)} \times 100$$

C1: No of mosquitoes bite/landing against 'control' mice at pretreatment

C2: No of mosquitoes bite/landing against 'control' mice at post-treatment

T1: No of mosquitoes bite/landing against 'treated' mice at pretreatment

T2: No of mosquitoes bite/landing against 'treated' mice at post-treatment

Preliminary dermal sensitization observation for the treated mice were carry out for a period of 7 days post-treatment, to study the effect of the DrBedBugs and Invisible DrMos Natural Bad Bugs Repellent against treated mice.

All tests to be conducted at a temperature of 26-28°C and relative humidity of 65-85%. A minimum of three tests is conducted.

### Harborage Choice Test (German cockroaches)

The first test system represents a laboratory choice test (Figure 3). It consisted of a Container (200mm x 200mm x 100 mm height) with two harborage for the German cockroaches, each consisting of two filter papers (0.018 m<sup>2</sup>, 60 ml/m<sup>2</sup>). The filter papers were fold up as cockroaches harborage. One of the harborage was treated with the repellent substance, and the other was untreated. The German cockroaches could move to the harborage or stay outside in the dish. The treated and untreated filter papers were swapped for the replicates to avoid side preferences of the German cockroaches choosing a harborage. For every substance, six replicates ( $n = 120$ -German cockroaches) were tested at the same time.

Top view of the harborage choice test setup. (a) Plastic container with German cockroaches; (b) treated, and (c) untreated filter paper harborage.



Figure 3: Plastic Container

Untreated controls were conducted to determine German cockroaches distribution in the two harborage. The German cockroaches distribution was documented 24 h after their release. The respective treatment experiments and controls were conducted simultaneously. With a total of  $n = 120$  German cockroaches.

At the beginning of the experiment, 20- German cockroaches (sex ratio 1:1) per replicate were put in the middle of the plastic container between the harborage. The number of German cockroaches in the treated and untreated harborage or outside in the container was documented every 60 min for 2 h. Afterward, the room was completely darkened. After 24 h, 48h and 72h, the position of all German cockroaches was counted again.

Table 1 indicated that DrBedBug provided 94.44±5.55%, 96.73±9.32%, 84.72±9.72%, 84.32±1.54%, 56.25±5.40% and 49.48±10.22% of landing / biting reduction at 0h, 0.5h, 1h, 2h, 3h and 4h, respectively. While Invisible DrMos showed 89.94±7.31%, 89.21±10.79%, 85.77±7.11%, 84.16±8.70%, 58.72±14.93% and 47.07±7.19% of landing / biting reduction at 0h, 0.5h, 1h, 2h, 3h and 4h, respectively. The average weight of the mice used for the tests are 27.93±0.29 g, 27.78±0.41 g and 27.90±0.27g for evaluation of control, DrBedBug and Invisible DrMos, respectively.

Table 1: Results showing the total mosquito landing/biting and percentage of reduction for DrBedBugs and Invisible by DrMos natural insect repellent treated mice adjusted with control mice in the Glass Chamber.

		Mean ± SE				
		Control	DrBedBug		Invisible DrMos	
	Time (h)	mosquito landing/biting	mosquito landing/biting	% Reduction	mosquito landing/biting	% Reduction
	Pre-treatment	17.33±0.33	16.00±0.58		15.67±0.88	
	Post-treatment 0h	16.33±0.88	1.00±1.00	<b>94.44±5.55</b>	1.33±0.88	<b>89.94±7.31</b>
	0.5h	15.00±0.58	2.00±1.53	<b>86.73±9.32</b>	1.33±1.33	<b>89.21±10.79</b>
	1h	15.67±0.67	2.33±1.45	<b>84.72±9.72</b>	2.00±1.00	<b>85.77±7.11</b>
	2h	16.00±0.58	2.33±0.33	<b>84.32±1.54</b>	2.33±1.33	<b>84.16±8.70</b>
	3h	14.00±1.15	5.66±0.88	<b>56.25±5.40</b>	5.00±1.53	<b>58.72±14.93</b>
	4h	13.33±0.88	6.00±0.58	<b>49.48±10.22</b>	6.33±0.27	<b>47.07±7.19</b>
	Mice weight (g)	27.93±0.29	27.78±0.41		27.90±0.27	

Figure 4 showed that the mice treated with DrBedBug and Invisible DrMos are stay healthy since day's 1 post treatment up to day's 7 observation. No treatment-related clinical signs were observed during the 7 days study period. Neither any erythema or oedema was present, nor the treated area of skin was abraded. Aberrations in the locomotor activity were also absent. The daily food and water consumption were normal. All mice had normal body weight, and appeared active and healthy.



Figure 4: Mice dermal sensitization observation at day's 7 post-treatment.

Table 2 indicated that the German cockroaches preferred to hide at control untreated harborage with 85.00±12.92% after 60 minutes and 89.33±8.65% after 2 hours until 3 days in comparison to DrBedBug treated harborage with 15.00±12.92% after 60 minutes and 10.67±8.65% after 2 hours until 3 days observation. Whilst for the Invisible DrMos treated harborage, the percentage of German cockroaches hid were 8.33±8.33%, 6.17±4.90%, 6.17±4.90%, 5.00±5.00% and 5.00±5.00% for 60 minutes, 2 hours, 1 day, 2 days and 3 days post evaluation respectively.

Table 2: Results showing the percentage of German cockroaches found in the harborage treated with DrBedBugs or Invisible by DrMos natural insect repellent compared to control untreated harborage in the container.

Post-treatment	% Mean ± SE		% Mean ± SE	
	Control	DrBedBug	Control	Invisible DrMos
60 min	85.00±12.92	15.00±12.92	91.67±8.33	8.33±8.33
120 min	89.33±8.65	10.67±8.65	93.83±4.90	6.17±4.90
1 day	89.33±8.65	10.67±8.65	93.83±4.90	6.17±4.90
2 days	89.33±8.65	10.67±8.65	95.00±5.00	5.00±5.00
3 days	89.33±8.65	10.67±8.65	95.00±5.00	5.00±5.00

## 6.0 CONCLUSION

Both DrBedBug and Invisible DrMos provided more than 84% repellent activity against *Aedes aegypti* for minimum of 2 hours post-treatment. For the *Blattella germanica*, German cockroaches harborage choice test, more than 89% dislike to hide in the harborage treated with DrbedBug or Invisible DrMos. As conclusion, both DrBedBug and Invisible DrMos Natural Bad Bug repellent are good mosquitoes repellent by provided minimum of 2 hours protection, and able to repel German cockroaches. No treatment-related clinical signs were observed for the treated mice for both DrBedBug and Invisible DrMos during the 7 days study period.

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