

Various Concentrations testing of an Essential Oil (Eucalyptus) to knock down Mosquitoes

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Article Info Volume 81 Page Number: 5854 - 5860 Publication Issue: November-December 2019

Abstract

This paper discusses about the effectiveness of essential oil in controlling dengue vectors. Dengue has been a huge challenge to curb since a very long time especially in Asia, as 70% of the type of mosquito (Ae. Aegypty) that is able to spread the viral infection are found here. However, many methods have been developed in order to control the spread of these viral infection including pesticides, aerosol sprays and many more which are currently being researched and developed. A breakthrough invention like the mosquito buster is discussed further in this paper. The mosquito buster is a vaporizer which has a high effectiveness in repelling mosquitoes. This paper focuses on the effectiveness of essential oil used in the vaporizer in repelling mosquitoes. Mosquitos were captured using the ovitrap for experimental purposes. An experimental setup rig was developed in order to study the knockdown rate of each concentration of essential oil. The concentration of essential oil varies from 1.25% to 10%. Based on the results obtained, it is known that at the maximum time the experiment was ceased which is at the 80th minute, a complete success rate is obtained at an essential oil concentration of 10%.

Article History Article Received: 5 March 2019 Revised: 18 May 2019 Accepted: 24 September 2019 Publication: 27 December 2019

Keywords: Dengue, Essential oil, Mosquitoes

1. Introduction

Dengue is the most quickly growing mosquito-borne viral infection influencing people around the world. Dengue is caused by an infection transmitted mainly by the Aedes aegypti mosquito. With the quantity of instances of dengue fever and serious dengue increment around the world, dengue is a significant risk to general wellbeing. Around 2.5 billion individuals in the more than 100 tropical and subtropical nations where the Ae.aegypti mosquito is found are in danger of contracting dengue. Of this in danger population, 1.8 billion are nations in Asia and the Pacific (WHO 2012a). The greater part of these nations might not have the assets expected to battle the rise of dengue fever and dengue pandemics. Controls of such genuine sicknesses are getting to be progressively troublesome in light of the high rate of multiplication and advancement of imperviousness to insecticides in mosquitoes. Synthetic control of vector mosquitoes is staying as a fundamental part of coordinated vector administration. Resistance has been accounted for to each compound class of bug spray utilized in vector control programs, including microbial bug sprays and insect development controllers.

The look for elective pesticides and control measures that represent no hazard or posturing insignificant hazard to human wellbeing and nature is of incredible enthusiasm from the preventive medication point of view. Pyrethrin-based mosquito fluid details are broadly utilized as a part of numerous nations, particularly in the household of provincial populations. In this setting plant, pesticides restored amid late years, because of the injurious impacts of engineered bug sprays, including absence of selectivity, effect on nature and the development and spread of vermin resistance. The



naturally used pesticide seem to have a promising part in the improvement of future business pesticides for security of the environment and public health. Applying mosquito and much of the time, it is the main approach to keep away from mosquito chomps. Mosquito coil is a standout amongst the most widely recognized family unit bug spray items utilized for individual assurance against mosquitoes in Asian nations. It seems like aerosols and mosquito soils are selling fast followed by liquid spray and vaporizers in the market. The majority of the mosquito curls enrolled and sold in market contain exclusively engineered pyrethroids, for illustration, dallethrin, d-transallethrin and transfluthrin as dynamic fixings. These curls give a high level of assurance from mosquitoes. In any case, many individuals still dislike the scent reminiscent of the mosquito coils containing manufactured pyrethroids when they are burned, and these individuals additionally feel that the coil might be unsafe for their wellbeing. The reality is that mosquito loops containing engineered pyrethroids and other organ phosphorus mixes cause many symptoms such as breathing issue, eye bothering, headache, asthma, itchy and sniffing to the people.

Assessment of essential oils against mosquitoes and confinement, identification and advancement of natural items from them are under the concentration of various research programs around the world. So far, just couple of bug sprays of plant source have reached the market. There is a re-established enthusiasm for plant essential oils items as wellsprings of new insect controlling agent, since they might be biodegradable to nontoxic mixes, hence limiting the collection of dangerous deposits, driving them to be more naturally agreeable contrasted with engineered mixes.

The knock down and slaughtering impact of the plant oils on the grown-up Aedes aegypti were contemplated and the estimations of the plant oils were taken note. Eucalyptus tereticornis (Myrtaceae) has for quite some time been perceived for its insecticidal properties particularly its mosquito repellent movement however presently can't seem to be broadly examined. Research on the utilization of plant-determined chemicals to control mosquitoes and other insect has expanded as of late. This is particularly valid for the utilization of natural product based on plant essential oils (EOs) as insecticides and anti-agents. The present investigation was embraced to assess the bio efficacy of some unpredictable oils against Aedes aegypti grown-up phase in distinctive bioassay techniques.

2. Literature Review

A research work by [1] regarding community perception towards dengue and dengue prevention Program among the residences of a rural settlement in Jempol, Negeri Sembilan is done for residents aged 18 and above. The subjects chosen were randomly done. Data collection was collected through monitored questionnaire that covered six aspects of the Health Belief Model (HBM) which are perceived susceptibility, barrier, benefits, and selfefficacy and cues to act. A total of 306 residents participated in the study of which 57.6 were females and the rest being males. Most respondents were Malay earning an average income of less than RM2000.00 per month.

Based on the survey conducted, most of the respondents had correct knowledge on the cause of dengue, symptoms of dengue and location of mosquito breeding ground. On the contrary however, most of the residents consider fogging as a harmful and inconvenient and that it can be totally monitored by the government authorities. It was concluded that healthy behavior among the residents is still an issue to be sorted and highlighted by the responsible party.



Figure 1: Dengue incidence rate and case fatality rate. [1] The following are the product from the International Conference of Dengue Prevention and Control & International Dengue Expert Consultation Meeting [2]. Based on the Dengue Incidence Rate and Case Fatality Rate which was conducted from the year 2000 till 2014, it can be clearly seen that the fatality rate has since gone down drastically while the incidence rate has reached an all-time peak.

The contributing factors for increased dengue cases are serotype shift (Den 1,2,3 and 4), climate change, poor environmental sanitation, human behavior, mobility of population and ineffectiveness vector control activities. The measures taken in new direction in dengue control are, it has to be a registered dengue case and confirmed by laboratory tests, source reduction activity has been increased and fogging activities has been reduced from two to one cycle only.

The Clinical Practice Guidelines are also being distributed, which mainly highlight on the main features such as early detection of dengue cases, standard and effective dengue management, public awareness on symptoms are signs of dengue and seek early treatment and medical practitioner awareness to detect dengue cases.

The new tools and strategy that has been used in the prevention and control of dengue are residual spray and larviciding activities using temephos EC or BTI. A new



Vaccination Program is also introduced by the Health Ministry of Malaysia As a conclusion, responsive measures are being taken by the responsible parties in the dengue outbreak; however more future research should be focused to enhance the effectiveness, cost effectiveness, sustainability and increasing the scale of existing interventions.

3. Methodology

In this section, there will be brief explanation on how this experiment will be conducted to obtain the result that satisfy the variables used in the experiment. Clear description of describe the materials and equipment used, how the samples were gathered, randomization techniques and how the samples were prepared, how the measurements were made and what calculations were performed upon the raw data and describe the statistical techniques used upon the data. These descriptions would simplify the scope of this project and making sure the developed procedure is satisfying the objectives of the project.

This project will be conducted to test whether the sample mosquito prepared able to withstand the sample organic insecticide prepared. The insecticide will be prepared with different level of concentration with the knock-down rate directly proportional to the rate of time change and concentration level of the insecticide. Initial step was to plan and setup the experiment layout, on how and what will be the constraints on conducting the experiment. The initial plan was to catch the mosquito using the confinement cage but as reviewed with lecturer but seems to be tedious in using that technique where the possibilities of the mosquito to escape from the cage is high. Nonetheless, the samples that need to be prepared which is the mosquito can't be stored for long-period in the cage.

So, there is a different method approach in containing the mosquito where by preparing the inverted placed bottle contained mixture of yeast and sugar solution on a cotton wool. This method seems to be working fine according to the review gathered from internet. As planned earlier, going to prepare few traps to be set around Kelompok Amanah as well as near the sewage processing area. The trap will be build using water bottle. As a mitigation plan, bi water bottle will be used to manually catch the mosquito. As for running the experiment in an enclosed area, the cage mosquito will be used to contain the mosquito and test it with insecticide vapor made of selected essential oil as a part of organic insecticide approach. Why is that so, is to reduce the harmfulness of the vapor and eco-logical friendly with acceptable odor.

The vaporizer will be mounted to the mosquito confinement cage by attaching hose from vaporizer to the container. The vaporizer is powered by electricity source and at the end of the cage, a blower will be added to increase the diffusion rate of the vapor and wide-spread of the vapor. There is two critical approach whereby with the attempt of space repellent technique in an enclosed space and another will be the observation of the mosquito knock-down by enhancing the knock-down rate value. So as mentioned earlier there are 4 critical methods has been implemented with the knowledge of turbomachinery as well mosquito repellency technique.

A. Confinement of Mosquito Samples: In order to contain the mosquito samples, simple cage made of Perspex with netted enclosure top. The prepared insecticide will be released in this cage through the hose connection.

B. Catching Mosquito Sample: There is line-up of 2 methods which is available to catch the mosquito samples. Yeast bait trap is an approachable method to be considered as the review obtained through the internet was satisfying with some tweaks. Next would the manual and hard way which is catching the mosquito with big bottles which has been split into two portion. When the mosquito approaches the target, the bottle will be quickly closed to contain the mosquito.

C. Conducting Test with Mosquito Samples Prepared: The test will be conducted inside the container by releasing the sample prepared. The vaporizer will be mounted to the mosquito confinement cage by attaching hose from vaporizer to the container. The vaporizer is powered by electricity source and at the end of the cage, a blower will be added to increase the diffusion rate of the vapor and wide-spread of the vapor.

Conduct of Experiment

Before conducting the experiment, the list of equipment and the procedure for the experiment was prepared. The list of equipment was basically formed from the procedure needed.

List of Equipment

- 1. Experimental Tank
- 2. Pipe Hose
- 3. Small Containers
- 4. Netting Cover
- 5. Cotton
- 6. Tape
- 7. Glucose
- 8. Vaporizer
- 9. Essential oil
- 10. Blower fan

Procedure

1. The tank was collected to be used to catch mosquitoes.

2. The glucose solution was poured into the small containers with the cotton to make it damp.

3. Once the sugar solution has been done, the sugar solution is then put into the tank.

4. A homemade ovitrap was made and inserted into the tank.

5. The tank was then brought to a specific location where there were a lot of mosquitos.

6. The tank was left there for a few hours to collect mosquitoes.



7. Once collected, the tank was brought back to our testing location which was located at UNITEN's campus called Amanah.

8. The test was then conducted on the same day after collecting the samples.

9. The tank was connected to a hose which was tapped to the mosquito vaporizer.

10. The vaporizer was connected to the essential oil selected which was Eucalyptus

11. The vaporizer was then connected to a power source in order to produce the vaporized solution.

12. 10 ml of acetone is used constantly in the vaporizer container.

13. The concentration of the Eucalyptus tereticornis (Myrtaceae) is added by the number of drops.

14. Each drop is equivalent to 0.05ml

15. The drops are dropped into the water containing

10 ml in the vaporizer container to achieve a total concentration of 1.25% of essential oil.

16. The vaporizer is the turned on and the knockdown time is recorded.

17. To better diffusion rate of the released vapor, at the end of the outlet, a fan was added to increase the rate of

diffusion.

18. The steps 13 to 17 was repeated with concentration of essential oil at 1.25%, 2.5%, 5% and 10%.

19. The results of the knockdown for each concentration is recorded and tabulated into a table of comparison.

Experimental Set Up



Figure 2: Experiment set up



Figure 3: Vaporizer



Figure 4: Hose inlet connection



Figure 5. Handmade ovitraps



Figure 6: Inside the tank



Figure 7 : Sugar solution

The experiment was set up based on Fig 2 above by using all the required equipment. Vaporizer is connected to a power source as shown in Fig 3. The vaporizer is also connected to a container which contains the essential oil solution. Hose inlet connection as shown in Fig 4. Homemade overtrip shown in Fig 5. Meanwhile Fig 6 shows the position of the material inside the tank. The sugar solution is poured into the container containing the cotton to produce a damp cotton to attract the mosquito as shown in Fig 7.Solution concentrations by percentage being used shown in Fig 8, 9, 10 and 11.



Figure 8: 1.25%





Figure 9: 2.5%



Figure 10: 5.0%



Figure 11: 10%

Location set up for ovitrap shown in Fig 12 and Fig 13. These locations were chosen due to the large number of mosquitoes which breeds in this area.



Figure 12: Ovitrap placed at the back of Block A6 (Hostel Amanah)

About 4 empty refills were prepared and each refill was filled with a constant volume of acetone which is about 10 ml. Then the refills labelled as A, B, C and D were differentiated by having different concentration of the Eucalyptus oil with a concentration value of 1.25%, 2.5%, 5% and 10% respectively as shown in Table 1. The concentration was determined or assumed by the number of drops of the Eucalyptus oil added to the refills. The formula (1) shown below was used in order to estimate

the number of drops of the Eucalyptus oil required to be added into the refill of the electric vaporizer.

Table 1: The number of drops required to achieve the specific concentration level

Concentration (%)	1.25%	2.50%	5%	10%
No. of drops	3	5	10	22

The Dimension of the Chamber:

Length, l = 250 mmWidth, w = 250 mmHeight, h = 250 mm

 $Volume = l \times w \times h$ $= 0.25 \times 0.25 \times 0.25 \times 0.25$ $= 0.01563 \text{ m}^3$

Knocked-down (%)) =
No. of mosquitoes knocked down (per interval)	v 100
No. of mosquitoes released	~ 100

(1)

4. Results and Discussions

Results

The results in Table 2, Table 3 and Table 5 tabulate the numbers of mosquitoes got knocked down observed and recorded for various concentration

Parameters Results

Table 2: The number of mosquitoes got knocked down observed and recorded for various concentration at each interval till 80 min.

Time	Concentration (%)			
(min)	1.25%	2.50%	5%	10%
5	0	0	0	0
10	0	0	0	0
15	0	0	0	0
20	0	1	0	2
25	0	1	1	2
30	0	2	1	2
35	1	2	3	2
40	1	2	3	3
45	1	3	3	4
50	2	4	3	4
55	2	5	4	5
60	4	5	4	5
65	4	5	4	7
70	4	5	5	7
75	4	6	5	7
80	4	6	6	7



Table 3: The time taken for the mosquitoes to get Knocked down completely tested with various oil concentrations.

Concentration	1.25%	2.50%	5%	10%
(%)				
Time (min)	180 ± 15	190 ± 15	205 ± 15	240 ± 15

Table 4: The time taken for the mosquitoes to completely recover after it got knocked down with various oil concentrations.

Concentration (%)	1.25%	2.50%	5%	10%
Time (min)	163 ± 0.5	140 ± 0.5	137 ± 0.5	65 ± 0.5

Table 5: Mosquitoes knocked-down rate of the mosquitoes calculated for various concentration at each interval till 80 min.

Time Internal (min)	Concentration (%)			
1 ime 1 nier vai (min)	1.25%	2.5%	5%	10%
5	0	0	0	0
10	0	0	0	0
15	0	0	0	0
20	0	14.29	0	28.57
25	0	14.29	14.29	28.57
30	0	28.57	14.29	28.57
35	14.29	28.57	42.86	28.57
40	14.29	28.57	42.86	42.86
45	14.29	42.86	42.86	57.14
50	28.57	57.14	42.86	57.14
55	28.57	71.43	57.14	71.43
60	57.14	71.43	57.14	71.43
65	57.14	71.43	57.14	100
70	57.14	71.43	71.43	100
75	57.14	85.71	71.43	100
80	57.14	85.71	85.71	100

**The total number of mosquitoes released into the chamber = 7 mosquitoes

5. Discussion

The experiment was done to see how many knockdowns were possible to happen if the mosquito was affected by the Eucalyptus oil. A total of 4 empty refills were prepared as there were 4 main concentrations to be observed at for this experiment. The concentration of the Eucalyptus oil which were chosen at a concentration rate of 1.25%, 2.5%, 5% and 10% respectively because these rates gives the best data to see how many mosquitoes can be knockdown. The volume of acetone that will be mixed with the essential oil will remain constant for every concentration which is at 10 ml. Thus, to conduct the experiment a formula of concentration was used to estimate the number of drops of the Eucalyptus oil that are needed to be added to the refills. This formula can be seen in the above data analysis calculation. By using the volume of Eucalyptus oil added and the total volume, the number of drops for all 4 concentrations were obtained. The number of drops for the concentration of 1.25%, 2.5%, 5%, 10% is 3 drops, 5 drops m 10 drops and 22 drops. With these number of drops, the experiment can be done easily as the Eucalyptus oil can be dropped into the water solution without any trouble.

After setting up the experiment, the time interval for every 5 minutes until 80 minutes was set to observe the knockdown for all 4 of the concentration. The test was done and the data was collected for each concentration which can be viewed at Table 1. From Table 1 it can be observed that the number of the mosquito knockdown increases with the number of time for every concentration. The number of mosquito that is knocked down for the concentration of 1.25%, 2.5%, 5%, and 10% are 4, 6, 6, and 7 number of mosquitoes. The time taken for the mosquito to get knockdown completely by the Eucalyptus oil concentration (Table 3) of 1.25%, 2.5%, 5%, 10% are 163 \pm 0.5, 140 \pm 0.5, 137 \pm 0.5, 65 \pm 0.5. The knockdown time for example 1.25% which is 163 represents the total minutes with a plus and minus of 0.5. From Table 4 which holds the value of time taken for the mosquitoes to completely recover after it gets knocked down can be observed. This table states that at concentration of 1.25%, 2.5%, 5%, 10%, the time taken



for the mosquitoes to get completely knockdown are 180 \pm 15, 190 \pm 15, 205 \pm 15, 240 \pm 15. This time the values of the time obtained have a fluctuating time of plus and minus of 15 minutes.

The dimension of the chamber was calculated using its length, width and height to obtain a total volume of 0.01563 m³. The knockdown rate of the mosquitoes at different concentration at each interval until 80 minutes was calculated using the formula of knockdown which is the number of mosquitoes knocked down per interval by the no of mosquitoes released was used. The number of mosquitoes captured for this experiment is 7 mosquitoes. Thus, from Table 5 it can be observed that at Eucalyptus oil concentration of 10% which is the highest concentration, the knock down rate is at full 100% starting from a time interval of 65 minutes and remained constant till 80 minutes. The rest of the data showed increasing order of knockdown rate as the time increases. For concentration 1.25% the knockdown rate reached a highest value of 57.14% starting from 60 minutes and remained constant till 80 minutes. At 2.5% the highest concentration obtained is 85.71 at 75 minutes and remained constant till 80 minutes. For the concentration of 5%, the highest knockdown rate obtained was also at 85.71 which was at 80 minutes. From the data obtained by the experiment, it can be said that as the number of Eucalyptus oil concentration increases with the interval time, the rate of knockdown (%) increases.

6. Conclusions

This project was conducted successfully by achieving all of its objective given in the required time scale. The effects of eucalyptus oil as a mosquito repellent is clearly is evident from this research. The effects of the eucalyptus oil were studied by varying its concentration from 0%, 1.25, 2.5%, 5% and 10%. An experimental rig was also constructed for this purpose, a container comprising of a hose which is connected to vaporizer ensures 100% effectiveness rate of the essential oil's effect towards the mosquitoes. Based on the experiment that was conducted, the knockdown rate of the mosquitoes is obtained. For concentration 1.25% the knockdown rate reached a highest value of 57.14% starting from 60 minutes and remained constant till 80 minutes. At 2.5% the highest concentration obtained is 85.71 at 75 minutes and remained constant till 80 minutes. For the concentration of 5%, the highest knockdown rate obtained was also at 85.71 which was at 80 minutes. From the data obtained by the experiment, it can be said that as the number of eucalyptus oil concentration increases with the interval time, the rate of knockdown (%) increases.

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