

Research Report

## THE EFFECTIVENESS OF HERBAL MOSQUITO COILS “MORIZENA” AGAINST *Aedes aegypti* DEATH

Rina Priastini Susilowati<sup>1a</sup>, Win Darmanto<sup>2</sup>, Nanik Siti Aminah<sup>3</sup>

<sup>1</sup> Department of Biology, Faculty of Medicine, Universitas Kristen Krida Wacana

<sup>2</sup> Department of Biology, Faculty of Science and Technology, Universitas Airlangga

<sup>3</sup> Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga

<sup>a</sup> Corresponding author: rina.priastini@ukrida.ac.id

### ABSTRACT

*Aedes aegypti* is a mosquito vector of Dengue Hemorrhagic Fever (DHF) which is one of endemic diseases in Indonesia. Until now the way of handling is to use synthetic insecticides. The mosquito coil is the most widely used insecticide by the community because it is cheap and easy to use. However, its continuous use can cause resistance to mosquitoes and disrupt the health of its users. The purpose of this study was to find alternative insecticide chemicals that are safer for use derived from Indonesian plants. Morizena is a natural mosquito coil which is a mixture of 40% of permot leaf extract (*Passiflora foetida*), 40% chrysanthemum extract (*Chrysanthemum cinerariaefolium*), 20% essential oil of citronella (*Cymbopogon nardus*). The herbal mosquito coil “Morizena” is as effective to kill *Ae. aegypti* as the use of Transflutrin synthetic chemical mosquito coil. Exposure to herbal mosquito repellent “Morizena” on *Ae. aegypti* for 8 hours / day with a concentration of 500 ppm (P<sub>1</sub>), 1000 ppm (P<sub>2</sub>), 2000 ppm (P<sub>3</sub>), 3000 ppm (P<sub>4</sub>), 4000 ppm (P<sub>5</sub>) and Transflutrin 2500 ppm (K<sub>1</sub>) synthetic fuel mosquito positive control group, and group without exposure to mosquito coils (K<sub>0</sub>) as negative controls. Animal models which are used in this experiment are 25 tail of *Ae. Aegypti* mosquitos for each treatment with 4 replications. The experimental design which are used was a probit test to calculate LC<sub>50</sub> and LC<sub>90</sub>. The results of herbal mosquito coils “Morizena” test given for 8 hours/day was yielded mortality of *Ae. aegypti* by 92% for a concentration of 3000 ppm (P<sub>4</sub>) and 100% to a concentration of 4000 ppm (P<sub>5</sub>) and 100% for test to synthetic mosquito coils transfluthrin 2500 ppm (K<sub>1</sub>). *Ae. aegypti* LC<sub>50</sub> and LC<sub>90</sub> value for treatment of exposure to herbal mosquito coils “Morizena” are 999 ppm and 2977 ppm. Treatment of herbal mosquito coils “Morizena” with graded doses up to 4000 ppm and synthetic mosquito coils Transfluthrin 2500 ppm was caused an increase in the enzyme acetylcholinesterase activity of *Ae. aegypti*. Based on the *Ae. Aegypti* LC<sub>90</sub> value is 2977 ppm. The effective dose of herbal mosquito coils “Morizena” to kill *Ae. aegypti* is 2977 ppm.

**Keywords:** Herbal mosquito coils, *Ae. aegypti*, Morizena, dengue

### ABSTRAK

*Aedes aegypti* adalah nyamuk vector penyakit Demam Berdarah Dengue (DBD) yang merupakan salah satu penyakit endemic di Indonesia. Hingga saat ini cara penanggulangannya adalah menggunakan insektisida sintetik. Obat nyamuk bakar adalah jenis insektisida yang paling banyak digunakan oleh masyarakat karena harganya murah dan mudah digunakan. Namun penggunaannya yang terus menerus dapat menyebabkan resistensi pada nyamuk dan mengganggu kesehatan penggunanya. Tujuan penelitian ini adalah menemukan alternative bahan kimia insektisida yang lebih aman untuk digunakan yang berasal dari tanaman Indonesia. Morizena adalah obat nyamuk bakar berbahan alami yang merupakan campuran dari 40% ekstrak daun permot (*Passiflora foetida*), 40% ekstrak bunga krisan (*Chrysanthemum cinerariaefolium*), 20% minyak atsiri daun-batang sereh (*Cymbopogon nardus*). Obat nyamuk bakar herbal “Morizena” sama efektifnya untuk membunuh *Aedes aegypti* seperti penggunaan obat nyamuk bakar berbahan kimia sintetik Transflutrin. Paparan obat nyamuk bakar herbal “Morizena” pada *Ae. aegypti* selama 8 jam/hari dengan konsentrasi 500 ppm (P<sub>1</sub>), 1000 ppm (P<sub>2</sub>), 2000 ppm (P<sub>3</sub>), 3000 ppm (P<sub>4</sub>), 4000 ppm (P<sub>5</sub>) dan paparan obat nyamuk bakar sintetik Transflutrin 2500 ppm (K<sub>1</sub>) sebagai kelompok kontrol positif, dan kelompok tanpa paparan obat nyamuk bakar (K<sub>0</sub>) sebagai kontrol negatif. Hewan coba yang digunakan adalah nyamuk *Ae. aegypti* dewasa sebanyak 25 ekor untuk setiap perlakuan dengan 4 ulangan. Rancangan percobaan yang digunakan adalah uji probit untuk menghitung LC<sub>50</sub> dan LC<sub>90</sub>. Hasil penelitian yang diperoleh dari paparan obat nyamuk bakar herbal “Morizena” selama 8 jam/

hari adalah mortalitas *Ae. aegypti* sebesar 92% pada konsentrasi 3000 ppm ( $P_4$ ) dan mortalitas 100% pada konsentrasi 4000 ppm ( $P_5$ ) dan 100% untuk obat nyamuk bakar sintetik Transfluthrin 2500 ppm ( $K_1$ ). Nilai  $LC_{50}$  and  $LC_{90}$  pada kelompok paparan obat nyamuk bakar herbal "Morizena" adalah 999 ppm dan 2977 ppm. Paparan obat nyamuk bakar herbal "Morizena" dengan dosis bertingkat hingga 4000 ppm dan obat nyamuk bakar sintetik Transflutrin 2500 ppm menyebabkan peningkatan aktivitas asetilkolinesterase *Ae. aegypti*. Kesimpulannya adalah nilai *Ae. aegypti*  $LC_{90}$  sebesar 2977 ppm, berarti dosis efektif obat nyamuk bakar herbal "Morizena" yang dapat membunuh *Ae. aegypti* adalah 2977 ppm.

**Kata kunci:** Obat nyamuk bakar herbal, *Ae. aegypti*, Morizena, dengue

## INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by the dengue virus and is transmitted through the bite of *Ae. aegypti* mosquito as the main factor.<sup>1</sup> The number of districts/cities in Indonesia infected by DHF in 2008 about 355 districts/cities (71.72%), in 2009 about 384 districts/cities (77.28%), in 2010 about 400 districts/cities (80.48%) and in 2011 about 374 districts/cities (76.5%). The number of patients with DHF are reached 65432 cases, about 596 of them dies. In 2014, 100347 cases of dengue were reported, and within the case, 907 people were dead, compared to the year 2013 as many as 112511 cases.<sup>2</sup> It can be seen from these data that the DHF prevention efforts in Indonesia has not been optimized yet because the number of cases are increasing every year.

One of the methods to break the chain of mosquitoes is by using insecticides to control the vector. This is because the synthetic insecticides are effective, practical, efficacious and economically more profitable. However, because the use of synthetic insecticides continuously will cause environmental pollution, the death of a wide variety of other living things and cause mosquitoes to become resistant, it can even cause mutations of genes in this species. Synthetic insecticides are bio active, contain chemicals which are difficult to be degraded in nature so the residue can contaminate the environment and can degrade the quality of the environment.<sup>3</sup> In addition, the presence of synthetic insecticides in the food chain can cause the death of some other living things and eventually will disrupt the ecosystem stability. Although before it is produced, synthetic insecticides have undergone very rigorous testing requirements regarding its safety. Because synthetic insecticide is bio active and a toxic substance, it will always create harmful effect to humans and environment.

Regarding to the side effects which caused by synthetic insecticides, it is necessary to find alternative materials which not only more environmentally friendly, but also effective in controlling mosquito population. The use of bioinsecticides as a substitute for synthetic insecticides is expected to reduce the problem of environmental pollution. This is due to the use of bioinsecticides does not cause environmental pollution because it contains material that is easily and rapidly degraded in nature and do not harm the environment, both animal and human. It has been also stated by Quarcoo *et al.* in 2014 that the bioinsecticides

has superior properties as biodegradable and harmless to humans and the environment.<sup>4</sup>

One of the chemical controls is mosquito coils. Mosquito coils are a material which produces insecticide fumes and it is widely used to reduce mosquito bites and reduce the density of mosquitoes either by the researchers and the community. Mosquito coils are easy to use, effective and cheap.<sup>5</sup> Therefore; this study is aimed to make mosquito coils made from plants called "Morizena". Herbal mosquito coils "Morizena" is made from a mixture of plant extracts of *Passiflora foetida* leaf, *Chrysanthemum cinerariaefolium* flower extract and essential oil of *Cymbopogon nardus* leaf-stem.

One of the medicinal plants which is assumed contains active ingredients that can be used as a bioinsecticides is *Passiflora foetida*. *Passiflora foetida* is a trailing plant from Indonesia which grows wildly between other plants and can be easily found in garden or trail on the wall and because of that it usually burned or thrown away.<sup>6-8</sup> Based on the previous research conduct by Susilowati in 2013 on how *Passiflora foetida* leaf extract affect the mortality of *Aedes aegypti* larvae in a various concentration start from 500 ppm, 1000 ppm, 1500 ppm to 2000 ppm. The results from the linear regression equation can achieve  $LC_{50}$  from *Passiflora foetida* leaf extract precise concentration of 1068 ppm.<sup>9</sup> However, after tested with the rate of concentration 500 ppm, 1000 ppm and 2000 ppm, it has not been able to reach mortality rate of *Ae. aegypti* more than 90%.<sup>10</sup>

Other plant that has been previously used as popular insect repellent active ingredients are pyrethrins found in *Chrysanthemum cinerariaefolium* flower seeds. Kardinan in 2000 was reported that one of the member of Asteraceae family is *Chrysanthemum cinerariaefolium* which contains bioactive compounds, pyrethrin.<sup>11</sup> These compounds are toxic contact that works as a neurotoxin to insects and can inhibit egg lying and egg hatching of insects. Furthermore, pyrethrin is a component that can kill mosquitoes and have low toxic levels for humans and other mammals and does not leave residue and safe for the environment.<sup>12</sup>

The leaves and stem extract of lemongrass (*Cymbopogon nardus*) has an active substance of citronellal, citronellol, and geraniol which can be used as mosquito repellent. Citronellal oil can also be used as an anti-bacterial, anti-fungal, anti-viral, cytotoxic, anti aflatoxigenic, perfume, food seasoning, the aroma of tea, and insect repellent

because it contains citronellal, citronellol, and geraniol. Citronellal substance has the characteristics of contact toxin. As a contact toxin, citronellal can cause death due to continuous fluid loss so that the mosquito is dehydrated.<sup>13</sup> According to Sakulku *et al.* in 2009 and Fradin in 1998 among the essential oils that exist, citronellal oil is the most effective which can serve as a repellent for 2 hours.<sup>14,15</sup> Citronellal oil showed good efficacy against 44 *Ae. aegypti* with the concentration ranging from 0.05% to 15% (w/v) or in combination with other natural materials or commercial products of insect repellent.

To get more than 90% of *Ae. aegypti*, by referring to some previous research results on bioinsecticides, herbal mosquito coils Morizena is made by mixing a few active ingredient: *Passiflora foetida* leaf extract containing harmaline, harmine and ermanin, *Chrysanthemum cinerariaefolium* flower seed extract containing pyrethrins and the essential oil of *Cymbopogon nardus* leaves-stems with active ingredient citronellal using the mixed composition 40% *Passiflora foetida* leaf extract, 40% *Chrysanthemum cinerariaefolium* flower seed extract and 20% of essential oil of *Cymbopogon nardus* leaves and stems.

## MATERIAL AND METHOD

### Herbal mosquito coils “Morizena”

After several stages of research to determine the percentage of the mixture, then herbal mosquito coils “Morizena” made by mixing a few active ingredient: 40% *Passiflora foetida* leaf extract with ethanol, 40% *Chrysanthemum cinerariaefolium* seed flower extract with ethanol and 20% of essential oil of *Cymbopogon nardus* leaves-stems. Adhesive materials for herbal mosquito coils “Morizena” are starch and honey, filler of mosquito coils is from the residue of *Passiflora foetida* leaf extract and the powder of coconut shell.

### Experimental animals

Experimental animals used were adult mosquitoes *Ae. aegypti*. *Ae. aegypti* eggs that will be bred for two generations to get a pure line for adult mosquitoes obtained from the Veterinary Faculty of Bogor Agricultural Institute. The number of mosquitoes used was 25 each treatment with 4 replications. Adult mosquitoes were used  $\pm$  14 days after instar IV phase.

### Treatment Dose

In this research, the treatment dose for *Ae. aegypti* are grouped as follows: negative control K<sub>0</sub> (without treatment of mosquito coils), positive control K<sub>1</sub> (synthetic mosquito coils transfluthrin 2500 ppm), P<sub>1</sub> (herbal mosquito coils “Morizena” 500 ppm), P<sub>2</sub> (herbal mosquito coils “Morizena” 1000 ppm), P<sub>3</sub> (herbal mosquito coils “Morizena” 2000 ppm), P<sub>4</sub> (herbal mosquito coils “Morizena” 3000 ppm)

and P<sub>5</sub> (herbal mosquito coils “Morizena” 4000 ppm). Each group was given the treatment for 8 hours/day. The treatment begins by burning mosquito coils at 8:00 to 16:00 in each mosquito cage with a multilevel dosage.

### The Bioassay Test

The bioassay testing was used *Ae. aegypti* aged 3–5 days that had previously been fed with sugar water, put in 12x12x12 cm<sup>3</sup> cage with 25 mosquitoes in each cage. The number of cage is matched up to 2 control groups and 5 treatment groups. Mosquito coils were placed next to the cage from 17.00 until 01.00. They were observed in the 5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup>, 60<sup>th</sup>, 120<sup>th</sup>, 180<sup>th</sup>, 240<sup>th</sup>, 300<sup>th</sup>, 360<sup>th</sup>, 420<sup>th</sup>, and 480<sup>th</sup> minute, by counted the number of mosquitoes which pass out and dead, and the calculated the percentage of the death. Temperature, pH and air relative humidity during the test period is also measured and recorded.

Mosquito knockdown rate recorded in 0.5 minutes at intervals of 1 minute up to 10 minutes for the *Ae. aegypti*. The criteria for knockdown mosquitoes are when they are not be able to keep their balance and not be able to fly any longer.<sup>16</sup> Efficacy criteria is taken based on the time of paralysis of the mosquitoes tested (calculated from the data corrected by the mortality and paralysis of tested mosquitoes) in the control group.

### The Acetylcholinesterase Activity Test

The analysis of acetylcholinesterase activity is performed based on the method from Ellman *et al.* in 1961 which applied on the *Ae. aegypti* larvae.<sup>17</sup> However, for this research, the method is modified so it can be applied to the adult *Ae. aegypti*. In a test tube containing potassium phosphate buffer 1.95 mL, with 0.1 M pH 7.5, added 200  $\mu$ L homogenate of *Ae. aegypti*, 150  $\mu$ L DTNB 0.0011 M in phosphate buffer and 100  $\mu$ L test insecticide solution in water and emulsifier alkylaryl polyglycol ether 400 mg/L 0.1%. The insecticide suspension control mixture was replaced with buffer solution. After perfectly whipped and left for 10 minutes, then in each test tube added 100  $\mu$ L of acetylcholine iodide 0.0105 M in phosphate buffer. The test was conducted on six concentrations of mosquito coils. Blank mixture contains the same components as tested mixture, except homogenates enzyme source was replaced by phosphate buffer. The reaction was left for 30 minutes, and then light absorption of the solution in each tube was measured at a wavelength of 412 nm using a spectrophotometer. Acetylcholinesterase activity expressed in molar substrate hydrolyzed per minute per mg.

### Data Analysis

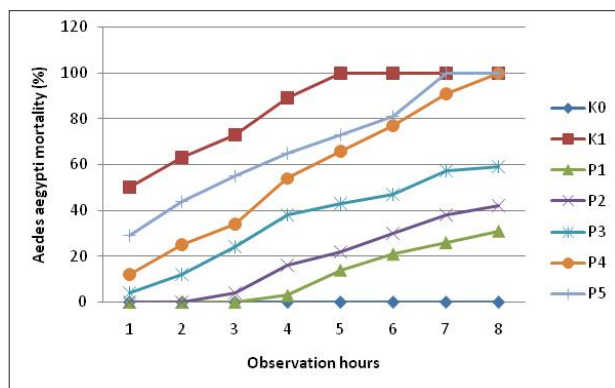
Data were analyzed by linear regression to the death of a mosquito mortality rate, LC<sub>50</sub> and LC<sub>90</sub> using probit test.<sup>18</sup> One way Anova test is used to determine the effect of mosquito coils in the activity of the acetylcholinesterase *Ae. aegypti*.

## RESULT AND DISCUSSION

### The Bioassay Test of *Ae. aegypti*

Toxicity test or bioassay is aimed to determine the killing power of each concentration which tested against *Ae. aegypti*. Toxicity test was conducted on mosquitoes by using herbal mosquito coils “Morizena” from the level of 500 ppm, 1000 ppm, 2000 ppm, 3000 ppm to 4000 ppm (P<sub>1</sub>-P<sub>5</sub>), and also using synthetic mosquito coils transfluthrin 2500 ppm (K<sub>1</sub>).

Determination tests are conducted on *Ae. aegypti* every minute for 8 hours/day reflects the increasing number of deaths (mortality) due to the use of herbal mosquito coils “Morizena” (P<sub>1</sub>-P<sub>5</sub>) and synthetic mosquito coils transfluthrin 2500 ppm (K<sub>1</sub>), the result can be seen in Figure 1.



**Figure 1.** Graph the number of deaths *Ae. aegypti* on treatment of herbal mosquito coils “Morizena” graded dose and synthetic mosquito coils transfluthrin 2500 ppm after 8 hours of observation.

From *one-way* Anova test results are showed that the probability value 0,000, means that there is a significant difference ( $p < 0.05$ ) of all treatment groups to the number of deaths (mortality) of *Ae. aegypti*. Furthermore, to compare the effects of each dose herbal mosquito coils “Morizena” (P<sub>1</sub>-P<sub>5</sub>), synthetic mosquito coils transfluthrin 2500 ppm (K<sub>1</sub>) with negative control (without treatment of mosquito coils) on the number of deaths of *Ae. aegypti* used least significant difference test (LSD). The least significant difference test showed was results that the number of deaths of *Ae. aegypti* without exposure to mosquito coils (K<sub>0</sub>) was significantly different with group exposed with synthetic mosquito coils transfluthrin 2500 ppm (K<sub>1</sub>) and all groups exposed with herbal mosquito coils Morizena graded doses (P<sub>1</sub>-P<sub>5</sub>). Meanwhile, among the group of synthetic mosquito coils transfluthrin 2500 ppm (K<sub>1</sub>) was not significantly different with herbal mosquito coils “Morizena” 3000 ppm (P<sub>4</sub>) and 4000 ppm (P<sub>5</sub>). This study has been showed that administration of synthetic mosquito coils transfluthrin 2500 ppm (K<sub>1</sub>) and a group of herbal mosquito coils “Morizena” 3000 ppm (P<sub>4</sub>) and 4000 ppm (P<sub>5</sub>) caused the death of *Ae. aegypti* more than 90%.

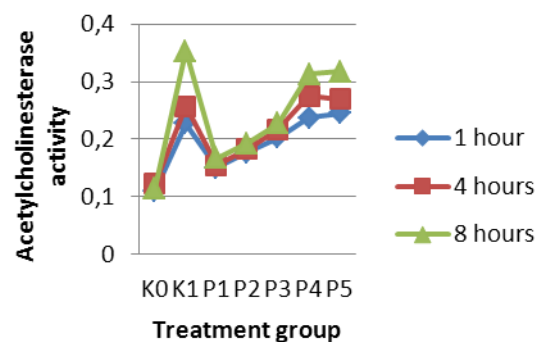
Probit test is commonly used in toxicology to determine the relative toxicity of chemicals, most common outcome

of a dose-response experiment in which probit test is used is the LC<sub>50</sub>. The probit test resulted regression equation  $y = 1.8372x + 0.0159$  ( $y$  is the mortality of *Ae. aegypti* and  $x$  is the dose of herbal mosquito coils “Morizena” with graded dose). From the regression equation the value of the effective dose of herbal mosquito coils “Morizena” that causes mortality of *Ae. aegypti* by 50% (LC<sub>50</sub>) is 999 ppm and by 90% (LC<sub>90</sub>) is 2977 ppm.

Based on the results of this research it can be concluded that herbal mosquito coils “Morizena” is more effective and environmentally friendly compared to synthetic mosquito coils transfluthrin 2500 ppm because the exposure of herbal mosquito coils “Morizena” up to a dose of 4000 ppm can cause 100% death of *Ae. aegypti*. *Ae. aegypti* mosquito LC<sub>90</sub> is 2977 ppm, which means the effective dose of herbal mosquito coils “Morizena” to kill *Ae. aegypti* is 2297 ppm and the effective dose of synthetic mosquito coils transfluthrin 2500 ppm.

### The Activity of the Acetylcholinesterase

After *Ae. aegypti* mosquitos which were used in this research dead, they were tested for the activity of the acetylcholinesterase. Each of *Ae. aegypti* mosquitos were homogenized in the appropriate buffer solution at a temperature of 4°C and 10 g supernatan is used to determine the activity of acetylcholinesterase. The activity of acetylcholinesterase was calculated in the 1<sup>st</sup>, 4<sup>th</sup>, 8<sup>th</sup> hour after the *Ae. aegypti* dead. Measurement of the acetylcholinesterase activity in the *Ae. aegypti* conducted using a modified method of Ellman et al. in 1961.<sup>17</sup> Acetylcholinesterase activity measurement results were expressed in molar substrate hydrolyzed per minute per mg protein as shown in Figure 2.



**Figure 2.** Acetylcholinesterase enzyme activity graph of *Ae. aegypti* on herbal mosquito coils “Morizena” graded dose and synthetic mosquito coils transfluthrin 2500 ppm on the hour to 1, 4 and 8 after the *Ae. aegypti* die.

The analysis of variants of acetylcholinesterase activity in *Ae. aegypti* which exposed with herbal mosquito coils “Morizena” graded dose (P<sub>1</sub>-P<sub>5</sub>) for 8 hours/day are showed that the probability value is 0.000. This means that there are significant differences of treatment effect of herbal mosquito coils “Morizena” graded dose (P<sub>1</sub>-P<sub>5</sub>),

positive control group which was treated with synthetic mosquito coils transfluthrin 2500 ppm ( $K_1$ ) and negative control group without treatment of mosquito coils on the activity of the acetylcholinesterase *Ae. aegypti*. Then the least significant difference test was performed to compare the significance of treatment effects of herbal mosquito coils “Morizena” graded dose ( $P_1$ - $P_5$ ), synthetic mosquito coils transfluthrin 2500 ppm ( $K_1$ ) and negative control group without treatment of mosquito coils on the activity of the acetylcholinesterase in *Ae. aegypti*. The least significant difference test results were showed that each concentration levels of herbal mosquito coils “Morizena” ( $P_1$ - $P_5$ ), synthetic mosquito coils transfluthrin 2500 ppm ( $K_1$ ) and without treatment of mosquito coils ( $K_0$ ) provide a significant difference between each concentration.

An attempt to control the population of *Ae. aegypti* is by spraying chemical insecticides, which can cause adverse effects to the environment, among others, the effects of the emergence of resistance in vectors and cause environmental pollution by chemical insecticides present in the form of a spray, mosquito coils, mats and others. The use of synthetic chemical insecticides with materials or insecticides to anticipate the negative effects that are more environmentally friendly which called biological insecticides (bioinsecticides) is required. Therefore, this research is tried to find new alternative of herbal mosquito coils “Morizena” which is mixture of permot leaf (*Passiflora foetida*), *Chrysantemum cinerariaefolium* flower seed and leaf-stem lemongrass (*Cymbopogon nardus*).

Toxicity test with the statistical approach is measured through  $LC_{50}$  and  $LC_{90}$  concentration of herbal mosquito coils “Morizena” to kill 50% of *Ae. aegypti*, the test used was probit test.<sup>18</sup> Probit test analysis of *Ae. aegypti* death after 8 hours/day of exposure showed that  $LC_{50}$  is 999 ppm and  $LC_{90}$  is 2977 ppm. Based on the toxicity test, the level of concentration applied is the main factors that affect the mortality of *Ae. aegypti* tested. It can be proved with the higher the concentration of treatment the greater the number of dead mosquitoes, with effective dose of herbal mosquito coils “Morizena” is 2977 ppm.

Heterogeneity seen in the probit test is due to the variation of the concentration of mosquito coils used in toxicity testing on *Ae. aegypti*. As Hartati in 2000 was stated that the power of each concentration of insecticide mosquito coils can affect the rate of oxygen consumption and metamorphosis of *Ae. aegypti*.<sup>19</sup> Killing power of biological insecticides (bioinsecticides) is because there is a toxic substance that is toxic to the stomach and contact toxin of the bodied animals. In addition, the use of bioinsecticide is not intended to put synthetic insecticides aside, but only as an alternative way with the aim that people should not rely on synthetic insecticides. Another goal is that the use of synthetic insecticides can be minimized so that the environmental damage can be reduced. Besides that, the use of bioinsecticides will help the development of agroindustries.<sup>20</sup>

Tarumingkeng in 1989 was stated that the first step in observing the effects of poisoning is to observe the physical response behavior of the tested animals.<sup>21</sup> The response is the basis for the classification of toxic materials. Furthermore, the symptoms shown due to the influence of the treatment given will show four stages of the animal's response include excitation, convulsions, paralysis and death.<sup>22</sup>

The stage of adult mosquitoes were stated by WHO in 2009 that the symptoms which has been showed as a result of the effect of insecticides which can stiffen the body of the mosquito. The insecticides attack the nervous system which was indicated by the mosquito's inability to fly, paralysis and knockdown for several times and eventually will die.<sup>24</sup> Death was caused by toxin in the digestive system (spiracles) that causes the mosquito dead.

Generally, a neurotoxin causing four stages of symptoms, namely: excitation, convulsions, paralysis and death. These stages are not necessarily complete and only a few that can be observed. The time between the toxin application and the appearance of first phase symptoms is called the latent period. Latent period is often found in the stomach toxin application. Excitation phase is often preceded by anxiety. At this anxiety stage, the mosquito is showed movements such as cleaning the body such as the antenna or the other body parts. The poisoning process raises signs such rapid movements, restless leg raise, rolling and head twitching. At the end of the excitation mosquitoes will lose balance and ataxia and finally falls.<sup>21</sup>

From this research,  $LC_{90}$  which can cause death of *Ae. aegypti* is 2977 ppm. From these results, it can be stated that herbal mosquito coils “Morizena” which were tested against *Ae. Aegypti*, have been showed high toxic effects (poison) so that it can be classified as a bioinsecticide. According to WHO in 2006, the insecticides is said to be good if it shows high level of concentration with the shorter amount of time.<sup>24</sup> The result is showed that the herbal mosquito coils “Morizena” with graded doses up to 3000 ppm capable in causing *Ae. aegypti* high mortality rate (more than 90%). From observation after exposure of herbal mosquito coils “Morizena”, the mortality of *Ae. aegypti* with the concentration of 3000 ppm ( $P_4$ ) and synthetic mosquito coils transfluthrin 2500 ppm ( $K_1$ ) occur simultaneously in a short time. However, *Ae. aegypti* at the concentration of 500 ppm and 1000 ppm, 2000 ppm were only paralyzed and then were recovered. This is because the amount of toxins in herbal mosquito coils “Morizena” less than 1000 ppm were not clinical enough to *Ae. aegypti*.<sup>25,26</sup>

In this case the target, which is *Ae. aegypti* mosquito, will die when inhaling bioinsecticides in this case is the herbal mosquito coils “Morizena” that contains toxic toxins in sufficient quantities (mixture of harmaline harmine, ermanine, pyrethrin and citronellol). As for the contact toxin due to direct contact with the insecticide through the skin (epidermal tissue). Pyrethrin compounds contained in extracts of chrysanthemum flower seeds and citronellol

of the leaves and stems lemongrass essential oil has a mechanism that inhibits the acetylcholinesterase, resulting in phosphorylation of the amino acid serine at the center of related synaptic enzyme. The toxicity symptoms appear due to accumulation of acetylcholine which causes central nervous system disorders, seizures, respiratory abilities obstruction and death. Mortality occurs in *Ae. aegypti* due to the ability of the active ingredient of herbal mosquito coils “Morizena” for instance harmaline, harmine and ermanine on permot leaf extract, pyrethrin on chrysanthemum flower seeds extract and citronellol in essential oils of lemongrass leaves and stems can interrupt the flow of Na<sup>+</sup> (sodium) in the nerve cells and neurotransmitters (chemical transmitter) in the synapse.<sup>26</sup>

Harmaline, harmine, ermanine and pyrethrin in nerves will extend Na<sup>+</sup> ions to flow into the membrane by slowing or blocking the channel closure. If harmaline and pyrethrin slow down the channel closure, the nerve will be depolarized in a long time, so there will be many Na<sup>+</sup> ions entering the membrane. This will cause symptoms of seizures and trembling. Harmaline, harmine, ermanine and pyrethrins are also able to block the closure of the channel, this situation will cause excess membrane ion Na<sup>+</sup> eventually become inactive nerves. Inactivity is occurring because the nerve is too positive and difficult to repolarize (back to its original state). Symptoms that will appear is paralysis.<sup>26</sup>

Pyrethrin presence at the synapse will interfere the transmitter chemicals or neurotransmitter which is acetylcholine.<sup>26,27</sup> Pyrethrin will increase acetylcholine and inhibit the enzyme from breaking down the acetylcholine. Acetylcholine function is to provide permeability properties at postsynaptic membranes that cause the displacement Na<sup>+</sup> ions, causing depolarization. Acetylcholines will be hydrolyzed by the acetylcholinesterase, which is present in large amounts at the synapse.<sup>25</sup> With pyrethrin, enzymes cannot break down the chemical transmitter acetylcholine so that acetylcholine will continue to increase as the result of the membrane excess positive ions. In these circumstances mosquitoes will paralyze and will eventually die.<sup>27</sup>

Problems of safe environmental pollution for Mosquito Coils Morizena can be done by the method of reducing the concentration of carbon dioxide released through the smoke.

## CONCLUSION

Exposure of herbal mosquito coils “Morizena” with graded doses can increase the activity of the acetylcholinesterase enzyme in *Ae. aegypti* and cause death of mosquito.

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