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Utilization of *Nicotiana Tobacum*'s Extract for Mosquito Extermination with Fogging Method

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Abstract. The number of Dengue Hemorrhagic Fever (DHF) victims in DKI Jakarta province continues to increase, despite many efforts have been made to control the spread of DHF. An example of permissive control to tackle DHF issue is the use of chemical-based fogging for fumigation. However, insecticide has an active compound that causes mosquito resistance and it has been discovered to have side effects on the human body. Its application in fogging also requires petroleum fuels as smoke-making agent. This study intends to substitute fluids formulation in fogging formula. This formulation aims to create liquid fogging products that are free from pyrethroid and petroleum, so fumigation becomes safer for its users. The research was done by formulating tobacco leaf extract as an active insecticide substance with a solvent from propylene glycol and glycerin as a smoke-making agent. The independent variables in this study were the concentration of the active substance and the ratio of solvent (PG:VG). Tests that will be conducted on the samples are LC_{50} toxicity test, physical stability test, organoleptic test, GCMS test. The results of the toxicity test with pyrolysis extract showed that with a concentration of 10%-20% has not reached LC50, the concentration of 30% reached LC_{50} in the 9th minute. The concentration of 40% reached LC_{50} in the 6th minute and the concentration of 50% reached LC_{50} in the 5th minute. For tobacco extract with extraction method, the concentration of 5%-20% has not reached LC_{50}. The concentration of 30% reaches LC_{50} in the 9th minute. The concentration of 40% reached LC_{50} in the 4th minute and the concentration of 50% in the 3rd minute.

Keywords: Aedes aegypti, fogging liquid, tobacco extract, LC_{50}

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a disease caused by a virus from the Arbovirus group which has identified by sudden high fever for no apparent reason, persists for 2-7 days without obvious causes, weak and languid, restless, heartburn, often accompanied by bleeding in the skin in form of red spots, sometimes nosebleeds, blood loss, blood vomit, and decreased consciousness.

One effort to control the spread of DHF is fogging or fumigation in an area with positive patient status, namely when 2 patients or more found in one area; 3 patients within a radius of 100 m from the residence of positive DHF patients; or when 1 dengue patient passed away. While using fogging as the way to control the spread of DHF, attention is taken to the active substance commonly used in liquid fogging specifically malathion.

The active ingredient of malathion in insecticides, even though it has low toxicity, is very easily absorbed by the body and can stimulate the production of malaoxon in the body, which is proven to be 61 times more toxic than malathion. In addition, exposure in high concentrations will lead to its side effects, include skin and eye irritation, cramps, diarrhea, excessive sweating, nausea, seizures, and even death. Malathion residue can also be converted into

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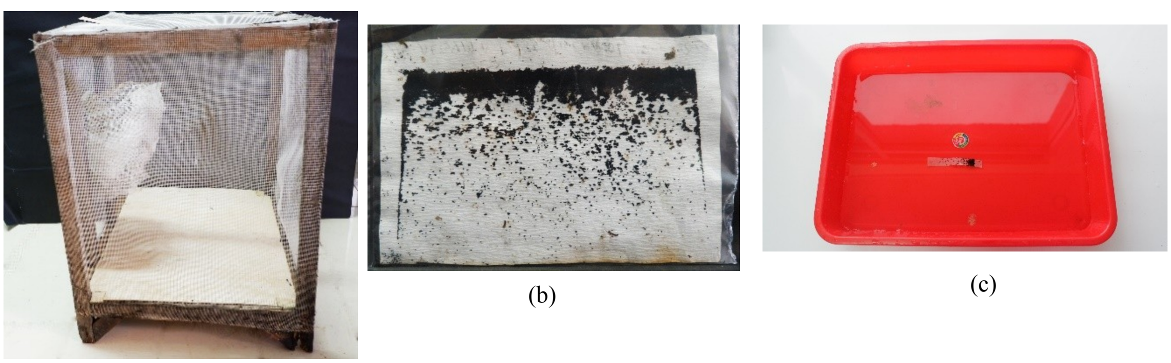
malaoxon when it enters a water body. According to a study in 2010, malathion also has a long-term effect on children, which causes interference with concentration and hyperactivity. In addition, malathion has been classified as carcinogenic class 2A by the US IARC and EPA. So, it is necessary to develop natural mosquito repellent as a substitute for these chemical compounds.

The alternative solution for this problem is by substitute pesticides that contain malathion to tobacco extract as a natural active ingredient in fogging formulations. In tobacco leaves, there are compounds such as nicotine, d-limonene, pyridine and indole which have effects as biopesticides and bio repellent (natural mosquito repellents). Besides being safer for humans, the use of tobacco-based insecticides can increase the value of tobacco and produce high-value tobacco-based products. Not only substitute its active compounds, this study also examined and formulate suitable solvents to replace diesel fuel which is commonly used as a fumigation agent on fogging machines. The type of solvent used is a combination of propylene glycol (PG) and glycerin (VG), a common solvent that is already known as a good solvent in the market. By replace solar solvents with PG and VG, it is expected that the smoke that the machine produced will not sting odor and cause health problems for the society.

MATERIALS AND METHODS

Breeding of *Aedes aegypti* Mosquitoes

Aedes aegypti larvae are bred according to guidelines issued by WHO (2005) for mosquitoes testing in laboratories. The stages begin with *Aedes aegypti* eggs placed on the surface of paper strips stored at room temperature in a tightly closed plastic container. Then the egg hatching container is prepared by adding distilled water and egg food. Paper strips containing eggs are immersed one-third in the distilled water. After 12 hours, the egg will hatch and develop into instar I, then transfer the instar I to a container containing low chlorinated water. Every 2 days, larvae are fed using fish food or mashed chicken stew. Larvae that are being cultured can be transferred to other containers if the water is dirty. In 5-7 days, the larvae will develop in the Instar III (old) stage or IV instar (young) with a length of about 4-5 mm. After reaching the third and fourth stage instars, transfer to a glass tube that has been added a sugar solution and covered with gauze, in 3-4 days the larvae will turn into mosquitoes that ready for testing.



(a) (b) (c)

FIGURE 1. (a) Cage of *Aedes aegypti* mosquito (b) eggs from the entomology lab FKH IPB (c) mosquito eggs strips in culture test containers that are in accordance with WHO

Insecticide Formulation

The formulation is carried out by varying the concentration of the active substance and varying the ratio of the solvent. The variation of the concentration of the active substance used is 5%, 10%, 20%, 30%, 40%,50%. The variation of the solvent ratio (PG:VG) used is 0:100 to 100:0 with a ratio interval for every 5%. Then, the formulation stirred by using a magnetic stirrer at 25°C for 10-15 minutes at a speed of 1000rpm. The sample is 200mL for each variation and stored in an airtight container.

Toxicity Testing

This method aims to determine LC_{50} and LC_{90} guidelines issued by WHO (2005) for mosquitoes testing in laboratories. First, prepare 100 - 200 ml water to containers with water levels between 5 - 10 cm. larvae in instar III and IV phases were prepared (avoid using larvae in small, unhealthy or damaged conditions). The fogging machine contains a sample of 25 mL for each concentration prepared. The larvae are put in a container filled with water, then placed in a closed room, until they become mosquitoes. The fogging machine is turned on and fumigation is carried out until the sample solution runs out. Each concentration was carried out with 2 or more repetitions and the control was prepared with fogging liquid made from active malathion and performed the same treatment as the sample. For longer observations, mosquito food must be added to the container. The test container is placed at 25 - 28 °C. Testing is done with three repetitions on different days. Dead mosquitoes counted when mosquitoes have fallen and are not moving anymore. Dead mosquitoes are observed and recorded in number.

Stability Testing

The physical stability test uses a centrifuge with a speed of 3750 rpm for 5 hours, then observe the separation of the phase. This mechanical test (centrifugation) can be equated with the effect of the gravitational force on the fogging liquid for about one year of storage. (Lachman, Lieberman, & Kanig, 1994).

Organoleptic Testing

Organoleptic testing was conducted to determine the physical changes that occurred in the sample. Organoleptic observation was carried out by observing changes in color, odor, clarity and separation of the fogging liquid phase for 4 weeks. Observations are conducted every week.

Insecticide was stored at 25 °C for 28 days. Every 1; 7; 14; 21 and 28 days after preparation, insecticides are evaluated physically including changes in color, odor, and texture and nicotine content. On parallel work, one of the samples from the formulation tested under the microscope to figure out its emulsion type.

RESULTS AND DISCUSSION

After all sample of PV:VG were formulated, each ratio tested by Fogging Burgess Bug Killer 1443 to find out its ability to create smoke. The analysis of fogging formulation experiment can be seen in Figure 2 and Table 1.

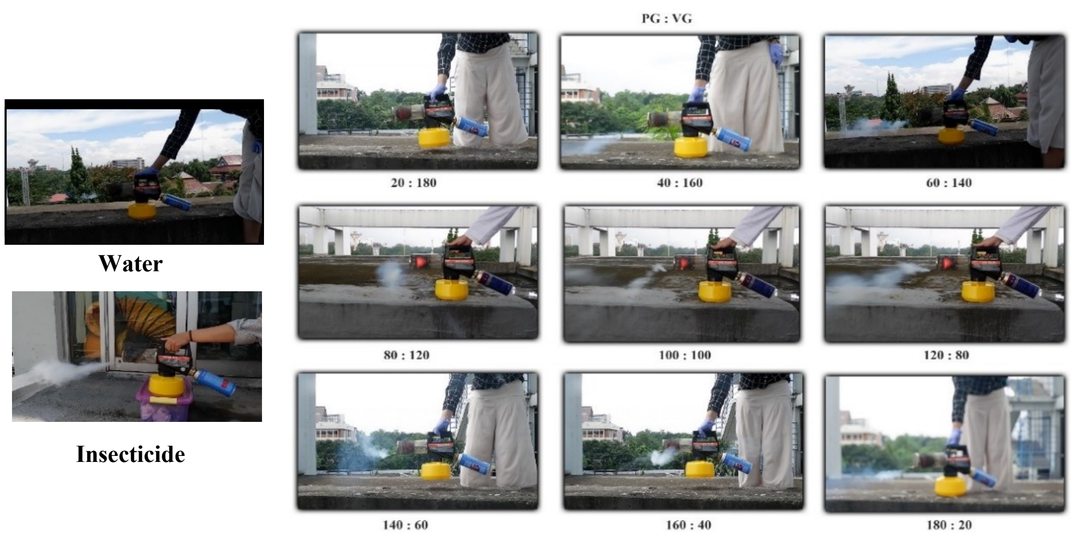


FIGURE 2. Fogging Formulation Experiment

TABLE 1. Fogging Formulation Experiment

Formulation	Time of smoke loss (s)	Smoke thickness
Water	1	Not thick
Insecticide	2	Thick
VG : PG (ml)		
20 : 180	1	Not thick
40 : 160	1	Not thick
60 : 140	1	Not thick
80 : 120	2	Thick
100 : 100	2	Thick
120 : 80	2	Thick
140 : 60	3	Very Thick
160 : 40	3	Very Thick
180 : 20	3	Very Thick

From the data, it concludes that the more Vegetable Glycerin used, the more smoke will be formed, and its smoke becomes thicker. Based on observation, the VG:PG formulations that has similar smoke form in comparison with usual insecticide are: VG:PG 80:120, 100:100, and 120:80. Furthermore, this formulation will be formulated with tobacco leaf extract at different concentrations, ranging from 5% to 50% and each of its formulations will be tested to find out its toxicity towards mosquitoes. The analysis of toxicity test can be seen in Figure 3 and 4.

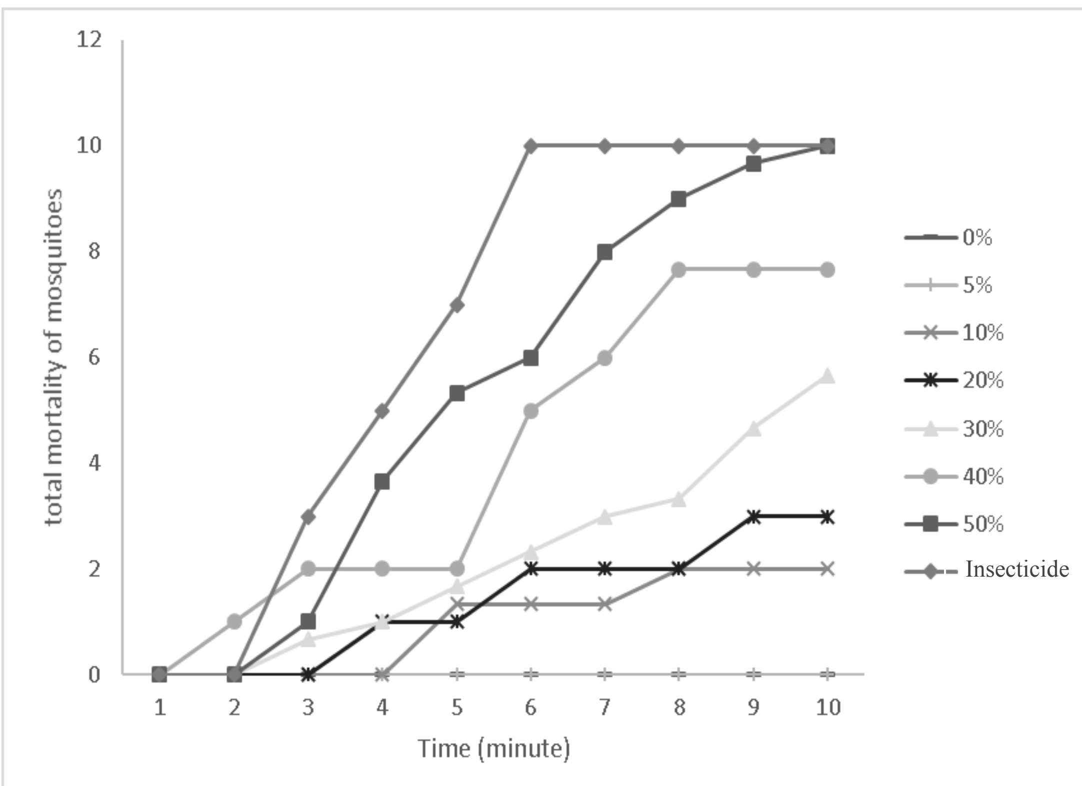


FIGURE 3. Concentration of Pyrolysis Tobacco Extract that Cause the Death of 50% of Experimental Mosquitoes (LC_{50}) in Specific Hours

The death rates of mosquitoes are calculated from the number of mosquitoes that fall or die; 10 mosquitos used in each concentration. In Figure 3, it can be seen that the results of toxicity test showed that the concentration of tobacco extract for 10 minutes between 5%-20% had not reached LC_{50}, the concentration of 30% reached LC_{50} in the 9th minute, the concentration of 40% reached LC_{50} in the minute 6, and the concentration of 50% in the 5th minute. Observations and concentrations of 50% tobacco extract at the 9th minute have met the criteria for the effectiveness of residual pesticides, namely a minimum of 90% of mosquitoes falling which are exposed to residues (Based on the Pesticide Commission).

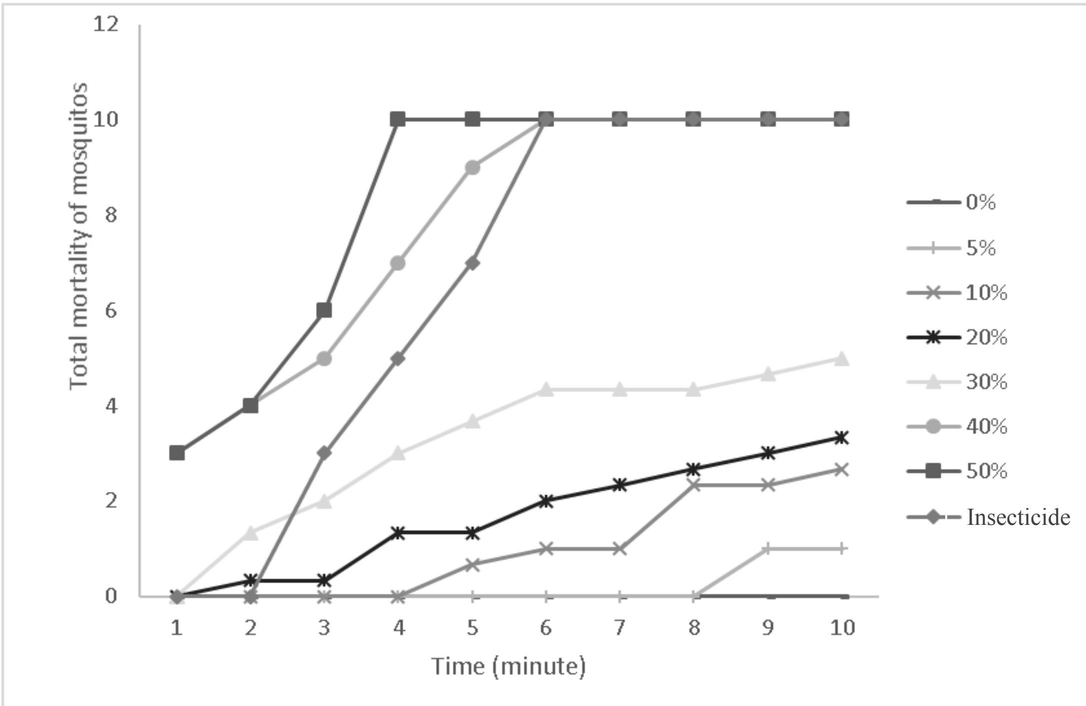
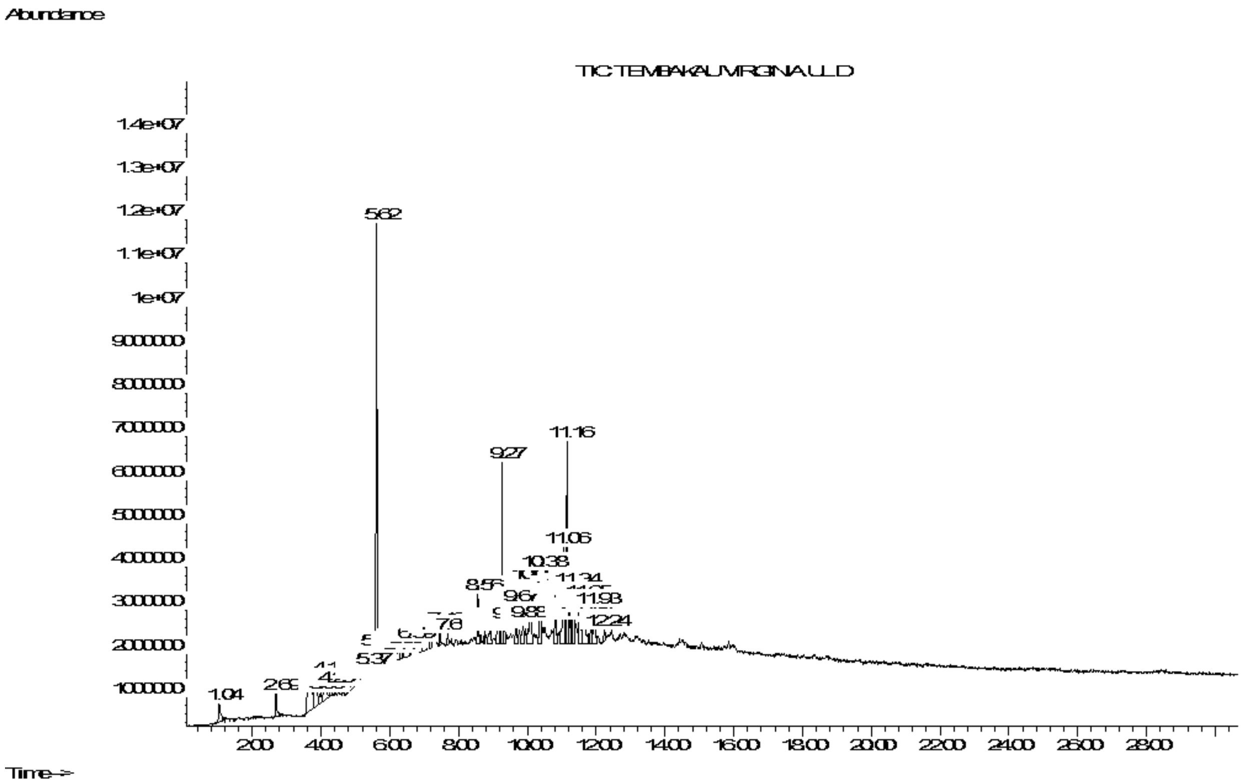
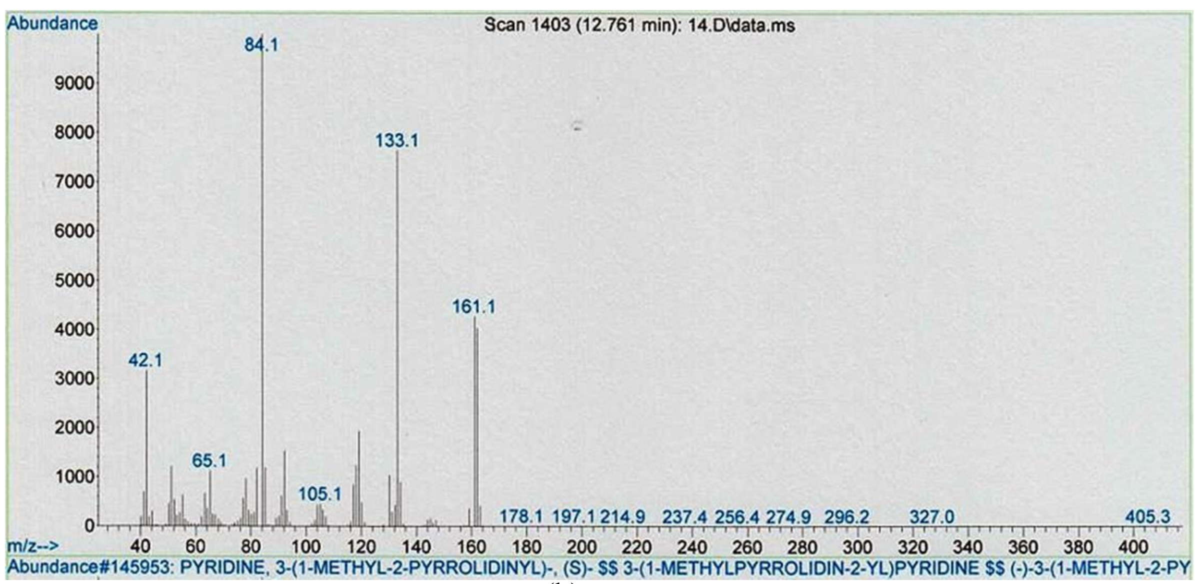


FIGURE 4. Concentration of Extraction Tobacco Extract that cause the Death of 50% of Experimental Mosquitoes (LC_{50}) in Specific Hours

Figure 4 shows the effectiveness of tobacco extract with the extraction method, the concentration of 5% -20% has not reached LC_{50}, the concentration of 30% reaches LC_{50} in the 9th minute. The concentration of 40% reached LC_{50} in the 4th minute and the concentration of tobacco extract was 50% in the 3rd minute. It was seen that the concentration of 40% pyrolysis tobacco extract in the 5th minute of observation and the concentration of 50% tobacco extract at the 4th minute had fulfilled the criteria for the effectiveness of residual pesticides, namely a minimum of 90% fall of mosquitoes exposed to residues (Based on the Pesticide Commission). Based on the data, tobacco extract with extraction method more effective in killing mosquitoes than tobacco pyrolysis extracts. Therefore, the amount of nicotine extract in tobacco extract with the extraction method is bigger than pyrolysis tobacco extract and can be proven through GC/MS test results. The analysis of GC/MS can be seen in Figure 5.



(a)



(b)

FIGURE 5. GC/MS Test in (a)Extraction Tobacco Extract (b) Pyrolysis Tobacco Extract

Based on GC-MS spectral analysis, it was observed that nicotine compounds (49.18%) and linoleic acid (21.27%) in extraction tobacco extract and nicotine compounds (18.01%) and acid 9.12.15-Octadecatrienoic acid (7.76%) in pyrolysis tobacco extract. This concludes that the active compound (nicotine) in extraction tobacco extract higher than pyrolysis tobacco extract. This causes a difference in the effectiveness of fogging, where the tobacco formulation for extraction much faster to reach LC_{50} compared to pyrolysis.

The analysis of stability test can be seen in Figure 6.

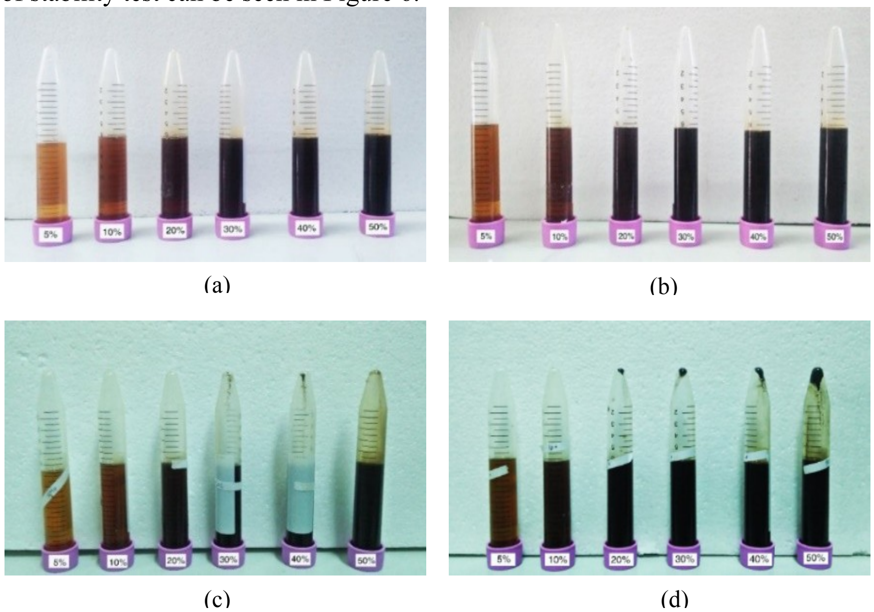


FIGURE 6. Stabilization test (a) liquid fogging pyrolysis before centrifuge (b) liquid fogging extraction before centrifuge (c) liquid fogging pyrolysis after centrifuge (d) liquid fogging extraction after centrifuge

Based on Figure 6, the sixteen fogging formula were not stable against gravity for one year by experiencing separation and precipitation (Lachman, Lieberman, & Kanig, 1994).

The analysis of organoleptic test can be seen in Figure 7.

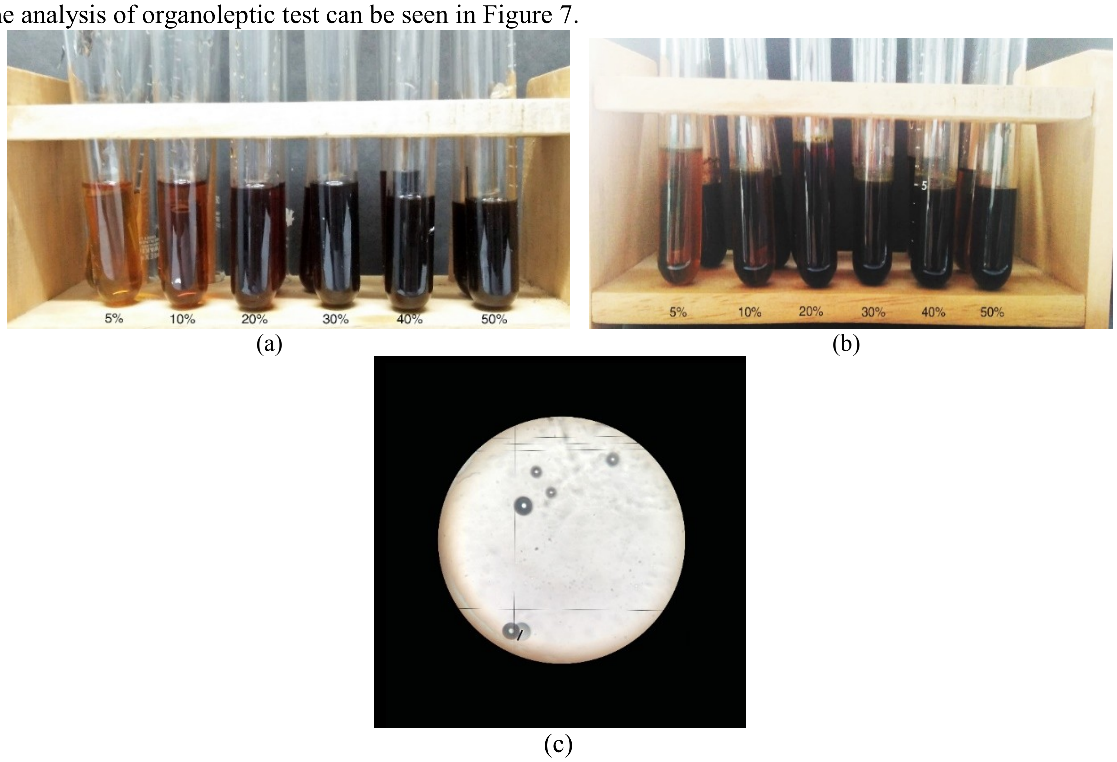


FIGURE 7. (a) liquid fogging at week 0 (b) liquid fogging at week 7 (c) liquid fogging under light microscope 40x magnification

The observation showed that at the 1st to 7th week there were no significant organoleptic changes in color, aroma, and texture. The emulsion of fogging formulation under light microscope seen was a water emulsion (indicated by a white inner circle) in oil (indicated by the outer circle of black tobacco liquid) (W / O) with the largest emulsion size of 14.3 µm and the smallest is 7.14 µm. Oil-based insecticides are known to have droplet sizes ranging from 2 - 110 µm.

CONCLUSION

The concentration for an effective fogging formulation is soluble PG: 50% VG: 50% with a tobacco extract concentration of 40-50%. The formulation of *Aedes aegypti* insecticide with 50% tobacco extract concentration can kill half the mosquito population (LC_{50}) for 6 minutes.40% tobacco extract concentrations can kill half the mosquito population (LC_{50}) for 5 minutes. 30% of tobacco extract concentrations can kill half the mosquito population (LC_{50}) for 9 minutes. Formulation with concentration of tobacco extract below 30% has not been able to kill mosquitoes up to LC_{50}. The organoleptic test results for the eight *Aedes aegypti* insecticide formulas did not experience significant changes in color and odor. While in terms of phase separation, the sixteen anti-mosquito insecticide formulations experienced phase separation at concentrations of 30%, 40%, 50% with pyrolysis method and separation at concentrations of 20%, 30%, 40%, 50% with extraction method. Tobacco leaf extract's droplet measured on a microscope ranging from size 7.14-14.3 µm.

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