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Use of *Nicotiana Tabacum L* Extract for Anti-*Aedes Aegypti* Mosquito Paint

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Abstract: This study intended to formulate mosquito repellent paints based tobacco leaf extracts-free pyrethroid substance which is safe for users. The active substance which was added to the paint as a mosquito repellent was an extract of tobacco leaves. The result of Anti-mosquito paint formulation produced was according to the Indonesia National Standard (SNI). The results of anti-*Aedes Aegypti* mosquito paint effectiveness test showed that 5% concentration of tobacco extract could kill half of the mosquito population (LC50) for 2 hours, the concentration of tobacco extract between 3-5% killed half the mosquito population (LC50) during 4 hours, while 1-3% and 0-1% concentration of tobacco extract killed half the mosquito population (LC50) for 6 and 24 hours, respectively.

INTRODUCTION

According to World Health Organization (WHO) data, Indonesia was reported as the 2nd country with the largest dengue cases among 30 endemic countries [1]. People should be aware of the mosquitoes around the house where the mosquitoes allows to be in the wall of the house. Innovation of anti-Aedes Aegypti products is growing, now has been presenting anti-Aedes Aegypti repellent products in the form of anti-Aedes Aegypti paint. The current anti-Aedes Aegypti paint products contain Pyrethroid substance which is middle-class toxins [2]. Therefore, the need for the latest innovation is a safer anti-Aedes Aegypti paint, without containing *Pyrethroid* substances.

Tobacco is the major raw material that contains nicotine. It is known to have a big potential as a pesticide or repellent. Many methods have been performed to extract nicotine from tobacco leaf. In this research, nicotine was provided by using ethanol extract of tobacco leaves. This method used column chromatography along with thin layer (TLC) to provide nicotine.

This research was a formulation of anti-Aedes Aegypti paint based on ethanol extract of tobacco leaf. Tobacco has a strong and lasting fragrance. The aroma is suspected of having repellent activity against mosquitoes [3]. This study determined the maximum percentage weight of extract from total weight of paint that does not change the paint color and repel mosquitoes effectively. This study also performed anti-Aedes Aegypti paint characterization based on SNI such as density test, viscosity, total solid, pH, alkali resistance.Organoleptic test, physical stability of anti-Aedes Aegypti paint used centrifuge, mold and bacteria sensitivity test with Kirby Bauer disc diffusion method, and effectiveness test of mosquito paint were also performed.

METHODS

Anti-Aedes Aegypti Paint Formulation

The anti-Aedes Aegypti decorative paint was made by mixing paint with the *Nicotiana Tabacum L* Ethanol Extract. After added tobacco ethanol extract, the paint color did not change. The extract was mixed into paint with sonicator at room temperature,1000 rpm for 15 minutes until tobacco extract was well-dispersed.

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Characterization of Anti-Aedes Aegypti Paint

The characterization of anti-Aedes Aegypti paint was conducted by density test, alkali resistance test, total solid test, viscosity test, pH test, organoleptic observation, physical stability test, microbial sensitivity test, and effectiveness test for mosquitoes. The results of mosquitoes repellent paint testing must fulfill the quantitative quality requirements of wall paint in accordance with SNI 3564:2009.

Density Test, Total Solid Test, pH Test, Alkali Resistance Test, and Viscosity Test

Density of Mosquitoes repellent paint was measured by using pycnometer. The density was calculated by the formula below:

$$D_m = \frac{(W-M)}{V} x100\% \quad (1)$$

with D_m density of mosquito; w is weight of empty pycnometer; M is weight of the filled pycnometer; V is the volume of the pycnometer.

The total solid test was performed by heating the aluminium foil plate for 30 minutes at 110°C±5°C. Then, it was cooled and weighed (W1). Then, the sample (S) was weighed and inserted into the aluminium foil plate. Sample dissolved with water 3 ml, stirred until well-dispersed. Then heated the aluminium foil plate that contained the sample for 60 minutes at 110°C±5°C, then it was cooled and weighed (W2). The total solid was calculated by the formula below:

$$N = \frac{(W_2-W_1)}{S} x100\% \quad (2)$$

pH was measured by using pH meter. The alkali resistance test was performed by applied the paint on the watch glass, then poured 0.1 N NaOH onto the mosquitoes repellent dry paint surface, after that it was left for about an hour. Then, the changes on the surface was observed. Viscosity test for Mosquitoes repellent paint was measured by using the viscometer brookfield.

Organoleptic Observation

Organoleptic was observed by the occurrence of color change, odor change, clarity, and phase separation in mosquitoes repellent decorative paint for 8 weeks. Observation was conducted every one week.

Physical Stability Test

The samples was centrifuged at 5000 rpm for 30 minutes, then samples were observed for phase separation.

Microbial Sensitivity Test

The method used for microbial sensitivity test was Kirby Bauer disc diffusion. Microbial sensitivity test is intended to examine the sensitivity of anti-Aedes Aegypti paint and tobacco ethanol extract to *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Aspergillus niger, Aspergillus versicolor, Penicillium rubens,* and *Cladosporium colocasiae*. The sensitivity test for bacteria and fungi used Nutrient agar (NA) medium and Potato Dextrose Agar (PDA) medium, respectively. The discs containing anti-Aedes Aegypti paint and tobacco ethanol extract were attached to the NA and PDA medium that contained the bacteria and fungus. The samples were incubated for 1-3 days at 37°C.

Effectiveness Test of Anti-Aedes Aegypti Paint

The method used for effectiveness test of anti-*Aedes Aegypti* paint was cone test. It used a transparent net to form a cone that allows to see the behavior of mosquitoes. At the bottom of cone was a plywood to be applied by paint. This test was performed to calculate the percentage of *Aedes aegypti* mosquitoes killed due to exposure to mosquitoes repellent paint-based tobacco ethanol paint.

RESULTS

Anti-Aedes Aegypti Paint Formulation

Tobacco ethanol extract and paint were mixed by using a sonicator in order to homogenous mixing results. After creating some formulas, maximum percentage of tobacco ethanol extract which does not change the paint base color was obtained. The maximum percentage by weight of tobacco ethanol extract is 0.1%. The percentage by weight of tobacco ethanol extract was then varied to determine whether the extract concentration of less than 0.1% remained effective against mosquitoes. Variations percentage by weight of tobacco ethanol extract were 0%, 0.025%, 0.05%, and 0.1%.

FIGURE 1. Anti-Aedes Aegypti Paint Formulation (Description of anti-mosquito paint from left to right; 2nd paint without extract (F5), 2nd paint with 0.025% extract (F6), 2nd paint with 0.05% extract (F7), 2nd paint with 0.1% extract (F8), 1st paint without extract (F1), 1st paint with 0.025% extract (F2), 1st paint with 0.05% extract (F3), 1st paint with 0.1% extract (F4)).

Based on Figure 1, it shows that there is no significant color change for both the first type (P-1) of paint and the second type (P-2) of paint. The resulting anti-mosquito paint formulation was then characterized by density test, alkali resistance test, total solid test, viscosity test, pH test, organoleptic observation, physical stability test, efficacy test against *Aedes Aegypti* mosquito, and microbial sensitivity test.

Characterization of Anti-Aedes Aegypti Paint

Density test, Total Solid Test, pH Test, Alkali Resistance Test, and Viscosity Test

The results of density test, alkali resistance test, total solid test, and pH test were appropriate with SNI 3564: 2009. Based on SNI 3564: 2009, density values, total solids, pH shall be min. 1.2 g / ml, min. 40%, and 7-9.5. Test results can be seen in table 1.

TABLE 1. Result of Density test, Total Solid Test, pH Test

Type of Paint	Concentration of Tobacco Extract	Density (g/ml)	Total Solid (%)	pH
P-1	0% (F1)	1.352	55.94	8.08
	0.025% (F2)	1.377	56.15	8.2
	0.05% (F3)	1.394	56.16	8.17
	0.10% (F4)	1.421	56.48	8.05
P-2	0% (F5)	1.377	57.03	9.16
	0.025% (F6)	1.406	56.61	9.09
	0.05%(F7)	1.484	57.28	9.06
	0.10%(F8)	1.611	56.27	9.04

The results of the alkali resistance test for the first type (P-1) of paint with 0.025% extract (F2) as shown in Fig. 2 b), shows the cracked anti-aedes aegypti mosquitoes paint. It means that the anti-mosquito paint with 0.025% extract and the EPSOPAINT INTERIOR ETNALUX/ OCTALITE paint type is less resistant to alkali. The results of the alkali resistance test for the second type (P-2) of paint with 0.025% extract (F6) are shown in Fig. 4 b) shows that the paint is slightly exfoliated, meaning that the anti-mosquito paint with 0.025% extract and MOWILEX paint type is less resistant to alkali. There was no color change, peeling, shrinking, liming, and bubbles for F1, F3, F4, F5, F7, and F8. That means the formula is quite alkaline. But small tobacco extract concentration was used in this research. It can be said that tobacco extract does not affect the resistance to alkali, but the type of wall paint used influences the alkali resistance.

a) b)

FIGURE 2. Alkali Resistance Test Results for a) First type (P-1) Paint without Extract, b) First type (P-1) Paint with 0.025% Extract

a) b)

FIGURE 3. Alkali Resistance Test Results for a) First type (P-1) Paint with 0.05% ekstract, b) First type (P-1) Paint with 0.1% Extract

a) b)

FIGURE 4. Alkali Resistance Test Results for a) Second type (P-2) Paint without Extract, b) second type (P-2) Paint with 0.025% Extract

a) b)

FIGURE 5. Alkali Resistance Test Results for a) Second type (P-2) Paint with 0,05% ekstract, b) Second type (P-2) Paint with 0.1% Extract

Organoleptic Observation

Organoleptic observations were performed for 7 weeks to determine changes in color, aroma, texture, and phase separation on the all formulations of the anti-*Aedes Aegypti* Mosquito paints. In first to seventh weeks there was no significant organoleptic change in color, aroma, and texture. In terms of phase separation, it can be seen in Figure 6 - 13. Based on Figure 6-13, phase separation was occurred in the first and second type (P-2) of paint. It occurred because the binding between the paint components was weak or lack of binder.

a) b)

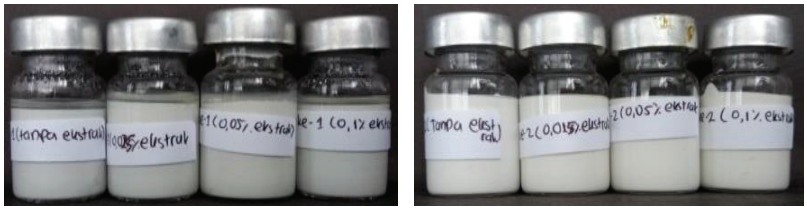
FIGURE 6. Organoleptic Test Result of Phase Separation Aspect a) First type (P-1) Paint on Week 0 b) Second type (P-2) Paint on Week 0

a) b)

FIGURE 7. Organoleptic Test Result of Phase Separation Aspect a) First type (P-1) Paint on First Week b) Second type (P-2) Paint on First Week

a) b)

FIGURE 8. Organoleptic Test Result of Phase Separation Aspect a) First type (P-1) Paint on Second Week b) Second type (P-2) Paint on Second Week



a) b)

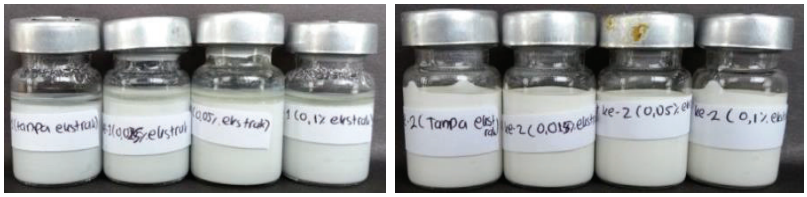
FIGURE 9. Organoleptic Test Result of Phase Separation Aspect a) First type (P-1) Paint on Third Week b) Second type (P-2) Paint on Third Week

a) b)

FIGURE 10. Organoleptic Test Result of Phase Separation Aspect a) First type (P-1) Paint on Fourth Week b) Second type (P-2) Paint on Fourth Week

a) b)

FIGURE 11. Organoleptic Test Result of Phase Separation Aspect a) First type (P-1) Paint on Fifth Week b) Second type (P-2) Paint on Fifth Week



a) b)

FIGURE 12. Organoleptic Test Result of Phase Separation Aspect a) First type (P-1) Paint on Sixth Week b) Second type (P-2) Paint on Sixth Week

a) b)

FIGURE 13. Organoleptic Test Result of Phase Separation Aspect a) First type (P-1) Paint on Seventh Week b) Second type (P-2) Paint on Seventh Week

Physical Stability Test

According to Fig. 14, it can be seen that all anti-Aedes Aegypti Mosquito paint formulations are unstable against gravity for one year with phase separation [4]. Therefore, it is necessary to add co-emulsifier to improve the stability of anti-mosquito paint.

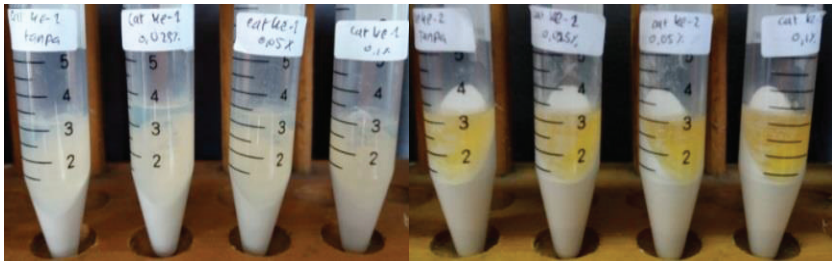


FIGURE 14. Differences in Physical Stability Test Results between First and Second type of Paint (From Left to Right, F1 to F8)

Microbial Sensitivity Test

The results of sensitivity test on anti-mosquito paint showed that paint without ethanol extract of tobacco leaf had growth inhibitory activity against *Aspergillus niger*, *Aspergillus versicolor*, *Penicillium rubens*, and *Cladosporium colocasiae* with inhibition zone diameter of 0.7-1.5 cm. This means that without or with the presence of ethanol extracts of tobacco leaves, anti-mosquito paints have growth inhibitory activity against *Aspergillus niger*, *Aspergillus versicolor*, *Penicillium rubens*, and *Cladosporium colocasiae*. It can be caused by

paint containing additive as *mildewcide* to prevent mold growing on the surface of the paint after being applied to the wall.

The same results of bacterial sensitivity test had been found on anti-mosquito paint. Both of the paint had growth inhibition activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. It occurred because the paint contains additives in the form of *biocides* that prevent bacterial growth. Whereas, the results of bacterial sensitivity test on anti-mosquito paint showed the paint with or without tobacco leaf ethanol extract did not have growth inhibition activity against *Pseudomonas aeruginosa*. It can be caused by Alginate, a viscous gel around the bacteria, which is exopolysaccharide contained polymer of glucuronic acid and mannuronic acid, Alginate allows bacteria to form biofilms. Alginate can protect bacteria from the host's body's defenses such as lymphocytes, phagocytes, cilia in the respiratory tract, antibodies, and complement. The ability of *Pseudomonas aeruginosa* to form biofilm makes its bacteria resistant to antibiotics [5].

In addition, the ability of tobacco extract inhibiting mold growth can be reduced using the solvent used in the extraction. Variations of solvent cause extracts different to inhibit mold growth, where samples extracted with ethyl acetate are more effective, followed by samples extracted with buthanol, while samples extracted with ethanol and buthanol did not have significant inhibitory effect [6]

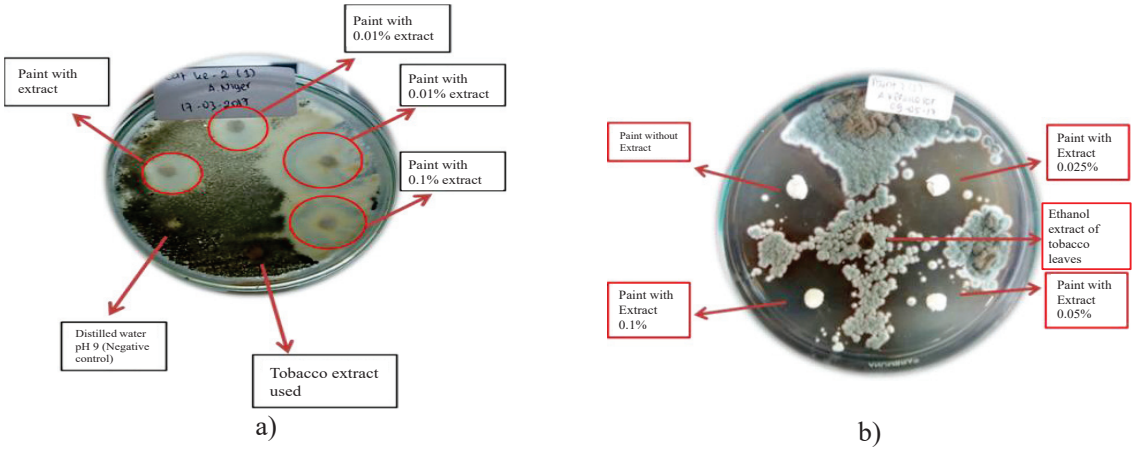


FIGURE 15. Measurement of inhibition zone for; a) Second type (P-2) Anti-Aedes Aegypti mosquito paint (repetition 1) on *A. Niger* culture, b) Second type (P-2) Anti-Aedes Aegypti mosquito paint (repeatation 2) on the *Aspergillus Versicolor* (Kirby-Bauer Method)

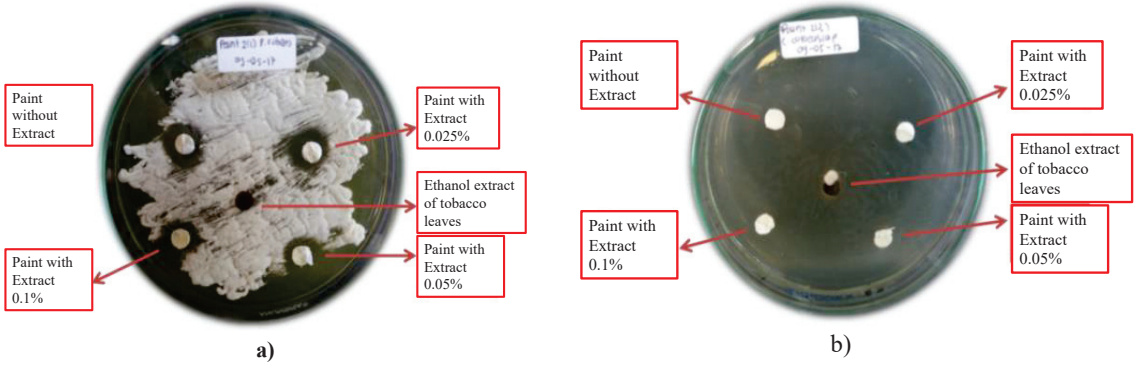


FIGURE 16. Measurement of inhibition zone for; a) Second type (P-2) Anti-Aedes Aegypti mosquito paint (repetition 1) on *Penicilium Rubens* culture, b) Second type (P-2) Anti-Aedes Aegypti mosquito paint (repeatation 3) on the *Cladosporium Colocasiae* (Kirby-Bauer Method)

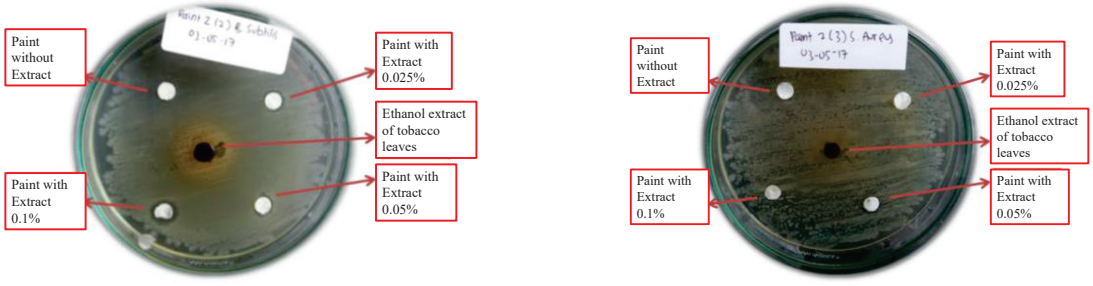


FIGURE 17. Measurement of inhibition zone for; a) Second type (P-2) Anti-Aedes Aegypti mosquito paint (repetition 2) on *Bacillus Subtillis* culture, b) Second type (P-2) Anti-Aedes Aegypti mosquito paint (repeatation 3) on the *Staphylococcus Aureus* (Kirby-Bauer Method)

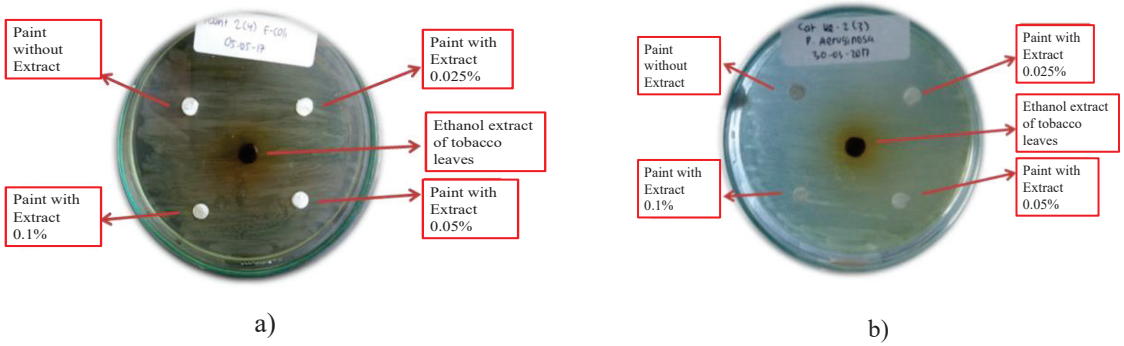
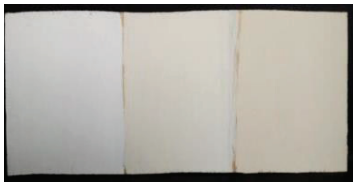


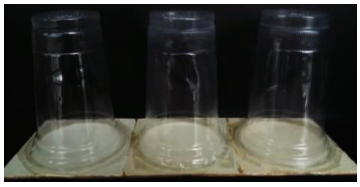
FIGURE 18. Measurement of inhibition zone for; a) Second type (P-2) Anti-Aedes Aegypti mosquito paint (repetition 4) on *Escheriachia coli* culture, b) Second type (P-2) Anti-Aedes Aegypti mosquito paint (repeatation 3) on the *Pseudomonas Aeruginosa* (Kirby-Bauer Method)

Effectiveness Test of Anti-Aedes Aegypti Paint

The purpose of conducting effectiveness test of Anti-*Aedes Aegypti* was to find the effectiveness of anti-*Aedes Aegypti* paint. The test was conducted using *Aedes Aegypti* mosquitoes aged 3-5 days for 6 hours. In the first hour, *Aedes Aegypti* mosquito's behavior was observed on every 10 minutes. Based on the results, the effectiveness of anti-mosquito paint against *Aedes Aegypti* mosquitoes showed that the mosquitoes were not killed by the paint containing tobacco extract, thus the formulation of anti-mosquito paint were not effective to expel/ kill mosquitoes. It might be caused by a small concentration of extract used (only 0.1%), where the use of the concentration did not change the basic color of paint. Tobacco has a strong and lasting aroma which might be from nicotine and pyridine in tobacco. It is expected to repel mosquitoes [3]. Because the concentration of tobacco extract was slightly added (0.1%) to the paint, so the aroma of tobacco extract did not cause anosmia or hyposmia so mosquitoes did not fall nor die. Since the concentration of 0.1% tobacco extract was not killed the mosquitoes, so the concentration of tobacco extracts was increased to determine the minimum concentration of extracts that can cause minimum of 90% death of mosquitoes. Variations of tobacco leaf extract concentration became 1%, 3%, and 5%.



a)



b)

FIGURE 19. a) Anti-mosquito paint that has been applied to the plywood (with variation of concentration from left to right, 1%, 3%, and 5%), b) Cone Used As Room for *Aedes Aegypti* Mosquito Contact

TABLE 2. Total Fall or death of *Aedes Aegypti* mosquitoes

Observation Time After Contact (Hour)	Total of the Fall or Death of Mosquitoes				Percentage of the Fall or Death of Mosquitoes			
	0%	1%	3%	5%	0%	1%	3%	5%
1/6	0	0	0	0	0	0	0	0
1/3	0	0	0	0	0	0	0	0
½	0	0	0	0	0	0	0	0
2/3	0	0	1	2	0	0	9%	18%
5/6	0	0	1	3	0	0	9%	27%
1	0	0	2	4	0	0	18%	36%
2	0	1	3	6	0	9%	27%	55%
3	0	2	3	9	0	18%	27%	82%
4	0	3	4	11	0	27%	45%	100%
5	0	4	4	11	0	36%	45%	100%
6	0	4	7	11	0	36%	64%	100%
24	0	11	11	11	0	100%	100%	100%

According to Table 2, for all the extract concentration variations, the mosquitoes was not killed until 30 minutes. The fall or death of mosquitoes just happened in the 40th minute for extract concentrations of 3% and 5%, each 1 and 2 mosquitos, respectively. Total mosquitoes killed in the experiment for 1 hour for 3% and 5% tobacco extract concentration were 2 and 4 mosquitoes. For 1% concentration of tobacco extract, one mosquito was killed in the 2nd hour of experiment. Falling or death of mosquitoes every minute or hour was always increased, all mosquitoes used for each concentration of extract (11 mosquitoes) was killed at 4th hour at 5% tobacco extract concentration, whereas at concentration of tobacco extract 1% and 3% at the 24th hour. During 6 hours of observation, 5% tobacco extract concentration was killed mosquitoes faster than 1% and 3% tobacco extract concentrations. Thus, the concentration of tobacco extract was 5% more effective than the concentration of tobacco extract of 1% and 3%.

The percentage of falling or death of mosquitoes for every minute and hour can also be seen in Table 2. The percentage of falling or mosquito death rates was calculated from the number of mosquitoes that fall or die on the number of mosquitoes used. Table 2 shows that the concentration of 5% tobacco extract at 4th hour of observation meets the effectiveness criteria of residual pesticide i.e. minimum 90% of mosquitoes fall exposed to residue (Based on Pesticide Commission). While the concentration of tobacco extract 1% and 3% meets the effectiveness criteria at 24 hours.

CONCLUSIONS

Anti-*Aedes Aegypti* mosquito paint with a 5% concentration of tobacco extract killed half the mosquito population (LC50) for 2 hours. Anti-*Aedes Aegypti* mosquito paint with a 3% concentration of tobacco extract killed half the mosquito population (LC50) for 4 hours. Anti-*Aedes Aegypti* mosquito paint with 1-3% tobacco extract concentration can kill half the mosquito population (LC50) for 6 hours. Meanwhile, anti-*Aedes Aegypti* mosquito paint with 0-1% tobacco extract concentration can kill half the mosquito population (LC50) For 24 hours. Anti- *Aedes Aegypti* mosquito paint formulation by mixing tobacco leaf extract with paint in the market. The density, viscosity, total solidity, pH, and alkali resistance values of Anti- *Aedes Aegypti* Mosquito Paint for the all formulas meet the standard from SNI 3564: 2009. The organoleptic test results for the all anti-Aedes Aegypti mosquito paint formulas did not change color and odor. Whereas, phase separation was occurred in the all formulas of Anti-*Aedes Aegypti* mosquito paint. The maximum concentration of tobacco extract that does not

change the color of the paint is 0.1%. However, that concentration is ineffective against mosquitoes, the concentration of tobacco extract which effectively repels mosquitoes is 1-5%.

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