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INSECTICIDA TEST OF ATTRACTIVE OIL ON CLOVE (*Syzygium aromaticum* (L.) Merr. & Perry.) LEAVES AGAINST *Aedes aegypti* MOSQUITO

Rekha Yulia Agustin[1], Selvi Marcellia[2*], Dwi Susanti[1]

[1]Program study of pharmacy, Faculty of health science, Malahayati university
[2]Program study of medical education, Faculty of medicine, Lampung university

[*)]Email Korespondensi: selvi.marcellia@fk.unila.ac.id

Abstract: Insecticida Test of Attractive Oil on Clove (Syzygium Aromaticum (L.) Merr. & Perry.) Leaves Against Aedes aegypti Mosquito. *Clove leaves (Syzygium aromaticum* (L.) Merr. & Perry.) *have eugenol compounds in essential oils which have the potential as natural insecticides. The purpose of this study was to determine the effectiveness of essential oil insecticides on clove leaves (Syzygium aromaticum* (L.) Merr. & Perry.) *in killing Aedes aegypti mosquitoes. This study used the distillation method with air as a solvent. The concentrations of clove leaf essential oil extract used were 0.5%s, 1%, 2.5% and 4%. The results of the LC_{50} test showed that clove leaf essential oil (Syzygium aromaticum) was effective in killing Aedes aegypti mosquitoes with an LC_{50} value of 0.993% and was included in the very toxic category. The higher the concentration of essential oils, the greater the killing activity and the lower the LC_{50} value, the higher the effectiveness in killing Aedes aegypti mosquitoes. It can be concluded from this study that clove leaf essential oil (Syzygium aromaticum* (L.) Merr. & Perry.) *has effectiveness as a natural insecticide against Aedes aegypti mosquitoes, can be seen from the mortality value in the 55th minute of 100% at a concentration of 4%.*

Keywords: *Aedes aegypti, Clove leaves, Effectiveness of essential oils, LC_{50}, Natural insecticides, LC_{50}.*

Abstrak: Uji Insektisida Minyak Atsiri Pada Daun Cengkih (*Syzygium Aromaticum* (L.) Merr. & Perry.) Terhadap Nyamuk *Aedes aegypti*. Daun cengkeh (*Syzygium aromaticum* (L.) Merr. & Perry.) memiliki senyawa eugenol didalam minyak atsiri yang berpotensi sebagai insektisida alami. Tujuan dari penelitian ini adalah mengetahui efektivitas insektisida minyak atsiri pada daun cengkeh (*Syzygium aromaticum* (L.) Merr. & Perry.) dalam membunuh nyamuk *Aedes aegypti*. Penelitian ini menggunakan metode destilasi dengan pelarut air. Konsentrasi ekstrak minyak atsiri daun cengkeh yang digunakan adalah 0,5%, 1%, 2,5% dan 4%. Hasil penelitian dari uji LC_{50} menunjukkan minyak atsiri daun cengkeh (*Syzygium aromaticum*) efektif dalam membunuh nyamuk *Aedes aegypti* dengan nilai LC_{50} sebesar 0,993% dan masuk dalam kategori sangat beracun. Semakin tinggi konsentrasi minyak atsiri maka semakin besar aktivitas membunuhnya dan semakin rendah nilai LC_{50} maka semakin tinggi efektivitas dalam membunuh nyamuk *Aedes aegypti*. Penelitian ini dapat disimpulkan bahwa minyak atsiri daun cengkeh (*Syzygium aromaticum* (L.) Merr. & Perry.) memiliki efektivitas sebagai insektisida alami terhadap nyamuk *Aedes aegypti*, dapat dilihat dari nilai mortalitas pada menit ke-55 sebesar 100% pada konsentrasi 4%.

Kata kunci: *Aedes aegypti*, Daun cengkeh, Efektivitas minyak atsiri, Insektisida alami, LC_{50}.

INTRODUCTION

Dengue Hemoragi Fever (DHF) is an acute febrile illness resulting in death caused by a virus that can be transmitted through the bite of Aedes genus mosquitoes, especially Aedes aegypti (Aulya et al., 2022). The main cause of DHF is the Aedes aegypti mosquito, which is transmitted by female Aedes aegypti mosquitoes that contain the Dengue virus in their bodies (Amelia et al., 2017). Various ways have been done in order to control the Aedes aegypti mosquito, starting from

several controls, namely, environmental control, biological and chemical control.

In Indonesia, the onset of DHF occurred in Surabaya in 1968 and spread worldwide in 2016 (Achmadi, 2010). The cumulative number of DHF cases in Indonesia reported in January 2022 was 87,501 cases (IR 31.38/100,000 population) and 816 deaths. DHF cases occurred in Lampung Province, especially the city of Bandar Lampung from January to August 2022, a total of 3,484 cases of dengue hemorrhagic fever (DHF) were reported, about 435 cases per month (Kementrian Kesehatan RI, 2022). Various ways have been done in order to control Aedes aegypti mosquitoes, starting from several controls, namely, environmental control, biological and chemical control. The use of chemical insecticides can be limited to prevent resistance to the mosquito. One of the efforts to control Aedes aegypti mosquitoes can be done by using natural insecticides. Natural insecticides made from plants can be the best choice as they are environmentally friendly. There are 30-40 types of mosquito species that can be reported as carriers of plasmodium parasites (Ridjal, 2017). One of the plants that can be used as a natural insecticide is the clove plant (*Syzygium aromaticum* (L.) Merr. & Perry.).

Based on the results of previous research, the concentration of clove leaf extract (*Syzygium aromaticum* (L.) Merr. & Perry.) which is most effective in killing mosquitoes, the LC50 value is obtained at a concentration of 16% (Aulya et al., 2022). Research conducted by (Ariwidiani et al., 2021) states that clove flower extract (*Syzygium aromaticum* L.) Merr. & Perry.) which contains essential oil can have potential as an insecticide for Aedes aegypti mosquitoes with an LC50 value at a concentration of 3.434%. Research that has been conducted (Aulya et al., 2022), shows that clove leaf extract contains compounds that are deadly to Aedes aegypti mosquitoes.

Based on the description above, researchers are very interested in conducting an Essential Oil Insecticide Test on Clove Leaves (*Syzygium aromaticum* (L.) Merr. & Perry.) against Aedes aegypti mosquitoes to determine the insecticidal effectiveness and concentration of essential oil in clove leaves (*Syzygium aromaticum* (L.) Merr. & Perry.) in killing Aedes aegypti mosquitoes.

METHOD

This research has been tested for ethical clearence number 3540/EC/KEP-UNMAL/V/2023, Malahayati University Ethics Commission. The samples used in this study were clove leaf plants (*Syzygium aromaticum* (L.) Merr. & Perry.) Figure 1. Clove leaves which were blackish green with good condition, taken from the stalk row 2- 3 rows from the base. With the sampling technique used in this study is random sampling, where each member of the population has the same opportunity or opportunity to be selected as a sample. The independent variable is the concentration of clove leaf essential oil (*Syzygium aromaticum* (L.) Merr. & Perry.). And the dependent variable is the death of Aedes aegypti mosquitoes.

Distillation is carried out using a distillator tool so that the essential oil can be taken. Essential oil distillation is carried out by distillation method for 20 days with a yield of 36 ml in 1 time distillation gets a yield of 1.5-2ml. The sample used was 8000 grams of clove leaves (*Syzygium aromaticum* (L.) Merr. & Perry.). The sample that has been prepared is weighed 1.99-2.50 grams and then cut into small pieces and then put into the distillation device, namely the heating flask and given additional water until it is submerged according to the weight of the sample used, then heated to a temperature of 100oC for 8 hours. Heating from water vapor the cells of the clove leaf simplisia will open so that there is binding, release and removal of essential oil compounds along with hot steam, then the vapor pressure containing essential oil begins to flow into the condenser unit at the 3rd hour. The results of distillation in the form of essential oil began to be

accommodated in erlenmayer. Essential oil and water vapor will be separated based on molecular weight after going through a condenser filled with cold water with a temperature of ≤ 40oC (Loppies et al., 2021). If the essential oil has been distilled, there is a yellowish-white liquid in the pipe / hose that drips into the container. The distillation process is declared complete when there is no distillate dripping from the condenser (Djau et al., 2022).

The test animals used in this study were Aedes aegypti mosquitoes. The number of samples to be used in this test is 25 adult mosquitoes per treatment with 4 repetitions for each treatment. Eggs are then hatched by preparing a tray containing 1.5-2 liters of water, then the paper egg is cut into 4 parts and each sheet of paper egg containing eggs is soaked in water in the tray that has been prepared then wait for the Aedes aegypti mosquito eggs to hatch within 24 hours. Adult Aedes aegypti mosquitoes with an age of 3 days and ready to be tested, before the 2-day treatment test mosquitoes were fed sugar water that had been dissolved by spraying on the screen as food, so that mosquito death was not caused by starvation. On the following day, Aedes aegypti mosquitoes were used as test animals. Table 1. Detail of Sample Treatments Used.

The study was conducted with a test cage consisting of a cube-shaped wooden frame with each side covered with tile cloth measuring 30 x 30 x 30 m3, inserted in a vacuum chamber, the mosquitoes used for the test were adult Aedes aegypti mosquitoes aged 3-5 days with each mosquito in the cage 25 mosquitoes. The test treatments were then sprayed with various concentrations of clove leaf essential oil, negative control and positive control. Spraying is done at maximum pressure, then the second, third and fourth spraying returns to the original spraying state (Wibawa, 2012). Spraying is also carried out under the condition that there are no mosquitoes that intersect directly with the straight line of the spraying direction so that the spraying pressure does not cause death due to pressure, then sprayed on the test cage 3 times and then inserted in the vacuum used in the test, which is a cube-shaped glass, with each test cage on its side covered with Styrofoam. Observations were then made every 5 minutes to count and record the number of Aedes aegypti mosquitoes that died within 1 hour of observation, with 4 repetitions for both control and treatment (Wahyuni & Yulianto, 2018). To calculate the percentage of mosquito mortality at each concentration, the formula was used :

$$\%\ Mortality = \frac{Total\ number\ of\ mosquitoes\ that\ died}{Number\ of\ test\ mosquitoes\ used} + 100\%$$

RESULTS

Clove leaf extract (*Syzygium acomaticum* (L.) Merr. & Perry.) obtained from the distillation extraction process is extract in the form of essential oil. After obtaining the extract in the form of essential oil, calculations are made to obtain the extract yield. According to (Armando, 2009), he higher the yield value, the greater the essential oil produced, this needs to be emphasized because the quality is inversely proportional to the yield. The higher the yield, the more essential oil meets the requirements of good quality. While good quality oil is usually characterized by a small amount of yield. The results of extraction in the distillation process carried out with a time of 8 hours are quite optimal and the samples used are wet leaves, so that the results affect the weight of the sample used in the calculation of the comparison (Syamsunur et al., 2019). The yield results can be seen in Table 2 The results of clove leaf extraction show that the yield of 0.45% is said to be good.

Table 1. Details of Sample Treatments Used

Treatment	Concentration (%)	Amount of Mosquitoes x Repetitions	Total Mosquitoes
Control (-) (Aquades)	0	25 Mosquitoes x 4	100
Control (+) (Transflutrin)	-	25 Mosquitoes x 4	100
F1	0,5	25 Mosquitoes x 4	100
F2	1	25 Mosquitoes x 4	100
F3	2,5	25 Mosquitoes x 4	100
F4	4	25 Mosquitoes x 4	100
Quantity			600

Table 2. Details of Yield Results

Solvent	Sample Weight (gram)	Volume Solvent (L)	Extract Weight (mL)	Percent Yield (%)
Water	8000	16	36	0,45

Table 3. Mosquito Death Effectiveness Test Results

Treatment Group	Average Mortality *Aedes aegypti* (%)					LC_{50} (%)minute to -55	*P. Value*
	Minute 40	Minute 45	Minute 50	Minute 55	Minute 60		
0,5%	6	7	9	10[a]	10	0,993	0,000
1%	34	38	51	60[b]	64		
2,5%	65	70	73	87[c]	87		
4%	93	93	99	100[d]	100		
Kontrol (+) (transflutrin)	100	100	100	100[d]	100		
Kontrol (-)	0	0	0	0[e]	0		

While the results of the mosquito mortality effectiveness test based on the results of the Shapiro-wilk data test show that the data is not normally distributed with a significant value ($p<0.05$), then further testing is carried out with the Kruskal Wallis test which is part of non-parametric statistics. The Kruskal wallis test was carried out because the normality distribution results were not normally distributed with a significant value obtained below 0.05 or ($p<0.05$) and then the Mann-Whitney Post Hoc Test will be carried out to determine the difference between each concentration of clove leaf essential oil (*Syzygium aromaticum* (L.) Merr. & Perry.) against Aedes aegypti mosquitoes. The results of the insecticide effectiveness test of clove leaf essential oil (*Syzygium aromaticum* (L.) Merr. & Perry.) against Aedes aegypti mosquitoes can be seen Table 2.

Based on Table 2, it can be seen that the difference between each treatment concentration of clove leaf essential oil extract (*Syzygium aromaticum* (L.) Merr. & Perry.) in influencing the number of dead mosquitoes. The higher the concentration of essential oil, the greater the mortality of Aedes aegypti mosquitoes. The results of the Mann-Whitney Post Hoc Test data show that clove leaf essential oil at concentrations of 0.5%, 1%, 2.5% and positive control (+) (Transflutrin) and negative control (-) have significant differences obtained ($p <0.05$), while positive control (+) with a concentration of 4% has no significant difference obtained ($p>0.05$), meaning it has comparable effectiveness in killing test animals. The

LC50 value obtained from the 55th minute was 0.993% with a linear regression equation y = -0.08 + 3.38x.

DISCUSSION

The sample used clove leaves and then distilled with water solvent so that the essential oil needed can be separated. The reason for using water can attract active substances contained in leaves that are polar and non-polar (Kodoatie & Sjarief, 2010). The distillation process carried out for 8 hours has been completed, the results that have been collected in erlenmayer are given the addition of Na2SO4, the separation process is carried out using a separating device, namely a separating funnel to get the results in the form of oil. The reason for using Na2SO4 is as a separator of a substance obtained to be free from water (Zuzani Fahira et al., 2015). Furthermore, calculations are carried out to obtain the yield of essential oil extracts. The yield calculation is a comparison of the amount (quantity) of oil produced from the extraction of aromatic plants. The unit used is percent (%) (Armando, 2009). The yield results in this study can be seen in table 3.

The number of test animals used was 25 mosquitoes according to (WHO, 2009) standards. The treatment was carried out when the mosquito was 3 days old, the age of the mosquito is a factor that greatly affects the mosquito's resistance to exposure to chemical compounds. The selection of mosquito age in the study ranges from 3-5 days because it has a strong physical condition, if the mosquito is under 3 days old it is said to be physically weak because it facilitates mosquito death. While at the age of 5 days the mosquito's resistance begins to decline so that it will result in the risk of death (Wibawa, 2012). After 3 days, the testing process was carried out using the spray method.

The spraying process in the clove leaf essential oil insecticide effectiveness test was carried out in a way that no mosquitoes intersected in the spraying direction. The length of contact time between Aedes aegypti mosquitoes and the concentration of clove leaf essential oil affects the effect of exposure between mosquitoes and essential oil. An effective exposure time application is within 1 hour, because if more than that time, the insecticide will be carried away by the wind. Too short a time will also cause a reduction in the length of interaction between chemical compounds and target mosquitoes which will result in a decrease in the number of mosquitoes that die (Wibawa, 2012).

Based on the observations in Table 4.2, it can be seen that the average percentage of mosquito mortality during the 60-minute observation time of each treatment group at concentrations of 0.5%, 1%, 2.5% cannot be said to be effective because the mortality value obtained is <90%. Based on the standards of the (Kementrian Pertanian, 2020), he effective mortality value can be seen starting from the 40th minute where the 4% concentration is able to kill 50% of Aedes aegypti mosquitoes with a mortality value obtained of 93%, this is in accordance with the standards of the (Ministry of Agriculture, 2020), where the mortality value can be said to be effective> 90% in the essential oil concentration group at a concentration of 4% reaching 100% mortality at the 55th minute in killing Aedes aegypti mosquitoes, while the treatment of concentration groups 0.5%, 1%. It can be concluded that the 4% essential oil concentration group has good mosquito killing activity because it is comparable to the positive control (+) (Transflutrin). Based on the results of this study, it shows that clove leaf essential oil has an insecticidal effect, where the concentration of the 40th minute has worked well, because the higher the concentration of clove leaf essential oil, the greater the mortality value of killing Aedes aegypti mosquitoes. The results of this study when compared to previous research obtained better results because in this study using clove leaf essential oil (*Syzygium aromaticum* (L.) Merr. & Perry.) with a lower concentration than the research of Ariwidiani (2021), whit a higher concentration used. In the

results of this study, effective results were obtained even at low concentrations.

Clove leaf essential oil (*Syzygium aromaticum* (L.) Merr. & Perry.) contains eugenol which gives a distinctive odor and aroma, has a spicy taste and is volatile when left in room air, allowing the compound to damage the respiratory system and has neurotoxic properties that can control damage to the motor nervous system (Sanjaya & Safaria, 2006). The mechanism of action of volatile essential oils allows the active compounds they contain to interact with the mosquito's sense of smell so as to repel mosquitoes. Essential oils inhaled by mosquitoes cause neurotoxic effects that lead to death. The compound inhibits the action of neurotransmitters that play a role in signal transmission in nerve cells. This causes the mosquito's nerve cells to be continuously depolarized, resulting in symptoms such as hyperactivity, spasticity, and tremors in the mosquito. As a result of continuous depolarization, nerve cells will eventually stop working resulting in coordination imbalance that leads to paralysis and death (Mossa, 2016).

The essential oil insecticide test on clove leaves (*Syzygium aromaticum* (L.) Merr. & Perry.) against Aedes aegypti mosquitoes, the next step is to test data analysis with SPSS software by conducting a normality test first using shapiro-wilk. This test aims to determine whether the data is normally distributed or not and continued with Levene's test. The results of the essential oil normality test using Shapiro wilk show that the data is not normally distributed with a significant value smaller than ($p<0.05$) so that it can be continued with the Kruskal wallis test. Based on the test results, it shows that the significant value obtained is below 0.05 or ($p<0.05$), meaning that each treatment concentration has a difference in killing Aedes aegypti mosquitoes. Data were further analyzed using the Post Hoc Mann-Whitney test conducted to determine the differences in various concentrations of essential oils in the differences of each treatment against the death of Aedes aegypti mosquitoes according to Table 3.

The probit analysis test was used to determine the LC50 of clove leaf essential oil. Lethal Concentration (LC50) is the concentration required to kill 50% of mosquitoes. Based on the results of probit analysis, the concentration needed to kill 50% of the test animals or LC50 was 0.993% in the highly toxic category. According to (Ismatullah et al, 2018) tates that the LC50 value is said to be highly toxic in the range of <1%, toxic 1-10%, moderately toxic 10-50%, slightly toxic 50-99%, and non-toxic in the range >100%. Based on the LC50 results obtained, it is included in the highly toxic category in killing mosquitoes. The lower the LC50 value of a substance means that the substance has a higher activity in killing experimental animals, because the substance needs a lower concentration to kill experimental animals for a long period of time. The correlation coefficient shows the degree of relationship between the concentration treatment and mosquito mortality. According to (Agung, 2020). the correlation coefficient (R^2) which has a value of >0.90-1.00 has a very strong correlation, >0.70-0.90 has a strong correlation, >0.40-0.70 has a moderate correlation, 0.20-0.40 has a weak correlation and <0.20 has a very weak correlation. The result of the correlation coefficient value is 0.945. Based on the results obtained, it is included in the criteria for a very strong correlation between the concentration treatment and mosquito mortality.

CONCLUSIONS

From the research that has been done, it can be concluded that clove leaf essential oil (*Syzygium aromaticum* (L.) Merr. & Perry.) has insecticidal activity against Aedes aegypti mosquitoes, the concentration of clove leaf essential oil *(Syzygium aromaticum* (L.) Merr. & Perry.) which is effective in killing Aedes aegypti mosquitoes at a concentration of 4% with 100% mortality in the 55th minute and the LC50 value of clove leaf essential oil *(Syzygium aromaticum* (L.) Merr. &

Perry.) as a natural insecticide against Aedes aegypti mosquitoes with a concentration of 50% can kill Aedes aegypti mosquitoes by 0.993%. Researchers hope that someone can help researchers when testing the activity of clove leaf essential oil (*Syzygium aromaticum* (L.) Merr. & Perry.) Aedes aegypti mosquitoes in order to minimize errors in observation, considering the number of tests and repetitions in large numbers. And for further research, it can be continued with a larger scale cage with a diameter of 180cm2 x 180cm2 x 180cm2 x 180cm2 and this research is expected to be continued on the formulation of preparations to find out whether the results of clove leaf essential oil (*Syzygium aromaticum* (L.) Merr. & Perry.) have a stable mosquito-killing effect on the preparation.

BIBLIOGRAPHY

Achmadi, U. (2010). Manajemen Demam Berdarah Berbasis Wilayah. *Buletin jendela, Bakti Husada*.

Armando, R. 2009. *Memproduksi 15 Minyak Atsiri Berkualitas.* Penebar Swadaya. Jakarta.

Amelia, T., Sumarmi, S., & Nuringtyas, T. (2017). Efektivitas Ekstrak Etanol Daun Mahoni (*Swietenia mahoni* L.) Terhadap Larva *Aedes aegypti. Jurnal Florea Volume, 4(2)*.

Aulya, M. S., Idris, S. A., & Prawibowo, E. (2022). Uji Efektivitas Ekstrak Daun Cengkeh *(Syzygium Aromaticum)* Pada Nyamuk *Aedes aegypti* Sebagai Anti Nyamuk Rapelan. Sulawesi Tenggara: Prodi D3 *Teknologi Laboratorium Medis Politeknik Bina Husada Kendari.*

Centers For Disease Control and Prevetion (CDC). (2012). *Dengue and the Aedes aegypti Mosquito, San Juan.*

Depkes RI, (2015). *Buku Pendoman Kesehatan Jiwa*. Jakarta: Derpatermen kesehatan Republik Indonesia.

Departemen Kesehatan RI, (2000), *Parameter Standar Umum Ekstrak Tumbuhan Obat, Cetakan Pertama*, 3-11, 17-19, Dikjen POM, Direktorat Pengawasan Obat Tradisional.

Ditjenbun. (2016). Pedoman Teknis Kegiatan Penanganan Organime Pengganggu Tumbuhan (OPT). *Direktorat Jendral Perkebunan, Kementrian Pertanian, Jakarta*, 86 hal.

Djau, I., Abdillah, A. S., Maulana, Y., & Limonu, M. (2022). Karakterisasi Hidrosol Berbahaya Dasar Daun Cengkeh (*Syzygium aromaticum*) Dengan Menggunakan Destilasi Uap. *Jurnal Program Stadi Teknologi Pangan*, 36-42.

Faris, M., & Lusiyana, N. (2021). Efek Adul Tisida Minyak Atsiri Bunga Cengkeh (Syzygium aromaticum) Sebagai Bahan Anti Nyamuk *Aedes aegypti*. *JIMKI*, 68-76.

Kementrian Kesehatan RI. (2022). *Profil Kesehatan Indonesia. Jakarta: Kementrian Kesehatan RI*.

Kermentan. (2020). Peraturan Menteri Pertanian Republik Indonesia Nomor 369/ KPTS/SR.330/M/6/2020. *Tentang Kriteria Teknis Pendaftaran Peptisida Pengendalian Vektor Penyakit Pada Manusia*. 14.

Loppies, J. E., Wahyudi, R., Ardiansyah, Rejeki, E. S., & Winaldi, A. (2021). Kualitas Minyak Atsiri Daun Cengkeh yang dihasilkan dari berbagai Waktu Penyulingan. *Jurnal Industri Hasil Perkebunan, 16 No.2*, 89-96.

Mossa, A.H. 2016. Green Pesticides: Essential Oils as Biopesticides in Insect-pest Management. *Journal of Environmental Science and Technology*. 9 (1): 354-378.

Sanjaya Y., Safaria T. 2006. *Toksisitas Racun Laba – laba Nephila sp. Pada Larva Aedes aegypti L.*

http://www.unsjournal.com/D?D0702/D70221.pdf.(11 April 2009).

Syamsunur, Baitanu, J. A., & Gani, S. A. (2019). Pengaruh Tempat Tumbuh dan Lama Penyulingan secara Hidrodestilasi terhadap Rendemen dan Profil Kandungan Kimia Minyak Atsiri Daun Kemangi (*Ocimuum canum sims L*). *Jurnal Fitofarmaka Indonesia,* V0l.6 No.2, 363-366.

WHO. (2009). *Guidelines For Effecacy Testing Of Moisquito Reppelens For Human Skin.*

WHO. (2011). *Specification and Evaluation Fgor Public Health Peticides, Themephos, Themephos Evaluation.*

WHO. (2016). *Test Procedures For Insecticide Resistance Monitoringinmalaria Vector Mosquitoes.* Geneva, Switzerland.

Wibawa, RR, (2012). *Potensi Ekstrak Biji Mahkota Dewa (Phaleria macrocarpa) Sebagai Insektisida Terhadap Nyamuk Aedes aegypti Dengan Metode Semprot. (Skripsi)*. Jember: Fakultas Kedokteran Universitas Jember.

World Health Organization & Regional Office for South-East Asia. (2011). Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever.

World Health Organization, Special Programme for Research, Training in Tropical Diseases, World Health Organization. Department of Control of Neglected Tropical Diseases, World Health Organization. Epidemic and Pandemic Alert, 2009. *Dengue: guidelines for diagnosis, treatment, prevention and control*. World Health Organization.