Volume 1 Issue 1 (2022):25-31
Tropical Marine Environmental Sciences
e-issn 2962-0759

Using *Rhizhopora apiculata* Extract for Mosquito Larvae Control

Soni Sanjaya[1*], Irwan Effendi[1], Nursyirwani[1]

[1]Faculty of Fisheries and Marine, Universitas Riau, Indonesia
Kampus Bina Widya KM. 12,5, Simpang Baru, Kec. Tampan, Kota Pekanbaru, Riau 28293
*soni.sanjaya@student.unri.ac.id

Article Info

Received
18 September 2022

Accepted
1 October 2022

Keywords:
Larvacide,
Mangrove Extract,
Mosquito Control,
LC50,
LT50.

Abstract

Temephos is the most commonly used synthetic larvicide to control vectors of several diseases. Currently in some areas there has been resistance of larvae to temephos, so natural larvicides are needed as an alternative. *Rhizophora apiculata* contains flavonoid compounds that are respiratory toxins to some larvae. This research was conducted in March - Juny 2022, to determine the inhibitory of mangrove extract against mosquito larvae in brackish water. Samples were collected from the Bakar Bakau Dumai, Riau Indonesia. The experiment was setted in a single factor experimental design. Mangrove leaves are finely ground and kneaded while mixed with water and deposited for 6 minutes and filtered. This extract solution was mixed with brackish water to obtain a test medium of 500 l with a concentration of 6 (a1), 9 (a2) and 12% (a3), positive control or 1 g of abate powder (a4) and negative control or brackish water (a0). A total of 20 mosquito larvae were put into the media and their mortality was observed at 0, 15, 30, 45 and 60 minutes after introduction. The mortality of larvae were then analyzed using probit analysis to obtain LC50 (Lethal Concentration 50) and LT50 (Lethal Time 50) values. *R. apiculata* extract is toxic to mosquito larvae, where larval death has been seen since 15 minutes and continues until 60 minutes after introduction. At the 60th minute the mortality rate is as follows; a1 (60 %), a2 (80 %), a3 (100 %), a4 (100 %), and a0 a1 (%). Based on the results of the above probit values in the LC50 and LT50 tests on *R. apiculata* leaves, the LC50 value is 9,732 while the LT50 value is estimated at 21,217.

1. Introduction

Larvasida is a type of insecticide group that is specified to kill larvae. Plant-based larvicides have also been widely pursued by research to minimize resistance. The advantage in using vegetable larvicides is that vegetable larvicides leave only a small residue on environmental components and foodstuffs, so they are safer than synthetic larvsides. Inaddition, pestisidic substances in vegetable larvicides decompose faster in nature, so they do not cause resistance to the target.

Rhizophora apiculata is one of the species of the rhizophoraceae family where oil mangroves are one of the most important species in mangrove forest ecosystems. *R.apiculata* has a very hard, *fast-growing* mangroove wood, has a breath root, opocyte leaf type, and reaches 15 meters in height. *R. apiculata* has a type of viviparous seedling where the lower surface of the leaves is yellowish-green. One of the distinctive features of *R. apiculata* that is different from other types of mangroves is that its leaves tend to be smaller (Kusmana *et al*., 2008)

Mosquitoes are one of the insects that carry diseases that are dangerous to humans such as dengue fever, malaria, zika, and chikunguya. *Dengue* Hemorrhagic Fever (DHF) is a disease caused by infection with the DEN-1, DEN-2, DEN-3, or DEN-4 virus (read: denggi virus type 1-4) which is transmitted

through the bite of mosquitoes that have previously been infected by *the dengue* virus from other dengue sufferers. Type this mosquito, found in almost all corners of Indonesia, except at an altitude of more than 1,000 meters above sea level. Mosquitoes are the most effective and main spreaders of dengue disease (vectors) because they live around residential areas (Ginanjar, 2007).

Mosquitoes are still the main vectors or carriers of dengue fever. The vector of DHF disease is the female mosquito. This mosquito has a special feature characterized by silvery-white ribbons or stripes on a black base, the size of the mosquito ranges around 3-4 mm with white cicin on its legs (Soegijanto, 2006).

The factor biotic or abiotic environment can influence the existence of mosquito vectors from the egg phase to the imago. Mosquito growth from eggs to adult mosquitoes is influenced by abiotic factors such as temperature rainfall and evaporation. Similarly, biotic factors such as predators, competitors, food in the place of habitation, organic matter, microbes, and water insects affect the pre-maturation survival of mosquitoes (Ananda, 2009).

One of the efforts to reduce the negative impact is to look for plant-based materials that are more selective, safe and environmentally sound. Plant-based insecticides leave no residue in the air, water and soil and have a higher level of safety when compared to inorganic toxins. This is due to the molecular arrangement of vegetable insecticides consisting mainly of carbohydrates, nitrogen, oxygen and hydrogen that are easily decomposed into environmentally safe compounds and also reduce the chances of animals that are not targeted for exposure to residues (Rochmat *et al.*, 2007).

In the previous study, Tukiran & Arianti (2012) reported that chloroform extract of mangrove bark oil is toxic to mosquito larvae, because it has an LC value of$_{50}$ of 338,364 mg/L. Halim *et al.* (2013) in their study also reported that in *R. apiculata* leaves was found to be rich with 2-(2-ethoxyethoxy) ethanol (26.45%) and kaur-16-ene (3.37%). The main components in flowers are 2-(ethoxyethoxy) ethanol (11.08%) and butyl cyclohexyl ester 1,2-benzenedicarboxylic acid (3.48%). While the stem contains octadecamethyl cyclononasiloxane (5.24%), kaurene (3.39%) and 1,2,3,4-tetramethoxy-5-(2- propenyl)-benzene (3.26%). Research on oil mangrove leaves (*R. apiculata*) is still very rarely done. Based on this, researchers are interested in conducting research on the larvicide activity test of oil mangrove leaf extract (*R. apiculata*) against mosquito larvae.

The purpose of this study was to test the inhibitory power of *Rhizophora apiculata* leaf extract against mosquito larvae in brackish water. Meanwhile, the results of this study are expected to increase knowledge and provide information about the potential of *Rhizophora apiculata* as a source of bioactive compounds, especially as insecticides

2. Methodology

2.1. Time, Place and Materials

This research has been carried out from March – June 2022 at the Marine Microbiology Laboratory and the Chemistry / Oceanography Laboratory, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Universitas Riau, Pekanbaru. Samples taken in the sea of Bandar Bakau Dumai, the sample was taken to the Marine Microbiology Laboratory for analysis.

2.2. Method Design

The ingredients used in this study are *R.apiculata* leaves. This *R. apiculata* plant was taken from Bandar Bakau Dumai, Pangkalan Sesai Village, Dumai, Riau Province. *R.apiculata* taken was taken to the Marine Microbiology Laboratory, Universitas Riau. Brackish water is used as a place for mosquito larvae, alcohol as a solvent for *R. apiculata* leaves that are tested, while aquades are used to clean all tools and materials used during research.

2.3. Water Quality

Water quality measurement is carried out by measuring physical and chemical aspects. Physical parameters include temperature and salinity, while the chemical parameters measured are pH. Measurement of water quality is carried out at three points of water.

2.4. Extract Preparation

The production of *R.apiculata* extracts was carried out at the Marine Microbiology Laboratory, and the Chemistry Laboratory Faculty of Fisheries and Marine, Riau University. The sample was then taken a few leaves to be weighed in laboratorium using

analytical scales. Furthermore, the sample that has been weighed as much as 10 g and then cut finely to be luskan using a pestle mortar.

The purified sample is then processed little by little enough alcohol to be used as a water sample solution. Furthermore, after the leaves were finely ground, then squeezed for the extraction of the *R. apiculata* leaf extract, the separation of the pulp from the *R. apiculata* leaf extract was carried out using a sieve.

The provision of mosquito larvae begins with looking for mosquito larvae in the area or ditch on the side of the Bina Krida road near the Riau University campus, Pekanbaru City. Mosquito larvae cultures were obtained from available places in Pekanbaru. The mosquito larvae culture obtained was then brought to the laboratory.

2.5. Media Preparation

The preparation of the test media begins with the manufacture of seawater media, and fresh water mixed to get the desired brackish water. Formost than 3 ppt forthe preparation of brackish water using 10 L of fresh water and ±1,200 mL of seawater is taken with a salinity of 25‰ which is put into a large container of water stirredso that it is perfectly mixed. Furthermore, the test media was poured into a sample bottlemeasuring 150 ml but only 100 mL of the test media was used. For this reason, the test media was used as many as 15 sample bottles to be put into the sample bottles. Each sample had 3 concentrations, namely 6, 9 and 12% while the nyes solution used was nipah extract, 1% Abate, and negative control. Furthermore, the removal of mosquito larvae is carried out using a drip pipette. Then put the mosquito larvae into the sample bottle of 20 mosquito larvae. To calculate the ratio of the amount of fresh water and seawater to obtain 3‰ maternity brackish water, it can be used by the formula (Nurdin, 2017).

2.6. Toxicity Test

Toxicity test of nipah extract against mosquito larvae, which is carried out by experimental methods. Toxicity in nipah extract (leaves, midrib, roots) with concentrations of 6%, 9% and 12 % as well as the presence of positive controls and negative controls as a comparison was put on the sample test and there was also control. Each sample contained 15-20 mosquito larvae with 3 replications. Each treatment was observed on the number of mosquito larvae mortality, each treatment group at each concentration within 1 hour in time intervals of 15, 30, 45, 60, by calculating the larvae of dead mosquitoes in each benchmark with the time the observation was carried out, namely n 3 times. The concentration figures taken were as much as 6%, 9% and 12% at the concentration, nipah extract can inhibit the death of mosquito larvae.

2.7. Data Analyzed

The data obtained are available in the form of tables and graphs. Furthermore, the data are analyzed descriptively. To see the differences between treatments, an ANOVA Test can then be carried out to see the certification value using the LSD advanced test. The LC50 value was analyzed using the probit test. All data obtained were analyzed using the SPSS *software* version 23 program.

3. Result and Discussion

3.1. Condition of Research Site

Dumai City is astronomically located between 1°23'00" - 1°24'23" N and 101°23'37" - 101°28'13" E. Dumai City area is bordered by Rupat Strait to the north, Bengkalis Regency to the east and south, and Rokan Hilir Regency to the west. The topography of Dumai City is partly composed of lowlands in the northern part and plateaus in the south. Mostly, the soil structure consists of red-yellow podzolic soils of sedimentary, alluvial, organosol soils, and humus glei in the form of marshes or wet soils. in Dumai city there are 7 sub-districts, namely Bukit Kapur District, Medang Kampai District, Sungai Sembilan District, Dumai Barat District, South Dumai District, Dumai Timur District, and Dumai City District.

Pangkalan Sesai Village is one of the villages located in west Dumai District of Dumai city. There are several tribes that inhabit, namely Malay, Acehnese, Minang, Batak, Javanese, Nias, Kubu Kelurahan Pangkalan Sesai has a coastal area with a coastline of 3 km starting from the district of the mouth of the West Dumai river. Bandar Bakau Dumai Forest or mangrove forest Bandar Bakau Kampung Tuo Kedondong is located in Pangkalan Sesai village, West Dumai District, Dumai City. This area is a mangrove conservation area that is a mangrove information center. The Mangrove Forest of Bandar Bakau Kampung Tuo Kodondong is bordered by the Rupat Strait in the north, in the

south by mascara settlements, in the west by the Dumai River Estuary and in the east by PT. Patra Dock Dumai.

3.2. Water Quality Parameter of Bandar Bakau Dumai

The condition of good and normal water will support the life of a condition of deep nipah forest. This measurement of water quality aims to see the condition of the mangrove forest that grows in these waters normally and there is no disease. In general, the growth of nipah and mangrove forests is influenced by salinity, pH, and temperature. The results of measurements of water quality parameters carried out at the sampling site can be seen in Table 1.

Table 1. Water Quality of Bandar Bakau Dumai

No	Parameters	Value Observations	Quality Standards
1	Salinity (ppt)	30	Natural
2	pH	6-7	6-9
3	Temperature (^{o}C)	28-30	Natural

Based on Table 1, it can be seen that the salinity of the waters at sampling is 30 ppt, while the degree of acidity (pH) with a range of 6-7, for water temperatures it ranges from 28-30 ºC.

3.3. Toxicity of *Rhizophora* Extract Against Mosquito Larvae

Toxicity is the relative property of a chemical substance in its ability to cause harmful effects or deviations of biological mechanisms in an organism. Toxicity is influenced by several factors, including the composition and type of toxicity, toxicance concentration, duration and frequency of exposure, environmental properties, and species of receiving biota. The degree of toxicity of a plant is assessed based on the mortality rate of the test material.

Observations were carried out for 1 hour from the beginning of the experiment, namely by calculating the time of 0 minutes, 15 minutes, 30 minutes, 45 minutes, and 60 minutes the number of larvae that died. The calculation of the number of larval deaths was carried out on each of the concentrations of *R.apiculata* extract, positive control, and negative control. The results of the calculation of the number of larval deaths after 1 hour of observation can be seen in Table 2.

Table 2. Cumulative Mortality Rate (%) Mosquito Larvae in Test Media Added *R. apiculata* Leaf Extract

Extract concentration	0 Min	15 Min	30 Min	45 Min	60 Min
6%	0	10	15	20	60
9%	0	15	45	55	80
12%	0	15	40	95	100
Kontrol (+)	0	70	90	95	100
Kontrol (-)	0	0	0	0	0

In Table 2, it can be seen that in each concentration it shows that *R. Apiculata* leaf extract has the ability as toxicity to mosquito larvae. At concentrations of 6%, 9% and 12% at the time of the 30th minute there was a difference in the percentage of deaths caused by several factors. The number of deaths of mosquito larvae is influenced by the length of time of observation and the consensuality given. The more solutions used the faster the death in mosquito larvae with an increase in the concentration of mangrove extract solution given.

In this observation, it can be said that the more concentration used the faster the mortality rate of the mosquito larvae. The mortality rate of mosquito larvae can clearly be seen in Figure 1.

In Figure 1, it can be seen that mosquito larvae die faster in the use of Abate with a positive concentration, namely at the time (minutes) obtained by 70, 90, 95 and 100%. Meanwhile, in the low concentration, there is a negative control of 0%. Deaths in *R. apiculata* leaf extract were obtained in the 45th minute, namely 20, 55, and 95%. Based on the results

of the grafik above, it is explained that the death of mosquito larvae by *R. apiculata* leaf extract every concentration at the 60th minute is 60, 80 and 100%. The use of mangrove extract is very effective against the death of mosquito larvae. So there is a difference between the treatments given at each concentration.

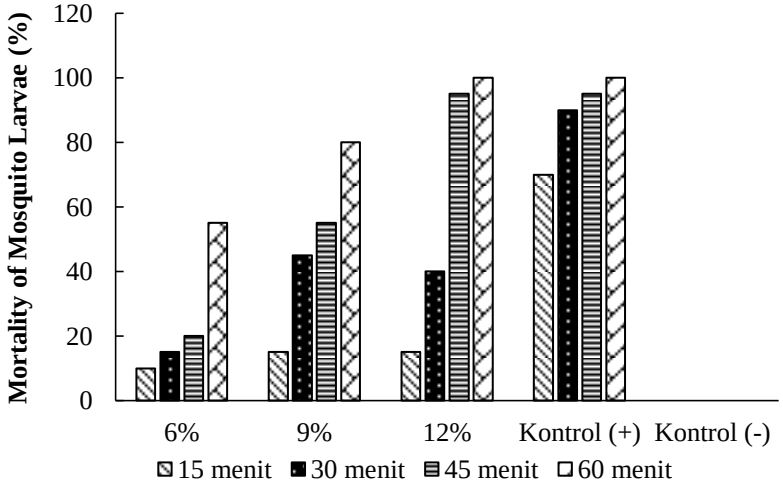

Figure 1. Mortality of Mosquito Larvae at Leaf *Concentrations (R. apiculata)*

This suggests that all mortality centimeters of the test solution concentration affect the mortality of mosquito larvae with observation time. There is treatment A3, A4, and A5 mosquito larvae already experienced death at a time of 60 minutes with a time of 1 hour of observation. In the test, solution treatment, larval mortality also had the highest number of larval mortality at the 60th minute with 1 hour of observation, and this can be assumed that the percentage of mosquito larval deaths contained in *R. apiculata* leaf extract and also positive control worked actively, causing many the larvae become dead.

The mean mortality of mosquito larvae was then analyzed using probit analysis to obtain values of LC50 (*Lethal Concentration* 50) and LT50 (*Lethal Time* 50). The results of the probit analysis can be seen in Table 3.

Table 3. Value of LC50 and LT 50 Leaf extract *(R. apiculata))* against mosquito larvae

		95% Confidence Limits for Extract	
Probability	*Estimate*	*Lower Bround*	*Upper Bround*
LC 50	9.732	7.946	13.307
LT 50	21.217	-	-

Based on the results of the probit value above in the LC50 and LT50 tests on *R.apiculata* leaves, the value for LC50 is 9,732 while in LT50 it is estimated that the value is 21,217. The leaf extract *of R. apiculata* contains active alkaloid-modified terpenoid compounds. The compound has the ability to work as a good contact poison due to its ability to penetrate the organs of insects. In the larvae of such compounds work both as contact poisons and stomach poisons, in the nervous system these active compounds act on the ganglia of the central nervous system. Alkaloids and terpenoids can also cause digestive system disorders because these compounds act as stomach toxins that enter through the mouths of larvae (Taher & Papuangan, 2015). According to Fayemiwo *et al*. (2014) this difference in LC50 is influenced by several factors, including the population used, methodology, concentration of essential oils used and the place of origin of plants used as essential oils.

Based on the probit analysis seen in Table 3, the LC50 from *R.apiculata* leaf extract was 9,732 with a lower limit of 7,946% and an upper limit of 13,307%. This value means that the concentration of *R.apiculata* leaf extract that can kill 50% of mosquito larvae is a concentration of 9,732% with an interval limit of 7,946% to 13,307%. Then obtained LT50 from *R.apiculata* leaf extract was 21.217%. This value means that the time it takes for R. apiculata leaf extract to kill 50% of mosquito larvae from a concentration of 6%, 9%, and 12% at time intervals of 15, 30, 45 and 60

minutes is 21.217%. This proves that the leaf extract *of R.apiculata is* effective as a larvicide.

Temephos has an LC50 against mosquito larvae of 0.001 ppm (Lariska *et al*., 2016). Although there are considerable differences in LC50 and LT50 between leaf extract and temephos, *R.apiculata* leaf solution can be used as an alternative as a natural larvicide against mosquito larvae. According to Ramayanti *et al.* (2016), death of mosquito larvae at various concentrations is thought to be caused by active compounds that have direct contact with larvae mosquito. In the ethanol extract of mangrove leaves *R.apiculata* has active compounds, namely, flavonoids, alkaloids, and tanin.

In positive control using, ABATE 0.01% was used as a positive control in this study. The administration of *Temephos* was 0.01% resulting in the death of 98- 100% of larvae, but in this study, the results of larval death were obtained with an average mortality that was very different in the concentration used. Abate showed a 100% mortality response after mosquito larvae were in contact for 1 hour. This suggests that the death of larvae in the test solution is likely due to the presence of compounds - compounds of a toxic nature capable of killing the mosquito larvae.

This study was conducted to determine the effectiveness of the extract on *R.apiculata* leaves in controlling mosquito larvae in the research test. In this study, *R.apiculata* leaf extract which has been extracted using the experiment method using a 70% alcohol solvent which is intended to obtain flavonoid and limonoid content contained in the extract of *R.apiculata* leaves which is suspected to have an effect on mosquito larvae. According to Isfarani *et al.* (2017) stated that in addition to that there are chemical factors, namely the extraction method and also the solvent used in the extraction will affect the condition of the extract and also its larvicide ability.

In this study, a reduction in the volume of containers was carried out by using containers of the same type as smaller mosquito larvae breeding sites which aimed to streamline the use of limited extracts. Then, measurements of temperature, pH and salinity are carried out at the place of the larval shelter. Solution temperature is an influencing factor in the growth and development of mosquito larvae, the measurement results in this study show an extract temperature of 28 - 30 ° C. According to Noorhajati *et al*. (2012), in testing this larval biolarvicide, a positive control was used, namely 100 ppm ABATE powder so that we can know directly the comparison of mosquito larval repellent power between ABATE as a positive control compared to the mangrove extract fractions

4. C**onclusion**

Based on the results of this study, it was found that *R. apicuata* leaf extract has effectiveness against msquito larvae by using a solution of *R. apiculata* leaf extract. The leaves of *R.apiculata* extract at concentrations of 6, 9, and 12% have the effectiveness to kill mosquito larvae. At a concentration of 12% it has the same effectiveness as the extract of abate solution to kill mosquito larvae. The results of this study showed that the higher the concentration of *R. apiculata* leaf extract given, the more deaths of mosquito larvae.

REFERENCES

Ananda, S. (2009). *Pengaruh Suhu, Kaporit, pH terhadap Pertumbuhan Cendawan Entomopatogen Transgenik Aspergillus Niger-GFP dan Patogenitasnya pada Larva Nyamuk Aedes aegypti. Skripsi.* Departemen Biologi FMIPA IPB.

Arianti & Tukiran. (2012). Biolarvasida dari Tumbuhan Bakau Minyak (*Rhizophora apiculata*). *Jurnal Penelitian Jurusan Kimia FMIPA-Universitas Surabaya.*

Fajri, N.E., Kasry, A. (2013). Kualitas Perairaian Muara Sungai Siak Ditinjau dari Sifat Fisika-Kimia dan Makrozoobentos. *Jurnal Berkala Perikanan Terubuk.* 41(1): 37-52.

Faraco, A., Araujo, D.O., Ribeiro-paes, J.T. and Deus, J.T.De. (2016). Larvicidal activity of *Syzygium aromaticum* (L) Merr and *Citrus sinensis* (L) Osbeck essential oils and their antagonistic effects with temephos in resistant populations of *Aedes aegypti. Memórias do Instituto Oswaldo Cruz*, 111(7): 443-449

Fayemiwo, K.A., Adeleke, M.A., Okoro, O.P., Awojide, S.H., Olufemi, I., Awoniyi. (2014). Larvicidal efficacies and chemical composition of essential oils of *Pinus sylvestris* and *Syzygium aromaticum* against mosquitoes. *Asian Pacific Journal of Tropical Biomedicine* 4(1): 30–34

Ginanjar, G. (2007). *Apa yang Dokter Anda Katakan tentang Demam Berdarah*. Bandung: B-First.

Hamuna, B., Paulangan, Y.P., Dimara, I. (2015). Kajian suhu permukaan laut menggunakan data satelit Aqua-MODIS diperairan Jayapura, Papua. *Depik*, 4(3):160-167.

Kusmana, C., Istomo, Wibowo, C., Budi S.W., Siregar, I.Z., Tiryana, T., Sukardjo, S. (2008). *Manual of mangrove silviculture in Indonesia*. Korea International Cooperation Agency (KOICA), Jakarta.

Lariska, S.S., Martini, Lintang D.S. (2016). Status resistensi *larva Aedes aegypti* (Linnaeus) terhadap Temephos (Studi di Kelurahan Jatiasih Kecamatan Jatiasih Kota Bekasi Provinsi Jawa Barat). *Jurnal Kesehatan Masyarakat*, 4(1): 142–152.

Mardina, P. (2011). Pengaruh Kecepatan Putar Pengaduk dan Waktu Operasi pada Ekstraksi Tannin dari Dewa. *Jurnal Kimia*: 5(2): 125-132.

Nadila, I., Istiana., Wydiamala, E. (2017). Aktivitas Larvasida Ekstrak Etanol Daun Binjai (*Mangifera caesia*) Terhadap Larva *Aedes aegypti*. *Berkala Kedokteran*, 13(1): 61-68.

Noor, Y.R., Khazali, M., Suryadiputra, I.N.N. (2001). *Panduan Pengenalan Mangrove di Indonesia.* Bogor: PHKA/WI-IP.

Nurdin, S. (2017). Optimasi Pembentukan Bioflok dari *Cheatoceros* sp., *Thalassiosira* sp, dan Bakteri Probiotik Melalui Variasi Salinitas Secara In Vitro. *Jurnal Bionature* 18(2).

Ramayanti, I., & Febriani, R. (2016). Uji Efektivitas Larvasida Ekstrak Daun Pepaya (*Carica papaya Linn)* terhadap Larva *Aedes aegypti*. *Fakultas Kedokteran Muhammadiyah Palembang*, 6(2), 79-88.

Rochmat, A., & Nuryoto, R. (2007). Isolasi dan Karakterisasi Senyawa Bioaktif Tanaman Ceraken *(Croton tiglium L.*) Sebagai Larvasida Pencegah Demam Berdarah Dengue.

Soegijanto, S. (2006). *Epidemiologi Demam Berdarah Dengue*. Surabaya: Airlangga University Press.

Taher, D. M., & N. Papuangan. (2015). Potensi cengkeh *(Syzygium aromaticum)* varietas Afo sebagai larvasida alami nyamuk *Anopheles subpictus* dan *Aedes aegypti*, *Biodiversitas Indonesia*. 1(6):1478–1482