LARVACIDAL EFFECT OF ETHANOL EXTRACT OF TORCH GINGER (ETLINGERA ELATIOR) LEAVES AGAINST CULEX QUINQUEFASCIATUS LARVAE

Rian Kurniawan Laksono[1*], Tri Baskoro Tunggul Satoto[2], Budi Mulyaningsih[2]
Faculty of Medicine, Duta Wacana Christian *University*, Yogyakarta[1], *Faculty of Medicine, Public Health and Nursing*, Universitas Gadjah Mada, Yogyakarta[2]
[*]*Corresponding Author* : rian.k.laksono@staff.ukdw.ac.id

ABSTRAK
Nyamuk adalah vektor dari banyak penyakit. Salah satu penyakit yang diperantarai oleh nyamuk adalah filariasis limfatik yang ditularkan oleh spesies *Culex quinquefasciatus* di daerah perkotaan dan semi-perkotaan. Salah satu cara untuk mengendalikan populasi nyamuk adalah dengan menggunakan insektisida. Pestisida sintetis dapat berdampak buruk pada tubuh manusia, sehingga insektisida alami atau yang lebih aman dapat menjadi solusi berkelanjutan. Kecombrang (*Etlingera elatior*) diketahui mengandung komponen insektisida dan telah diteliti sebagai larvasida pada spesies nyamuk lainnya. Dalam penelitian ini, ekstrak etanol dari daun *E. elatior* digunakan untuk menyelidiki potensi efek larvasida terhadap larva *Cx. quinquefasciatus*. Konsentrasi ekstrak yang bervariasi mulai dari 0,1 hingga 10000 ppm ekstrak etanol daun *E. elatior* diperkenalkan kepada larva *Cx. quinquefasciatus* di dalam air selama 24 jam di bawah kondisi laboratorium yang terstandarisasi sebagai uji awal. Pengujian lebih lanjut dilakukan dengan konsentrasi ekstrak bertingkat mulai dari 600 hingga 6750 ppm dan kemudian mortalitas larva dinilai untuk masing-masing konsentrasi. Menggunakan analisis probit, didapatkan LC50 dan LC90 dari ekstrak daun kecombrang adalah 1767.591 ppm dan 6744.573 ppm. Ekstrak etanol dari daun *E. elatior* memiliki efek larvasida terhadap larva *Cx. quinquefasciatus* dalam kondisi laboratorium, dengan peningkatan konsentrasi ekstrak menyebabkan peningkatan mortalitas, namun dosis larvasida tersebut masih belum mencapai dosis yang efisien untuk penggunaan sehari-hari.

Kata kunci : aktivitas larvasida, daun *etlingera elatior*, larva *culex quinquefasciatus*

ABSTRACT
Mosquito is a vector of many diseases. One of them is lymphatic filariasis which is transmitted by Culex quinquefasciatus in urban and semi-urban areas. One way to control the population of mosquito is through the use of insecticide. Synthetic pesticide may have harmful effect to the human body and natural or safer insecticides may be a sustainable solution. Torch ginger (Etlingera elatior) are known to contain insecticidal components and has been studied as larvacide in other species of mosquito. In this research, ethanol extract of E. elatior leaves were used to investigate any potential larvacidal effect against Cx. quinquefasciatus larvae. Varying concentrations from 0.1 to 10000 ppm of ethanol extract of E. elatior leaves were introduced to larvae of Cx. quinquefasciatus for 24 hours under standardized laboratory condition as preliminary test. Further division of the extract concentration from 600 to 6750 ppm was used and larval mortality was assessed. Using probit analysis, the LC_{50} and LC_{90} of the torch ginger leaves extract are 1767.591 ppm and 6744.573 ppm, respectively. The ethanol extract of E. elatior leaves have larvacidal effect against Cx. quinquefasciatus larvae in laboratory settings, with increasing concentrations causing increasing mortality, but still at an inefficient dosage for everyday use.

Keywords *: culex quinquefasciatus larvae, etlingera elatior leaves, larvacidal activity*

INTRODUCTION

Mosquitoes are insects that continuously affect the health of human and animals. The itch caused by the bites of female mosquito irritates humans and also animals. Not only that the bites decrease the quality of life, it is also a mode of disease transmission (Beaty & Marquardt,

1996). Mosquito-borne diseases can become a problem when these four living elements are present: Pathogen, the organism that causes the disease; reservoir & host, the animals in which the pathogen lives and which serve as the source of the pathogen for the mosquitoes that transmit it; susceptible hosts, the people and/or other animals that can be infected by the pathogen; vectors, the mosquito species that can transmit the pathogen, either mechanically or biologically, from it reservoirs to the susceptible hosts. All four of these living elements must be present for a mosquito-borne disease to continue to occur and cycle in a geographic location (Beaty & Marquardt, 1996).

One of the mosquito species that is a vector is *Culex quinquefasciatus*. It is a vector of lymphatic filariasis across urban and semi-urban areas (WHO, 2012). Another disease spread by *Cx. quinquefasciatus* is Japanese encephalitis (Tan *et al.*, 1993). Humans tried to control the population of the vectors, in this case mosquitoes, as effective vaccines or drugs were not always available for the prevention or treatment of the disease. Humans have used various chemicals to control the pests to crops, humans and animals. The developments of synthetic pesticides were done by scientist to achieve this goal (Khan and Ahmad, 2019). However, the use of synthetic pesticides brought their own consequences. The World Health Organization estimated that around 200,000 people are killed every year, worldwide, as a direct result of pesticide poisoning. Added to that, synthetic based larvacide is said to be able to increase the resistance of the vector (Khater, 2012). In northern India, *Cx. quinquefasciatus* mosquitoes are highly resistant to DDT (dichlorodiphenyltrichloroethane) and malathion, while the larvae were found to be resistant to temephos (Kumar *et al.*, 2011). In Macha, Zambia, *Cx. quinquefasciatus* population is also highly resistant to DDT and deltamethrintreated-LLIN material, which is used on mosquito nets, along with some degree of resistance to pyrethroids and malation (Norris & Norris, 2011)

Khater (2012), states that pesticidal plants were used widely until the 1940s, before they are replaced by the synthetic pesticides. Plant based insecticides or bioinsecticides has the property of “hit and run” in which after they are applied to kill the insects, their residue will degrade quickly as they are made from natural products (Kardinan, 2004). One of the plants that are considered to be a bioinsecticide is torch ginger (*Etlingera elatior*). Torch ginger is known to contain insecticidal compounds. The active ingredients that can be found in *E. elatior* are saponin, flavonoid and essential oils (Sulaiman, 2013). Saponin can decrease the surface tension of the mucous membrane of the gastrointestinal tract so the walls of the tract became corrosive. Flavonoid is a compound of the plant defense system that has the quality of inhibiting the insect preying and is toxic (Edmi & Kurniawan, 2012).

The ecological role of essential oils in nature is to protect plants by defense mechanisms against pathogens and predators. The literature review on essential oil had been done and showed that more than 60% of them are effective against *Aedes aegypti* (Dias & Moraes, 2013). Studies on *E. elatior* and its individual parts, mainly leaves and flower, on *Aedes aegypti* larvae shows its larvacidal effects. *E. elatior* flowers extracts has shown a larvacidal effect on *Aedes aegypti* larvae similar to to other plant extracts such as *Phyllantus acidus*, aloe vera, and *Vetiveria zizanoides* (Siregar *et al*, 2019). Another study on the larvacidal effect of *E. elatior* flower on *Aedes aegypti* larvae also shows a promising result, obtaining an LC_{50} at a low concentration. Other than mosquito, E. elatior essential oil has been shown to have an insecticidal effect on termites (*Coptotermes curvignathus sp*.) (Zuzani *et al.*,2015). This study aims to explore the larvacidal effect of *E. elatior* leaves on *Cx. quinquefasciatus* mosquito larvae.

METHODS

The research is done through a phase 1 simple experimental study to test the biopotency of formulated larvacide. The larvae of *Cx. quinquefasciatus* is exposed to extract of *E. elatior*

leaves with varying concentrations in laboratory settings. The subject used is larvae of *Cx. quinquefasciatus* mosquito at the stage of instar III, which are taken from the ecosystem of Yogyakarta and colonized in Laboratory of Parasitology, Faculty of Medicine, Gadjah Mada University. *E. elatior* leaves extract was prepared by maceration extraction method and stock solutions with concentrations of (in ppm) 0.01, 0.1, 1.0, 10.0, 100.0, 1000.0, and 10000.0 are made along with negative controls of aquadest and aquadest with tween solution at 0.5%. The *Cx. quinquefasciatus* larvae are exposed to different concentrations of *E. elatior* leaves extract prepared through serial dilution and placed under room temperature and observed after 24 hours for larval mortality. The concentration causing larva mortality in between 20% to 80% is determined and seven different concentrations within this range are prepared to determine the LC_{50} and LC_{90}. Probit analysis is used to assess the toxicity. The LC50 and LC90 can be determined, based on the regression line, with confidence limit of 95%, made using the results obtained from the final test of the larvacidal activity.

RESULT

The study consisted of preliminary test and final test. The preliminary test was conducted to determine the range of concentration that will have 20% to 80% larval mortality. In the preliminary test, seven groups of 10 larvae were exposed to prepared dosage of ethanol extract of *E. elatior* leaves. The concentrations used (in ppm) are 0.01, 0.1, 1.0, 10.0, 100.0, 1000.0, and 10000.0. The mortality of the larvae is observed after a period of 24 hours. The exposure of larvae to the concentrations of 0.01 ppm, 0.1 ppm, 1.0 ppm, 10 ppm and 100 ppm resulted in 0% larval mortality each respectively. Larval mortality in concentration of 1000 ppm showed 50% mortality of the larvae. The concentrations of 10000 ppm yield 100% larval mortality. The following Table 1 illustrates the result obtained.

Table 1. Larval Mortality After 24 Hours Exposure To Ethanol Extract Of *E. Elatior* Leaves In Preliminary Test

Concentration (ppm)	Total Larvae Tested	Total Mortality of Larvae	Percentage of Larval Mortality (%)
Control	10	0	0.00
0.01	10	0	0.00
0.1	10	0	0.00
1.0	10	0	0.00
10.0	10	0	0.00
100.0	10	0	0.00
1000.0	10	5	50.00
10000.0	10	10	100.00

Based on the preliminary results, nine different concentrations are made to be used in the final test. The increment of the concentrations is calculated with the formula

$$F = \sqrt[n-1]{\frac{LD}{SD}}$$

Where F is the multiplier factor, SD and LD are the doses estimated to produce around 20% and 80% larval mortality and n is the preferred amount of concentration variations. These are 600.0 ppm, 900.0 ppm, 1350.0 ppm, 2000.0 ppm, 3000.0 ppm, 4500.0 ppm, and 6750.0 ppm.

Based on the result of the final test, the lowest concentration that showed larval mortality is at 10.0 ppm with 10% average mortality. The concentration that showed the highest mortality of 93.3% is at 6750 ppm. It can be concluded that the ethanol extract of *E. elatior* leaves have

larvacidal activity against *Cx. quinquefasciatus* larvae in laboratory setting. Using the results in Table 2, it can be observed that increasing the concentration of the extract of *E. elatior* leaves produces increasing larval mortality. Based on the results obtained, there is a proven relationship between the concentration of the extract used and the resulting mortality of the larvae. In accordance to the Laboratory and Field Testing of Mosquito Larvacides released by WHO in 2005, attest result is valid if the relative standard deviation is less than 25% (WHO, 2005). The controls produce 3.3% mortality, so the experiment does not require corrections using the Abbot formula. The result can be analyzed using probit analysis to calculate LC50 and LC90 values for the extract.

Table 2. Larval Mortality After 24 Hours Exposure To Ethanol Extract Of *E. Elatior* Leaves In The Final Test

Concentration (ppm)	Total Larvae Tested	Total Mortality of Larvae			Percentage of Average Larval Mortality (%)
		N1	N2	N3	
Control	10	0	1	0	3.3
600	10	2	1	0	10.0
900	10	3	3	2	26.7
1350	10	6	3	5	46.7
2000	10	7	7	5	63.3
3000	10	6	7	7	66.7
4500	10	7	6	8	70.0
6750	10	9	10	9	93.3

Table 3. Value Of LC50 And LC90 Of Ethanol Extract Of *E. Elatior* Leaves Against *Cx. Quinquefasciatus* Larvae

Mortality Percentage	Concentration (ppm)	95% Confidence Limits		Variance
		Lower	Upper	
50	1767.591	1488.524	2156.94	1.94571
90	6744.572	4551.265	9994.844	7.595821

y = 2.203914x – 2.156951

Regression coefficient = 2.203914
Intercept = -2.156951
Chi square (X2) = 5.032734
Degree of freedom = 5

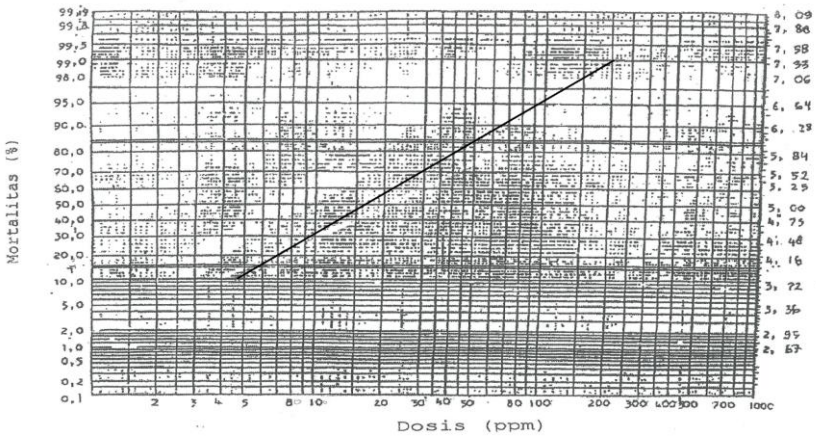

Figure 1. The Percentage Death Of *Cx. Quinquefasciatus Larvae* Against Concentrations Of Ethanol Extract Of *E. Elatior* Leaves

Based on the data in table 3, a dose-response graph can be plotted. The left y-axis showa percentage of mortality and the right shows probit unit. The x-axis shows the dosage concentrations in ppm. The equation derived is y = 2.203914x – 2.156951. The y-intercept of the line is – 2.156951. The gradient of the line is 2.203914, a positive value which means that increasing the concentration of the extract will cause increasing larval mortality. The LC50 is 1767.591 ppm and LC90 is 6744.572 ppm.

DISCUSSION

This research is conducted with the aim to establish the larvacidal effect of ethanol extract of *E. elatior* leaves on *Cx. quinquefasciatus* larvae. This study can be developed into phase II laboratory testing or field testing of the extract. It is to be used as the base for further researches to develop bioinsecticide that serves as an alternative to the insecticides currently used. The results obtained from the experiment shows that ethanol extract of *E. elatior* leaves has larvacidal effect against *Cx. quinquefasciatus* larvae. Larval mortality can be observed starting from the concentration of 600 ppm with 10% mortality in average. The other concentrations of 900 ppm, 1350 ppm, 2000 ppm, 3000 ppm, 4500 ppm and 6750 ppm produces 26.7%, 46.7%, 63.3%, 66.7%, 70.0% and 93.3% larval mortality in average respectively. The standard parameter used to evaluate larvacidal properties is Lethal Concentration 50 (LC_{50}) (WHO, 2005). It provides an estimation of the toxicity of the insecticide and represents the tolerance of the insect (Heong *et al.*, 2011).

Using probit analysis, the dosage response relationship sigmoid curve can be converted into regression line, making it possible to find the LC_{50}. An effective larvacide needs to have a small number of LC_{50} on the tested larvae. Any type of substance must have the LC50 below 100 ppm to be used as commercial larvicide (Tarumingkeng, 2001). The LC_{50} of the ethanol extract of *E. elatior* leaves on *Cx. quinquefasciatus* larvae is 1767.591 ppm. This value is higher than 100 ppm, which means that the extract will not make an effective larvacide. The LC_{90} was recorded at 6744.572 ppm. The LC_{50} of a substance is a parameter of the toxicity towards mammals and human. The toxicity of a specific substance is considered high if the LC_{50} is below 100 ppm and considered low if the LC_{50} is above 1000 ppm (Tarumingkeng, 1992).

Suryanto *et al* (2018) reported an LC50 of 1.216% (12160 ppm) at 24 hours and 0.595% (5950 ppm) at 48% for *E. elatior* leaves ethanol extract on *Cx. quinquefasciatus* larvae. This value is siginificantly higher than the value obtained in this study. The larvacidal effect of E. elatior leaves has been studied on other species of mosquito in Indonesia, *Ae. aegypti*. Sulaiman (2013) tested the larvacidal activity of ethanol extract of the leaves against *Ae. aegypti* larvae. The result of this study shows an LC_{50} of 0.47% which is equivalent to 4700 ppm showing a significant difference to this study. Koorag *et al*. (2016) also tested ethanol extract of *E. elatior* leaves on *Ae. aegypti* larvae and obtained a higher LC_{50} of 1.204% (12040 ppm). Siregar *et al* (2019) reported a similar result, which is an LC_{50} of 1.112% for *E. elatior* leaves extract on *Ae. aegypti* larvae. Other studies have been conducted to test the larvacidal effect of other part of *E. elatior*. The extract of the flower was tested against *Cx. quinquefasciatus* larvae and produces an LC_{50} of 52.087,360 ppm (Astuti, 2011). Another study by Awang *et al* (2019) showed an LC_{50} of around 25 ppm, which is far lower than the result obtained by Astuti (2011).

The flower extract of *E. elatior* was also studied as a larvacide for another species, *Ae. aegypti*, and showed an LC_{50} of 0.334% or equivalent to 3340 ppm (Destriani *et al.*, 2023), 0.053% (530 ppm) (Koraag *et al*., 2016) and 0.862% (8240 ppm) (Siregar *et al*, 2019) on *Ae. aegypti* larvae. Despite having better results on *Ae aegypti*, a contradicting result was obtained by Annashr *et al* (2024) which tested *E. elatior* flower extracted with 70% ethanol and resulted in LC50 of 6.577%. One of the factors that may cause the difference in these values is the source of plantation, which may affect the chemical content of the plant. Leaves of highland

populations of *E.* species were found to have higher Total Phenolic Content (TPC) than lowland counterparts (Chan *et al.*, 2011). *E. elatior* grown in three different locations also contain three different total phenolics (TPC), total flavonoids (TF), and total tannin content (TTC) levels showing different antibacterial and antioxidant and anticancer properties (Ghasemzadeh *et al*., 2015).

Aside from the leaves and flower, the stem of *E. elatior* has been studied as a larvacide but done on *Ae. aegypti* instead of *Cx. quinquefasciatus* and showed an LC_{50} of 0.77% or 770 ppm (Edmi and Kurniawan, 2012). Hidayatulloh *et al* (2013) studied the root extract of *E. elatior* and reported an LC_{50} of 0.54% on *Ae aegypti* larvae. Various parts of *E. elatior* contain different chemicals which are responsible for the larvacidal property. Phytochemical screening of inflorescences showed the presence of flavonoids, terpenoids, saponins, tannins, and carbohydrates (Wijekoon *et al*., 2010). The leaves contain flavonoids of kaempferol 3-glucuronide, quercetin 3-glucuronide, quercetin 3-glucoside, and quercetin 3-rhamnoside (Abdelwahab *et al.*, 2010). Flavonoid can decrease the capability to digest food by decreasing the activity of protease and amylase enzymes. As a result, the growth of the insect is disturbed (Tarigan *et al.*, 2014). There were a few limitations to this research which as follows Inaccuracy in the preparation of the stock solution and the concentrations for the tests due to human error or instrumental error. The damage on the active compound in the leaves during drying and preparation process could not be identified. The larvae chosen for the test may be unhealthy from the start and the mortality is not caused by the extract. The selection of larvae was difficult due to the limitation of equipments and time. This increases the chance of older larvae to be chosen and increases the chance of pupation.

CONCLUSION

The ethanol extract of *E. elatior* leaves has an LC_{50} of 1767.591 ppm and LC_{90} of 6744.572 ppm against *Cx. quinquefasciatus* larvae. Increasing the concentration of ethanol extract of *E. elatior* leaves increases the *Cx. quinquefasciatus* larvae mortality. Ethanol extract of *E. elatior* leaves have larvacidal effect against *Cx. quinquefasciatus* larvae, but it is not effective to be used as an insecticide.

ACKNOWLEDGMENT

The researcher would like to express his gratitude for the support, inspiration and assistance to all parties in helping the researcher complete this research, including the participants who were willing to participate in the research until it was completed.

REFERENCES

Abdelwahab, S. I., Zaman, F. Q., Mariod, A. A., Yaacob, M., Abdelmageed, A. H. A., & Khamis, S. (2010). Chemical composition, antioxidant and antibacterial properties of the essential oils of *Etlingera elatior* and *Cinnamomum pubescens* Kochummen. *Wiley Online Library*.

Annashr, N. N., Anissa Permata Sari, & Dian Saraswati. (2024). Effectiveness Extract Larvacides of Kecombrang Flower (Etlingera elatior) on Death of Aedes aegypti Mosquito Larvae. *Miracle Get Journal*, *1*(4), 16–25. https://doi.org/10.69855/mgj.v1i4.53

Astuti, M. A. W. (2011). Uji daya bunuh ekstrak bunga kecombrang (*Nicolaia speciosa*(Blume) Horan.) terhadap larva nyamuk *Culex quinquefasciatus* Say. [Undergraduate thesis, Universitas Atma Jaya Yogyakarta].

Awang, Z., Husin, M. N. H., & Abdullah, S. S. A. (2019). *Potensi Larvasid Nyamuk daripada Ekstrak Bunga Kantan (Etlingera Elatior) terhadap Culex Quinquefasciatus*. *4*(1).

Beaty, B. J., & Marquardt, W. C. (1996). *The biology of disease vectors*. University Press of Colorado.

Chan, E. W. C., Lim, Y. Y., & Wong, S. K. (2011). Phytochemistry and pharmacological properties of *Etlingera elatior*: A review. *Pharmacognosy Journal*, 3, 6-10.

Destriani, N. P. I., Risma, Pasaribu, I. A., & Rohmah, E. A. (2024). Effectiveness Of Kecombrang Flower (*Etlingera elatior*) As A Larvacide For *Aedes aegypti* Instar III Mosquitoes. *E-Jurnal Medika Udayana*, *13*(2), 26. https://doi.org/10.24843/MU.2024.V13.i02.P06

Dias, C. N., & Moraes, D. F. C. (2013). Essential oils and their compounds as *Aedes aegypti* L. (Diptera: Culicidae) larvicides: Review.

Edmi, F., & Kurniawan, B. (2012). Uji Efektivitas Fraksi N-Heksana Ekstrak Batang Kecombrang (*Etlingera elatior*) Sebagai Larvasida Terhadap Larva Instar III *Aedes aegypti*. *MAJORITY Medical Journal Lampung University)*, *1*(1), 50–61.

Finney, D. J. (1971). *Probit analysis* (3rd ed.). Cambridge University Press.

Gerberg, E. J., Barnard, D. R., & Ward, R. A. (1994). Manual for mosquito rearing and experimental techniques. *American Mosquito Control Association Bulletin*, No. 5, 61-62.

Ghasemzadeh, A., Jaafar, H. Z. E., Rahmat, A., & Ashkani, S. (2015). Secondary metabolites constituents and antioxidant, anticancer and antibacterial activities of Etlingera elatior (Jack) R.M.Sm grown in different locations of Malaysia. *BMC Complementary and Alternative Medicine*, *15*(1), 335. https://doi.org/10.1186/s12906-015-0838-6

Heong, K. L., Tan, K. H., Fabellar, L. T., & Garcia, C. P. F., Lu, Z. (2011). Research methods in toxicology and insecticide resistance monitoring of rice planthoppers. The International Rice Research Institute.

Hidayatulloh, N., Kurniawan, B., & Wahyuni, A. (2013). Efektivitas pemberian ekstrak ethanol 70% akar kecombrang (*Etlingera elatior*) terhadap larva instar III *Aedes aegypti*sebagai biolarvasida potensial. [Undergraduate thesis, Universitas Lampung].

Hidayatulloh, N., Kurniawan, B., & Wahyuni, A. (2013). *Efektivitas Pemberian Ekstrak Ethanol 70% Akar Kecombrang (Etlingera elatior) Terhadap Larva Instar III Aedes aegypti sebagai Biolarvasida Potensial*.

Kardinan, A. (2004). *Pestisida nabati, ramuan dan aplikasi*. Penerbit Swadaya.

Khater, H. F. (2012). Prospects of botanical biopesticides in insect pest management. *Journal of Applied Pharmaceutical Science*, JAPS.2012.2546.

Khan, Md. A., & Ahmad, W. (2019). Synthetic Chemical Insecticides: Environmental and Agro Contaminants. In Md. A. Khan & W. Ahmad (Eds.), *Microbes for Sustainable Insect Pest Management* (pp. 1–22). Springer International Publishing. https://doi.org/10.1007/978-3-030-23045-6_1

Koraag, M. E., Anastasia, H., Isnawati, R., & Octaviani, O. (2016). Efikasi Ekstrak Daun dan Bunga Kecombrang (Etlingera elatior) terhadap Larva Aedes aegypti. *ASPIRATOR - Journal of Vector-borne Disease Studies*, *8*(2), 63–68. https://doi.org/10.22435/aspirator.v8i2.4615.63-68

Kumar, K., Sharma, A. K., Kumar, S., Patel, S., Sarkar, M., & Chauhan, L. S. (2011). Multiple insecticide resistance/susceptibility status of *Culex quinquefasciatus*, principal vector of bancroftian filariasis from filarial endemic areas of northern India. *Asian Pacific Journal of Tropical Medicine*, 426-429.

Norris, L. C., & Norris, D. E. (2011). Insecticide resistance in *Culex quinquefasciatus*mosquitoes after the introduction of insecticide-treated bed nets in Macha, Zambia. *Journal of Vector Ecology: Journal of the Society for Vector Ecology*, 36(2), 411–420.

Siregar, A. Z., Pradana, M. G., & Rahmi, D. (2020). Effect of Kecombrang (Etlingera elatior) as Larvacide to Control Aedes aegypti (Diptera: Culicidae). *Proceedings of the International Conference and the 10th Congress of the Entomological Society of Indonesia (ICCESI 2019)*. International Conference and the 10th Congress of the Entomological Society of Indonesia (ICCESI 2019), Kuta, Bali, Indonesia. https://doi.org/10.2991/absr.k.200513.006

Sulaiman. (2013). Efektivitas pemberian ekstrak ethanol 70% daun kecombrang (*Etlingera elatior*) terhadap larva instar III *Aedes aegypti* sebagai larvasida potensial. [Undergraduate thesis, Universitas Lampung].

Suryanto, Santoso, L. M., & Suratmi. (2018). Pengaruh Penggunaan Ekstrak Daun Kecombrang (Etlingera Elatior) Sebagai Larvasida Nabati Larva Nyamuk Culex Quinquefasciatus Dan Sumbangannya Pada Pembelajaran Biologi Sma. *Jurnal Pembelajaran Biologi*, *5*(2).

Tarumingkeng, R. C. (2001). Pestisida dan penggunaannya. http://rudyct.tripod.com/TOX/PESTISIDA.htm

Tarumingkeng, R. C. (1992). *Insektisida: Sifat, mekanisme kerja dan dampak penggunaannya*. Ukrida Press.

Tarigan, L. A., Batubara, R., & Sumardi. (2014). Pemberian variasi konsentrasi maserat bunga kecombrang (*Etlingera elatior* Jack R. M. Sm) sebagai bioinsektisida terhadap nyamuk *Aedes* spp. [Undergraduate thesis, Universitas Sumatera Utara].

Virgianti, D., & Masfufah, S. (2015). Efektivitas ekstrak daun kecombrang (*Etlingera elatior*) sebagai antioviposisi nyamuk *Aedes aegypti*. *Jurnal Kesehatan Bakti Tunas Husada: Jurnal Ilmu-ilmu Keperawatan, Analis Kesehatan dan Farmasi*, 14, 108. https://doi.org/10.36465/jkbth.v14i1.117

Wijekoon, M. M. J. O., Bhat, R., & Karim, A. A. (2010). Effect of extraction solvents on the phenolic compounds and antioxidant activities of bunga kantan (*Etlingera elatior*Jack) inflorescence. *Journal of Food Composition and Analysis*.

World Health Organisation (WHO). (2005). *Guidelines for laboratory and field testing of mosquito larvacide*. http://whqlibdoc.who.int/hq/2005/who_cds_whopes_gcdpp_2005.13.pdf

World Health Organisation (WHO). (2012). *Lymphatic filariasis factsheet*. http://www.wpro.who.int/mvp/topics/ntd/20120308/en/

Zuzani, F., Harlia, & Idiawati, N. (2015). Aktivitas Termitisida Minyak Atsiri Dari Daun Cekalak (Etlingera elatior (JACK) RM. SM.) TERHADAP RAYAP Coptotermes Curvignathus Sp PADA TANAMAN KARET. *Jurnal Kimia Khatulistiwa*, *4*(3), 16–21.