KEMAS 17 (1) (2021) 24-30

Jurnal Kesehatan Masyarakat

http://journal.unnes.ac.id/nju/index.php/kemas

Z. mauritiana Leaves as Larvasidal Alternatives

Amaliyah Nurul✉, Aryanto Purnomo, Khayan Khayan, Slamet Wardoyo, Taufik Anwar
Department of Environmental Health, Poltekkes Kemenkes Pontianak, 78241, Pontianak, Indonesia

Article Info

Article History:
Submitted May 2020
Accepted September 2020
Published July 2021

Keywords:
Z. mauritiana leaves extract; Aedes aegypti, larvae; mortality

DOI
https://doi.org/10.15294/kemas.v17i1.24377

Abstract

The development of resistance to chemical insecticides among mosquitos has been considered as a setback in vector control. However, the use of chemical insecticides is often toxic to both human and non-target animals, thus it needs the development of natural insecticides. This study aims to analyze the larvicidal effect of Z. mauritiana leaves as a natural larvicidal that is friendly to the environment. This research will outline the larvicidal effect of Z. mauritiana leaves and discuss the significance of addressing this natural larvicide against Aedes aegypti larvae. Methods and Material in this study, we developed a mosquito larvacide by extracting Z. mauritiana leaves with the maceration method. We extracted Z. mauritiana leaves into 5 different concentrations (1%, 3%, 5%, 7%, 9%) and its effect compared to the current chemical larvicide (1% of temephos). Data analysis was performed by using the computer and statistical of variance test One Way Anova. This experiment was successfully 100% killed Aedes aegypti in 24 hours of the intervention of 9% extract of Z. mauritiana leaves which was a powerful effect compare to recent studies. The intervention of concentration variations was significantly associated with larvae mortality ($p \leq 0,001$).

Introduction

Mosquitoes can transmit a number of diseases than any other group of arthropods and affect more than 700 million people worldwide annually, including arboviruses responsible for yellow fever, dengue hemorrhagic fever, epidemic polyarthritis, several forms of encephalitis and bancroftianfilariases (Kazembe and Makusha 2012) and pathogens which continue to have devastating effect on human beings (Maheswaran, Sathish, and Ignacimuthu 2008). One of the concerned disease which cause a high number of mortality is Dengue Hemorrhagic Fever (Jain et al., 2017). Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by Aedes aegypti (World Health Organization 2009; Pham et al., 2011; World Health Organization 2016; Sucipto, Raharjo, and Nurjazuli 2015). In Province of West Borneo, for the last five years, there were fluctuative Dengue Fever cases starting from 2011, were 784 cases (CFR 1,3%), 1.614 cases in 2012 (CFR 1,4%), 2013 recorded 838 cases (CFR 1,7%), 5.049 cases were noted in 2014 (CFR 1,3%), 2015 were 951 cases (CFR 1,6%) and 1.210 cases in 2016 (CFR 1,4%) (Kementerian Kesehatan 2016; Dinas Kesehatan Kalimantan Barat 2015).

Baseline cause of DHF is the high population of Aedes aegypti. This mostly due to the presence of uncovered water reservoirs and less implementation of larvicides other than low Clean and Healthy Living Behavior (Sucipto, Raharjo, and Nurjazuli 2015). Most community in West Borneo use water reservoir like crocks inside or outside of their houses. Based on research, the highest OR value (8.8) as the causes of mosquitos breeding are water reservoir and Clean and Healthy Living Behavior, Especially hanging the clothes behavior with OR value (8.3) (Kementerian Kesehatan 2016). Personal protection from mosquito bites is currently the most important way to prevent transmission of these disease (Fradin 1998). To prevent

✉ Correspondence Address:
Department of Environmental Health, Poltekkes Kemenkes Pontianak, 78241, Pontianak, Indonesia
Email : amaliyah760@gmail.com

pISSN 1858-1196
eISSN 2355-3596

proliferation of this mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential.

Chemical control like the use of larvacides in line with WHO is as the best choice to control vectors specifically in endemic or the high number of outbreaks areas (World Health Organization 2009), meanwhile based on a research in Brazil, chemical larvicides such as themepos has experienced resistance (Marcome et al., 2012; Abou-elnaga 2014), side effect towards environment (non-target organisms involved) (Aguirre-obando et al., 2015; Tennyson et al., 2013) and people tend to dislike the smell (Tennyson et al., 2013). There is now a strong consensus among scientists and public health workers that alternative tools (insecticides) and strategies are urgently needed to ensure effective and sustainable control of dengue in the tropics, for example natural ingredients which can be accepted by the community and safe to use (Marcome et al., 2012; Pamungkas, Syafei, and Soeroto 2017). The new tools need to be specific, cost-effective, and safe for the environment and non-target fauna. Unfortunately, the research and development of new chemical classes for use in public health control has been rather limited in the past 20 years because it involves a long, expensive, and non-profitable process for private companies.

Different methods have been offered by recent studies in the control of larvicides and mosquitos. The use of natural compounds such as lemongrass, citrus leaves, betel leaf as larvacides has been carried out by several researchers and the results are quite effective to be used as larvacides, but based on research has not been applied in the community due to lack of socialization about the use and change of taste natural as mentioned above, so it needs an alternative to other natural ingredients such as Z. mauritiana leaves (Pratiwi 2012). Z. mauritiana leaves contain compounds such as flavonoid, pektin, glikosida, alkaloid, triterpenoat acid, saponin, tanin and lipid (Ghosh, Chowdhury, and Chandra 2012; Dureja and Dhiman 2012). Furthermore, it also contains triterpenoat acid, oleanolat acid, betulinat acid, oleanonat acid, zizyberenalat acid and betulinat acid. Saponin and tannin are larvicides which can destroy tracture digestive membrane and epiphytic and binds the larvae of Aedes aegypti, more over Z. mauritiana leaf has carotene source, vitamins A and C and can be used as vegetables (Ma et al., 2017; Lamien-Meda et al., 2008). This statement encouraged us to use Z. mauritiana leaves as natural larvicide by extracting in order to isolate its chemical compounds.

The objective of this study was to determine the larvicide effect of Z. mauritiana leaves extract towards Aedes aegypti larvae by using the variations of Z. mauritiana leaves extract and to determine the value of LC50 and LC90. In addition, this study is important to identify the effectivity of Z. mauritiana leaves compare to temephos. Researchers state whether there is a difference mortality of Aedes aegypti larvae after giving interventions with Z. mauritiana leaves extract.

Methods

Z. mauritiana leaves extract were obtained from local producers in Pontianak, and its extract were obtained from Tanjung Pura University Laboratory by using maceration method and ratio 1:1. Maceration results are filtered by using filter paper, then the existing maserate is concentrated at 500C in oven so Z. mauritiana leaves extract becomes more concentrated. Concentration of Z. mauritiana . Eggs of Aedes aegypti was collected from researchers house, especially in a humid and dark rooms such as under the stairs in Pontianak. Aedes aegypti was obtained as egg rafts on the filter paper and were reared in trays containing tap water and maintained at 28 ± 2`C. The average of eggs trapped in each ovitrap around 30-50 eggs. In the main experiment, the larvae were reared in 15 × 20 cm plastic trays. When the eggs were hatched out into first instar larvae, they were fed with a mixture of yeast powder and dog biscuits in the ratio of (1:3). On the third day after hatching the first instar larvae moulted into second instar larvae on the fifth day, third instar larvae observed, which moulted into fourth instar larvae on the seventh day (Mohan and Ramaswamy 2007). The 1st and 2nd instar of Aedes aegypti was experimented for the present study.

The larvicidal assay were conducted according to WHO (Fuadzy and Marina 2012).

The larvacidal effects of the Z. mauritiana leaves formulation were tested on Aedes aegypti under room conditions. Baseline tests were initially run in distilled water to determine the range of lethal doses of the formulation. Each of the six beaker glasses had the ordinary larval rearing medium supplemented with the bidara leaves extract formulation at the different concentrations (i.e. 1%, 3%, 5%, 7% and 9%). Counting the number of larvae mortality by using shooter count was conducted to determine the effects of different concentration of bidara leaves extract and 1% of temephos. Terminate mortality was assessed at 24 hours. Dead larvae were identified when they failed to move after touching with tip of thin brush. This experiment was repeated four times.

Data analysis was performed by using computer and statistical of variance test One Way Anova and means were separated using LSD at p<0,05. Probit analysis of larvae mortality was carried out to determine LC50 and LC90. Significant difference in LC50 and LC90 were determined by the overlapping and non-overlapping of the 95% confidence interval (CI).

Results and Discussion

The study began by making Z. mauritiana leaves extract using maceration with a ratio of 1:1, then examined the content of alkaloids, flavonoids and saponins by phytochemical tests which conducted in the laboratory of Tanjung Pura University with positive results for those parameters. After becoming an instar III / IV a preliminary test is performed to determine the LC50 and LC90 values. In this study, physical environment is considered by measuring temperature and humidity by using 4 in 1 multifunction. The temperature and pH are measured in each repetition, and the average results can be seen in the table 1.

Table 1. Measurement result of temperature and pH

Repetition	Temperature [^{0}C]	pH
I	29	6.5
II	28	6.5
III	28	6.4
IV	29	6.4
Average	28.5	6.45

Source: Primary data, 2018

The average of temperature in all repetition was 28,50C and pH was about 6,45. These result similar with the optimum condition of environment where Aedes aegypti larvae lives.

After turning into instar III/IV, a preliminary test is carried out in order to determine LC50 and LC90 value. The mean, minimum and maximum lethal concentration (LC50 and LC90) on Aedes aegypti larvae at 24 hours exposure in interventing Z. mauritiana leaves extract is shown in Table 2.

Based on the result of equation analysis, it can be concluded that concentration (Y) = 4.1667X + (-2.6667), where X is the amount of mortality larvae.

The highest mortality of larvae is recorded in 9% of Z. mauritiana leaves extract at 24 hours application which was significantly higher than average mortality of larvae in 1%-7% (Table 1). In addition, at 24 hours after Z. mauritiana leaves extract exposure to larvae, larvae mortalities at concentration of 7% to 9% were not significantly but they were significantly higher than mortalities at 0%, 1%, 3% and 5% (Table 2). To compare, in temephos 1%, all larvae found dead, was significantly higher than average mortalities in 0%, 1%, 3% and 5% of Z. mauritiana leaves extract.

Table 2. The analysis result of LC_{50} and LC_{90} of *Z. mauritiana* Leaves as Larvacide

Lethal Concentration	Mean	Minimum	Maximum
LC_{50}	4.697	1.306	6.472
LC_{90}	8.648	6.136	12.113

Source: Primary data, 2018

Table 3. Effect of *Z. mauritiana* Leaves Extract Concentration on Larval Mortality of *Aedes aegypti*

Repetition	Larvae Mortality							P value
	Control	1%	3%	5%	7%	9%	Temephos 1%	
I	0	2	8	14	19	20	20	≤0.001
II	0	3	8	13	20	20	20	
III	0	2	7	14	18	20	20	
IV	0	2	8	14	19	20	20	
Mean	0	2.75	7.75	13.75	19	20	20	
%	0	13.75	38.75	68.75	95	100	100	

Source: Primary data, 2018

It can be seen that the Z. mauritiana leaves concentration at 9% cause the highest larvae mortality than others, and it had same mortality average with 1% temephos intervention. In all cases, higher larvae mortality was recorded with increase in the concentration of Z. mauritiana leaves extract which exposure to larvae. Statistical analysis proved that there was a difference of larvae mortality between concentration variations of Z. mauritiana leaves extract. Z. mauritiana leaves extract was an effective larvacide against Aedes aegypti larvae; it was highly toxic to mosquito larvae. The high rated of larvae mortality observed at higher concentrations (7% and 9% of the Z. mauritiana extract concentrations) within 24 hour after exposure indicate the high toxicity of the product. Surprisingly, this product had the same potency as temephos did, which cause 100% of dead larvae.

To see the difference effect towards larvae mortality, Anova test was performed. It proved that there was a difference between bidara leaves concentration and the mortality larvae of Aedes aegypti with p value ≤0.001 and it was found significant difference between 2 variations, except between 7% and 9% Z. mauritiana leaves extract, and between 7% and 9% bidara leaves extract with temephos 1%, by meaning that 7% and 9% bidara leaves extract had similar potency with temephos 1% for larvicide. Based on the average of larvae mortality after giving intervention 9% similar with abate 1%, it caused 100% larvae died in 24 hours, or in another words it can be stated that the most effective consentration of Z. mauritiana leaves extract to kill Aedes aegypti larvae in 24 hours was 9%. Life cycle of Aedes aegypti larvae in brood water successfully survive to hatch into adult mosquitos in a range pH 4,4 to 9,3 (Fuadzy and Marina 2012). Meanwhile light has indirect effect to the larvae growth. Light will also affect the temperature, while the optimal temperature for those larvae is in range 250C–350C (World Health Organization 1981). The result obtained the average of temperature in all repetition was 28,50C and pH was 6,45. Thus, this result is similar with the optimum condition of environment where Aedes aegypti larvae live in order to reduce confounding variables.

As per the preliminary phytochemical investigation, the constituents like flavanoids, tannins, alkaloids, saponins, phenolic compounds, coumarins and carbohydrates are equally present in leaf, root, bark and seed of aqueous and ethanolic extracts. These findings were in agreement of similar nature of study conducted by Okaye and Carol (Palejkar et al., 2012; Ek et al,. 2018).Those compounds like saponnin might cause destruction of tracture digestivus membrane and epicuticula and binds the larvae of Aedes aegypti (Abalaka, Daniyan, and Mann 2010). Moreover, it is stated that alkaloids could catalyst and improve the speed of neurosecretory cells to secretion exdison hormone that might cause failed in moulting process of mosquito larvae and flavonoid in form of glycoside to inhibit their move to the surface in order to inhale oxygen (Perumal et al., 2012). It is in line with a recent study, that Z. mauritiana leaves contained higher alkaloids compare to other species of Ziziphus (Perumal et al., 2012).

A survey of literature on control of different phytochemicals obtained from various plants has been carried out by number of researchers in the field of vector control (Sukumar, Perich, and Boobar 1991). There are many studies of toxicity carried out with other plants that reflect a similar behavior against Aedes aegypti. Plant could be an alternate source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Many of the defensive components of plants are biodegradable with non-residual effects on the biological environment. Hence an attempt has been made in the present investigation to identify the larvicidal potential of the locally available plant Z.mauritiana.

Public acceptance of the use of larvicides from plants, in general, was accepted by the community as research on the acceptance of larvicides using lemongrass. Larvicide from plant material is generally accepted by the public because of the appearance (color and odor) aspects, ease of use, application in mosquito breeding sites, and availability of larvicidal material (Pratiwi 2012). Biolarvicides can be another alternative in controlling Aedes aegypti to break the chain of transmission of Dengue Fever. Control using natural ingredients to replace insecticides can maintain the carrying capacity of the environment. Controlling using an insecticide is a last resort in controlling fever based vectors. The most important control efforts are through environmental management, physical control, and biological control(Boesri 2010).

From the foregoing, it is clear that the concentrations of Z. mauritiana leaves extract were effective for the larvae mortality or control of mosquitos larvae, but 9% of bidara leaves extract was the most effective followed by others. It should however be noted that though, all the concentration of bidara leaves were toxic to larvae; meanwhile the degree of toxicity depends on the concentration applied and also the period of exposure. Extracts of Z. mauritiana leaves have been reported to be effective as temephos at concentration 9%. It can conclude from this study that in totality, the data collected show that Z. mauritiana indeed has larvicidal potential when treated to larvae in low concentrations, and can be used as substitute for commercial insecticides, like temephos. Though the presence of phytochemicals in Z. mauritiana, it could be studied further in detail and its beneficial effect to control mosquitoes. It is suggested for community to use Z. mauritiana leaves as natural larvicides by giving 9% of Z. mauritiana leaves extract in clear water, which estimated 1 ml extract for 1000 ml clean water or 5 ml (1 spoon) for 5 litre of water, especially clean water which stored in containers, thus reducing Aedes aegypti larvae. For further research, it will give significant improvement by considering the effectivity length of Z. mauritiana leaves extract compared to temephos which can be stayed as larvicides for 3 months.

Conclusion

The use of natural chemical compounds in many plants offers a solution to this limitation through new and innovative approaches to reducing mosquito larvae. This study showed that larvae of Aedes aegypti can be killed by chemical compounds in Z. mauritiana leaves. The implementation of Z. mauritiana leaves is suit with situation today where most community in West Borneo used water reservoir such as crock or container. Thus, it is clearly that issue deserves to be appointed in this study.

References

Abalaka, M.E., Daniyan, S.Y., & Mann, A., 2010. Evaluation of the Antimicrobial Activities of Two Ziziphus Species (Ziziphus Mauritiana L. and Ziziphus Spinachristi L.) on Some Microbial Pathogens. African Journal of Pharmacy and Pharmacology, 4(4), pp.135–39.

Abou-elnaga., & Zeinab, Sh., 2014. Insecticidal Bioactivity of Eco-Friendly Plant Origin Chemicals against Culex Pipiens and Aedes Aegypti (Diptera: Culicidae). Journal of Entomology and Zoology Studies, 2(6), pp.340–47.

Aguirre-obando., Alexander, O., Pietrobon, A.J., Caroline, A., Bona, D., & Navarro-silva, M.A., 2015. Contrasting Patterns of Insecticide Resistance and Knockdown Resistance (Kdr) in Aedes Aegypti Populations from Jacarezinho (Brazil) after a Dengue Outbreak. Revista Brasileira de Entomologia, 181(8), pp.1–13.

Boesri, H., 2010. Prospek Pengelolaan Lingkungan Dalam Upaya Pengendalian Serangga

Kesehatan. Aspirator, 2(1), pp.32–36.
Dinas Kesehatan Kalimantan Barat., 2015. Profil Kesehatan Provinsi Kalimantan Barat.
Dureja., Gupta, A., & Dhiman, K., 2012. Free Radical Scavenging Potential and Total Phenolic and Flavonoid Content of Ziziphus Mauritiana and Ziziphus Nummularia Fruit Extracts. International Journal of Green Pharmacy, 2012, pp.187–92.
Ek, N., Joseph, S., Rajeev, K. R., Kavya, V., Anjali, K.M., & Bharathi, M.S., 2018. Preliminary Phytochemical and Biochemical Analysis of Carica Papaya Linn. (Seed). International Journal of Research in Pharmacy and Pharmaceutical Sciences, 3(3), pp.19–22.
Fradin, M.S., 1998. Mosquitoes and Mosquito Repellents: A Clinician's Guide. Annals of Internal Medicine, 128(11), pp.931–40.
Fuadzy, H., & Marina, R., 2012. Potensi Daun Dewa (Gynura Pseudochina(L.) DC Sebagai Larvasida Aedes Aegypti (LINN.). Aspirator, 4(1), pp.7–13.
Ghosh, A., Chowdhury, N., & Chandra, G., 2012. Plant Extracts as Potential Mosquito Larvicides. The Indian Journal of Medical Research, 135, pp.581–98.
Jain, S., Mittal, A., Sharma, S.K., Upadhyay, A.D., Pandey, R.M., Sinha, S., Soneja, M., Biswas,A., Jadon, R.S., Kakade, M.B., & Dayaraj, C., 2017. Predictors of Dengue-Related Mortality and Disease Severity in a Tertiary Care Center in North India. Open Forum Infectious Diseases 4(2), pp.1–8.
Kazembe, T., & Makusha, C., 2012. Evaluation of Mosquito Repellencies of Capsicum Frutescens , Carica Papaya and Cyanodon Dactylon Extracts and Extract Mixtures. Bulletin of Environment, Pharmacology and Life Sciences, 1(7), pp.34–40.
Kementerian Kesehatan. 2016. Situasi DBD Di Indonesia. In InfoDATIN.
Lamien-Meda, A., Lamien, C.E., Compaoré, M.M.Y., Meda, R.N.T., Kiendrebeogo, M., Zeba, B., Millogo, J.F., & Nacoulma, O.G., 2008. Polyphenol Content and Antioxidant Activity of Fourteen Wild Edible Fruits from Burkina Faso. Molecules, 13, pp.581–94.
Ma, R., Hussain, T., Rafay,M., Ia, M., Ma, G., & Guangchun, L., 2017. Desert Dwelling Trees : Forage Suitability and Ethnobotany , Pakistan. Journal of Biodiversity & Endangered Species, 5(3).
Maheswaran, R., Sathish, S., & Ignacimuthu, S., 2008. Larvicidal Activity of Leucas Aspera (Willd.) against the Larvae of Culex Quinquefasciatus Say. and Aedes Aegypti L. Borders, International Journal of Integrative Biology, 2(3), pp.1–5.
Marcome, S., Mathieu, R.B., Pocquet, N., Riaz, M., Pourpardin, R., Se´ lior, S., Darriet, F., Reynaud, S., Yébakima, A., Corbel, V., David, J., Chandre, F., 2012. Insecticide Resistance in the Dengue Vector Aedes Aegypti from Martinique : Distribution, Mechanisms and Relations with Environmental Factors. Plos One, 7(2).
Mohan, D.R., & Ramaswamy, M., 2007. Evaluation of Larvicidal Activity of the Leaf Extract of a Weed Plant , Ageratina Adenophora , against Two Important Species of Mosquitoes , Aedes Aegypti and Culex Quinquefasciatus. African Journal of Biotechnology 6(5), pp.631–38.
Palejkar, C.J., Palejkar, J.H., Patel, A.J., & Patel, M.A., 2012. A Plant Review on Ziziphus Mauritiana. International Journal of Universal Pharmacy and Life Sciences, 2(2), pp.2249–6793.
Pamungkas, R.W., Syafei, N.S., & Soeroto, A.Y., 2017. Perbandingan Efek Larvasida Minyak Atsiri Daun Cengkeh (Syzygium Aromaticum L.) Varietas Zanzibar Dengan Temephos Terhadap Larva Nyamuk Aedes Aegypti Abstrak. Pharm Sci Res, 4(1), pp.139–44.
Perumal, S., Mahmud, R., Piaru, S.P., Cai, L.W., & Ramanathan, S., 2012. Potential Antiradical Activity and Cytotoxicity Assessment of Ziziphus Mauritiana and Syzygium Polyanthum. International Journal of Pharmacology, 8(6), pp.535–41.
Pham, H.V., Doan, H.T.M., Phan, T.T.T., & Minh, N.N.T., 2011. Ecological Factors Associated with Dengue Fever in a Central Highlands Province , Vietnam. BMC Infectious Diseases, 11(172), pp.1–6.
Pratiwi, A., 2012. Penerimaan Masyarakat Terhadap Larvasida Alami. Jurnal Kesehatan Masyarakat, 8(1), pp.88–93.
Sucipto, P.T., Raharjo, M., & Nurjazuli., 2015. Faktor – Faktor Yang Mempengaruhi Kejadian Penyakit Demam Berdarah Dengue (DBD) Dan Jenis Serotipe Virus Dengue Di Kabupaten Semarang Factors Related to The Occurence of Dengue Hemorrhagic Fever (DHF) And Dengue Virus Serotipe in Semarang District. Jurnal Kesehatan Lingkungan Indonesia, 14(2), pp.51–56.
Sukumar, K., Perich, M.J., & Boobar, L.R., 1991. Botanical Derivatives In Mosquito Control : A Review. Journal of American Mosquito Control Association, 7(2), pp.210–37.
Tennyson, S., Samraj, D.A., Jeyasundar, D., & Chalieu, K., 2013. Larvicidal Efficacy of

Plant Oils Against the Dengue Vector Aedes Aegypti (L.) (Diptera: Culicidae). Middle-East Journal of Scientific Research, 13(1), pp.64–68.

World Health Organization., 1981. Intructions for Determining the Susceptibility or Resistance of Mosquito Larvae to Insecticides.

———. 2009. Debue Guidlines For Diagnosis, Treatment, Prevention and Control. In New Edition.

———. 2016. Dengue Guidlines for Patient Care in The Region of The Americas. In Second Edition.