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Original Article

Formulation, Dosage, and Exposure Time of Natural Substances in Controlling Aedes aegypti Larvae

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ABSTRACT

The incidence of vector-borne diseases in Indonesia, particularly Dengue Hemorrhagic Fever (DHF), continues to escalate. This surge correlates with climate change, the extreme transition from hot to rainy seasons. The use of synthetic chemicals for control measures can also poses environmental risks; hence, there is a necessity to explore natural control methods by harnessing the biodiversity of plant species within the environment. This research aims to assess the effectiveness of botanical extract mixture on the mortality of Aedes aegypti larvae. Method: This study utilizes an experimental design with a complete factorial random arrangement, which aimed at elucidating the effectiveness of plant extract as a bio-larvacide against Aedes aegypti mosquitoes. The effectiveness is measured through larval mortality rates based on a formulated equation, with dosage and exposure time as the research variables. The research was conducted in the Environmental Health Departement Laboratory at Health Polytechnic of Tanjungkarang from March to July 2023. The observational sheet serves as the instrument The collected data are processed and analysed using ANOVA to discern variations in larva mortality based on the formula, linear regression is applied to explore the influence of dosage and exposure time on larva mortality. Result: The result of this study exhibits that the most efficacious formulations to terminate larvaes were determined to be the 9th, 10th, and 11th formulations. Furthermore, an extended exposure time correlates with the escalating rate of larval demise. The statistical model prosperously accounts for 88,59% of the variability in the response pertaining to Aedes larval mortality. Conclusion: This study discerns that individually, the formula, dosage, and exposure time, also demonstrates an impact on larval death. This study unveils that an insecticidal formula derived from soursop leaves yields a higher mortality effect compared to formulations based on other materials.

Keywords: *Aedes, Natural Substances, Effectiveness, Larvacidal, Vector*

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INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a condition caused by the dengue virus transmitted by the Aedes aegypti mosquito, serving as the main vector[1]. As of now, DHF remains a global health concern due to its high mortality rate, especially among children[2]. It is estimated that 3.6 million people are at risk, 230 million people are infected, and 21.000 deaths[1–3]. From 1968-2010, Indonesia maintained the highest number of DHF cases in Southeast Asia[4]. In 2015-2020, DHF cases exhibited fluctuations[5]. In Bandar Lampung, during the 2017 period, the incidence rate (per 100.000 population) was 103,97 (35,30-230,90), surpassing both the provincial rate of 42,37 (16,37 - 68,44) and the national rate of 54,21

(27,7 - 78,6) [6]. The stakes on DHF cases often coincide with the climate changes, transitioning from dry season to the rainy season.

Vector mosquito control programs have been implemented in Indonesia, encompassing activities directed at modifying the physical environment to prevent the creation of breeding grounds, including attempts such as draining stagnant water, sealing water storage containers, and burying discarded items. However, the efficacy of those attempts measures is deemed insufficient. Meanwhile, the practice of fogging which utilize chemical agents, remains prevalent among sanitation attempts. Sedangkan sanitarian masih mengandalkan *fogging*. Kegiatan *fogging* dilakukan dengan bahan kimia. The adverse environmental impacts resulting from the use of such chemicals are noteworthy. The development of natural insecticides is imperative given Indonesia's diverse plant species that can serve as natural insecticidal agents[7]. Within a plant extract, aside from several main active compounds, there exist other less active compounds that can enhance the overall extract activity through synergy. This characteristic reduces the probability of insects developing resistance. The ability of insects to develop defense mechanisms against multiple different compounds simultaneously is smaller than it against a singular insecticidal compound[8].

The implementation of plant extract as insecticidal agent poses no adverse effects on the environment and human health, as they can be easily degraded, leaving no residues in soil, water, and air[9]. Based on a review of research conducted over the past decade, no studies have been identified that examine the formulation of various plants as bio-larvicides against mosquito larvae.

Mosquito control can be categorized into three methods, these are mechanic, biological, and chemical. Inappropriate application of insecticides for dengue infection prevention and control should be avoided. During periods of minimal or no dengue virus activity, the routine reduction of larval sources in the environment can be complemented by using larvicides in containers that cannot be discarded, sealed, filled, or handled in other specified ways[10]. Plants derived from nature with a potential as botanical insecticides, generally exhibit bitter taste (due to the presence of alkaloids and terpenes), a putrid odor, and slightly pungent flavor. These plants, or botanicals, are seldom attacked by pests, making them widely utilized as extracts for organic pesticide applications in agriculture[11]. When considering insecticide use for control purposes, it is crucial to opt for environmentally friendly, natural insecticides. More than 24.000 plant series belonging to 255 families are reported to contain pesticide compounds. Certain plants emerge as promising insecticides due to the presence of diverse bioactive compounds, including saponins, flavonoids, alkaloids, tannins, and alkenyl phenols. These compounds are readily accessible and are prevalent in various plant parts, including root, stems, leaves, flowers, fruits, and seeds of green plants across the diverse region of Indonesia[12]. It has been proven that flavonoids and saponins possess biolarvicidal capabilities against adult flies, achieving 100% mortality rate at a concentration at 25% [13].

Several studies have conducted efficacy tests of plants on larval mortality. The effectiveness of betel leaves against larvae has been demonstrated in trials [14,15]. In 2021, the study was undertaken to assess the capabilities of leaves from 14 plants species which identified as having potential as bio-larvicides. These plants were selected based on their highest content of flavonoids and saponins, as determined in prior research on bio-larvicidal properties. The purpose of this study is to evaluate the effectiveness of a mixture of plant extracts on the mortality of Aedes larvae.

METHOD

The design of this study is experimental with the aim of investigating the impact of bio-larvicides on larval mortality. The approach utilized a complete factorial random design. The study subjects were self-cultivated Aedes mosquito larvae. The variables under examination include the bio-larvicide, dosage and exposure time concerning the mortality of Aedes larvae. These variables were chosen based on prior studies suggesting that the effectiveness of a substance on laval mortality can be measured through the formula, dosage, and duration of the exposure[16–18]. The formula consist of 6 levels, the concentration comprises of 66 levels, and exposure time is employed across 9 levels. The experimental design include two repetitions, following the Foreder formula replication in experimental research.

Stage I of the research commenced with the extraction of plant extracts. Leaves from the Soursop, Periwinkle Flower, Averrhoa Blimbi, and Guava plants were collected. These leaves were air-dried for 7 days and subsequently pulverized. Each set of leaves, weighing 1 kg, was soaled in 4 liters of 96% ethanol for 1x24 hours and filtered using a sieving device. The filtrate was then evaporated using a rotary evaporator at a temperature of 60 °C until 1 liter remained (100% concentration). Subsequently, six different combinations of plant extract mixtures were prepared (AB = Periwinkle Flower-Soursop leaf, AC = Periwinkle Flower-Guava leaf, AD = Periwinkle Flower- Averrhoa Blimbi, BC = Soursop-Guava leaf, BD = Soursop-Averrhoa Blimbi leaf, and CD = Averrhoa Blimbi-Guava leaf). Eleven dosage combinations were established based on the ratio of the first ingredients to the second ingredients. These combinations are as follows: 1 = 0% : 100%, 2 = 10% : 90%, 3 = 20% : 80%, 4 = 30% : 70%, 5= 40% : 60%, 6 = 50% : 50%, 7 = 60% : 10%, 8 = 70 : 30%, 9 = 80% : 20%, 10 = 90% : 10%, 11 = 100% : 0%.

Stage II involved an inspection of the active ingredient in the mixture of extracts. A total 60 formulations were scrutinized at the Laboratory of Agricultural Technology at the Lampung State Polytechnic.

Stage III required the preparation of mosquito larvae. A total of 2.640 larvae were requited (66 variations x 20 larvae x 2 replications). The larvae eggs were obtained from the Research and Development Center for Health in Baturaja, South Sumatera. The eggs were allowed to hatch for 3 days and identified as Aedes aegypti based on their siphon shape. The selected larvae were reared until reaching their adulthood and egg-laying maturity. Larvae were fed with finely ground fish feed, while adult mosquitoes were provided with marmot blood and sugar water. Hatched eggs were nurtured until reaching the third instar larvae stage.

Stage IV involved testing the susceptibility of the larvae. The vulnerability test procedure entailed exposing mosquito larvae to various concentration and control test to determine the activity range of the tested substance. After determining the larval mortality within a broad concentration range, the narrower range was employed to establish the LC50. Batches from 20 instar larvae were transferred through a sieve. Subsequently, glasses (containers) with a height of 5 cm were prepared (deeper levels could induce uintended mortality)[19]. Alongside, add 3 ml of the insecticide to each glass. Observations began at 15 minutes, 30 minutes, 60 minutes, 120 minutes, and continued hourly until 48 hours. After period of time the research activity, the deceased larvae were in the soil, while the surviving larvar were utilized as fish feed. Environmental conditions for larval life were maintained at a temperature of 25ºC and water pH of 7. Data were collected through hourly observation and recorded using observation instruments. Subsequently, the data were processed and analysed univariately to interpret the characteristics of each variable. Further analysis involved bivariate assessments using One-way Anova to examine variable effects, and multivariate analysis employing Two-Way Classification of Variance and linear regression to explore the combined variable on larval mortality. All analyses were conducted using computer applications.

The mortality of the treatment groups is adjusted using the formula:

$$\text{Mortality (\%)} = \frac{x-y}{x}\ 100$$

Annotation:
x = The percentage of the control group that did not receive any treatment
y = The percentage of sample group with treatment

Deceased larvae were examined under a microscope to observe the damage incurred due to the exposure to the biopesticide. The research findings are presented in the form of images and tables. This study has obtained ethical clearance certification from the Ethics Commission of the Tanjungkarang Health Polytechnic, Ministry of Health, with Certificate Number 259/KEPK-TJK/IV/2023.

RESULTS

The examination of the active ingredients in plant revealed flavonoid concentrations in the extract as follows: Periwinkle Flower leaves extract at 22,07 Mg QE/g extract, Soursop leaves extract at 9,49 Mg QE/g, extract Guava leaves extract at 33,16 Mg QE/g, and extract Averrhoa Blimbi at 34,84 Mg QE/g.

The result in Figure 1 illustrate larval mortality based on formulas. In formulas AB and BC, across all mixtures, larvae exhibit

100% mortality at the 16-hour mark. For formulas AC, AD, BD, and CD, 100% mortality occurs at the 43rd hour. Figure 2 explains that formula 1 (Periwinkle Flower leaves – Soursop leaves) yields the highest mortality effect.

Figure 3 shows that dosage 9, 10, and 11 result in increasing mortality effects. Figure 4 indicates that as the exposure time lengthens, the mortality of larvae proportionally increases as well.

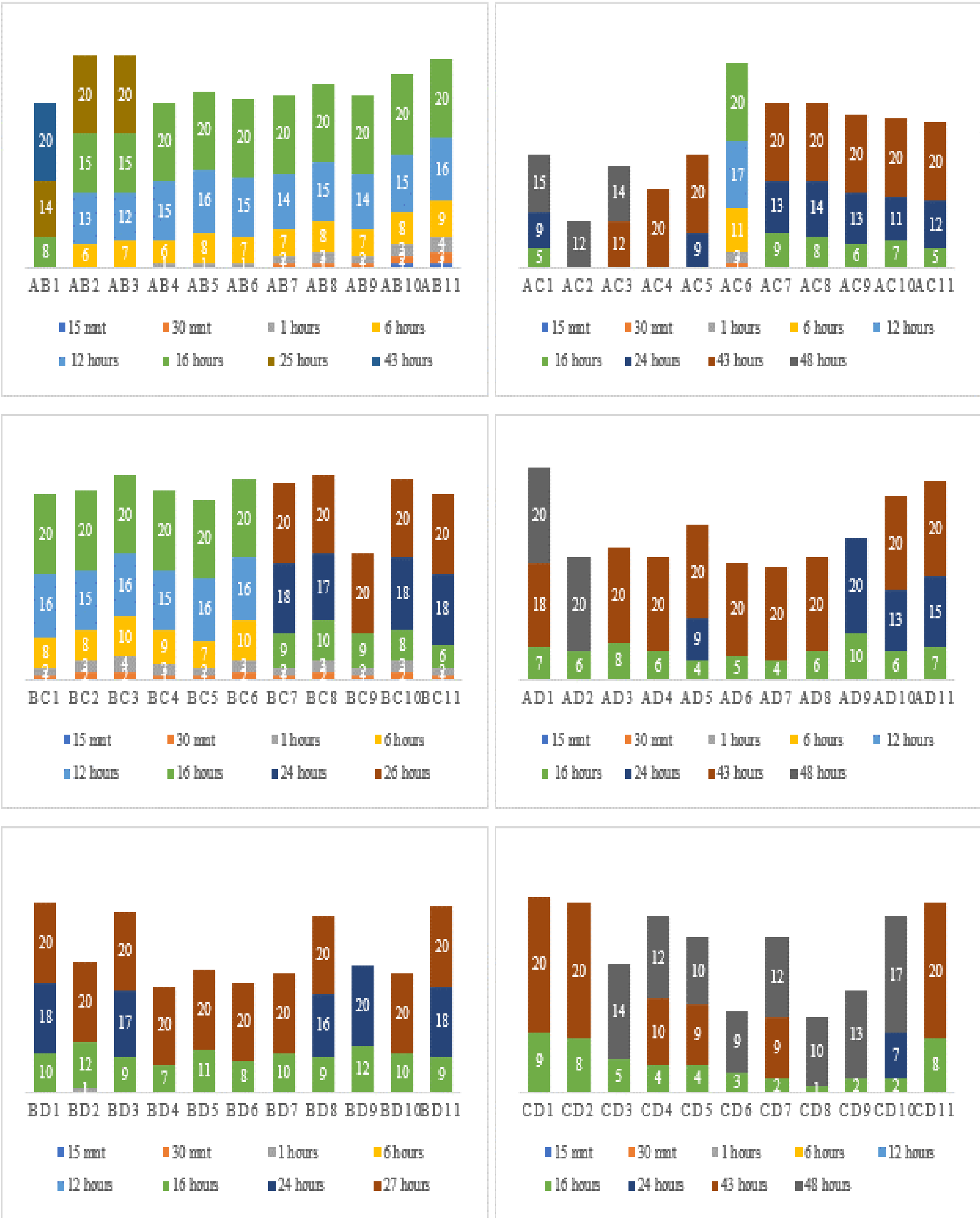

Figure 1. Mortality of Aedes Larvae Based on Observation Time

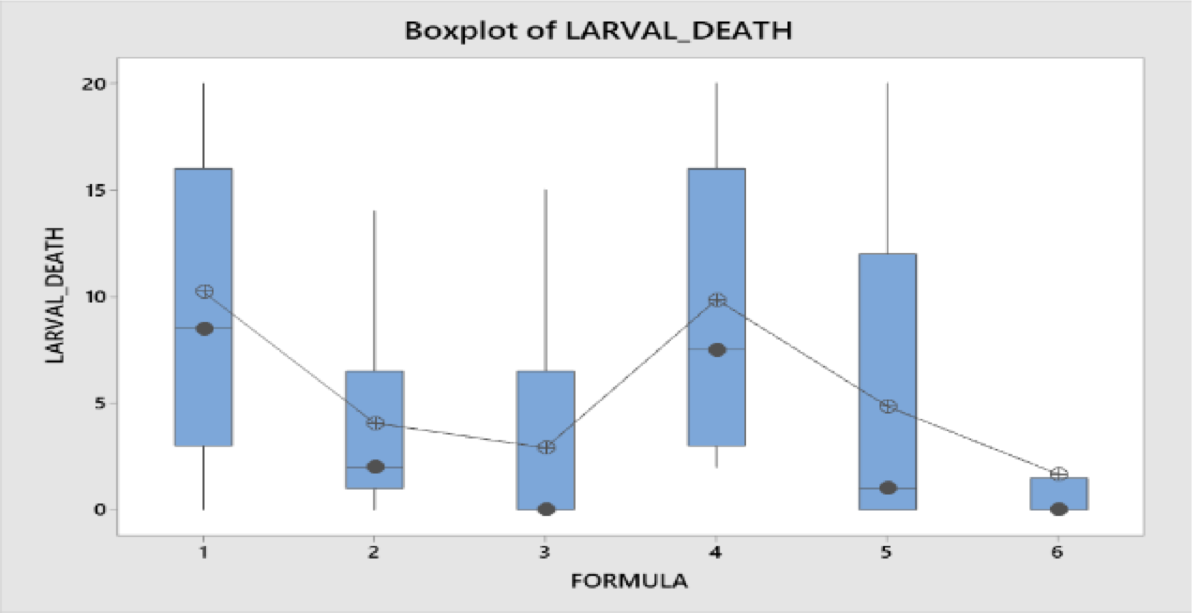

Figure 2. Mortality of Aedes Larvae Based on the Formula

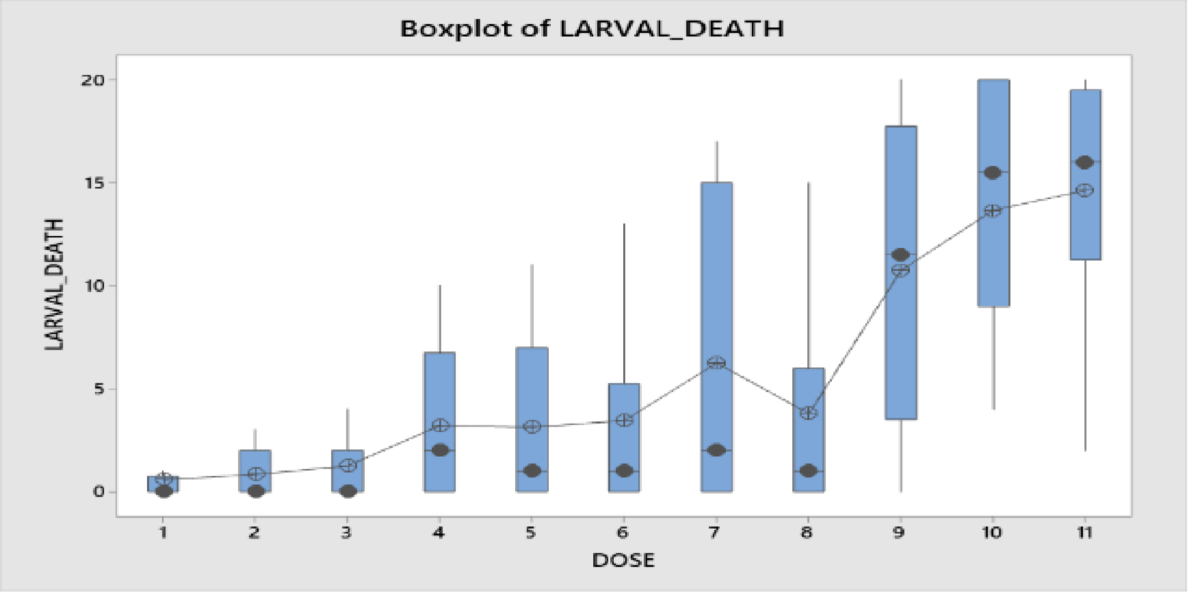

Figure 3. Mortality of Aedes Larvae Based on the Dosage Exposure

Table 1. Mortality of Aedes Larvae Based on Formula, Dosage, and Exposure Time

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formula	5	2872.5	574.504	109.57	0.000
Dosage	10	6239.5	623.951	119.00	0.000
Time	3	112.7	37.580	7.17	0.000
Formula*Dosage	50	1523.9	30.477	5.81	0.000
Dosage*Time	30	471.9	15.730	3.00	0.000
Error	165	865.1	5.243		
Total	263	12085.6			
R Square (Adjusted)		88.59			

DISCUSSION

1. Formula

Formula AB, consisting Periwinkle Flower leaves and Soursop leaves, exhibited a mortality effect at the 16th hour, as did formula BC, composed of Soursop leaves and Guava leaves. This could be attributed to the high concentration of active ingredients acting as toxins for the larvae. The study indicates that Soursop leaves contain 11,85 Mg QE/g extract of flavonoidsm while Guava leaves contain 9,49 Mg QE/g extract. It is also established that flavonoids possess toxic properties against insects[20-22].

In formula AC (Periwinkle Flower leaves- Guava leaves), AD (Periwinkle Flower leaves –Averrhoa Blimbi leaves), and CD (Guava leaves-Averrhoa Blimbi leaves) 100 % mortality occurred at the 43rd hour. Prior studies has suggested that the suspected active ingredients as insecticide is Flavonoid [23]. Flavonoid compounds exhibit selective characteristics against pests, are non-harmful to human, environmentally degradable, leave no toxic residues on hosts that have developed resistance to insecticides, and are compatible with other pest control technologies [20,24,25]. Figure 2 has proved that formula 1 which consist of a blend of Periwinkle Flower leaves and Soursop leaves, yields the highest mortality effect. This could be attributed to the presence of flavonoids in the Periwinkle Flower leaves – Soursop leaves combination, coupled with the larvicidal role of alkaloid. The mechanisms involves acting as an antifeedant, inhibiting the larvae's ability to feed, which leads to larval death due to nutrient deficiency [26–28].

2. Dosage

The 9th mixture has a first ingredient to second ingredients ratio of 80% to 20%, the 10th mixture consists of 90% to 10%, and the 11th mixture consist of 0% to 100%. All three mixtures exhibit an escalating trend in mortality rates (Figure 3). The finding is corroborated by the study conducted by Kusumawati, et al (2018), asserting that the average larval mortality increases with each dosage [29]. The highe dosage correlate with a greater amount of larval deaths, assigned to the presemce of chemicals in natural larvicide extracts containing compounds with high toxicity.

3. Exposure Time

The result of the statistical analysis indicates that the longer the exposure time, the higher the mortality rate of the larvae. Individually, the formula, dosage, and exposure time significantly influence larval mortality (p-value = 0,000). Despite exhibiting diverse outcome, all four plant species share the presence of Flavonoids, which play a crucial role as natural insecticides [30–32]. The toxic impact of Flavonoid that proceeds as an antifeedant against insects. Insect feeding activity diminishes after exposure to the insecticide, leading to larval body contraction and over time larval death [33–35]. Additionally, Flavonoids disrupt the nervoud system of larvae by inhibiting the respiratory tract. Oxygen limitation causes larvae to grow smaller than the larvae under normal conditions [36,37]. Larval growth ceases due to insufficient oxygen intake.

4. Formula, Dosage, and Exposure Time

This study has proven the correlation between formula and dosage, as well as dosage and exposure time, indicating an impact on larval mortality. The statistical test results model reveals an adjusted R square of 88,59%, signifying that the formula, dosage, exposure time, and their interactions collectivel contribute to an 88,59% influence on Aedes larvae mortality. The remaining percentage is influences by other factors.

The use of this larvacides in places serving as breeding grounds for Aedes larvae, such as bathubs, stored clean water for daily use, and areas outside the house flooded with water but not directlt connected to the soil, proves to be environmentally friendly. This, in addition to being safe for the environment, the application of this bio-larvicide is also harmless to other ecosystems. However, it is noted that the water may become discoloured and turbid. The introduction of water clarifying agents in natural insecticides is necessary to address this issue. This is intented to ensure the communities to not find the water inadmissibile, considering that Aedes larvae thrive in clear water typically used for daily activities.

CONCLUSION

This study reveals that insecticides formula derived from Soursop leaves elicits higher mortality effect compared to formulations using other materials. This observation is attributed to the influence of the formula dosage, and exposure time on larval mortality. Similarly, the combination of formula with dosage, as well as dosage and the exposure time, demonstrated significant effects. The findings of this study propose potential recommendation for the formulation's utilization as an alternative bio-larvacide. Further investigations are suggested, particularly if the larvacide is intended for application in clean water reservoirs designated for cooking purposes.

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REFERENCES

1. Naish S, Dale P, Mackenzie JS, McBride J, Mengersen K, Tong S. Climate change and dengue: a critical and systematic review of quantitative modelling approaches. BMC infectious diseases. 2014 Dec;14(1):1-4. https://doi.org/10.1186/1471-2334-14-167
2. Gubler DJ. The economic burden of dengue. The American journal of tropical medicine and hygiene. 2012 May 5;86(5):743. doi:10.4269/ajtmh.2012.12-0157
3. Halasa YA, Shepard DS, Zeng W. Economic cost of dengue in Puerto Rico. *Am J Trop Med Hyg*. 2012;86(5):745-752. https://doi.org/10.4269/ajtmh.2012.12-0157
4. Kemenkes RI. Demam Berdarah Dengue. *Bul Jendela Epidemiol*. 2010;2:48.
5. Mufarida B. Kemenkes Catat 84.734 Kasus DBD, Sebaran Tertinggi Ada di Pulau Jawa. Okenews.
6. Yushananta P. Dengue Hemorrhagic Fever and Its Correlation with The Weather Factor In Bandar Lampung City: Study From 2009-2018. *J Aisyah J Ilmu Kesehat*. 2021;6(1):117-126.
7. Fathonah A. *Uji Toksisitas Ektrak Daun Dan Biji Cariya Papaya Sebagai Larvasida Anopheles Aconitus*. Program Studi Biologi Fakultas Sain dan Teknologi Universitas Islam Negeri Sunan Kalijaga Yogyakarta; 2013.
8. Murdani R. *Keefektifan Daya Bunuh Ekstrak Daun Jeruk Nipis (Citrus Aurantifolia) Terhadap Larva Nyamuk Aedes Aegypti Instar III*. Program Studi Kesehatan Masyarakat Ilmu Kesehatan Unversitas Muhammadiyah Surakarta; 2014.
9. Yuniar N, Majid R, Ode L, Zety M, Wildan E. Larvacidal effect of papaya leaf extracts (Carica papaya l.) toward the larvae of Anopheles aconitus donits mosquitoes as an effort to prevent malaria disease in Rural Areas of Southern Konawe. *Proceeding Int Semin Glob Heal 2017*. 2017;1(1):333-343.
10. Purnama SG. Diktat Pengendalian Vektor. Prodi IKM FK Universitas Udayana. 2017:4-50.
11. Ahdiyah I, Purwani KI. Pengaruh ekstrak daun mangkokan (Nothoponax scutellarium) sebagai larvasida Nyamuk culex sp. *Sain dan Seni ITS*. 2015;3(1).
12. Ramayanti I, Febriani R. Uji Efektivitas Larvasida Ekstrak Daun Pepaya (Carica papaya Linn) terhadap Larva Aedes aegypti Pendahuluan Nyamuk yang ada . Spesies ini dapat ditemukan aegypti di Indonesia . Bisa dikatakan sebagai yang telah resisten , salah satunya Metode Penelitian. *Syifa'MEDIKA*. 2016;6(2):79-88.
13. Yushananta P, Mei. The Effectiveness of Betle Leaf (Piper betle L.) Extract as a Bio- pesticide for Controlled of Houseflies (Musca domestica L.). *Open Access Maced J Med Sci*. 2021;9(E):895-900. https://doi.org/10.3889/oamjms.2021.6886
14. Adibah A, Dharmana E. Larvicidal Effectiveness Test of Boiled Betle Leaf (Piper betle L.) Against Aedes aegypti Larvae : Studies in Value of Lc50, Lt50, and The Rate of Larvae Mortality. *Diponegoro Med J*. 2017;6(2):244-252.
15. Anisah, Sukesi T. Uji Efektifitas

Ekstrak Daun Sirih (Piper betle L) S ebagai Larvasida Larva Lalat Rumah (Musca domestica) Effectiveness of Sirih Leaf Extract (Piper b etle L) as A House Fly Larvae (Musca Domestica) Larvicidal. *J Vektor Penyakit*. 2018;12(1):39-46. https://doi.org/10.22435/vektorp.v12i1.283

16. Praulins G, McDermott DP, Spiers A, Lees RS. Reviewing the WHO tube bioassay methodology: Accurate method reporting and numbers of mosquitoes are key to producing robust results. Insects. 2022 Jun 14;13(6):544. **https://doi.org/10.3390/insects13060544**
17. Sayono, Nurullita U. Situasi Terkini Vektor Dengue (Aedes Aegypti) Di Jawa Tengah. *KEMAS J Kesehat Masy*. 2016;11(2):96-105.
18. Efriadi H, Mutiara D, Emilia I. Uji Toksisitas Akar Tuba (Derris eliptica) terhadap Mortalitas Benih Ikan Nila (Oreochromis sp). *Sainmatika J Ilm Mat dan Ilmu Pengetah Alam*. 2018;15(1):56. doi:10.31851/sainmatika.v15i1.1783
19. WHO. *Guidelines for Laboratory and Field Testing of Mosquito Larvicides*. World Health Organization Communicable Disease Control, Prevention and Eradication, WHO Pesticide Evaluation Scheme; 2005. http://whqlibdoc.who.int/hq/2005/WHO_CDS_WHOPES_GCDPP_2005.13.pdf?ua=1
20. Ahyanti M, Yushananta P. Kombinasi Ekstrak Daun Tapak Dara (Catharanthus roseus) Dan Daun Sirsak (Annona muricata) Sebagai Bio-larvasida. *Ruwa Jurai J Kesehat Lingkung*. 2022;16(3):113. doi:10.26630/rj.v16i3.3611
21. Melliska CE. Efektivitas Larvasida Ekstrak Daun Sirsak (Annnona Muricata Lina) Terhadap Kematian Jentik Culex Sp (Studi Kasus di-Gampong’’Purwodadi, Kecamatan: Kuala*Pesisir,_Kabupaten&Nagan!Raya). *PREPOTIF J Kesehat Masy*. 2022;6(2):1782-1786.
22. Syazana N. Kajian Literatur Efektivitas Biolarvasida Ekstrak Daun Sirsak Terhadap Jentik Nyamuk Aedes Aegypti. *Environ Occup Heal Saf J •*. 2022;2(2):133.
23. Ahyanti M, Yushananta P. Kandungan Saponin dan Flavonoid Pada Tanaman Pekarangan serta Potensinya Sebagai bioinsektisida Lalat Rumah (Musca domestica). *Ruwa Jurai J Kesehat Lingkung*. 2023;17(1):31-43. http://dx.doi.org/10.26630/rj.v17i1.3763
24. Saputri AE, Hariyanti DB, Ramadhani IA, Harijani WS. Potensi Daun Lamtoro (Leucaena leucocephala) Sebagai Biopestisida Ulat Grayak (Spodoptera litura F.). *Agritrop J Ilmu-Ilmu Pertan (Journal Agric Sci*. 2021;18(2):209-216. doi:10.32528/agritrop.v18i2.3740
25. Jannah NAM, Yuliani Y. Keefektifan Ekstrak Daun Pluchea indica dan Chromolaena odorata sebagai Bioinsektisida Terhadap Mortalitas Larva Plutella xylostella. *LenteraBio Berk Ilm Biol*. 2021;10(1):33-39.
26. Laksono FW, Sari NLS, Salsabila S, Kurniasari L. Pengaruh Insektisida Alami Ekstrak Daun Jelatang (Urtica Dioica L.) Terhadap Mortalitas Larva Aedes Aegypti. *Pros Sains Nas dan Teknol*. 2022;12(1):1-8.
27. Sitohang M, Mamahit JME, Pakasi SE. Inovasi bomb fizzies antifeedant dari ekstrak daun pangi (Pangium edule Reinw.) untuk pengendalian hama kubis Plutella xylostella L. *J Agroteknologi Terap*. 2022;3(2):124-130.
28. Asfahani F, Halimatussakdiah, Amna U. Analisis Fitokimia Ekstrak Daun Sirsak (Annona muricata Linn .) dari Kota Langsa. *Quim J Kim Sains dan Terap*. 2022;4(2):18-22.
29. Kusumawati WD, Subagiyo A, Firdaust M. Pengaruh Beberapa Dosis dan Jenis Ekstrak Larvasida Alami Terhadap Kematian Larva Nyamuk Aedes aegypti. *Bul Kesehat Lingkung Masy*. 2018;37(3):283-295.
30. Fajriyati AF, Anwar S, Kusmiyati F. Aplikasi ekstrak daun tapak dara (Catharanthus roseus l.) terhadap pertumbuhan dan morfologi tanaman bawang daun (Allium fistulotum l.). *J Ilm Pertan*. 2022;19(1):29-37.
31. Andriani T, Fikri EN, budi IS. Uji

Efektifitas Serbuk Tanaman Tapak Dara Uji Efektivitas Serbuk Tanaman Tapak Dara (Catharanthus roseus L.) Terhadap Serangan Nematoda Puru Akar Meloidogyne spp. pada Tomat (Lycopersicum esculentum Mill.). *Prot Tanam Trop*. 2018;1(2):36-39.
32. Dewi IALP, Damriyasa IM, Dada IKA. Bioaktivitas Ekstrak Daun Tapak Dara (Catharanthus Roseus) Terhadap Periode Epitelisasi Dalam Proses Penyembuhan Luka Pada Tikus Wistar. *Indones Med Veterinus*. 2013;2(1):58-75.
33. Sayono, U N, M S. Pengaruh konsentrasi flavonoid dalam ekstrak akar tuba (Derris eliptica) terhadap kematian larva Aedes aegypti. *Kesehat Masy Indones*. 2010;6(1):38-47.
34. Fauzana H, Faradilla N. Uji Konsentrasi Ekstrak Daun Krinyuh (Eupatorium odoratum L.) sebagai Racun Perut terhadap Mortalitas Ulat Grayak (Spodoptera litura F.). *Agroteknologi Trop*. 2018;7(2):108-115.
35. Maheswari PP, Wijaya IN, Sritaman M. Uji Efektivitas Beberapa Jenis Ekstrak Daun Tanaman Terhadap Perkembangan Ulat Daun Kubis (Plutella xylostella L.) di Laboratorium. *E-Jurnal Agroekoteknologi Trop*. 2018;7(3):392-399. Retrieved from https://ojs.unud.ac.id/index.php/JAT392
36. Callier V, Nijhout HF. Control of body size by oxygen supply reveals size-dependent and size-independent mechanisms of molting and metamorphosis. *Proc Natl Acad Sci U S A*. 2011;108(35):14664-14669.
37. Ahyanti M, Yushananta P, Usman S. Efektifitas Beberapa Tanaman dalam Mengendalikan Lalat Rumah (Musca domestica). Poltekita: Jurnal Ilmu Kesehatan. 2022 Feb 21;15(4):398-402.