International Journal of Scientific Research and Management (IJSRM)
||Volume||12||Issue||08||Pages||1168-1172||2024||
Website: www.ijsrm.net ISSN (e): 2321-3418
DOI: 10.18535/ijsrm/v12i08.mp05

The Use of Eco-Enzyme as a Larvacide for *Aedes aegypti* Mosquito Larvae

Hariyono Hariyono[1*], Sri Pantja Madyawati[1], Shifa Fauziyah[2], P Muthu[3], Teguh Hari Sucipto[4]

[1]Postgraduate School, Universitas Airlangga
[2]Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga
[3]SRM Institute of Science and Technology, Kattankulathur, Tamilnadu, India
[4]Institute of Tropical Disease, Universitas Airlangga

Abstract
Nearly half of the world's population is at risk of dengue infection, an arboviral infection that is typically spread by the mosquito *Aedes aegypti*. Insecticide use and community awareness campaigns are the main vector control strategies currently in use in some countries, and they didn't done many ways to reduce dengue. Eco-enzyme is the product of fermentation from organic waste such as vegetable, fruit, and sugar. Eco-enzyme can be used in agriculture, medicine, and others. This study aims to analyze the toxicity level of ecoenzyme as the larvicide for *Aedes aegypti* larvae. An analytical experimental study were conducted in Bionas Parasitology Laboratory from February to March 2024. As many as 20 *Aedes aegypti* larvae were used in each of exposure. Each exposure were replicated four times. The mortality of *Aedes aegypti* larvae was analyzed by a two-way ANOVA test, followed by a Fisher's least significant defference (LSD) post hoc test with a significance level of ($p<0.01$). This data was analysed by Statistical Product and Service Solutions(SPSS)Inc. 25.0. The concentrations of ecoenzyme induce mortality of the third instar of *Aedes aegypti* larvae were started from 2% concentration, and the highest was found in 10% concentrations which resulted in average of 50% mortality. In this context, the analysis results show that time, concentration, and the interaction between time and concentration have a significant influence on the observed quantities. These findings can have important implications in the context of studies or experiments related to the variables being tested. Below are the profile plots of each variable (concentration and time of exposure) which give significant influence on the mortality of *Aedes* larvae.

Keywords: *Aedes*, larvicide, ecoenzyme, side-eye

Introduction

Nearly half of the world's population is at risk of dengue infection, an arboviral infection that is typically spread by the mosquito *Aedes aegypti*. Dengue is hyperendemic in tropical and subtropical climates area and has been more frequent in last decade. While most of dengue patients stay asymptomatic, some of them experience an acute febrile illness that can progress from an undifferentiated fever to shock and dengue hemorrhagic fever (DHF). The modality of test depends on the day of illness. Laboratory confirmation can be performed either directly by detecting viral components in the blood or indirectly by using serological measures. There are three phases to the clinical course: the febrile, critical, and recovery phases. The hallmark of DHF is plasma leakage, which happens shortly after the end of the febrile phase, whereas dengue fever avoids the critical phase (Kularatne et al., 2022).

Vector control is the key to preventing dengue outbreaks. Insecticide use and community awareness campaigns are the main vector control strategies currently in use in some countries, and they didn't done many ways to reduce dengue. Cost, delivery, pesticide resistance, sustainability, and environmental safety are just a few of the problems in dengue controlling program. Vector proliferation and persistence are caused by a variety of factors, including widespread infrastructure development without first conducting health impact assessments, poor irrigation and water systems, inadequate solid waste management, deforestation, an increase in international travel and trade, and climate change. Effective, efficient, and sustainable is the main goals of IVM strategy, which prioritizes biological control and environmental management along with the continuous use of chemical methods (NVBDCP, 2017).

One of the larvacides that currently developing is biolarvacide, because of the compound of larvacides from plants and didn't resulting of residue, easily decomposes in the environment, soil, and water (Listyorini, 2012). One of the plants that can be used as biolarvacides namely banana plants (Haditomo, 2010). in phytochemical tests that banana leaves contain chemical compounds tannins, alkaloids, terpenoids, saponins, flavonoids, glycosides heart, deoxy sugar, and carbohydrates (Asuquo and Udobi, 2016). Several studies shows the use of biolarvacide for *Aedes aegypti* control, and also showing various toxicity levels. Eco-enzyme is the product of fermentation from organic waste such as vegetable, fruit, and sugar. Eco-enzyme can be used in agriculture, medicine, and others. This study aims to analyze the toxicity level of ecoenzyme as the larvicide for *Aedes aegypti* larvae.

Methods

Study Design

An analytical experimental study were conducted in Bionas Parasitology Laboratory from February to March 2024. As many as 20 *Aedes aegypti* larvae were used in each of exposure. Each exposure were replicated four times.

Ethical Clearance

This study design already approved by Ethical Commitee from Faculty of Medicine, Universitas Airlangga with the registered number 24-934/UN3.14/PPd/2013

Preparation of Ecoenzyme

The procedure of the ecoenzyme preparation starting by mixing 1 part sugar/molasses, 3 parts organic waste and 10 parts of clear water. The mixture was left for 3 months in an airtight plastic container. If the pH is below 4.0, it means the eco enzyme is ready to be harvested. Before use, filter it first.

Larvae Rearing

Eggs of *Aedes aegypti* were reared in Bionas Parasitology Laboratory under the laboratory condition. Eggs were reared till emerge into first instar and then developed to third instar. Only third instar larvae that were used for larvicide test. Larvae rearing were conducted in optimum laboratory condition (room humidity 65-80% and room temperature 28-30°C).

Larvicide test

Larvicide test in this study was conducted based on the WHO Standard. Third instar larvae were used for larvicide test. Ecoenzyme solution was made into several concentrations: 2%, 5%, 8%, and 10%. Negative control used aquadest. For each treatment, 100 mL of each solution was filled to plastic container. Then, as many as 20 third-instar larvae of *Aedes aegypti* were placed in every container and then observed for 30 hours. The number of mortality was observed till 30 hours, with the detail of 1 hour, 2 hour, 4 hour, 8 hour, and 30 hour. Larva mortality was evaluated at the end of 30-h exposure. Each exposure were replicates as many as 4 times.

Data Analysis

The mortality of *Aedes aegypti* larvae was analyzed by a two-way ANOVA test, followed by a Fisher's least significant defference (LSD) post hoc test with a significance level of ($p<0.01$). This data was analysed by Statistical Product and Service Solutions(SPSS)Inc. 25.0

Results

Larvae Mortality

Mortality of the third-instar of *Ae.aegypti* larvae were calculated at the end of 30h exposure. The percentage of mortality was found below:

Table 1. Percentage of the mortality of *Aedes aegypti* larvae after exposed by ecoenzyme solution

Concentration of ecoenzyme/side eye solution	The Number of Larvae that were tested	Average Number of Dead Larvae	Percentage (%)
Negative Control/Aquadest	20	0	0%
2%	20	7	35%
5%	20	7	35%
8%	20	7	35%
10%	20	10	50%

The concentrations of ecoenzyme induce mortality of the third instar of *Aedes aegypti* larvae were started from 2% concentration, and the highest was found in 10% concentrations which resulted in average of 50% mortality.

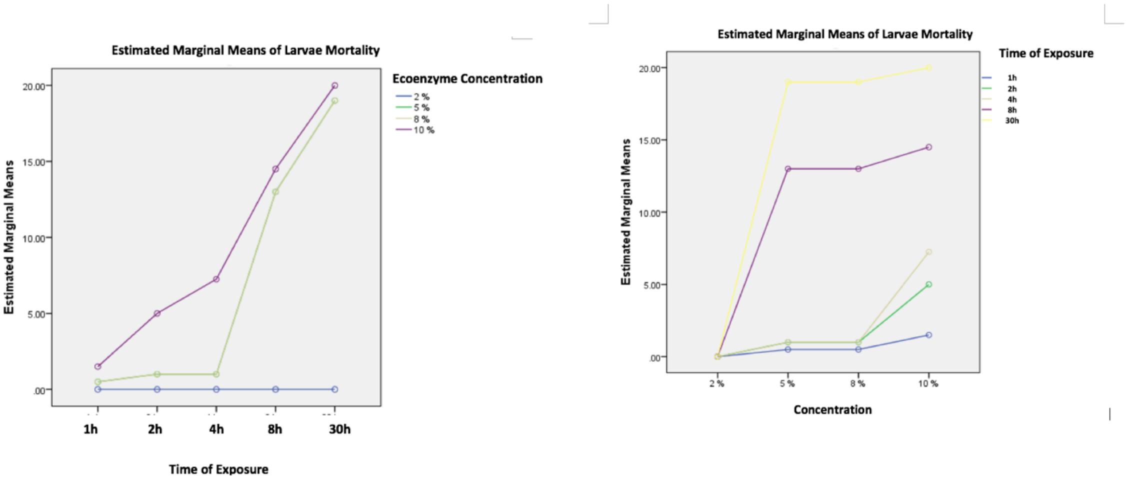
Table 2. Test Between Subjects Result

Source	Type III Sum of Squares	df	Mean Square	F	Sig
Corrected Model	4268.738[a]	19	224.670	128.689	.000
Intercept	2749.512	1	2749.512	1574.900	.000
Time Variable	2395.550	4	598.888	343.038	.000
Concentration	1017.338	3	339.113	194.241	.000
Time*Concentration	855.850	12	71.321	40.852	.000

The results of the between-subject effect analysis show that there is a significant influence on the larvae mortality with a sum of squares value of 4268.738 (df = 19, mean square = 224.670, F = 128.689, p < 0.001). These results indicate that at least one of the observed independent variables has a significant influence on the dependent variable. Time variable shows a significant effect with a sum of squares of 2395.550 (df = 4, mean square = 598.888, F = 343.038, p < 0.001), indicating that changes in time have a significant impact on the dependent variable. The concentration level also has a significant influence with a sum of squares of 1017.338 (df = 3, mean square = 339.113, F = 194.241, p<0.001).

From the results of the analysis, there is also an interaction between the variables time and concentration, shown by the sum of squares of 855,850 (df = 12, mean square = 71,321, F = 40,852, p < .001). This shows that the combination of changes in time and concentration also has a significant influence on the larvae mortality. Overall, the resulting model was able to explain most of the variability in the data, with an R-squared value of 0.976. This means that approximately 97.6% of the variation in the dependent variable can be explained by the tested model. In addition, the Adjusted R-squared being almost that high indicates that the model is not experiencing overfitting.

In this context, the analysis results show that time, concentration, and the interaction between time and concentration have a significant influence on the observed quantities. These findings can have important implications in the context of studies or experiments related to the variables being tested. Below are the profile plots of each variable (concentration and time of exposure) which give significant influence on the mortality of *Aedes* larvae.



(1a) (1b)

Figure 1. (1a) Plot of Time of Exposure; 1b) Plot of Concentration

Discussion

Our results shows that *Side-Eye* or biolarvicide from ecoenzyme can lead to the mortality in *Aedes aegypti* third-instar larvae. Larvae shows low number of mortality in 2% concentration, while the highest shows in 10% concentration. Time of exposure also can affect to the increasing of mortality. The study results for the effect of ecoenzyme from the activity test which shows an increase in the mortality of third instar of *Aedes aegypti* larvae along with the increase in the concentration of ecoenzyme. It is proven that the larval mortality percentages at concentrations of 2%, 5%, 8%, and 10% (Table 1) so that ecoenzyme solution is proven to have a larvicidal effects on the third instar of *Aedes aegypti* larvae.

Ecoenzyme can made from the fermentation of 4 types of fruit waste or all of organic waste (dragon, pineapple, banana, and orange), then fermented for three month. Ecoenzyme from those aforementioned of fruit waste contains the highest lipase enzyme activity. The pH of ecoenzyme shows pH<4.0. Ecoenzyme contains phenol compounds and their derivatives, except alkaloids, lipase activity is quite high, pH is low, and total bacterial plate numbers are low (Widjanarko et al., 2023).

Ecoenzyme generated through fruit or vegetable waste fermentation. Fruit peel eco-enzymes from Nepal have been tested for antibacterial activity against *Salmonella typhi*, *E. coli* (ATCC 25922), *Bacillus* spp., *Shigella* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (ATCC 25923) (Neupane & Khadka, 2019). Furthermore, the growth of *Enterococcus faecalis* bacteria was inhibited by the ecoenzyme, which was made from a mixture of orange peel (*Citrus aurantium*) and pineapple peel (*Ananas comosus*) at a ratio of 4:6 (Mavani et al., 2020). Due to its antibacterial qualities, ecoenzyme is widely used in Indonesia as a hand sanitizer and disinfectant liquid (Alkadri & Asmara, 2020). It has also been used as a bioactive component in liquid soap formulas produced by the home industry (Saifuddin, et al, 2021).

The phytochemical screening results of the eco-enzyme contains tannins and saponins. According to Wafa, Sofiane, and Mouhamed (2016), tannins are secondary metabolites of the polyphenol group that have the ability to act as antifungals (*Aspergillus niger*, *Aspergillus flavius*, and *Candida albicans*) and antibacterials (*Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*) (Ramadani et al., 2022).

Previous study from other researcher shows that the Annonaceae and Piperaceae plant families were found to have the most potential effects as larvicide against *Aedes* larvae. This raises the possibility that it would be profitable to use these plants to make pesticides for sale. Since acetogenins isolated from Annonaceae have demonstrated strong larvicidal activity, more research needs to be done to confirm these compounds suitability as larvicides for commercial use. It is already known how to synthesize acetogenins with strong larvicidal activity, such as muricatetrocin, sylvatacin, and annonacin, among many others, all of which can be utilized to produce these substances (Rodriguez et al., 2020).

When larvicide components reach high concentrations in the larvae, they begin to suppress the nervous and respiratory systems activity and can break down the cells in the digestive tracts walls more quickly, which causes the larvae to lose appetite (Steinwascher, 2018). The larvae will not only grow more slowly, but they will also pass away more quickly. The larvae that were given a low concentration exhibited faster functioning of the active compound content. If it was low, it could be argued that it had no appreciable impact on larval mortality, or even that poisoning was not experienced (Araujo et al., 2018). Our study can be used as the preliminary data to reveal the potential of ecoenzyme as larvicide against *Aedes aegypti*.

Conclusion

Ecoenzyme has potential effects as larvicide against *Aedes aegypti* larvae. Ecoenzyme can be used as an alternative of larvicide which can be made by the fermentation of organic waste. So that, organic waste can be used into something useful for vector control program.

References

1. Alkadri SPA & Asmara KD. Pelatihan Pembuatan Eco-Enzyme Sebagai Hand sanitizer dan Desinfektan Pada Masyarakat Dusun Margo Sari Desa Rasau Jaya Tiga Dalam Upaya Mewujudkan Desa Mandiri Tangguh Covid-19 Berbasis Eco-Community. Jurnal Buletin Al-Ribaath, 2020. 17(2): 98. https://doi.org/10.29406/br.v17i2.2387
1. Araujo I, Ferreira R, Sena I, Lima A, Carvalho JC. Larvicidal Effect of Hydroethanolic Extract from the Leaves of Acmella oleracea L. R. K. Jansen in Aedes aegypti and Culex quinquefasciatus. South African Journal of Botany, 2018. 2(1), pp.134–140

2. Asuquo EG & Udobi CE. Antibacterial and toxicity studies of the ethanol extract of *Musa paradisiaca* leaf. Cogent Biology, 2016. 2(1): 1–10. doi : 10.1080/23312025.2016.1219248
3. Haditomo I. 2010. Efek larvasida ekstrak daun cengkeh (Syzygium aromaticum L.) terhadap Aedes aegypti. Skripsi. Universitas Sebelas Maret. Surakarta
4. Kularatne SA, Dalugama C. Dengue infection: Global importance, immunopathology and management. Clin Med (Lond). 2022 Jan;22(1):9-13. doi: 10.7861/clinmed.2021-0791. PMID: 35078789; PMCID: PMC8813012.
5. Listyorini IP. 2012. Uji keamanan ekstrak kayu jati (Tectona Grandis L.F) sebagai bio-larvasida Aedes Aegypti terhadap mencit. Skripsi. Universitas Negeri Semarang. Semarang
6. Mavani HAK, Tew IM, Wong L, Yew HZ, Mahyuddin A, Ghazali RA, Pow, E. H. N. Antimicrobial Efficacy of Fruit Peels Eco-Enzyme against Enterococcus faecalis:An In Vitro Study. International Journal of Enviromental Research and Public Health, 2020: 17(5107), 1–12
7. National Vector Borne Diseases Control Programme (NVBDCP), Government of India. Manual on integrated vector management. [accessed on June 3, 2017]. Available from: http://www.nvbdcp.gov.in/Doc/IVM-Manual-Draft-2015.pdf .
8. Neupane K & Khadka R. Production of Garbage Enzyme from Different Fruit and Vegetable Wastes and Evaluation of Its Enzymatic and Antimicrobial Efficacy. TUJM, 2019. 6(1): 113–118
9. Rodrigues AM, Martins VEP, Morais SM. Larvicidal efficacy of plant extracts and isolated compounds from Annonaceae and Piperaceae against Aedes aegypti and Aedes albopictus. Asian Pacific Journal of Tropical Medicine, 2020. 13(9), 384-396.
10. Saifuddin S, Syahyadi R, Nahar N, Bahri S. Peningkatan Kualitas

Utilization of Domestic Waste for Bar Soap and Enzym Cleanner (Ecoenzym) Sebagai Bahan Baku Pembuatan Sabun. Jurnal Hasil-Hasil Penerapan IPTEKS Dan Pengabdian Kepada Masyarakat, 2021. 5(1): 45–56. https://doi.org/10.30811/vokasi.v5i1.2158

11. Steinwascher K. Competition among *Aedes aegypti* Larvae Kurt Steinwascher. Journal PloS ONE, 2018. 13(11): pp.1–41.
12. Widjanarko SB, Aulia LP, Khoirunnisa Y. Profil Sifat Fisiko-Kimia dan Mikrobiologi Cairan Eco-Enzyme dari Beberapa Jenis Buah dan Daun Cemara Udang. Jurnal Teknologi Pertanian. 2023 Aug 28;24(2):117-26.