



Effectiveness of *Ricinus communis* as Natural Larvicide for *Aedes aegypti* Mosquito Larvae in Medan City

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Abstract

Abstract. Dengue Hemorrhagic Fever, a disease caused by *Aedes aegypti*, can be prevented by reducing larval density using natural larvicides. This research aimed to investigate the natural larvicide effectiveness in reducing the *Aedes aegypti* larvae density in Medan City in 2023. This quasi-experimental research conducted a pre-test and post-test on the Container Index (CI). The population in this study was 1,057 houses in Ladang Bambu Village, the sample being 150 houses. The treatments were control and *Ricinus communis*-seed powder at 40 mg, 80 mg, 100 mg, and 120 mg/l doses in ovitraps, placed next to the water reservoir. Consider specifying the unit of measurement for the container index. The container index was calculated before (day 0) and after treatment (day 7). Data were analyzed using paired T-test, one-way ANOVA, and LSD. The results were that the modified ovitrap with the seed of *Ricinus communis* was effective in reducing the *Aedes aegypti* mosquito larvae density. The analysis showed a significant difference in the mean CI between the control and the modified ovitrap, the most effective dose in reducing CI being 100 mg/l of water. The community can use the 100 mg/l seed of *Ricinus communis* in ovitraps as an eco-friendly vector control.

Introduction

Dengue fever is a viral infection that spreads from mosquitoes to humans and ranks among the top ten global health threats. Dengue fever incidence has increased dramatically worldwide in recent decades, with cases reported to the World Health Organization (WHO) increased from 505,430 cases in 2000 to 5.2 million in 2019. Dengue fever is an endemic disease in more than 100 countries in Africa, the Americas, the East Mediterranean, Southeast Asia, and West Pacific Regions. The Americas, Southeast Asia, and the Western Pacific are the worst affected regions, with Asia representing approximately 70% of the global disease burden (World Health Organization, 2023). Bhatt *et al.* (2013) estimate that 390 million dengue infections occur every year with 96 million of them having clinical manifestations with

varying levels of disease severity. Untreated dengue can trigger extraordinary events, severe dengue, and even death.

The number of dengue fever cases in Indonesia has been increasing every year. There were 73,518 cases of dengue fever with 705 deaths and 143,266 cases of dengue fever with 1,237 deaths respectively in 2021 and 2022. In 2023, dengue cases increased from 1,259 cases in the 5th week to 57,884 cases in the 33rd week (Minister of Health Republic of Indonesia, 2023). DHF cases in North Sumatra Province in 2019 amounted to 7,584 cases of illness and 37 cases of death. This number has increased compared to 2018, which recorded 5,786 cases with 28 deaths (Minister of Health Republic of Indonesia, 2020).

The target or indicator for dengue hemorrhagic fever set in the 2020-2024 Ministry

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of Health the Republic of Indonesia Strategic Plan is that 90% of districts/cities have a dengue incidence rate (IR) $\leq 49/100,000$ population in 2024 which will be achieved in stages. This target is expected to be achieved through activities such as: (1) increasing innovation (integrated and biological vector control); (2) strengthening case management; (3) increasing advocacy and communication; (4) strengthening the public health laboratory system to strengthen surveillance; (5) strengthening reporting and real-time surveillance; (6) building an early warning system; and (7) increasing regional capacity (Minister of Health Republic of Indonesia, 2020).

Vector control is crucial for the prevention and control of dengue fever. The use of insecticides in vector control plays a crucial role in the dengue control program, especially to break the chain of transmission. It is essential to follow WHO recommendations and national guidelines for the safe use of insecticides to prevent the development of vector resistance, as chemical insecticides can cause such resistance. According to the research conducted by Goindin *et al.* (2017), the larvae of *Ae. aegypti* exhibited high resistance levels to temephos (from 8.9 to 33.1-fold) according to the resistance ratios (RR50) computed. This pattern of resistance to insecticides requires serious attention and the implementation of national guidelines for the use of insecticides needs to be closely monitored. Thus, biological vector control is one of the strategies outlined in the 2020-2024 Strategic Plan of the Ministry of Health Republic of Indonesia.

Long-term application of insecticides to the same targets puts unprecedented pressure on the population of *Aedes aegypti*, causing it to develop more quickly and become resistant. The development of a safer and more specialized biological insecticide is one approach to solving this issue. A biological insecticide is defined as an insecticide derived from plants and contains chemicals (bioactive) that are poisonous to insects but biodegradable, meaning they do not harm the environment and are generally harmless to humans (Wahyuni *et al.*, 2019). One of the natural larvicide is *Ricinus communis* (castor seed). Castor plants are toxic to nematodes, insects, and fungi

because they contain the bioactive ricin. Ricin is an enzyme protein that can inhibit protein synthesis causing cell death (Sowa-Rogozńska *et al.*, 2019). Ricin is a highly toxic ribosome-inactivating lectin found in castor seeds (Sousa *et al.*, 2017). In the research conducted by Sogan *et al.* (2018), *Ricinus communis* leaf and seed extracts showed significant mortality of *Aedes aegypti*. The research of Wamakot *et al.* (2018) carried out can conclude that the ethanol extract of *Ricinus communis* seed and leaves was effective as a larvicide against *Aedes aegypti* mosquito larvae. As a more efficient larvicide against mosquitoes, *Ricinus communis* seed extract exhibits higher larvicidal activity than leaf extract (Sogan *et al.*, 2018).

Therefore, the seed of *Ricinus communis* to be studied in the form of powder was put into a small container (ovitrap) and placed near a water reservoir. In line with this, mosquito egg traps (ovitrap) are an effective way to control *Aedes aegypti* mosquito larvae. Ovitrap was effective in reducing the density of *Aedes sp.* mosquitoes and can control dengue fever in a simple and environmentally friendly. Ovitrap has been modified to maximize its function in reducing larval density. Modifications Rekattidiri ovitrap has undergone significant in comparison to the larval density index (HI p-value: 0.025, CI p-value: 0.052, BI value of p: 0.04). Additionally, there are variations in the mean number of larvae trapped in Rekattidiri ovitrap and standard ovitrap (p-value: 0.001) (Saepudin *et al.*, 2017). Modifying the shape and adding natural ingredients is one way to improve the function of the ovitrap. Ovitrap modified with natural ingredients can attract and inhibit the growth of larvae. According to the research results of Cahyati *et al.* (2017), hay infusion and papaya leaf juice can be natural attractants when using modified ovitrap.

The latest national data in 2022 showed that Medan City with 2,262 cases was among the 5 cities with the highest number of dengue fever cases in Indonesia (Minister of Health Republic of Indonesia, 2023). The study conducted by Purba *et al.* (2022) showed that Medan Tuntungan District is one of the areas with a high vulnerability to dengue fever analyzed spatially. Ladang Bambu Urban Village in Medan Tuntungan District is a risk

area for dengue fever transmission. The pre-survey showed that 62% of the 135 houses examined were positive for mosquito larvae in Ladang Bambu Urban Village. This research aimed to investigate the effectiveness of *Ricinus communis* (castor seed) in an ovitrap to reduce the density of *Aedes aegypti* larvae in Medan City.

Method

This type of research was quasi-experimental research with a nonequivalent control group design where pre-test and post-test are carried out on the container index for ovitrap without treatment and ovitrap modification with *Ricinus communis* seed powder. This research was conducted in the residential area of Ladang Bambu Urban Village, Medan Tuntungan District in September 2023. The population in this study was 1,057 houses, with a sample size of 150 households chosen using purposive sampling. In this study, observation sheets and questionnaires were used to collect data. The treatments in this study were controlled and *Ricinus communis* seed powder at doses of 40 mg, 80 mg, 100 mg, and 120 mg/l water was put into ovitraps placed next to the water reservoir. Two containers were examined for each house, one inside and one outside, and observed for 7 days. These treatments had been in 6 different houses at the same time. Container Index (CI) was obtained from 5 differently inspected houses at each repetition, resulting in as many as 30 CI data. CI was calculated as the number of containers with any larvae or pupae, divided by the total number of inspected containers, multiplied by 100. The data was observed on day 0 (before treatment) and day 7 (after treatment).

The independent variable in this study was the modification of the ovitrap with *Ricinus communis* seed powder and the dependent variable was the density of *Aedes sp* mosquitoes, which was measured by the CI. The tools and materials used in this research included 1.3 litre as many as 300 buckets, small plastic, stirrers, *Ricinus communis* seed powder, and water. The data analysis technique uses the paired t-test, one-way ANOVA, and the LSD multiple comparison test. Previously, the data assumptions were carried out using

the Shapiro-Wilk test and homogeneity test. Ethical approval was received from The Ethics Committee, Universitas Sumatera Utara with registration number 937/KEPK/USU/2023.

Result And Discussion

The results showed that the average CI after treatment decreased, of which the highest decrease occurred at a dose of 100 mg/l water, which amounted to 33.33%. The lowest decrease occurred at a dose of 0 mg/l water, with no CI change (0%) (Table 1). Based on the results of the normality test using the Shapiro-Wilk test, it was found that the average CI after treatment with ovitrap placement with a dose of 0 mg/l of water (control), 40 mg/l of water, 80 mg/l of water, 100 mg/l of water, and 120 mg/l of water is normally distributed with p values respectively 0.078; 0.091; 0.110; 0.212; 0.101 ($p\text{-value} \geq 0.05$). The test was continued with the paired T-test (Table 2) which showed that each dose (40 mg, 80 mg, 100 mg, and 120 mg/l of water) was effective in reducing the average CI after treatment ($p\text{-value} < 0.05$).

The overall density of *Aedes aegypti* mosquito larvae (CI) after the ovitrap modification treatment decreased compared to the container index before treatment. The decrease in the average container index after ovitrap treatment occurred because the ovitrap attracted mosquitoes to lay eggs inside it so they no longer lay eggs in the water reservoir at home. The ovitrap used in this study was dark-colored and made of plastic. This is based on the bionomics of the *Aedes aegypti* mosquito, which prefers dark colors for egg-laying (Windyaraini *et al.*, 2020). Furthermore, mosquitoes tend to lay their eggs in plastic containers and do not tend to dislike soil (Cahyati & Siyam, 2019). The statement relevance of *Aedes* mosquitoes inhabiting plastic materials has also been reinforced by a study conducted in Zanzibar City, Tanzania, where both larvae and pupae *Aedes aegypti* preferred plastic containers, followed by metal containers, ceramic or cement containers, rubber (tires), and natural habitats. Similar findings were found in Dar es Salaam, Tanzania, indicating that *Aedes aegypti* is the primary dengue vector and breeds primarily in medium-sized plastic containers (Mboera *et al.*, 2016).

Ovitrap in this study was modified by adding *Ricinus communis* as a natural larvicide which is lethal to larvae. Following the other research results it was stated that *Ricinus communis* seed extracts showed significant mortality of *Aedes aegypti* (Sogan *et al.*, 2018; Wamaketa *et al.*, 2018). This causes the number of containers that are positive for larvae to decrease so that the CI also decreases. According to the findings of Wamaketa *et al.* (2018), which affirms that Among the five concentrations that were investigated, the highest dosage (100 ppm) exhibited the highest effectiveness against third-instar larvae, leading to an 85.33% mortality rate. This is additionally substantiated by the study carried out by Sogan *et al.* (2018), at concentrations of 32 ppm and 64 ppm, the seed extract resulted in 100% mortality for *An. culicifacies* and *Ae. aegypti*, respectively.

Ricinus communis is one such plant that has the potential to be utilized in vector control programs, attributed to its castor oil and ricinine (Sogan *et al.*, 2018). Ricin is a toxin that is naturally contained in *Ricinus communis* seed. Ricin can be created from leftover material from the processing of *Ricinus communis* seed. It can be made in the form of powder, mist, or pellets, or it can also be dissolved in water or weak acid (Centers for Disease Control and Prevention, 2018). Furthermore, numerous bioactive phytochemical elements of *Ricinus communis* have been identified in methanol extract, including alkaloids, flavonoids, tannins, terpenoids, and others. The research conducted by Alugah and Ibraheem (2014) revealed the presence of flavonoids and tannins in the methanol extract of *Ricinus communis* seeds. The phytochemical contents found in the methanol extract of *Ricinus communis* seeds are also elucidated in other studies, encompassing alkaloids, steroids, triterpenoids, tannins, flavonoids, saponins, coumarins, and emodins (More & Pandhure, 2015; Rahman *et al.*, 2022). The phytochemical content exhibits larvicidal

properties against *Aedes aegypti* larvae by impeding the transition of eggs into larvae and enhancing the rate of larval mortality (Panche *et al.*, 2016; Shymanovich *et al.*, 2015).

Table 1. Container Index Before and After Treatment

Doses (mg/l)	Repetition	Container Index (%)		Diff
		D-0	D-7	
0	1	60.00	60.00	0.00
	2	60.00	60.00	0.00
	3	80.00	80.00	0.00
	4	50.00	50.00	0.00
	5	60.00	60.00	0.00
	6	50.00	50.00	0.00
	Average		60.00	60.00
40	1	50.00	30.00	20.00
	2	50.00	40.00	10.00
	3	50.00	30.00	20.00
	4	50.00	40.00	10.00
	5	50.00	40.00	10.00
	6	50.00	20.00	30.00
	Average		50.00	33.33
80	1	40.00	40.00	0.00
	2	50.00	20.00	30.00
	3	40.00	20.00	20.00
	4	50.00	50.00	0.00
	5	50.00	30.00	20.00
	6	50.00	20.00	30.00
	Average		46.67	30.00
100	1	40.00	10.00	30.00
	2	40.00	50.00	10.00
	3	50.00	20.00	30.00
	4	50.00	10.00	40.00
	5	50.00	0.00	50.00
	6	40.00	10.00	30.00
	Average		45.00	11.67
120	1	30.00	20.00	10.00
	2	40.00	30.00	10.00
	3	50.00	10.00	40.00
	4	40.00	20.00	20.00
	5	40.00	20.00	20.00
	6	30.00	20.00	10.00
	Average		38.33	20.00

Table 2. Mean Difference of Container Index Before and After Treatment

Doses (mg/l)	Mean CI Pre-test (%)	Mean CI Post-test (%)	Mean Diff	t	p
0	60.00	60.00	0.00	-	-
40	50.00	33.33	16.67	5.00	0.004
80	46.67	30.00	16.67	2.99	0.031
100	45.00	11.67	33.33	7.91	0.001
120	38.33	20.00	18.33	3.84	0.012

Table 3. Data Homogeneity

		Statistics	df1	df2	p
Container	Based on Mean	1.047	4	25	0.403
Index Post-Test	Based on Median	0.873	4	25	0.494
	Based on the Median and with adjusted df	0.873	4	19.6	0.498
	Based on trimmed mean	1.065	4	25	0.394

Table 4. Influence of Ricinus communis Seed Dosage on Container Index

Sources of Variation	Sum of Squares	Df	Mean Square	F	p
Between groups	8053.333	4	2013.333	22.707	0.0001
Within groups	2216.667	25	88.667		
Total	10270.000	29			

Table 3 of the homogeneity test above shows that the average CI after treatment is homogeneous with a p-value of 0.403 (p-value < 0.05) and can proceed with the ANOVA test. The results of the one-way ANOVA test in Table 4 showed that the difference in the average CI for the various doses of *Ricinus communis* seed powder used had a p-value of 0.0001 (p-value < 0.0001), meaning that there was a significant difference in the average CI between the doses. This shows that there was an influence of *Ricinus communis* seed dosage on CI. This is in line with research conducted by Wamakot *et al.* (2018) in Thailand which showed that among the five doses examined, the highest dosage (100 ppm) was the most effective against third-instar larvae, with a mortality rate of 85.33%.

Table 5. Effective Ricinus communis Seed Dosage in Reducing Container Index

Observation Group		Mean Diff	p
Doses (I)	Doses (J)	I - J	
0 mg/l	40 mg/l	26.67	0.0001
	80 mg/l	30.00	0.0001
	100 mg/l	48.33	0.0001
	120 mg/l	40.00	0.0001
40 mg/l	80 mg/l	3.33	0.545
	100 mg/l	21.67	0.001
	120 mg/l	13.33	0.022
80 mg/l	100 mg/l	18.33	0.002
	120 mg/l	10.00	0.078
100 mg/l	120 mg/l	-8.33	0.138

Based on the results of the multiple comparison test (Table 5), there is a difference in the average CI after treatment between the control ovitrap and ovitrap at various doses

with a p-value of 0.0001 (p-value < 0.05), which means that dosing *Ricinus communis* seed is effective in reducing CI. Then, based on the results of the average difference in CI, a dose of 100 mg/l water is the most effective dose in reducing CI. The results showed that seed powder of *Ricinus communis* in an ovitrap with a dose of 100 mg/l was more effective than a dose of 120 mg/l water in reducing CI. It happens because, at a dose of 120 mg/l water, the water in the ovitrap becomes more turbid. *Aedes aegypti* likes clear water reservoirs and has a low level of turbidity. Research by Dalpadado *et al.* (2022) showed that *Aedes sp.* tends to like water with low turbidity and TDS.

The density of *Aedes sp.* mosquito larvae in an area must be controlled and handled appropriately by breaking the chain of transmission and controlling the dengue vector population. The incidence of DHF was substantially correlated with *Aedes aegypti* larvae presence in a water container (Panggabean *et al.*, 2019; Surendran *et al.*, 2021; Yuanita *et al.*, 2019). Mosquito Nest Eradication (PSN) is a government program that can be carried out to control mosquito populations, including by using larvicides. Research by Retnaningrum *et al.* (2019) showed that 85.5% of respondents did not use temephos powder, potentially increasing the risk of dengue vector mosquitoes exploding. However, temephos resistance has been discovered in several locations (Goindin *et al.*, 2017; Haziqah-Rashid *et al.*, 2019; Ikawati *et al.*, 2017). This pattern of resistance to insecticides requires serious attention hence it is necessary to modify the use of natural ingredients as insecticides that do not cause resistance and are biodegradable (environmentally friendly).

The use of appropriate technology to prevent dengue that can be applied in the community is the manufacture of ovitrap hence the use of modified ovitrap with natural larvicide rather than chemical. The use of modified ovitrap with *Ricinus communis* seed larvicide at a dose of 100 mg/l can be used as an environmentally friendly vector control activity because chemical larvicide can trigger mosquito resistance. In line with this study, natural ingredients from ethanolic preparation of *C. papaya* leaf possess larvicidal action against *Aedes spp.* (Ilham *et al.*, 2019). The seed of *Ricinus communis* also were determined to be non-harmful to non-target organisms, such as Guppy fish (*P. reticulata*), as it did not show any discernible impact on *P. reticulata* even after 24 or 48 hours of exposure at their LC50 and LC90 values against fourth-instar larvae of *Ae. aegypti* (Sogan *et al.*, 2018).

Integrated vector control can be carried out in conjunction with other vector control measures. The current methods for *Aedes* larval source reduction employed in dengue (DENV) control efforts in the Jaffna Peninsula face significant challenges, including issues with littering practices, non-compliance with established dengue control guidelines, vertical transmission of DENV in vector mosquitoes, and the development of larvae in brackish water, open surface drains, and domestic wells used for potable water supply (Surendran *et al.*, 2021). As a result, the use of natural larvicides should be complemented by promoting community involvement in dengue prevention activities, such as emptying water containers four times a month and regularly monitoring for the presence of larvae in water containers (Sarumpaet *et al.*, 2017; Windiyaningsih & Nurhasuti, 2018). The community's role in vector control is of utmost importance, as the health of households and proper sanitation presence of facilities in the community are closely related to the container index (Sukesi *et al.*, 2023).

Conclusion

Ricinus communis seed powder in an ovitrap effectively reduced the density of *Aedes aegypti* mosquito larvae at an optimal concentration of 100 mg/l. The modified

ovitrap employed in this study represents an advancement in effective technology involving the modification of a small dark-colored plastic container filled with *Ricinus communis* seed larvicide. This method principle combines the creation of a breeding site for *Aedes aegypti* egg deposition with the prevention of egg and larvae development, ultimately leading to mortality. This innovative approach serves as an environmentally friendly method for mosquito vector control.

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