Jurnal Biolokus: Jurnal Penelitian Pendidikan Biologi dan Biologi
Vol. 5, Issue 2, December 2022
Pages : 99 – 106

p-ISSN: 2621-3702
e-ISSN: 2621-7538

Toxicological assessment of lemongrass (*Cymbopogon nardus* L.) liquid waste: Biolarvicides againts the third instar larvae of *Aedes aegypti*

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ABSTRACT

Liquid waste generated from the distillation process of lemongrass (*Cymbopogon nardus* L.) in a relatively short time will cause a foul odor, ammonia or phosphine that occurs due to the fermentation process. These problems can be minimized by reusing liquid waste of lemongrass (*C. nardus* L.) into biolarvicides. This study aims to determine the value of Lethal Concentration 50 (LC50) from the liquid waste of lemongrass (*C. nardus* L.) which can kill the third instar larvae of *A. aegypti* mosquitoes for 24 hours. The liquid waste of lemongrass (*C. nardus* L.) used in this study is waste obtained from the lemongrass (C. nardus L.) distillery located on Jalan Masjid Ulayat, Percut Sei Tuan District, Deli Serdang Regency, North Sumatra. A total of 10 mosquito larvae of *A. aegypti* instar III were used for each different concentration of 6,982%, 7,607%, 8,287%, 9,027% and 9,833%. Each concentration consisted of five repetitions and was observed for 24 hours. After 24 hours, larvae that died were analyzed using probit analysis via SPSS 25. The results showed that the LC50 of lemongrass (*C. nardus* L.) liquid waste can kill larvae for 24 hours was 8,763%.

Keywords: *Aedes aegypti*, biolarvicides, LC50, lemongrass, toxicity

INTRODUCTION

Indonesia is a country thas has abundant natural resources (Jeshika, 2019). The wealth of natural resources is one of the factors that support Indonesia currently engaged in the industrial sector. Most of industrial activities in Indonesia are currently derived from plant utilization activities. This can be seen from Indonesia's ability to supply 85% of the world's essential oil needs. Lemongrass (*Cymbopogon nardus* L.) is a plant that can produce essential oils and can be used as a pesticide and anti-mosquito drug (Anwar et al., 2016). One of the industrial centers for the production of lemongrass (*C. nardus* L.) essential oil can be found in the Deli Serdang area, to be precise, in Percut Sei Tuan District (Gultom et al., 2020).

The production of lemongrass (*C. nardus* L.) essential oil is generally carried out through a distillation process using the steam-hydro distillation method (Gotama & Ashadi, 2020). In addition to getting benefits, this activity is also expected to have a negative impact on the environment. Discharge from the production of industrial activities can produce waste that can pollute the environment around industrial sites (Supraptini, 2002). Based on its characteristic, waste can be classified into three types, namely solid, liquid and gaseous waste (Sunarsih, 2014). Liquid waste is waste that is produced in the form of water. The high negative impact that can be caused by liquid waste is influenced by the type and characteristics of the liquid waste (Fisma & Bhayu, 2020).

Liquid waste generated from the distillation process of lemongrass (*C. nardus* L.) in relatively short time will cause a foul odor, ammonia or phosphine that occurs due to the fermentation process. The environmental imbalance that receives the waste load every day from the lemongrass

(*C. nardus* L.) distillation process will certainly affect the quality of water and the environment around the distillery (Susanti & Fitria, 2020). Therefore, to minimize the problems, one of the solutions that can be done is to reuse the liquid waste of lemongrass (*C. nardus* L.) into natural larvicide. Natural larvicides can be an alternative that can be used to reduce the use of synthetic larvicides. The use of synthetic larvicide has been widely used to eradicate larvae and has proven to be affective but often pollutes the environment (Widyastuti et al., 2019).

Lemongrass (*C. nardus* L.) liquid waste is estimated to contain a little more essential oil from the heavy fraction (high boiling point). Typical chemical compounds that are still contained in it are volatile and non-volatile compounds such as terpenes used for insecticides (Usmiati et al., 2005). This estimate is reinforced by Syair (2020) which says that are liquid waste from the distillation of lemongrass (*C. nardus* L.) contains terpenoid and sesquiterpene compounds consisting of citronella, citronellol and geraniol compounds. It is estimated that almost all of the active compounds contained in lemongrass (*C. nardus* L.) liquid waste have a toxic effect at certain concentrations. Therefore, to find out how much an organism's ability to accept the effects of these wastes, it is necessary to carry out an initial test known as a toxicity test. Toxicity tests aim to determine toxic effects and the threshold for using a substance to be used as a drug or larvicide (Sinaga et al., 2018).

Toxicity tests can be carried out using *Aedes aegypti* mosquito larvae (Boesri et al., 2015). The *A. aegypti* mosquito is a vector of dengue fever caused by the dengue virus through the mosquito bite (Agustin et al., 2017). The easiest mosquito eradication to do is in the aquatic phase, where *A. aegypti* mosquito is in its larval phase. The larvae used as test larvae were third instar larvae. The selection of third instar larvae was carried out because in this phase the larvae already have complete organs and have a more stable ability to neutralize toxic compounds compared to first and second instar larvae. Therefore, it can be assumed that the concentration that can kill third instar larvae is also capable of killing first and second instar larvae (Utomo et al., 2010). Based on this, it is necessary to conduct a LC_{50}-24 hours toxicity test of lemongrass (*C. nardus* L.) liquid waste against third instar larvae of *A. aegypti* mosquitos.

METHOD

This study was a *true experimental design* which was included in the *postteest-only control design* because there were two groups, namely the control group and the experimental group, which were randomly selected to test the toxicity of lemongrass (*C. nardus* L.) liquid waste to third instar larvae of *A. aegypti*. The research design used a completely randomized design which consisted of five treatments with certain concentrations. Treatment was only given to the experimental group. Each treatment was carried out five times repetition.

Time and location of research

This research was conducted from May 2022 to July 2022 at the Biology Laboratory, State University of Medan. Lemongrass (*C. nardus* L.) liquid waste was obtained from the lemongrass (*C. nardus* L.) oil refinery located on Jalan Masjid Ulayat, Percut Sei Tuan District, Deli Serdang Regency, North Sumatra.

Tools and materials

The tools used in this study were aqua cups, filter paper, black duct tape, plastic containers, glass plates, 10 ml measuring cups, 50 ml measuring flasks, dropping pipettes, cellphone flashlights, light microscope Yazumi XSZ-107 BN, stereo microscope Olympus CX 21, object glass, cover glass, needles, tissue, label paper, cellphone camera, stationery and laptop.

The materials used in this study were lemongrass (*C. nardus* L.) liquid waste obtained from a lemongrass (*C. nardus* L.) distillery, third instar lavae of *A. aegypti* mosquitoes, ground water and larvae feed.

Test larvae

Egg culture

Culture was carried out to obtain *A. aegypti* mosquito eggs so they could be bred into third instar larvae. Third instar larvae are larvae that experience growth for ± 2 days with a slightly larger size than second instar larvae, which is above 3,8 mm (Ditjen PPPL, 2017). The eggs are obtained from water that has been previously prepared in an egg trap (ovitrap) in the form of an aqua cup covered with black duct tape which is placed behind the Biology laboratory building. Medan State Uniersity.

Egg identification

Egg identification was carried out through macroscopic and microscopic observations by observing the morphology of the eggs according to Adrianto (2020). *A. aegypti* eggs that were identified were placed in a container to be bred into third instar larvae.

Maintenance of test larvae

Eggs obtained from egg traps (ovitrap) from the field were incubated in plastic containers measuring (20 x 14 x 10) cm^3 with a water level of ¾ of the size of the container. The stocking density of the larvae in a container is ± 15 individuals/liter of water. During the hatching process, the container where the larvae are kept is illuminated with a light to speed up the life cycle. After hatching, the larvae are transferred to another plastic container which has been filled with water ¾ high from the plastic container and given food (Gama et al., 2010).

Lemongrass (C. nardus L.) liquid waste

The lemongrass (*C. nardus* L.) liquid waste used in the study is waste obtained from the lemongrass (*C. nardus* L.) distillery located on Jalan Masjid Ulayat, Percut Sei Tuan District, Deli Serdang Regency, North Sumatra. The sorted leaves and stems of lemongrass (*C. nardus* L.) are put into a katel to obtain essential oils and liquid waste resulting from essential oil distillation (hydrosol). Lemongrass (*C. nardus* L.) liquid waste needs to go through the pre-treatment stage before being used, namely by cleaning floating objects such as sand (Sugiharto, 2005).

Toxicity test lethal concentration 50 (LC_{50}-24 Hours)

Preliminary Test

This study consisted of one control group and five treatment groups using different concentrations. The concentrations used were 6%, 8%, 10%, 12% and 14%. Each treatment contained 10 larvae and was repeated three times. Observation of the number of dead larvae was carried out every 4 hours for 24 hours. The number of dead larvae is recorded to obtain the percentage of mortality with the formula:

$$M = \frac{No - Nt}{No} \times 100\%$$

Note:
M = Mortality
Nt = Number of test larvae at the end of rearing (tails)
No = Number of test larvae at the start of rearing (tails)

The percentage results obtained were then analyzed using probit analysis to obtain a Lethal Concentration 50 (LC_{50}), lower threshold value and upper threshold value. These values are the basis for determining the concentration variations used in the actual test through the logarithmic scale formula using Busvine's (1971) calculations as follows:

$$\text{Log}\frac{N}{n} = k \log + \frac{a}{n} \ldots (1)$$

Note:
N = Upper threshold concentration
n = Lower threshold concentration
k = Number of concentration intervals

$$\frac{a}{n}=\frac{b}{a}=\frac{c}{b}=\frac{d}{c}=\frac{e}{d}\ldots(2)$$

Note:

a, b, c, d, e are the concentrations obtained from the calculation results of formulas (1) and (2).

Real test

This study consisted of a control group of five treatments using different concentrations. The concentrations used were 6,982%, 7,607%, 8,287%, 9,027% dan 9,833%. This concentration is the concentration obtained from the results of probit analysis and calculations by Busvine (1971) in the previous test. Each treatment contained 10 larvae and was repeated five times. Observation of the number of dead larvae was carried out every 4 hours for 24 hours.

Observations of morphological characteristics of poisoined test larvae

The poisoned test larvae were observed using a Yazumi XSZ 107-BN light microscope and an Olympus CX 21 stereo microscope to see the morphological characteristics seen after being exposed to lemongrass (*C. nardus* L.) liquid waste.

Furthermore, the differences in the characteristics that are seen after being given treatment will be described based on the results of observations that have been made in this study.

Data Analysis

Data were analyzed through probit analysis using SPSS 25 to determine the value of Lethal Concentration 50 (LC_{50}). The research data are presented in the form of tables and graphs to see the toxic effects produced from lemongrass (*C. nardus* L.) liquid waste.

RESULTS AND DISCUSSION

Observations were made using several treatments (Table 1). One treatment as a control and five treatments using several different concentration variations, namely 6%, 8%, 10%, 12% and 14%. Each treatment was carried out three times repetition. The number of test larvae used in each treatment was 10.

Table 1. The results of observing the death of *A. aegypti* larvae in the preliminary test

Concentration	Test Larvae	Replication			Number of Dead Larvae	Average	(%)
		I	II	III			
Control	10	0	0	0	0	0	0%
6%	10	3	0	0	3	1	10%
8%	10	5	4	5	14	4,6	46%
10%	10	6	7	5	18	6	60%
12%	10	9	10	7	26	8,6	86%
14%	10	10	10	10	30	10	100%

Observations were made using several treatments (Table 1). One treatment as a control and five treatments using several different concentration variations, namely 6%, 8%, 10%, 12% and 14%. Each treatment was carried out three times repetition. The number of test larvae used in each treatment was 10. Based on these data, it is known that the highest concentration that can kill 100% of the test larvae is the concentration in the fifth treatment (P5), which is 14% (v/v). Meanwhile, the lowest concentration that killed 10% of test larvae was the concentration in the first treatment (P1), which was 6% (v/v). In addition, it can also be seen that the higher the concentration given to the treatment, the greater the percentage of mortality obtained. A graph of the percentage of third instar larvae mortality of *A. aegypti* for 24 hours after being treated with lemongrass

(*C. nardus* L.) liquid waste with different concentration variations in the preliminary test (Figure 1).

Based on Table 2, observations were made using several treatments. One treatment as a control and five treatments using several different concentration variations, namely 6.982%, 7.607%, 8.287%, 9.027% and 9.833%.

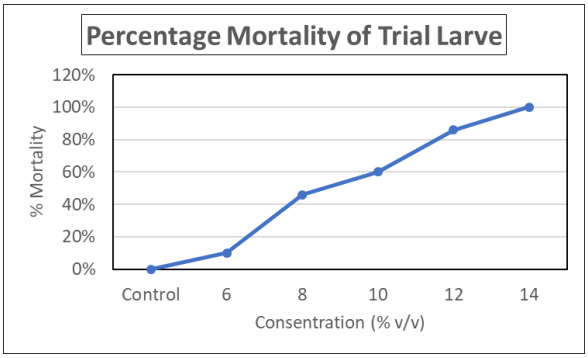

Figure 1. Graph of the percentage of third instar larvae mortality of *A. aegypti* in the preliminary test.

Based on Table 2, observations were made using several treatments. One treatment as a control and five treatments using several different concentration variations, namely 6.982%, 7.607%, 8.287%, 9.027% and 9.833%. Each treatment was carried out five times repetition. The number of test larvae used in each treatment was 10.

Based on these data, it is known that the highest concentration that can kill 58% of the test larvae is the concentration in the fifth treatment (P5), which is equal to 9.833% (v/v). While the lowest concentration that killed 30% of test larvae was the concentration in the first treatment (P1) which was 6.982% (v/v). In addition, it can also be seen that the higher the concentration given to the treatment, the greater the percentage of mortality obtained.

Table 2. Results of observing the death of *A. aegypti* larvae in the real test

Concentration	Test Larvae	Replication					Number of Dead Larvae	Average	(%)
		I	II	III	IV	V			
Control	10	0	0	0	0	0	0	0	0%
6,982%	10	4	3	2	3	3	15	3	30%
7,607%	10	4	3	4	5	4	20	4	40%
8,287%	10	5	4	5	6	4	24	4,8	48%
9,027%	10	6	5	5	6	4	26	5,2	52%
9,833%	10	6	5	7	6	5	29	5,8	58%

A graph of the percentage of third instar larvae mortality of *A. aegypti* for 24 hours after being treated with lemongrass (*C. nardus* L.) liquid waste with different concentration variations in the real test (Figure 2).

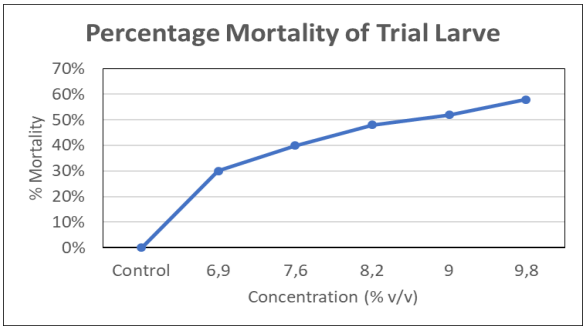

Figure 2. Graph of the percentage of third instar larvae mortality of *A. aegypti* in the real test

The results of temperature measurements during the toxicity test ranged from 28°C - 30°C, this temperature range is still within normal limits so it is unlikely that the test larvae in the study died due to the influence of room temperature. This is supported by the statement of Yunita et al., (2009) which stated that a temperature of 28°C - 30°C is the normal temperature limit that can be used to conduct research on toxicity tests.

A preliminary test of lemongrass (*C. nardus* L.) liquid waste on third instar larvae of *A. aegypti* mosquitoes had a Lethal Concentration of 50 (LC_{50}-24 hours) of 8.816

with a lower threshold of 6.408 and an upper threshold of 9.947, while the Lethal Concentration 50 (LC_{50}) in the real test was 8.763. Noerbaeti (2001) defines that Lethal Concentration 50 (LC_{50}) is a concentration calculation that can statistically kill 50% of the test animals for a certain period of time and can be estimated graphically. The Lethal Concentration 50 (LC_{50}) value obtained between the treatment of lemongrass (*C. nardus* L.) liquid waste and the Lemongrass extract (*C. nardus* L.) extract is of course different. This can be compared with previous research. Based on research by Astuti & Handoko (2014) regarding the effect of varying doses of the lemongrass leaf solution on the mortality of *Aedes* sp mosquito larvae, the concentration that could kill 52% of the tested larvae was at a concentration of 20 percent (v/v), while in this study it was sufficient for 9.027% (v/v) only. This is because there is a difference in the number of chemical compounds contained in the lemongrass (*C. nardus* L.) liquid waste and the compounds contained in the lemongrass (*C. nardus* L.) extract. Based on this study, it can be assumed that the use of lemongrass (*C. nardus* L.) liquid waste is more lethal to the third instar larvae of *A. aegypti* than the use of lemongrass (*C. nardus* L.) extract in previous studies.

A. aegypti larvae exposed to lemongrass (*C. nardus* L.) liquid waste gave a response showing symptoms of poisoning. These symptoms are in the form of uncontrolled movement of the larvae, swimming in circles and emerging to the surface of the water until they lose their balance. Furthermore, the larvae sink to the bottom. The larvae that are susceptible to exposure to lemongrass (*C. nardus* L.) liquid waste subsequently die due to the toxic effects produced from the waste. This is of course very different from the untreated test larvae (control), which showed proper movement and were much quieter. Morphologically, larvae that have been poisoned over time will excrete fluids from their bodies due to dehydration (continuous lack of fluids). This can be seen from the physical stickiness to the wall of the treatment medium (aqua cup). The thorns located on the thorax were also damaged and detached from the thorax.

The symptoms and morphological characteristics seen in the larvae are comparable to some statements which say that poisoned *A. aegypti* larvae will show symptoms of anxiety (Astuti & Handoko, 2014). These symptoms are in the form of up and down movements in the medium (telescopic) (Tarumingkeng, 1992). The head of the larva will eventually break off because it tries too often to surface to meet its oxygen needs. The intestines of the larvae turn black, this is because there are bioactive compounds that enter the digestive system and cause a burning feeling, do not react when touched (Astuti & Handoko, 2014). The same thing was said by Moehammadi (2005) that dead larvae have a darker body, the size of the body looks longer and stiffer and the head is almost broken off. Aisiah (2009) states that poisoned larvae have weak and slow body movements and the body size of the larvae increases in length, which is about 6 mm from the previous size which was only 5 mm.

Terpene compounds in waste are thought to consist of citronella and geraniol. Syair (2020). Citronella causes the larvae to experience contact poison which results in the larvae experiencing a continuous lack of fluids, disrupting the respiratory system which can lead to death (Astuti & Handoko, 2014).

Figure 3. Observation of larvae poisoned by lemongrass (*C. nardus* L.) liquid waste,

seen macroscopically and microscopically using a Yazumi XSZ -107 BN light microscope, magnification 5x/0.12 and an Olympus CX 21 stereo microscope, 40x magnification (Source: Personal Documentation).

Whereas geraniol can inhibit the cholinesterase enzyme which results in motor nerves being stimulated resulting in the larvae experiencing seizures and fatigue (Pinardi et al., 2010). Observation of the test larvae that were poisoned by lemongrass (*C. nardus* L.) liquid waste was carried out using a Yazumi XSZ -107 BN light microscope with a magnification of 5x/0.12 and an Olympus CX 21 stereo microscope with a magnification of 40x (Figure 3).

CONCLUSION

The Lethal Concentration 50 (LC_{50}) value of lemongrass (*C. nardus* L.) liquid waste which can kill 50% of third instar larvae of *Aedes aegypti* is 8.763% in 24 hours of observation. The larvae of *A. aegypti* exposed to lemongrass (*C. nardus* L.) liquid waste gave a response in the form of uncontrolled larval movements, swimming in circles and rising to the surface of the water until they lost their balance. Furthermore, the larvae sink to the bottom. The larvae that are susceptible to exposure to lemongrass (*C. nardus* L.) liquid waste then die due to the toxic effects produced from the waste.

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