

# THE EFFECTIVENESS OF MIXED LEAVES FROM PANDANUS LEAVES (*Pandanus amaryllifolius*) AND LEMONGRASS STEMS (*Cymbopogon nardus* Linn. Rendle) AS BIOLARVICIDES AGAINST Aedes Aegypti LARVA

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## ABSTRACT

Using of chemical larvicides continuously triggers resistance in larvae and side effects for humans. Larvicides from natural ingredients are safer for health, materials derived from plants are called biolarvicides. Fragrant pandanus and lemongrass stems have the potential to be biolarvicides because they contain saponins, flavonoids, alkaloids, and polyphenols. The purpose of this study was to analyze the effectiveness of a mixture of biolarvicides from fragrant pandan leaves (*Pandanus amaryllifolius*) and lemongrass stems (*Cymbopogon nardus* Linn. Rendle) on the mortality of *Aedes aegypti* larvae.

This type of research is experimental with post test only control group research design, using 5 treatments. The treatments used were a mixture of fragrant pandan extract and lemongrass stems with concentrations of 10%, 15%, 20%, 25%, and 1 control. The sample used was 500 larvae of *Aedes aegypti* divided into 20 larvae for each group with 6 repetitions and exposure time for 24 hours and room temperature, water temperature, water pH, and humidity as control variables. Data on larval mortality was analyzed using the One Way Anova statistical test.

The mortality of *Aedes aegypti* larvae at concentrations of 10%, 15%, 20%, and 25%, respectively, was 28.3%, 39.1%, 50%, and 65.8% of the test larvae. The higher the concentration of the mixture of fragrant pandan leaves extract (*Pandanus amaryllifolius*) and lemongrass stems (*Cymbopogon nardus* Linn. Rendle) given, the greater the average death of *Aedes aegypti* larvae.

A mixture of fragrant pandan leaves extract and lemongrass stems has the potential as a biolarvicide of *Aedes aegypti*, but has not been effective in killing 90-100% of larvae. Further researchers can increase the concentration of the extract mixture which allows killing 90-100% of test larvae.

**Keywords:** Dengue haemorrhagic fever; biolarvicide; pandan fragrance (*Pandanus amaryllifolius*); lemongrass (*Cymbopogon nardus* Linn. Rendle).

## INTRODUCTION

Indonesia is a tropical country that is suitable as a breeding ground for vectors. Mosquitoes are an example of a vector that can breed in tropical climates. Diseases transmitted by mosquitoes are a type of disease that is classified as dangerous and deadly. Dengue Hemorrhagic Fever is a disease that is transmitted through mosquitoes which is currently still a health problem in Indonesia. DHF is a disease caused by the dengue virus and is transmitted through *Aedes aegypti* and *Aedes albopictus*.

Indonesia's health profile data in 2019 the number of dengue cases was 138,127 cases, the morbidity rate was 51 per 100,000 population, the case fatality rate was 0.67%, and the number of deaths was 919 people. The main step taken to control dengue hemorrhagic fever is to control *Aedes aegypti*, because vaccines to prevent and drugs to eradicate the dengue virus are still not available. The physical method that can be done is by eradicating mosquito nests through the 3M plus movement. The biological method that can be done is by spreading fish that are predatory predators of *Aedes aegypti* larvae. Chemical control of *Aedes aegypti* larvae, namely providing larvicides in water storage areas. Giving larvicides in air storage is a method that is often used in controlling *Aedes aegypti* larvae [1].

The type of larvicide that is often used by the community is temefos. Continuous use of temefos can trigger resistance in larvae and side effects for humans can cause poisoning [2]. Judging from the side effects, it is necessary to innovate by making biolarvicides or natural larvicides derived from plants that have toxic effects on insects, but will not cause side effects on the environment and humans. The plants that contain natural larvicides are fragrant pandan leaves (*Pandanus amaryllifolius*) and lemongrass stems (*Cymbopogon nardus* Linn. Rendle).

Fragrant pandan leaves was effective as a larvicide, as evidenced by the highest larval mortality in *Aedes aegypti* larvae that had been exposed to fragrant pandan leaves extract at a

concentration of 15% [3]. Lemongrass extract from stem part was effective in killing 50% of the *Aedes aegypti* larva population at a concentration of 36.48% and the time required to kill 50% of the larvae population for 10.45 hours [4]. Research on pandan leaves and lemongrass stems as larvicides has been carried out by several researchers, but only limited to the use of one of these plants. In this study, mixed pandanus and lemongrass stems as biolarvicides to determine whether the two plants were effective in killing the larvae or otherwise making the larvae resistant.

## RESEARCH METHODS

This type of research is an experiment using a post test only control group design. The object of this study was divided into two, namely the first as an experimental group using a mixture of fragrant pandan leaves extract and lemongrass stem extract, the second group as an untreated control. The treatments in this study didn't considered the incubation condition of larvae were 5 treatments using a mixture of fragrant pandan leaves extract and lemongrass stems at concentrations of 10%, 15%, 20%, and 25% and aquadest as a control. The number of larvae used in this study was 20 in each treatment according to WHO provisions [5]. Each of these studies required 6 times of contact testing, so that the total required larvae of *Aedes aegypti* on third instar were 500 individuals.

Data on mortality of *Aedes aegypti* larvae were analyzed using the One Way Anova statistical test which was then followed by the Post Hoc Least Significance Difference (LSD) test to determine the differences between groups that had significant differences.

## RESULT AND DISCUSSION

The study was conducted to determine the effectiveness of a mixture of fragrant pandan leaves alcoholic extract (*Pandanus amaryllifolius*) and lemongrass stem (*Cymbopogon nardus* Linn. Rendle) as *Aedes aegypti* biolarvicides. Fragrant pandan leaves and lemongrass stems contain active compounds that have the potential to kill *Aedes aegypti* mosquito larvae.

### 1. Number of *Aedes aegypti* larvae that died in the treatment group for 24 hours

Based on Table 1, it can be seen that at a concentration of 10% the average mortality percentage of *Aedes aegypti* mosquito larvae is 28.3%, at a concentration of 15% the average percentage of *Aedes aegypti* mosquito larvae mortality is 39.1%, at a concentration of 20% the average the percentage of mortality of *Aedes aegypti* mosquito larvae is 50%, at a concentration of 25% the average percentage of mortality of *Aedes aegypti* mosquito larvae is 65.8 %. On control group (0%) has a mortality percentage of 0%, which means that there is no mortality of *Aedes aegypti* larvae in the control group.

In the control group (0%) from the results of research conducted for 24 hours with the observation of the number of deaths recorded every certain time period, namely after 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes, 6 hours, and 24 hours, it was found that the average number of deaths for the control group was 0%, which means that there were no dead *Aedes aegypti* larvae. The absence of larva mortality in the control group was due to the fact that the control group only used distilled water without any mixture. Aquades does not have a larvicidal effect that causes death in test larvae because aquades or water is a habitat for *Aedes aegypti* mosquito larvae and does not contain toxic substances [6]. In addition, controlled physical environmental conditions like temperature and humidity are also one of the causes of the absence of dead larvae.

The results obtained that the average percentage of larval mortality was 28.3%, 39.1%, 50%, and 65.8%, respectively. These results prove that there is a synergistic effect larvicidal effect on the administration of a mixture of extracts of fragrant pandan leaves (*Pandanus amaryllifolius*) and lemongrass stems (*Cymbopogon nardus* Linn. Rendle) on test larvae, higher concentration also high larval mortality too.

The death of *Aedes aegypti* larvae was caused by the active compounds contained in the fragrant

pandan leaves extract and lemongrass stems extract. Based on the results of phytochemical tests, the active compounds contained in fragrant pandan leaves are Alkaloids (7.05%), Polyphenols (4.05%), Tannins (6.11%), Saponins (3.51%) and Flavonoids (1.90%). The active compounds contained in lemongrass stems are saponins (4.68%), polyphenols (7.96%), flavonoids (4.10%) and essential oils (1.48%). The active compounds contained in each of these extracts were proven to kill *Aedes aegypti* larvae. Alkaloid compounds, tannins, saponins, and polyphenols act as stomach poisons, while flavonoid compounds and essential oils act as respiratory poisons. In the process, the active compounds contained in fragrant pandan leaves extract and lemongrass stems extract were only able to kill 28-66% of *Aedes aegypti* larvae.

The results obtained are not in line with the research which stated that fragrant pandan leaves (*Pandanus amaryllifolius*) with a concentration of 15% with observation for 24 hours effectively killed 100% of *Aedes aegypti* larvae and lemongrass stems extract (*Cymbopogon nardus* linn. rendle) with concentrations of 50% and 60% with observation for 24 hours effectively killed 100% of *Aedes aegypti* larvae [3,4].

### 2. Physical environment measurements

Based on Table 2, it can be seen that the results of temperature and pH measurements of water media. The result of the average temperature of the test media is 29°C and the average pH of the test media is 5.86.

Measurements of the physical environment that can affect the death of *Aedes aegypti* mosquito larvae include water media temperature, water media pH, room temperature and humidity. The average result of the research room temperature is 29°C and the average humidity of the research room is 74%. The results of measurements of the physical environment showed that the temperature of the water medium, pH of the water medium, room temperature and humidity according to physical conditions of larval habitat.

**Table 1. The number of *Aedes aegypti* larvae that died in the treatment group for 24 hours**

Replication	Concentration			
	10%	15%	20%	25%
1	5	7	9	12
2	6	7	8	10
3	4	6	9	13
4	5	8	10	13
5	7	9	11	14
6	7	10	13	17
Average	5,6	7,8	10	13,1
Percentage	28,3%	39,1%	50%	65,8%

**Table 2. The measurements of the physical environment for 24 hours**

Concentration	Temperature	pH
Control	29°C	6
10%	29°C	6,3
15%	29°C	6
20%	29°C	5,6
25%	29°C	5,4
Average	29°C	5,86

The death of *Aedes aegypti* larvae was caused by the administration of various concentrations of a mixture of fragrant pandan leaves extract (*Pandanus amaryllifolius*) and lemongrass stem (*Cymbopogon nardus* linn. Rendle). This can be seen from the results of measurements of physical environmental factors which can be said that the temperature of the water medium, pH of the water medium, room temperature, and humidity are not factors that can affect the mortality rate of *Aedes aegypti* larvae.

### 3. Analysis of differences in the concentration of the mixture of fragrant pandan leaves extract (*Pandanus Amaryllifolius*) and lemongrass stems (*Cymbopogon Nardus* Linn. Rendle)

Based on Table 3, means that there is a difference in the concentration of the mixture of fragrant pandan leaves extract (*Pandanus Amaryllifolius*) and lemongrass stems (*Cymbopogon Nardus* Linn. Rendle) on the mortality of *Aedes aegypti* larvae as a biolarvicide on the mortality of *Aedes aegypti* larvae for 24 hours ( $P=.000$ ).

Based on Table 4, the results can be seen that the results of the Post Hoc Mann Least Significance Difference test in the treatment for 24 hours it was found that each concentration had  $P = .000 < (.05)$ , then  $H_0$  was rejected, which means there is a

difference in concentration a mixture of extracts of fragrant pandan leaves (*Pandanus amaryllifolius*) and lemongrass stems (*Cymbopogon nardus* linn rendle) on the mortality of *Aedes aegypti* larvae. It means that there is a significant difference in the number of *Aedes aegypti* larvae mortality in various concentration groups of a mixture of fragrant pandan leaves extract (*Pandanus amaryllifolius*) and lemongrass stems (*Cymbopogon nardus* Linn. Rendle).

The active compounds contained in a mixture of fragrant pandan leaves extract (*Pandanus amaryllifolius*) and lemongrass stem (*Cymbopogon nardus* Linn. Rendle), namely alkaloids, polyphenols, tannins, saponins, flavonoids, and essential oils caused the death of *Aedes aegypti* larvae. The larvicidal potency of fragrant pandan leaves extract at 4% concentration is equivalent to temephos [7]. Essential oils of lemongrass stem have toxic influence to kill mosquito larvae because of the content in it [8,9]. The higher the concentration given, the more active compounds are received. The active compounds contained in a mixture of fragrant pandan leaves extract (*Pandanus amaryllifolius*) and lemongrass stems (*Cymbopogon nardus* Linn. Rendle) have larvicidal properties. Mixed of two essential oil plants are possible application in synergistic blend, develop new eco-friendly, safe and effective herbal insecticides [10].

**Table 3. One Way Anova Test**

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	587.667	4	146.917	60.212	.000
Within Groups	61.000	25	2.440		
Total	648.667	29			

**Table 4. Post Hoc Least Significance Difference Test**

Concentration	Sig.	Result
10 – 15	.024	There was a significant difference for larval mortality
10 – 20	.000	There was a significant difference for larval mortality
10 – 25	.000	There was a significant difference for larval mortality
15 – 20	.024	There was a significant difference for larval mortality
15 – 25	.000	There was a significant difference for larval mortality
20 – 25	.002	There was a significant difference for larval mortality

## CONCLUSION

The average mortality of *Aedes aegypti* larvae with a mixture of fragrant pandan leaves extract (*Pandanus amaryllifolius*) and lemongrass stems (*Cymbopogon nardus* Linn. Rendle) at concentrations of 10%, 15%, 20%, and 25%, respectively, was 28.3%, 39.1%, 50%, and 65.8% of the test larvae. A mixture of extracts of fragrant pandan leaves (*Pandanus amaryllifolius*) and lemongrass stems (*Cymbopogon nardus* Linn. Rendle) has been still not effective in killing all of *Aedes aegypti* larvae but potential as larvacide.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

- Iskandar, Iwan, Hevi Horiza, Nanang Fauzi. Efektivitas Bubuk Biji Pepaya (*Carica Papaya Linnaeus*) Sebagai Larvasida Alami Terhadap Kematian Larva *Aedes aegypti* Tahun 2015. *Jurnal Kesehatan Lingkungan*. 2017;18(1):12–18.
- Huljani, Riska Ahsanunnisa. Pemanfaatan Ekstrak Buah Ketumbar (*Coriandrum Sativum* L.) sebagai Larvasida Nabati Nyamuk *Aedes aegypti*. *Jurnal Sains dan Teknologi Terapan*. 2019;2(1):1 – 6.
- Muftiah, Andi Tilka, Andi Yulia Kasma, Renaldi. Efektivitas Ekstrak Daun Pandan Wangi (*Pandanus amaryllifolius*) Terhadap Mortalitas Larva *Aedes* sp dan *Anopheles*. *Jurnal Vektor Penyakit*. 2019;13(2):107-114.
- Makkiah, Makki, Christina Salaki, Berty Assa. Efektivitas Ekstrak Serai Wangi (*Cymbopogon nardus* L.) sebagai Larvasida Nyamuk *Aedes aegypti*. *Jurnal Bios Logos*. 2019;10(1):1-6.
- World Health Organization. Pencegahan dan Pengendalian Dengue dan Demam Berdarah Dengue. Panduan Lengkap. Alih bahasa: Palupi Widyastuti. Editor Bahasa Indonesia: Salmiyatun. Cetakan I. Jakarta, Penerbit Buku Kedokteran EGC; 2005.
- Krissanti, Oktaviana, Setiawan, Koerniasari. Efektivitas Air Perasan Daun Alpukat (*Persea Americanamill.*) Terhadap Kematian Larva Nyamuk *Aedes aegypti*. *Gema Kesehatan Lingkungan*. 2018; 16(1):213- 220.
- Putri, Regina, Teresa Liliana Wargasetia, Susy Tjahjani. Efek Larvasida Ekstrak Etanol Daun Pandan Wangi (*Pandanus amaryllifolius* Roxb.) terhadap Larva Nyamuk *Culex* sp. *Global Medical and Health Communication*. 2017;5(2):103-107.
- Kurniasih N, Nuryadin W, Harahap MN, Supriadin A, Kinarsih I. Toxicity of essential oils from orange (*Citrus sinesis* L. Obbeck) and lemongrass (*Cymbopogon nardus* L. Rendle) on *Aedes aegypti* a vector of Dengue Hemorrhagic Fever (DHF). *Journal of Physics: Conference Series*1869; 2021.
- Ayidé C. Ahouansou, Sèdami R, Medegan Fagla, Jean M. Tokoudagba, Habib Toukourou, Yvette K. Badou and Fernand

- A. Gbaguidi. Chemical composition and larvicidal activity of the essential oil of *Cymbopogon nardus* (L.) Rendle on *Anopheles gambiae*. Int. J. Biol. Chem. Sci. 2019;13(3):1861-1869
10. Pavela, Roman, Filippo Maggi, Guilio Lupidi, Hélène Mbuntcha, Verlainé Woguem, Hilaire Macaire Womeni, Luciano Barboni, Léon Azefack Tapondjou & Giovanni Benelli. *Clausena anisata* and *Dysphania ambrosioides* essential oils: from ethno-medicine to modern uses as effective insecticides. Environmental Science and Pollution Research. 2018;25:10493-10503.