

Phytochemical Test and Effectiveness of Alang-alang Stolon Extract (*Imperata cylindrica* L.) Against Mortality of Dengue Fever Mosquito Larvae (*Aedes* Sp.)

Ray Natalia Antou^{1, a*}, Rudi Alexander Repi^{2, b}, Marthy L.S. Taulu^{3, c}

¹Tatengesan 1, Kecamatan Pusomaen, Indonesia

²Alexa School Lotta Pineleng 2, Indonesia

³Mountain View Residence Jln Anggrek 2 No. 1 Paniki Bawah, Indonesia

*Corresponding author: nataliaantou@gmail.com, rudirepi63@gmail.com, stellataulu16260@gmail.com

Received: August 21, 2022

Accepted: September 19, 2022

Abstract

Aedes sp. is a species of mosquito that acts as a vector for transmitting dengue. The use of chemical insecticides to control *Aedes* sp. result in negative impacts on the environment. One vector control of dengue fever is using plant-derived insecticides, namely stolon Alang-Alang extract *Imperata cylindrica* L. which is known to be more effective against dengue fever mosquitoes *Aedes* sp. This study aims to determine the effectiveness of stolon alang-alang extract against the mortality of dengue mosquito larvae in *Aedes* sp. The method in this study is to analyze mortality data using Microsoft Excel 2016 probit analysis using seven treatments and three replications with a concentration of 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, and 0.5%. Larvae were tested at each concentration of as many as ten tails. The effectiveness of stolon alang-alang extract on larval mortality was observed every 12-72 hours. The data were analyzed by calculating the LC50 value using probit analysis. The results showed that alang-alang stolon extract effectively killed the mosquito larvae of *Aedes* sp. ranging from 0.25-0.5% concentration. The highest mortality rate was obtained at a concentration of 0.3-0.5%, capable of killing 100% of the mosquito larvae of *Aedes* sp. A concentration of 0.3-0.35% can cause 100% mortality of larvae at 60 hours after application (JSA), a concentration of 0.4% can cause 100% mortality in 48 (JSA) and a concentration of 0.45-0.5% causes 100% mortality of larvae in 36 (JSA). Probit analysis showed the LC50 result was 0.2116% (2115.5 ppm) with a toxic category (500-5000 ppm).

Keywords: *Aedes* sp. mosquito, alang-alang stolons, vegetable insecticides, secondary metabolites, phytochemical identification.

INTRODUCTION

Alang-alang (*Imperata cylindrica* L.) is a type of grass that can grow in tropical and subtropical regions. Reeds in Indonesia are widespread from the sea to the mountains at an altitude of 2700 m asl (asl). The area of *Imperata* plants is ± 16,000,000 ha. Reeds have compounds: groups of organic acids, sugars, amino acids, pectates, gibberellic acids, tannins, alkaloids and phenolic acids (Cahyati et al., 2018).

Mosquitoes are classified as insects that have an important role in human life. *Aedes* sp. is a species

of mosquito as a carrier of vectors that cause DHF (Dengue Hemorrhagic Fever), yellow fever (yellow fever) and chikungunya. The population of *Aedes* sp mosquitoes. Significantly increases in the rainy season. The population explosion was caused by the availability of breeding places for *Aedes* sp. where the rainwater stagnated, such as used cans, used tires, used pieces of bamboo, holes in trees, bird drinking places, and so on. Mosquitoes belong to the Nematocera sub-order, family Culicidae and consist of several genera, one of which is the *Aedes* genus which consists of 500 species, *Aedes aegypti* and *Aedes albopictus* which are the most important species (Hadi & Soviana, 2000).

The Spread of *Aedes* sp. in a place is influenced by weather conditions, ambient temperature, humidity and breeding media. There is a significant difference between the rainy season and the dry season. In the rainy season, the development of *Aedes* sp. experienced high fluctuations and decreased during the dry season (Dian, 2004). Until now, the cure for dengue fever has not been found, so vector control is still used. Habitats of *Aedes* sp. as a transmitter of DHF, being close to a place of residence will increase direct contact with humans (Focks, 2003). dengan tempat tinggal akan meningkatkan kontak langsung dengan manusia (Focks, 2003).

Chemical insecticides used to control *Aedes* sp. result in a negative impact on the environment. Therefore, controlling preferences using more environmentally friendly insecticides is urgently needed, namely insecticides made from plants or bio-insecticides (Kasmara, 2004). Plants are similar to large organic compounds (compounds of bioactive natural products), which are selected as a source of new insecticides (Schoonhoven, 1982 in Munandar et al., 2002). Various plants have been known to contain bioactive compounds, such as reeds. According to Takahashi (1981), bioactive compounds can be divided into three parts: a). Antiphytopathogenic compounds (agricultural antibiotics); b). Compounds that are phytotoxic (regulate plant growth such as phytotoxins, plant hormones, and the like); c). Active compounds against insects (hormones, pheromones, antifeedants, repellents, attractants, and insecticides).

Extracts of alang-alang stolons, used as food ingredients and traditional medicines, are known to contain bioactive compounds that have the potential to be developed as vegetable pesticides (Hutapea, 1994 in Melanie et al., 2004). Bioactive compounds in Imperata plants are present in all parts of the plant. However, a literature study found that the Stolons of Reeds have many biological activities with essential oils and phenolic compounds such as cyperene, alkaloids, oligostilbenoids, phenolic acids and triterpenes. In addition to compounds that act as pesticides, reeds also contain high protein, which reaches 15% and low fiber and nitrogen, phosphorus and potassium content (William and Smith, 1984 in Melanie et al., 2004).

Current control has been mainly carried out by fogging and eradicating mosquito nests (PSN) using 3M. However, this has yet to be done optimally, so there are still many cases of DHF (WHO, 2005). One of the alternative controls that can be carried out besides synthetic insecticides is the use of stolon extract of *I. cylindrica* L., which can kill the larvae of the *Aedes* sp. dengue fever mosquito. by inhibiting the development of the mosquito.

RESEARCH METHODS

Tools and materials

The tools used are a separatory funnel, volume pipette, stationery, scissors, test tube, ethanol, cotton, aluminium foil, filter paper, Erlenmeyer flask, petri dish, analytical balance, spatula, and rotary evaporator. The materials used in the study were stolons of *Imperata cylindrica* L. 6 liters of Imperata extract and 240 larvae of *Aedes* sp. dengue mosquitoes. 75% ethanol, distilled water, wire mesh filter.

Sample preparation

Alang-alang stolon extract samples were taken from Tatengesan Village, Pusomaen District. Samples were cleaned and then dried and then mashed, and sieved.

Extract Production

First, provide the tools and materials to be used. The reeds are crushed by pounding and then blended until smooth. The material powder is filtered using a wire mesh filter and weighed as much as 100 grams using an analytical balance. The fine powder that is weighed is put into a measuring cup and then added solvent using 75% ethanol solvent. The ratio of the weight of the material and the solvent, namely (1:1), the mixture obtained is filtered first using filter paper. The next step is to separate the water from the solution using evaporation which is carried out using a rotary evaporator at a temperature of 55°C-60°C at a pressure of 580-600 mmHg. The resulting crude extract is stored in a separatory funnel.

Larvae Propagation

The larvae of the *Aedes* sp. dengue fever mosquito. They were taken to the village of Tatengesan, Pusomaen District and maintained in a bucket by giving fish food and changing the water every 24 hours. Food is given every day, and the waste is cleaned. Maintenance is carried out until the final instar. After hatching the eggs, the larvae are maintained until the third instar with a larva length of more than 3 mm, which is 5-7 days old (Sastriawan, 2015).

Research design

This study used extracts of reed stolons which were treated with concentration levels with seven treatments and three replications with preliminary tests carried out first. The concentration of reed stolon extract: 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, and 0.5%.

Table 1. Experimental design with seven treatments and three replications

Concentration	0,2%	0,25%	0,3%	0,35%	0,4%	0,45%	0,5%
1	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10
Total larvae	30	30	30	30	30	30	30

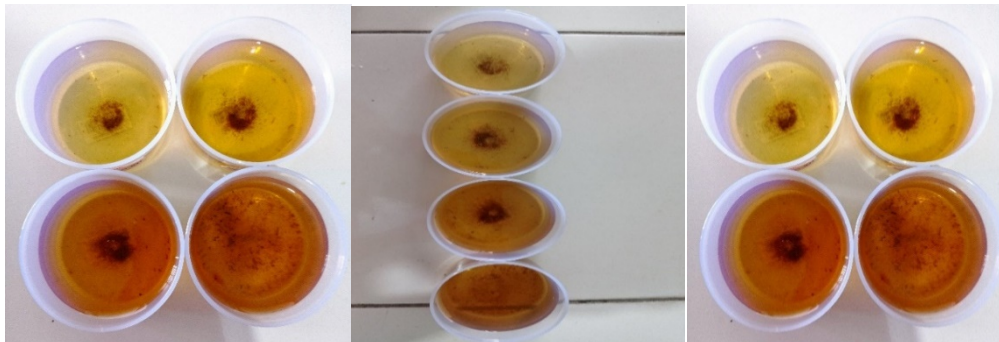


Figure 1. Mortality Test (personal documentation)

Testing on Larvae

The finished extract was tested for its activity on the *Aedes* sp. dengue fever mosquito. Third instar larval stage. Extract testing was carried out using a test solution with a concentration of 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, and 0.5% each in a pipette according to the concentration and put into a plastic cup added ten larvae of dengue fever *Aedes* sp. Each concentration used seven treatments, three repetitions and preliminary tests. Then observed at time 12-72 (JSA).

Observation

Observations were made on the number of dead larvae at each 12, 24, 36, 48, 60, and 72 (JSA) concentration of alang-alang stolon extract.

Data analysis

Mortality data of *Aedes* sp. dengue mosquito larvae. After the application is calculated using the formula for calculating the percentage of larval death (Abbott, 1925) as follows:

$$P = \frac{Po - Pc}{100 - Pc} \times 100\%$$

information: P = Corrected mortality rate (%)

Po = Mortality rate (%) in the treatment group

Pc = Mortality rate (%) in the control group

Observations were made every (JSA).

Probit analysis data by calculating the LC50 value using Microsoft Excel 2016.

RESULTS AND DISCUSSION

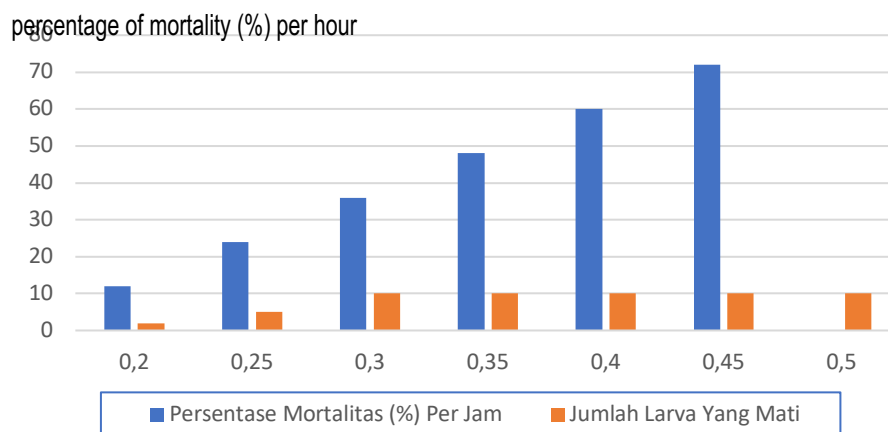
Mortality indicates the toxicity of *Imperata cylindrica* stolon extracts against *Aedes* sp. dengue mosquito larvae. The larvicidal activity was observed every 12-72 hours after inserting the larvae into the plastic cup, and the percentage of mortality of *Aedes* sp. dengue mosquito larvae. Instar III was applied with stolon extract of *I. cylindrica* using 96% ethanol solvent, which was observed for 12, 24, 36, 48, 60, and 72 JSA (hours after application).

Table 2. The average number of deaths of *Aedes sp.* dengue mosquito larvae. at concentration data of 0.2-0.5% at 12-72 hours after treatment

Concentration (%)	Amount Larvae	Percentage Mortality % Per Hour						Number of Dead Larvae (%)
		12	24	36	48	60	72	
0,2	10	0	0	0	0	0	20	20
0,25	10	0	0	0	0	20	50	50
0,3	10	0	0	20	50	100		100
0,35	10	0	0	40	90	100		100
0,4	10	10	30	60	100			100
0,45	10	10	60	100				100
0,5	10	20	60	100				100

Stolon extract of *Imperata cylindrica* L. can cause death in *Aedes sp.* dengue mosquito larvae. From the lowest concentration of 0.25% to the highest concentration of 0.5%. The length of treatment time also determines the percentage value of mortality from *Aedes sp.* dengue mosquito larvae. Instar III, which can be seen in table 2.

The concentration of 0.2% at 12-60 hours after application, there was no death of larvae applied with reed stolon extract. At 72 hours after application, the mortality is 20%. At a concentration of 0.25%, within 12-48 hours after application, the larvae had no death. At 60-72 hours after application, mortality is 20%-50%. At a concentration of 0.3%, within 12-24 hours after application, there was no death of the larvae. At 36, 48, and 60 hours after application, the mortality was 20%, 50%, and 100%. At a concentration of 0.35%, within 12-24 hours after application, the larvae had no death. At 36, 48, and 60 hours after application, the mortality was 40%, 90%, and 100%. At a concentration of 0.4%, there was an increase in the average presentation of mortality at 12, 24, 36, and 48 hours after successive application of 10%, 30%, 60%, and 100%. Likewise, concentrations experienced an increase in the average presentation of mortality of *Aedes sp.* dengue mosquito larvae. Third instar at 0.45% concentration at 12, 34, and 36 hours after successive application mortality of 10%, 60%, and 100%. At a concentration of 0.5% at 12, 24, and 36 hours after application, the mortality rate was 20%, 60%, and 100%, respectively.

**Figure 2.** Diagram of probit analysis of the percentage of mortality (%) per hour and the number of dead larvae in the probit analysis data

The potential of stolon extract of *I. cylindrica* L. stolons in killing *Aedes* sp. dengue mosquito larvae. At various concentrations within 12-72 hours, there was an increase in the average mortality as the concentration increased. The higher the concentration applied, the higher the average mortality of *Aedes* sp. dengue mosquito larvae.

The potency of various concentrations of alang-alang stolon extract in killing *Aedes* sp. dengue mosquito larvae. There was an increase in the average mortality. The higher the concentration and treatment time applied, the higher the average mortality of *Aedes* sp. dengue mosquito larvae. (Prabowo, 2010). In Figure 2, it can be seen that an increase in concentration indicates a toxic compound that enters the body of the test larvae resulting in disrupted larval life and causing mortality. The death of the larvae when treated with concentrations of stolon extract from reeds, there was a change in activity where when the larvae were about to die, there was movement up and down on the surface. The death of the larvae if the larvae do not move and do not respond and float above the surface indicates that the larvae are dead.

Extract of reed stolons is effective against *Aedes* sp. dengue mosquito larvae. Because the content of compounds and solvents used is very influential on mortality, it works to produce pure extracts for use in killing larvae. Kardina (2011) stated that vegetable pesticides contain active ingredients (single active ingredient) and (multiple active ingredients). This study used ethanol to produce pure particles from extracts of reed stolons (Arianti, 2012). The solvent chosen in the extraction process results in high effectiveness by observing the solvent compounds present in the natural product. Ethanol can dissolve all secondary metabolites (Harborne, 1987).

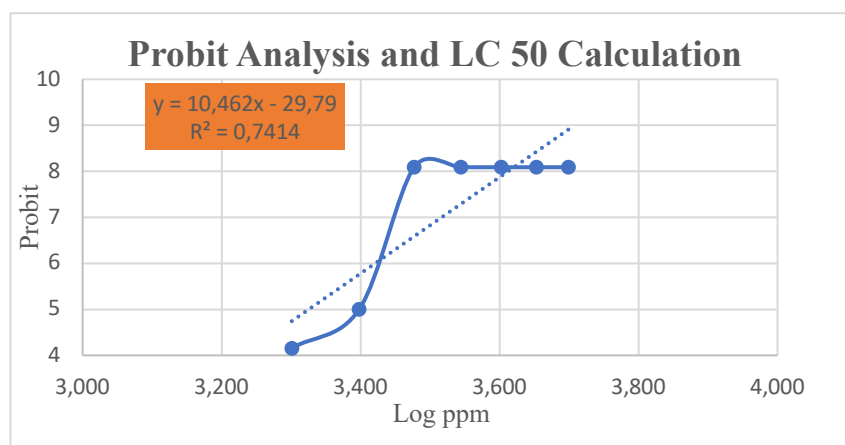


Figure 3. Graph of probit analysis calculations

The stolon extract of alang-alang proved to be effective against the mortality of *Aedes* sp. dengue mosquito larvae. The results of the phytochemical tests showed that the extract of alang-alang stolons could be used as a vegetable insecticide containing alkaloid compounds and triterpenoids (Arianti, 2012). Using chemical larvicides results in losses such as environmental pollution, death of other non-target animals, and insect resistance.

CONCLUSION

Stolon extract of *Imperata cylindrica* L. is a potential source of insecticide to control *Aedes* sp. dengue mosquito larvae. The stolon extract of *I. cylindrica* L. stolons proved effective in causing *Aedes* sp. dengue mosquito larvae's death, starting from a 0.25-0.5% concentration. The highest mortality rate was obtained in treatment with a concentration of 0.3-0.5%, killing 100% of the *Aedes* sp. dengue mosquito larvae. Concentrations of 0.3-0.35% can cause 100% mortality of larvae at 60 hours after application (JSA), and concentrations of 0.4% can cause 100% mortality at 48 (JSA). A concentration of 0.45-0.5% causes 100% death of larvae at 36 (JSA). Probit analysis showed that the LC50 was 0.2116% (2115.5 ppm) in the toxic category (500-5000 ppm).

REFERENCES

- Abbot. (1925). *Penggunaan Analisis Probit Untuk Pendugaan Tingkat Kepekaan Populasi*. (<https://www.litbang.pertanian.go.id/warta-ip/pdf-file/abdinegara-12.pdf>)
- Kemenkes. (2020). *Data kasus terbaru DBD di Indonesia*. Kemenkes RI. <https://www.kemkes.go.id/article/view/2012030000/data-kasus-terbaru-dbd-di-indonesia.html>.
- Cahyati, N. (2018). *Pengaruh Ekstrak Alang-Alang (Imperata cylindrical L.) terhadap Pertumbuhan Tanaman Gulma Ageratum conyzoides L.* Skripsi. Fakultas Tarbiyah dan Keguruan. Universitas Islam Negeri Raden Fatah.
- Dian, R. (2004). *Jumlah dan Daya Tetas Telur, serta Perkembangan Pradewasa Aedes aegypti di Laboratorium*. (Skripsi). Bogor: Institut Pertanian Bogor.
- Focks, DA. (2003). *A Review of Entomological Sampling Methods and Indicators for Dengue Vectors*. Geneva: WHO special Programme for Research and Training in Tropical Disease.
- Hadi, UK; S, Soviana. 2000. *Ektoparasit: Pengenalan, Diagnosis dan Pengendaliannya*. Bogor: *Laboratorium Entomologi Bagian Parasitologi dan Patologi FKH-IPB*.
- Hutapea, J.R. (1994). *Inventaris Tanaman Obat Indonesia III*. Badan Penelitian dan Pengembangan Kesehatan, Jakarta.
- Kasmara, H. (2004). *Toksisitas dan Daya Hambat Ekstrak Biji Mimba (Azadirachta indica A. juss) Terhadap Larva Nyamuk Aedes aegypti L.* *Jurnal biotika*, Vol. 3, No. 2.
- Sastriawan. (2015). *Structure of Mangrove Community and It's Relation to Macroepifauna Abudance in Intertidal Area of Rupert Straits Riau Province*. *Jurnal Online Mahasiswa*. Vol 2(2).