

POTENTIAL OF BAY LEAF (*SYZYGium POLYANTHUM*) EXTRACT AS BIOINSECTICIDE AGAINST *Aedes Aegypti*

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ABSTRACT

Potential of Bay Leaf (*Syzygium polyanthum*) Extract as Bioinsecticide Against *Aedes aegypti*. Pesticides contribute to environmental pollution, health problems, and resistance. Bioinsecticides offer an alternative solution. This study evaluated the potential of bay leaf juice (*Syzygium polyanthum*) as a bioinsecticide in the 2024 efficacy test against *Aedes aegypti* mosquitoes. This research is pseudo-experimental, with a post-test only control group design. The object of this study was female *Aedes aegypti* mosquitoes aged 2–5 days. This study used replication 5 times. The variables of this study were the variation of bay leaf juice 12%, 17%, 22%, 27%, 32%, and the number of deaths of *Aedes aegypti* mosquitoes after one hour. Data were analysed using One Way Anova. Bay leaf juice has the potential to kill *Aedes aegypti* mosquitoes. Variations in the concentration of bay leaf juice caused significant differences in the mortality of *Aedes aegypti* mosquitoes. The concentration of bay leaf juice at 31.136% is LC50, and the concentration of bay leaf juice at 52.803% is LC95. Other researchers can test the active ingredients of bay leaf juice that have the highest effect on killing *Aedes aegypti* mosquitoes.

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INTRODUCTION

Pesticides can cause adverse effects, such as environmental contamination, health disorders in humans, and resistance. Environmental degradation refers to soil pollution caused by pesticides, which in turn reduces soil quality and its capacity to support plant nutrition [1]. Chronic illnesses resulting from pesticide exposure include cancer, neurological disorders, and respiratory complications [2]. Resistance is a condition where vectors enhance their immune system against frequently used insecticides. Resistance in mosquitoes has been demonstrated in studies showing that *Aedes aegypti* mosquitoes in Ngawonggo, Kajen, and Meger sub-districts of Klaten Regency were resistant to cypermethrin [3].

Dengue Hemorrhagic Fever (DHF) in Indonesia remains a significant vector-borne disease and has yet to be fully addressed. The number of DHF cases in Indonesia continues to rise—from 73,518 cases in 2021 to 143,184 cases in 2022—with 35,694 cases and 270 deaths recorded by May 2023. Prevention is essential to reduce the population of *Aedes aegypti* mosquitoes [4]. Mosquito vector control methods include physical or mechanical, chemical, and biological approaches. While insecticide use can yield rapid and convenient results, excessive application can have negative consequences for both the environment and human health [4]. Improper usage may also lead to insect resistance to insecticides [5]. According to

a statement by the World Health Organization cited in a study by Haidah et al., chemical insecticides cause stress in mosquitoes, prompting them to adapt for survival—even under varying dosage conditions [5]. An alternative approach involves the use of natural insecticides, or bioinsecticides.

Bioinsecticides are derived from natural ingredients, such as plants. Bay leaf (*Syzygium polyanthum*) is one such plant, containing active compounds like flavonoids, tannins, eugenol, citric acid, carbohydrates, steroids, alkaloids, triterpenoids, and essential oils. Bay leaves contain tannins (21.7%), flavonoids (0.4%), and essential oils (0.05%) [7]. Saponins, flavonoids, tannins, and alkaloids are the active ingredients in bay leaves that make them effective as bioinsecticides.

A study by Chandra Bagaskara Putra and Ngadino (2022) [8] using bay leaf (*Syzygium polyanthum*) filtrate on houseflies (*Musca domestica*) showed an LC50 concentration of 9.64%. Another study using powdered bay leaves as a cockroach repellent showed a repellency rate of 91.6% at a concentration of 10 grams, with an average of 9.1 cockroaches [9]. A study by Umami and Ahsanunnisa (2019) using bay leaf (*Syzygium polyanthum*) extract as a bioinsecticide for *Aedes aegypti* mosquitoes found that a concentration of 0.8% resulted in the highest mosquito mortality, with an average of 95%. Research by Haidina Ali and Mulyati (2021) using bay leaf extract as a larvicide for *Aedes aegypti* larvae showed that a 5% concentration was most effective, with a larval mortality rate of 89.6%.

Excessive use of chemical pesticides can pollute the environment, trigger chronic diseases in humans, and lead to resistance in disease vectors. The continuous rise in Dengue Hemorrhagic Fever (DHF) cases in Indonesia highlights the need for safer control of *Aedes aegypti* mosquitoes. Natural-based bioinsecticides such as bay leaf (*Syzygium polyanthum*) offer a promising alternative due to their active compounds (flavonoids, tannins, saponins, and alkaloids) that are effective in killing insects. Several previous studies have demonstrated the efficacy of bay leaves against various insect species, including mosquitoes. Based on preliminary tests, different concentrations of bay leaf extract showed varying levels of effectiveness in killing *Aedes aegypti* mosquitoes. Therefore, this study aims to analyze the potential of bay leaf extract as an eco-friendly bioinsecticide in the efficacy test against *Aedes aegypti* mosquitoes in 2024.

MATERIALS AND RESEARCH METHODS

This study employed a quasi-experimental design using a post-test-only control group approach. The research was conducted from November 2023 to April 2024 at the Entomology Laboratory of the East Java Provincial Health Office. The test subjects were female *Aedes aegypti* mosquitoes aged 2 to 5 days [12]. A total of 25 mosquitoes were used in each treatment unit. Each concentration of bay leaf extract was tested with five replications.

The variables of the study were as follows:

Independent variable: variations in the concentration of bay leaf extract (12%, 17%, 22%, 27%, and 32%), Dependent variable: the number of *Aedes aegypti* mosquito deaths within 1 hour, Controlled variables: temperature and humidity of the testing chamber

Study procedures included:

Preparation of Bay Leaf Extract, Wash the prepared bay leaves, Weigh the bay leaves according to the desired concentration, using the following formula:

$$\text{Concentration (\%)} = \frac{X}{V} \times 100\%$$

Grind the bay leaves using distilled water (aquadest). Strain the blended mixture using gauze and transfer the liquid into a spray bottle.

Experimental Procedure

Prepare a test cage made of glass measuring 60 cm × 60 cm × 60 cm, Use a hygrometer to measure the temperature and humidity of the testing room, Collect 25 mosquitoes using an aspirator, then place them into the test cages labeled with 12%, 17%, 22%, 27%, and 32% concentrations, Use a sprayer filled with the bay leaf extract and spray it into the mosquito test cage, Observe and record the number of mosquitoes that fall (paralyzed or dead) every minute until the 10th minute after spraying, Continue observing and recording the fallen mosquitoes (paralyzed or dead) every 10 minutes until the 60th minute. Observe the mosquito mortality at the second and fourth hours, Collect both live and dead mosquitoes from the test cage into a container covered with gauze and provide moist cotton soaked in sugar solution, Conduct final observations and record the mosquito mortality rate 24 hours after the bioinsecticide application.

This study employed a quasi-experimental method with a post-test only control group design. The research was conducted at the Entomology Laboratory of the East Java Provincial Health Office from November 2023 to April 2024. The study subjects were female *Aedes aegypti* mosquitoes aged 2 to 5 days, with 25 mosquitoes per treatment unit and five replications. The independent variable was the concentration of bay leaf extract (12%, 17%, 22%, 27%, 32%), the dependent variable was the number of mosquito deaths after 1 hour, while the controlled variables included room temperature and humidity. The bay leaf extract was prepared by washing the leaves, weighing them according to the required concentration, grinding them using distilled water, and filtering the mixture to obtain a solution, which was then transferred into a spray bottle. The testing was carried out by spraying the solution into mosquito cages, followed by observation of mosquito mortality every minute up to the 10th minute, every 10 minutes up to the 60th minute, and again at the second, fourth, and 24th hours post-treatment. The mortality data were analyzed using One-Way ANOVA, followed by Post-Hoc LSD test, and Probit analysis to determine the LC50 and LC95 values.

RESEARCH RESULTS AND DISCUSSION

The application of **bay leaf extract (*Syzygium polyanthum*)** as a bioinsecticide showed mosquito mortality rates of 12%, 17%, 22%, 27%, and 32% within one hour of exposure. The data are presented in the following table:

Table 1. Percentage of *Aedes aegypti* Mosquito Mortality Due to Bay Leaf Extract (*Syzygium polyanthum*) as a Bioinsecticide at the 1st Hour

Concentration	Number of Mosquito Deaths					Number of Test Mosquitoes	Averange	Percentage (%)
	I	II	III	IV	V			
Control (-)	0	0	0	0	0	25	0	0
12%	2	3	1	1	2	25	1,8	7,2
17%	4	4	3	4	5	25	4	16
22%	5	6	6	7	6	25	6	24
27%						25	8,2	32,8
32%						25	14	56

Source: Primary Data, 2024

The test results showed that increasing the concentration of bay leaf extract led to a higher mortality rate of *Aedes aegypti* mosquitoes. After 1 hour of observation, the average mortality rates at concentrations of 12%, 17%, 22%, 27%, and 32% were 7.2%, 16%, 24%, 32.8%, and 56%, respectively, while the negative control showed no mortality. This study also measured temperature and humidity as control variables to determine whether mosquito mortality was influenced by environmental conditions in the test chamber. The data on temperature and humidity measurements are presented in the following table:

Table 2. Results of Temperature and Humidity Measurements in the Test Chamber

Measurement	Optimum Temperatur	Room Temperature	Optimum Humidity	Room Humidity
I	25-30°C	28°C	70-90%	73%
I	25-30°C	28°C	70-90%	73%
II	25-30°C	28°C	70-90%	73%
IV	25-30°C	28°C	70-90%	73%
V	25-30°C	28°C	70-90%	73%
Average		28		73

Source: Primary Data, 2024

Table 2 shows that the average room temperature in the Entomology Laboratory of the East Java Provincial Health Office was 28°C. This temperature can still be categorized as the optimum temperature for the growth and development of mosquitoes, which ranges between 25–30°C. The average room humidity in the laboratory was 73%, which also falls within the optimum humidity range for mosquito growth and development, typically between 70–90%.

The data on the number of *Aedes aegypti* mosquito deaths caused by bay leaf (*Syzygium polyanthum*) extract were then analyzed using One-Way ANOVA. The analysis was conducted to determine whether there were significant differences in mosquito mortality rates across the different concentrations of bay leaf extract. The results are presented in the following table:

Table 3. One-Way ANOVA Results on the Number of *Aedes aegypti* Mosquito Deaths at Each Concentration of Bay Leaf Extract

Note	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	436.400	4	109.100	111.327	.000
Within Groups	19.600	20	.980		
Total	456.000	24			

Source: Primary Data, 2024

Based on Table 3, the statistical analysis using One-Way ANOVA yielded a significance value of $p = 0.000$ (< 0.05), indicating that there were significant differences in mosquito mortality across the different concentrations of the extract.

Table 4. Post Hoc Test Results on *Aedes aegypti* Mortality at Each Concentration of Bay Leaf (*Syzygium polyanthum*) Extract

Concentration Difference Test	Sig
12%	.002
	.000
	.000
	.000
17%	.005
	.000
	.000
22%	.002
	.000
27%	.000

Source: Primary Data, 2024

Table 4 shows that the significance value from the Post Hoc LSD test method must not exceed 0.05. Therefore, the significance value meets the criteria for rejecting H_0 if $p < \alpha$ (0.05). The LSD Post Hoc test confirms that each concentration difference has a significant effect on mosquito mortality.

The next step is to perform a Probit analysis to determine the concentration of bay leaf juice that can cause 50% and 95% mortality in test organisms. The data are presented in the following table:

Table 5. LC₅₀ and LC₉₅ Values of Bay Leaf (*Syzygium polyanthum*) Extract on *Aedes aegypti* Mortality

Lethal Concentration	Estimate	Lower Bound	Upper Bound
LC ₅₀	31,136	29,240	
LC ₉₅	52,803	47,395	

Source: Primary Data, 2024

Based on Table 5 of the Probit analysis, the LC₅₀ concentration was determined at 31.136% and LC₉₅ at 52.803%, indicating that a concentration higher than 32% is still required to achieve optimal mortality rates.

Bay leaf extract (*Syzygium polyanthum*) exhibits potential as a bioinsecticide. This is evidenced by the mortality of *Aedes aegypti* mosquitoes in response to varying concentrations of the extract. Mortality occurred due to direct contact between the mosquitoes and the active compounds within the aerosolized particles of the sprayed extract. The alkaloid content in bioinsecticides can be used to control and even eliminate insect populations in the environment [6]. Plant-based bioinsecticides are derived from organs such as roots, leaves, and stems. Active compounds with bioinsecticidal potential include flavonoids, tannins, steroids, alkaloids, and terpenoids [7]. The results of this study confirm that bay leaf extract has bioinsecticidal properties, with mosquito mortality increasing proportionally with concentration.

The mortality of *Aedes aegypti* mosquitoes can also be influenced by the testing environment. The glass chamber restricts mosquito flight, increasing contact between the test mosquitoes and the aerosolized particles. It also limits the escape of aerosol particles, especially smaller ones that remain suspended in the air longer [8]. The mode of action of this natural insecticide stems from active compounds such as flavonoids, tannins, saponins, alkaloids, and essential oils, which can disrupt the respiratory, digestive, and nervous systems of insects, ultimately causing death. These findings align with previous studies demonstrating the insecticidal effectiveness of bay leaf against various insect species.

Environmental variables such as temperature and humidity can interfere with experimental outcomes and were therefore measured in the test room. *Aedes aegypti* mosquitoes may die due to inappropriate temperature and humidity levels [9]. The optimal temperature for mosquito development is between 25–30°C. Temperatures above 40°C can lead to adult mosquito death and developmental failure in eggs and larvae. At extreme temperatures below 11°C and above 36°C, *Aedes aegypti* mosquitoes struggle to survive. Female *Aedes aegypti* mosquitoes are unable to blood-feed at temperatures of 14–15°C and will die within 2–3 days without a blood meal [10]. These mosquitoes can fly at 15–32°C, with an optimum of 21°C. The gonotrophic cycle shortens with increasing temperature [11]. The optimum humidity range for mosquito development is 70–90% [12]. Mosquito lifespan is affected by humidity; they can survive at a minimum of 60% humidity. Below this threshold, their life expectancy decreases. Mosquitoes are ineffective as vectors when virus transfer from the gut to the salivary glands is impaired. A humidity level of 80–85% supports adult mosquito growth and egg production [13]. Increased humidity and temperature fluctuations affect mosquito breeding, adult survival and reproduction, parasite development, and human-mosquito contact duration [14].

One-Way ANOVA was used to assess differences in *Aedes aegypti* mortality across extract concentration variations. This test determines whether the various concentrations of *Syzygium polyanthum* extract result in statistically different mosquito mortality rates within a 1-hour period. According to Table 3, One-Way ANOVA confirmed significant differences in mortality between concentration groups. Post-Hoc analysis was conducted to determine which specific concentrations differed significantly. The Least Significant Difference (LSD) test was chosen to assess all pairwise comparisons between group means [21]. As presented in Table 4, the Post-Hoc test results indicate significant differences in mosquito mortality among the various extract concentrations. This confirms that the number of *Aedes aegypti*

deaths was influenced by the concentration level used—the higher the concentration, the greater the mortality observed. This finding is supported by Sidauruk et al. (2020) [15], who stated that higher chemical concentrations contain greater amounts of active substances, thereby increasing insecticidal potency. Statistical analysis further confirms significant differences in mosquito mortality at each concentration level, with LC₅₀ and LC₉₅ values of 31.136% and 52.803%, respectively. These results indicate that even the highest concentration tested in this study did not achieve full effectiveness (95% mortality).

Flavonoids used as bioinsecticides can act as respiratory inhibitors or toxins by disrupting the respiratory muscles and nerves of *Aedes aegypti* mosquitoes [23]. Alkaloids, found in plant parts such as leaves, branches, flowers, seeds, bark, and roots [24], act as stomach poisons for insects, impairing their digestive tracts and inhibiting gustatory receptors in the mouthparts [25]. Saponins function as respiratory and contact poisons. Exposure to saponins can damage mosquito cuticle mucosa, leading to blood hemolysis, enzyme disruption, and ultimately death [26]. This is corroborated by Kurniawan et al. (2021) [27], who reported that saponins act as contact toxins that damage insect cell membranes. Tannins protect certain woody plants by impairing the insect digestive process [16]. In insect digestive systems, tannins bind to growth-related proteins and reduce feeding due to their bitter taste [17]. Steroids are active compounds used in pest control due to their bioactivity, including antifeedant, antiparasitic, and toxic properties that suppress feeding, digestion, and metabolism [18]. Essential oils from various plants have demonstrated toxicity against *Aedes aegypti*. The volatile bioactive compounds in these oils interact with mosquito odor receptors (ORNs), and inhalation can lead to neurotoxic effects resulting in death [19].

The LC₅₀ and LC₉₅ values were estimated using Probit analysis. In this study, the concentrations of *Syzygium polyanthum* extract capable of killing 50% and 95% of *Aedes aegypti* mosquitoes within 1 hour were 31.136% and 52.803%, respectively. A pesticide is considered effective if it causes at least 80% mortality among test organisms [20]. Based on this criterion, none of the five tested concentrations of bay leaf extract reached the threshold required to effectively eliminate the mosquito population. The highest concentration used was 32%, which did not achieve LC₉₅. Toxicity tests should ideally induce less than 95% mortality to ensure the test concentrations are within acceptable survival ranges [21]. Thus, this study does not require a repetition of the toxicity test, as mosquito mortality did not exceed 95%. Overall, bay leaf extract demonstrates potential as an environmentally friendly bioinsecticide for controlling dengue vector mosquitoes. However, further investigation into higher concentrations or optimized formulations of the active compounds is necessary to develop an effective and widely applicable product.

CONCLUSIONS AND RECOMMENDATIONS

Bay leaf extract (*Syzygium polyanthum*) shows potential as a bioinsecticide against *Aedes aegypti* mosquitoes. The percentage of mosquito mortality after 1 hour of exposure to extract concentrations of 12%, 17%, 22%, 27%, and 32% was 7.2%, 16%, 24%, 32.8%, and 56%, respectively. Significant differences in *Aedes aegypti* mortality were observed across the tested concentration levels. The LC₅₀ and LC₉₅ values at 1-hour exposure were 31.136% and 52.803%, respectively.

Higher extract concentrations are needed to determine the effective dose required to kill at least 80% of *Aedes aegypti* mosquitoes. A dosage of 31.136 grams of bay leaf extract may be used as a bioinsecticide to help reduce dengue transmission in the community. This study demonstrates that bay leaf extract has environmentally friendly bioinsecticidal potential for controlling *Aedes aegypti*. Although mortality increases with concentration, the current formulation has not yet achieved optimal effectiveness. Therefore, further research is recommended to explore the active compounds in bay leaf, as well as to develop more effective concentrations and application methods for broad-scale community use in dengue prevention.

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