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Mortality and Morphological Changes in *Aedes aegypti* Larvae by *Bacillus thuringiensis* and *Serratia marcescens*

Mortalitas dan Perubahan Morfologi Larva *Aedes aegypti* oleh *Bacillus thuringiensis* dan *Serratia marcescens*

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Abstrak

Penggunaan biolarvasida merupakan salah satu alternatif sebagai agensia hayati pengganti insektisida sintetik, yaitu bakteri entomopatogen. Tujuan penelitian ini untuk mengetahui potensi ekstrak *Bacillus thuringiensis* dan *Serratia marcescens* terhadap mortalitas dan perubahan morfologi larva *Aedes aegypti*. Penelitian ini merupakan penelitian eksperimen 2 faktor dengan menggunakan Rancangan Acak Kelompok (RAK). Faktor pertama, jenis bakteri entomopatogen yaitu *B. thuringiensis* dan *S. marcescens*. Faktor kedua, konsentrasi ekstrak bakteri (60 mL, 70 mL, 80 mL), kontrol negatif (aquadest steril). Data mortalitas diolah dengan uji ANOVA pada taraf 5%. Hasil penelitian menunjukkan bahwa pemberian ekstrak *B. thuringiensis* dan *S. marcescens* menyebabkan mortalitas serta memberikan perubahan morfologi larva *Ae. aegypti*. *B. thuringiensis* pada konsentrasi 60 mL, 70 mL, 80 mL menyebabkan persentase mortalitas larva 100%, sedangkan *S. marcescens* pada konsentrasi 60 mL sebesar 70%, konsentrasi 70 mL sebesar 53%, dan konsentrasi 80 mL sebesar 50%. Perubahan morfologi larva ditunjukkan dengan perubahan warna, tekstur, dan kerusakan sistem pencernaan. Disimpulkan bahwa *B. thuringiensis* memiliki potensi paling tinggi sebagai agen entomopatogen dibandingkan *S. marcescens* dalam menyebabkan mortalitas dan perubahan morfologi larva *Ae. aegypti.*

Kata kunci: *B. thuringiensis, S. marcescens,* bakteri entomopatogen, larva *Ae. aegypti*

Abstract

The use of biolarvicides is an alternative biological agent to replace synthetic insecticides, namely entomopathogenic bacteria. The purpose of this study was to determine the potential of Bacillus thuringiensis and Serratia marcescens extracts on mortality and morphological changes in Aedes aegypti larvae. This study was a 2-factor experimental study using a Randomized Block Design (RBD). The first factor, the type of entomopathogenic bacteria, namely B. thuringiensis and S. marcescens. The second factor, the concentration of bacterial extracts (60 mL, 70 mL, 80 mL), negative control (sterile distilled water). Mortality data were processed using ANOVA at the 5% level. The results showed that the administration of B. thuringiensis and S. marcescens extracts caused mortality and morphological changes in Ae. aegypti larvae. B. thuringiensis at concentrations of 60 mL, 70 mL, and 80 mL caused 100% larval mortality, while S. marcescens at concentrations of 60 mL resulted in 70%, 53%, and 50% at concentrations of 70 mL and 80 mL, respectively. Morphological changes in larvae were indicated by changes in color, texture, and damage to the digestive system. It was concluded that B. thuringiensis has the highest potential as an entomopathogenic agent compared to S. marcescens in causing mortality and morphological changes in Ae. aegypti larvae.

Keywords: *B. thuringiensis, S. marcescens, entomopathogenic bacteria, Ae. aegypti larvae*

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INTRODUCTION

The World Health Organization (WHO) estimates that the global risk of dengue fever is increasing every year. The dengue virus is primarily transmitted through the bite of the *Aedes aegypti* mosquito [1]. Indonesia is one of the countries in Southeast Asia with a high number of dengue fever cases [2]. To reduce the number of cases of this disease, efforts must be made to prevent the transmission of dengue fever.

Efforts to control dengue fever include eradicating mosquito breeding sites through the 3-methods (draining, covering, and recycling) as well as the Plus method (installing wire mesh screens, using mosquito nets while sleeping, and not hanging clothes inside the house). The 3-methods Plus mosquito breeding site control method involves draining water storage containers at least once a week, tightly covering water containers, and recycling items that could serve as breeding grounds for mosquitoes that transmit dengue fever [3]. In addition, many chemical control measures have been implemented, including fogging, applying Abate, and using synthetic larvicides and insecticides. However, the continuous use of chemical insecticides can cause mosquitoes to become resistant and have other adverse effects, such as leaving behind harmful residues and disrupting the environmental balance by killing beneficial insects, including pets [4].

Entomopathogenic bacteria are microorganisms with great potential as a source of toxins and metabolites for pest control. Using entomopathogenic bacteria as pest control agents has several advantages: they are environmentally friendly and harmless to humans and non-target species [5]. These bacteria infect insects through their digestive systems [6]. Entomopathogenic agents cause rapid mortality in hosts within less than 48 hours [7].

Although prevention through vector control programs, such as mechanical and chemical eradication, has been widely implemented, it has not yielded optimal results. There has been little research directly comparing the potential of *B. thuringiensis* and *S. marcescens* in causing mortality and morphological changes in *Ae. aegypti* larvae. One environmentally friendly alternative for controlling pests is the use of entomopathogenic bacteria as biological agents. We used *B. thuringiensis* and *S. marcescens* to determine their potential to cause mortality in *Ae. aegypti* larvae. We also examined the potential of entomopathogenic bacteria to induce morphological changes in larvae, thereby determining the extent of damage caused by the bacteria's mechanism of action. Previous research on *B. thuringiensis* [8] examined its effect on *Ae. aegypti* larval mortality through LC50 and LC90 values within 24 hours. [9]

examined the use of *S. marcescens* to determine the mortality of *Ae. aegypti* larvae.

METHODS

This research was conducted from February to April of 2025. The bacterial suspension was prepared in the Microbiology Laboratory, and the potency test was conducted in the Zoology Laboratory in the Department of Biology at the Faculty of Mathematics and Natural Sciences at the University of Lampung.

This study used the following tools: subculture tubes, glass beakers, measuring cups, erlenmeyer flasks, stirring rods, volumetric pipettes, rubber bulbs, bacterial incubators, autoclaves, analytical balances, hot plate stirrers, binocular microscopes, digital microscopes, brushes, tissues, thin-walled plastic boxes for hatching and testing larvae, label paper for labeling each treatment, and writing instruments. The materials used were Nutrient Agar (NA) medium, Nutrient Broth (NB) medium, distilled water, 70% alcohol, and *Ae. aegypti* eggs, larval food (Takari), and well water.

Research Procedure

Preparation of Test Animals

Ae. aegypti eggs were obtained from the Baturaja Public Health Laboratory in South Sumatra. For the initial preparation, thin-walled plastic boxes were set up to serve as incubation chambers for the eggs. The boxes were filled with healthy water, and then the eggs were placed inside. The eggs were left for four to five days until they became third-instar larvae. The larvae were fed Takari fish pellets at a dose of 0.22% (w/v) [10] once a day in the corners of the container [11].

Bacterial Isolate Rejuvenation

Pure isolates of *B. thuringiensis* and *S. marcescens* were obtained from the Microbiology Laboratory in the Department of Biology at the Faculty of Mathematics and Natural Sciences at the University of Lampung. The isolates were revived on slanted NA media using a single bacterial loop streak. The bacteria were then incubated at 37°C for 24 hours [12].

Preparation of Bacterial Stock Solution

A bacterial isolate was collected with a 1-inch loop and inoculated into 10 mL of NB medium as a starter culture. The culture was then incubated in a shaker incubator for 24 hours. Then, the starter culture was inoculated into 90 mL of NB medium, maintaining the same inoculum volume for each treatment to ensure consistent initial bacterial density. The cultures were incubated again for 72 hours at the optimal growth temperature of each active, homogeneous bacterium before use in pathogenicity tests on *Ae. aegypti* larvae [13].

Testing the Potential of Bacterial Extracts

The bacterial stock solution, obtained from the bacterial culture, was concentrated by homogenizing the culture with sterile

distilled water. Each concentration was placed in a thin-walled box and tested for pathogenicity against third instar larvae. After homogenizing the stock solution with sterile distilled water, 10 larvae were placed in each treatment [8]. This process was repeated three times for each concentration.

Observation of Larval Mortality

The mortality rate and morphological changes in *Ae. aegypti* larvae were observed after the administration of *B. thuringiensis* and *S. marcescens*. The observations were made at 12, 24, 36, and 48 hours after administration in two stages:

a. Observe the mortality rate of *Ae. aegypti* larvae after administering an entomopathogenic bacterial extract suspension into thin-walled plastic boxes.
b. Observe morphological changes in *Ae. aegypti* larvae after administration, including texture, body shape, and body color.

The larvae were declared dead if they did not respond to stimulation, such as movement, when touched with a brush or stick. The percentage of larval mortality was then calculated using formula [14], as follows:

$$\text{Mortality Rate (\%)} = \frac{\text{number of dead larvae}}{\text{number of test larvae}} \times 100\%$$

RESULTS AND DISCUSSION

***Aedes aegypti* Larval Mortality**

Over a period of 48 hours, observations showed that *B. thuringiensis* and *S. marcescens* had the potential to cause mortality and morphological changes in *Ae. aegypti* larvae. Table 1 shows the percentage of larval mortality at a concentration of 60 mL.

Based on a concentration of 60 mL after administration of *B. thuringiensis* extract at 24 hours, the mortality rate was 100%. Furthermore, treatment with *S. marcescens* at 12 hours resulted in a mortality rate of 36%. At 36 hours, the mortality rate increased to 56%, and by 48 hours, it had risen to 70%.

Table 1. Percentage of larval mortality at a concentration of 60 mL

Types of Bacteria	Observation Time (Hours)	Larval Mortality (%)
Bacillus thuringiensis	12	0
	24	100
	36	100
	48	100
Serratia marcescens	12	36
	24	36
	36	56
	48	70

The percentage of larval mortality at a concentration of 70 mL is shown in Table 2.

Table 2. Percentage of larval mortality at a concentration of 70 mL

Types of Bacteria	Observation Time (Hours)	Larval Mortality (%)
Bacillus thuringiensis	12	0
	24	76
	36	100
	48	100
Serratia marcescens	12	16
	24	20
	36	33
	48	53

Based on a concentration of 70 mL after administering *B. thuringiensis* extract at 24 hours, the mortality rate was 76%, increasing to 100% at 36 hours. Furthermore, treatment with *S. marcescens* showed a mortality rate of 16% at 12 hours, which continued to increase to 20% at 24 hours. Then, at 36 hours, it increased to 33%, and at 48 hours to 53%. The percentage of larval mortality at a concentration of 80 mL is shown in Table 3.

Table 3. Percentage of larval mortality at a concentration of 80 mL

Types of Bacteria	Observation Time (Hours)	Larval Mortality (%)
Bacillus thuringiensis	12	0
	24	100
	36	100
	48	100
Serratia marcescens	12	50
	24	50
	36	50
	48	50

Based on a concentration of 80 mL, 100% mortality was observed after 24 hours following the administration of *B. thuringiensis* extract. Furthermore, the administration of 80 mL of *S. marcescens* extract resulted in 50% mortality after 12 hours, with no further increase observed within the subsequent 48 hours. These results suggest that the larvae were able to survive because they had the physiological ability to neutralize the toxin or had adapted to increase their resistance to infection.

The mortality rate may have been influenced by the fact that the larvae used in the study were in the third instar stage. At this stage, their digestive systems are fully developed, enabling the *B. thuringiensis* toxin to work effectively. A higher concentration of the *B. thuringiensis* suspension results in a higher mortality rate, and increasing the exposure time strengthens this effect [15]. The toxin ingested by the larvae reacts with their alkaline intestines, producing protease enzymes that break down protein crystals into smaller, non-toxic components. These proteins damage the abdomen and thorax of the larvae, disrupting the osmotic balance of their intestines and ultimately leading to death [16]. [15] Conducted a study on the pathogenicity of *B. thuringiensis* against *Ae. aegypti* larvae. The study found a 100% mortality rate at a concentration of 50 µL.

Bacterial infection by *S. marcescens*, an entomopathogenic bacterium, began 12 to 72 hours after application. Currently, the use of *S. marcescens* as a biological agent is not well understood for its ability to target specific pests [17]. *S. marcescens* is an insect pathogen that can cause bacteremia. Bacteremia is a condition in which bacteria are present in the bloodstream and can quickly spread to other parts of the body, potentially causing severe illness or death. *S. marcescens* produces various toxins and enzymes, including extracellular degradative enzymes such as protease, lipase, carbohydrase, and chitinase [5]. [9] studied the effects of *S. marcescens* extract on *Ae. aegypti* larval activity, observing a 1%

mortality rate at concentrations of 500 and 1000 ppm.

Data Analysis Results

ANOVA tests were conducted to determine the significant effects of the bacterial species and concentrations used in the study on larval mortality. The results of these tests are shown in Table 4.

Table 4. ANOVA Test Results on *Ae. aegypti* Larval Mortality

Treatment	F value	p value	Significance
Bacteria Type	14,019	0,003	Yes (p < 0,05)
Concentration	0,301	0,746	No (p > 0,05)
Type × Concentration	0,301	0,746	No (p > 0,05)

The ANOVA for suspension concentration was not significant (p = 0.746 > 0.05), indicating that suspension concentration did not significantly affect larval mortality. The ANOVA results for concentration also did not show a significant effect. It is possible that the toxin reached its effective threshold at low concentrations (60 mL), so increasing the concentration to 80 mL did not result in a significant increase in mortality. Additionally, the results of the interaction analysis between bacterial type and concentration were not significant (p = 0.746 > 0.05), indicating that the two factors did not interact to affect the mortality of *Ae. aegypti* larvae. Therefore, no further analysis was conducted.

Morphological Changes in *Ae. aegypti* Larvae

The morphology of *Ae. Aegypti* instar III larvae in the control (-) or untreated group are shown in Figure 1.

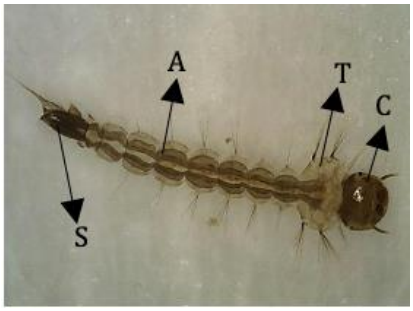

Figure 1. Morphology of control *Ae. aegypti* larvae (-)

Image description:

C = caput (head)

T = thorax (chest)

A = abdomen (stomach)

S = siphon

Morphological changes in *Ae. aegypti* larvae after application of *B. thuringiensis* extract, based on observation time at various concentrations, are shown in Figure 2.

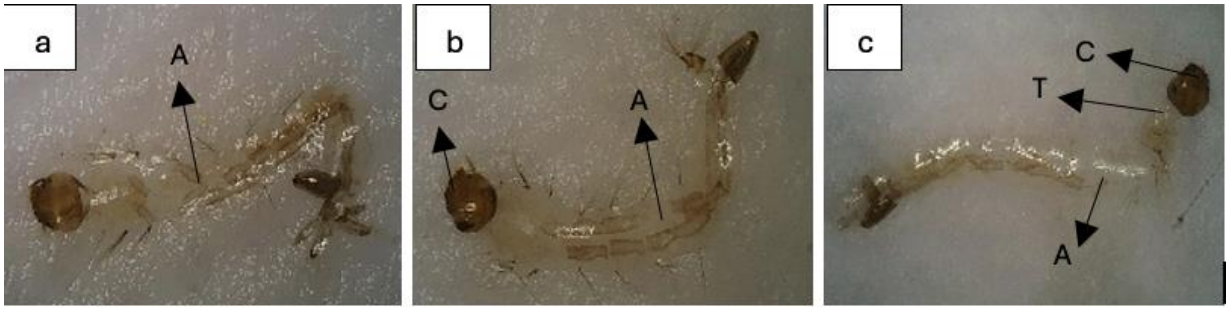

Figure 2. Morphological changes in *Ae. aegypti* larvae observed over 24 hours at various concentrations

Figure 2(a) shows morphological changes in larvae at a concentration of 60 mL, including a brittle texture, a milky white (pale) color from the thorax to the abdomen, and an incomplete intestinal tract. Figure 2(b) shows changes in larvae at a concentration of 70 mL: a brittle texture; a milky white (pale) color from the thorax to the abdomen; a damaged digestive tract (not intact); and one broken antenna. Figure 2(c) shows larvae at a concentration of 80 mL with a brittle texture, a milky white color from the thorax to the abdomen, a damaged digestive tract, both antennae broken off, and the head nearly detached.

Figure 3 shows morphological changes in *Ae. aegypti* larvae after the application of *B. thuringiensis* extract at various concentrations over time.

After 36 hours of observation, morphological changes in *Ae. aegypti* larvae at a concentration of 70 mL exhibited a fragile body texture, a milky white (pale) color extending from the thorax to the abdomen, and an incomplete intestinal tract.

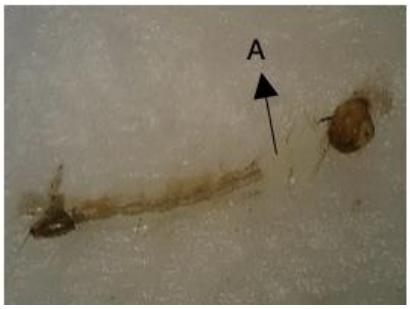

Figure 3. Morphological changes in *Ae. aegypti* larvae observed after 36 hours at a concentration of 70 mL

Figure 4 shows morphological changes in *Ae. aegypti* larvae after the administration of *S. marcescens* extract at various concentrations over time.

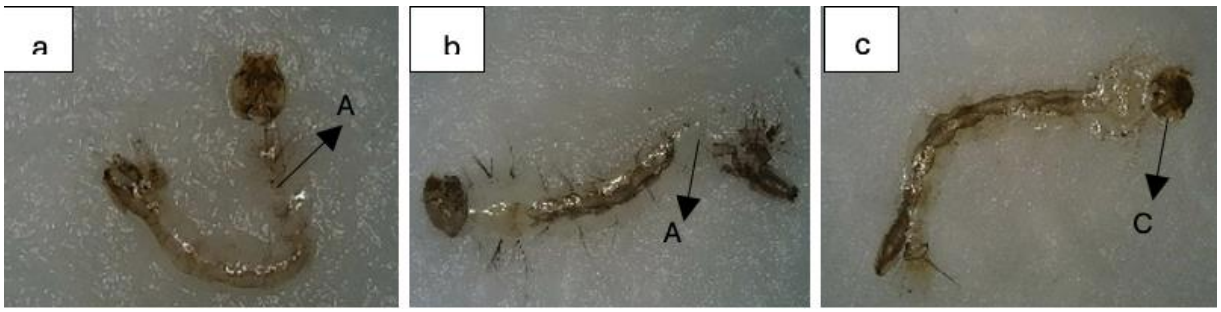

Figure 4. Morphological changes in *Ae. aegypti* larvae observed after 12 hours at various concentrations

Figure 4(a) shows morphological changes in larvae at a concentration of 60 mL, including a softer texture, a yellowish-white color from the thorax to the abdomen, and an incomplete intestinal tract. Figure 4(b) shows changes in larvae at a concentration of 70 mL: a softer texture and a damaged digestive tract. Figure 4(c) shows morphological changes in larvae at a concentration of 80 mL, a smooth texture, and one broken antenna.

Morphological changes in *Ae. aegypti* larvae after administration of *S. marcescens* extract, based on observation time at various concentrations, are shown in Figure 5.

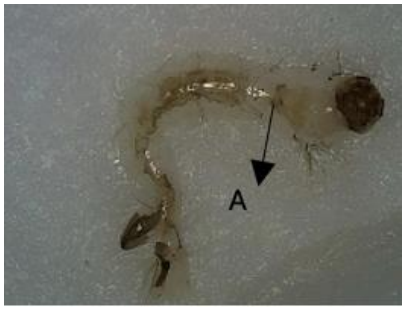

Figure 5. Morphological changes in *Ae. aegypti* larvae observed after 24 hours at a concentration of 70 mL

Morphological changes include a softer texture, a yellowish-white color from the thorax to the abdomen, and damage to the digestive tract (not intact).

Morphological changes in *Ae. aegypti* larvae after administration of *S. marcescens* extract, based on observation time at various concentrations, are shown in Figure 6.

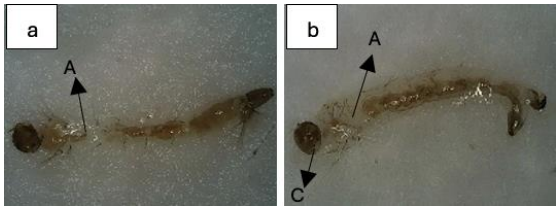

Figure 6. Morphological changes in *Ae. aegypti* larvae observed after 36 hours at various concentrations

Figure 6(a) shows morphological changes in larvae at a concentration of 60 mL, namely a softer texture and a yellowish-white colour from the thorax to the abdomen. The digestive tract is also damaged and not intact. Figure 6(b) shows changes in larvae at a concentration of 70 mL, a softer texture, a yellowish-white colour from the thorax to the abdomen, a damaged digestive tract, and both antennae broken off.

Morphological changes in *Ae. aegypti* larvae after administration of *S. marcescens* extract, based on observation time at various concentrations, are shown in Figure 7.

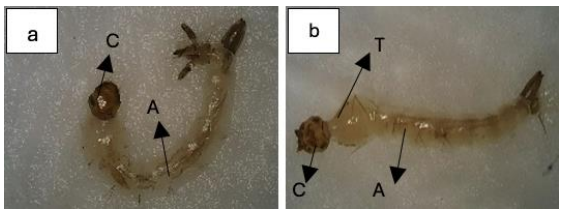

Figure 7. Morphological changes in *Ae. aegypti* larvae observed after 48 hours at various concentrations

Figure 7(a) shows morphological changes in the larvae at a concentration of 60 mL, including a softer texture, a yellowish-white colour from the thorax to the abdomen, a damaged intestinal tract, and broken

antennae. Figure 7(b) shows the morphological changes in larvae at a concentration of 70 ml. These changes include a softer texture, a milky white colour from the thorax to the abdomen, a damaged digestive tract, both antennae broken off, and the head almost broken off.

B. thuringiensis is a Gram-positive, rod-shaped bacterium that can be found in various ecosystems. It is selective and non-pathogenic to other animals or humans. It contains a protein compound known as delta-endotoxin, which causes the death of target insects when ingested [16]. Morphological changes were observed in *Ae. aegypti* larvae that died after the administration of a *B. thuringiensis* extract [18]. These changes included the formation of pores in the midgut epithelium, resulting in leakage. This facilitates the entry of *B. thuringiensis* spores. Cell swelling and lysis then occur, resulting in infection and death of the insect. The bacterium *B. thuringiensis* produces a protein compound called Cry or δ-endotoxin. This compound is toxic to certain types of insects but not to non-target insects or organisms [19].

S. marcescens is a Gram-negative, rod-shaped, motile bacterium belonging to the Enterobacteriaceae family. It can be found on leaf surfaces, in soil and water, and even inside insects and humans. This bacterium produces the secondary metabolite prodigiosin [20]. According to [9], morphological changes observed in *Ae. aegypti* larvae that died after being treated with *S. marcescens* extract included the absence of all body parts except the abdomen and digestive system. The cuticle has detached from the larva's body, becoming transparent in the process. *S. marcescens* bacteria contain compounds such as prodigiosin, alkaloids, and saponins. Alkaloids act as stomach poisons that reduce the appetite of the larvae by affecting the cuticle cell membrane. Saponins act as contact poisons, damaging the outer part of the larvae's body by washing away its protective wax layer.

CONCLUSION

The potency test of *B. thuringiensis* against *Ae. aegypti* larvae revealed a 100% mortality rate at concentrations of 60, 70, and 80 mL. In contrast, the *S. marcescens* extract exhibited a 70% mortality rate at 60 mL, 53% at 70 mL, and 50% at 80 mL. Morphological changes in larvae after the administration of *B. thuringiensis* extract include a change in color to pale milky white, damage to the digestive system (disintegration), and broken antennae. Meanwhile, morphological changes in larvae infected with *S. marcescens* include a shift in color to yellowish white, damage to the digestive tract, broken antennae, and an almost severed head.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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