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Biological Control Agent of *Spodoptera frugiperda* Using *Bacillus thuringiensis* Bacteria

Indriyani[1], **Kusuma Handayani**[2*], **Alvian Firmansyah**[3], **Gina Dania Pratami**[4]

[1,2,3,4] Department of Biology, Faculty of Marhematics and Natural Sciences, Universitas Lampung

Abstract

The main obstacle to the development of food crops and horticultural production is the attack of *Spodoptera frugiperda* larvae. Pest control using chemical insecticides has long-term negative impacts. Biological control of *S. frugiperda* larvae can be achieved using *Bacillus thuringiensis* bacteria. This study aimed to determine the most effective *B. thuringiensis* isolate for killing *S. frugiperda* larvae, identify the most effective concentration of *B. thuringiensis* suspension, and assess larval mortality rates. The study employed a randomized block design (RBD) with two factors. The first factor was the type of *B. thuringiensis* isolate, consisting of four isolates. Larval mortality data were analyzed using Analysis of Variance (ANOVA), followed by Duncan's multiple range test. The results showed that the highest average mortality of *S. frugiperda* larvae was achieved using isolates Bt3BP14 and Bt4TSR6. These isolates resulted in the highest average mortality on the third day. Bt3BP14 and Bt4TSR6 demonstrated high potential for controlling *S. frugiperda* larvae, with average mortality rates of 86.67% and 66.67%, respectively, observed over three days. The most effective doses of *B. thuringiensis* suspension for killing *S. frugiperda* larvae were 15 ml and 20 ml. It can be concluded that *B. thuringiensis* isolates can be used as natural biological control agents against *S. frugiperda* larvae attacking food crops and horticultural plants.

Keywords: *Bacillus thuringiensis, Spodoptera frugiperda larvae*, *Effectiveness, Mortality, Suspension Dosage*

Introduction

Food crops and horticulture are sub-sectors of agriculture that have considerable potential for economic development. However, the production of food crops and horticultural commodities in Indonesia still faces several obstacles, one of which is pest infestation. Various types of pests have been reported to attack food crops and vegetables, but a few have emerged as the main pests causing significant economic losses. Among these is the armyworm, *Spodoptera frugiperda* (Apriani et al., 2021).

S. frugiperda larvae originate from the tropical regions of the Americas (Septian et al., 2021). The larvae were first discovered in Indonesia, in West Sumatra and Lampung, in 2019 (Trisyono et al., 2019). *S. frugiperda* is considered a highly destructive pest because it can attack more than 80 species of plants, including corn, rice, sorghum, sugarcane, vegetables, and cotton. These larvae feed on young leaves, often leaving only the upper

*Corresponding Author: Kusuma Handayani, Email: indriy783@gmail.com. Jalur dua Univeristas Lampung, Jl. Lintas Sumatera No.1, Kota Bandar Lampung, Lampung 35141

epidermis and leaf veins, which can prevent the development of new leaves (Mafazah & Zulaika, 2017). Umboh et al. (2017) also reported that infestations by these larvae can cause yield losses of more than 41.42% in leek crops. According to a survey by Trisyono et al. (2019), *S. frugiperda* infestations were found to cause severe damage to corn plants as young as two weeks old, with attack rates reaching 100% in Lampung Province.

The infestation of *S. frugiperda* larvae causes significant losses to food crops. In general, farmers use chemical insecticides to control the spread of these pests (Septian et al., 2021). However, the continuous use of chemical insecticides can have detrimental long-term effects, including the death of non-target organisms, the development of resistance, and the resurgence of target pests (Khamid & Siriyah, 2018). Due to these risks, alternative pest control methods are needed. Biological control, which utilizes natural enemies of the larvae, offers a promising solution.

Entomopathogenic bacteria are an alternative biological control method for larval pests. *Bacillus thuringiensis* is one of the entomopathogenic bacteria commonly used for biological control, particularly against pests in the order Lepidoptera (Apriyana et al., 2021). This bacterium produces protein crystals that are toxic to insects and larvae. The protein crystals formed by *B. thuringiensis* can cause swelling, lysis, and damage to the epithelial cells of the caterpillar's midgut. The toxins produced can create pores in the cell membranes of the digestive tract, disrupting the osmotic balance of these cells (Mafazah & Zulaika, 2017). This makes *B. thuringiensis* highly potential as a biological control agent for *S. frugiperda* larvae, especially in food crops. The purpose of this research was to determine the most effective dosage of *B. thuringiensis* suspension for killing *S. frugiperda* larvae, and to ascertain the mortality rate of *S. frugiperda* larvae infected with *B. thuringiensis* bacteria.

Research Methods

The materials used in this research were pure isolates of *B. thuringiensis*, *S. frugiperda* larvae, Sapporo insecticide 52 EC as a positive control, Nutrient Agar (Merck) for bacterial rejuvenation on solid media, and Nutrient Broth (Merck) as a medium for bacterial growth in liquid culture. The experimental design used was a randomized block design (RBD) with two factors. The first factor was the type of bacterial isolate, consisting of two types of *B. thuringiensis* isolates. The second factor was the variation in suspension dose for each bacterial isolate. The doses of *B. thuringiensis* used were 10 ml, 15 ml, and 20 ml. Sterile distilled water was used as the negative control, and Sapporo 52 EC insecticide served as the positive control.

Larval Sample Collection of *Spodoptera frugiperda*

Larval samples of *S. frugiperda* were collected from maize fields showing signs of infestation. The larvae used as samples were those in the 3rd and 4th instar stages (Suby et al., 2020).

Isolate Rejuvenation of *Bacillus thuringiensis*

B. thuringiensis isolates were obtained from the soil of the Liwa Botanical Garden and coded as Bt1TBA4 and Bt2TSR6. Rejuvenation was carried out by taking one bacterial isolate and purifying it using the streak method (Rohmawati, 2020) on a Petri dish containing Nutrient Agar (NA). The plates were incubated for 72 hours at 37°C.

Preparation of *Bacillus thuringiensis* Stock Solution

Each rejuvenated *B. thuringiensis* isolate was used to inoculate 90 ml of Nutrient Broth (NB) in an Erlenmeyer flask and incubated for 72 hours on a rotary shaker. This culture was used as the *B. thuringiensis* stock solution (Handayani et al., 2025). The bacterial cell density used for testing was 10^7 cells/ml (Krishanti et al., 2017), calculated using a hemocytometer.

Preparation of *Bacillus thuringiensis* Suspension Dosages

Based on Indriani (2020), the dose treatments were as follows: P1 = 10 ml, taken from the stock solution and diluted with 200 ml of sterile distilled water; P2 = 15 ml of stock solution, diluted with 200 ml of sterile distilled water; P3 = 20 ml of stock solution, diluted with 200 ml of sterile distilled water. The negative control (K–) received only sterile distilled water without *B. thuringiensis* suspension. The positive control (K+) used 5 ml of Sapporo 52 EC insecticide added to 200 ml of sterile distilled water.

Application of *Bacillus thuringiensis* Suspension to *Spodoptera frugiperda* Larvae

The application was performed using the leaf-dipping method (Hrithik et al., 2022). Young corn leaves, serving as larval feed, were cut into 4 x 4 cm pieces (five per treatment), dipped in each suspension for 10 minutes, and air-dried. The treated corn leaves were then placed into experimental jars. Five *S. frugiperda* larvae were introduced into each jar, and each treatment was replicated three times. Leaves were replaced with fresh ones daily, and untreated leaves were provided one day after treatment. Observations of *S. frugiperda* larvae were carried out daily for three days after the application of the *B. thuringiensis* suspension. Observations aimed to determine the percentage of larval mortality due to infection with *B. thuringiensis*.

Data Analysis

Larval mortality data for *S. frugiperda* were analyzed using Analysis of Variance (ANOVA). If a significant difference was found, further analysis was conducted using Duncan's Multiple Interval Test at a 5% significance level. Data are presented in the form of tables and bar charts.

Research Results and Discussion

Larval Mortality of *Spodoptera frugiperda*

Based on the research conducted, the percentage mortality of *S. frugiperda* following application of various doses of the entomopathogenic bacterium *B. thuringiensis* was further analyzed using Duncan's test at a significance level of 5%. The results of the Duncan test for larval mortality of *S. frugiperda* at different suspension doses of *B. thuringiensis* are presented in Table 1.

Table 1
Duncan's Test Results for Spodoptera frugiperda *Larval Mortality*

Isolate	T	Mortality rate (% ± SD)

		D1	D2	D3
Bt3BP14	P1	33.33±11.547b	73.33±11.547c	86.67±11.547c
	P2	26.67±11.547b	26.67±11.547b	40.00±0.000b
	P3	26.67±11.547b	20.00±0.000b	46.67±11.547b
	K+	100.00±0.000c	100.00±0.000d	100.00±0.000d
	K-	0.00±0.000a	0.00±0.000a	0.00±0.000a
Bt4TSR6	P1	0.00±0.000a	0.00±0.000a	60.00±0.000a
	P2	0.00±0.000a	33.33±11.547b	46.67±11.547c
	P3	0.00±0.000a	40.00±20.000b	66.67±11.547c
	K+	100.00±0.000b	100.00±0.000c	100.00±0.000d
	K-	0.00±0.000a	0.00±0.000a	0.00±0.000a
Bt6TB7	P1	6.67±11.547a	26.67±11.547b	46.67±11.547b
	P2	13.33±11.547a	60.00±0.000c	66.67±11.547b
	P3	13.33±11.547a	33.33±11.547b	46.67±30.551b
	K+	100.00±0.000b	100.00±0.000d	100.00±0.000c
	K-	0.00±0.000a	0.00±0.000a	0.00±0.000a
Bt7TBA7	P1	0.00±0.000a	6.67±11.547a	20.00±20.000ab
	P2	0.00±0.000a	0.00±0.000a	26.67±11.547b
	P3	6.67±11.547a	13.33±23.094a	33.33±11.547b
	K+	100.00±0.000b	100.00±0.000b	100.00±0.000c
	K-	0.00±0.000a	0.00±0.000a	0.00±0.000a

Note: Values are presented as mean ± standard deviation (SD). Means followed by the same superscript letter within the same column are not significantly different according to Duncan's multiple range test (α = 0.05).

T : Treatment
D1, D2, D3 : Days 1, 2, and 3
P1 : Suspension dose 10 mL + 200 mL sterile distilled water
P2 : Suspension dose 15 mL + 200 mL sterile distilled water
P3 : Suspension dose 20 mL + 200 mL sterile distilled water
K(+) : Positive control (Sapporo 52 EC, 5 mL + 200 mL sterile distilled water)
K(-) : Negative control (sterile distilled water)

Based on Duncan's test results, the highest average mortality of *S. frugiperda* larvae infected with protein crystals from *B. thuringiensis* was observed in isolates Bt3BP14 and Bt4TSR6. Both isolates showed the highest average mortality on the third day. Bt3BP14 and Bt4TSR6 thus have strong potential for controlling *S. frugiperda* larvae. These isolates yielded average mortality rates of 86.67% and 66.67% at doses of 15 ml and 20 ml, respectively, after three days of observation. The third isolate is also likely to be *B. thuringiensis* and shows great potential as an entomopathogenic bacterium.

Differences in the mortality rates of each isolate are influenced by the varying protein crystal structures present. These protein crystals are formed when the bacteria undergo sporulation. Bt Cry proteins are produced as inactive protoxins within crystal inclusions. After ingestion, the crystals dissolve in the alkaline larval midgut and are proteolytically processed by midgut proteases, generating shorter active toxin fragments (e.g., ~65 kDa for Cry1Ac and ~50 kDa for Cry2Ab). This proteolytic activation is essential for insecticidal activity (Liu et al., 2020).

Nair et al. (2018) also reported that bipyramidal protein crystals encode Cry I genes, which are specific to the order Lepidoptera. Crystals that are cuboidal in shape encode Cry II genes, which are specific to Lepidoptera and Diptera. Flat or irregular protein crystals encode Cry III genes, which are specific to Coleoptera. Cry IV, also bipyramidal, is specific to Diptera. Each gene encoding these protein crystals has a different molecular weight, influencing the level of toxicity: Cry I (130–138 kDa), Cry II (69–71 kDa), Cry III (74–74 kDa), and Cry IV (73–134 kDa) (Djenane et al., 2017). According to Hammer et al. (2017), the pH in the larval digestive tract is highly alkaline (up to pH 10–12), which facilitates activation of these protein crystals (Hammer et al., 2017). The size of the protein crystals also greatly affects the concentration of toxins dissolved in the insect midgut; larger crystals result in higher concentrations of dissolved toxins (Mafazah & Zulaika, 2017) . Other factors, such as larval age, also influence the toxicity of *B. thuringiensis*, with younger larvae being more susceptible than older ones (Poopathi & Tyagi, 2016).

Morphological Changes in *Spodoptera frugiperda* Larvae

Morphological changes in *S. frugiperda* larvae after the application of entomopathogenic *B. thuringiensis* isolates for three consecutive days are shown in Figure 1.

Figure 1

Morphological changes in S. frugiperda *larvae over three days: (a) first day, (b) second day, (c) third day; (K+) positive control; (K-) negative control.*

On the first day, there were no significant morphological changes observed in *S. frugiperda* larvae, but some larvae died, likely due to the initial poisoning by protein crystals produced by *B. thuringiensis*. On the second day, the larvae's bodies began to turn black, probably as their digestive systems started to break down, again caused by the bacterial protein crystals. By the third day, the larval bodies became watery, very soft, and black, with some body parts secreting a foul-smelling fluid, most likely due to the disintegration of the digestive tract.

These findings are consistent with Arsi et al. (2019), who reported that larvae dying on the first day showed little change, while those dying on the second day displayed reddish-brown discoloration. On the third day, the larval bodies turned black, emitted a foul odor, and secreted a milky-white liquid. When examined, the larvae were observed to shrink and become thinner. Devi et al. (2022) further found that symptoms of bacterial infection in larvae include inactivity, decreased appetite, weakness, diarrhea, and discharge from various body parts. Larvae infected with *B. thuringiensis* became swollen and brownish-black, with the carcasses smelling rotten and shrinking over time. The larvae eventually disintegrated. Enzymes such as chitinase, produced by entomopathogenic bacteria, cause cuticle thinning in *S. frugiperda* larvae, resulting in softened bodies and eventual death (Castro et al., 2019).

Infected larvae experience loss of body fat and weakened tissues until the bacteria enter the hemocoel, leading to septicemia. Activated Cry toxins bind to receptors on midgut epithelial cells and form pores in the cell membrane, causing cell lysis and severe midgut damage, which ultimately leads to feeding cessation and larval death (Liu et al., 2020).

Conclusion

Based on the research conducted, it can be concluded that the most effective *B. thuringiensis* isolates in killing *S. frugiperda* larvae were Bt3BP14 and Bt4TSR6. Isolate Bt3BP14 showed a mortality rate of 86.67%, while isolate Bt4TSR6 showed a mortality rate of 66.67%. The most effective doses of *B. thuringiensis* bacterial suspension for each isolate in killing *S. frugiperda* larvae were 15 mL and 20 mL.

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