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Efficacy of Endophytic Bacteria as Entomopathogens against *Spodoptera frugiperda* (Lepidotera: Noctuidae) on Corn (*Zea mays* L.)

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ABSTRACT

The fall armyworm (Spodoptera frugiperda) poses a problem for corn plants (Zea mays L.). The use of endophytic bacteria Bacillus sp. as an entomopathogen is expected to control the S. frugiperda pest. This study aims to determine the effective concentration of the bacteria and the active compounds produced by Bacillus sp. strain Bth 22 in controlling S. frugiperda. The research was conducted from August to October 2024 at the Plant Health Laboratory of the Faculty of Agriculture, UPN "Veteran" East Java, and the Airlangga Research Hub in Surabaya. The study was designed using a completely randomized design (CRD) with bacterial concentration treatments of 0% (control), 10%, 15%, 20%, 25%, 30%, and 35%, repeated five times. Observational parameters included mortality rate, number of pupae and imago formed, as well as the mechanisms and compounds produced by Bacillus bacteria based on HPLC and FT-IR tests. The application of Bacillus sp. Bth-22 affected the mortality parameters, the number of pupae formed, and the number of imago formed. The Bacillus sp. Bth-22 bacteria produced metabolites in the form of hydrocarbon derivatives and amide group compounds, disrupting metabolism and digestion, leading to the mortality of S. frugiperda.

1. INTRODUCTION

Corn productivity in Indonesia varies depending on several factors, including the type of variety planted, cultivation techniques used, weather conditions, and general farm management. Corn production in Indonesia, in 2020 increased by 2.6 million tons or 11.52% from 2019, and East Java is one of the centers of Indonesian corn production with a production of 5.73 million tons and contributing to national corn production of 25.26%, but this can be higher or lower depending on the influencing factors (Komalasari, 2024).

One of the causes of the fluctuation in corn plant productivity is due to attacks by the armyworm *Spodoptera frugiperda*. Armyworms are also known as "Fall Armyworms" causing significant damage to corn plants by eating leaves, cobs, and even parts of the plant as a whole. Pest attacks at the early vegetative stage can cause more leaf damage and yield losses than infestations at the late vegetative stage. The impact is that armyworm attacks can cause major losses in corn production if not controlled properly (Nonci *et al*., 2019).

Conventionally, farmers apply chemical insecticides to overcome the spread of *S. frugiperda* larval populations. Chemical insecticides are one way for farmers to control pest attacks on plants because chemical insecticides have a very fast reaction in controlling insect pests (Septian *et al*., 2021). Continuous use of chemical insecticides can result in quite detrimental impacts in the long term. The negative impacts caused by the continuous use of chemical insecticides include pest resistance, reduced biodiversity, residues in food and environmental damage. Therefore, alternative control efforts are needed that have the lowest negative impact, namely biological control.

The application of biological control techniques against plant pests and diseases using natural enemies, such as predators, parasitoids, pathogens, and antagonists has long been proposed as one of the components of integrated pest and disease control. Biological control, especially endophytic bacteria, has been developed and applied along with the increasing public attention to health and environmental sustainability (Yulianti, 2013). Biological agents in the form of endophytic bacteria have relatively efficient ability in controlling pest attacks. Endophytic bacteria live in plant tissue and can be isolated from all parts of the plant, namely seeds, leaves, stems and roots. Endophytic bacteria live in mutualistic symbiosis with host plants both in forming passive and active plant resistance so that bacteria and plants can survive in a complex ecosystem (Sianipar *et al*., 2020). Based on these benefits, there is potential that endophytic bacteria can be used as pest control, especially *S. frugiperda* in corn plants.

Research on the use of endophytic bacteria in shallots against *Spodoptera litura* in vegetable plants showed symptoms of infection occurred in the third instar of *S. litura* larvae, toxic compounds produced by bacteria caused symptoms in the form of slower larval growth, shrinking larval bodies, blackening larvae, larvae secreting fluid, and diarrhea Rahman *et al*. (2023). This happens because bacterial toxins damage the digestive system of the larvae, causing death. Krishanti *et al*. (2017), explained that infected larvae shrink, their body color becomes blacker and shrinks. The mechanism of infection in the form of interference with the digestive system of the larvae causes death.

When reviewed from previous research, there has been no in-depth analysis of chemical compounds, especially compounds produced by endophytic bacteria in controlling insect pests. So that an advanced analysis method is needed for the type of poison or chemical compound in endophytic bacteria through the HPLC method and the FTIR (Fourier Transform Infrared Spectroscopy) method. This method is generally used to identify chemical compounds based on the absorption pattern of infrared radiation by chemical bonds in the compound (Bendrianis, 2024).

Currently, the utilization of endophytic bacteria, especially endophytic bacteria *Bacillus* sp. as entomopathogens against *S. frugiperda* and the chemical compounds produced have not been widely published. Based on this, a study on the efficacy of endophytic bacteria as entomopathogens against *Spodoptera frugiperda* (Lepidotera: Noctuidae) in corn plants needs to be performed. In this case, the endophytic bacteria are *Bacillus* sp. Bth-22, which comes from the roots, stems and leaves of eggplant plants (collection from the Plant Health Laboratory of the Agriculture Faculty, UPN "Veteran" East Java) (Purnawati *et al*., 2024). This study aims to determine the effective concentration and the types of compounds produced by endophytic bacteria *Bacillus* sp. strain Bth-22. This study is also useful in informing the effective concentration and types of compounds produced by endophytic bacteria *Bacillus* sp. strain Bth-22.

2. MATERIALS AND METHODS

2.1. Research Location

The research was conducted at the Plant Health Laboratory of the Faculty of Agriculture, UPN “Veteran” East Java and Airlangga Research Hub Surabaya geographically located at 7° 9' - 7° 21' South Latitude and 112° 36' - 112° 57' East Longitude. The research was conducted from August to October 2024.

2.2. Tools and Materials

The tools used in this study include an autoclave, oven, ose needle, tweezers, bunsen lamp, analytical balance, laminar air flow, microscope, test tube, petri dish, beaker, erlenmeyer, tip, vortex, hot plate, magnetic stirrer, cellphone camera, tweezers, matches, label paper, ruler, pipette, glass funnel, slide glass, cover glass, rotary shaker and microscope.

The materials used in this study include NA (Nutrient Agar) media, NB (Nutrient Broth) media, pure isolates of biological control agents of endophytic bacteria *Bacillus* sp. strain Bth-22 from eggplant plants collected by Dr. Ir. Arika Purnawati, MP., spirits, aquades, plastic wrap, cotton, rubber bands, tissues.

2.3. Research Design

This research was arranged in a completely randomized design (CRD) with endophytic bacterial concentration treatments, namely Concentrations of 10%, 15%, 20%, 25%, 30%, and 35% and controls without *Bacillus* which were repeated 5 times, and 10 instar 3 *Spodoptera frugiperda* larvae in each treatment.

2.4. Research Implementation

2.4.1. Rejuvenation and Propagation of Endophytic Bacterial Isolates

The endophytic bacterial isolate Bth-22 was a collection of Dr. Ir. Arika Purnawati which was isolated from healthy eggplant (*Solanum melongena*) plants, grown on Nutrient Agar (NA) media and stored in paraffin oil. For this study, the endophytic bacterial isolate Bth-22 was rejuvenated by growing on new NA media, incubated at 37°C for 24 h (Purnawati *et al*., 2024). After growing, it was used for research. Propagation of endophytic bacterial isolates aged 24 h, taken 1000 µl, inoculated into 100 mL of Nutrient Broth (NB) media, incubated on a rotary shaker for 48 h (Rohma & Wahyuni, 2022).

2.4.2. Calculation of Population Density of Endophytic Bacteria Bth-22

Calculation of population density endophytic bacterial isolates aged 24 hours were taken 1000 µl using a micropipette and made dilutions from 10^{-1} to 10^{-8}. At a dilution of 10^{-8}, 100 µl was taken using a micropipette and dripped onto Nutrient agar (NA) media, leveled until homogeneous using an L glass and left for ± 15 minutes, then incubated at 28°C for 24 hours. The endophytic bacteria used were a population density of 1010 CFU/mL. The population density calculation was carried out using the standard plate count method with the provision of 30-300 colonies per plate, using the formula according to Rosmania & Yuniar (2021) as the following:

$$\text{Number of colonies per mL} = \frac{\text{Number of colonies} \times 1}{\text{Dilution}} \quad (1)$$

2.4.3. Maintenance and Propagation of Instar 3 Larvae

The larvae used were obtained from the Sweetener and Fiber Plant Instrument Standard Testing Center (BSIP-TAS), then maintained and propagated. The larvae used in the study were instar 3, which are invasive larvae so that during maintenance and propagation, separation was carried out for each tail to avoid the cannibalistic nature of *S. frugiperda* larvae. The feed used was baby corn (Fitriani *et al*., 2023).

2.4.4. Application of Endophytic Bacteria

The application was carried out using the feed dipping method (Balfas, 2009). The feed used was baby corn. Baby corn was thinly sliced and weighed as much as 5 grams per jar, dipped in each suspension, left for 10 minutes and dried by airing. The baby corn that had been treated was put into the experimental jar. Each experimental jar was invested with 10 *S. frugiperda* larvae. The feed was changed every day (Alvian, 2023).

2.4.5. Observation Parameters

Observation parameters for insects treated with endophytic bacteria include larval mortality, number of pupae formed, number of imago formed. Analysis parameters of compounds produced by endophytic bacteria *Bacillus* sp. Bth-22 include the types of compounds detected during testing.

2.5. Data Analysis

The research data from the pathogenicity test were analyzed using R studio software with the ANOVA procedure. If the conclusion is obtained that the sig value <alpha (0.05), then the average difference between treatments is tested using the DMRT (Duncan Multiple Range Test) with a real level of 5%.

3. RESULTS AND DISCUSSION

3.1. Larval Mortality

Symptoms of endophytic bacterial infection in *S. frugiperda* larvae include reduced feeding activity, slower movement and less sensitivity to touch. Larvae that die due to endophytic bacterial infection experience changes in color and shape. The body color of the larvae changes to reddish, dark brown to blackish, shrivels, and the body of the larvae releases fluid. fluid and smells bad. The larvae then dry out and shrink with all integuments (Figure 1).

Figure 1. Died *S. frugiperda* larvae due to: (a) control, (b) infected with *Bacillus* sp. strain Bth-22, (c) dry, shrink larvae

Senewe *et al*. (2012) stated that the initial symptoms of caterpillars that have eaten the treated feed are changes in caterpillar behavior, caterpillar movement becomes slow, feces are somewhat liquid or diarrhea, different from control feces which remain in the form of granules. Caterpillars that have been infected will eventually die, their body color becomes blackish and their bodies become soft. The condition of *S. frugiperda* is thought to be caused by the presence of *Bacillus* sp. bacteria already in the intestinal epithelial tract so that they produce toxin compounds in the form of protein crystals that can cause death to *S. frugiperda*. This is in accordance with the statement Novizan (2002), that larval death can occur within a few hours after the first infection.

Based on the results of the analysis, the administration of concentrations has not shown any effect until the fourth day. The fifth day of observation showed the effect of administering the *Bacillus* sp. strain Bth-22 suspension (Table 1). This is in accordance with Dara (2017), that in corn caterpillar pests, midgut paralysis occurs after delta-endotoxin is swallowed followed by cessation of feeding. Insects can move actively because general paralysis will not occur. Death occurs within 48-96 hours.

Treatment of 15% concentration of bacteria caused the highest mortality rate of *Spodoptera frugiperda* larvae (Table 1), compared to treatments of 20%, 25%, 30%, 35% and control, this can be seen from the fifth to eighth day the percentage of larval mortality at a concentration of 15% increased dominantly, namely 48%, 74%, 80% and 86%. Giving endophytic bacteria with a concentration of 15% was sufficient to affect the death of *S. frugiperda* larvae. This is suspected that at a concentration of 15%, the entire physiological system of the larvae has been optimally exposed. Increasing the concentration of toxin no longer increases its effectiveness because the number of receptors has reached maximum capacity. As stated by Pardo-López *et al*. (2013), the effectiveness of cry toxin in *Bacillus thuringiensis* is influenced by the ability of the protein to bind optimally to specific receptors in the insect intestine. The limited number of receptors allows saturation to occur, where too high a concentration of toxins does not increase their effectiveness and can even decrease the performance of the toxin due to protein aggregation or non-specific binding that can inhibit the activity of the toxin.

Table 1. *Bacillus* sp. Test Results on the Mortality Level of *Spodoptera frugiperda*

Treatment	Larval Mortality (%) on Day -							
	1	2	3	4	5	6	7	8
10%	22.00	28.00	34.00	42.00	60.00 a	66.00 a	68.00 a	70.00 ab
15%	22.00	30.00	34.00	40.00	48.00 a	74.00 a	80.00 a	86.00 a
20%	12.00	22.00	26.00	34.00	52.00 a	54.00 ab	60.00 a	62.00 b
25%	24.00	30.00	30.00	32.00	44.00 a	58.00 ab	64.00 a	64.00 b
30%	20.00	20.00	22.00	22.00	36.00 a	40.00 bc	62.00 a	64.00 b
35%	30.00	36.00	38.00	38.00	38.00 a	68.00 a	68.00 a	70.00 ab
Control	0.00	4.00	6.00	6.00	10.00 b	20.00 c	38.00 b	40.00 c
DMRT 5%	ns	ns	ns	ns	25.54	21.79	20.54	19.34

Note: Mean values accompanied by the same letter in each treatment in the same column show no significant difference in the 5% DMRT test.

Table 2. Results of LC50 and LT50 Tests of *Bacillus* sp. against *Spodoptera frugiperda*

Treatment	LC50 (%)	LT50 (Day)
10%	9.17	4.01
15%	9.17	3.61
20%	9.17	5.47
25%	9.17	5.23
30%	9.17	7.16
35%	9.17	4.14

Note: Mean numbers accompanied by the same letter in each treatment in the same column shows no significant difference in the 5% DMRT test.

Based on the results of the LC50 test, the concentration of *Bacillus* bacterial suspension of 9.17% was able to control 50% of the insect pest population. The lowest LT50 value was found in the treatment of *Bacillus* sp. strain Bth-22 bacteria at a concentration of 15%, which was 3.61 days, meaning that the bacteria could cause the death of 50% of *S. frugiperda* larvae within three days.

3.2. Number of Pupae Formed

Based on the results of the analysis, the provision of concentration variations affects the number of pupae formed as presented in Table 3. The pupae formed are influenced by the level of mortality during the larval stage caused by *Bacillus* sp. infection. The higher the larval mortality rate, the fewer the number of pupae formed. After going through the larval phase, the armyworm undergoes metamorphosis into a pupa. The number of pupae formed on the 4th to 11th day varies based on concentration. From a plant protection perspective, the treatment of endophytic bacteria *Bacillus* sp. Bth-22 at a concentration of 15% produced the lowest pupa population, which was 54%. This is because the 15% concentration caused the highest mortality compared to other treatments, so the high larval mortality value caused the low number of pupae formed.

Table 3. *Bacillus* sp. Test Results on the Number of Pupae Formed

Treatment	Number of Pupae Formed (%) on Day-						
	4	5	6	7	8	9	10
10%	18.00	42.00	50.00	58.00 bc	60.00 bc	60.00 c	60.00 c
15%	22.00	32.00	50.00	50.00 c	52.00 c	52.00 c	54.00 c
20%	14.00	50.00	58.00	70.00 abc	72.00 abc	72.00 abc	72.00 abc
25%	10.00	38.00	56.00	64.00 abc	64.00 bc	64.00 bc	64.00 bc
30%	20.00	56.00	74.00	82.00 ab	84.00 ab	86.00 ab	86.00 ab
35%	18.00	42.00	54.00	62.00 bc	64.00 bc	64.00 bc	64.00 bc
Control	28.00	54.00	74.00	90.00 a	90.00 a	90.00 a	92.00 a
DMRT 5%	ns	ns	ns	24.43	22.60	22.86	22.76

Note: Mean numbers accompanied by the same letter in each treatment in the same column shows no significant difference in the 5% DMRT test.

The low number of pupae formed is thought to be caused by the toxin compound produced by *Bacillus* sp. According to Nelly *et al*. (2024), the toxin compound causes larval mortality which has an impact on the number of pupae formed. *Bacillus* treatment of *S. frugiperda* insects also has an impact on the condition of the pupae formed. Larvae treated with *Bacillus* produced abnormal pupae and normal pupae formed. Normal pupae are reddish brown in color, the tail moves when touched, and parts of the antennae, wings and legs appear faintly attached to the body (Figure 2A). This is in accordance with the research results of Taufika *et al*. (2022), the type of lepidoptera pupa is obtecta, where the appendages are tightly attached to the body.

Meanwhile, in abnormal pupae, the shape of the pupa produced is not perfect. The condition of the abnormal pupae found is either partially pupae, and some are still in the form of larvae (Figure 2B). Abnormal pupae can also resemble normal pupae but with conditions such as small size, wrinkled body surface, flattened in two, do not move when

touched, there is fluid, or the body is perforated and smells bad. This is in line with the research of Arsi *et al*. (2019), where the larvae in the study formed abnormal pupae. The pupae found experienced various abnormalities including: the thorax condition remained in the shape of a larval head, but the abdomen managed to form a pupa; the pupae had an abnormal size or were smaller than normal pupae; the pupae changed color to black and rotted; and the abdomen of the pupa did not produce movements like normal pupae. In addition, pupae that successfully became imagos were short-lived and would die then release a light brown liquid and smell bad.

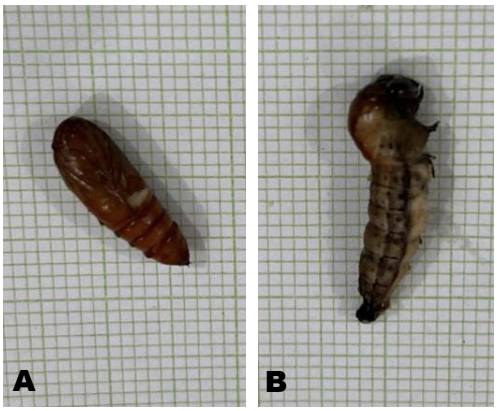

Figure 2. Condition of *Spodoptera frugiperda* Pupae (A) Normal, (B) Abnormal

3.3. Number of Imago

Based on the results of the analysis, the provision of variations in the concentration of *Bacillus* sp. affects the number of imago formed as presented in Table 4. The imago formed is influenced by the level of mortality during the pupal stage caused by *Bacillus* sp. infection. The lower the number of pupae formed, the fewer the number of imago formed.

Table 4. Results of the *Bacillus* sp. Test on the number of larvae that successfully become imago

Treatments	Number of Imago Formed (%) on Day -						
	11	12	13	14	15	16	17
10%	0.00	2.00	14.00 bc	14.00 b	14.00 b	14.00 b	14.00 b
15%	4.00	6.00	8.00 c	10.00 b	10.00 b	12.00 b	12.00 b
20%	2.00	2.00	12.00 bc	22.00 b	24.00 b	24.00 b	24.00 b
25%	2.00	14.00	28.00 b	28.00 b	28.00 b	30.00 b	30.00 b
30%	6.00	18.00	24.00 bc	28.00 b	28.00 b	30.00 b	30.00 b
35%	2.00	6.00	12.00 bc	14.00 b	14.00 b	14.00 b	14.00 b
Control	4.00	16.00	48.00 a	56.00 a	56.00 a	58.00 a	58.00 a
DMRT 5%	ns	ns	16.68	17.31	16.68	16.46	16.46

Description: The average figures accompanied by the same letter in each treatment in the same column show no significant difference in the 5% DMRT test.

Imago is the final stage in metamorphosis where the surviving pupae form adult insects that will continue the reproductive cycle. Based on the results of the analysis, the administration of *Bacillus* sp. at various concentrations had a significant effect compared to the control. The lowest number of imagos formed until the end of the observation period was found in the treatment at a concentration of 15% *Bacillus*, which was 12% of the total population of larvae tested. This is because at a concentration of 15%, the number of pupae formed was lower than the other treatments, thus affecting the number of imagos formed. The low number of pupae formed is thought to be caused by the activity of *Bacillus* sp. in decomposing the internal organs of insects during the pupal phase.

According to Arsi *et al*. (2019), *Bacillus* sp. bacteria, in addition to causing mortality of *S. frugiperda* at the larval stage, can also cause mortality and toxicity at the pupal stage. *S. frugiperda* pupae infected by *Bacillus* sp. bacteria experience varying damage such as being black, dry, or imperfectly shaped (defective). As a result, larvae infected by

entomopathogens from *Bacillus* sp. bacteria. when entering the pupal stage, it will experience interference from bacteria which is indicated by the pH in the larval intestine not being in a neutral or normal condition. This condition indicates that bacteria live in the insect's body, causing failure of imago formation.

Bacillus treatment of *S. frugiperda* insects also has an impact on the condition of the imago that is formed. Larvae treated with *Bacillus* and then become pupae, produce abnormal imago and normal imago that are formed (Figure 3A). Normal pupae are reddish brown, the tail moves when touched, and parts of the prospective antennae, wings and legs appear faintly attached to the body.

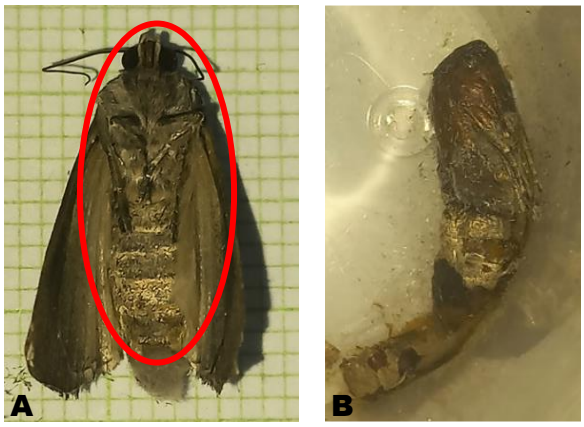

Figure 3. Condition of *Spodoptera frugiperda* Imago (A) Normal, (B) Abnormal

The formation of abnormal pupae and imago is thought to be caused by a lack of nutrients and the presence of toxins secreted by endophytic bacteria, then tissue damage occurs. This is in accordance with research by Nelly *et al.* (2024) which states that disruption of the body's metabolism of larvae due to the bacterial toxins produced causes the larvae to lack energy to enter the pupal stage, if the larvae do not die from the toxins, an abnormal pupa (defect) will form.

3.4. *Bacillus* sp. Bth-22 Metabolite Compounds and their Effects on *Spodoptera frugiperda*

The results of metabolite compound testing using the HPLC method specifically for Ninhydrin Postcolumn-reaction detection using the High Speed Amino Acid Analyzer-Hitachi Model LA 8080, with a flow rate of 0.001-1,000 mL/minute are presented in Table 5. It can be seen that there are three most dominant compounds, namely Aspartate, Glycine, and Alanine. The aspartate content in healthy larvae and *Bacillus* sp. bacteria shows a high number compared to symptomatic larvae, in healthy larvae it is 1.898%, while in *Bacillus* sp. it is 5.165%. Aspartic acid is one of the amino acids that plays a role in synthesizing proteins and regulating hormones. According to Holeček (2023), L-aspartic acid is part of the protein synthesized in the body and is responsible for encouraging the production of antibodies that support the function of the immune system. In symptomatic larvae, the aspartic acid content is very small, namely 0.792%, this is thought to be because the infecting *Bacillus* sp. bacteria are able to damage the larvae's immune system. This is in accordance with the statement of Jani *et al.* (2023), that the larvae experience diarrhea, their body color becomes dark, and they die after a few hours of swallowing *Bacillus* sp.

Table 5. Comparison of Compounds in Endophytic Bacterial Isolate, Healthy Larvae, and Symptomatic Larvae

Compound	Metabolite Content (%)		
	Healthy Larvae	*Bacillus sp.* Strain Bth-22	symptomatic larvae
Aspartate	1.898	5.165	0.792
Glycine	1.529	3.671	1.025
Alanine	2.689	5.514	0.670

Glycine compound is a non-essential amino acid with a function as a specific receptor and transporter binder expressed in many types of cells throughout the organism to provide its effects. Glycine is an amino acid that is synthesized endogenously and has been described many activities involving it that cover various systems. Glycine compound found in *Bacillus* sp is 3.671% while in healthy *S. frugiperda* larvae it is 1.529%. This content is greater than in larvae infected with bacteria which is 1.025%, this is thought to be because in larvae infected with *Bacillus* sp., intestinal damage occurs.

According to the research conducted by Dara (2017), when *Bacillus* sp. is ingested, the alkaline conditions in the insect gut (pH 8–11) activate toxic proteins (delta-endotoxins) that bind to receptor sites in the midgut and create pores in the midgut cells. This leads to the loss of osmoregulation, midgut paralysis, and cell lysis. Consequently, gut contents leak into the insect's body cavity (hemocoel), and blood (hemolymph) leaks into the gut, disrupting the pH balance. The bacteria that enter the body cavity cause septicemia and ultimately result in the death of the host insect. Midgut paralysis occurs after the ingestion of delta-endotoxins, followed by the cessation of feeding. Insects may remain actively mobile because general paralysis does not occur. Death typically occurs within 48–96 hours.

Alanine, also known as α-alanine, α-aminopropionic acid, β-alanine, or β-aminopropionic acid, is an amino acid used in the synthesis of proteins. Alanine serves as an energy source for muscles and the central nervous system (Tan *et al*., 2003). Alanine, one of two amino acids—specifically, L-alanine or alpha-alanine (α-alanine)—is a constituent of proteins. L-alanine is a precursor to D-alanine, which is a key component of the cell wall (National Center for Biotechnology Information, 2025).

In larvae infected with bacteria, the Alanine content is very small, namely 0.670%, while in healthy larvae and *Bacillus* sp. bacteria, it is quite high, namely 2.689% and 5.514%. This is thought to be because bacteria are able to damage cell walls and disrupt the body's resistance of *S. frugiperda* larvae. In line with the opinion of Jani *et al.* (2023), *Bacillus* sp. bacteria have a protein crystal content which is the main toxin that causes death in Lepidoptera larvae. Protein crystals contain endotoxins that can cause damage to the intestinal wall cells of larvae, causing paralysis in the digestive system of Lepidoptera larvae. *Bacillus* sp. bacteria have a protein crystal content which is the main toxin that causes death in Lepidoptera larvae.

The results of the FTIR test of endophytic bacterial isolate samples, healthy larvae and symptomatic larvae (Figure 5) showed the detection of the same or adjacent waves between symptomatic larvae and *Bacillus* sp. bacteria strain Bth 22, which was not detected in healthy larval samples. The detected waves were at 1651.07 (C=C) symptomatic larvae and 1649.14 (C=C) in *Bacillus* sp. then wave 1462.04 (C-H) symptomatic larvae and 1454.33 (C-H) in *Bacillus* sp. In addition, there was also one wave point that was detected the same in the symptomatic larvae and *Bacillus* sp. samples, although it was classified as weak, namely at wave 1242.16 (C-N).

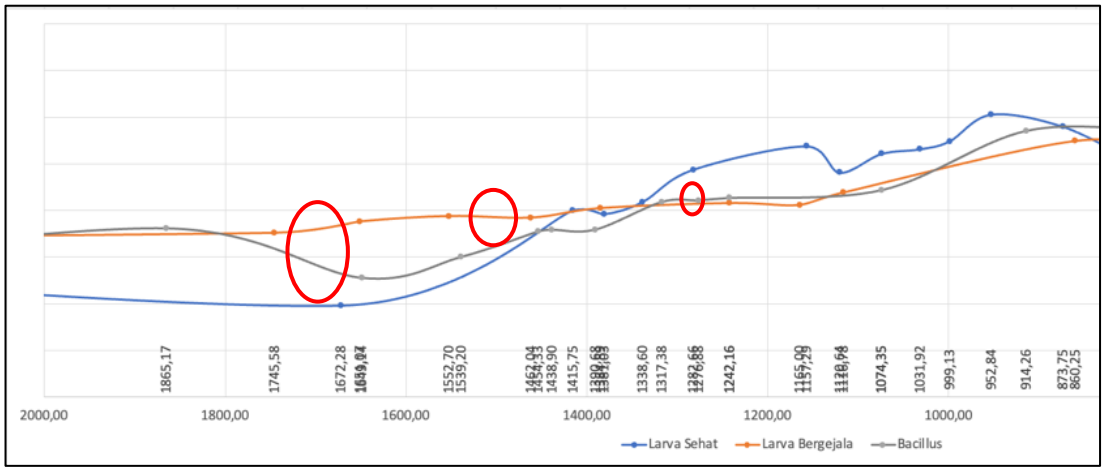

Figure 4. FT-IR analysis results

There are several similarities in the detection of compound waves between symptomatic larvae and *Bacillus* sp. strain Bth 22 but not detected in healthy larva samples, it is suspected that symptomatic larvae are indeed infected with *Bacillus* sp. strain Bth 22. Carbon-hydrogen (C-H) and carbon-carbon (C-C) bonds are forms of simple hydrocarbon compounds. Based on the bonds contained, aliphatic hydrocarbon compounds are divided into three types, namely alkanes, alkenes, and alkynes. Alkane derivative compounds are compounds derived from alkanes in which one or more H atoms are substituted by certain functional groups. The functional group is a group of substitute atoms which means it is a group that determines the properties of alkane derivative compounds. Examples of alkane derivative compounds are Alcohol (Alkanol), Ether (Alkoxy Alkane), Aldehyde (Alkanal), Ketone (Alkanone), Carboxylic Acid (Alconic Acid), Alkyl Halide (Haloalkane) (Setiowati, 2023). The C-N bond is an amide functional group consisting of a central carbonyl group with a nitrogen atom singly bonded to the carbonyl carbon. This nitrogen is called "amide nitrogen", and can have carbon or nitrogen bonded to it (Smith, 2019).

Based on the results of compound detection in the above test, it can be seen that *Bacillus* sp. bacteria can produce metabolite compounds. This is in accordance with the results of the study by Karačić *et al*. (2024), that *Bacillus* sp. as a biological control agent produces many volatile secondary metabolites with a wide spectrum. Volatile substances produced by *Bacillus* spp. involving various organic compounds (alcohols, alkenes, benzenoids, ketones, pyrazines, terpenes) and inorganic (eg, NH_3, HCN, H_2S, NO_2, CO_2). This is in line with research by Kai (2020), that the majority of volatile secondary metabolites detected in *Bacillus* sp. are classified as ketones, nitrogen-containing compounds, hydrocarbons, aromatic compounds and alcohols. In addition, volatile secondary metabolites also include aldehydes, acids, and esters. At a lower level, the compounds produced can be sulfur-containing compounds, silicon-containing compounds, ethers, halogenated compounds, naphthalene, and pyranones. In line with the research of Prihatiningsih & Djatmiko (2016), which states that *Bacillus* sp. is able to produce compounds of the alkane, aldehyde, ketone, carboxylic acid, ester, amine, and amide groups.

4. CONCLUSION

The conclusion drawn from the results of this study is that the application of *Bacillus* sp. Bth-22 affects the mortality parameters and the number of larvae that become pupae and the number of imago. *Bacillus* sp. Bth-22 bacteria produce metabolite compounds in the form of organic hydrocarbon derivatives and amide group compounds in disrupting metabolism and digestion, causing the death of *S. frugiperda*. It is recommended to conduct field tests to determine the effectiveness of *Bacillus* sp. Bth-22 on *Spodoptera frugiperda* with more complex agroecosystem conditions, as well as its potential impact on non-target organisms and the environment.

REFERENCES

Alvian, F. (2023). Uji efektivitas kandidat bakteri entomopatogen *Bacillus thuringiensis* terhadap larva *Spodoptera frugiperda*. [*Undergraduated Thesis*]. Universitas Lampung, Bandar Lampung.

Arsi, A., Pujiastuti, Y., Herlinda, S., Suparman, S.H.K., & Gunawan, B. (2019). Efikasi bakteri entomopatogen *Bacillus thuringiensis* Barliner sebagai agens hayati *Spodoptera litura* Fabricus pada lahan pasang surut dan rawa lebak. *Prosiding Seminar Nasional Lahan Suboptimal 2019*, 254–263.

Bendrianis, D. (2024). Identifikasi molekuler dan potensi metabolit sekunder antioksidan isolat jamur endofit tumbuhan mangrove (*Rhizophora apiculata*). [*Master's Thesis*]. Universitas Andalas.

Dara, S.K. (2017). Entomopathogenic microorganisms: modes of action and role in IPM. *Strawberries and Vegetables*, 1-7. https://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=24119

Fitriani, A.A., Dulbari, D., & Nuryanti, N.S.P. (2023). Uji keefektifan insektisida Spinetoram terhadap ulat grayak (*Spodoptera frugiperda* J.E.Smith). *J-Plantasimbiosa*, ***5***(2), 51–61. https://doi.org/10.25181/jplantasimbiosa.v5i2.3322

Holeček, M. (2023). Aspartic Acid in Health and Disease. *Nutrients*, ***15***(18), 4023. https://doi.org/10.3390/nu15184023

Jani, R., Soedijo, S., & Liestiany, E. (2023). Kemampuan *Bacillus thuringiensis* untuk mengendalikan *Spodoptera frugiperda* J.E. Smith. *Jurnal Proteksi Tanaman Tropika*, ***6***(2), 630–637. https://doi.org/10.20527/jptt.v6i2.1844

Kai, M. (2020). Diversity and distribution of volatile secondary metabolites throughout *Bacillus subtilis* isolates. *Frontiers in Microbiology, **11***, 559. https://doi.org/10.3389/fmicb.2020.00559

Karačić, V., Miljaković, D., Marinković, J., Ignjatov, M., Milošević, D., Tamindžić, G., & Ivanović, M. (2024). *Bacillus* Species: Excellent biocontrol agents against tomato diseases. *Microorganisms*, ***12***(3), 457. https://doi.org/10.3390/microorganisms12030457

Komalasari, W.B. (2024). *Analisis Kinerja Perdagangan Jagung* (Vol. 14, Issue 1B). Pusat Data dan Sistem Informasi Pertanian Sekretariat Jenderal - Kementerian Pertanian, Jakarta.

Krishanti, N.P.R.A., Wikantyoso, B., Zulfitri, A., & Zulfiana, D. (2017). Bakteri entomopatogen sebagai agen biokontrol terhadap larva *Spodoptera litura* (F.). *Berita Biologi*, ***16***(1), 13–21.

National Center for Biotechnology Information. (2025). *PubChem compound summary for CID 5950, Alanine*. PubChem. https://pubchem.ncbi.nlm.nih.gov/compound/5950

Nelly, N., Hamid, H., Lina, E.C., Yunisman, Rusli, R., Yanti, Y., & Kairunisa, M. (2024). Effectiveness of *Bacillus* spp. from West Sumatra, Indonesia in controlling *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Biodiversitas*, ***25***(4), 1472–1478. https://doi.org/10.13057/biodiv/d250415

Nonci, N., Kalgutny, H.S., Mirsam, H., Muis, A., Azrai, M., & Aqil, M. (2019). *Pengenalan Fall Armyworm (Spodoptera frugiperda J.E. Smith) Hama Baru pada Tanaman Jagung di Indonesia*. Badan Penelitian dan Pengembangan Pertanian Balai Penelitian Tanaman Serealia.

Novizan. (2002). *Membuat dan Memanfaatkan Pestisida Ramah Lingkungan*. Agromedia Pustaka.

Pardo-López, L., Soberón, M., & Bravo, A. (2013). *Bacillus thuringiensis* insecticidal three-domain Cry toxins: Mode of action, insect resistance and consequences for crop protection. *FEMS Microbiology Reviews*, ***37***(1), 3–22. https://doi.org/10.1111/j.1574-6976.2012.00341.x

Prihatiningsih, N., & Djatmiko, H.A. (2016). Enzim amilase sebagai komponen antagonis *Bacillus subtilis* B315 terhadap *Ralstonia solanacearum* kentang. *Jurnal Hama dan Penyakit Tumbuhan Tropika*, ***16***(1), 10–16. https://doi.org/10.23960/j.hptt.11610-16

Purnawati, A., Triwahyu P, E., & Fari A, H. (2024). Potential of encapsulation *Bacillus cereus* BTH-22 againts bacterial wilt disease on eggplant. *Jurnal Bioteknologi dan Biosains Indonesia*, ***11***(1), 155–161. https://ejournal.brin.go.id/JBBI/article/view/3736

Rahman, A., Hamid, H., & Resti, Z. (2023). Pathogenicity of endophytic bacteria as entomopathogens against *Spodoptera litura* fabricius. (Lepidoptera: noctuidae). *CROPSAVER: Journal of Plant Protection*, ***6***(1), 10-18. https://doi.org/10.24198/cropsaver.v6i1.44665

Rohma, M., & Wahyuni, W.S. (2022). Pengendalian penyakit layu *Fusarium oxysporum* f.sp cepae pada tanaman bawang merah dengan air rebusan serai dapur (*Cymbopogon citratus*). *Berkala Ilmiah Pertanian*, ***5***(2), 65. https://doi.org/10.19184/bip.v5i2.28856

Rosmania, R., & Yuniar, Y. (2021). Pengaruh waktu penyimpanan inokulum *Escherichia coli* dan *Staphilococcus aureus* pada suhu dingin terhadap jumlah sel bakteri di Laboratorium Mikrobiologi. *Jurnal Penelitian Sains*, ***23***(3), 117-124. https://doi.org/10.56064/jps.v23i3.624

Senewe, E., Maramis, R., & Salaki, C.L. (2012). Pemanfaatan bakteri entomopatogenik *Bacillus cereus* terhadap hama *Spodoptera litura* pada tanaman kubis. *Eugenia*, ***21***(3), 134-144. https://doi.org/10.35791/eug.18.2.2012.3567

Septian, R.D., Afifah, L., Surjana, T., Saputro, N.W., & Enri, U. (2021). Identifikasi dan efektivitas berbagai teknik pengendalian hama baru ulat grayak *Spodoptera frugiperda* J.E. Smith pada tanaman jagung berbasis PHT-Biointensif. *Jurnal Ilmu Pertanian Indonesia*, ***26***(4), 521–529. https://doi.org/10.18343/jipi.26.4.521

Setiowati, E.D. (2023). Skrining aktivitas antioksidan tanaman obat tropis di Taman Nasional Meru Betiri (Tnmb) berdasarkan hasil Gc-Ms serta pemanfaatannya sebagai book chapter. [*Undergraduated* Thesis]. Fakultas Keguruan dan Ilmu Pendidikan. Universitas Jember.

Sianipar, G.W.S., Sartini, S., & Riyanto, R. (2020). Isolasi dan karakteristik bakteri endofit pada akar pepaya (*Carica papaya* L). *Jurnal Ilmiah Biologi UMA (JIBIOMA)*, ***2***(2), 83–92. https://doi.org/10.31289/jibioma.v2i2.312

Smith, B.C. (2019). Organic Nitrogen compounds VI: Introduction to Amides. *Spectroscopy*, ***34***(11), 30–33.

Tan, C.Y., Wainman, D., & Weaver, D. (2003). N-, α-, and β-Substituted 3-Aminopropionic acids: design, syntheses and antiseizure activities. *Bioorganic & Medicinal Chemistry*, ***11***(1), 113–121. https://doi.org/10.1016/S0968-0896(02)00330-9

Taufika, R., Sumarmi, S., & Hartatie, D. (2022). Pemeliharaan ulat grayak (*Spodoptera litura* Fabricius) (Lepidoptera: Noctuidae) menggunakan pakan buatan pada skala laboratorium. *AGROMIX*, ***13***(1), 47–54. https://doi.org/10.35891/agx.v13i1.2866

Yulianti, T. (2013). Pemanfaatan endofit sebagai agensia pengendali hayati hama dan penyakit tanaman. *Buletin Tanaman Tembakau, Serat dan Minyak Industri*, ***5***(1), 40–49. https://doi.org/10.21082/bultas.v5n1.2013.40-49