Vol. 15, No. 2 (2026): 608 - 617 http://dx.doi.org/10.23960/jtepl.v15i2.608-617

JURNAL TEKNIK PERTANIAN LAMPUNG

ISSN 2302-559X (print) / 2549-0818 (online)
Journal homepage : https://jurnal.fp.unila.ac.id/index.php/JTP

The Potential for Controlling Maize Pest *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Using Biological Agents *Bacillus* spp.

Muhammad Qoyyimul Amri[1], Yenny Wuryandari[1,✉], Noni Rahmadhini[1]

[1] Department of Agrotechnology, Faculty of Agriculture, UPN "Veteran" Jawa Timur, Surabaya, INDONESIA.

Article History:

Received : 9 June 2025
Revised : 25 December 2025
Accepted : 14 January 2026

Keywords:

Bacteria,
Biological agent,
Fall armyworm,
Maize pest,
Mortality.

Corresponding Author:
✉ yennywuryandari@upnjatim.ac.id
(Yenny Wuryandari)

ABSTRACT

Maize production is still facing many challenges nowadays. One of the main challenges in maize production is pest attacks. S. frugiperda has been causing significant damage to maize farming in Indonesia recently. This research was conducted to determine the potential of Bacillus spp. isolates Bcz-20 and Bcz-30 are identified as biological agent candidates to control the S. frugiperda population, with bacterial population densities of 10^9 CFU/mL, 10^8 CFU/mL, and 10^7 CFU/mL, respectively, as determined by in vitro and in vivo application tests. The Bcz-20 treatment with a population density of 10^7 CFU/mL was capable of suppressing the S. frugiperda larvae population in an in vitro application test, resulting in a mortality percentage of 47.5%. The Bcz-20 treatment with a population density of 10^9 CFU/mL was capable of suppressing the S. frugiperda larvae population by in vivo application test, resulting in a mortality percentage of 70 %. The conclusion, Bacillus spp. Bcz-20 isolates with a population density of 10^7 CFU/mL are efficient and effective for controlling the S. frugiperda larvae population.

1. INTRODUCTION

Maize (*Zea mays* L.) is included in the main food ingredient group in Indonesia (Fatikhasari *et al.*, 2022). National maize production in 2021 reached 57.09 Qt/ha, Java Island tends to have a higher average production yield compared to outside Java, which was 60.09 Qt/ha (Astuti *et al.*, 2022). National corn demand in 2021 reached 14.37 tons (Minarsih *et al.*, 2022), equaling 143.7 Qt.

Maize production nowadays still faces many challenges. One of the main challenges in maize production is pest attacks. *Spodoptera frugiperda* are lately attacking maize crops in Indonesia massively. This pest is an invasive insect that has become the main pest in maize crops in Indonesia (Dudurang *et al.*, 2023). This pest attack can cause a significant loss of yield, up to 80 % (Sari, 2020). This pest has been reported to attack Indonesian maize crops in the regions of Sumatra, West Java, East Java, and Yogyakarta. *S. frugiperda* attacks early maize crops and leaf shoots that are marked by the presence of bite marks (Megasari *et al.*, 2022).

Serious and ongoing control is needed to deal with this pest. One of the alternatives to sustainable control is by utilizing biological agents. Pest control with this method is considered an environmentally friendly way of control, because it does not produce residues that endanger to environment. Control using biological agents was very effective in integrated pest management (IPM) systems (Lugito *et al.*, 2023). Alternatives to biological agents that can be used in controlling pests come from various types. A biological agent that has the potential to control *S. frugiperda* is *Bacillus* spp. Sutriono & Zahar (2022) research obtained a result of 100 % *S. litura* larval mortality on 5–8 days after application of *Bacillus thuringiensis*, with application concentrations of 10 g/L, 20 g/L, and 30 g/L.

Some *Bacillus* spp. Isolates are also known to play a role as a biological agent. The result of the research conducted by Zinidin (2022) mentioned *Bacillus* spp. Isolates Bcz-20 and Bcz-30 have a bactericidal antibiotic mechanism against the pathogen *Ralstonia solanacearum* causes diseases in chili crops. The diameter of the inhibition zone from the result of the research for Bcz-20 and Bcz-30 isolates against *R. solanacearum,* respectively, was 34.17 mm and 34.67 mm. Another research conducted by Heriyati (2023) mentioned that *Bacillus* spp. with a population density of 10^8 CFU/mL can inhibit fungal growth of *Fusarium* sp. by 20.02% in vitro. More research by Anjarsari *et al.* (2022) obtained results that *Bacillus* sp. with a population density of 10^9 CFU/mL can inhibit the development of pathogenic fungi *Phytophthora palmivora,* causing cocoa fruit rot, with an inhibition percentage of 31.5–61 % in vitro. This research was conducted to determine the potential of *Bacillus* spp. isolate Bcz-20 and Bcz-30 as candidates of biological agents as control *S. frugiperda* larvae, using bacteria population density of 10^9 CFU/mL, 10^8 CFU/mL, and 10^7 CFU/mL.

2. MATERIALS AND METHODS

2.1. Instruments and Materials

The research was carried out at the Plant Health Laboratory and the Screen House of the Faculty of Agriculture, UPN "Veteran" Jawa Timur. The instruments used to support this research include: 16 mm × 100 mm test tube, 9 cm, petri dish, inoculation loop, 200 µL micropipette, yellow tip, erlenmeyer flask 35 mL plastic cup, 32 cm × 25 cm, plastic basket, tweezer, fine brush number 2, measuring tape 5 m, beaker glass 50 mL, beaker glass 100 mL, vortex mixer, hoe, shovel, crop marker, 100 mL sprayer, cover made from organza fabric.

The materials used to support this research activity include: *Bacillus* spp. Bcz-20 and Bcz-30 isolates collection of Dr. Ir. Yenny Wuryandari, M.P., nutrient agar (NA), *S. frugiperda* instar 2–3 larvae, maize leaves, millimeter block paper, 70% alcohol, sterile aquadest.

2.2. Research Design

Equal to research by Anjani *et al.* (2025), Completely Randomized Design (CRD) Factorial with two factors was used in this research. The two factors are the type of bacterial isolates (B) and the density of the bacterial population (K). The type of isolates used are *Bacillus* spp. isolate Bcz-20 (B_1) and Bcz-30 (B_2). The density of the bacterial population (K) used are of 10^9 CFU/mL (K_1), 10^8 CFU/mL (K_2), 10^7 CFU/mL (K_3). This population density refers to research conducted by Anjarsari *et al.* (2022), who use isolates of *Bacillus* sp. with a population density of 10^9 CFU/mL. More research was conducted by Heriyati (2023), who used isolates of *Bacillus* spp. with a population density of 10^8 CFU/mL. In vitro and in vivo application tests were carried out with 8 treatments (6 main treatments and 2 controls) and 4 repetitions. The number of repetitions is calculated using Gomez's equation with the formula $t\,(r-1) \geq 15$ (Gunawan *et al.*, 2023).

There are two controls used in this research for each repetition. The first control is a negative control (KO_1). KO_1 uses only sterile aquadest. The second control is the positive control (KO_2). This KO_2 uses Turex WP insecticide with a dose of 2 g/L. Turex WP is a biological insecticide that works as a light brown flour-shaped stomach poison that can be suspended, used to control armyworms, *S. litura* on soybean crops Wibawa (2018).

2.3. Preparation

2.3.1. *S. frugiperda* Larvae Preparation

S. frugiperda eggs were obtained from the area of Balai Penelitian Tanaman Pemanis dan Serat di Kabupaten Malang (Sweetener and Fiber Crops Research Institute, Malang Regency). The eggs will be hatched and maintained until it can be confirmed that those are *S. frugiperda* larvae. The larvae used as test materials came from eggs of *S. frugiperda* larvae that had been reared previously. The total number of larvae used for treatment was 640, for both in vitro and in vivo application tests.

Fitriana *et al.* (2020) research using *S. frugiperda* instar 2 larvae for a total of ten per cup. Ten instar 3 *S. frugiperda* larvae in each treatment also used by Kurniawati *et al.* (2025). Based on both researches, the larvae used in this research are *S. frugipeda* instar 2–3, ten larvae per treatment. In vitro application test using ten larvae per treatment repetition,

divided into five plastic cups, each cup containing two *S. frugiperda* larvae. In vivo application test using ten *S. frugiperda* larvae per maize crop.

2.3.2. Preparation of *Bacillus* spp. Suspension

The series of processes to prepare *Bacillus* spp. suspensions of Bcz-20 (B_1) and Bcz-30 (B_2) isolates in this research began with preparing five test tubes of *Bacillus* spp. culture on nutrient agar media for each isolate. The cultures were then harvested at the age of less than 24 hours by adding 10 mL of sterile aquadest to each test tube. The surface where the bacteria grew was then scraped using an inoculation loop, then homogenized using a vortex mixer and collected in one Erlenmeyer flask with a total suspension volume of 50 mL. 10 mL suspension was taken from the stock suspension, then poured into an Erlenmeyer flask containing 50 mL of sterile aquadest, and homogenized with a vortex mixer. 10 mL suspension is taken out of a total of 60 mL. The homogeneous suspension then take another 10 mL to be dilute nine times. A suspension of 0.2 mL, each grown on nutrient agar media in a petri dish. Based on preliminary observations that have been made, colony count can be done in the range of 16–21 h before the growth of bacterial colonies is too widespread. The bacterial population density of 10^9 CFU/mL was obtained through this method.

The bacterial population density of 10^8 CFU/mL and 10^7 CFU/mL was also obtained by the same measures as the bacterial population density of 10^9 CFU/mL. The only difference is the volume of sterile aquadest mixed with 10 mL of stock suspension. The bacterial population density of 10^8 CFU/mL was obtained by homogenizing 10 mL of stock suspension and 70 mL of sterile aquadest. The bacterial population density of 10^7 CFU/mL was obtained by homogenizing 10 mL of stock suspension and 90 mL of sterile aquadest.

The counting of bacterial colonies was carried out using the Total Plate Count (TPC) and stated as Colony Forming Unit (CFU/mL) Laili *et al.* (2022). This calculation is to find out if the density of the bacterial population is as desired, which is 10^9 CFU/mL (K_1), 10^8 CFU/mL (K_2), and 10^7 CFU/mL (K_3). TPC calculated using the formula:

$$\text{Total Plate Count (TPC)} = Number\ of\ colonies\ per\ petri\ dish \times \frac{1}{Dilution\ factor} \quad (1)$$

2.3.3. Maize Crop Preparation

Deden *et al.* (2023) conducted research using 17 days after planting (DAP) maize crops. Based on this research, maize crops with an age close to 17 DAP were used in this research, namely maize crops of 21 DAP. Maize planting was carried out in a 20 cm × 27 cm polybag. The planting medium consists of a combination of soil and compost with a ratio of 2 : 1. The planting medium was sterilized by stirring well with formalin (5 % concentration), with a dose of 2.5 mL/kg, then wrapped in plastic to let it sit for 7 days, followed by drying for 7 days (Sianipar *et al.*, 2019). Three maize seeds were planted into each polybag. The crops used for the in vivo application test are one of the best crops of each polybag, while the remaining two were discarded.

The maize leaves used for the in vitro application test were the same as the leaves of the maize crop used for the in vivo application test, which comes from the same type of seed. Based on Fitriana *et al.* (2020) research, maize leaf feed is required in the amount of 5 grams per day for ten larvae, so that each cup contains 1 gram of maize leaves for two larvae. The maize leaves were first dipped with Bcz-20 (B_1) and Bcz-30 (B_2) suspensions, respectively, according to the desired population density, 10^9 CFU/mL (K_1), 10^8 CFU/mL (K_2), 10^7 CFU/mL (K_3). Leaves were dipped in suspension for the in vitro application test, and sprayed with suspension for the in vivo application test.

2.4. Procedure

2.4.1. In Vitro Application Test of *Bacillus* spp. to *S. frugiperda* Larvae

The in vitro application test was carried out in a laboratory environment with the main components of *S. frugiperda* larvae and corn leaves that have been dipped in *Bacillus* spp. suspension. This application test uses a plastic cup for every two individual *S. frugiperda* larvae. A total of 20 cups with a total of 40 *S. frugiperda* larvae were placed in one plastic box, consisting of four treatments divided into five rows in one box. The plastic box used totals 9 units, so there are 320 larvae for in vitro application tests. 5 grams of maize leaves were given for each treatment every day, each cup filled with 1 gram of maize leaves according to the treatment. The leaves were first dipped in Bcz-20 (B_1) and Bcz-30

(B_2) suspensions according to the population density K_1 – K_3 (10^9 – 10^7 CFU/mL). Incubation is carried out within 7 days. Data collection of *S. frugiperda* larval mortality was carried out for 7 days of observation.

2.4.2. In Vivo Application Test of *Bacillus* spp. to *S. frugiperda* Larvae

The in vivo application test was carried out on a screen house scale. The main components of the in vivo application test were maize crops with leaves that had been sprayed with *Bacillus* spp. suspension and *S. frugiperda* larvae. The maize crops used are 32 crops. Spraying of Bcz-20 (B_1) and Bcz-30 (B_2) suspensions with a dose of 2.4 mL per leaf was carried out first according to the population density of K_1 – K_3 (10^9 – 10^7 CFU/mL). Ten larvae of *S. frugiperda* were then infested on each maize crop. A cover sheathes each crop to the base of the polybag to prevent crops from being disturbed by other pests, and prevent *S. frugiperda* larvae from moving to another crop. Data collection of larval mortality and apparent symptoms of larval attack in the maize crop was carried out for 7 days of observation.

2.5. Parameter

2.5.1. Larval Mortality of *S. frugiperda*

Larval mortality of *S. frugiperda* observation observed in in vitro and in vivo application tests. Observation of the mortality percentage was carried out by dividing the number of dead larvae by the total number of larvae, multiplied by 100 % (Putri *et al.*, 2022).

$$\%\ Mortality = \frac{Number\ of\ dead\ larvae}{Total\ number\ of\ larvae} \times 100\% \quad (2)$$

2.5.2. Attack Symptoms of *S. frugiperda* Larvae

Symptoms of *S. frugiperda* larval attack observed during in vivo application tests. It was done by observing the presence of holes in the leaves that are still buds or have not been fully opened, parallel transparent spots on the leaves, and the presence of feces on and/or around the crops. These three types of symptoms are signs of the presence and attack of *S. frugiperda*, especially instar 2–3 observed. The signs are based on statements by Maharani *et al.* (2019), Trisyono *et al.* (2019), and Putra *et al.* (2024).

Affected crop shoots (by *S. frugiperda*), if the leaves have not opened fully (buds), appear hollow, and there are many larval feces. It can be seen that many parts of the leaves are damaged, with holes on the leaves that have been opened and attacked by larvae (Maharani *et al.*, 2019). Early *S. frugiperda* instar (1–2 instar) attack in the form of parallel transparent spots on the leaves, while in the advanced instar (3–6 instar), the bite holes on the leaves form parallel (Trisyono *et al.*, 2019; Putra *et al.*, 2024).

2.6. Data Analysis

The data from the research was analysed using ANOVA (Analysis of Variance). If the conclusion is obtained under the condition calculated F > F table 5%, then the average difference between treatments is tested using the DMRT (Duncan Multiple Range Test) at a real level of 5 % Hariyanto *et al.* (2022), using SPSS 16.0 software.

3. RESULTS AND DISCUSSION

3.1. Mortality of *S. frugiperda* Larvae as an In Vitro Application Test

The results of *Bacillus* spp. in vitro application tests against *S. frugiperda* larvae show two conditions, namely healthy or asymptomatic larvae, and dead larvae with symptoms of poisoning (Figure 1). Healthy *S. frugiperda* larvae, based on preliminary observations, show bright green to brown body surface features, dense body texture, shiny clear caput, and fast mobility and voracious eating activities (Figure 1. (a)). Research conducted by Putri *et al.* (2024) also describes a similar trait, namely a green to dark brown body during the instar phases 3–5. Dead larvae show characteristics of skin discoloration to become darker and opaque, as well as body fluids out of the body (exocytosis) so that the larvae's body texture is not too dense or tends to be soft (Figure 1. (b)). Research result by da Costa *et al.* (2020), stated that the *S. frugiperda* larvae those tested showed symptoms of poisoning, such as low mobility, blurred colour, and soft texture.

These symptoms were also similar to the results obtained by Karshanal & Kalia (2023), that the larvae are found dead on the feed surface and become mushy after death. The results of the same research also mentioned that in the Bt treatment, the intestinal part of the larvae turned black.

Karshanal & Kalia (2023) research results were also strengthened by the results of Mohammad *et al.* (2024) research, which stated that *B. thuringiensis* toxicity is likely caused by the Cry protein produced by the bacteria, causing larval death. Research conducted by Knaak *et al.* (2010) has confirmed that strains and proteins synthesized by *B. thuringiensis thuringiensis* 407 (pH 408) and *B. thuringiensis kurstaki* HD-73 are efficient in controlling *S. frugiperda*, and the Cry1Ac protein was the most effective, with an LC_{50} value of 1.79 against instar 1 *S. frugiperda* larvae.

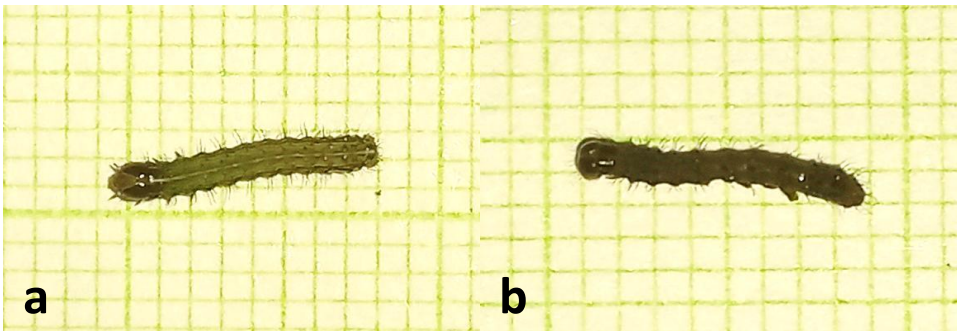

Figure 1. *S. frugiperda* larvae in vitro application tests result: (a) healthy larvae; (b) dead larvae with poisoning symptoms

The results of the ANOVA test on *S. frugiperda* larval mortality data from the in vitro application test stated that there was no difference between the mortality data. The results of the ANOVA test also showed no interaction between the bacterial isolates of *Bacillus* spp. (Bcz-20 and Bcz-30) and the bacterial population density (10^9 CFU/mL, 10^8 CFU/mL, and 10^7 CFU/mL) on the mortality of *S. frugiperda* larvae by an in vitro application test. This conclusion was obtained from the significant value in the results of the ANOVA Test of 0.322 for the type of bacterial isolate; 0.693 for bacterial population density, as well as 0.125 for a combination of bacterial isolate type and bacterial population density, where the significant values are greater than 0.05.

Based on the data listed in Table 1, it is known that the highest mortality percentage of the in vitro application test reached 47.5 %. The highest mortality percentage occurred in treatment with *Bacillus* spp. Bcz-20 isolates with a population density of 10^7 CFU/mL (B_1K_3). The lowest mortality percentage of the in vitro application tests was only 30 %. The lowest mortality percentage occurred in treatment with *Bacillus* spp. Bcz-30 isolates with a population density of 10^9 CFU/mL (B_2K_1). These mortality percentages were lower when compared to the mortality percentage in positive controls using Turex WP insecticide dose 2 g/L (KO_2), which reached 60 %. Turex WP is an insecticide containing *Bacillus thuringiensis* var. Aizawai GC-91: 3.8 %.

Table 1. Mortality of *S. frugiperda* larvae by in vitro application tests

Treatment Code	Treatment Description	% Mortality
KO_1	Aquades sterile (negative control)	27.5
KO_2	Turex WP Insecticide dosage 2 g/L (positive control)	60
B_1K_1	Bcz-20 population density 10^9 CFU/mL	42.5
B_1K_2	Bcz-20 population density 10^8 CFU/mL	32.5
B_1K_3	Bcz-20 population density 10^7 CFU/mL	47.5
B_2K_1	Bcz-30 population density 10^9 CFU/mL	30
B_2K_2	Bcz-30 population density 10^8 CFU/mL	42.5
B_2K_3	Bcz-30 population density 10^7 CFU/mL	35

Information: The results of the ANOVA test showed that the data between treatments were not significantly different; Controls were not included in the ANOVA Test

Bcz-20 (B_1) isolates were able to cause *S. frugiperda* larval mortality to be close to 50 %, namely at a population density of 10^7 CFU/mL (K_3) of 47.5 % and 10^9 CFU/mL (K_1) of 42.5 %. These results support the fact that *Bacillus* spp. isolate Bcz-20 (B_1) can cause *S. frugiperda* larvae mortality. Considering the mortality data in Table 1, with a lower

population density, namely 10^7 CFU/mL (K_3), *Bacillus* spp. was able to kill *S. frugiperda* larvae. With no different result from the higher population density of *Bacillus* spp. The application test of both *Bacillus* spp. isolates were more efficient and effective using a population density of 10^7 CFU/mL (K_3) by in vitro application tests.

3.2. Symptoms of *S. frugiperda* Larval Attack on Maize Crops

Attack symptoms of *S. frugiperda* larvae were observed to ensure the presence and life of the *S. frugiperda* larvae on maize crops as a test material for an in vivo application test. Three attack symptoms of *S. frugiperda* were observed in this research. The three symptoms of the attack were the presence of holes in the leaves that are still buds or have not been fully opened, parallel transparent spots on the leaves, and the presence of feces on and/or around the crops. These three types of symptoms are based on statements by Maharani *et al.* (2019), Trisyono *et al.* (2019), and Putra *et al.* (2024). Figure 2 shows three symptoms observed in this research. Figure 2a to 2c respectively show symptoms of maize plant attacked by *S. frugiperda* in form of holes in the partly opened leaf, parallel transparent spots on the leaf, and feces of *S. frugiperda* on the leaf.

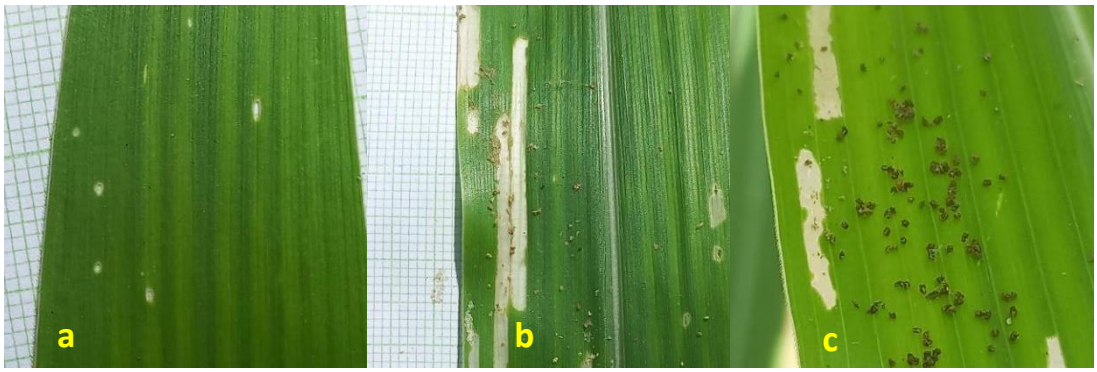

Figure 2. Symptoms of *S. frugiperda* larval attack on maize crops: (a) holes in the partly opened leaf; (b) parallel transparent spots on the leaf; (c) feces on the leaf

Table 2. Symptoms of *S. frugiperda* larval attack on maize crops

Treatment Code	Treatment Description	Holes in partly opened leaves	Parallel transparent spots on leaves	Feces on and/or around crops
KO_1	Aquades sterile (negative control)	√	√	√
KO_2	Turex WP Insecticide dosage 2 g/L (positive control)	√	√	√
B_1K_1	Bcz-20 population density 10^9 CFU/mL	√	√	√
B_1K_2	Bcz-20 population density 10^8 CFU/mL	-	√	√
B_1K_3	Bcz-20 population density 10^7 CFU/mL	√	√	√
B_2K_1	Bcz-30 population density 10^9 CFU/mL	-	√	√
B_2K_2	Bcz-30 population density 10^8 CFU/mL	-	√	√
B_2K_3	Bcz-30 population density 10^7 CFU/mL	√	√	√

Information: Observation of the *S. frugiperda* symptoms attack was carried out on the 1st to 7th days after application.

Mulyani *et al.* (2024) describe the attack that begins with the larvae feeding on the tissue on the leaf surface, so that the leaves appear transparent with a diameter of less than 5 mm. The next attack of the larvae will create an increasingly large bored hole of irregular shape, on the leaf surface or around the feeding area there was dirt such as sawdust. More severe damage the larvae attack the shoots of the plant, eating the inside, causing damage to the shoots.

Based on the data in Table 2, known that all three types of *S. frugiperda* larval attack symptoms occur in almost all treated maize crops. The absence of holes in the bud leaves or not fully opened leaves only occurs in plants with Bcz-20 treatment with a population density of 10^8 CFU/mL, Bcz-30 with a population density of 10^9 CFU/mL, and Bcz-

30 with a population density of 10^8 CFU/mL. The results of the treatment were better when compared to the two controls who experienced three attack symptoms according to the desired parameters.

3.3. Mortality of *S. frugiperda* Larvae as an In Vivo Application Test

Results of biological agent *Bacillus* spp. in vivo application tests against *S. frugiperda* larvae show two conditions, namely healthy or asymptomatic larvae, and dead larvae with poisoning symptoms (Figure 3). Healthy *S. frugiperda* larvae, based on preliminary observations, show the characteristics of a bright green to brown body surface, a dense body texture, a shiny and clear caput, and fast mobility and gluttonous eating activities (Figure 3 (a)). Larvae that have died show the characteristics of skin discoloration to become darker and opaque, as well as body fluids out of the body (exocytosis), so the larvae body texture is not too dense or tends to be soft (Figure 3 (b)). da Costa *et al.* (2020) in their research, stated that the *S. frugiperda* larvae those tested showed symptoms of poisoning, such as low mobility, blurred color, and soft texture.

More specific larval mortality symptoms are shown in Figure 3 (b), namely, the colour of the caput is feculent, the body texture is soft, and the digestive part looks blackish or darker than other parts of the body. These symptoms are supported by the results obtained by Karshanal & Kalia (2023) that in Bt treatment, the intestinal part of the larvae turns black. The larvae are found dead on the feed surface and become mushy after death. These results are also strengthened by the Mohammad *et al.* (2024) research result, which stated that larvae fed with bacteria show severe changes in the part of the midgut involving separation and destruction basement membrane and degeneration of vacuoles severe in epithelial lining in the midgut and malpighian tubules, with degradation and folding peritrophic matrix. *B. thuringiensis* toxicity is also mentioned, likely caused by the Cry protein produced by the bacteria, causing death in the larvae. In a research conducted by Knaak *et al.* (2010) it was confirmed that strains and proteins synthesized by *B. thuringiensis thuringiensis* 407 (pH 408) and *B. thuringiensis kurstaki* HD-73 are efficient in controlling *S. frugiperda*, and the Cry1Ac protein was the most effective, with an LC50 value of 1.79 against first instar *S. frugiperda* larvae.

Figure 3. *S. frugiperda* larvae in vivo application tests result: (a) healthy larvae; (b) dead larvae with poisoning symptoms

The results of the ANOVA test on *S. frugiperda* larval mortality data by the in vivo application test stated that there was no difference between the mortality data. The results of the ANOVA test on the mortality of *S. frugiperda* larvae by the in vivo application test also showed no interaction between the bacterial isolates of *Bacillus* spp. (Bcz-20 and Bcz-30) and the bacterial population density (10^9 CFU/mL, 10^8 CFU/mL, and 10^7 CFU/mL) on the mortality of *S. frugiperda* larvae by an in vivo application test. This conclusion was obtained from the significant value in the results of the ANOVA Test of 0.249 for the type of bacterial isolate; 0.699 for bacterial population density, as well as 0.971 for a combination of bacterial isolate type and bacterial population density, where the significant values are greater than 0.05.

Based on the data listed in Table 3, known that the highest percentage of mortality by in vivo application test reached 70 %. The highest mortality percentage occurred in treatment with *Bacillus* spp. Bcz-20 isolates with a population density of 10^9 CFU/mL (B_1K_1). The lowest mortality percentage by in vivo application test was only 52.5 %. The

lowest mortality percentage occurred in treatment with *Bacillus* spp. Bcz-30 isolates with a population density of 10^8 CFU/mL (B_2K_2). These percentages were higher when compared to the percentage of larval mortality in positive controls using Turex WP insecticide dose of 2 g/L (KO2), which reached 50 %. Turex WP is an insecticide containing *Bacillus thuringiensis* var. Aizawai GC-91: 3.8 %.

Table 3. Mortality of *S. frugiperda* larvae by in vivo application tests

Treatment Code	Treatment	% Mortality
KO_1	Aquades sterile (negative control)	27.5
KO_2	Turex WP Insecticide dosage 2 g/L (positive control)	50
B_1K_1	Bcz-20 population density 10^9 CFU/mL	70
B_1K_2	Bcz-20 population density 10^8 CFU/mL	62.5
B_1K_3	Bcz-20 population density 10^7 CFU/mL	67.5
B_2K_1	Bcz-30 population density 10^9 CFU/mL	62.5
B_2K_2	Bcz-30 population density 10^8 CFU/mL	52.5
B_2K_3	Bcz-30 population density 10^7 CFU/mL	55

Information: The results of the ANOVA test showed that the data between treatments were not significantly different; Controls were not included in the ANOVA Test

Research conducted by Nelly *et al.* (2024) using *Bacillus* spp. suspension with a concentration of 10^8 mol/mL shows the ability to suppress *S. frugipeda* larval populations significantly, with mortality on the fourth day reaching 61.67 %. This percentage is not much different when compared to the results of the in vivo application test at a population density of 10^8 CFU/mL, which is 62.5 % in the Bcz-20 (B_1K_2).

Isolates Bcz-20 (B_1) are capable of causing *S. frugiperda* larval mortality of more than 50 %. Highest mortality in population density 10^9 CFU/mL (K_1) by 70% and population density 10^7 CFU/mL (K_3) by 67.5 %. These results support the fact that *Bacillus* spp. isolate Bcz-20 (B_1) can cause *S. frugiperda* larval mortality. These results are also supported by Zakqy *et al.* (2024) research result, which stated that the treatment with *Bacillus* spp. Bcz-20 isolate at a dose of 30 mL/plant (population density 10^9 CFU/mL) shows a suppression of pathogen intensity *Fusarium* sp. with an emphasis of 58.94 %. Considering the mortality data in Table 3, with a lower population density, namely 10^7 CFU/mL (K_3), all isolates of *Bacillus* spp. capable of killing larvae of *S. frugiperda*. With results that do not differ from the higher population density of *Bacillus* spp. The application test of both *Bacillus* spp. isolates are more efficient and effective by using a population density of 10^7 CFU/mL (K_3) in an in vivo application test.

4. CONCLUSION

The research prove that Bacillus spp. can be used as an effective biological agent to control Spodoptera frugiperda pests in corn plants. The results of this research concluded that *Bacillus* spp. Bcz-20 isolates with a population density of 10^7 CFU/mL efficiently and effectively caused mortality of *S. frugiperda* larvae by in vitro and in vivo. At this density, *Bacillus* spp. Bcz-20 results in 67.5% mortality of *S. frugiperda.* Furthermore, *Bacillus* spp. Bcz-20 isolate has the potential to be developed as a biological agent that can be widely used for controlling pest *S. frugiperda* in maize crop.

AUTHOR CONTRIBUTION STATEMENT

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
MQA	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓
YW	✓	✓		✓	✓		✓	✓		✓	✓	✓		✓
NR	✓	✓		✓	✓		✓	✓		✓	✓	✓		✓

C: Conceptualization
M: Methodology
So: Software
Va: Validation
Fo: Formal Analysis
I: Investigation
D: Data Curation
R: Resources
O: Writing - Original Draft
E: Writing - Review & Editing
Vi: Visualization
Su: Supervision
Fu: Funding Acquisition
P: Project Administration

REFERENCES

Anjani, A.D., Wuryandari, Y., & Lestari, S.R. (2025). Optimization of bioencapsulation of *Bacillus* sp. to increase resistance to bacterial wilt and growth of cayenne pepper. *Jurnal Teknik Pertanian Lampung (Journal of Agricultural Engineering)*, ***14***(4), 1325–1333. https://doi.org/10.23960/jtepl.v14i4.1325-1334

Anjarsari, D.T., Prasetyawati, E.T., & Wuryandari, Y. (2022). Inhibitory test of *Bacillus* sp. against phytophthora palmivora causes cocoa fruit rot disease. *Nusantara Science and Technology Proceedings*, 14–21. https://doi.org/10.11594/nstp.2022.2003

Astuti, K., Ramdhani, D.M., & Khasanah, I.N. (2022). *Analisis produktivitas jagung dan kedelai di Indonesia 2021 (Hasil Survei Ubinan)* (W. P. Buana, H. Ferdinan, S. Haryanto, S. Suwarti, & I. Wirawati (eds.)). BPS - Statistics Indonesia. https://www.bps.go.id/id/publication/2022/12/16/9e87d65dae851717a1af5784/analisis-produktivitas-jagung-dan-kedelai-di-indonesia--2021.html

da Costa, F.S.S., Praça, L.B., Gomes, A.C.M.M., dos Santos, R.C., Soares, C.M.S., & Monnerat, R.G. (2020). *Bacillus thuringiensis* effect on the vegetative development of cotton plants and the biocontrol of *Spodoptera frugiperda*. *Agronomy,* ***10***(12), 1889. https://doi.org/10.3390/agronomy10121889

Deden, D., Umiyati, U., & Dukat, D. (2023). Preferensi dan intensitas serangan *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) pada berbagai varietas tanaman jagung manis (*Zea mays* saccharata). *Jurnal Agrotek Tropika*, ***11***(2), 173–179. http://dx.doi.org/10.23960/jat.v11i2.5067

Dudurang, B., Rante, C.S., & Wanta, N.N. (2023). Persentase serangan hama *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) pada tanaman jagung manis di Kecamatan Matuari Kota Bitung. *ENFiT: Jurnal Entomologi Dan Fitopatologi*, ***3***(1), 31–36. https://ejournal.unsrat.ac.id/v3/index.php/enfit/article/view/51123

Fatikhasari, Z., Lailaty, I.Q., Sartika, D., & Ubaidi, M.A. (2022). Viabilitas dan vigor benih kacang tanah (*Arachis hypogaea* L.), kacang hijau (*Vigna radiata* (L.) R. Wilczek), dan jagung (*Zea mays* L.) pada temperatur dan tekanan osmotik berbeda. *Jurnal Ilmu Pertanian Indonesia*, ***27***(1), 7–17. http://dx.doi.org/10.18343/jipi.27.1.7

Fitriana, I.N., Suryaminarsih, P., & Mujoko, T. (2020). Potential of multientomopa *Streptomyces* sp. and *Trichoderma* sp. in potato extract broth and glucose nitrate broth media on pests (*Spodoptera litura*) eating behavior by in vitro test. *Nusantara Science and Technology Proceedings*, 270–276. https://doi.org/10.11594/nstp.2019.0438

Gunawan, S., Kahar, K., & Karmini, M. (2023). Rancang bangun alat peredam kebisingan berbasis variasi ketebalan busa dakron dan egg tray terhadap penurunan intensitas kebisingan di industri farmasi tahun 2023. *Jurnal Pengendalian Pencemaran Lingkungan (JPPL)*, ***5***(2), 118–125. https://doi.org/10.35970/jppl.v5i2.1981

Hariyanto, A.L., Wuryandari, Y., & Suryaminarsih, P. (2022). Efektivitas metabolit sekunder *Pseudomonad fluorescent* sebagai antimikroba patogen *Fusarium oxysporum* secara in vitro. *Jurnal AGROHITA*, *7*(3), 514–518.

Heriyati, S. (2023). Kemampuan beberapa isolat *Bacillus* spp. dalam menghambat perkembangan penyakit layu fusarium dan memacu pertumbuhan tanaman cabai merah (*Capsicum annuum* L.). [*Undergraduate thesis*]. UPN "Veteran" Jawa Timur

Karshanal, J., & Kalia, V.K. (2023). Efficacy of native *Bacillus* isolates against different larval instars of fall armyworm, *Spodoptera frugiperda* alone and in combination. *Egyptian Journal of Biological Pest Control*, ***33***(102), 1–11. https://doi.org/10.1186/s41938-023-00743-7

Knaak, N., Franz, A.R., Santos, G.F., & Fiuza, L.M. (2010). Histopathology and the lethal effect of cry proteins and strains of *Bacillus thuringiensis* erliner in *Spodoptera frugiperda* J.E. Smith Caterpillars (Lepidoptera, Noctuidae). *Brazilian Journal of Biology*, ***70***(3), 677–684. https://doi.org/10.1590/s1519-69842010000300028

Kurniawati, K., Purnawati, A., & Windriyanti, W. (2025). Efficacy of endophytic bacteria as entomopathogens against *Spodoptera frugiperda* (Lepidotera: Noctuidae) on corn (*Zea mays* L.). *Jurnal Teknik Pertanian Lampung (Journal of Agricultural Engineering)*, ***14***(1), 1040–1050. https://doi.org/10.23960/jtep-l.v14i3.1040-1050

Laili, N.H., Abida, I.W., & Junaedi, A.S. (2022). Nilai total plate count (TPC) dan jumlah jenis bakteri air limbah cucian garam (bittern) dari tambak garam Desa Banyuajuh Kecamatan Kamal Kabupaten Bangkalan. *Juvenil: Jurnal Ilmiah Kelautan dan Perikanan*, ***3***(1), 26–31. http://doi.org/10.21107/juvenil.v3i1.15075

Lugito, R., Yunus, M., & Asrul, A. (2023). Pengendalian hayati larva *Spodoptera frugiperda* J.E. Smith dengan menggunakan cendawan entomopatogen metarhizium anisopliae di laboratorium. *Agrotekbis: Jurnal Ilmu Pertanian*, ***11***(2), 429–436.

Maharani, Y., Dewi, V.K., Puspasari, L.T., Rizkie, L., Hidayat, Y., & Dono, D. (2019). Cases of fall army worm *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) attack on maize in Bandung, Garut and Sumedang District, West Java. *CROPSAVER - Journal of Plant Protection*, ***2***(1), 38–46. https://doi.org/10.24198/cropsaver.v2i1.23013

Megasari, D., Putra, I.L.I., Martina, N.D., Wulanda, A., & Khotimah, K. (2022). Biologi *Spodoptera frugiperda* JE Smith pada beberapa jenis pakan di laboratorium. *Agrovigor: Jurnal Agroekoteknologi*, ***15***(1), 63–67.

https://doi.org/10.21107/agrovigor.v15i1.11978

Minarsih, S., Samijan, S., Supriyo, A., Praptana, R.H., & Komalawati, K. (2022). Efektivitas pupuk organik cair hasil aktivasi molekul dalam meningkatkan pertumbuhan dan hasil jagung. *Jurnal Pangan*, ***31***(2), 125–134.

Mohammad, A.M., Elham, A.R., Khalil, A.F., & Ahmed, H.Y. (2024). The potency of the green algae *Chlorella vulgaris* and *Bacillus thuringiensis* as biocides against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). *Functional Foods in Health and Disease*, ***14***(6), 445–454. https://doi.org/10.31989/ffhd.v14i6.1359

Mulyani, M., Syafi'i, M., Afifah, L., & Irfan, B. (2024). Intensitas serangan dan fluktuasi populasi hama ulat grayak (*Spodoptera frugiperda* J.E Smith) pada beberapa galur tetua jagung manis (*Zea mays* saccharata Sturt) MS-UNSIKA Mutan Generasi M7. *Jurnal Agrotech*, ***14***(1), 63–69. https://doi.org/10.31970/agrotech.v14i1.163

Nelly, N., Hamid, H., Lina, E.C., Yunisman, Y., 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

Putra, I.L.I., Aulia, D.R., & Hanafi, Y. (2024). Bentuk serangan *Spodoptera frugiperda* J.E. Smith pada tanaman selain jagung di Kabupaten Kulon Progo. *Jurnal Sains dan Edukasi Sains*, ***7***(1), 58–67. https://doi.org/10.24246/juses.v7i1p58-67

Putri, R.C., Megasari, D., & Rahmadhini, N. (2024). Photoperiod effects on the growth stages of fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) under laboratory conditions. *Jurnal Ilmiah Pertanian (JIP)*, ***21***(3), 198–208. https://doi.org/10.31849/jip.v21i3.20216

Putri, S.N.S., Susanto, A., Bari, I.N., & Santriyani, A.S. (2022). Pengaruh beberapa pelarut ekstrak daun *Nerium oleander* L. terhadap mortalitas, konsumsi makan dan kelulushidupan *Spodoptera frugiperda*. *Jurnal Agrikultura*, ***33***(3), 369–378. https://doi.org/10.24198/agrikultura.v33i3.41671

Sari, K.K. (2020). Viral hama invasif ulat grayak (*Spodoptera frugiperda*) ancam panen jagung di Kabupaten Tanah Laut Kalsel. *Proteksi Tanaman Tropika*, ***3***(03), 244–247.

Sianipar, H.F., Sijabat, A., & Pane, E.P. (2019). Pengaruh pemberian berbagai tingkat mikoriza arbuskula pada tanah terakumulasi logam Pb terhadap pertumbuhan tanaman belimbing wuluh (*Averrhoa bilimbi*). *JBIO: Jurnal Biosains*, ***5***(2), 53–58.

Sutriono, S., & Zahar, I. (2022). Perbandingan efektivitas bacillus thuringiensis dengan teknologi ozon dalam pengendalian hama *Spodoptera litura* pada daun cabai (*Capsicum annum*). *Jurnal Rona Teknik Pertanian*, ***15***(2), 13–22. https://doi.org/10.17969/rtp.v15i2.28350

Trisyono, Y.A., Suputa, S., Aryuwandari, V.E.F., Hartaman, M., & Jumari, J. (2019). Occurrence of heavy infestation by the fall armyworm *Spodoptera frugiperda*, a new alien invasive pest, in Corn Lampung Indonesia. *Jurnal Perlindungan Tanaman Indonesia*, ***23***(1), 156–160. https://doi.org/10.22146/jpti.46455

Wibawa, I.P.A.H. (2018). Perbandingan efektivitas beberapa pestisida organik pada budidaya brokoli (*Brassica rapa* L.) di Bedugul, Bali. *Agro Bali: Agricultural Journal*, ***1***(1), 1–9.

Zakqy, N., Wuryandari, Y., & Purnawati, A. (2024). Potential of the biological agent *Bacillus* spp in inhibiting fusarium wild disease and its effects on the growth and production of cayenne papper (*Capsicum frutescens* L.). *Jurnal Pembelajaran Dan Biologi Nukleus*, ***10***(1), 38–51. https://doi.org/10.36987/jpbn.v10i1.5366

Zinidin, M. (2022). Eksplorasi *Bacillus* spp. pada rhizosfer cabai merah (*Capsicum annuum* L.) dataran tinggi dan potensinya sebagai agensia pengendali hayati patogen *Ralstonia solanacearum* secara in vitro. [*Undergraduate thesis*]. UPN "Veteran" Jawa Timur. https://repository.upnjatim.ac.id/id/eprint/6876