

## Effectiveness of *Bacillus* spp. from West Sumatra, Indonesia in controlling *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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**Abstract.** Nelly N, Hamid H, Lina EC, Yunisman, Rusli R, Yanti Y, Kairunisa M. 2024. Effectiveness of *Bacillus* spp. from West Sumatra, Indonesia in controlling *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Biodiversitas* 25: 1472-1478. *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) is an invasive pest widely recognized for attacking corn plants. This pest was first discovered in early 2019 in West Sumatra, Indonesia, posing a significant threat to agricultural productivity. To control *S. frugiperda*, local biological agents, namely *Bacillus* spp., originating from West Sumatra, have been explored as a potential control method. Therefore, this study aimed to obtain *Bacillus* spp isolates from rhizosphere origin and assess their effectiveness in controlling *S. frugiperda* in the laboratory. A completely randomized design was used, consisting of five treatments with three replications. The treatments included *Bacillus cereus* strain MRPLUMBE1.3, *Bacillus myocytes* strain MRRZLL 2.2, and *Bacillus* sp. MRTE strain 1.3.3. For comparison, there was a synthetic insecticide (active ingredient chlorantraniliprole) and a control (distilled water). The application of isolate suspension was carried out through larvae feed. The parameters observed included larvae mortality, percentage of formed pupae and adults (imago), number of eggs laid by females, and survival time of adults. The data obtained were analyzed using Analysis of Variance (ANOVA) and tested with LSD at the 5% level. The results showed that *Bacillus* spp. isolates originating from the rhizosphere effectively controlled *S. frugiperda* larvae. The best isolate that can be used to control *S. frugiperda* is *Bacillus* spp. from Solok. Specifically, *Bacillus* sp. strain MRTPE 1.3.3 showed larvae mortality ranged between 28.33 and 41.67%, and the suspension affected the percentage of formed pupae (51.66%) and the survival time of adults (4.5-5.8 days).

**Keywords:** *Bacillus*, biocontrol, fall armyworm, indigenous isolates

### INTRODUCTION

*Spodoptera frugiperda* J.E. Smith or Fall Armyworm (FAW) (Lepidoptera: Noctuidae) is an invasive pest originating from South America (Nagoshi et al. 2017). This pest was first discovered in March 2019 in Pasaman Barat Regency, West Sumatra, Indonesia (Sartiami et al. 2020). *S. frugiperda* is a polyphagous pest capable of attacking 353 host plant species from 75 families, including corn plants (Montezano et al. 2018). In Africa, the negative impacts of *S. frugiperda* on corn plants are significant, causing losses ranging from 8.5 to 21 million tons, with a value of 250 to 630 million US dollars (Bateman et al. 2018). Meanwhile, the percentage of *S. frugiperda* attacks in Indonesia ranges from 60.12 to 87.05%, with an average larvae count between 0.74 and 3.00 individuals per plant (Kalqutny et al. 2021). *S. frugiperda* is capable of attacking all stages of corn plants, from vegetative to generative (Prasanna et al. 2018), and the damage commonly found at the growing point leads to harm in the formation shoots (Maharani et al. 2019; Nelly et al. 2021). Furthermore, it is polyphagous, and some of the main hosts include food crops from the Graminae group, such as corn, rice, wheat, sorghum, and sugarcane, which require vigilance in monitoring and population development.

The control of *S. frugiperda* is predominantly carried out using synthetic insecticides (Kansiiime et al. 2019;

Houngbo et al. 2020). Excessive use of synthetic insecticides, such as carbamates, organochlorines, organophosphates, and pyrethroids (Gutiérrez-Moreno et al. 2019), can lead to resistance in *S. frugiperda* (Tambo et al. 2020). Using synthetic insecticides also has environmental impacts, including killing natural enemies, resulting in resurgence, environmental pollution, or residue effects. To mitigate this negative impact, there is a need to explore local biological agents as environmentally friendly pest control alternatives. Moreover, an environmentally friendly pest control method that plays a crucial role in Integrated Pest Management (IPM) programs is entomopathogens (Ahissou et al. 2022). Some reported effective agents for controlling *S. frugiperda* included the fungi *Beauveria bassiana* and *Metarhizium anisopliae* (Ayudya et al. 2019; Ramanujam et al. 2020; Gustianingtyas et al. 2021; Idrees et al. 2022) as well as *Bacillus* sp. (Burtet et al. 2017; Handayani et al. 2023).

Several *Bacillus* spp. species have been widely reported as effective in controlling *S. frugiperda*, including *Bacillus thuringiensis* (Fontana Capalbo et al. 2001; Monnerat et al. 2006; Burtet et al. 2017; Gutiérrez-Moreno et al. 2019). Recent studies also reported that *Bacillus* sp. isolates other than *B. thuringiensis* could control pests such as *Bemisia tabaci* (Hamid et al. 2020), *Aphis gossypii* (Ali and Ibrahim 2023), and *S. frugiperda* (Nelly 2022; Handayani et al. 2023). Applying *Bacillus* spp. can increase larvae mortality

when treatment is applied over an extended period. The active compounds present in *Bacillus* spp. do not function as stomach toxins. Biological agents such as bacterial isolates from local sources can also act as stomach toxins or anti-feedants against larvae to control pests in a particular region. Several types of entomopathogenic isolates have been proven effective in killing insect larvae, indicating the effectiveness of controlling *S. frugiperda*. *Bacillus* isolates from several locations in West Sumatra and three isolates from 3 different locations have been obtained. In initial tests, *Bacillus* spp. isolates from District Tanah Datar, Agam, and Solok can kill insects. Therefore, this study aimed to assess the effectiveness of several *Bacillus* spp. isolates from different locations are used to control *S. frugiperda*.

## MATERIALS AND METHODS

### Study area

This study was conducted in the Insect Bioecology and Microbiology Laboratory, Department of Plant Protection, Faculty of Agriculture, Universitas Andalas, Padang, Indonesia. Bacterial isolates were obtained from 3 locations in West Sumatra, Indonesia namely Tanah Datar, Agam, and Solok districts.

### Procedures

This study used a completely randomized design with five treatments and three replications. The treatments consisted of 3 *Bacillus* isolates, with a control and synthetic insecticide as comparators. These included *Bacillus cereus* (MRPLUMBE 1.3) isolates from Tanah Datar, *Bacillus mycoides* (MRRZLL.2.2) from Agam, and *Bacillus* sp. (MRTPE1.3.3) from Solok. Isolates are from the collection of the Laboratory of Microbiology, Department of Plant Protection, Universitas Andalas.

### *Bacillus* spp. isolates reculture

*Bacillus* spp. isolates were refreshed using a streak method and transferred with the ose needle to TSA medium provided in a Petri dish, followed by incubation for 24 hours (Figure 1).

### Hypersensitivity reaction (HR)

*Bacillus* spp. isolates were confirmed with gram and hypersensitive reaction tests to prove that the recultured isolates still had the same characteristics as the original. Subsequently, a gram test for *Bacillus* spp. was performed using a 3% KOH solution dropped onto a glass slide, which was added to a single colony of pure culture of actinobacterial isolates aged 7 x 24 hours and homogenized. The one needle was slowly lifted, and when no mucus formed based on the tests conducted, the bacteria were identified as gram-positive (Schaad et al. 2001). The Hypersensitive Reaction (HR) was performed to ensure that the used isolates were not pathogenic to plants. *Bacillus* spp. Suspension with a density of  $10^8$  spores/mL was drawn using a 1 mL syringe and infiltrated onto the lower surface of the leaves of *Mirabilis jalapa* flowers

(Klement et al. 1990). Subsequently, the infiltrated leaves portion was covered with plastic, and plants were incubated for 2 x 24 hours. When the infiltrated leaves portion did not show necrotic symptoms, the tested isolate was considered non-pathogenic to plants (negative).

### Preparation of *S. frugiperda* larvae

The feed used for *S. frugiperda* larvae was the leaves of Paragon corn plants. To initiate cultivation, seeds were planted in polybags filled with soil and manure in a 1:1 ratio. Using the furrow method at a hole depth of 2-5 cm, three corn seeds were planted, covered, and watered in each hole. Subsequently, corn leaves were ready for use as feed when plants reached approximately one month old.

*Spodoptera frugiperda* larvae were obtained from corn plants in the Belimbing area, Kuranji Village, West Sumatra. Larvae were placed in a 14 x 10 cm plastic box, taken to the laboratory, and raised in 4 cm diameter and 3.5 cm high plastic cups. Corn leaves were placed in plastic cups as feed, and larvae were fed until the pre-pupae stage was reached. Pupae were separated into 6 cm diameter and 11 cm high plastic cups and raised until they became adults (imago) stage. Newly adults from pupae were given a 10% (v/v) honey solution, which was dipped in cotton as feed until mated adults laid eggs. *S. frugiperda* eggs obtained from breeding were transferred to plastic cups until hatching. Subsequently, larvae were raised to reach the second instar, and fresh corn leaves were provided as feed. Larvae to be treated were placed in plastic cups, each containing one larva, with 20 larvae in each replication.

### Preparation of *Bacillus* spp. suspension

*Bacillus* spp. proliferation was carried out in a liquid culture, where a single colony of pure culture aged 2 x 24 hours was taken. The sample collected was placed in 10 mL NB medium in a 50 mL culture bottle and incubated on a rotary shaker for 1 x 24 hours. Subsequently, 1 mL pre-culture was transferred to 49 mL sterile coconut water in a 100 mL culture bottle and incubated for 2 x 24 hours at 150 rpm. The population density was determined by comparing the turbidity of bacterial suspension with a McFarland scale  $10^8$  solution (population density  $10^8$  CFU/mL) (Yanti et al. 2017).



**Figure 1.** *Bacillus* spp. isolate reculture: A. Pure isolate in a microtube, B. Isolate culture on TSA medium

### Effect of *Bacillus* spp. on *S. frugiperda* larvae

Corn leaves were cut into 4 × 4 cm size, dipped into each 2 x 24-hour bacterial suspension, and air-dried. The treatment included applying the bacterial suspension to feed the leaves of larvae. Subsequently, treated leaves were placed in plastic cups containing 1 *S. frugiperda* larvae. The experiment consisted of 3 replications, each with 20 larvae second-instar. Larvae with corn leaves treated with the suspension were raised to adults, and several observations were made daily, including:

**Larvae mortality:** Dead *S. frugiperda* larvae for each treatment after *Bacillus* spp. The application was counted and observed daily for 14 days post-application. Larvae mortality was calculated using the following formula:

$$M = n/N \times 100\%$$

Where: M: Larvae mortality, n: Number of dead larvae, and N: Number of larvae treated

**Percentage of formed pupae (%):** The number of formed pupae in *S. frugiperda* was observed daily, and the percentage was calculated using the following formula:

$$P_p = \frac{p}{N} \times 100\%$$

Where: Pp = Percentage of formed pupae, p: number of pupae, N: Number of larvae treated

**All treatment percentages of emergence adults,** including males and females, were calculated and observed daily until all pupae fully developed. The formula for calculating the percentage of adult emergence is as follows:

$$P_i = \frac{I}{N} \times 100\%$$

Where: P<sub>i</sub> is the percentage of emerging adults, I is the number of emerging adults, and N is the number of treated larvae

**Number of eggs laid by adults:** The number of eggs laid was counted each day until the 12<sup>th</sup> day after the adults were transferred to plastic containers used for maintenance.

**Preoviposition period (days):** The pre-oviposition period was calculated from the formation of female adults to the laying of the first egg.

**Oviposition period (days):** The oviposition period was calculated from when female adults laid the first egg to the last egg was laid.

**Post-oviposition period (days):** The post-oviposition period was calculated from when female adults stopped laying eggs to death.

**Survival time of adults:** The survival time of adults was calculated from the emergence of adults from pupae until the moth's death.

### Data analysis

The data were analyzed using Analysis of Variance (ANOVA) with Stat. 8.0 (Software program for Windows) and subjected to LSD test at a 5% level.

## RESULTS AND DISCUSSION

### Hypersensitivity reaction (HR)

The hypersensitivity reaction test showed that actinobacteria was negative, as shown in Figure 2. The results of hypersensitive test on *Bacillus* spp. isolates were also negative. There was no mucus in the isolate. The reaction on the plants did not show necrotic symptoms. The isolate found can be used for biological control.

### Effect of treatments on larvae mortality

*Spodoptera frugiperda* larvae fed with *Bacillus* spp. compared to other treatments, it showed a significantly different effect ( $P = 0.004$ ). Treatment using synthetic insecticides causes 100% mortality. Meanwhile, no larvae died in the control, and mortality was 0%. Larvae mortality due to using *Bacillus* ranged from 28.33 to 41.67%, and the treated larvae mortality is presented in Table 1.

Larvae that consumed corn leaves treated with *Bacillus* isolates showed different morphologies. Normal larvae were characterized by more extended size, which differed significantly from abnormal due to the treatment. The observation of second-instar larvae that died after treatment is shown in Figure 3.



**Figure 2.** Gram and HR test results (a) No mucus formation in *Bacillus* spp. (gram-positive) (b) No necrotic symptoms (negative HR)

**Table 1.** *S. frugiperda* larvae mortality after the application of various bacterial suspensions

Treatments	Mortality (%) ± SD
Insecticide (chlorantraniliprole)	100.00 ± 0.00 a
<i>Bacillus</i> sp. (MRTPE 1.3.3)	41.67 ± 0.57 b
<i>Bacillus mycoides</i> (MRRZLL 2.2)	30.00 ± 4.35 b
<i>Bacillus cereus</i> (MRPLUMBE 1.3)	28.33 ± 2.51 b
Control	0.00 ± 0.00 c

Note: Numbers followed by the same lowercase letter in the same column are not significantly different based on the LSD test at the 5% level

Observations on *S. frugiperda* larvae mortality after treatment showed a high prevalence on the second and third days. All larvae fed with corn leaves containing *Bacillus* sp. isolates on the second day showed signs of mortality, which continued to the fourth day after the treatment. Furthermore, treatment with synthetic insecticide (chlorantraniliprole as the active ingredient) significantly differed from *Bacillus* sp. strain MRTPE 1.3.3, as shown in Figure 4. A day after treatment resulted in mortality: the rate reached 61.67 % on the fourth day, while untreated feed or control showed no larvae mortality.

### Percentage of formed pupae (%)

The application of various treatments to *S. frugiperda* larvae affected the percentage of formed pupae ( $P=0.000$ ). The control showed that the percentage of formed pupae was 100%. However, no pupae were formed due to treatment with synthetic insecticide, as presented in Table 2.

The observation results of formed pupae after treatment of larvae fed with various *Bacillus* isolates are shown in Figure 5. Pupae is considered normal and healthy, showing an elongated and shiny brown, as presented in Figure 5.A. After treatment, the prospective pupal head still resembled the larvae and showed non-formed pupae, as indicated in Figure 5.B. Meanwhile, Figure 5.C shows that pupae became rotten and were characterized by turning black instead of reddish-brown.

### Percentage of adults emergece

The application of various treatments to *S. frugiperda* affected the percentage of emerging adults, indicating a significant difference between treatments ( $P = 0.000$ ), as presented in Table 3. In control, the number of adults who emerged was 100%. Treatment of larvae fed with *Bacillus* sp causes the adults to emerge on average less than 55%.

The observation results of emerging adults showed both normal and abnormal adults. Generally, normal adults have similar morphologies, and females are larger than males. Abnormal adults showed abnormalities or damage, particularly in the wings, as shown in Figure 6.

### Number of eggs laid by *S. frugiperda* adults

The application of various treatments to *S. frugiperda* larvae affected the number of eggs laid by adults. The observation results showed that the highest number of egg clusters of female adults originating from untreated larvae or the control significantly differed compared to other treatments ( $P = 0.000$ ), as presented in Table 4.

**Table 2.** Percentage of formed pupae after treatment

Treatment	Pupae (%) $\pm$ SD
Control	100.00 $\pm$ 0.00 a
<i>Bacillus cereus</i> (MRPLUMBE 1.3)	71.66 $\pm$ 2.51 b
<i>Bacillus mycoides</i> (MRRZLL 2.2)	63.33 $\pm$ 4.61 b
<i>Bacillus</i> sp. (MRTPE 1.3.3)	51.66 $\pm$ 1.52 b
Insecticide (chlorantraniliprole)	0.00 $\pm$ 0.00 c

Note: Numbers followed by the same lowercase letter in the same column are not significantly different based on the LSD test at the 5% level

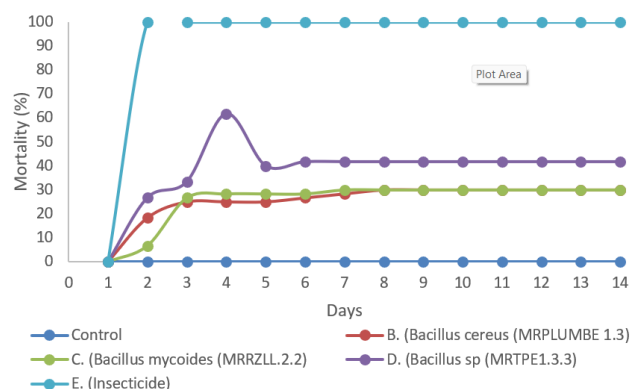
**Table 3.** Percentage of adults emergece after treatment on larvae

Treatment	Adults (%) $\pm$ SD
Control	100.00 $\pm$ 0.00 a
<i>Bacillus cereus</i> (MRPLUMBE 1.3)	71.66 $\pm$ 2.51 b
<i>Bacillus mycoides</i> (MRRZLL 2.2)	48.33 $\pm$ 2.08 c
<i>Bacillus</i> sp. (MRTPE 1.3.3)	45.00 $\pm$ 2.64 c
Insecticide (chlorantraniliprole)	-

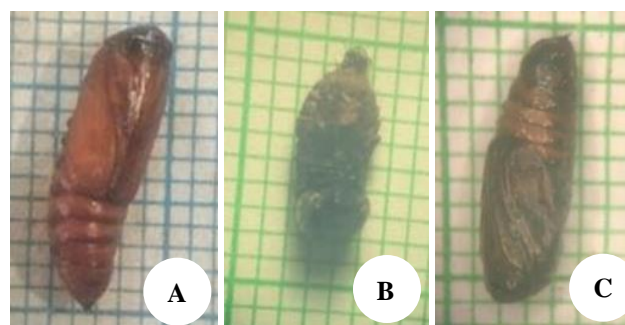
Note: Numbers followed by the same lowercase letter in the same column are not significantly different based on the LSD test at the 5% level



**Figure 3.** Observations of *S. frugiperda* larvae mortality after the application with *Bacillus* spp. A. Larvae before the application, B. Dead larvae after the application



**Figure 4.** Cumulative curve of *S. frugiperda* larvae mortality



**Figure 5.** Observations of formed pupae after treatment: A. Normal pupae, B. Non-formed pupae, C. Abnormal pupae



The number of eggs laid by *S. frugiperda* adults varied daily, with the control showing the highest number on day 1. Eggs were laid by female adults from untreated larvae or the control to day 8, while those from larvae treated with *Bacillus* continued to lay eggs until day 5, as shown in Figure 7.

#### Preoviposition, oviposition, and pasca oviposition periods

Female adults passed through pre-, oviposition, and post-oviposition periods after copulation, with significant variations observed among larvae treated with different treatments ( $P = 0.000$ ), as presented in Table 5.

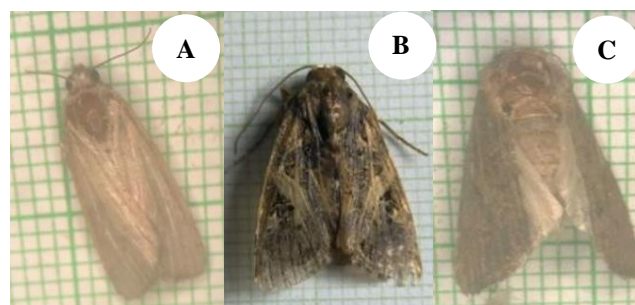
The average pre-oviposition period of female adults in control was 3.1 days, differing from those treated with *Bacillus*, which ranged from 2.44 to 2.66 days. Similarly, the highest oviposition period in the control was 6.46 days, compared to other treatments ranging from 2.54 to 3.72 days. Post oviposition period between the control and other treatments I have had the same average, ranging from 1.45 to 2.42 days.

#### Survival time of adults

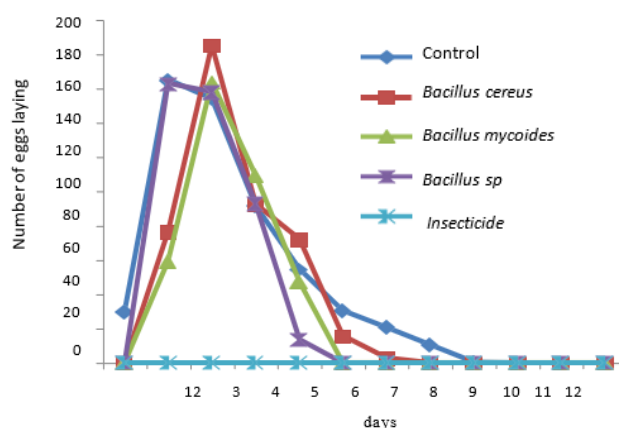
The application of various treatments to feed *S. frugiperda* larvae affected the survival time of adults. The significantly lowest survival time of adults was observed in *Bacillus* sp. (MRTPE 1.3.3) compared to *Bacillus mycoides* (MRRZLL 2.2), *Bacillus cereus* (MRPLUMBE 1.3), and control ( $P = 0.000$ ). The observation results for the survival time of adults from larvae treated with *Bacillus* are presented in Table 6.

The survival time of adults from larvae treated with *Bacillus* significantly differed from the control, where female adults reached 13 days, while males survived for seven days. The shortest survival time of females was in *Bacillus* sp. (MRTPE 1.3.3) treatment. However, no adults emerged due to treatment with synthetic insecticide because larvae mortality reached 100% at the commencement of the treatment.

Application of *Bacillus* to the leaves of corn plants as fed for *S. frugiperda*, causes the death of the larvae. The development of *S. frugiperda* will be disrupted due to death in the larvae phase, no pupae formation, or the emergence of an abnormal adult from the pupae.



**Figure 6.** Formed *S. frugiperda* adults: A. Female adults, B. Male adults, C. Abnormal female adults after bacterial application



**Figure 7.** Laying pattern of female *S. frugiperda* adults from larvae treated with five different treatments

**Table 4.** The average number of egg clusters and eggs laid by female *S. frugiperda*

Treatments	Egg clusters $\pm$ SD	Eggs $\pm$ SD
Control	12.07 $\pm$ 2.14 a	588.11 $\pm$ 87.43 a
<i>Bacillus cereus</i> (MRPLUMBE 1.3)	7.44 $\pm$ 1.88 b	443.06 $\pm$ 144.66 b
<i>Bacillus</i> sp. (MRTPE 1.3.3)	7.00 $\pm$ 1.48 b	426.36 $\pm$ 156.26 b
<i>Bacillus mycoides</i> (MRRZLL 2.2)	4.70 $\pm$ 1.70 c	370.30 $\pm$ 102.82 b
Insecticide (chlorantraniliprole)	-	-

Note: Numbers followed by the same lowercase letter in the same column are not significantly different based on the LSD test at the 5% level

**Table 5.** Average pre-oviposition, oviposition, and post-oviposition periods of female *S. frugiperda* adults from larvae treated with different treatments

Treatments	Pre oviposition (days) $\pm$ SD	Oviposition (days) $\pm$ SD	Post oviposition (days) $\pm$ SD
Control	3.10 $\pm$ 0.83 a	6.46 $\pm$ 0.96 a	2.42 $\pm$ 1.10 a
<i>Bacillus cereus</i> (MRPLUMBE 1.3)	2.66 $\pm$ 0.59 b	3.72 $\pm$ 1.22 b	2.38 $\pm$ 0.84 a
<i>Bacillus mycoides</i> (MRRZLL 2.2)	2.50 $\pm$ 0.52 b	2.80 $\pm$ 0.63 c	2.30 $\pm$ 0.67 a
<i>Bacillus</i> sp. (MRTPE 1.3.3)	2.45 $\pm$ 0.52 b	2.54 $\pm$ 0.52 c	1.45 $\pm$ 0.93 b
Insecticide (chlorantraniliprole)	-	-	-

Note: Numbers followed by the same lowercase letter in the same column are not significantly different based on the LSD test at the 5% level

**Table 6.** Survival time of adults after treatment on feed of *S. frugiperda* larvae

Treatments	Adults (days)	
	Female $\pm$ SD	Male $\pm$ SD
Control	11.59 $\pm$ 1.41 a	6.96 $\pm$ 0.47 a
<i>Bacillus cereus</i> (MRPLUMBE 1.3)	8.38 $\pm$ 1.38 b	5.94 $\pm$ 0.82 b
<i>Bacillus mycoides</i> (MRRZLL 2.2)	7.68 $\pm$ 1.20 b	5.80 $\pm$ 0.78 b
<i>Bacillus</i> sp. (MRTPE 1.3.3)	5.80 $\pm$ 1.37 c	4.50 $\pm$ 0.79 c
Insecticide (Chlorantraniliprole)	-	-

Note: Numbers followed by the same lowercase letter in the same column are not significantly different based on the LSD test at the 5% level

## Discussion

The *Bacillus* spp isolates found showed negative HR test results. Tests on plant leaves also did not produce necrotic symptoms. This isolate is safe to use as a biological agent to control *S. frugiperda*. Treating bacterial suspensions on *S. frugiperda* larvae affected mortality and development. *Bacillus* spp. suspensions with a concentration of  $10^8$  mol/mL showed a significant capacity to suppress the larvae population, with the highest mortality observed in *Bacillus* spp. (MRTPE 1.3.3) treatment at around 41.67%. Similarly, (Burtet et al. 2017) stated that the administration of *Bacillus* spp. The infected larvae to shrivel, darken in color, and shrink. In this study, larvae fed with corn leaves and treated using bacterial suspensions experienced decreased appetite, became weak, had difficulty moving, and died. The body parts changed color to dark brown and became soft and watery, but larvae started to dry and shrink after a few days.

*Bacillus* spp. part from causing death in larvae, suspensions affected the pupae of *S. frugiperda*. Although infected larvae survived and developed into pupae, the formation was abnormal due to bacterial infection during the larvae stage. Larvae still harbor the bacteria without toxicity due to the non-neutral pH in the large intestine.

In this study, the morphology of formed pupae varied, as the abdominal part was observed to have a complete shape, while the prospective head resembled larvae. Additionally, formed pupae are abnormal, with different sizes and darker colors than the control. Arsi et al. (2019) stated that pupae normally formed in treatment-experienced decay, characterized by turning black instead of reddish-brown. Normal pupae, when touched, would move and pass through the process to adults. Based on the result, the adults observed were from normal pupae, which produced eggs and were abnormally characterized by abnormal wing or leg morphology. Furthermore, different treatments were found to affect the number of eggs produced by females.

Results showed that the number of eggs produced by female adults in all treatments was fewer than that of the control. This was attributed to the application of bacterial suspensions to corn leaves, causing a loss of larvae feeding ability and reduced nutrition for development into adults. Consequently, a significant effect was observed on the female's ability to lay eggs. Insufficient feeding assimilation can hinder female adult egg production (de Bortoli and Jurat-Fuentes 2019).

Female adults showed longer lifespans than males due to the distinct preoviposition, oviposition, and post-

oviposition phases, as presented in Table 6. The survival time of adults in the control group was longer than that of other treatments due to the amount of feed consumed during the larvae stage. The length of the female oviposition period significantly affected the number of eggs produced. (Hutasoit et al. 2020) Stated that a more extended oviposition period resulted in a larger number of eggs, while a shorter oviposition period led to fewer eggs.

The results of survival time of adults exhibited that all treatments allow adults to develop and grow properly. However, synthetic insecticide treatment (active ingredient chlorantraniliprole) killed 100% of *S. frugiperda* larvae. This was consistent with Willing et al. (2020), where chlorantraniliprole-based insecticides at a dose of 2 cc/l suppressed the *S. frugiperda* population with 100% mortality within five days of the application. The average survival time for female adults ranged from 5 to 11 days, while males had 4 to 6 days. The best *Bacillus* spp. Isolates for controlling *S. frugiperda* are *Bacillus* sp. strain MRTPE 1.3.3, *Bacillus mycoides* strain MRZLL 2.2, and *Bacillus cereus* strain MRPLUMBE 1.3, which showed the potential of killing 28.33-41.67% of *S. frugiperda* larvae. These isolates affected the percentage of formed pupae (51.66%) and survival time of adults (5.80-4.50 days). Moreover, determining lethal doses for each *Bacillus* isolate was necessary to apply against *S. frugiperda* larvae.

In conclusion, several *Bacillus* isolates originating from various locations can control *S. frugiperda* larvae. Larval mortality ranged between 28.33 and 41.67%.

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