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Concentration-Related Mortality of *Spodoptera frugiperda* Larvae Treated With *Bacillus thuringiensis* and *Beauveria bassiana***Henny Vianne Gretha Makal¹, Christina L. Salaki^{1*}, Yefta Pamandungan²**¹Plant Protection Study Program, Faculty of Agriculture, Sam Ratulangi University²Agronomy Study Program, Faculty of Agriculture, Sam Ratulangi University*Corresponding author:
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Abstract. The fall armyworm, *Spodoptera frugiperda*, is a serious crop pest for which microbial control agents warrant laboratory evaluation. This study described larval mortality associated with three concentrations of *Bacillus thuringiensis* spore suspensions and *Beauveria bassiana* conidial suspensions. The study was conducted from June to August 2026 at the Plant Disease Laboratory, Faculty of Agriculture, Sam Ratulangi University, Indonesia. The reported completely randomized design comprised one control and six microbial treatments, with three replicates of ten larvae per treatment (210 larvae in total). Suspensions were applied by direct spraying at 1×10^7 , 1×10^8 , and 1×10^9 spores/mL for *B. thuringiensis* or conidia/mL for *B. bassiana*. The bioassay lasted seven days. Across the three concentrations, reported mortality was 53.3%, 76.7%, and 93.3% after *B. thuringiensis* treatment, compared with 46.7%, 66.7%, and 83.3% after *B. bassiana* treatment. Thus, mortality increased numerically with concentration within each microbial treatment series. However, control mortality, the basis for aggregation across replicates, and details of the microbial preparations were not reported in the available study record. Consequently, these results should be interpreted descriptively and do not establish statistical superiority or equivalent biological potency between bacterial spores and fungal conidia. The observations support further, more completely documented bioassays of these microbial preparations for fall armyworm management.

Keywords: *Bacillus thuringiensis*; *Beauveria bassiana*; biological control; larval mortality; *Spodoptera frugiperda*.

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a highly polyphagous invasive insect that threatens maize and other crops. Its extensive host range, high dispersal capacity, and ecological adaptability have facilitated its spread across agricultural regions (Tay et al., 2023). Larval feeding damages foliage and growing points, making sustainable management an important concern in crop production.

Frequent reliance on synthetic insecticides can increase selection pressure for insecticide resistance and may have undesirable effects on non-

target organisms. Microbial agents are therefore of interest as possible components of integrated pest management, although their effectiveness depends on the microorganism, isolate, host developmental stage, and application conditions (Kumar et al., 2021; Mahmood & Parwez, 2024).

Bacillus thuringiensis is an entomopathogenic bacterium associated with insecticidal proteins, including Cry proteins. In susceptible insects, ingestion of an appropriate toxin-containing preparation can disrupt the midgut epithelium (Kumar et al., 2021). However, spore number does not by itself quantify Cry-protein content or biological potency.



Studies of native *Bacillus* isolates indicate that insecticidal responses may vary with isolate and larval instar (Karshanal & Kalia, 2023a, 2023b); variation among bacterial toxins and secondary metabolites has also been documented (Apirajkamol *et al.*, 2025).

Beauveria bassiana is an entomopathogenic fungus whose conidia can adhere to an insect cuticle, germinate, and initiate infection under suitable conditions. Its activity is influenced by fungal strain, conidial viability, larval instar, and exposure method (Mahmood & Parwez, 2024). Variable pathogenicity toward *S. frugiperda* has been reported among fungal isolates and experimental conditions (Idrees *et al.*, 2022; Gao *et al.*, 2022; Apirajkamol *et al.*, 2023). Research in Indonesia has also examined locally collected *Beauveria* isolates and indigenous *Bacillus* preparations as potential biological control resources (Lakshita *et al.*, 2024; Nelly *et al.*, 2024).

Investigations of entomopathogenic agents against fall armyworm under laboratory and field conditions support continued development of microbial control approaches (Cokola *et al.*, 2023; Khanal *et al.*, 2024; Azazy *et al.*, 2025; Frimpong-Anin *et al.*, 2025; Wakil *et al.*, 2026). Nevertheless, direct comparisons of numerical spore and conidial concentrations require caution because they represent distinct propagules and may not correspond to equivalent active insecticidal doses.

The present study provides a descriptive laboratory assessment of *S. frugiperda* larval mortality following direct spraying with three nominal concentrations each of *B. thuringiensis* and *B. bassiana*. The objective was to describe the concentration-related pattern of observed mortality within each

treatment series during a seven-day bioassay, without assuming that the preparations have equivalent biological potency.

MATERIAL AND METHODS

Study Area and Duration

The study was conducted from June to August 2026 at the Plant Disease Laboratory, Faculty of Agriculture, Sam Ratulangi University, Indonesia.

Test Insects and Microbial Preparations

The test organism was the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). The design specified ten larvae per replicate. *Bacillus thuringiensis* was evaluated at 1×10^7 , 1×10^8 , and 1×10^9 spores/mL, while *Beauveria bassiana* was evaluated at 1×10^7 , 1×10^8 , and 1×10^9 conidia/mL. The original report did not specify larval provenance or instar, microbial strain or product source, or procedures for preparation and confirmation of the suspensions; these details require verification against laboratory records.

Experimental Design and Treatment Application

The reported completely randomized design comprised seven treatment groups: one control and six microbial treatments. Each group was replicated three times, with ten larvae assigned to each replicate, yielding 21 replicate groups and 210 larvae in the declared design. The microorganism-treated groups and the control are listed in Table 1. The replicate group containing ten larvae is treated as the reporting unit for the design description; its physical arrangement and independence require author confirmation.



Table 1. Treatment structure for the laboratory bioassay of *Spodoptera frugiperda* larvae.

Code	Treatment	Concentration	Replicates	Larvae per replicate
A	<i>B. thuringiensis</i>	1×10^7 spores/mL	3	10
B	<i>B. thuringiensis</i>	1×10^8 spores/mL	3	10
C	<i>B. thuringiensis</i>	1×10^9 spores/mL	3	10
D	<i>B. bassiana</i>	1×10^7 conidia/mL	3	10
E	<i>B. bassiana</i>	1×10^8 conidia/mL	3	10
F	<i>B. bassiana</i>	1×10^9 conidia/mL	3	10

Note. The source manuscript identifies a control but does not specify its composition. Bacterial spores and fungal conidia are different propagules, not directly equivalent insecticidal doses.

The microbial suspensions were administered to assigned larval groups by direct spraying. The source manuscript does not provide the spray volume, sprayer characteristics, substrate, treatment handling, or control application procedure. These details must be recovered from the original experiment records before the procedure can be regarded as fully reproducible.

Mortality Observation

The bioassay was conducted for seven days, and larval mortality was the reported outcome. The available manuscript does not specify a daily observation schedule, the criterion used to classify a larva as dead, or whether the percentages represent cumulative mortality over seven days or mortality observed at the endpoint. Accordingly, Table 2 reports the mortality values exactly as provided without assigning an unverified temporal definition.

Data Analysis

Mortality percentages were summarized descriptively for the three concentrations of each microbial agent. Although the declared experimental design included three replicate groups per treatment, the manuscript does not state whether the percentages were calculated by pooling larvae across replicates or by averaging replicate-level percentages. No measures of replication-level variability or inferential tests were provided. Therefore, the results are compared descriptively, without statistical significance claims or estimates of relative biological potency.

RESULTS AND DISCUSSION

Results

The reported treatment-level mortality values increased with concentration for both microbial suspensions during the seven-day laboratory bioassay (Table 2). The control-group mortality was not reported in the supplied dataset.

Table 2. Reported larval mortality during the seven-day bioassay with microbial treatments.

Code	Treatment	Concentration	Mortality (%)
A	<i>B. thuringiensis</i>	1×10^7 spores/mL	53.3
B	<i>B. thuringiensis</i>	1×10^8 spores/mL	76.7
C	<i>B. thuringiensis</i>	1×10^9 spores/mL	93.3
D	<i>B. bassiana</i>	1×10^7 conidia/mL	46.7
E	<i>B. bassiana</i>	1×10^8 conidia/mL	66.7
F	<i>B. bassiana</i>	1×10^9 conidia/mL	83.3

Note. Treatment mortality values were transcribed from the original manuscript. Control mortality and replication-level variability were unavailable. The source did not specify whether percentages were cumulative or endpoint values; no inferential tests are indicated.

Mortality in the *B. thuringiensis* series increased from 53.3% at 1×10^7 spores/mL to 93.3% at 1×10^9 spores/mL,

a difference of 40.0 percentage points. For *B. bassiana*, corresponding values increased from 46.7% at 1×10^7



conidia/mL to 83.3% at 1×10^9 conidia/mL, a difference of 36.6 percentage points based on the rounded percentages. The highest observed value within each microbial series occurred at its highest tested concentration.

Discussion

The concentration-related pattern observed with *B. thuringiensis* is compatible with the known insecticidal potential of this bacterium against susceptible lepidopteran pests. Cry proteins can damage the insect midgut following ingestion (Kumar et al., 2021), but neither Cry-protein content nor infection or toxin mechanisms were measured in this experiment. Isolate-dependent variation in activity has been reported for *Bacillus* preparations tested against fall armyworm (Karshanal & Kalia, 2023a, 2023b; Apirajkamol et al., 2025). Accordingly, the mechanism provides biological context rather than a demonstrated explanation for the recorded mortality values.

Mortality likewise rose numerically across the tested concentrations of *B. bassiana*. Fungal infection commonly involves conidial adherence and penetration of the insect cuticle; however, mycosis, conidial viability, and the infection sequence were not measured in the present study (Mahmood & Parwez, 2024). Previous bioassays demonstrate differences associated with fungal isolate, concentration, insect stage, and application method (Idrees et al., 2022; Gao et al., 2022; Apirajkamol et al., 2023; Zhang et al., 2024).

Studies of entomopathogenic microorganisms in Indonesian and international contexts support the practical relevance of further investigations (Lakshita et al., 2024; Nelly et al., 2024; Cokola et al., 2023; Frimpong-Anin et al., 2025). Nevertheless, numerical mortality differences between the bacterial and fungal series cannot establish that one agent is more biologically potent. Their

measured concentrations describe different propagules, and the study did not provide parallel estimates of toxin amount, conidial viability, control mortality, or replicate variability. These limitations also preclude conclusions about statistically significant treatment differences or efficacy under field conditions.

The experimental observations are therefore most useful as preliminary descriptive evidence of mortality patterns within each microbial series. Confirmation of larval origin and instar, microbial preparation identity, control conditions, administration parameters, mortality timing, and replicate-level data would enable more rigorous interpretation of the existing bioassay. Subsequent standardized laboratory and field assessments could evaluate whether the patterns remain consistent under broader crop-protection conditions (Khanal et al., 2024; Wakil et al., 2026).

CONCLUSION

Across the three reported concentration levels, mortality of *Spodoptera frugiperda* larvae increased numerically after treatment with either *Bacillus thuringiensis* spore suspensions or *Beauveria bassiana* conidial suspensions during a seven-day laboratory bioassay. The highest reported mortalities were 93.3% at 1×10^9 spores/mL for *B. thuringiensis* and 83.3% at 1×10^9 conidia/mL for *B. bassiana*. These are the largest observed values among the tested concentrations, not established optimal doses. The absence of reported control mortality, replication-level variation, and detailed preparation information limits causal and between-agent interpretation. The findings provide preliminary descriptive evidence to inform better-documented laboratory and field studies of microbial approaches to fall armyworm management.



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