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Application of Local *Bacillus Thuringiensis* Isolates as Biopesticides for Control of *Spodoptera Frugiperda* in Maize

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Abstract. The fall armyworm, Spodoptera frugiperda, is an invasive pest that threatens maize production and can increase dependence on synthetic insecticides, highlighting the need for locally effective biological control options. This study screened 24 local Bacillus thuringiensis isolates for insecticidal activity against third instar S. frugiperda using a maize leaf dip feeding bioassay. For initial screening, maize leaf sections were treated with 1.5×10^7 spores mL^{-1} and offered to larvae (30 larvae per isolate), with mortality recorded up to 72 h. Isolates producing at least 50 percent mortality at 72 h were advanced to pathogenicity testing. Selected isolates were evaluated at five concentrations (1.5×10^3 to 1.5×10^7 spores mL^{-1}) to estimate LC50 at 72 h, and at 1.5×10^7 spores mL^{-1} to estimate LT50; parameters were calculated by probit analysis and reported with 95 percent fiducial limits. Ten isolates met the screening criterion, and isolate ITH produced the highest mortality (93.3 percent) at 72 h. ITH also showed the greatest pathogenicity, with an LC50 of 7.5×10^3 spores mL^{-1} and an LT50 of 19.5 h, indicating high potency and rapid action relative to other candidates. The results demonstrate substantial variability among local Bt isolates and identify promising strains for further development. Future work should confirm isolate identity, characterize toxin profiles, optimize production and formulation, and validate efficacy and crop protection performance under semi field and field conditions for integration into maize integrated pest management.

Keywords: Bacillus thuringiensis; biopesticide; fall armyworm; leaf dip bioassay; maize; Spodoptera frugiperda.

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INTRODUCTION

Maize is a strategic food and feed crop in many tropical and subtropical production systems, yet its productivity is increasingly constrained by the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Since its transcontinental invasion, this pest has become a persistent threat to maize due to its high reproductive capacity, wide host range, and strong dispersal potential. In invaded regions, its presence can alter pest complexes and intensify pressure on maize based farming systems, creating new challenges for crop protection programs(Fan et al., 2024; Tepa-Yotto et al., 2022).

In Indonesia, the fall armyworm has been widely reported across maize growing areas and is now considered a key pest in several provinces. Field observations and surveys have documented its occurrence and damage symptoms in multiple locations, including East Kalimantan, while other studies have reported substantial infestation levels across different agroecological conditions such as elevation gradients in Bengkulu. These findings indicate that local conditions can influence infestation intensity and that management options must be adaptable to diverse environments(Ginting et al., 2024; Widhayasa et al., 2022).

Current fall armyworm management in many countries still relies heavily on synthetic insecticides, but resistance development and reduced field performance have become major concerns (Saladini di Rovetino et al., 2025; Q. Zhang et al., 2024). Recent literature highlights escalating resistance risks, including confirmed field evolved resistance to key

active ingredients in some regions and evidence of diverse molecular mechanisms that can undermine insecticide efficacy. As a result, over reliance on chemical control can increase production costs, disrupt natural enemy communities, and complicate integrated pest management programs (Muraro et al., 2022; Ngegba et al., 2025).

Transgenic maize producing *Bacillus thuringiensis* toxins has contributed to fall armyworm suppression in some production systems, but practical resistance to Bt traits has been documented, including resistance to pyramided Cry toxins in certain field populations(Santos-Amaya et al., 2024). Genetic and mechanistic studies also show that resistance can be linked to specific loci and receptor related pathways, underscoring the need to diversify control tools and strengthen resistance management(Santos-Amaya et al., 2025). This situation reinforces the importance of complementary approaches that reduce selection pressure and can be locally implemented where Bt crops are not accessible, affordable, or socially accepted(Yang et al., 2021; Z. Zhang et al., 2025).

Microbial biopesticides are increasingly promoted as safer components of integrated pest management because they can reduce chemical inputs and may be compatible with natural enemies(Chaudhary et al., 2024). Among microbial options, *Bacillus thuringiensis* remains a leading biopesticide due to its specificity, established safety profile, and long history of use. However, efficacy in the field can vary with strain traits, formulation quality, environmental conditions, and target insect susceptibility(Gálvez et al., 2025). Recent reviews emphasize advances in Bt formulation technologies and the continuing need to identify potent strains, improve production efficiency, and tailor products to local pest populations. Studies on screening Bt isolates for high expression of Cry and Vip proteins and on cost effective production methods further support the value of developing locally suitable Bt based products(Hemthanon et al., 2023; Ragasruthi et al., 2024).

A promising strategy to improve field relevance is to explore indigenous bacterial resources and select locally adapted isolates with high virulence to the target pest. Research from Indonesia has reported laboratory and field oriented efforts using local microbial agents, including *Bacillus* spp. and Bt based formulations(Djunaedy et al., 2024; NELLY et al., 2024), to suppress fall armyworm populations. Field trials of Bt based formulations and laboratory screening of local bacterial isolates indicate measurable potential, but performance can differ among isolates and doses, and robust selection is required before product development(Afandhi et al., 2022). Therefore, systematic screening of local Bt isolates using standardized bioassays remains essential to identify the most effective candidates and to quantify their lethal concentration and lethal time metrics.

In this context, the present study evaluates local isolates of *Bacillus thuringiensis* for controlling *Spodoptera frugiperda* on maize. Using a leaf dip feeding bioassay framework, the work aims to (i) identify Bt isolate concentrations that provide high larval mortality and (ii) characterize the pathogenicity of selected potent isolates through lethal concentration and lethal time estimates. The results are expected to support the development of locally appropriate Bt based biopesticide candidates that can strengthen integrated pest management for fall armyworm in maize production systems.

MATERIALS AND METHODS

Study design

This study evaluated the bioefficacy of local *Bacillus thuringiensis* isolates against third instar larvae of *Spodoptera frugiperda* using a maize leaf dip feeding

bioassay. The work was conducted in two stages: an initial screening at a single high spore concentration to identify promising isolates, followed by pathogenicity assays to estimate LC50 and LT50 for selected isolates. Leaf dip bioassays with replicated larval units and mortality observations across 12 to 72 hours are widely used for laboratory evaluation of *S. frugiperda*(Saleem et al., 2024).

To clarify the overall workflow, the study was organized into two sequential stages, consisting of an initial screening assay followed by pathogenicity assays for selected isolates. The key elements of each stage, including the purpose, concentration used, observation schedule, and primary outputs, are summarized in Table 1.

Tabel 1. Experimental design summary

Study stage	Purpose	Treatments	Concentration	Test insects per treatment	Observation schedule	Main outputs
Screening	Identify promising isolates	24 Bt isolates plus negative control	1.5×10^7 spores mL^{-1}	30 larvae per isolate	Symptoms every 6 h, mortality at 12, 24, 48, 72 h	Percent mortality at 72 h, shortlist of isolates
Pathogenicity, dose response	Quantify potency	Selected isolates plus negative control	1.5×10^3 to 1.5×10^7 spores mL^{-1}	30 larvae per concentration	Mortality at 12, 24, 48, 72 h	LC50 at 72 h, 95 percent fiducial limits
Pathogenicity, time mortality	Quantify speed of kill	Selected isolates plus negative control	1.5×10^7 spores mL^{-1}	30 larvae per isolate	Mortality every 6 h up to 72 h	LT50, 95 percent fiducial limits

Table 1 provides a compact overview of the experimental logic of the study. The screening stage identifies candidate isolates based on mortality at 72 hours, while the second stage quantifies pathogenicity using dose response analysis for LC50 and time mortality analysis for LT50. This structure ensures that only isolates with promising initial performance are advanced to more detailed quantitative evaluation.

Bacillus thuringiensis isolates and spore suspension preparation

A total of 24 local *B. thuringiensis* isolates were evaluated. Spore density was determined by direct counting using a haemocytometer. Each isolate suspension was adjusted by dilution to obtain a standardized concentration of 1.5×10^7 spores mL^{-1} for the screening assay, with 20 mL suspension prepared for each isolate.

Spore density was determined by direct counting using a haemocytometer, and each suspension was adjusted by serial dilution to the required concentration. For pathogenicity assays, suspensions were prepared at five concentrations: 1.5×10^3, 1.5×10^4, 1.5×10^5, 1.5×10^6, and 1.5×10^7 spores mL^{-1}. Similar Bt bioassay studies against *S. frugiperda* report spore or inoculum standardization and dose response analysis using probit based approaches(Gemmeda et al., 2023; Noran et al., 2024).

Insect source and rearing conditions

Larvae of *S. frugiperda* were collected from maize fields and maintained in the laboratory to obtain a continuous supply of test insects. Adults were allowed to mate and oviposit, egg masses were collected until hatching, and larvae were reared on fresh maize leaves until the third instar.

Third instar larvae were selected to ensure a uniform test stage across all treatments. Rearing and bioassays were conducted under controlled environmental conditions set to 27 ± 2 °C, relative humidity 70 ± 10 percent, and a 12hour light and 12hour dark photoperiod.

Leaf dip screening bioassay

Fresh maize leaves were washed with clean water and air dried. Leaf sections were dipped into a spore suspension of each isolate at 1.5×10^7 spores mL^{-1} for a short-standardized immersion period, then air dried at room temperature until the surface was no longer wet. Treated leaves were placed into rearing containers and offered as food to larvae.

Each isolate was tested on 30 third instar larvae. To support statistical reliability, larvae should be distributed into replicates, for example three replicates of 10 larvae per isolate, with replicates handled independently. Mortality and symptoms were monitored as follows: larval symptoms and behavior were checked at 6 hour intervals, and cumulative mortality was recorded at 12, 24, 48, and 72 hours after exposure. A larva was considered dead when it did not respond to gentle prodding. A negative control was included using leaf sections dipped in distilled water and processed identically. Leaf dip bioassays using water dipped controls and replicated larval groups are commonly used in laboratory evaluations involving *S. frugiperda*(El-Solimany et al., 2024; Saleem et al., 2024).

Selection of isolates for pathogenicity testing

Isolates were considered promising and selected for further pathogenicity testing when they produced at least 50 percent larval mortality by 72 hours in the screening bioassay. This type of screening threshold is commonly used to narrow a large isolate set to a smaller group of candidates for dose response and time mortality characterization(Gemmeda et al., 2023; Noran et al., 2024).

Concentration response bioassay and LC50 estimation

Selected isolates were evaluated at the five spore concentrations listed above, from 1.5×10^3 to 1.5×10^7 spores mL^{-1}, using the same leaf dip feeding procedure. For each concentration, larvae were exposed to treated leaves and mortality was recorded at 12, 24, 48, and 72 hours.

Median lethal concentration values were calculated using probit analysis with 95 percent fiducial limits. Probit based LC50 estimation is routinely used in *S. frugiperda* susceptibility and efficacy studies and is also reported in Bt isolate bioassay work. For the concentration response bioassay, five spore concentrations were prepared to capture a wide range of larval responses from low to high exposure levels. The concentration series used for LC50 estimation is presented in Table 2.

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Code	Concentration	Unit
C1	1.5×10^3	spores mL^{-1}
C2	1.5×10^4	spores mL^{-1}
C3	1.5×10^5	spores mL^{-1}
C4	1.5×10^6	spores mL^{-1}
C5	1.5×10^7	spores mL^{-1}

Table 2 shows a tenfold serial dilution series from 1.5×10^3 to 1.5×10^7 spores mL^{-1}, which is suitable for fitting probit models and estimating LC50 values. Using a broad concentration range increases the likelihood that mortality will span from low to high levels, improving the precision and

interpretability of the dose response relationship.

Time mortality bioassay and LT50 estimation

For estimation of median lethal time, LT50, larvae were exposed to leaves treated with 1.5×10^7 spores mL^{-1} of each selected isolate. Mortality was checked at 6 hour intervals up to 72 hours. LT50 values were estimated using probit based time mortality analysis and reported with 95 percent fiducial limits. Time mortality analysis combined with probit estimation is commonly reported alongside LC50 to describe speed of kill and overall pathogenicity.

Correction for control mortality and data quality rules

Observed mortality was expressed as percent mortality for each isolate, concentration, and time point. When mortality occurred in the control group, corrected mortality was calculated using Abbott correction. Several recent laboratory studies explicitly apply Abbott correction when control mortality is low to moderate, and discard tests when control mortality exceeds 20 percent.

The following quality control rules were applied. If control mortality was 5 to 20 percent, Abbott corrected mortality was used. If control mortality was greater than 20 percent, the bioassay was discarded and repeated.

Statistical analysis and reporting

Screening mortality results were summarized as mean percent mortality by isolate and observation time. For pathogenicity assays, dose response probit models were fit to estimate LC50 with 95 percent fiducial limits, and time mortality probit models were used to estimate LT50 with 95 percent fiducial limits. Model reporting should include slope, standard error, and a goodness of fit indicator where available because many Scopus indexed journals ask for these details. Probit based LC50 reporting and inclusion of slope parameters are explicitly described in Bt related bioassay work against *S. frugiperda*.

RESULTS AND DISCUSSION

Screening bioassay at 1.5×10^7 spores mL^{-1}

The initial screening of 24 local *Bacillus thuringiensis* isolates identified a subset of candidates with high larvicidal activity against third instar *Spodoptera frugiperda*. To make the selection process transparent, mortality at 72 h for all isolates, and the isolates that met the advancement criterion of at least 50 percent mortality, are presented in Table 3.

Table 3 shows that 10 isolates achieved at least 50 percent mortality by 72 h. The highest screening mortality was observed for ITH (93.3 percent), followed by ITD (86.7 percent), ITF (83.3 percent), and ITW (80.0 percent). This pattern is consistent with the general expectation that Bt activity can vary substantially among isolates due to differences in toxin profiles, sporulation performance, and strain level traits that influence ingestion, midgut damage, and septicemia development.

Note on isolate labels: two isolate codes appeared more than once in the original dataset, so they are shown here as ITK1 and ITK2, and ITX1 and ITX2, to keep every tested entry uniquely identifiable. Before submission, the laboratory isolate log should confirm whether these were truly different isolates or a transcription issue.

Pathogenicity of selected isolates, LC50 and LT50

After screening, the 10 selected isolates were quantified using dose response analysis to estimate LC50 at 72 h and time mortality analysis to estimate LT50. The results are summarized in Table 4, which allows direct comparison of potency and speed of kill among candidate isolates

Table 3. *Screening mortality*

No.	Isolate code	Mortality at 72 h (percent)	Selected for pathogenicity
1	ITK1	56.7	Yes
2	ITH	93.3	Yes
3	ITA	33.3	No
4	ITC	40	No
5	ITD	86.7	Yes
6	ITG	30	No
7	ITW	80	Yes
8	ITX1	46.7	No
9	ITB	36.7	No
10	ITE	33.3	No
11	ITX2	46.7	No
12	ITY	56.7	Yes
13	ITF	83.3	Yes
14	ITK2	66.7	Yes
15	ITU	50	Yes
16	ITM	53.3	Yes
17	ITS	26.7	No
18	ITR	46.7	No
19	ITN	73.3	Yes
20	ITQ	43.3	No
21	ITP	40	No
22	ITT	36.7	No
23	ITO	40	No
24	ITV	46.7	No

Table 4. LC50 at 72 h and LT50

Isolate code	LC50 at 72 h (spores mL^{-1})	95 percent fiducial limits (spores mL^{-1})	LT50 (h)	95 percent fiducial limits (h)
ITK1	2.9×10^4	2.3×10^4 to 9.7×10^4	28.2	22.2 to 35.5
ITH	7.5×10^3	1.1×10^2 to 1.9×10^4	19.5	15.9 to 33.9
ITD	1.9×10^4	1.3×10^4 to 7.7×10^4	29.5	23.9 to 36.6
ITW	2.1×10^4	0.4×10^4 to 3.1×10^4	22.4	17.4 to 28.8
ITY	1.1×10^5	4.2×10^4 to 2.8×10^5	30.2	25.1 to 36.3
ITF	5.4×10^4	0.7×10^4 to 0.6×10^5	22.9	18.6 to 28.2
ITK2	6.6×10^4	3.6×10^4 to 1.2×10^5	34.7	28.2 to 42.7
ITU	1.5×10^5	4.7×10^4 to 4.5×10^5	24.6	19.9 to 30.2
ITM	2.5×10^5	1.1×10^5 to 5.8×10^5	36.5	30.2 to 43.7
ITN	6.5×10^4	2.9×10^4 to 6.8×10^4	37.2	30.9 to 44.7

Table 4 indicates that ITH was the most potent isolate, with the lowest LC50 (7.5×10^3 spores mL^{-1}) and the fastest median lethal time (LT50 19.5 h). In practical terms, an isolate that combines low LC50 with short LT50 is a strong candidate for further development because it can provide high mortality at lower doses and act more quickly after ingestion. Similar selection logic is commonly used when advancing Bt isolates toward formulation development, and safety screening against undesirable metabolites is often recommended at this stage as well.

The pathogenicity of the selected *Bacillus thuringiensis* isolates was quantified using probit analysis to estimate LC50 at 72 h and LT50 at 1.5×10^7 spores mL^{-1}, and the results are reported together with 95 percent fiducial limits to describe the precision of each estimate. Overall, isolate ITH showed the highest potency and fastest killing activity, with the lowest LC50 at 72 h of 7.5×10^3 spores mL^{-1} and the shortest LT50 of 19.5 h, indicating strong

potential for further development as a Bt based biopesticide candidate against *Spodoptera frugiperda*. Several other isolates also performed well based on their LC50 and LT50 profiles, suggesting that locally sourced Bt isolates can provide valuable candidates for integrated pest management. The fiducial limits generally bracket the point estimates, supporting internal consistency of the reported LC50 and LT50 values and enabling more reliable comparison among isolates.

Implications for integrated management

The identification of locally effective Bt isolates is relevant because fall armyworm management increasingly requires diversification of tools, especially given documented resistance challenges in some regions for both insecticides and Bt crops. Evidence of practical resistance to pyramided Bt maize in field populations highlights the importance of integrated approaches that reduce selection pressure and provide multiple compatible options. In this context, locally sourced Bt based biopesticide candidates can serve as an important component of integrated pest management, potentially complementing cultural control, biological control, and targeted insecticide use.

Field oriented studies of Bt formulations against *S. frugiperda* also show that Bt based products can reduce larval survival and crop injury under applied conditions, supporting the translational value of laboratory isolate screening when followed by formulation and field validation.

CONCLUSION

This study demonstrated that local *Bacillus thuringiensis* isolates vary substantially in their larvicidal activity against third instar *Spodoptera frugiperda* when evaluated using a maize leaf dip bioassay. Screening at 1.5×10^7 spores mL^{-1} identified 10 isolates that achieved at least 50 percent mortality by 72 h, indicating that several local isolates have promising bioinsecticidal potential and merit further development. Among the selected isolates, ITH showed the highest pathogenicity, characterized by the lowest LC50 at 72 h of 7.5×10^3 spores mL^{-1} and the fastest killing activity with an LT50 of 19.5 h. Other isolates also showed strong performance based on their mortality, LC50, and LT50 profiles, supporting the feasibility of selecting locally sourced Bt candidates for fall armyworm management. Overall, the results provide a foundation for subsequent steps, including confirmation of isolate identity, characterization of toxin profiles, optimization of production and formulation, and validation under semi field and field conditions to determine efficacy, persistence, and compatibility within integrated pest management programs for maize.

Future work should focus on steps required to translate these laboratory findings into a practical biopesticide option. First, isolate identity should be verified and traceable through standardized coding and molecular confirmation, followed by characterization of insecticidal toxin profiles and relevant virulence markers. Second, production parameters and formulation should be optimized to improve spore and crystal yield, stability during storage, and persistence under environmental conditions. Third, efficacy should be validated under semi field and field trials using appropriate application methods and dose rates, while monitoring impacts on crop injury and yield. Finally, compatibility with integrated pest management components should be evaluated, including interactions with commonly used insecticides, natural enemies, and other biological agents, as well as the potential role of these isolates in resistance management programs for sustainable fall armyworm control.

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