ASIAN JOURNAL OF AGRICULTURE
Volume 10, Number 1, June 2026
Pages: 10

E-ISSN: 2580-4537
https://doi.org/10.13057/asianjagric/g100181

Laboratory screening of three local entomopathogenic fungal isolates against third-instar *Spodoptera frugiperda*

LILIAN RIZKIE[1,2], TJANDRA ANGGRAENI[3,♥], I NYOMAN PUGEG ARYANTHA[3], AGUS SUSANTO[2]

[1]Biology Doctoral Program, School of Life Sciences and Technology, Institut Teknologi Bandung. Jl. Ganesa 10, Bandung 40132, West Java, Indonesia
[2]Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran. Jl. Raya Bandung-Sumedang Km. 21, Jatinangor 45363, West Java, Indonesia
[3]School of Life Sciences and Technology, Institut Teknologi Bandung. Jl. Ganesa 10, Bandung 40132, West Java, Indonesia. Tel.: +62-022-2511575, ♥email: tjandra@itb.ac.id

Manuscript received: 2 March 2026. Revision accepted: 26 June 2026.

Abstract. *Rizkie L, Anggraeni T, Aryantha INP, Susanto A. 2026. Laboratory screening of three local entomopathogenic fungal isolates against third-instar* Spodoptera frugiperda*. Asian J Agric 10: g100181. https://doi.org/10.13057/asianjagric/g100181.* The fall armyworm (*Spodoptera frugiperda*) is an important pest of maize in tropical production systems, including Indonesia. In the field, control of this pest still relies mainly on synthetic insecticides, making the evaluation of local biological control agents necessary. This study tested three local entomopathogenic fungal isolates, morphologically assigned to *Beauveria*, *Metarhizium*, and *Lecanicillium*, against third-instar *S. frugiperda* larvae at 10^6-10^9 conidia mL^{-1}. Day-7 mortality and sublethal responses were analyzed with generalized linear models, while LT and LC values were estimated by probit analysis. At 10^9 conidia mL^{-1}, mortality reached 85.0% for the *Beauveria* isolate and 90.0% for the *Metarhizium* isolate, compared with 63.3% for the *Lecanicillium* isolate. LT_{50} values at this concentration were 2.75, 2.56, and 5.08 days, respectively. LC estimates outside the tested concentration range were treated as assay-based comparative indices, not as absolute toxicity thresholds. After 48 h of sublethal exposure, relative growth rate, relative consumption rate, and efficiency of conversion of ingested food were reduced, especially in the *Beauveria* and *Metarhizium* treatments. Overall, the *Beauveria* and *Metarhizium* isolates showed stronger laboratory performance against *S. frugiperda* larvae than the *Lecanicillium* isolate. Molecular confirmation and greenhouse or field validation are still required before practical application can be considered.

Keywords: Biological control, fall armyworm, maize pest management, sublethal effects, virulence bioassay

INTRODUCTION

The fall armyworm (*Spodoptera frugiperda*, Lepidoptera: Noctuidae) is an important invasive pest of maize outside the native range in the Americas (Overton et al. 2021; Kenis et al. 2023). During the past decade, this polyphagous insect has spread across Africa, Asia, and Oceania, where outbreaks have caused serious crop losses (Chen et al. 2023). The larvae can attack many host plants, including maize, rice, and sorghum, making the pest difficult to manage in tropical and subtropical production areas (Dessie et al. 2024; Yaméogo et al. 2024).

In Indonesia, *S. frugiperda* was first reported in 2019 and quickly became an important pest of maize. Within two years, outbreaks had caused yield losses of 20-50% in several maize-growing regions (Asfiya et al. 2020; Lestari et al. 2024). The larvae usually damage the maize whorl, but feeding can also extend to the stem and cob, reducing crop productivity (Trisyono et al. 2024). Surveys in Yogyakarta showed that the pest was already widespread in smallholder farming systems (Putra et al. 2024), while reports of corn and rice strains indicate genetic variation that may complicate management (Puu et al. 2024). Its rapid dispersal, adaptability, and high reproductive capacity make *S. frugiperda* difficult to manage in maize production systems (Montezano et al. 2019).

Farmers still depend largely on chemical insecticides when larval damage is visible in the field. This approach can provide rapid suppression, but intensive use has accelerated resistance, reduced efficacy, and raised environmental concerns (Togola et al. 2018; Van den Berg and du Plessis 2022). Reduced susceptibility to several insecticide classes has also been reported in *S. frugiperda* populations, so chemical control alone cannot be relied on as a long-term strategy (Bird et al. 2022; Khan and Ali 2025).

Entomopathogenic fungi are biological control agents used against insect pests, including lepidopteran larvae. Unlike many microbial agents that must be ingested, these fungi can infect insects through the cuticle, followed by host colonization and, in some cases, metabolite production that may affect immunity, metabolism, or feeding behavior (Karthi et al. 2024; Shahbaz et al. 2024). Fungal infection has been associated with changes in gut microbiota and digestive enzyme activity in *S. frugiperda* (Liu et al. 2023; Zhang et al. 2023). Pathogenicity of *Beauveria* and *Metarhizium* species has been reported against lepidopteran pests, including *Helicoverpa armigera*, *Plutella xylostella*, and *S. frugiperda* (Bathina and Bonam 2020; Gao et al. 2022; Idrees et al. 2022; Alwaneen et al. 2024). *Lecanicillium* is more often reported from hemipteran hosts, although infection of lepidopteran larvae has also

been documented (Abdel-Raheem et al. 2020; Anshary et al. 2020). Virulence can differ among fungal isolates, even within the same genus, because isolates may differ in origin, host association, and infection ability (Shang et al. 2022; Ullah et al. 2022). Local isolates therefore, need to be tested under laboratory conditions before further evaluation.

After *S. frugiperda* was found in Indonesia, local entomopathogenic and endophytic fungi began to be tested against this pest. In South Sumatra, endophytic fungi from plant tissues around maize ecosystems, including *Beauveria*, *Metarhizium*, and *Paecilomyces*, were evaluated against fall armyworm larvae (Gustianingtyas et al. 2021). Herlinda et al. (2020) reported pathogenicity of local fungal isolates under regional climatic conditions, and Minarni et al. (2022) recorded reduced feeding, fecundity, and fertility after fungal exposure. In East Java, soil-inhabiting *Beauveria* and *Metarhizium* isolates were pathogenic to *Spodoptera litura* (Afandhi et al. 2022). Endophytic fungi from South Sumatra also reduced *S. frugiperda* populations and leaf damage in inoculated maize (Rindiani et al. 2024). These studies indicate that local fungal isolates can affect lepidopteran pests, but direct comparisons against *S. frugiperda* under similar laboratory bioassay conditions remain limited.

Comparative laboratory information is needed for locally sourced isolates against third-instar *S. frugiperda*, especially when lethal and sublethal responses are assessed together. The present study tested three individual local isolates morphologically assigned to *Beauveria*, *Metarhizium*, and *Lecanicillium*. These isolates were treated as isolate-level materials rather than as representatives of genus-wide performance, without examining local adaptation, ecological matching, or performance relative to non-local isolates. Mortality, LT and LC estimates, survival patterns, and sublethal nutritional responses were evaluated to provide an initial comparative basis for further assessment of these candidate isolates.

MATERIALS AND METHODS

Procedures

Laboratory rearing of *Spodoptera frugiperda*

Larvae of *S. frugiperda* were collected from a maize field in Jatinangor, West Java, Indonesia, and used to establish a laboratory colony at the Pesticides and Environmental Toxicology Laboratory, Universitas Padjadjaran, West Java. The colony was maintained on fresh baby corn under controlled conditions of 25±2°C, 70±5% relative humidity, and a 12:12 h light:dark photoperiod. To minimize cannibalism, larvae were reared at low density and separated individually from the third instar onward. Pupae were transferred to mesh cages, where adults were supplied with 10% honey solution and provided with maize plants for oviposition. Eggs were collected to establish successive generations. Uniform third-instar larvae from the F10-F11 laboratory generation were used in the bioassays.

Preparation of fungal isolate and spore suspension

Three local entomopathogenic fungal isolates representing the genera *Beauveria*, *Metarhizium*, and *Lecanicillium* were obtained from the Microbial Pesticide Subdivision, Pesticides and Environmental Toxicology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran (Table 1). These isolates were originally recovered from *Tenebrio molitor* cadavers and were considered locally available fungal resources for comparative laboratory screening.

Each genus was represented by a single isolate only; accordingly, the present study compares three individual local isolates rather than replicated taxonomic groups. Biological interpretation is therefore restricted to isolate-level performance within genus-level identification.

Identification was conducted to the genus level based on standard morphological characteristics, including conidial morphology, conidiogenous structures, and colony features, following Samson et al. (1988) and Humber (2012). Molecular identification, such as ITS or TEF1-α sequencing, was not performed because the work was designed as an initial laboratory bioassay-based screening within the available project resources. Because identification was based on morphology alone, the isolates are treated here at genus-level resolution. Their taxonomic identity therefore remains limited, which reduces reproducibility across studies and restricts interpretation to the tested isolates. The cultures are retained in the laboratory collection for possible molecular confirmation in future work.

For each isolate, conidia were collected from a 1 cm diameter sporulating colony region. The cultures were grown on Potato Dextrose Agar (PDA) at 25±2°C for 30 days before conidia were harvested. This incubation period was used to obtain conidia from colonies of similar maturity. To harvest conidia, 10 mL of sterile distilled water containing 0.05% Tween 80 was added to each plate, and the colony surface was scraped gently (Bugeme et al. 2008). The suspension was filtered through sterile gauze to separate conidia from hyphal fragments.

Table 1. Local fungal isolates used in the bioassay against *Spodoptera frugiperda*

Isolate	Isolate code	Host	Site of origin	Coordinate	Year of isolation
Beauveria	BCLSJS 2	*Tenebrio molitor*	Cileles, Jatinangor	6°54'40.3"S, 107°46'39.1"E	2024
Metarhizium	MCBSJS 1	*Tenebrio molitor*	Cibeusi, Jatinangor	6°56'29.0"S, 107°45'30.8"E	2024
Lecanicillium	VCBSJS 2	*Tenebrio molitor*	Cibeusi, Jatinangor	6°56'29.0"S, 107°45'30.8"E	2024

Conidial concentration was counted with a hemocytometer after serial dilution and adjusted to 1×10^9 conidia mL^{-1}. The stock suspension was then diluted tenfold to obtain 1×10^8, 1×10^7, and 1×10^6 conidia mL^{-1}. These four concentrations, ranging from 10^6 to 10^9 conidia mL^{-1}, were used for comparative screening across isolates. Concentrations below 10^6 conidia mL^{-1} were not included because the bioassay was designed to compare isolate performance within a practical laboratory range. It was not intended to define the lower boundary of the lethal concentration response.

Spore viability was checked once before the bioassays using a direct germination method, with three replicate plates for each isolate. A 150 µL aliquot of the stock suspension was plated onto water agar and incubated at 25°C for 24 h. Germination was assessed by examining at least 300 conidia per isolate under a microscope. A conidium was recorded as viable when the germ tube length was equal to or greater than the conidium length (Oliveira et al. 2015). Only suspensions with germination rates above 85% were used in the bioassays.

Bioefficacy evaluation of fungal isolates against *Spodoptera frugiperda*

Third-instar *S. frugiperda* larvae were used to test the three fungal isolates using a contaminated-feed leaf-dip assay. Maize leaf sections were dipped in conidial suspension, and larvae contacted the conidia while feeding and moving on the treated leaf surface. Exposure therefore involved surface contact and incidental ingestion. Because leaves, not larvae, were treated, the exact number of conidia contacting each larva was not measured. The suspension concentration in conidia mL^{-1} was used as the exposure level for the assay. It was not treated as a measured topical dose per larva.

Fresh maize leaves were cut into 4×4 cm pieces and dipped for 30 s in the conidial suspensions, following a modified method of Louw et al. (2025). The dipped leaves were kept under sterile conditions for about 20 min until the surface was dry. The *Beauveria*, *Metarhizium*, and *Lecanicillium* isolates were tested at 1×10^9, 1×10^8, 1×10^7, and 1×10^6 conidia mL^{-1}. Control leaves were treated in the same way using sterile distilled water containing 0.05% Tween 80.

For each treatment, three independent biological replicates were prepared. Each replicate contained 20 larvae, giving 60 larvae per treatment. The replicates were kept separate to maintain independence and avoid pseudoreplication. This replication level was used for preliminary comparison among isolates, although bounded LC estimates and LT_{80} values beyond the observation window have limited precision. Larvae were placed individually in sterile Petri dishes lined with moist filter paper. Treated leaves were given once at the start of the test and left for 48 h. After this period, larvae were fed untreated fresh maize leaves, which were replaced daily until the end of observation. Individual larvae served as biological observation units, while replicate-level data were used for statistical inference.

Mortality was checked daily for seven days. The larvae were considered dead when they no longer responded to gentle probing with a fine brush. Dead larvae were surface-sterilized with 70% ethanol and transferred to moist chambers to check for external fungal sporulation. This observation was used only as qualitative postmortem confirmation. The proportion of sporulating cadavers was not analyzed as a quantitative endpoint. Cumulative daily mortality was used to estimate LT_{50} and LT_{80}, whereas day-7 mortality was used to estimate LC_{50} and LC_{90}. The bioassays were maintained at 25±2°C, 75±5% relative humidity, and a 12:12 h light:dark photoperiod. Because larvae were exposed through treated leaves rather than by direct application, LC values were interpreted as comparative indices for this laboratory system, not as absolute topical-dose toxicity values.

Evaluation of sublethal effects and nutritional indices

Sublethal effects were assessed during the first 48 h after treatment because larvae were still actively feeding during this period, allowing growth and food consumption to be measured. The 48-h period also limited the loss of larvae available for measurement as mortality increased after longer exposure. Nutritional indices were calculated following Waldbauer (1968).

Third-instar larvae were weighed before treatment (W_1) and after 48 h of feeding (W_2). Maize leaf sections used as diet were weighed before being offered to the larvae (W_i) and after feeding (W_f) to determine food consumption (F). Leaf sections without larvae were kept under the same conditions to correct for weight loss caused by desiccation. Corrected food consumption was calculated as follows:

$$F = (W_i - W_f) - (C_i - C_f)$$

Where, W_i and W_f are the initial and final weights of leaves offered to larvae (g), and C_i and C_f are the initial and final weights of control leaves (g). Feeding duration (t) was expressed in days. Relative Growth Rate (RGR), Relative Consumption Rate (RCR), and Efficiency of Conversion of Ingested Food (ECI) were calculated as:

$$RGR = (W_2 - W_1) / (t\times W_1),$$

$$RCR = F / (t\times W_m),$$

$$ECI\ (\%) = (W_2 - W_1) / F\times100$$

Where, W_1 = initial larval weight (g), W_2 = final larval weight (g), F = amount of food consumed (g), W_m = mean larval weight during the feeding period ($W_m = (W_1 + W_2)/2$), and t = feeding duration (days). Accordingly, RGR and RCR are expressed in g g^{-1} day^{-1}, and ECI in %. Nutritional indices were calculated for individual larvae that remained alive and evaluable at the end of the 48-h feeding period. For each treatment replicate, individual values were averaged to obtain one replicate-level mean, and these replicate means were used as the inferential statistical unit for ANOVA.

Data analysis

Before analysis, control mortality was corrected with Abbott's formula. Day-7 mortality was analyzed with a quasibinomial generalized linear model. Isolate, conidial concentration, and the isolate×concentration interaction were included in the model. Estimated marginal means were compared among isolates at each concentration using Sidak adjustment.

LT_{50} and LT_{80} were estimated from cumulative mortality from day 1 to day 7. For LT estimation, a probit model was fitted to each biological replicate. LC_{50} and LC_{90} were estimated from day-7 mortality by fitting probit regression to $\log_{10}$-transformed conidial concentration. Replicates were kept separate and were not pooled, so that independence was maintained and pseudoreplication was avoided. LT and LC values are reported as mean±standard error across biological replicates. The 95% confidence intervals were calculated from among-replicate variation. LT_{80} values beyond the 7-day observation period were regarded as model-based extrapolations, not observed endpoints. Model fit was evaluated using deviance-based diagnostics.

Survival analysis was kept separate from the probit-based LT estimates. Kaplan-Meier curves and log-rank tests were used to compare time-to-death among treatments. These analyses provided the main survival comparison. Probit-derived LT values were used only as descriptive lethal-time summaries.

Nutritional indices were analyzed by two-way ANOVA with isolate and dose as fixed factors. Individual larvae were the biological observation units, and replicate-level means were used for statistical inference. Residual normality and homogeneity of variance were checked for the ANOVA models. Control treatments were analyzed separately by one-way ANOVA followed by Dunnett tests. All analyses were performed in R version 4.4.1.

RESULTS AND DISCUSSION

Spore viability

Before the bioassays, conidial germination was assessed for all three isolates, and each showed germination above 85%. Mean germination rates were 90.41±0.86% for the *Beauveria* isolate, 91.67±0.52% for the *Metarhizium* isolate, and 89.23±0.79% for the *Lecanicillium* isolate. One-way ANOVA detected no significant differences among isolates ($F_{2,6} = 2.74$, $p = 0.143$). Given that inoculum quality was comparable across treatments at the time of application, initial conidial viability is unlikely to have been a primary driver of the differences in mortality and sublethal responses observed subsequently.

Mortality of *Spodoptera frugiperda*

Larval mortality of *S. frugiperda* increased with conidial concentration across all treatments, indicating a clear dose-related response. At each tested concentration, the *Beauveria* and *Metarhizium* isolates consistently produced higher mortality than the *Lecanicillium* isolate. At 1×10^9 conidia mL^{-1}, day-7 mortality reached 85.0% and 90.0% in the *Beauveria* and *Metarhizium* treatments, respectively, compared with 63.3% in the *Lecanicillium* treatment.

A quasibinomial generalized linear model revealed significant main effects of isolate ($F_{2,24} = 20.37$, $p < 0.001$) and conidial concentration ($F_{3,24} = 8.45$, $p < 0.001$), whereas the isolate×concentration interaction was not significant ($F_{6,24} = 0.78$, $p = 0.591$). This indicates that the relative ranking of the three isolates remained broadly consistent across the tested concentration range. The same pattern was visible in Figure 1, where *Lecanicillium* showed only limited overlap in 95% confidence intervals with the other two isolates at the higher concentrations. Control mortality was low, with 1/20, 0/20, and 2/20 larvae dying in the three biological replicates, with a mean mortality of 5.0% and a range of 0-10%. Mortality values were then corrected using Abbott's formula before analysis. After incubation in moist chambers, external sporulation was used as a qualitative postmortem check for fungal infection. Mycosis frequency was not recorded as a quantitative endpoint and was excluded from inferential analysis.

Lethal time estimates (LT_{50} and LT_{80})

At the highest concentration, lethal-time estimates separated the three isolates more clearly. At 1×10^9 conidia mL^{-1}, the *Metarhizium* and *Beauveria* isolates reached LT_{50} at 2.56 and 2.75 days, respectively, compared with 5.08 days for the *Lecanicillium* isolate (Table 2). LT_{80} values showed the same tendency across the concentration series, with lower estimates for the *Beauveria* and *Metarhizium* isolates.

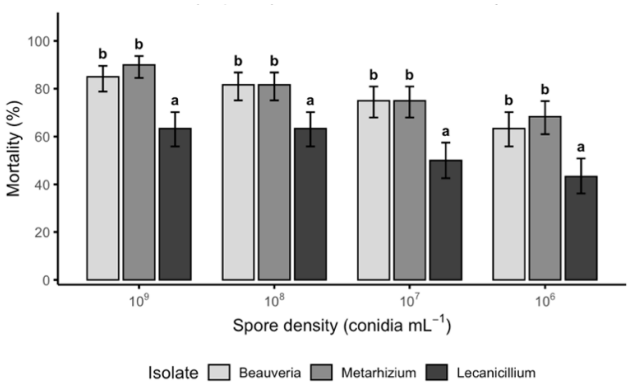

Figure 1. Mortality (%) of third-instar *S. frugiperda* larvae after exposure to the local *Beauveria*, *Metarhizium*, and *Lecanicillium* isolates at four conidial concentrations (10^6-10^9 conidia mL^{-1}). The isolates were assigned to genus level based on morphology. Each treatment comprised three biological replicates of 20 larvae (n = 60 per treatment). Bars show estimated marginal means with 95% confidence intervals. Different letters indicate significant differences among isolates within each concentration based on Sidak-adjusted comparisons

Table 2. Day-7 mortality (%) and lethal time estimates (LT_{50} and LT_{80}) of third-instar *S. frugiperda* larvae exposed to three entomopathogenic fungal isolates at four conidial concentrations

Isolate	Concentrations	Mortality (%)±95% CI	LT_{50}±SE (days)	LT_{80}±SE (days)
Beauveria	1×10^9	85.0 (78.8-89.6)^b	2.75±0.25	5.75±0.45
	1×10^8	81.7 (75.2-86.8)^b	3.96±0.26	6.95±0.39
	1×10^7	75.0 (68.0-80.9)^b	4.48±0.15	7.22±0.26^x
	1×10^6	63.3 (55.9-70.2)^b	5.12±0.17	8.67±0.49^x
Metarhizium	1×10^9	90.0 (84.5-93.7)^b	2.56±0.20	5.57±0.46
	1×10^8	81.7 (75.2-86.8)^b	3.93±0.04	6.63±0.23
	1×10^7	75.0 (68.0-80.9)^b	4.04±0.14	7.33±0.35^x
	1×10^6	68.3 (61.0-74.9)^b	4.61±0.14	7.87±0.33^x
Lecanicillium	1×10^9	63.3 (55.9-70.2)^a	5.08±0.22	8.43±0.19^x
	1×10^8	63.3 (55.9-70.2)^a	5.51±0.21	8.50±0.25^x
	1×10^7	50.0 (42.6-57.4)^a	6.61±0.29	9.80±0.74^x
	1×10^6	43.3 (36.1-50.9)^a	6.69±0.20	10.08±0.41^x

Note: Values represent mean±SE from three biological replicates. Mortality values are Abbott-corrected estimated marginal means with 95% confidence intervals. Different letters within each concentration indicate significant differences among isolates based on Sidak-adjusted comparisons. LT_{80} values marked with superscript x were extrapolated from fitted probit models because the estimated LT_{80} exceeded the 7-day observation period and should therefore be interpreted as model-based comparative summaries rather than directly observed endpoints

Table 3. Time-to-death estimates (LT_{50} and LT_{80}) dataset of *Spodoptera frugiperda* larvae infected with three entomopathogenic fungal isolates (*Beauveria, Metarhizium*, and *Lecanicillium*) across four spore concentrations (10^6-10^9 conidia mL^{-1})

Isolate	Concentrations	LT_{50} ± SE (days)	LT_{50} 95%CI (days)	LT_{80} ± SE (days)	LT_{80} 95%CI (days)	Goodness-of-fit		
						χ^2	df	p-value
Beauveria	1×10^9	2.75 ± 0.25	[2.23, 3.28]	5.75 ± 0.45	[4.87, 6.64]	2.14-8.48	5	0.132-0.829
Beauveria	1×10^8	3.96 ± 0.26	[3.44, 4.47]	6.95 ± 0.39	[6.28, 7.61]	0.23-1.57	5	0.905-0.999
Beauveria	1×10^7	4.48 ± 0.15	[4.17, 4.84]	7.22 ± 0.26	[6.79, 7.26]	0.80-1.95	5	0.856-0.977
Beauveria	1×10^6	5.12 ± 0.17	[4.79, 5.46]	8.67 ± 0.49	[7.70, 9.64]	0.24-2.33	5	0.802-0.999
Metarhizium	1×10^9	2.56 ± 0.20	[2.21, 2.92]	5.57 ± 0.46	[4.76, 6.38]	1.63-2.70	5	0.747-0.898
Metarhizium	1×10^8	3.93 ± 0.04	[3.84, 4.02]	6.63 ± 0.23	[6.11, 7.19]	0.56-2.94	5	0.709-0.990
Metarhizium	1×10^7	4.04 ± 0.14	[3.77, 4.31]	7.33 ± 0.35	[6.84, 7.82]	0.59-3.45	5	0.631-0.988
Metarhizium	1×10^6	4.61 ± 0.14	[4.32, 4.89]	7.87 ± 0.33	[7.49, 8.20]	0.90-2.38	5	0.794-0.970
Lecanicillium	1×10^9	5.08 ± 0.22	[4.64, 5.51]	8.43 ± 0.19	[8.06, 8.81]	0.60-1.69	5	0.890-0.988
Lecanicillium	1×10^8	5.51 ± 0.21	[5.10, 5.93]	8.50 ± 0.25	[8.01, 8.99]	0.24-2.00	5	0.850-0.999
Lecanicillium	1×10^7	6.61 ± 0.29	[6.05, 7.18]	9.80 ± 0.74	[8.36, 11.25]	0.85-1.26	5	0.939-0.974
Lecanicillium	1×10^6	6.69 ± 0.20	[6.29, 7.09]	10.08 ± 0.41	[9.40, 10.76]	0.97-1.74	5	0.884-0.965

Note: Values are means ± Standard Error (SE) from three independent biological replicates analyzed without pooling. LT_{50} and LT_{80} were estimated by probit regression fitted separately for each replicate, and treatment means are summarized across replicates; 95% Confidence Intervals (CI) are shown. Goodness-of-fit statistics (χ^2, df, p) are reported for the replicate-level probit models and were non-significant ($p > 0.05$), indicating adequate model fit. Day-7 mortality (%) was analyzed using a quasibinomial GLM (logit link) including isolate × dose and replicate effects; different superscript letters within each dose indicate significant differences among isolates ($\alpha = 0.05$; Sidak-adjusted pairwise comparisons). Control was excluded from probit model fitting

Replicate-level probit models gave acceptable goodness-of-fit values (χ^2 = 0.23-8.48, df = 5, $p > 0.05$). Several LT_{80} estimates fell outside the 7-day observation period. They are reported as fitted probit estimates rather than observed endpoints and are used only to compare treatments. For this reason, they require more caution than LT values estimated within the observation period. Kaplan-Meier curves were analyzed with log-rank tests, and survival differed significantly among treatments ($p < 0.05$). The Kaplan-Meier curves and pairwise log-rank results are provided in Table 3. The probit-derived LT_{50} and LT_{80} values are reported as descriptive lethal-time benchmarks, not as the primary basis for survival inference.

Lethal concentration estimates (LC_{50} and LC_{90})

Dose-response summaries also indicated clear differences among isolates. For *Beauveria* and *Metarhizium*, day-7 mortality already exceeded 50% at the lowest tested concentration (1×10^6 conidia mL^{-1}), so LC_{50} could not be resolved numerically within the tested range and is therefore reported as $< 1\times10^6$ conidia mL^{-1}. In contrast, *Lecanicillium* showed lower virulence, with an LC_{50} on the order of 10^6 conidia mL^{-1} that was bracketed within the present concentration series (Table 4).

Table 4. Lethal concentration estimates (LC_{50} and LC_{90}) of *S. frugiperda* larvae following exposure to three entomopathogenic fungal isolates

Isolate	LC_{50} (conidia mL^{-1})±95% CI	LC_{90} (conidia mL^{-1})±95% CI	Slope±SE	χ^2 (df)	p-value
Beauveria	$< 1\times10^6$ (NR)	8.4×10^9 (1.1×10^9-6.8×10^{10})	0.238±0.046	0.76 (2)	0.716
Metarhizium	$< 1\times10^6$ (NR)	2.9×10^9 (2.9×10^8-3.0×10^{10})	0.259±0.064	0.52 (2)	0.777
Lecanicillium	1.0×10^6 (1.0×10^5-1.1×10^7)	$> 1\times10^9$ (NR)	0.141±0.039	0.42 (2)	0.829

Note: LC values were estimated from replicate-level probit models based on day-7 mortality. Values reported as $< 1\times10^6$ indicate estimates below the lowest tested concentration, whereas values reported as $> 1\times10^9$ indicate estimates above the highest tested concentration. LC values should be interpreted as comparative indices within the present leaf-dip laboratory system

Table 5. Nutritional indices (RGR, RCR, and ECI) of *Spodoptera frugiperda* larvae exposed to entomopathogenic fungal isolates, including untreated control

Isolate	Dose	RGR (g/g/day)		RCR (g/g/day)		ECI (%)	
		Mean ± CI 95%		Mean ± CI 95%		Mean ± CI 95%	
Beauveria	10^9	0.121 ± (0.025-0.217)	ab	0.460 ± (0.373-0.546)	a	20.14 ± (13.00-27.29)	ab
Beauveria	10^8	0.199 ± (0.102-0.295)	abc	0.545 ± (0.458-0.631)	ab	28.74 ± (21.60-35.88)	bc
Beauveria	10^7	0.266 ± (0.170-0.362)	bcd	0.716 ± (0.630-0.802)	c	28.54 ± (21.40-35.69)	bc
Beauveria	10^6	0.300 ± (0.203-0.396)	cd	1.210 ± (1.123-1.296)	e	19.12 ± (11.97-26.26)	ab
Metarhizium	10^9	0.094 ± (-0.003-0.190)	a	0.480 ± (0.393-0.566)	a	14.74 ± (7.60-21.88)	a
Metarhizium	10^8	0.192 ± (0.095-0.288)	abc	0.556 ± (0.469-0.642)	ab	27.73 ± (20.58-34.87)	bc
Metarhizium	10^7	0.264 ± (0.168-0.360)	bcd	0.952 ± (0.866-1.039)	d	20.22 ± (13.08-27.37)	ab
Metarhizium	10^6	0.332 ± (0.236-0.429)	cde	1.208 ± (1.122-1.294)	e	20.47 ± (13.33-27.62)	ab
Lecanicillium	10^9	0.323 ± (0.227-0.419)	cde	0.555 ± (0.469-0.641)	ab	41.92 ± (34.77-49.06)	d
Lecanicillium	10^8	0.362 ± (0.266-0.458)	de	0.688 ± (0.601-0.774)	bc	36.94 ± (29.80-44.09)	cd
Lecanicillium	10^7	0.474 ± (0.378-0.571)	e	1.131 ± (1.045-1.218)	e	28.63 ± (21.49-35.78)	bc
Lecanicillium	10^6	0.401 ± (0.304-0.497)	de	1.206 ± (1.119-1.292)	e	23.90 ± (16.76-31.04)	ab
Control	—	0.809 ± (0.712-0.905)	f	1.086 ± (1.000-1.172)	de	41.34 ± (34.20-48.48)	d
Group df		12		12		12	
Residual df		26		26		26	
F value		33.079		115.920		13.447	
p-value		< 0.001		< 0.001		< 0.001	

Note: Values represent means ± 95% Confidence Intervals (CI) from three independent biological replicates (n = 3). Statistical comparisons between infected treatments and the untreated control were conducted using Dunnett's post hoc test following one-way ANOVA ($\alpha = 0.05$). Different superscript letters indicate significant differences relative to the control. Two-way ANOVA and Sidak-adjusted pairwise comparisons for infected treatments only are presented in Figure 2 of the main manuscript

At the LC_{90} level, *Beauveria* and *Metarhizium* required concentrations on the order of 10^9 conidia mL^{-1} to achieve high mortality, whereas LC_{90} for *Lecanicillium* was not resolved within the tested range and is therefore reported as $> 1\times10^9$ conidia mL^{-1}. Because several LC estimates were bounded outside the tested concentration range, these values should be interpreted primarily as comparative assay-based indices for relative isolate performance rather than as absolute toxicological thresholds. The bounded LC_{50} values for *Beauveria* and *Metarhizium* further indicate that the concentration range used here was more suitable for comparative mortality screening than for precise calibration of lethal concentration boundaries.

Replicate-level probit models showed acceptable goodness-of-fit statistics ($\chi^2 = 0.42$-0.76, df = 2, $p > 0.71$). Estimated slopes are presented descriptively only and were not subjected to formal statistical comparison.

Sublethal impacts on larval performance

At 10^9 conidia mL^{-1}, the *Beauveria* and *Metarhizium* isolates produced marked reductions in larval nutritional performance (Table 5). RGR was reduced to 0.121 and 0.094 g g^{-1} day^{-1}, and ECI decreased to 20.1% and 14.7%, respectively. Larvae exposed to the *Lecanicillium* isolate at the same concentration showed higher values, with RGR of 0.323 g g^{-1} day^{-1} and ECI of 41.9% (Figure 2).

Two-way ANOVA detected significant effects of fungal isolate, spore density, and their interaction on all three nutritional indices ($p < 0.001$). Under the *Beauveria* and *Metarhizium* treatments, RCR decreased from values above 1.0 g g^{-1} day^{-1} at 10^6 conidia mL^{-1} to 0.46-0.48 g g^{-1} day^{-1} at 10^9 conidia mL^{-1}. In the *Lecanicillium* treatment, RCR remained higher across the concentration range, at 0.55-1.13 g g^{-1} day^{-1}. Compared with the Tween-only control, infected larvae had significantly lower RGR, RCR, and ECI across multiple concentrations based on Dunnett tests ($p < 0.001$). Full pairwise statistics are reported in Table 5. Residual normality and homogeneity of variance were adequate for the ANOVA models.

Food intake and conversion of ingested food were lower in treated larvae than in control larvae. This reduction was more pronounced in the *Beauveria* and *Metarhizium* treatments than in the *Lecanicillium* treatment. However, this assay could not determine whether the response was caused by fungal infection, reduced feeding on conidia-coated leaves, or both. Table 6 summarizes the lethal and sublethal indicators used to compare isolate performance.

Table 6. Integrated performance summary of three entomopathogenic fungal isolates against third-instar *Spodoptera frugiperda*

Isolate	Mortality (%) (10^9 conidia mL^{-1})	LT_{50} (days) (10^9 conidia mL^{-1})	LC_{50} (conidia mL^{-1})	RGR (g g^{-1} day^{-1}) (10^9 conidia mL^{-1})	RCR (g g^{-1} day^{-1}) (10^9 conidia mL^{-1})	ECI (%) (10^9 conidia mL^{-1})	Comparative summary
Metarhizium	90.0	2.56	$< 1 \times 10^6$	0.094	0.480	14.7	Higher mortality, shorter LT_{50}, stronger sublethal reduction
Beauveria	85.0	2.75	$< 1 \times 10^6$	0.121	0.460	20.1	Higher mortality, shorter LT_{50}, stronger sublethal reduction
Lecanicillium	63.3	5.08	1.0×10^6	0.323	0.555	41.9	Lower mortality, longer LT_{50}, weaker sublethal reduction

Note: Mortality, LT_{50}, RGR, RCR and ECI values are shown at the highest tested concentration (10^9 conidia mL^{-1}) to facilitate direct comparison of isolate performance under the present laboratory assay. LC_{50} values were derived from dose-response analysis across the tested concentration range. Values for *Beauveria* and *Metarhizium* were bounded below the lowest tested concentration

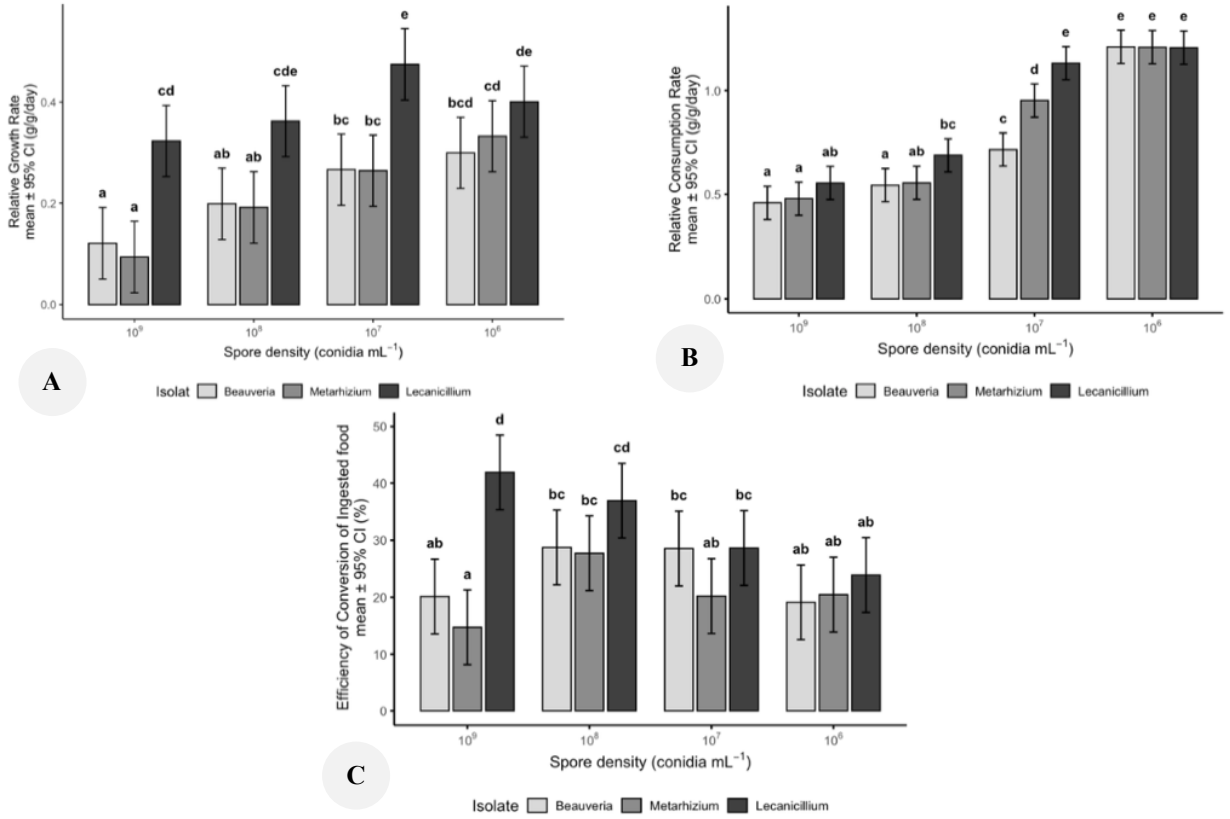

Figure 2. Nutritional indices of *S. frugiperda* larvae after exposure to the *Beauveria*, *Metarhizium*, and *Lecanicillium* isolates: A. Relative Growth Rate (RGR), B. Relative Consumption Rate (RCR), and C. Efficiency of Conversion of Ingested Food (ECI). Bars show estimated marginal means with 95% confidence intervals. Different letters denote significant differences among isolates within the same spore density

Discussion

The *Beauveria* and *Metarhizium* isolates gave higher day-7 mortality, shorter lethal-time summaries, and stronger effects on larval performance than the *Lecanicillium* isolate. Initial conidial viability was similar among isolates, so the pattern is unlikely to be a simple artifact of inoculum quality. It is best treated as an isolate-level difference in laboratory performance. With only one isolate representing each genus, however, the result does not support a genus-level interpretation of virulence.

All three isolates originated from agricultural environments in Jatinangor, West Java, and were originally recovered from *T. molitor* cadavers. Host of origin, however, does not reliably predict cross-host performance.

Pathogenicity toward *S. frugiperda* is shaped by infection biology, host immune responses, and ecological compatibility, and these factors can produce divergent outcomes even among isolates sharing the same recovery host. The present results describe screening outcomes for three locally sourced isolates and are not evidence of host-specialized adaptation.

The local origin of the isolates is still relevant, but only as context. These fungi were available from the same broad agroecological setting in which future biological control resources may be explored. The assay does not show whether geographic origin gave them any performance advantage, since non-local isolates were not tested and local adaptation was not examined. Its main contribution is a comparative laboratory baseline for more targeted evaluation of the tested isolates.

The stronger performance of the *Beauveria* and *Metarhizium* isolates could reflect faster conidial germination, more efficient cuticle penetration, or greater extracellular enzyme production during host invasion, although none of these traits were measured directly. Rapid establishment may be especially important against fast-developing lepidopteran larvae, since molting can interrupt fungal attachment or penetration when infection progresses too slowly (Hong et al. 2024). The short LT values observed in this study are consistent with previous laboratory studies showing rapid mortality in *S. frugiperda* and other lepidopteran larvae after exposure to entomopathogenic fungi (Ramanujam et al. 2020; Apirajkamol et al. 2023).

The *Lecanicillium* isolate acted more slowly and produced weaker sublethal effects than the *Beauveria* and *Metarhizium* isolates. This may reflect lower compatibility with *S. frugiperda* or less efficient infection under the assay conditions used here, although germination dynamics, enzyme activity, and cuticle penetration were not measured. Reports on *Lecanicillium* species more often involve soft-bodied pests, whereas outcomes against lepidopteran larvae appear more variable (Saruhan 2018; Ullah et al. 2022). The weaker response observed here should therefore be treated as an isolate-specific result, not as a genus-level conclusion.

The LC estimates followed the same isolate-level order as the mortality and lethal-time data. LC estimation was limited by the concentration series used. For the *Beauveria* and *Metarhizium* isolates, day-7 mortality was already above 50% at 1×10^6 conidia mL^{-1}, so LC_{50} fell below the tested range. For the *Lecanicillium* isolate, LC_{90} remained above 1×10^9 conidia mL^{-1}, and confidence intervals became wider at higher response levels. A broader concentration series would improve LC estimation, especially for isolates that caused substantial mortality even at the lowest tested concentration.

The lethal concentration estimates should also be interpreted with the leaf-dip method in mind. Larvae contacted conidia while feeding and moving on treated maize leaves, rather than through a measured topical inoculation. For that reason, the number of viable conidia contacting each larva could not be determined. The nominal suspension concentration was only an operational exposure index. LC values from this assay are useful for comparing the three isolates within the leaf-dip system, but they should not be treated as absolute toxicity thresholds.

This also affects comparison with topical bioassays. Zalte et al. (2024) reported an LC_{50} of 1.42×10^7 conidia mL^{-1} at 120 h in a topical bioassay of *Beauveria* against third-instar *S. frugiperda*, together with a comparatively steep probit slope. The shallower slopes in the present assay may reflect greater variation in actual conidial contact under leaf-dip exposure, producing a more gradual mortality increase across nominal concentrations. Direct numerical comparison between the two systems is therefore not warranted.

Mortality did not capture all effects of fungal exposure. Lower RGR, RCR, and ECI values show that treated larvae also had reduced feeding performance and poorer conversion of ingested food. These effects were strongest under the *Beauveria* and *Metarhizium* treatments, matching their higher mortality and shorter lethal-time values. Similar reductions in larval growth and feeding have been found in other lepidopteran larvae exposed to entomopathogenic fungi (de Souza et al. 2020; Russo et al. 2020). The lower food intake and poorer conversion observed here may be related to early infection stress, reduced feeding on conidia-coated leaves, or both. Because these effects could not be separated in the assay, the nutritional results indicate reduced feeding performance but do not identify the underlying mechanism.

The *Lecanicillium* treatment gave weaker sublethal effects than the *Beauveria* and *Metarhizium* treatments. At several concentrations, its nutritional-index values remained higher, indicating a smaller reduction in larval feeding performance. This may be related to slower infection or a weaker early effect on feeding and food conversion. Partial compensatory feeding under moderate infection stress has been reported in other lepidopteran larvae (Ullah et al. 2023; Tesari et al. 2024). In this assay, feeding may also have been affected by larval behavior or by the conidia-coated leaf surface.

Several points limit the interpretation of this study. The isolates were identified by morphology and retained at the genus level. Each genus was also represented by only one isolate. For this reason, the results apply to the tested isolates and should not be used to compare the genera as a whole. Molecular confirmation and testing of more isolates within each genus are still needed. The leaf-dip method also affected dose interpretation because larvae were exposed through treated maize leaves, not by direct inoculation. The exact number of conidia contacting each larva was not measured. Therefore, bounded LC estimates and LT_{80} values extending beyond the 7-day observation period should be read carefully. The LT_{80} values beyond this period are model-based estimates, not observed endpoints. Postmortem mycosis was checked only as qualitative confirmation and was not used as a quantitative endpoint. Even with these limits, the assay allowed the three local isolates to be compared under the same laboratory conditions.

The next step should include molecular confirmation, testing of more isolates within each genus, wider

concentration ranges, and greenhouse or field evaluation. Formulation work or wider application should wait until these steps are completed. For now, the results give an initial basis for selecting locally sourced isolates for further evaluation against *S. frugiperda* in maize.

In conclusion, the tested *Beauveria* and *Metarhizium* isolates showed stronger laboratory performance than the *Lecanicillium* isolate against third-instar *S. frugiperda* under laboratory conditions. They caused higher mortality, shorter LT values, and stronger reductions in larval feeding performance. These findings are still preliminary and isolate-specific because each genus was represented by only one isolate and identification was based on morphology. Molecular confirmation, testing of more isolates, and greenhouse or field evaluation are needed before the results are used for genus-level interpretation, formulation development, or practical application.

ACKNOWLEDGEMENTS

This study was supported by the Indonesian Education Scholarship, the Center for Higher Education Funding and Assessment, and the Indonesian Endowment Fund for Education.

REFERENCES

Abdel-Raheem MA, Youssif MAI, Helaly SMMY. 2020. Use of *Verticillium lecanii* and *Beauveria bassiana* against tomato leaf miner, *Tuta absoluta* (Meyrick) and *Bemisia tabaci* (Genn.) in tomato crop. Plant Arch 20 (Suppl 1): 479-482.

Afandhi A, Choliq FA, Fernando I, Marpaung YM, Setiawan Y. 2022. Occurrence of soil-inhabiting entomopathogenic fungi within a conventional and organic farm and their virulence against *Spodoptera litura*. Biodiversitas 23 (2): 1172-1180. https://doi.org/10.13057/biodiv/d230263.

Alwaneen WS, Tahir M, Avery PB, Wakil W, Kavallieratos NG, Eleftheriadou N, Boukouvala MC, Rasool KG, Husain M, Aldawood AS. 2024. Initial evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium robertsii*, and the entomopathogenic nematode *Heterorhabditis bacteriophora*, individually and in combination against *Helicoverpa armigera* (Lepidoptera: Noctuidae). Agronomy 14 (7): 1395. https://doi.org/10.3390/agronomy14071395.

Anshary A, Saleh S, Pasaru F. 2020. Influence of the Entomopathogenic Fungus, *Verticillium lecanii* (Zimm.) on the Cocoa Pod Borer, *Conopomorpha cramerella* (Snellen) under In-Vitro Conditions. Intl Conf 10th Congres Entomol Soc Indones (ICCESI 2019) (pp. 54-58). Atlantis Press, Dordrecht. https://doi.org/10.2991/absr.k.200513.009.

Apirajkamol N, Hogarty TM, Mainali B, Taylor PW, Walsh TK, Tay WT. 2023. Virulence of *Beauveria* sp. and *Metarhizium* sp. fungi towards fall armyworm (*Spodoptera frugiperda*). Arch Microbiol 205: 328. https://doi.org/10.1007/s00203-023-03669-8.

Asfiya W, Subagyo VN, Dharmayanthi AB, Fatimah F, Rachmatiyah R. 2020. Attack intensity of *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) on maize crops in Garut and Tasikmalaya Regencies, West Java. J Entomol Indones 17 (3): 163-170. https://doi.org/10.5994/jei.17.3.163.

Bathina P, Bonam R. 2020. Effect of endophytic isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin on *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in cabbage. Egypt J Biol Pest Control 30: 142. https://doi.org/10.1186/s41938-020-00342-w.

Bird LJ, Miles MM, Quade A, Spafford H. 2022. Insecticide resistance in Australian *Spodoptera frugiperda* (J.E. Smith) and development of testing procedures for resistance surveillance. PLoS One 17 (2): e0263677. https://doi.org/10.1371/journal.pone.0263677.

Bugeme DM, Maniania NK, Knapp M, Boga HI. 2008. Effect of temperature on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates to *Tetranychus evansi*. Exp Appl Acarol 46 (1-4): 275-285. https://doi.org/10.1007/s10493-008-9179-1.

Chen HL, Hasnain A, Cheng QH, Xia LJ, Cai YH, Hu R, Gong CW, Liu XM, Pu J, Zhang L, Wang XG. 2023. Resistance monitoring and mechanism in the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) for chlorantraniliprole from Sichuan Province, China. Front Physiol 14: 1180655. https://doi.org/10.3389/fphys.2023.1180655.

de Souza TD, Fernandes FO, Sanches AC, Polanczyk RA. 2020. Sublethal effects of different fungal isolates on *Helicoverpa armigera* (Lepidoptera: Noctuidae). Egypt J Biol Pest Control 30: 141. https://doi.org/10.1186/s41938-020-00327-9.

Dessie B, Ferede B, Taye W, Shimelash D. 2024. Field infestation of the invasive fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on maize in Southern Ethiopia. Crop Prot 178: 106587. https://doi.orG/10.1016/j.cropro.2024.106587.

Gao YP, Luo M, Wang XY, He XZ, Lu W, Zheng XL. 2022. Pathogenicity of *Beauveria bassiana* PfBb and immune responses of a non-target host, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Insects 13 (10): 914. https://doi.org/10.3390/insects13100914.

Gustianingtyas M, Herlinda S, Suwandi S. 2021. The endophytic fungi from South Sumatra (Indonesia) and their pathogenicity against the new invasive fall armyworm, *Spodoptera frugiperda*. Biodiversitas 22 (2): 1051-1062. https://doi.org/10.13057/biodiv/d220262.

Herlinda S, Noni O, Suwandi S, Hasbi H. 2020. Exploring entomopathogenic fungi from South Sumatra (Indonesia) soil and their pathogenicity against a new invasive maize pest, *Spodoptera frugiperda*. Biodiversitas 21 (7): 3071-3080. https://doi.org/10.13057/biodiv/d210711.

Hong S, Shang J, Sun Y, Tang G, Wang C. 2024. Fungal infection of insects: Molecular insights and prospects. Trend Microbiol 32 (3): 302-316. https://doi.org/10.1016/j.tim.2023.09.005.

Humber RA. 2012. Identification of entomopathogenic fungi. In: Lacey LA (eds.). Manual of Techniques in Invertebrate Pathology. 2nd Ed. Academic Press, San Diego. https://doi.org/10.1016/B978-0-12-386899-2.00006-3.

Idrees A, Afzal A, Qadir ZA, Li J. 2022. Bioassays of *Beauveria bassiana* isolates against the fall armyworm, *Spodoptera frugiperda*. J Fungi 8 (7): 717. https://doi.org/10.3390/jof8070717.

Karthi S, Vasantha-Srinivasan P, Senthil-Nathan S, Han YS, Shivakumar MS, Murali-Baskaran RK, Kalaivani K, Radhakrishnan N, Park KB, Malafaia G. 2024. Entomopathogenic fungi promising biocontrol agents for managing lepidopteran pests: Review of current knowledge. Biocatal Agric Biotechnol 58: 103146. https://doi.org/10.1016/j.bcab.2024.103146.

Kenis M, Benelli G, Biondi A, Calatayud PA, Day R, Desneux N, Harrison RD, Kriticos D, Rwomushana I, Berg JVD, Verheggen F. 2023. Invasiveness, biology, ecology, and management of the fall armyworm, *Spodoptera frugiperda*. Entomol Gen 43 (2): 187-241. https://doi.org/10.1127/entomologia/2022/1659.

Khan HAA, Ali A. 2025. First report of field-evolved resistance to insecticides in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) from Punjab, Pakistan. PLoS One 20 (6): e0324857. https://doi.org/10.1371/journal.pone.0324857.

Lestari P, Swibawa IG, Fitriana Y, Suharjo R, Utomo SD, Hartaman M. 2024. Population dynamics of *Spodoptera frugiperda* after its invasion in Lampung Province, Indonesia. J Hama Penyakit Tumbuhan Trop 24 (1): 98-108. https://doi.org/10.23960/j.hptt.12498-108.

Liu YC, Chen TH, Huang YF, Chen CL, Nai YS. 2023. Investigation of the fall armyworm (*Spodoptera frugiperda*) gut microbiome and entomopathogenic fungus-induced pathobiome. J Invertebr Pathol 200: 107976. https://doi.org/10.1016/j.jip.2023.107976.

Louw S, Paradza VM, Berg JVD, Plessis HD. 2025. Susceptibility of *Spodoptera frugiperda* to commercial entomopathogenic fungi formulations in South Africa. Insects 16 (7): 656. https://doi.org/10.3390/insects16070656.

Minarni EW, Nurtiati N, Istiqomah D. 2022. Biological effects of indigenous entomopathogenic fungi and their application methods on *Spodoptera frugiperda*. J Perlindung Tanam Indones 26 (2): 107-118. https://doi.org/10.22146/jpti.70816.

Montezano DG, Specht A, Soja E, Sosa-Gomez DR, Roque-Specht VF, Malaquias JV, Paula-Moraes SV, Peterson JA, Hunt TE. 2019. Biotic

potential and reproductive parameters of *Spodoptera frugiperda*. J Agric Sci 11 (13): 240-253. https://doi.org/10.5539/jas.v11n13p240.

Oliveira DGP, Pauli G, Mascarin GM, Delalibera I. 2015. Protocol for determination of conidial viability of *Beauveria bassiana* and *Metarhizium anisopliae* from commercial products. J Microbiol Method 119: 44-52. https://doi.org/10.1016/j.mimet.2015.09.021.

Overton K, Maino JL, Day R, Umina PA, Bett B, Carnovale D, Ekesi S, Meagher R, Reynolds OL. 2021. Global crop impacts, yield losses and action thresholds for fall armyworm (*Spodoptera frugiperda*): A review. Crop Prot 145: 105641. https://doi.org/10.1016/j.cropro.2021.105641.

Putra ILI, Saputra WH, Hanafi Y. 2024. Abundance, attack intensity, and distribution of *Spodoptera frugiperda* in Kulon Progo, Yogyakarta. Planta Trop 12 (2): 149-158. https://doi.org/10.18196/pt.v12i2.21296.

Puu YMSW, Daud ID, Nassrudin A, Melina. 2024. *Spodoptera frugiperda* in East Nusa Tenggara Province of Indonesia: Genetic characterization and strain detection. Caraka Tani 39 (2): 439-450. https://doi.org/10.20961/carakatani.v39i2.91748.

Ramanujam B, Poornesha B, Shylesha AN. 2020. Effect of entomopathogenic fungi against invasive pest *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in maize. Egypt J Biol Pest Control 30: 100. https://doi.org/10.1186/s41938-020-00291-4.

Rindiani DE, Herlinda S, Suwandi S. 2024. Population and attacks of *Spodoptera frugiperda* on corn inoculated with endophytic entomopathogenic fungi from South Sumatra. J Suboptim Land 13 (1): 87-93. https://doi.org/10.36706/jlso.13.1.2024.707.

Russo ML, Jaber LR, Scorsetti AC, Vianna F, Cabello MN, Pelizza SA. 2020. Effect of entomopathogenic fungi introduced as corn endophytes on the development, reproduction, and food preference of the invasive fall armyworm *Spodoptera frugiperda*. J Pest Sci 94 (3): 859-870. https://doi.org/10.1007/s10340-020-01302-x.

Samson RA, Evans HC, Latgé JP. 1988. Atlas of entomopathogenic fungi. Springer, Berlin. https://doi.org/10.1007/978-3-662-05890-9.

Saruhan I. 2018. Efficacy of some entomopathogenic fungi against *Aphis fabae* Scopoli (Hemiptera: Aphididae). Egypt J Biol Pest Control 28: 89. https://doi.org/10.1186/s41938-018-0096-2.

Shahbaz M, Palaniveloo K, Tan YS, Palasuberniam P, Ilyas N, Wiart C, Seelan JS. 2024. Entomopathogenic fungi in crop protection with an emphasis on bioactive metabolites and biological activities. World J Microbiol Biotechnol 40 (1): 217. https://doi.org/10.1007/s11274-024-04022-x.

Shang J, Tang G, Lu M, Wang C. 2022. Host and environmental sensing by entomopathogenic fungi to infect hosts. Curr Clin Microbiol Rep 9 (4): 69-74. https://doi.org/10.1007/s40588-022-00185-z.

Tesari T, Leksono AS, Mustafa I. 2024. Effectiveness of botanical pesticide combined with *Beauveria bassiana* on mortality, nutritional index and fecundity of *Spodoptera litura* L. Cogent Food Agric 10 (1): 2320816. https://doi.org/10.1080/23311932.2024.2320816.

Togola A, Meseka S, Menkir A, Badu-Apraku B, Boukar O, Tamò M, Djouaka R. 2018. Measurement of pesticide residues from chemical control of the invasive *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in a maize experimental field in Mokwa, Nigeria. Intl J Environ Res Public Health 15 (5): 849. https://doi.org/10.3390/ijerph15050849.

Trisyono YA, Hendrayanti HN, Yuantomoputro AP, Setyaningrum AV, Harjanto S, Aryuwandari VE. 2024. An update on the fall armyworm: Severity of maize damage and susceptibility to emamectine benzoate and chlorantraniliprole. J Perlindung Tanam Indones 28 (2): 134-140. https://doi.org/10.22146/jpti.95262.

Ullah MI, Qadeer M, Arshad M, Khalid S, Saleem U, Riaz MA, Mumtaz A, Rizwan M, Sayed SM, Alhelaify SS, Alharthy OM. 2023. Insights into sublethal effects of *Metarhizium anisopliae* on the biotic potentials of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) on maize. Egypt J Biol Pest Control 33: 96. https://doi.org/10.1186/s41938-023-00741-9.

Ullah S, Raza AB, Alkafafy M, Sayed S, Hamid MI, Majeed MZ, Riaz MA, Gaber NM, Asim M. 2022. Isolation, identification and virulence of indigenous entomopathogenic fungal strains against the peach-potato aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), and the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). Egypt J Biol Pest Control 32: 2. https://doi.org/10.1186/s41938-021-00500-8.

Ullah SA, Khan I, Khan MS, Jahangir K, Habib A, Mohammad D, Khpalwak W, Aziz H, Sardar MU, Qazi I, Elahi ME. 2022. Effectiveness of selected entomopathogenic fungi against the tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Pak J Med Health Sci 16 (1): 507-510. https://doi.org/10.53350/pjmhs22161507.

Van den Berg J, du Plessis H. 2022. Chemical control and insecticide resistance in *Spodoptera frugiperda*. J Econ Entomol 115 (6): 1761-1771. https://doi.org/10.1093/jee/toac108.

Waldbauer GP. 1968. The consumption and utilization of food by insects. Adv Insect Physiol 5: 229-288. https://doi.org/10.1016/S0065-2806(08)60230-1.

Yaméogo IS, Latévi K, Ouattara D, Agboyi LK, Dabiré RA, Zallé S, Kenis M, Gnankiné O, Nacro S. 2024. Level of damage and yield losses associated with the fall armyworm (*Spodoptera frugiperda*) on maize (*Zea mays*), millet (*Pennisetum glaucum*), and sorghum (*Sorghum bicolor*) on station in Burkina Faso. Crop Prot 182: 106743. https://doi.org/10.1016/j.cropro.2024.106743.

Zalte OP, Panchbhai PR, Pillai T, Lavhe NV, Deshmukh V, Shah V, Fand BB, Pande R, Prabhulinga T, Nikoshe A, Thube S. 2024. Bio-efficacy evaluation of *Beauveria bassiana* against fall armyworm, *Spodoptera frugiperda* (J.E. Smith). Hexapoda 31 (1&2): 44-48. https://doi.org/10.55446/hexa.2024.12.

Zhang C, Teng B, Liu H, Wu C, Wang L, Jin S. 2023. Impact of *Beauveria bassiana* on antioxidant enzyme activities and metabolomic profiles of *Spodoptera frugiperda*. J Invertebr Pathol 198: 107929. https://doi.org/10.1016/j.jip.2023.107929.