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Pathogenicity of Indigenous Entomopathogenic Fungi from West Sumatera, Indonesia, against Fall Armyworm *Spodoptera frugiperda* and Their Effects on Post-Larval Development

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Abstract

Spodoptera frugiperda is an invasive maize pest requiring control strategies. Entomopathogenic fungi offer biological control options, but information on indigenous isolates from West Sumatra, Indonesia, and their effects on postlarval development remains limited. This study evaluated the pathogenicity of six fungal isolates against *S. frugiperda* under laboratory conditions. A completely randomized design comprised eight treatments and three replications: *Beauveria bassiana* BBWS and BBPD, *Metarhizium anisopliae* B22C and KRJ, *Trichoderma asperellum* A116 and S2D11, sterile distilled water, and cypermethrin. Fungal suspensions were applied to second-instar larvae at 1×10^8 conidia mL^{-1}. Colony growth was summarized descriptively. Other response variables were analyzed using ANOVA followed by the LSD test at the 5% significance level, while LT_{50} was estimated using probit regression. Fungal treatments caused larval mortality ranging from 40.00% to 70.00% and reduced the proportions of larvae reaching the pupal and adult stages. Among fungal treatments, *B. bassiana* BBWS produced the numerically highest larval mortality (70.00%) and the shortest estimated LT_{50} (4.00 days). Its mortality did not differ significantly from that of BBPD (66.67%). These findings demonstrate that the tested fungal isolates affected larval survival and development of *S. frugiperda*, with BBWS showing potential for further evaluation as a biological control agent. Further research should investigate pathogenicity mechanisms, conidial viability during storage, formulation stability, and efficacy under greenhouse and field conditions.

Keywords: Biological control, *Beauveria bassiana* BBWS, fungal pathogenicity, integrated pest management, larval mortality

Introduction

Maize (*Zea mays* Linnaeus) is a strategic agricultural commodity that plays an important role as a source of food and feed. However, maize productivity is seriously threatened by the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera:Noctuidae).

In Indonesia, the infested area has increased by 15%, from 80,913.33 ha to 93,050.33 ha (DFCP, 2026). This pest is polyphagous, highly adaptable, and has a rapid reproductive rate, enabling it to cause severe damage from the early vegetative stage to ear formation (Goergen et al., 2016; Day et al., 2017; Ginting et al., 2024; Nurkomar et al., 2023).

Control of *S. frugiperda* is still largely dominated by synthetic insecticides. Although these insecticides provide rapid control, continuous reliance on them may lead to resistance, environmental pollution, and negative effects on non-target organisms and human health (Gutierrez-Moreno et al., 2019; Tudi et al., 2021; Ediagbonya et al., 2025). Therefore, effective, environmentally friendly, and sustainable control alternatives are urgently needed in maize cultivation systems.

Biological control using entomopathogenic fungi (EPF) is a promising approach because these fungi can infect insects through cuticle penetration, enzymatic degradation, and secondary metabolite activity (Litwin et al., 2020; Pedrini, 2022). *Beauveria bassiana* and *Metarhizium anisopliae* have been investigated against a range of insect pests (Trizelia et al., 2016; Trizelia et al., 2018; Flawerina et al., 2021; Hendra et al., 2022a; Hendra et al., 2022b; Trizelia et al., 2023; Gomes et al., 2023; Syahrawati et al., 2025). Endophytic colonization and plant-mediated protection provide additional routes for their use in pest management (Bamisile et al., 2018; Vajri et al., 2024; Vajri et al., 2026a). Studies have also investigated effects on *S. frugiperda* eggs and larval development (Akutse et al., 2019; Ramanujam et al., 2020; Vajri et al., 2026b). However, efficacy varies with the fungal isolate and insect host, and low mortality has also been reported in other noctuid larvae (Loureiro et al., 2024).

In addition to classical EPF, *Trichoderma* spp. are widely known as biological control agents against plant pathogens and as plant growth-promoting fungi (Chen et al., 2025; Srivastava et al., 2026; Joo and Hussein, 2022; Peng et al., 2021; Zin and Badaluddin, 2020). Several studies have reported that *Trichoderma* spp. affect insect pests through secondary metabolites or indirectly through induced plant resistance mechanisms (Anwar et al., 2016; Mona et al., 2016; Islam et al., 2022; Rodriguez-Gonzalez et al., 2017; Poveda et al., 2020; Poveda, 2021). However, unlike *B. bassiana* and *M. anisopliae*, *Trichoderma* spp. are not primarily recognized as classical entomopathogenic fungi; therefore, their direct pathogenicity and biological effects on *S. frugiperda*, particularly *T. asperellum*, remain insufficiently understood.

Although the potential of common EPF has been widely reported, information on indigenous fungal isolates from West Sumatra, particularly those originating from specific ecological niches such as infected insects, endophytic tissues, and rhizospheres, remains limited. Most previous studies have focused on general EPF species or commercially known isolates, whereas information on local West Sumatra isolates and their effects on larval mortality and post-larval development remains scarce. Therefore, this study aimed to evaluate the pathogenicity of indigenous *B. bassiana*, *M. anisopliae*, and *T. asperellum* isolates from West Sumatra against *S. frugiperda* larvae. The novelty of this study lies in the comparative evaluation of indigenous fungal isolates from different ecological origins by assessing not only acute larval mortality but also post-larval developmental responses, including pupation success and the emergence of normal and abnormal adults.

Methods

This research was conducted at the Biological Control Laboratory, Faculty of Agriculture, Andalas University, from July to October 2024.

Research Design

The study used a Completely Randomized Design (CRD) with eight treatments and three replications. The isolates consisted of *Beauveria bassiana*, *Metarhizium anisopliae*, and *Trichoderma asperellum*. Cypermethrin insecticide was used as a chemical positive control, whereas sterile distilled water was used as a negative control (Table 1). The fungal isolates used in this study were obtained from the verified collection of the Biological Control Laboratory, Department of Plant Protection, Faculty of Agriculture, Universitas Andalas, Indonesia. The species identity of the fungal isolates had been confirmed through DNA sequencing analysis of ITS region, conducted by Genetika Science, followed by sequence matching against the NCBI database (unpublished data).

Table 1. Fungal isolates and control treatments used in the pathogenicity bioassay against *Spodoptera frugiperda* larvae

Treatment	Code	Origin
Beauveria bassiana	BBWS	Infected stink bug
Beauveria bassiana	BBPD	Chili leaf endophyte
Metarhizium anisopliae	B22C	Oil palm rhizosphere
Metarhizium anisopliae	KRJ	Corn rhizosphere
Trichoderma asperellum	A116	Chili root endophyte
Trichoderma asperellum	S2D11	Shallot leaf endophyte
Positive control	K(+)	Cypermethrin insecticide
Negative control	K(-)	Sterile distilled water

Rejuvenation of Fungal Isolates

All fungal isolates were rejuvenated by transferring mycelial plugs from actively growing colonies using a sterile corkborer onto Sabouraud Dextrose Agar Yeast (SDAY) medium in Petridishes. The transferred isolates were incubated at approximately 25 °C for 14 days. Fungal characteristics were observed macroscopically based on colony morphology, particularly colony surface color, and microscopically based on hyphal and conidial morphology under a binocular microscope. The macro- and microscopic characteristics of the fungal isolates are presented in Table 2 and Figure 1.

Table 2. Macroscopic and microscopic characteristics of fungal isolates grown on SDAY medium

Treatment code	Isolate source	Region of origin	Colony surface color	Hyphae	Conidia
BBWS	Infected stink bug	Padang City, West Sumatra	White, cottony	Septate	Round
BBPD	Chili leaf endophyte	Padang City, West Sumatra	White, cottony	Septate	Round
B22C	Oil palm rhizosphere	Pasaman Regency, West Sumatra	Light green	Branched	Cylindrical
KRJ	Corn rhizosphere	Padang City, West Sumatra	Light green	Branched	Cylindrical
A116	Chili root endophyte	Agam Regency, West Sumatra	Dark green	Septate	Round
S2D11	Shallot leaf endophyte	Solok Regency, West Sumatra	Dark green	Septate	Round

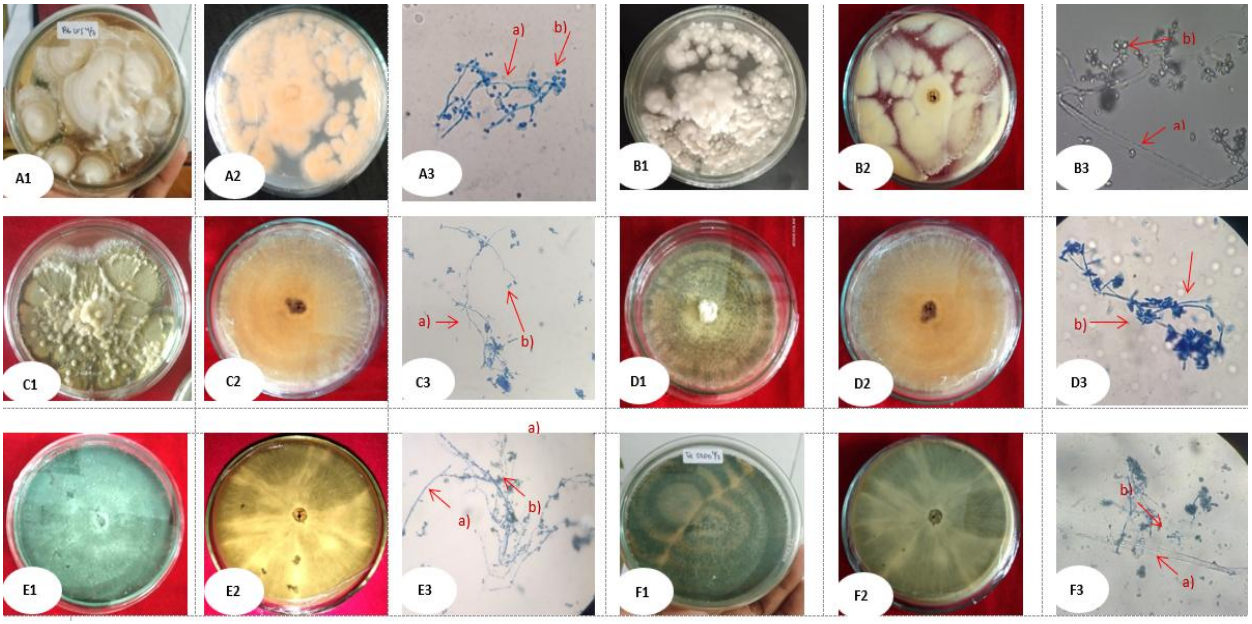


Figure 1. Morphology of fungal isolates grown on SDAY medium at 15 days after inoculation (DAI): *Beauveria bassiana* (A. BBWS; B. BBPD), *Metarhizium anisopliae* (C. B22C; D. KRJ), and *Trichoderma asperellum* (E. A116; F. S2D11). For each isolate: 1 = macroscopic top view, 2 = macroscopic bottom view, and 3 = microscopic characteristics showing (a) hyphae and (b) conidia.

Colony Growth

Colony growth was observed for each fungal isolate. Seven-day-old fungal colonies were transferred onto Petri dishes containing SDAY medium using mycelial plugs with a diameter of 0.7 cm. After transfer, the isolates were incubated at 25 °C. The colony diameter of each isolate was measured every two days until 8 days after inoculation.

Conidial Density and Viability Measurement

Conidial density and viability were measured using conidial suspensions prepared from 15-day-old fungal cultures. Conidial density was determined by counting the number of conidia using a hemocytometer under a light microscope at 400× magnification. Conidial viability was assessed based on the percentage of germinated conidia. A drop of conidial suspension was placed onto 2% water agar or a thin layer of PDA medium on a sterile glass slide and incubated at 25 °C, with germination assessed at 24 and 48 h. Each treatment was replicated three times, and 100 conidia were observed per replicate under a binocular microscope at 400× magnification. Conidia were considered germinated when the germ tube length exceeded the conidial diameter.

Propagation of *S. frugiperda*

Larvae of *S. frugiperda* were collected from corn cultivation areas in Kuranji District, Padang City, West Sumatra. The collected larvae were third to fourth instars. Each larva was individually reared in a plastic container measuring 5 cm × 5 cm × 5 cm and fed with fresh corn leaf pieces, which were replaced every 24 h. Larvae that entered the pupal stage were transferred into plastic jars lined with filter paper. After adult emergence, the adults were placed in insect cages measuring 45 cm × 45 cm × 45 cm, with corn leaf pieces provided as an oviposition substrate. The adults were fed with 10% honey solution absorbed onto cotton and hung inside the cage. The eggs produced by the adults were collected and used as the source of larvae for the bioassay.

Preparation of Fungal Suspension

Fifteen-day-old fungal colonies were harvested by adding 10 mL of sterile distilled water containing 0.01% Tween 80 as a surfactant to each petridish. The colony surface was gently scraped using a sterile soft brush to dislodge the conidia. The resulting suspension was transferred into a test tube and homogenized using a vortex mixer (Gemmy VM-300, Taiwan). Serial dilutions were prepared using sterile distilled water to obtain a final concentration of 1×10^8 conidia mL^{-1}. Conidial density was determined using a hemocytometer (Improved Neubauer, Minitub, Germany) according to Lacey (2012).

Application to *S. frugiperda* Larvae

Fungal pathogenicity tests were conducted to evaluate the ability of the fungal isolates to cause mortality in *S. frugiperda* larvae. The bioassay was carried out using a modified method of Trizelia (2005). The conidial concentration used for each fungal isolate was 1×10^8 conidia mL^{-1}. The experiment consisted of eight treatments with three replications. The treatment and replication arrangement was considered using the Mead Resource Equation (Mead, 1988). Each replication unit consisted of 10 second-instar *S. frugiperda* larvae (Ramanujam et al., 2020), resulting in a total of 240 larvae.

Treatment was applied by spraying 3 mL of fungal conidial suspension onto second-instar *S. frugiperda* larvae using a micro hand sprayer at a distance of 15 cm to ensure uniform coverage. The positive control was sprayed with cypermethrin insecticide at a concentration of 1 mL L^{-1}, whereas the negative control was sprayed with sterile distilled water. Both controls were applied using the same volume and spraying distance. After spraying, the larvae were allowed to air dry for several minutes and then transferred aseptically into clean rearing containers. Each larva was placed individually in one container and provided with fresh corn leaf pieces, which were replaced daily. The containers were incubated in the laboratory at 25 °C, 80% RH, and a photoperiod of 12:12 h (L:D). Larval mortality was observed daily for 7 days, from 2 to 8 days after application. The percentage of larval mortality (M) was calculated using the following formula:

$$M = (n / N) \times 100 \quad \ldots\ldots (1)$$

Where M = Larval mortality, n = Number of dead larvae, N = Number of larvae treated.

The LT_{50} value was estimated only for treatments that resulted in total mortality exceeding 50% by the end of the observation period. Meanwhile, fungal efficacy (E) was calculated as corrected mortality using Abbott's formula (Abbott, 1925), as follows:

$$E = [(T - C) / (100 - C)] \times 100 \quad \ldots\ldots (2)$$

Where E = Fungal efficacy, T = treatment mortality, C = control mortality

The development of *S. frugiperda* was observed until the adult stage. The percentage of pupae formed (P) was calculated using the following formula:

$$P = (b / N) \times 100 \quad \ldots\ldots (3)$$

Where P = Pupae formed, b = Number of pupae formed, N = Number of larvae treated.

The percentage of adults formed (I) is calculated using the formula:

$$I = (d / N) \times 100 \quad \ldots\ldots (4)$$

Where I = Adults formed, d = Number of adults formed, N = Number of larvae treated

Data Analysis

Data on fungal colony growth, observed based on the increase in colony diameter, were analyzed descriptively to describe the growth pattern of each isolate. Data on conidial density, conidial viability, larval mortality, pupal formation, pupal weight, pupal duration, and adult formation were analyzed using analysis of variance (ANOVA). When significant differences were detected, mean comparisons were performed using the

Least Significant Difference (LSD) test at the 5% significance level. The time to death of *S. frugiperda* larvae was recorded to estimate the lethal time parameter. Mortality data were analyzed using probit regression to calculate LT_{50} values, and LT_{50} was estimated only for treatments with mortality exceeding 50% by the end of the observation period. All statistical analyses were performed using Statistix 8.0.

Results

Colony Growth

Observation of colony growth from 2 to 8 days after inoculation showed variation in colony expansion among fungal isolates. *T. asperellum* isolates S2D11 and A116 showed the fastest colony growth compared with the other isolates, reaching colony diameters of 4.76 cm and 4.00 cm, respectively, at 2 days after inoculation. Both isolates completely filled the Petri dishes, reaching 9.00 cm in diameter, by 4 days after inoculation and maintained this diameter until day 8. In contrast, *B. bassiana* BBPD showed the slowest colony growth, with a colony diameter of 0.88 cm at 2 days after inoculation and gradually increasing to 2.37 cm by day 8. The other isolates, BBWS, B22C, and KRJ, showed moderate colony growth, with colony diameters ranging from 2.48 to 3.48 cm at day 8. These differences indicate variation in physiological growth characteristics among isolates, which may influence their competitiveness and adaptability under specific environmental conditions (Table 3).

Table 3. Colony diameter (cm) of fungal isolates grown on SDAY medium from 2 to 8 days after inoculation (DAI)

Treatments	Colony diameter (cm)			
	2 DAI	4 DAI	6 DAI	8 DAI
S2D11	4.76	9.00	9.00	9.00
A116	4.00	9.00	9.00	9.00
BBWS	1.20	2.04	2.73	3.48
B22C	1.15	1.64	2.17	2.64
KRJ	0.99	1.55	1.93	2.48
BBPD	0.88	1.33	1.78	2.37

Density and Viability of Conidia

The analysis of variance showed that fungal isolate significantly affected conidial density and viability. *T. asperellum* isolates A116 and S2D11 produced the highest conidial densities, reaching 13.00×10^8 and 12.83×10^8 conidia mL^{-1}, respectively. These isolates also showed high conidial viability after 24 h of incubation, with germination of 77.00% and 74.33%, respectively. After 48 h of incubation, conidial viability increased in all isolates. The highest viability was observed in *B. bassiana* BBWS, reaching 93.67%, followed by *T. asperellum* A116 and S2D11, each with 90.33%, and *B. bassiana* BBPD with 87.33%. These values were not significantly different from one another based on the LSD test at the 5% level. In contrast, *M. anisopliae* isolates showed lower viability at 48 h, particularly KRJ and B22C, with germination of 72.33% and 79.67%, respectively. Data on conidial density and viability are presented in Table 4.

Although *T. asperellum* A116 and S2D11 produced the highest conidial densities, their germination percentages at 48 h were statistically comparable to those of *B. bassiana* BBWS and BBPD. Furthermore, extending incubation to 48 h increased germination in all isolates, but the lower final values in *M. anisopliae* indicated that differences among isolates persisted.

Table 4. Conidial density and viability of fungal isolates used in the pathogenicity bioassay

Treatments	Conidial dencity ($1x10^8$ conidia mL^{-1}) ± SD	Viability (%) ± SD	
		1 x 24 h	2 x 24 h
A116	13.00 ± 2.78 a	77.00 ± 3.00 a	90.33 ± 2.08 a
S2D11	12.83 ± 4.80 a	74.33 ± 5.51 a	90.33 ± 3.51 a
BBWS	5.17 ± 1.26 b	51.00 ± 3.00 b	93.67 ± 8.50 a
KRJ	4.17 ± 2.89 b	48.00 ± 5.20 b	72.33 ± 4.16 b
BBPD	3.50 ± 1.00 b	43.67 ± 2.08 b	87.33 ± 3.79 a
B22C	2.67 ± 0.76 b	50.67 ± 7.51 b	79.67 ± 3.79 b

Numbers followed by the same letter in the same column are not significantly different in the LSD test at the 5% level.

Pathogenicity against *S. frugiperda* larvae

The results showed that the application of fungal isolates significantly affected the mortality of *S. frugiperda* larvae. All fungal treatments caused higher larval mortality than the negative control, which showed only 3.33% mortality. The chemical positive control, cypermethrin, caused the highest mortality at 90.00% and was used as a reference for the effect of a conventional insecticide. Among the fungal treatments, *B. bassiana* isolates showed the highest efficacy, ranging from 65.52% to 68.97%. The highest larval mortality was recorded in *B. bassiana* BBWS at 70.00%, followed by *B. bassiana* BBPD at 66.67%. *M. anisopliae* isolates B22C and KRJ caused moderate mortality, each reaching 60.00%, whereas *T. asperellum* isolates A116 and S2D11 showed lower mortality, at 43.33% and 40.00%, respectively. The mortalities caused by BBWS and BBPD did not differ significantly.

The fastest LT_{50} values among the fungal treatments were recorded for *B. bassiana* BBWS and BBPD, at 4.00 and 4.60 days, respectively. The LT_{50} values of *M. anisopliae* B22C and KRJ were 4.90 and 5.00 days, respectively. In contrast, LT_{50} values were not determined for *T. asperellum* A116 and S2D11 because their mortality did not exceed 50% by the end of the observation period. Infected larvae showed typical symptoms, including reduced movement, loss of responsiveness, darkened body color, and death. Fungal colonization was indicated by the appearance of mycelial growth on the surface of larval cadavers during the post-mortem phase and by the recovery of fungal colonies with similar morphology after re-isolation. Larval mortality and estimated LT_{50} values are presented in Table 5, and cumulative mortality is shown in Figure 2.

Table 5. Mortality, LT_{50}, and corrected efficacy of fungal isolates against second-instar *Spodoptera frugiperda* larvae

Treatments	Larval mortality (%) ± SD	LT_{50} (day)	Fungal efficacy (E)
K(+)	90.00 ± 1.00 a	1.90	89.66
BBWS	70.00 ± 1.00 b	4.00	68.97
BBPD	66.67 ± 0.58 b	4.60	65.52
B22C	60.00 ± 1.00 c	4.90	58.62
KRJ	60.00 ± 1.00 c	5.00	58.62
A116	43.33 ± 0.58 d	-	41.38
S2D11	40.00 ± 1.00 d	-	37.93
K(-)	3.33 ± 0.58 e	-	-

Numbers followed by the same letter in the same column are not significantly different in the LSD test at the 5% level.

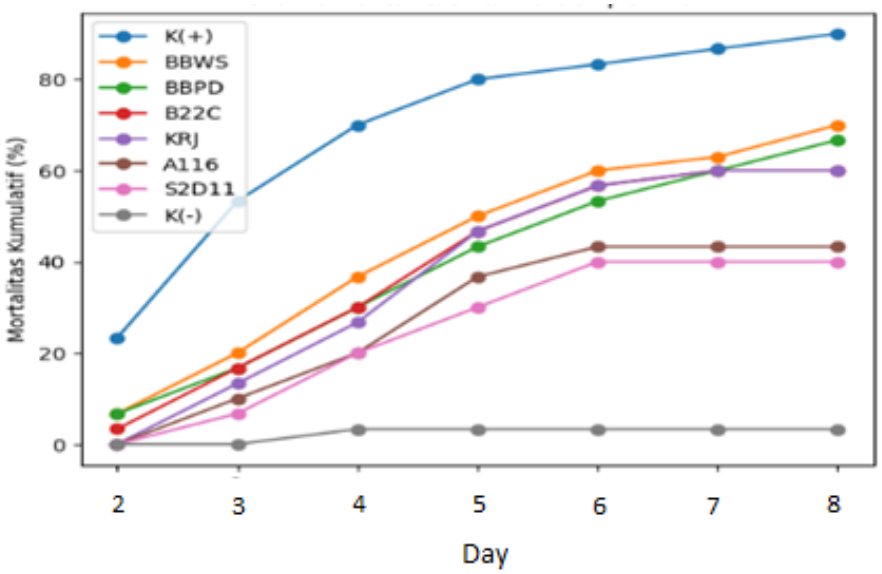


Figure 2. Cumulative mortality of *Spodoptera frugiperda* larvae from 2 to 8 days after application of fungal isolates

Pupal Formation

The results showed that fungal isolate treatments significantly affected total and normal pupal formation and pupal weight of *S. frugiperda*. The negative control showed the highest total pupal formation, reaching 86.67%, with 80.00% normal pupae and an average pupal weight of 0.19 g. In contrast, all fungal isolate treatments reduced pupal formation compared with the negative control. Among the fungal treatments, *B. bassiana* BBWS and BBPD resulted in the lowest total pupal formation, each reaching only 26.67%. These treatments also produced lower percentages of normal pupae, at 20.00% and 23.33%, respectively. The chemical positive control resulted in the lowest pupal formation overall, with only 3.33% of larvae reaching the pupal stage. However, this treatment should be interpreted cautiously because larval survival was very low. Data on pupal formation, pupal weight, and pupal duration are presented in Table 6.

Table 6. Pupal formation, pupal weight, and pupal duration of *Spodoptera frugiperda* after application of fungal isolates and control treatments

Treatment	Pupal formed (%) ± SD						Pupal weight (g)		Pupal duration (days)	
	Total pupae		Normal pupae		Abnormal pupae					
K(-)	86.67 ± 1.15	a	80.00 ± 1.00	a	6.67 ± 0.58	a	0.19 ± 0.006	a	7.48 ± 0.13	a
S2D11	53.33 ± 0.58	b	40.00 ± 1.00	b	13.33 ± 1.53	b	0.15 ± 0.028	bc	8.39 ± 0.33	a
A116	40.00 ± 1.73	bc	33.33 ± 1.15	bc	6.67 ± 0.58	bc	0.16 ± 0.008	b	8.17 ± 0.35	a
KRJ	33.33 ± 1.15	cd	23.33 ± 0.58	cd	10.00 ± 1.00	cd	0.15 ± 0.027	bc	8.17 ± 1.04	a
B22C	30.00 ± 1.00	cd	16.67 ± 2.08	d	13.33 ± 1.15	d	0.13 ± 0.033	cd	7.75 ± 0.66	a
BBPD	26.67 ± 0.58	d	23.33 ± 0.58	cd	3.33 ± 0.58	cd	0.15 ± 0.013	bc	8.06 ± 1.19	a
BBWS	26.67 ± 0.58	d	20.00 ± 1.00	cd	6.67 ± 1.15	cd	0.12 ± 0.027	cd	8.10 ± 0.17	a
K(+)	3.33 ± 0.58	e	3.33 ± 0.58	e	0.00 ± 00.00	e	0.06 ± 0.098	e	6.00 ± 3.46	b

Numbers followed by the same letter in the same column are not significantly different in the LSD test at the 5% level.

Although the chemical positive control showed the strongest inhibition of pupal formation, this treatment represented the effect of a conventional insecticide and should be interpreted separately from the fungal treatments. Among the fungal isolates, *B. bassiana* BBWS and BBPD showed the strongest effect on pupal formation, with only

26.67% of larvae developing into pupae. *M. anisopliae* isolates B22C and KRJ resulted in pupal formation of 30.00% and 33.33%, respectively. In contrast, *T. asperellum* isolates A116 and S2D11 showed relatively higher pupal formation, at 40.00% and 53.33%, respectively, but these values remained lower than that of the negative control.

Abnormal pupae were recorded in all fungal treatments. The numerically highest percentages occurred in *T. asperellum* S2D11 and *M. anisopliae* B22C (13.33% each), compared with 6.67% in the negative control. Pupal weight in fungal treatments ranged from 0.12 to 0.16 g, compared with 0.19 g in the negative control. Pupal duration did not differ significantly between the fungal treatments and the negative control, with mean values ranging from 7.75 to 8.39 days in the fungal treatments compared with 7.48 days in the negative control. The reported pupal weight and duration for the positive control require cautious interpretation because very few larvae reached the pupal stage.

Adult Formation

The results showed that fungal isolate treatments significantly affected adult formation of *S. frugiperda*. The negative control, showed the highest adult formation, reaching 80.00%, with most adults developing normally (76.67%) and only 3.33% showing abnormalities. In contrast, all fungal isolate treatments reduced adult formation compared with the negative control. Data on adult formation are presented in Table 7.

Among the fungal treatments, *T. asperellum* S2D11 and A116 resulted in relatively higher adult formation, at 40.00% and 33.33%, respectively, although both were still significantly lower than the negative control. *M. anisopliae* and *B. bassiana* isolates, including KRJ, BBPD, B22C, and BBWS, showed lower adult formation, ranging from 16.67% to 23.33%. The chemical positive control, K(+), resulted in the lowest adult formation, with only 3.33% of larvae developing into adults, indicating strong developmental inhibition by the conventional insecticide. The proportion of abnormal adults increased in several fungal treatments, particularly in *T. asperellum* S2D11, which produced 10.00% abnormal adults. Observed abnormalities included wing deformation and incomplete eclosion. In general, lower normal adult formation indicated stronger developmental inhibition by the fungal isolates.

Table 7. Adult formation of *Spodoptera frugiperda* after application of fungal isolates and control treatments

Treatment	Adults formed (%) ± SD		
	Total	Normal	Abnormal
K(-)	80.00 ± 1.00 a	76.67 ± 0.58 a	3.33 ± 0.58 a
S2D11	40.00 ± 1.00 b	30.00 ± 0.00 b	10.00 ± 1.00 b
A116	33.33 ± 1.15 b	26.67 ± 0.58 b	6.67 ± 0.58 b
KRJ	23.33 ± 0.58 bc	16.67 ± 0.58 bc	6.67 ± 1.15 bc
BBPD	23.33 ± 0.58 bc	16.67 ± 0.58 bc	6.67 ± 0.58 bc
B22C	20.00 ± 1.00 bc	13.33 ± 0.58 bc	6.67 ± 0.58 bc
BBWS	16.67 ± 2.08 bc	13.33 ± 1.53 bc	3.33 ± 0.58 bc
K(+)	3.33 ± 0.58 c	3.33 ± 0.58 c	0.00 ± 0.00 c

Numbers followed by the same letter in the same column are not significantly different in the LSD test at the 5% level

Discussion

This study showed that vegetative growth and conidial production were not the only determinants of pathogenicity against *S. frugiperda*. Although *T. asperellum* isolates showed the fastest colony growth and

produced the highest conidial density, the isolates did not cause the highest larval mortality (Tables 3 and 4). In contrast, *B. bassiana* BBWS showed the highest conidial viability after 48 h and produced the strongest larval mortality response (Tables 4 and 5). These observations suggest that colony growth alone did not predict mortality; a relationship between conidial viability and infection success was not tested statistically. Viable conidia are important for germination, attachment, and penetration of the insect cuticle. The virulence of entomopathogenic fungi is closely related to conidial germination, host colonization, and the production of extracellular enzymes and secondary metabolites during infection (Pedrini, 2022). Similarly, Gebremariam et al. (2022) stated that the effectiveness of entomopathogenic fungi is influenced by the quality of infective propagules and by the fungus's ability to colonize the host.

Among the fungal isolates, *B. bassiana* showed the strongest pathogenicity against second-instar *S. frugiperda* larvae. The BBWS isolate produced the numerically highest larval mortality, reaching 70.00%, with the shortest estimated LT_{50} value of 4.00 days (Table 5). *M. anisopliae* isolates showed moderate pathogenicity, whereas *T. asperellum* isolates showed lower mortality and did not reach the threshold required for LT_{50} estimation (Table 5). The stronger effect of *B. bassiana* and *M. anisopliae* is consistent with their well-known role as classical entomopathogenic fungi. Their infection process may involve conidial germination, cuticle penetration, and the activity of extracellular enzymes such as proteases, chitinases, and lipases, as reported in previous studies (Pedrini, 2022; Maisarah et al., 2025). *B. bassiana* infection may also involve internal host colonization and physiological disruption, although these mechanisms were not directly measured in this study (Litwin et al., 2020). In *M. anisopliae*, host mortality has been associated with toxin production, including destruxins, which may contribute to host paralysis and death (Vajri et al., 2024).

The lower mortality rate caused by *T. asperellum* should be interpreted with caution. *Trichoderma* spp. are more widely recognized as antagonists of plant pathogens and plant growth-promoting fungi rather than as classical entomopathogenic fungi (Chen et al., 2025; Srivastava et al., 2026). However, the mortality and developmental responses observed in this study indicate that *T. asperellum* still had biological effects on *S. frugiperda* larvae (Tables 5–7). Several studies have reported that some *Trichoderma* strains may affect insect pests through secondary metabolites or plant-mediated resistance mechanisms (Mukherjee et al., 2013; Poveda, 2021). However, because this study used direct application to larvae and did not evaluate secondary metabolites or plant resistance responses, the effect of *T. asperellum* should be regarded as potential biological activity rather than confirmed classical entomopathogenic activity.

Fungal exposure during the larval stage was associated with lower subsequent pupal formation. *B. bassiana* BBWS and BBPD each produced 26.67% pupal formation, compared with 86.67% in the negative control. Pupal weights were also generally lower in fungal treatments (Table 6). Because pupal formation was calculated relative to the initial number of larvae, these percentages reflect both larval mortality and subsequent developmental success; they do not independently demonstrate sublethal effects among surviving larvae. Reduced feeding, nutrient depletion, and physiological stress are possible explanations that require direct measurement. Research on *B. bassiana*-infected *S. frugiperda* has documented changes in protective and

detoxifying enzyme activities and metabolomic profiles (Gao et al., 2022; Zhang et al., 2023).

Adult formation was reduced by all fungal treatments relative to the negative control. *B. bassiana* BBWS produced the numerically lowest adult formation among fungal isolates (16.67%; Table 7). Abnormal pupae and adults, including wing deformation and incomplete eclosion, were also observed. Pupal duration, however, did not differ significantly between the fungal treatments and the negative control (Table 6). Reduced normal adult formation could limit the number of individuals contributing to the next generation, but reproductive performance was not measured in this study.

Overall, this approach enhances the study's novelty by evaluating both lethal and postlarval developmental responses of *S. frugiperda* to indigenous fungal isolates from West Sumatra. However, since this study was conducted under controlled laboratory conditions, greenhouse and field studies are needed to evaluate formulation stability, persistence, and practical effectiveness of these isolates against *S. frugiperda* under corn production conditions.

Conclusion

This study showed that the tested fungal isolates affected the survival and subsequent development of *S. frugiperda* under laboratory conditions. Fungal treatments caused larval mortality of 40.00–70.00% and reduced pupal and adult formation relative to the negative control. *B. bassiana* BBWS was a promising isolate, producing the numerically highest larval mortality (70.00%) and the shortest estimated LT_{50} among fungal treatments (4.00 days). Its larval mortality did not differ significantly from that of BBPD (66.67%). BBWS produced 26.67% pupal formation and 16.67% adult formation. Further research should evaluate infection mechanisms, conidial viability during storage, formulation stability, and efficacy under greenhouse and field conditions.

Declaration

Author contribution

Indri Yanil Vajri designed the study, conducted experiments, analyzed the data, and drafted the manuscript. Trizelia supervised the study, contributed to methodology and manuscript revision, and served as the corresponding author. Haliatur Rahma and Yusniwati contributed to data interpretation and manuscript revision. All authors read and approved the final manuscript.

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Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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