Virulence of the endophytic fungus *Trichoderma asperellum* against larvae of *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae)

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ABSTRACT

Spodoptera frugiperda JE Smith, known as the fall armyworm (FAW), is a major pest of maize crops. This bug damages maize plant leaves, leading to yield declines of up to 79.9%. An alternative approach to controlling *S. frugiperda* entails the use of entomopathogenic fungi. Many entomopathogenic fungi are present, including *Trichoderma asperellum*, which resides endophytically within plants. This study aims to evaluate the pathogenicity of T. asperellum in controlling *S. frugiperda* larvae. Five isolates of *T. asperellum* (A116, PC21, S2D11, SD34, and AB2B3) were procured from various plant tissues. Two milliliters of conidia suspension, with a concentration of 10^8 conidia/ml, and sterile distilled water as a control were supplied to S. frugiperda larvae. The measured variables encompassed the mortality rate of *S. frugiperda* larvae, the percentage of pupae that matured, and the percentage of adults that emerged. The results demonstrated that the endophytic fungus T. asperellum can eradicate *S. frugiperda* larvae. The mortality rate of *S. frugiperda* larvae ranged from 34.67% to 57.33%. The utilization of *T. asperellum* on *S. frugiperda* led to a 44.00% reduction in pupae formation and a 36.00% reduction in adult formation. *T. asperellum* S2D11 is the most efficacious isolate for infecting S. frugiperda larvae.

Keywords: Endophytes, fungus, isolates, larvae, mortality

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INTRODUCTION

Spodoptera frugiperda (Lepidoptera: Noctuidae) is a significant pest affecting corn crops worldwide (Russo *et al.,* 2021). In Indonesia, it was first reported in West Sumatra, specifically in West Pasaman Regency, in 2019 (Sartiami *et al.,* 2020). The damage caused by this pest primarily affects young corn leaves, where the larvae feed on the leaves and may even reach the plant's growing point (Nonci *et al.,* 2019). The yield loss from fall army-worm (FAW) infestation in sweet corn has been recorded as 24.35 ± 6.28%, with the potential to increase up to 76.9% in severe cases of infestation (Overton *et al.,* 2021). Economic losses due to *S. frugiperda* infestation are estimated to be between US$2.5 and 6.2 billion annually (FAO and CABI, 2019). Currently, the most common control method involves the use of chemical insecticides. However, prolonged use of these insecticides has led to resistance in *S. frugiperda* populations and may cause negative impacts on the environment and non-target organisms (Berg and Plessis, 2022; Bird *et al.,* 2022; Boaventura *et al.,* 2020).

As an alternative to chemical control, biological control agents such as entomopathogenic fungi have become increasingly popular. Various fungal species, including *Beauveria bassiana*, *Metarhizium anisopliae, Penicillium citrinum*, and *Cladosporium* sp., have been reported to have the ability to infect and inhibit the growth and development of *S. frugiperda* in laboratory conditions (Idrees *et al.,* 2022; Idrees *et al.,* 2023; Herlinda *et al.,* 2020).

Trichoderma can act as an entomopathogen through mechanisms like parasitism and the production of secondary metabolites that are toxic, antifeedant, and repellent (Poveda, 2021). Several studies have

indicated that Trichoderma can function as an entomopathogen against various pests. For example, a survey by Trizelia *et al.* (2024) using *T. asperellum* on *Nilaparvata lugens* achieved 48% mortality in the nymph stage and 46% in the adult stage. In another study (Anwar *et al.,* 2016) used *T. longibrachiatum* on *Bemisia tabaci*, resulting in 73% mortality in nymphs and 40% in adults. Nawaz *et al.* (2020) reported that *Trichoderma* spp. at a concentration of 10^8 conidia/mL induced mortality in *Aphis gossypii*, achieving 73.33% in laboratory conditions and 87.77% in field conditions. Putri *et al.* (2025) also reported that *T. asperellum* could infect *C. pavonana* larvae. Larval mortality reached 93.3%. Application of *T. asperellum* also inhibited the formation of pupae and adults. Therefore, further development of *T. asperellum* as an entomopathogenic fungus is essential to evaluate its effectiveness as an alternative control method for *S. frugiperda* in corn fields. This study aims to explore the endophytic fungus *T. asperellum*'s potential as an entomopathogen and obtain the most effective isolates in controlling *S. frugiperda* larvae.

MATERIALSANDMETHODS

Preparation of *T. asperellum* isolate

This research employed an endophytic fungus from the Biological Control Laboratory at Universitas Andalas. *T. asperellum* isolates were acquired as endophytes from diverse plant species throughout West Sumatra (Table 1). The five isolates were cultivated and revitalized on Sabouraud Dextrose Agar Yeast (SDAY) medium in Petri dishes, subsequently incubated for 14 days at ambient temperature until the conidia encompassed the whole surface of the medium.

Mass rearing of insects

Larvae of *S. frugiperda* were collected from cornfields in the Kuranji sub-district of Padang city. The larvae were maintained in plastic cups measuring 6 cm in diameter and 5 cm in height for cultivation. Each larva was provided with maize leaves as sustenance, which were substituted every day. As the larvae approached pupation, they were relocated to gauze enclosures. Adults derived from pupal development were provided with cotton wool saturated with a 10% honey solution in the raising box as sustenance, along with corn plants designated as oviposition locations. The collected larvae were transported to petri dishes for use as subjects for tests.

Table 1. Isolates used in this study, their code, plant source and origin

Isolate Code	Plant source	Origin
T. asperellum A116	Chili roots	Parabek, Banuhampu District, Agam Regency
T. asperellum PC21	Rice stem	Kuranji District, Padang City
T. asperellum S2D11	Shallot leaves	Sungai Pua, Sungai Pua District, Agam Regency
T. asperellum SD324	Chili leaves	Batu Bagiriak, Lembah Gumanti District, Solok Regency
T. asperellum AB2B3	Shallot stem	Air Batumbuk, Gunung Talang District, Solok Regency

Preparation of conidia suspension

Fungal conidia suspension was obtained by adding 10 ml of sterile distilled water and 0.05% Tween 80 as a grading agent into a petri dish. Conidia were released from the media using a fine brush. The concentrated suspension was diluted, and the conidia density was calculated using a haemocytometer to convert it into a uniform conidia concentration in all isolates. The concentrated suspension was diluted, and the conidia density was calculated using a haemocytometer to 108 conidia/ml.

Virulence test

The virulence test was conducted on second-instar *S. frugiperda* larvae. A total of 15 larvae were infested in Petri dishes lined with sterile tissue paper. The conidial suspension was applied by spraying 2 ml of a manual spray bottle onto the test larvae in the Petri dishes lined with moist tissue paper. As a control, the larvae were sprayed with the same volume of sterile distilled water. This experiment was repeated five times. Each larva was then transferred to a plastic cup to prevent cannibalism, and healthy corn leaves were provided as food. Larval mortality was recorded daily until the seventh day after treatment. Dead larvae were incubated in Petri dishes lined with filter paper

moistened with sterile distilled water to confirm that the mortality was caused by *T. asperellum.* Live larvae were transferred to plastic cups until they became pupae and adults, and the number of pupae and adults formed was counted.

Data analysis

The data obtained was analyzed using analysis of variance (ANOVA) and LSD using STAT 8. Lethal period of Lethal Time 50 T. asperellum against test insects was calculated using SPSS software

DISCUSSION

Larvae mortality

The virulence test results showed that all isolates were able to cause mortality of *S. frugiperda* at 32-54.67%. The mortality of each isolate is presented in Table 2.

Table 2. Mortality of *S. frugiperda* larvae after *T. asperellum* inoculation

Treatment	Larvae mortality (%) ± SE	Lehal Time $_{50}$ (Day)
T. asperellum S2D11	54.67 ± 4.42 a	3.34
T. asperellum AB2B3	45.33 ± 6.80 ab	9.52
T. asperellum SD324	41.33 ± 9.04 ab	9.11
T. asperellum A116	37.33 ± 9.80 ab	25.95
T. asperellum PC21	32.00 ± 9.29bc	16.86
Control	12.00 ± 3.89c	

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

Table 2 shows that larval mortality in all isolates is higher than in the control; four isolates, S2D11, AB2B3, SD324, and A116, significantly differ from the control. At the same time, each isolate showed no significant differences. Lethal time 50 value of *T. asperellum* ranges from 3.34 to 25.95 days. The highest and fastest mortality rate occurred in S2D11 at 54.67% and 3.34 days. However the other fungal isolates were also found infected *S. frugiperda* larvae. Daily mortality is presented in Figure 1. It shows that the daily mortality of larvae in all isolates occurred 1 day after inoculation, and increased after 6 days of inoculation, reaching 30 percent mortality. Larval mortality did not appear on the tenth day and continued until the pupal stage. The results showed that *T. asperellum* can infect insect bodies; dead larvae showed blackish symptoms as shown in the picture. Dead larvae overgrown with fungal mycelia after 3 days in humid conditions, the larval body is greenish-white.

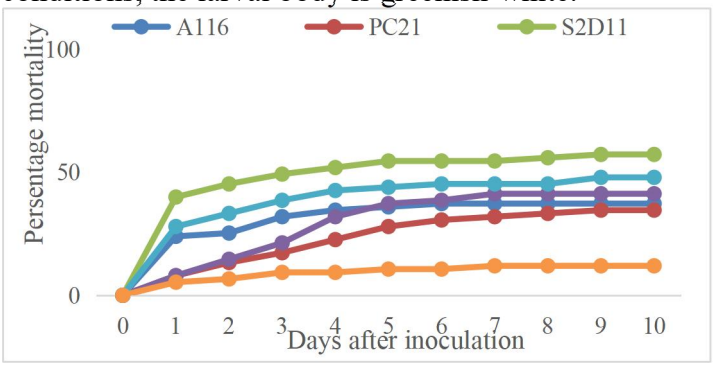

Figure 1. Daily mortality of *S. frugiperda* after inoculation

Trichoderma can infect *S. frugiperda* directly in several ways, namely through parasitism, where the fungus develops in the body of the host insect, entomopathogenic fungi infect insects through direct penetration of the cuticle, and the third is to produce toxin compounds that can kill insects (Salwa & Kottb, 2017).

Mortality rates are relatively low because the fungal conidia that enter and infect are still not optimal. After all, entomopathogenic fungi that enter and infect insects have a long lifespan. In addition, entomopathogenic fungi that infect and kill insects highly depend on the number of environmental conidia and nutrients needed (Nasution *et al.,* 2018). Symptoms of larval mortality are shown in Figure 2.

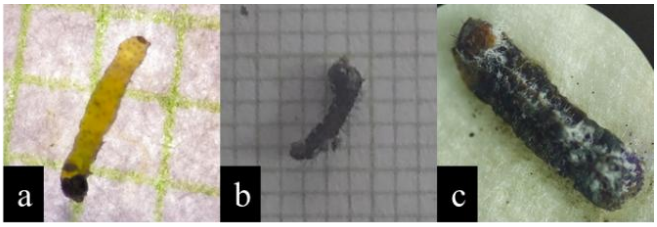

Figure 2. *S. frugiperda* larvae after *T. asperellum* inoculation. a) normal second instar larvae, b) dead larvae after inoculation, c) *T. asperellum* hyphae growing on dead larvae.

Symptoms of infected insects due to entomopathogenic fungus infection vary depending on favorable environmental conditions, such as higher relative humidity (Herlinda *et al.,* 2020).

Death caused by entomopathogenic fungi occurs because the fungi can grow inside the cells of the insect's body, and absorb the insect's body fluids, resulting in the insect dying in a hardened body state

like a mummy (Vega and Kaya, 2012). Hyphae from spores can enter the inner cavity of the host body due to the help of enzymes and mechanical pressure, the entire body of the host insect is full of propagules, and the soft part of the body will be penetrated and reveal the growth of hyphae outside the host insect body (Harun *et al.,* 2022). The infection process is thought to be assisted by the help of enzymes contained in Trichoderma secondary metabolites, including cellulase, polygalacturonase (PG), chitinase, β-1,3 glucanse, and protease (Gajera and Vakharia, 2012). Other studies reported that trichoderma fungi can kill insect pests by a parasitism mechanism on *Bemisia tabaci* (Anwar *et al.,* 2016), *Aphis gossypii* (Nawaz *et al.* 2020), and *Nilaparvata lugens* (Trizelia *et al.,* 2024).

Pupae formation

Pupae formation from surviving larvae in this study was in the range from 42.67 to 60.00%. That showed that *S. frugiperda* inoculation can reduce the incidence of pupae formation. Results presented in Table 3 revelas that applying *T. asperellum* to *S. frugiperda* larvae can reduce the incidence of pupae formation compared to the control. All five *T. asperellum* isolates exhibited markedly distinct outcomes from the control, with isolate S2D11 demonstrating the lowest percentage results relative to the other four isolates. Symptoms of pupae not forming are characterized by the failure of the 6th instar larvae to form pupae, and thereafter display death, as in Figure 3.

The pupae that had not formed showed an incomplete sixth instar larva that transformed into pupae. The larvae turned black and died. Pupae that are sick or die after being inoculated with conidia of endophytic fungi generally become stiff, shrink, shrivel, harden, and gradually turn black but have no smell (Gustianingtyas *et al.,* 2021).The pupae that had not formed showed an incomplete sixth instar larva that transformed into pupae. The larvae turned black and died. Pupae that are sick or die after being inoculated with conidia of endophytic fungi generally become stiff, shrink, shrivel, harden, and gradually turn black but have no smell (Gustianingtyas *et al.,* 2021).

Adult formation

The results showed that *S. frugiperda* inoculation can reduce the formation of adults by 36%. Table 4 shows that the five isolates showed significantly different results from the control.

Table 3. Percentage of pupae formed after *T. asperellum* inoculation

Treatment	Pupae formed (%) ± SE
Control	88.00 ± 3.89 a
T. asperellum PC21	60.00 ± 8.16 b
T. asperellum A116	58.67 ± 5.33 bc
T. asperellum SD324	52.00 ± 7.72 bc
T. asperellum AB2B3	44.00 ± 4.99 bc
T. asperellum S2D11	42.67 ± 3.40 c

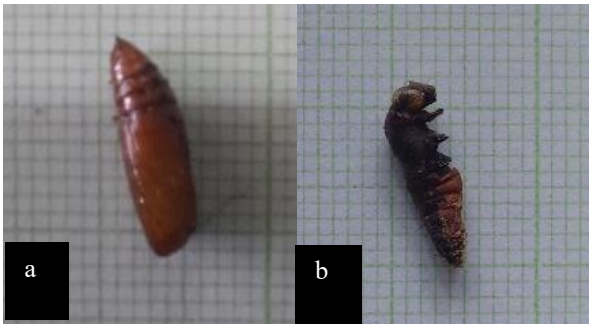

Figure 3. *S. frugiperda* pupae after *T. asperellum* inoculation: a) pupae formed, b) pupae not formed

All isolated showed lower results than the successfully formed pupae, as in Table 3. Symptoms of an adult not formed can be shown in Figure 4.

Table 4. Percentage of adult formed after *T. asperellum* inoculation

Treatment	Adult formed (%) ± SE
Control	88.00 ± 3.89 a
T. asperellum PC21	60.00 ± 8.16 b
T. asperellum A116	54.67 ± 4.42 bc
T. asperellum AB2B3	44.00 ± 4.99 bcd
T. asperellum SD324	42.67 ± 6.18 cd
T. asperellum S2D11	36.00 ± 3.40 d

The numbers followed by the same letter in the same column are not significantly different in the LSD test at 5% level. An unformed adult can be seen from an abnormal adult after exiting the pupa (Figure 4c) and then dying. Some abnormal adults remain alive with incompletely formed wings (Figure 4d). Insects exhibit abnormalities and malformations due to entomopathogenic fungal infections, which are caused by proteases and chitinases that degrade proteins and chitin within their bodies. Adults with incomplete

wings are unable to reproduce, as they cannot fly to mate, thereby diminishing their offspring's population (Herlinda *et al.*, 2020).

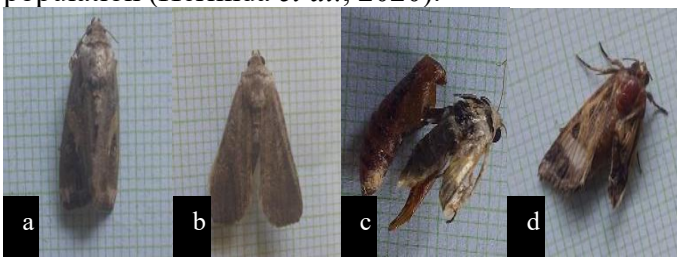

Figure 4. Adults of *S. frugiperda* after *T. asperellum* inoculation. a) normal male adults, b) normal female adults, c) unformed adults, d) abnormal adults.

Results indicated that the endophytic fungus *T. asperellum* can infect *S. frugiperda* larvae and limit pupae and adult production. *T. asperellum* S2D11 had the most significant virulence effect on *S. frugiperda* larvae among the five isolates.

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