Jurnal Ilmu Pertanian Indonesia (JIPI), October 2025
(Indonesian Journal of Agricultural Sciences)
ISSN 0853-4217 EISSN 2443-3462

Vol. 30 (4): 741–746
http://journal.ipb.ac.id/index.php/JIPI
DOI: 10.18343/jipi.30.4.741

Endophytic Fungus *Trichoderma asperellum*'s Virulence on *Spodoptera frugiperda* J. E. Smith (Lepidoptera, Noctuidae) Eggs

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(Received November 2024/Accepted July 2025)

ABSTRACT

Spodoptera frugiperda J.E. Smith, sometimes known as armyworm, is a serious pest in maize crops. This pest affects maize plants' leaves, resulting in yield losses of up to 79.9%. Entomopathogenic fungi is one alternate method for controlling the pest. Various entomopathogenic fungus can be found in nature, one of which thrives endophytically on plants, such as Trichoderma asperellum. The purpose of this study was to obtain and evaluate T. asperellum's pathogenicity in suppressing S. frugiperda eggs. This investigation used five T. asperellum isolates (A116, PC21, S2D11, SD34, and AB2B3) obtained from diverse plant tissues. S. frugiperda larvae were treated with 2 mL of a conidia suspension containing 108 conidia/mL and sterile distilled water as a control. The observation variables comprised S. frugiperda egg mortality, first-instar larvae mortality, pupae formation percentage, and imago formation percentage. The findings indicated that the endophytic fungus T. asperellum can infect S. frugiperda eggs. The mortality rate for S. frugiperda eggs ranged from 43.27 to 78.34%. The mortality rate of first instar larvae ranged between 36.94% and 60.22%. The application of T. asperellum to S. frugiperda decreased pupae and imago production by 39.78% and 37.87%, respectively. T. asperellum SD324 is the most effective isolate for infecting S. frugiperda eggs.

Keywords: endophytes fungus, entomopathogenic fungus, larvae, maize, Spodoptera frugiperda

INTRODUCTION

Spodoptera frugiperda (Lepidoptera: Noctuidae) is a serious pest of maize crops in several countries, including Indonesia. This insect severely damages maize crops by attacking leaves, stems, and growth points, resulting in production losses of up to 8.3−20.6 million tons per year (FAO and CABI 2019). The pest is predicted to harm 12,137 hectares of maize crops in Indonesia by 2024, accounting for 34.5% of total maize plantation area across all provinces (Balai Besar Peramalan Organisme Pengganggu Tumbuhan 2024). Farmers most usually utilize synthetic pesticides as a control tool. However, intensive pesticide usage can cause resistance in *S. frugiperda* populations, lowering control effectiveness (Boaventura *et al*. 2020; Bird *et al*. 2022; Berg and Plessis 2022). One solution is to use biological control agents, such as entomopathogenic fungi. Under laboratory conditions, various fungal species, including *Beauveria bassiana*, *Metarhizium anisopliae*, *Penicillium citrinum*, *Cladosporium* sp., and *Trichoderma* sp., have been shown to be effective in controlling *S. frugiperda* (Idrees *et al.* 2022; Idrees *et a*l. 2023; Herlinda *et al*. 2020; Afandhi *et al*. 2022).

The employment of biological agents such as entomopathogenic fungus is an alternate pest management method. Entomopathogenic fungi have distinct modes of action than synthetic pesticides, giving them a promising option for combating autumn armyworms. Under ideal conditions, entomopathogenic fungus spores that come into touch with the insect host will germinate and penetrate the insect cuticle enzymatically and mechanically, entering the insect's body. After infecting the insect tissues, the fungi multiply quickly and exit from the dead insect carcass, producing new fungal spores (Parjane *et al*. 2023). *Metarhizium anisopliae* and *Beauveria bassiana* are two entomopathogenic fungi that have been shown to be efficient against *S. frugiperda* in the laboratory. These fungi have also been shown in numerous countries to be effective in lowering insect populations (Herlinda *et al.* 2020).

Trichoderma. is a fungus that can operate as an entomopathogen by parasitizing insects and producing harmful secondary metabolites (Poveda 2021). The fungus can grow as endophytes in a variety of plant species (Trizelia *et al.* 2024). According to some research, Trichoderma can operate as an entomopathogen on certain pest species, including *Nilaparvata lugens* (Trizelia *et al*. 2024), *Bemisia tabaci* (Anwar *et al*. 2016), *Aphis gossypii* (Nawaz *et al*. 2020), and *Oryctes rhinoceros*.(Nasution *et al.* 2018).

Trichoderma as an entomopathogenic fungus should be studied to determine its potential to control *S. frugiperda* at different stages. Eggs have been

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identified as the most efficient stage for suppressing *S. frugiperda* with entomopathogenic fungi (Idrees *et al*. 2022). *T. asperellum* has also been used to regulate the eggs *of Crocidolomia pavonana* (Putri *et al*. 2025). The study of entomopathogenic fungi on the egg stage of *S. frugiperda* has received little attention. The purpose of this study was to look at the endophytic fungus *T. asperellum's* ability to act as an entomopathogen and govern *S. frugiperda* eggs. Endophytic fungus should connect with plants to inhibit *S. frugiperda*.

METHODS

From July to September 2024, this study was carried out at Andalas University's Biological Control Laboratory, Faculty of Agriculture. The study used an experimental approach with a completely randomized design (CRD) that included six treatments and five replications. This study used five *T. asperellum* isolates acquired endophytically from distinct plant species in different locations of West Sumatra as treatments: A116 (isolates from chili roots, Parabek, Banuhampu District, Agam Regency), PC21 (isolates from rice stem, Kuranji District, Padang City), S2D11 (isolates from Sungai Pua, Sungai Pua District, Agam Regency), SD324 (isolates from chili leaves, Batu Bagiriak, Lembah Gumanti District, Solok Regency), and AB2B3 (isolates from shallot stem, Air Batumbuk,Gunung Talang District, Solok Regency).

All isolates were grown and regenerated in Petri dishes using Sabaround Dextrose Agar Yeast (SDAY) media before being cultured at room temperature for 14 d until the conidia covered the whole surface of the medium (Mwikali *et al*. 2024).

Insect Rearing

S. frugiperda larvae were obtained from corn fields in Kuranji Subdistrict, Padang City. The larvae were raised individually in plastic containers measuring 6 cm in diameter and 5 cm in height, and they were given fresh maize leaves that were replenished every day. After pupation, the larvae were placed in rearing cages of 40 × 40 × 50 cm and coated with gauze. The emerging adults were fed cotton soaked in a 10% honey solution, and maize plants served as oviposition sites. The eggs were transported to Petri dishes and utilized as test insects (Peter *et al*. 2023).

Preparation for Conidial Suspension

T. asperellum conidial suspensions were prepared by adding 10 mL of sterile distilled water with 0.05% Tween 80 to the Petri dishes containing the fungal isolates. The conidia were gently scraped off the medium with a soft brush. The dense suspension was then diluted, and the conidial density was measured using a hemocytometer to ensure consistent conidial concentration across all isolates. This investigation employed a concentration of 10^8 conidia/mL (Idrees *et al*. 2021).

Virulence Test

Virulence experiments were carried out on *S. frugiperda* eggs. To apply the conidial suspension, 2 mL was spray at a concentration of 10^8 conidia/mL into test groups in Petri dishes lined with moist tissue paper using a manual spray bottle. In the control group, the same volume of sterile distilled water was sprayed. Each therapy was repeated five times.

First-instar larvae hatching from the eggs were separately transferred to plastic cups to avoid cannibalism and fed fresh maize leaves. To confirm that *T. asperellum* infection was the cause of death, larvae were re-incubated in Petri dishes lined with moistened filter paper. Surviving larvae were raised to pupation and adult emergence, and the quantity of pupae and adults produced was documented (Idrees *et al*. 2021).

Data Analysis

The data were analyzed using STAT 8 software, which included analysis of variance (ANOVA) and Least Significant Difference (LSD) tests. *T. asperellum's* lethal time fifty (LT_{50}) values against the test insects were computed using SPSS software.

RESULTS AND DISCUSSION

Egg Mortality

The inoculation of *T. asperellum* on *S. frugiperda* eggs revealed that all isolates could cause egg mortality, with mortality rates ranging from 43.27% to 78.54% (Table 1). Values followed by the same letter in the same column indicate no significant differences at the 5% level, according to the LSD test results. All *T. asperellum* treatments resulted in significantly increased egg mortality than the control, with the SD324 isolate showing the highest mortality rate. As illustrated in Figure 1, hyphal development was detected after three days of incubation under humid conditions, demonstrating that *T. asperellum* could infect *S. frugiperda* eggs. The virulence experiments revealed that applying *T. asperellum* at the egg stage influenced not only egg development but also the development of first-instar larvae hatching from the eggs, with mortality rates ranging from 36.94% to 60.22%.

Trichoderma's infection strategies against insects may include parasitism, toxin synthesis, or the formation of antifeedant chemicals that cause insect death (Poveda 2021). Insect eggs have three layers: the exochorion, which contains carbohydrates, the endochorion, and the crystalline layer, which contains proteins (Prayogo 2010). These layers include

Table 1 Mortality of eggs after inoculation of *T. asperellum* on *S. frugiperda*

Treatments	Eggs mortality (%) ± SE
T. asperellum SD324	78.54 ± 3.35 a
T. asperellum S2D11	73.05 ± 8.51 ab
T. asperellum AB2B3	72.63 ± 8.94 ab
T. asperellum PC21	55.31 ± 3.35 bc
T. asperellum A116	43.27 ± 5.25 c
Control	12.53 ± 1.21 d

Remarks: Numbers followed by the same letter in the same column are not significantly different in the LSD test at the 5% level. SE: Standard error.

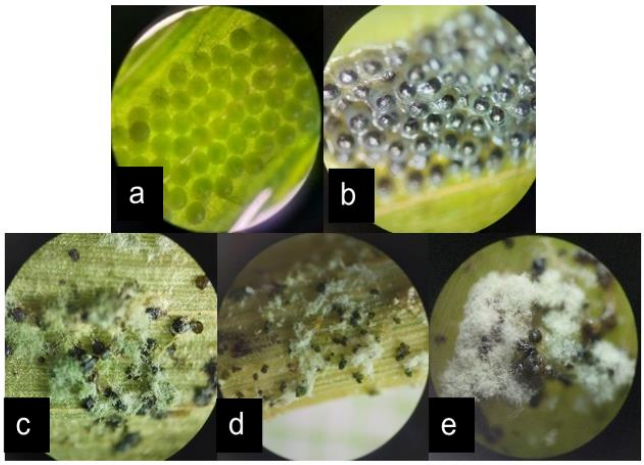

Figure 1 Eggs of *S. frugiperda* after inoculation with *T. asperellum:* (a) Normal eggs; (b) Eggs that did not hatch (SD324); (c) Eggs filled with hyphae after inoculation with *T. asperellum* SD324; (d) Eggs filled with hyphae after inoculation with *T. asperellum* S2D11; and (e) Eggs filled with hyphae after application of *T. asperellum* AB2B3.

substances that can be used by entomopathogenic fungal conidia, which produce enzymes such proteases, lipases, amylases, and chitinases to destroy cell wall components made up of proteins, lipids, carbohydrates, and chitin (Wang *et al*. 2005). Following infection, the fungus can exploit the resources within the egg through hyphal development. At this point, the embryo in the egg has perished, allowing the fungus to enter the saprophytic phase.

First-Instar Larval Mortality

The mortality rate of first-instar larvae after infection with *T. asperellum* ranged from 36.94% to 60.22% (Table 2). All isolates were capable of infecting first-instar larvae, with substantial changes from the control, but no significant differences were identified amongst the isolates. The first-instar larvae were observed, ingesting eggshell remnants that had previously been exposed to *T. asperellum* solutions, promoting fungal infection of the larval body. Mortality symptoms included rigid and darker larval bodies, as depicted in Figure 2. The mortality rate in first-instar larvae was lower than in eggs. This conclusion is consistent with Idrees *et al*. (2022) finding, that first-instar larvae have high feeding activity and mobility, rendering them less susceptible to fungal infection than the stationary egg stage, which allows for fungal attachment and infection.

Pupal and Adult Formation

The study found that *T. asperellum* inhibited pupal development by up to 42.67% and adult emergence by up to 37.87%. Tables 3 and 4 show the percentages of pupae and adults generated from each isolate. *T. asperellum* treatment on *S. frugiperda* larvae inhibited pupal development compared to the control. Two isolates, AB2B3 and PC21, had significantly poorer pupal development than the control. Failed pupation symptoms included sixth-instar larvae being unable to pupate, followed by mortality (Figure 3). Table 4 shows that all five *T. asperellum* isolates produced significantly different outcomes than the control. Four isolates, A116, AB2B3, SD324, and S2D11, had reduced adult emergence percentages compared to pupae (Table 3), with unformed adults displayed in Figure 4.

T. asperellum treatment also influenced the percentages of pupal and adult development in *S. frugiperda*. Pupal formation rates ranged from 39.78% to 58.51%, and adult emergence rates ranged from 37.87% to 51.13%. In addition to mortality at the egg and first-instar larval stages, the failure of late-instar larvae to transition into pupae and then into adults contributed to the decrease in pupal and adult emergence. Larvae that failed to pupate exhibited symptoms of darkening and death. Infected or dead pupae following entomopathogenic fungal application

Table 2 Mortality of first-instar larvae after inoculation of *T. asperellum* on eggs

Treatments	Mortality of first-instar larvae (%) ± SE
T. asperellum AB2B3	60.22 ± 9.86 a
T. asperellum S2D11	46.29 ± 9.63 a
T. asperellum A116	43.44 ± 8.30 a
T. asperellum SD324	37.97 ± 11.82 ab
T. asperellum PC21	36.94 ± 9.79 ab
Control	8.32 ± 1.90 b

Remarks: Numbers followed by the same letter in the same column are not significantly different in the LSD test at the 5% level. SE: Standard error.

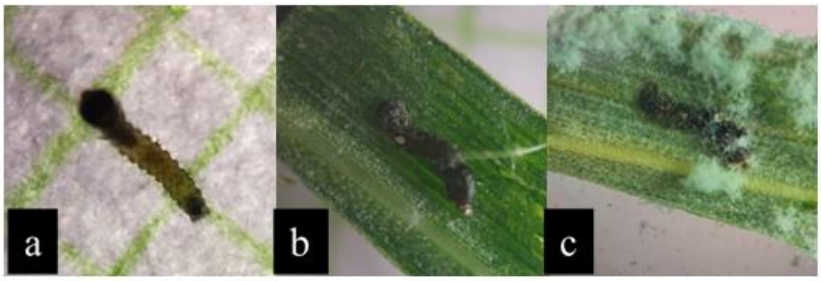

Figure 2 First-instar larvae of *S. frugiperda* after inoculation with *T. asperellum*: (a) Normal larvae, (b) Dead larvae, and (c) Hyphae of *T. asperellum* growing on the larvae's bodies.

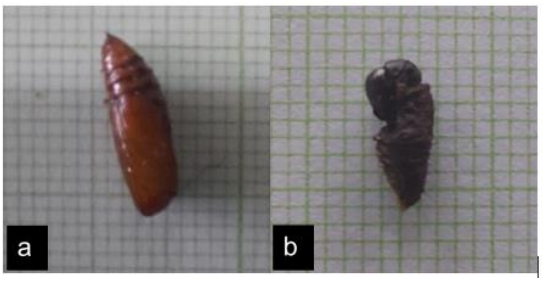

Figure 3 *S. frugiperda* pupae after inoculation of *T. asperellum* on eggs: (a) Formed pupae, and (b) Unformed pupae.

generally displayed rigid, shrunken, wrinkled, and hardened bodies that turned black without emitting any odor (Gustianingtyas *et al.* 2021). These symptoms are similar to those reported by Herlinda *et al.* (2020) in the application of *Metarhizium anisopliae*, where pupae were smaller, darker, and showed no abdominal movement when touched with a brush.

Adults that failed to emerge were indicated by unsuccessful eclosion from the pupae. Some adults that managed to emerge displayed underdeveloped wings, preventing them from flying and mating. Additionally, entomopathogenic fungi may disrupt the normal growth of insects, reduce fecundity, and decrease adult vitality (Herlinda *et al.* 2020).

CONCLUSION

The study's findings show that the endophytic fungus *T. asperellum* can infect *S. frugiperda* eggs, and that applying the fungus inhibits the production of pupae and adults. All five isolates have similar virulence effects, but *T. asperellum* isolate S2D11 had the strongest effect on *S. frugiperda* larvae.

ACKNOWLEDGMENTS

The author would like to take this opportunity to thank Andalas University's Research and Community Service Institution (93/UN16.19/KPT/PT.01.00/2024) for funding this study, which allowed it to move forward effectively.

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Table 3 Pupae formed after inoculation with *T. asperellum* on eggs

Treatments	Pupae formed (%) ± SE
Control	78.54 ± 2.61 a
T. asperellum PC21	73.05 ± 9.09 ab
T. asperellum SD324	72.63 ± 11.76 ab
T. asperellum A116	55.31 ± 7.68 bc
T. asperellum S2D11	43.27 ± 9.92 c
T. asperellum AB2B3	12.53 ± 9.86 d

Remarks: Numbers followed by the same letter in the same column are not significantly different in the LSD test at the 5% level. SE: Standard error.

Table 4 Adult formed after inoculation with *T. asperellum* on eggs

Treatments	Adult formed (%) ± SE
Control	72.82 ± 7.86 a
T. asperellum SD324	51.59 ± 10.13 ab
T. asperellum S2D11	50.47 ± 9.96 ab
T. asperellum A116	49.99 ± 7.66 ab
T. asperellum PC21	45.13 ± 4.98 b
T. asperellum AB2B3	37.87 ± 10.67 b

Remarks: Numbers followed by the same letter in the same column are not significantly different in the LSD test at the 5% level. SE: Standard error.

Figure 4 Adult S. frugiperda after application of T. asperellum: (a) Normal male adult, (b) Normal female adult, (c) Malformed adult, and (d) Abnormal adult.

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