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Colonization Ability of *Trichoderma asperellum* And Its Effect on Oviposition Preference of *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) in Maize

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ABSTRACT

Fall armyworm (Spodoptera frugiperda J.E. Smith) is a major pest of maize crops. This pest has the ability to lay eggs and reproduce quickly. One option to control this pest is to improve plant resistance using biological agents. The Trichoderma asperellum fungus is one of the biological agents that can develop in plant tissues. This study aims to see the colonization ability of T. asperellum on maize and its effect on S. frugiperda oviposition. The research was conducted experimentally using a completely randomized design using endophytic fungi from several types of plants in several regions in West Sumatra. Treatment application was carried out by soaking maize seeds with a suspension of T. asperellum fungus then planted in polybags. The plants from the introduction of the fungus were used as a test site for S. frugiperda oviposition preference and colonization tests. Observations included the percentage of colonization on maize leaves and the number of eggs oviposited by S. frugiperda on maize. The results showed that T. asperellum can live as an endophyte on maize leaves at 21 DAI with a low colonization ability of 10%. The introduction of T. asperellum in maize through seed soaking did not affect the oviposition of S. frugiperda adult.

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KEYWORDS: Colonization, Endophytic fungi, Oviposition preference, Spodoptera frugiperda, Trichoderma asperellum

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INTRODUCTION

Fall Armyworm (*Spodoptera frugiperda* J.E. Smith) is a major pest of maize crops. This pest easily spreads in Indonesia because it has a wide host that can survive on a variety of plants, has the ability to lay eggs and reproduce quickly (Andini, & Triyuliana, 2023). One approach to controlling this pest is to increase plant resistance.

Increased plant resistance can be induced through the mechanism of Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR). SAR induction occurs through the addition of chemical compounds or elicitors that activate the plant defense system, while ISR is triggered by the administration of non-pathogenic biotic agents, such as endophytic fungi (Munawara and Haryadi, 2020).

Trichoderma fungi are commonly found in the rhizosphere can be endophytic, which has been widely studied as a control agent for fungi, bacteria and plant pathogenic nematodes (Contreras-Cornejo et al., 2018). The use of *Trichoderma* in controlling pests has also been reported in several studies. Research by Vijayakumar et al. (2016) reported that *T. viride* produces a chitinase enzyme that can act as a repellent for *Corcyra cephalonica*. The research results of Alınç et al. (2021) stated that colonization of *T. harzianum* on tomato plants can increase plant defense and reduce the consumption rate of *Nezara viridula*. Some Trichoderma species such as *T. harzianum*, *T. viride* and *T. citrinoviride* produce volatile organic compounds (VOCs) that can act as insect repellents.

In addition to its role as an entomopathogenic fungus, the fungus *T. asperellum* can develop in plant tissues without causing symptoms of infection in plants. Previous research reported that the introduction of the endophytic fungus *T. asperellum* from several types of plants through seed soaking, was able to colonize plant tissue and had a significant effect on reducing the

development of *Mycus persicae* populations in chili (Trizelia et al., 2020). Another study found that colonization of entomopathogenic fungi in plant tissues can affect the oviposition behavior of insect pests (Hendra et al., 2022). Colonization of *T. asperellum* on tomato can affect the oviposition preference of tuta absoluta (Agbessenou et al., 2022).

The use of endophytic fungi in inducing plant resistance is expected to colonize plant tissues and can increase plant resistance by becoming an unpreferred plant for insect pest oviposition. This study aims to see the colonization ability of *T. asperellum* on corn plants and its effect on *S. frugiperda* oviposition.

MATERIAL AND METHODS

This research was conducted in the biological control laboratory of the faculty of agriculture, Andalas University. The research was conducted experimentally using a complete randomized design.

Preparation of T. asperellum isolate

This study used endophytic fungi collection of biological control laboratory Universitas Andalas. *T. asperellum* was obtained endophytically from several types of plants in several areas in West Sumatra as shown in Table 1. The five isolates were propagated and rejuvenated on Sabaround Dextrose Agar Yeast (SDAY) media in petri dishes, then incubated for 14 days at room temperature until full conidia covered all petri dishes.

Table 1. *Trichoderma asperellum* isolates used in the study

Isolate Code	Plant origin of isolate	Region of origin
A116	Chili roots	Parabek, Banuhampu sub-district Agam district
PC21	Rice stems	Kuranji sub-district Padang Municipality
S2D11	Shallot leaves	Sungai pua, Sungai pua sub-district Agam district
SD324	Chili leaves	Batu bagirik, Lembah gumanti sub-district Solok district
AB2B3	Onion stalks	Air batumbuk, Gunung talang sub-district Solok district

Preparation of conidia suspension

Fungal conidia suspension was obtained by adding 10 ml of sterile distilled water and 0.05% Tween 80 as a levelling agent into a petri dish. Conidia were released from the media using a fine brush. The concentrated suspension was then diluted and the conidia density was calculated using a haemocytometer so that it could be converted into a uniform conidia concentration in all isolates. The conidia concentration used was 10^8 conidia/ml.

Introduction of *T. asperellum* in maize

Before treatment, maize seeds were surface sterilized by soaking the seeds in sterile distilled water and 70% alcohol for two minutes each steps. The seeds were rinsed three times with sterile distilled water and dried in a laminar air flow blower. Next, the seeds were soaked in a 10^8 conidia suspension of *T. asperellum* for 5 hours (Kiarie et al., 2020). Seeds that have been treated are then aerated for 20 minutes before planting. The seeds for control plants were only soaked in sterile distilled water. Seeds that had been soaked with the suspension were planted on polybags measuring 40 x 17 cm^2 at a planting distance of 75 cm x 25 cm between polybags. plant maintenance included watering, weeding and mechanical pest control. The plants were aged 21 Days After Planting (DAP) can be used as test plants for colonization tests and insect egg-laying sites for oviposition preference tests.

T. asperellum colonization assay on maize

Colonization assay of *T. asperellum* on maize plant tissues was observed on plants 21 and 35 days after introduction. The plant part observed was the corn leaf (Kiarie et al., 2020) were cut into small pieces with a size of ±1 cm, then sterilized using 1% NaOCl solution for 1 minute, washed with sterile distilled water, and dried in a laminar air flow cabinet on filter paper at room temperature (Tefera & Vidal, 2009). After drying, the leaf pieces were planted on PDA media in petri dishes, with each dish containing five leaf pieces. Each treatment was carried out as many as five replicates. After 4 days of incubation, the presence of *T. asperellum* was evidenced by the emergence of mycelium or conidia from the tip of the leaf tissue. Colonization success was calculated based on the ratio between the number of plant pieces showing *T. asperellum* fungus growth and the total number of pieces tested.

Insect rearing

S. frugiperda larvae were collected from corn fields in Kuranji District, Padang City. Larvae were reared in plastic cups (6 cm in diameter and 5 cm high). Each larva was given corn leaves as larval food, which was replaced daily. Imago obtained from pupal and larvae development were used as test insects.

Oviposition preference assays of *S. frugiperda*

Oviposition preference assays were conducted using the choice test method to see the most preferred corn for egg laying following the method of Deden et al. (2023). Corn plants that had been treated with *T. asperellum* by seed soaking and control plants that were 14 HST old were moved into cages with a size of 40 x 40 x 50 cm3. Six corn plants of all treatments were placed in one cage and 5 pairs of *S. frugiperda* imago were infested in each gauze box. Groups of eggs laid by imago on corn leaves were transferred to plastic cups to count the number of eggs laid, observations were made every day until the imago died.

Data analysis

The data obtained were analyzed using analysis of variance (ANOVA) and LSD using STAT 8 software.

RESULTS AND DISCUSSION

Colonization ability of *T. asperellum* on maize leaves

The introduction of *T. asperellum* fungus showed a significant effect on the colonization of T. asperellum on maize leaves at 21 DAI but had no significant effect at 35 DAI. The colonization ability of *T. asperellum* fungus at 21 DAI ranged from 0 to 10% and 0 to 3.33% at 35 DAI. The percentage of colonization of each isolate can be seen in Table 2.

Tabel 2. Colonization ability of T. asperellum on maize leaves at 21 and 35 DAI

Treatment	Kolonisasi pada daun (%)	
	21 DAI*	35 DAI*
T. asperellum S2D11	10,00 a	0,00
T. asperellum PC21	3,33 b	0,00
T. asperellum SD324	0,00 b	3,33
T. asperellum AB2B3	0,00 b	0,00
T. asperellum A116	0,00 b	0,00
Control	0,00 b	0,00

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

Table 2 shows that the colonization ability of T. asperellum on 21 DAI plants is low, the percentage of colonization found in isolate S2D11 by 10%, significantly different from other treatments. While the colonization ability of *T. asperellum* on 35 DAI plants shows results that are not different in all treatments. The successful colonization of T. asperellum was marked by the presence of green mycelia that grew spreading from the corn leaves in the petri dish as shown in Figure 1.

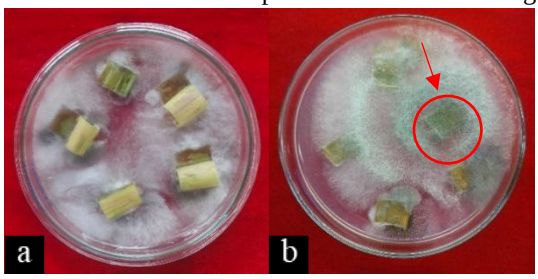

Figure 1. Colonization of T. asperellum on 21-day-old maize leaves: (a) Control, (b) mycelia of T. asperellum from colonization of isolate S2D11.

Other studies reported that Trichoderma can colonize several plants such as tomatoes (Agbessenou et al., 2022), chili peppers (Trizelia et al., 2020), and cocoa seedlings (Sriwati et al., 2015). According to Sriwati et al. (2015) the results of *Trichoderma spp*. colonization on leaves tend to be lower than on the roots, this fungus is saprophytic and more adaptive to live in the rhizosphere of plants. Some strains of *Trichoderma spp*. are able to colonize endophytically in root tissue, which contributes to increased plant resistance. Research by Kiarie et al. (2020) similarly reported that the colonization ability of *T. harzianum* was able to colonize the leaves of corn plants only 10% at 14 days after planting and 0% at 35 days after planting while T. atroviridae was only able to colonize 5 HSI by 90%, no sign of colonization of the two types of fungi was found at 63 days after planting. Kiarie et al. (2020) reported that T. harzianum and T. atroviridae were able to colonize the roots and stems of corn plants up to 90%.

The presence of entomopathogenic fungi in plant tissues depends on the host plant. The presence of nutrients is related to plant physiology, such as the process of photosynthesis that produces carbohydrates carbohydrates needed by fungi for survival.

(Vega, 2008). Safavi et al. (2007) explained that endophytic fungi require nutrients for biosynthesis and energy release as the main factor to support the ability to live, and the development of their colonies.

Oviposition preference of *S. frugipeda* on corn plants that have been introduced with *T. asperellum*

The introduction of T. asperellum on maize plants did not show a significant effect on the number of egg groups and the total number of eggs in the oviposition of S. frugiperda adult. Egg groups placed ranged from 1.8 to 3.2 per plant, while the total number of eggs placed on plants ranged from 104 to 270 eggs per plant. The average egg groups and number of eggs per plant can be seen in Table 3.

Table 3. Number of egg groups oviposited by S. frugiperda on plants introduced with T. asperellum 21 DAI

Treatment	Number of egg groups ± SE*[*]	Effectiveness of egg groups laid (%)	Number of ovipositioned eggs ± SE*	Effectiveness of number of eggs laid (%)
T. asperellum PC21	3,8 ± 1,20	-18,75	240,4 ± 71,72	10,96
Kontrol	3,2 ± 0,73		270,0 ± 76,78	
T. asperellum SD324	2,4 ± 0,75	25,00	105,4 ± 40,19	60,96
T. asperellum S2D11	2,2 ± 0,58	31,25	146,0 ± 35,58	45,93
T. asperellum A116	2,0 ± 0,45	37,50	143,4 ± 34,07	46,89
T. asperellum AB2B3	1,8 ± 0,37	43,75	104,0 ± 67,66	61,48

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

Table 3 shows that there is no significant difference in the number of egg groups and the number of eggs produced from the oviposition of S. frugiperda in all corn treatments, but isolates AB2B3, SD324, S2D11 and A116 were found to have fewer egg groups than the control with an effectiveness of 25.00 to 43.75%. While the number of eggs laid by S. frugiperda imago showed lower results in all isolates than the control with an effectiveness of 10.96 to 61.48%. In general, all treated plants showed lower oviposition results than the control.

Another study found that reported that T. asperellum inoculation affected the oviposition preference of female Tuta absoluta imago on tomato plants in a Y-tube test (dual choice), female imago preferred uninoculated tomato plants. This difference in host preference may be due to volatile compounds produced by plants after T. asperellum inoculation (Agbessenou et al., 2022). The difference in isolate strains is a factor that gives a different response to pests, the effect of fungi that can develop in plant tissue has a different response depending on the fungal isolate used and the host plant (Hendra et al., 2022).

The interaction between plants and endophytic fungi is interconnected and can occur symbiotic mutualism (mutual benefit). Plants fulfill nutritional needs for fungi so that fungi can develop in certain tissues of the host plant, then fungi produce secondary metabolite compounds from various different biosynthetic pathways (Schulz et al., 2002). Most of the secondary metabolite compounds produced by endophytic fungi are toxic and antibiotic, it can be used as an additional defense system. However, based on this study we found that the ability of the fungus T. asperellum to live in plant tissue did not increase plant resistance in terms of oviposition preference.

CONCLUSIONS

T. asperellum can live as an endophyte on maize plant leaves at 21 HST with a low colonization ability of 10%. The introduction of *T. asperellum* in corn plants through seed soaking did not affect the oviposition of S. *frugiperda* imago.

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