IIAJ INTERNATIONAL INTEGRATED AGRICULTURAL JOURNAL
Volume 02 Number 02
November 2026
e-ISSN: 3124-8179
p-ISSN: 3124-7865

In Vitro Antagonistic Activity of *Trichoderma* sp. against *Fusarium* sp. Causing Maize Stalk Rot

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Abstract:
Fusarium stalk rot reduces maize (*Zea mays* L.) yields, and heavy fungicide use harms the environment. This study evaluated the inhibitory ability of *Trichoderma* sp. against *Fusarium* sp. and described the colony growth of both fungi in vitro. The experiment was conducted from January to February 2025 at the Plant Protection Laboratory, Politeknik Negeri Jember, Indonesia, using the dual culture method in a completely randomized design with three treatments (control without *Trichoderma*, and conidial densities of 10^7 and 10^8 conidia mL^{-1}) and three replications. Colony growth was measured at 24, 48, and 72 hours after incubation, and inhibition was analyzed by ANOVA and the LSD test at 5%. *Trichoderma* sp. inhibited *Fusarium* sp. at all observation times. Inhibition reached 69–72% at 72 hours, and the two conidial densities did not differ significantly. *Trichoderma* sp. grew faster than the pathogen, reaching 7.7 cm (10^7) and 6.8 cm (10^8) at 72 hours, while *Fusarium* sp. was restricted to 2.4–2.6 cm compared with 8.6 cm in the control. *Trichoderma* sp. is therefore a promising biological control agent against *Fusarium* sp. of maize, and a density of 10^7 conidia mL^{-1} is sufficient for further greenhouse and field evaluation.

Keywords:
biological control, dual culture, *fusarium* sp., *trichoderma* sp.

Check for updates

Article info:
Submitted:
10-08-2026
Revised:
13-11-2026
Accepted:
13-11-2026
Published:
21-11-2026

https://doi.org/10.53713/iiaj.v2i2.466

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INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops in Indonesia. It is a staple food in several regions and the main raw material for the animal feed industry, so the demand for maize grows every year. Maize production, however, is constantly threatened by pests and diseases, and a large share of the national maize area is affected by plant-disturbing organisms every season. Among the diseases, stalk rot caused by *Fusarium* species is one of the most destructive soil-borne problems. The disease attacks the roots and lower stalk, causes discoloration and decay of the pith, and leads to lodging and premature death of plants after flowering (Asiedu and Miedaner, 2025). Yield losses due to *Fusarium* stalk rot typically range from 10% to 30–50% in severely affected areas (Gai et al., 2018).

Fusarium verticillioides and related species are the most common causal agents of stalk and ear rot in maize worldwide. These fungi survive in soil and crop residues, infect plants through roots and seeds, and move systemically from roots to the stalk and ear (Blacutt et al., 2018; Gai et al., 2018). In addition to reducing yield, they produce mycotoxins such as fumonisins, which contaminate grain and threaten the health of humans and livestock (Yazid et al., 2023). Because the pathogen lives in the soil and inside the plant, the disease is difficult to control once established, and no maize

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e-ISSN: 3124-8179
p-ISSN: 3124-7865

hybrids are fully resistant to it (Asiedu and Miedaner, 2025).

Farmers usually control *Fusarium* diseases with synthetic fungicides. Excessive and repeated use of these chemicals, however, pollutes soil and water, harms non-target organisms, leaves residues in food, and can select for resistant pathogen strains. Fungicides also give only limited protection against soil-borne pathogens that live inside the roots and stalk, because the chemicals rarely reach the infection sites in sufficient amounts for long enough. For example, a strain of *F. verticillioides* from maize was found to be resistant to carbendazim (Yassin et al., 2021). Biological control using antagonistic microorganisms is therefore needed as an environmentally friendly alternative or complement to chemical control (Thambugala et al., 2020).

Fungi of the genus *Trichoderma* are the most widely studied and used biological control agents against fungal plant pathogens (Thambugala et al., 2020; Guzmán-Guzmán et al., 2023). They are common soil inhabitants, grow rapidly, use a wide range of substrates, and tolerate a wide range of environmental conditions (Tyśkiewicz et al., 2022). *Trichoderma* suppresses pathogens through several mechanisms: competition for space and nutrients, mycoparasitism (coiling around and penetrating the hyphae of the pathogen), and antibiosis through the production of volatile and diffusible antifungal metabolites and cell wall-degrading enzymes such as chitinases and glucanases (Sood et al., 2020; Mukhopadhyay and Kumar, 2020; Rajani et al., 2021). In addition, *Trichoderma* colonizes roots, promotes plant growth, and induces systemic resistance in the host plant (Zin and Badaluddin, 2020; Alfiky and Weisskopf, 2021).

Several studies have shown that *Trichoderma* can suppress *Fusarium* pathogens of maize. *T. harzianum* and *T. viride* inhibited the mycelial growth of *F. verticillioides* and *F. proliferatum*, the causal agents of maize stalk rot, by 60–80% in dual culture (Yassin et al., 2021). Selected *Trichoderma* strains inhibited *F. graminearum* and *F. verticillioides* by more than 70% in vitro and reduced stalk rot in the field by over 65% (Lu et al., 2020). Native *Trichoderma* isolates from Ethiopian maize fields inhibited *F. verticillioides* by 34–78% in vitro and reduced kernel colonization and fumonisin contamination in the field (Tsehaye et al., 2026). In Indonesia, *Trichoderma* and *Gliocladium* have been used to control *Fusarium* stem rot in other crops (Pardede et al., 2022). However, the antagonistic ability of *Trichoderma* differs widely among isolates and pathogens, so each local isolate must be tested against the target pathogen before it is developed as a biological control agent (Ferreira and Musumeci, 2021; Olowe et al., 2022).

The density of the inoculum is another factor that determines the effectiveness of a biological control agent. A higher conidial density is expected to lead to faster colonization and stronger suppression of the pathogen; in chili pepper, a density of 10^7 spores mL^{-1} inhibited *F. oxysporum* more than lower densities (Nabila et al., 2021). Commercial *Trichoderma* products in Indonesia are usually required to contain at least 10^8 spores mL^{-1} (Amara et al., 2025). Whether a density of 10^8 conidia mL^{-1} is more effective than 10^7 conidia mL^{-1} against *Fusarium* from maize has not been clearly established, and the answer has practical value because lower densities reduce the cost of mass production.

The dual culture test is a simple and widely used in vitro method for the initial screening of antagonists. It allows the pathogen and the antagonist to grow on the same medium and enables measurement of the pathogen's inhibition and the growth rates of both fungi (Yassin et al., 2021; Olowe et al., 2022). Therefore, the aims of this study were (1) to determine the percentage of inhibition of *Fusarium* sp. from maize by *Trichoderma* sp. at conidial densities of 10^7 and 10^8 conidia mL^{-1} using the dual culture method, and (2) to describe the colony growth of *Trichoderma* sp. and *Fusarium* sp. during the interaction. The results provide an initial basis for developing *Trichoderma* sp. as a biological control agent for *Fusarium* stalk rot of maize.

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p-ISSN: 3124-7865

METHOD

The research was conducted from January to February 2025 at the Plant Protection Laboratory, Department of Agricultural Production, Politeknik Negeri Jember, East Java, Indonesia. All work with fungal cultures was carried out under aseptic conditions in a laminar airflow cabinet. Before use, the working surface was wiped with 70% alcohol, and all glassware and tools were sterilized by autoclaving or flaming.

The materials used were potato dextrose agar (PDA, instant medium), an isolate of *Trichoderma* sp., an isolate of *Fusarium* sp. obtained from diseased maize, sterile distilled water, 70% alcohol, tissue paper, labels, and plastic wrap. The equipment included a laminar airflow cabinet, a hot plate, an autoclave, beakers, Erlenmeyer flasks, Petri dishes (9 cm in diameter), a Bunsen burner, micropipettes, an inoculating needle, a cork borer, tweezers, a scalpel, microscope slides, a light microscope, a hemocytometer, and a ruler.

The *Fusarium* sp. isolate was obtained from the stalks of maize plants showing typical stalk rot symptoms, including brown discoloration and soft decay of the pith, collected from the sampling location. Small pieces of tissue from the margin between diseased and healthy parts were surface sterilized with 70% alcohol and 1% NaOCl, rinsed with sterile distilled water, placed on PDA, and incubated at room temperature. Fungal colonies growing from the tissue were purified by subculturing hyphal tips onto fresh PDA. The *Trichoderma* sp. isolate was obtained from the source (e.g., laboratory collection or maize rhizosphere soil) and purified in the same way. Both isolates were identified to the genus level based on macroscopic colony characteristics (color, texture, and growth pattern) and microscopic characteristics (conidiophores, phialides, conidia, and macroconidia) observed under a light microscope using an identification key. Pure cultures were maintained on PDA and subcultured 7 days before the experiment. The PDA medium was prepared by dissolving instant PDA in 1 L of distilled water, heating on a hot plate until dissolved, and sterilizing in an autoclave at 121 °C for 15 min. After cooling to about 45–50 °C, approximately 15–20 mL of medium was poured into each sterile Petri dish and allowed to solidify.

The conidial suspension of *Trichoderma* sp. was prepared from a 7-day-old culture on PDA. Sterile distilled water (10 mL) was poured onto the culture surface, and the conidia were gently scraped with a sterile spatula. The suspension was filtered through sterile cloth to remove mycelial fragments and homogenized. The conidial density was counted with a hemocytometer under a light microscope and calculated as $C = t/(n \times 0.25) \times 10^6$, where C is the conidial density (conidia mL^{-1}), t is the total number of conidia counted in the sample squares, n is the number of squares counted, and 0.25 is a correction factor for the small squares of the hemocytometer. The suspension was then diluted with sterile distilled water to obtain densities of 10^7 and 10^8 conidia mL^{-1}.

The antagonism test was arranged in a non-factorial completely randomized design (CRD) with three treatments and three replications, resulting in nine experimental units (Table 1). Each experimental unit consisted of one Petri dish. The treatments were T1 = control (*Fusarium* sp. grown alone without *Trichoderma* sp.), T2 = *Trichoderma* sp. at 10^7 conidia mL^{-1}, and T3 = *Trichoderma* sp. at 10^8 conidia mL^{-1}. The control was used to measure the pathogen's normal growth and as the reference for calculating the percentage of inhibition.

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e-ISSN: 3124-8179
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Table 1. Treatments of the dual culture antagonism test

Code	Treatment	Description
T1	Control (0)	Fusarium sp. alone, without Trichoderma sp.
T2	Trichoderma sp. 107 conidia mL−1	Fusarium sp. + Trichoderma sp.
T3	Trichoderma sp. 108 conidia mL−1	Fusarium sp. + Trichoderma sp.

Note: Each treatment was replicated three times (one Petri dish per replication; nine experimental units in total).

The antagonism test followed the dual culture method. A mycelial disc (5 mm in diameter) taken from the edge of an actively growing *Fusarium* sp. colony was placed on PDA 3 cm from the edge of the Petri dish. *Trichoderma* sp. was placed 3 cm from the opposite edge of the same dish, on the same diameter line, using the application method, e.g., a 5-mm sterile paper disc soaked in 10 µL of conidial suspension at the respective density. In the control, *Fusarium* sp. was placed in the same position, and sterile distilled water was applied at the opposite position instead of the conidial suspension. The dishes were sealed with plastic wrap, labeled, and incubated at room temperature for 72 h.

Colony growth of *Trichoderma* sp. and *Fusarium* sp. was measured with a ruler at 24, 48, and 72 hours after incubation (HAI) as the radius/diameter of each colony along the line connecting the two inoculation points (Table 2). The percentage of inhibition of radial growth (PIRG) of *Fusarium* sp. was calculated using the formula PIRG (%) = (R1 − R2)/R1 × 100, where R1 is the colony growth of *Fusarium* sp. in the control, and R2 is the colony growth of *Fusarium* sp. towards the *Trichoderma* sp. colony in the dual culture. The PIRG ranges from 0% (no inhibition) to 100% (complete inhibition). In addition, the growth increment of each colony between consecutive observations (cm per 24 h) was calculated from the mean colony growth to describe the growth dynamics of both fungi.

Table 2. Observed variables, units, time, and method of observation

Variable	Unit	Time	Method
Colony growth of Fusarium sp.	cm	24, 48, 72 HAI	Measured with a ruler
Colony growth of Trichoderma sp.	cm	24, 48, 72 HAI	Measured with a ruler
Percentage of inhibition (PIRG)	%	24, 48, 72 HAI	(R1 − R2)/R1 × 100
Growth increment	cm 24 h−1	24–48, 48–72 HAI	Difference between consecutive means

Note: HAI = hours after incubation; R1 = colony growth of Fusarium sp. in the control; R2 = colony growth of Fusarium sp. in dual culture.

The percentage of inhibition at each observation time was subjected to analysis of variance (ANOVA) according to the CRD model $Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$, where Y_{ij} is the observation for the *i*-th treatment and *j*-th replication, µ is the general mean, τ_i is the effect of the *i*-th treatment, and ε_{ij} is the experimental error. When the treatment effect was significant ($p < 0.05$), the means were compared using the least significant difference (LSD) test at the 5% level. Data were analyzed using software on a computer. Colony growth data are presented as means of three replications and were used to calculate the growth increments and the ratio of *Trichoderma* sp. to *Fusarium* sp. colony growth.

RESULTS

Trichoderma sp. inhibited the growth of *Fusarium* sp. at both conidial densities and at all observation times (Table 3). At 24 HAI, inhibition was 58.59% at 10^7 conidia mL^{-1} and 43.60% at 10^8 conidia mL^{-1}. Inhibition decreased at 48 HAI (41.15% and 30.47%, respectively) and then increased

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e-ISSN: 3124-8179
p-ISSN: 3124-7865

sharply at 72 HAI, when it reached 69.18% at 10^7 conidia mL^{-1}. According to the LSD test, the two densities shared the same letter at every observation time, which means that the difference between 10^7 and 10^8 conidia mL^{-1} was not statistically significant.

Table 3. Percentage of inhibition of Fusarium sp. by Trichoderma sp. at two conidial densities

Observation	Conidial density (conidia mL−1)	Inhibition (%)	Calculated from means (%)
24 HAI	10^7	58.59 a	59.38
	10^8	43.60 ab	46.88
48 HAI	10^7	41.15 a	40.54
	10^8	30.47 a	37.84
72 HAI	10^7	69.18 a	69.77
	10^8	104.14 a	72.09

The value reported for 10^8 conidia mL^{-1} at 72 HAI (104.14%) exceeds the theoretical maximum of the PIRG formula and therefore cannot be correct. Calculated from the mean colony growth in Table 4, the inhibition at this time was 72.09%, which is close to the value at 10^7 conidia mL^{-1} (69.77% calculated from the means). The values calculated from the means agreed closely with the replicate-based values at the other observation times (within about 1–7 percentage points), which supports the use of 72.09% as the best estimate for this treatment. Thus, at 72 HAI, both densities inhibited *Fusarium* sp. by about 70%.

Fusarium sp. grown alone (control) reached 3.2, 3.7, and 8.6 cm at 24, 48, and 72 HAI, respectively, and covered almost the entire surface of the medium by the end of the observation (Table 4; Figure 1). In dual culture, the growth of *Fusarium* sp. was strongly restricted, reaching only 1.3–1.7 cm at 24 HAI and 2.4–2.6 cm at 72 HAI. *Trichoderma* sp. grew rapidly at both densities: it reached 2.6 cm at 24 HAI, 3.6–5.1 cm at 48 HAI, and 6.8–7.7 cm at 72 HAI. At 72 HAI, the colony of *Trichoderma* sp. at 10^7 conidia mL^{-1} was the largest (7.7 cm).

Table 4. Colony growth (cm) of Trichoderma sp. and Fusarium sp. in dual culture

Observation	Treatment	Trichoderma sp. (cm)	Fusarium sp. (cm)
24 HAI	Control	–	3.2
	10^7	2.6	1.3
	10^8	2.6	1.7
48 HAI	Control	–	3.7
	10^7	3.6	2.2
	10^8	5.1	2.3
72 HAI	Control	–	8.6
	10^7	7.7	2.6
	10^8	6.8	2.4

IIAJ INTERNATIONAL INTEGRATED AGRICULTURAL JOURNAL
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e-ISSN: 3124-8179
p-ISSN: 3124-7865

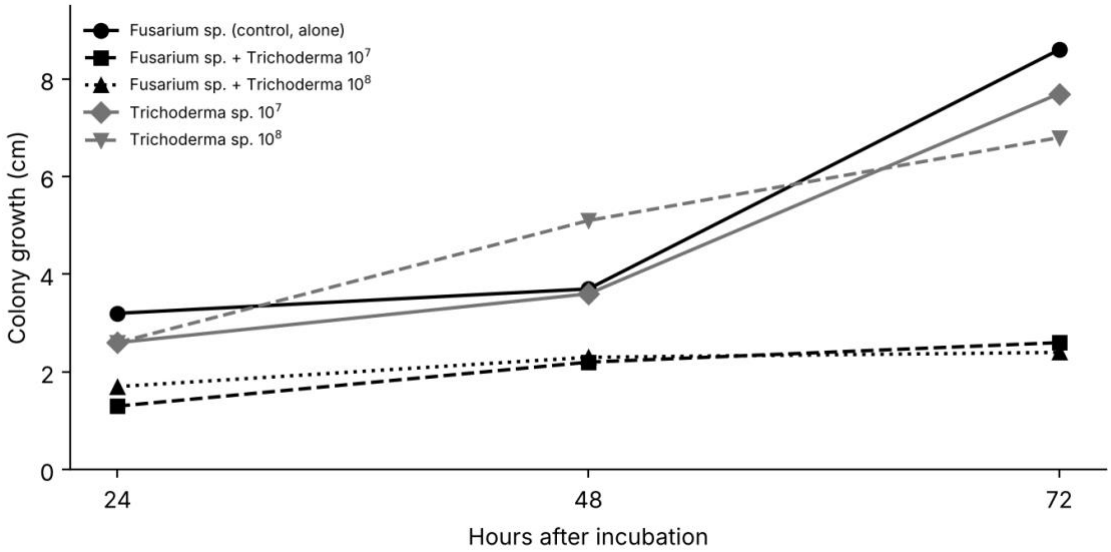


Figure 1. Colony growth of *Trichoderma* sp. and *Fusarium* sp. at 24, 48, and 72 hours after incubation (means of three replications)

The growth increments in Table 5 show the different dynamics of the two fungi. In the control, *Fusarium* sp. grew slowly between 24 and 48 HAI (0.5 cm) and then very rapidly between 48 and 72 HAI (4.9 cm). In dual culture, the increments decreased steadily and were only 0.1–0.4 cm between 48 and 72 HAI, indicating that the pathogen's growth had almost stopped. *Trichoderma* sp. continued to grow during the whole period, with increments of 1.0–2.5 cm between 24 and 48 HAI and 1.7–4.1 cm between 48 and 72 HAI. At 72 HAI, the colony of *Trichoderma* sp. was 2.8–3.0 times larger than that of *Fusarium* sp. in the same dish, and the growth of *Fusarium* sp. was reduced by 6.0 cm (10^7) to 6.2 cm (10^8) compared with the control.

Table 5. Growth increments of Trichoderma sp. and Fusarium sp. and colony ratio in dual culture

Colony	0–24 HAI	24–48 HAI	48–72 HAI	Ratio T/F at 72 HAI
Fusarium sp. (control)	3.2	0.5	4.9	–
Fusarium sp. + 10^7	1.3	0.9	0.4	–
Fusarium sp. + 10^8	1.7	0.6	0.1	–
Trichoderma sp. 10^7	2.6	1.0	4.1	2.96
Trichoderma sp. 10^8	2.6	2.5	1.7	2.83

Table 6. Summary of the antagonistic performance of *Trichoderma* sp. at 72 hours after incubation

Conidial density (conidia mL^{-1})	Inhibition (%)	Reduction of *Fusarium* sp. (cm)	*Trichoderma* sp. colony (cm)	Ratio T/F
10^7	69.18 a	6.0	7.7	2.96
10^8	72.09 a	6.2	6.8	2.83

Table 6 summarizes the antagonistic performance of *Trichoderma* sp. at the end of the observation. Both densities reduced *Fusarium* sp. colony growth by about 70% compared with the control, and both produced *Trichoderma* colonies that were almost three times larger than the pathogen colonies. The density of 10^7 conidia mL^{-1} resulted in a slightly larger *Trichoderma* colony and a slightly smaller reduction of the pathogen compared with 10^8 conidia mL^{-1}, but these differences were small and not statistically significant.

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p-ISSN: 3124-7865

DISCUSSION

This study showed that the tested *Trichoderma* sp. isolate has strong antagonistic activity against *Fusarium* sp. isolated from maize. The pathogen was inhibited at every observation time, and by 72 hours its colony had almost stopped growing, reaching only about 2.5 cm, compared with 8.6 cm in the control. The 72-hour inhibition of about 70% falls within the range reported for effective antagonists. Yassin et al. (2021) obtained 60–80% inhibition of *F. verticillioides* and *F. proliferatum* by *T. harzianum* and *T. viride*; Lu et al. (2020) selected *Trichoderma* strains that inhibited maize *Fusarium* pathogens by more than 70%, and Tsehaye et al. (2026) reported 34–78% inhibition of *F. verticillioides* by native isolates. *Trichoderma* species also showed 73–100% inhibition of mycotoxigenic *Fusarium* species from maize in Malaysia (Yazid et al., 2023). These comparisons indicate that the local isolate is a good candidate for further development.

The main mechanism suggested by the growth data is competition for space and nutrients. *Trichoderma* sp. reached 2.6 cm within 24 hours and continued to grow rapidly, whereas *Fusarium* sp. grew slowly when it shared the medium with the antagonist. Rapid growth is a key trait of *Trichoderma* as a biological control agent, as a fast-growing colony occupies the medium and takes up available nutrients before the pathogen can use them (Tyśkiewicz et al., 2022; Guzmán-Guzmán et al., 2023). In the dual culture, the pathogen therefore faced a shrinking area and a declining nutrient supply, and its growth increment fell to only 0.1–0.4 cm between 48 and 72 hours. In contrast, *Fusarium* sp. grown alone had access to the whole dish and expanded by 4.9 cm in the same period.

Competition alone, however, does not fully explain why the pathogen was inhibited as early as 24 hours, before the two colonies had yet to meet. At this time, *Fusarium* sp. in dual culture was already 47–59% smaller than in the control. This early inhibition suggests that *Trichoderma* sp. released diffusible or volatile compounds that suppressed the pathogen at a distance. *Trichoderma* species produce a wide range of antifungal secondary metabolites, including peptaibols, pyrones, and volatile organic compounds, which inhibit pathogen germination and hyphal growth (Sood et al., 2020; Rajani et al., 2021). Yassin et al. (2021) showed that culture filtrates and extracts of *T. harzianum* and *T. viride* inhibited the mycelial growth of *Fusarium* pathogens in maize, confirming that antibiosis contributes to the antagonism observed in dual culture.

After the colonies came into contact, the large size of the *Trichoderma* colony relative to the pathogen (about 3 times larger at 72 hours) indicates that *Trichoderma* sp. grew over the *Fusarium* colony. Overgrowth is typically accompanied by mycoparasitism, in which the hyphae of *Trichoderma* coil around the hyphae of the pathogen and degrade their cell walls with chitinases, glucanases, and proteases, causing the hyphae to lyse (Mukhopadhyay and Kumar, 2020; Alfiky and Weisskopf, 2021). Mycoparasitic coiling of *T. harzianum* around *Fusarium* hyphae from maize has been observed under the microscope (Yassin et al., 2021). Microscopic observation of the interaction zone was not performed in this study; therefore, the occurrence of mycoparasitism should be confirmed in future work.

The temporary decrease in inhibition at 48 hours can be explained by the control colony's growth pattern. Between 24 and 48 hours, *Fusarium* sp. grown alone increased by only 0.5 cm, so the difference between the control and the dual culture became relatively smaller. After 48 hours, the control colony expanded rapidly, whereas the pathogen in dual culture was blocked by *Trichoderma*, with inhibition increasing to about 70%. This pattern shows that the percentage of inhibition depends not only on the antagonist but also on the pathogen's growth phase, and that observations should be continued until the control colony has covered the dish to obtain a stable

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e-ISSN: 3124-8179
p-ISSN: 3124-7865

estimate of antagonism.

An important practical finding is that the two conidial densities did not differ significantly. Although the density of 10^7 conidia mL^{-1} yielded numerically higher inhibition at 24 and 48 hours and the largest *Trichoderma* colony at 72 hours, both densities resulted in about 70% inhibition at the end of the observation. Once *Trichoderma* has established a colony, its subsequent growth depends on the medium and the environment rather than on the number of conidia applied. A very high density may even increase competition among germinating conidia for the limited nutrients at the inoculation point, which could explain the slightly smaller *Trichoderma* colony at 10^8 conidia mL^{-1} at 72 hours. Similarly, Nabila et al. (2021) found that inhibition of *F. oxysporum* increased with spore density only up to 10^7 spores mL^{-1}. For practical use, this means that a density of 10^7 conidia mL^{-1} may be sufficient to achieve effective inhibition in vitro, thereby reducing the amount of inoculum required for mass production. Nevertheless, under field conditions, a higher density may still be needed to compensate for reduced viability and competition with native soil microflora (Ferreira and Musumeci, 2021; Amara et al., 2025).

The reported value of 104.14% inhibition at 10^8 conidia mL^{-1} after 72 hours warrants attention. The PIRG formula compares the pathogen's growth in dual culture with its growth in the control, so the result cannot exceed 100% unless the pathogen colony is recorded as zero or negative. A value above 100% therefore indicates an error in calculation or data entry. When recalculated from the mean colony growth, the inhibition was 72.09%, consistent with the other treatments and the colony growth data. Reporting these values accurately is important because the percentage of inhibition is the primary criterion used to select antagonists for further testing (Olowe et al., 2022).

The results of this study support the use of *Trichoderma* as part of an integrated strategy for managing *Fusarium* stalk rot of maize. Because the pathogen survives in soil and crop residues and infects the plant through the roots (Blacutt et al., 2018; Gai et al., 2018), a soil-inhabiting antagonist that grows rapidly and suppresses the pathogen early has clear advantages. *Trichoderma* can be applied as a seed treatment or soil amendment, colonize the roots, and promote plant growth and induce resistance in plants (Zin and Badaluddin, 2020; Tyśkiewicz et al., 2022). In field studies, *Trichoderma* agents reduced maize stalk rot by more than 65% and increased yield (Lu et al., 2020), and seed biopriming with native isolates reduced kernel infection and fumonisin contamination (Tsehaye et al., 2026). Such approaches are also consistent with efforts to reduce fungicide use in maize production (Thambugala et al., 2020; Asiedu and Miedaner, 2025).

Suppressing *Fusarium* growth is also important for food safety. *F. verticillioides* and related species produce fumonisins in infected stalks and grains, and the toxin level is closely related to fungal biomass (Blacutt et al., 2018). Several *Trichoderma* species not only inhibited the growth of mycotoxigenic *Fusarium* species from maize but also reduced the production of fumonisins B_1 and B_2 to very low levels in dual culture (Yazid et al., 2023), and seed biopriming with *Trichoderma* reduced fumonisin contamination of maize grain by up to about 90% in the field (Tsehaye et al., 2026). Because the *Fusarium* colony in the present study stopped growing almost completely after 48 hours, the tested isolate may also limit toxin production. However, mycotoxin production was not measured, and some interactions between antagonists and pathogens can stimulate toxin synthesis under stress, so this possibility should be tested directly (Yazid et al., 2023).

Compared with other local studies, the inhibition obtained here is relatively high for a short observation period. In Indonesian studies, *Trichoderma* isolates inhibited *F. oxysporum* by about 55–59% in dual culture (Nabila et al., 2021; Amara et al., 2025), and *Trichoderma* and *Gliocladium* suppressed *Fusarium* stem rot of vanilla (Pardede et al., 2022). The level of about 70% reached within 72 hours in the present study suggests that the isolate exhibits both rapid growth and early

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e-ISSN: 3124-8179
p-ISSN: 3124-7865

antibiosis. In practice, isolates that colonize the medium quickly and inhibit the pathogen early are preferred because, in the soil, the antagonist must establish itself on the roots before the pathogen infects the plant (Ferreira and Musumeci, 2021; Guzmán-Guzmán et al., 2023).

From an applied perspective, the results suggest a simple pathway for using the isolate. *Trichoderma* can be mass-produced cheaply on local substrates such as rice or maize grain, harvested as a conidial suspension, and applied to maize seed or to the planting hole at sowing, so that the antagonist is already established when the roots begin to grow (Zin and Badaluddin, 2020; Ferreira and Musumeci, 2021). Because 10^7 conidia mL^{-1} was as effective as 10^8 conidia mL^{-1} in vitro, the lower density can be used as a starting point for dose–response trials in pots, which will show whether the advantage of a lower inoculum also holds in soil. Combining *Trichoderma* with other practices, such as crop rotation, removal of infected residues, and balanced potassium fertilization, is likely to give more stable control of stalk rot than any single method (Asiedu and Miedaner, 2025).

This study has several limitations. The isolates were identified only to the genus level, only one isolate of each fungus was tested, and the observation period was limited to 72 hours. In addition, the dual culture test measures antagonism on an artificial medium, and the results may differ in soil or in plants. Future research should identify the isolates to the species level using molecular methods, observe the interaction zone microscopically, test the antifungal activity of culture filtrates and volatile compounds, and evaluate the effectiveness of the *Trichoderma* isolate in reducing stalk rot in greenhouse and field experiments.

CONCLUSION

Trichoderma sp. effectively inhibited *Fusarium* sp. from maize in vitro, with inhibition increasing to about 70% at 72 hours after incubation (69.18% at 10^7 and 72.09% at 10^8 conidia mL^{-1}), and the two conidial densities did not differ significantly. *Trichoderma* sp. grew faster than the pathogen, reaching 7.7 cm (10^7) and 6.8 cm (10^8) at 72 hours, while *Fusarium* sp. was restricted to 2.4–2.6 cm compared with 8.6 cm in the control. These results show that *Trichoderma* sp. has good potential as a biological control agent for *Fusarium* stalk rot of maize, and that a density of 10^7 conidia mL^{-1} is sufficient for further greenhouse and field evaluation.

ACKNOWLEDGEMENT

The authors thank the Plant Protection Laboratory, Department of Agricultural Production, Politeknik Negeri Jember, for providing the facilities used in this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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