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In vitro growth assessment of *Trichoderma* spp. from paddy rhizosphere on glyphosate- and fipronil- containing medium

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Abstract. *Simamora AV, Nenotek PS, Hahuly MV, Ishaq LF, Widinugraheni S, Kana YR, Pasi SCU, Ola ARB, Hosang EY, Fitriadi BR. 2025. In vitro growth assessment of* Trichoderma *spp. from paddy rhizosphere on glyphosate- and fipronil- containing medium. Biodiversitas 26: 2028-2038.* Agricultural practices worldwide increasingly rely on synthetic pesticides to enhance crop productivity and manage pest infestations. Glyphosate, a widely used herbicide, and fipronil, a broad-spectrum insecticide, are extensively applied due to their effectiveness. However, prolonged use of these agrochemicals raises significant environmental concerns, particularly soil contamination, which disrupts microbial diversity and degrades soil health. The persistence of glyphosate and fipronil in soil ecosystems challenges sustainable agriculture, necessitating the exploration of bioremediation strategies. Research on the ability of *Trichoderma* spp. to degrade these pesticides remains limited, highlighting the need for this study. This research aimed to (i) isolate *Trichoderma* spp. from paddy fields with frequent glyphosate and fipronil application; (ii) assess their growth on PDA media supplemented with glyphosate and fipronil; and (iii) evaluate their in vitro potential for pesticide degradation. Growth assessments were conducted on PDA media supplemented with glyphosate at 0 mL/L (control), 5.0 mL/L (recommended concentration), and 12.5 mL/L, and with fipronil at 0 mL/L (control), 2.0 mL/L (recommended concentration), and 4.0 mL/L. The degradation potential was evaluated using a modified dual-culture method with *Fusarium oxysporum* as the indicator pathogen. The results showed four *Trichoderma* isolates were obtained, namely *T. harzianum* 01, *T. camerunense* 02, *T. harzianum* 03, and *Trichoderma* 04. Growth analysis indicated that *T. harzianum* 01 had the highest colony diameter (9.0 cm) and exhibited complete resistance at recommended glyphosate and fipronil concentrations, while *T. camerunense* 02 was the most sensitive. Relative inhibition rate analysis confirmed that glyphosate had a stronger inhibitory effect than fipronil. Antagonism tests revealed that all isolates suppressed *Fusarium oxysporum*, with inhibition rates ranging from 70.59 to 83.96%, indicating strong biocontrol potential. These findings demonstrate the resilience of *Trichoderma* spp. under pesticide exposure and their capacity to inhibit phytopathogens, supporting their potential as bioremediation and biological control agents for sustainable soil management.

Keywords: Bioremediation, biodegradation, dual culture, *Fusarium oxysporum*, mycoparasitism, *Trichoderma*

INTRODUCTION

Pesticides have become essential tools in modern agriculture, helping farmers effectively manage weeds and insect pests while significantly improving crop yields. During the 20th century, pesticide used played a major role in increasing global food production to meet the demands of a rapidly growing population. Currently, approximately one-third of global agricultural output relies on pesticides, preventing yield losses of up to 78% in fruits, 54% in vegetables, and 32% in cereals (Tudi et al. 2021). As the global population is estimated to reach 8.5 billion by 2030 and 9.7 billion by 2050, peaking at around 10.4 billion in the 2080s—ensuring food security remains a critical challenge. While advancements in genetics, biotechnology, and agricultural practices have boosted productivity, pesticide use has also increased to support this demand (Khan et al. 2021; Sălceanu et al. 2022).

However, the large and frequent application of pesticides raises serious concerns regarding environmental sustainability, soil health, and human well-being. In Indonesia, glyphosate and fipronil are among the most commonly used pesticides, supported by national data and field observations (Effendy et al. 2021; Mordor Intelligence 2025). Glyphosate, a broad-spectrum herbicide, is prevalent in oil palm plantations, accounting for more than 60% of its national usage (Brookes 2019), but it is also frequently used by rice farmers, especially during land preparation and fallow periods. Fipronil is widely used by rice farmers—often under commercial names like “Regent” to control insect pests. Field surveys in the study area confirmed that both glyphosate and fipronil are regularly applied by local farmers.

Frequent use of these pesticides has raised concerns about their accumulation in soils and long-term impacts on soil biology. Studies have shown that glyphosate and fipronil can alter microbial communities, reduce biodiversity, and contribute to soil degradation (Singh et al. 2020; Prado et al. 2023). In Indonesia, residues have been detected not

only in agricultural soils but also in nearby aquatic environments (Hendriadi et al. 2020; Effendy et al. 2021), highlighting the urgent need for sustainable strategies to mitigate pesticide contamination. Several remediation techniques have been explored, including physical removal, chemical treatments, and phytoremediation. However, such approaches often have limitations, including high costs, reduced effectiveness in complex soil systems, and potential risks to non-target organisms. In contrast, mycoremediation—a biological method using fungi to degrade or transform contaminants—has emerged as an eco-friendly alternative (Vaksmaa et al. 2023; Pujiati et al. 2024; Swathy et al. 2024).

Various fungal genera have shown potential for mycoremediation, including *Aspergillus*, *Penicillium*, *Fusarium*, and white-rot fungi such as *Phanerochaete chrysosporium* (Bogale 2020; El Sayed and El-Sayed 2020; Gugel et al. 2024; Oladipo and Salami 2024). Among these, *Trichoderma* spp. stand out due to their multiple functional roles. Besides their effectiveness as biocontrol agents, *Trichoderma* are widely distributed in Indonesian agricultural soils—including paddy fields—and are already utilized by farmers to enhance plant health and suppress disease. Certain strains have been shown to tolerate and degrade glyphosate, using it as a carbon source and contributing to the restoration of soil microbial balance (Arfarita et al. 2016; Spinelli et al. 2021; Conte et al. 2025).

Although research on fipronil degradation by *Trichoderma* remains limited, emerging studies suggest that it may influence fungal community composition and that some *Trichoderma* strains can tolerate fipronil-contaminated soils (Budd et al. 2015; Ramirez-Olier et al. 2021; Soesanto et al. 2022; Tall and Puigbò 2022). Due to their adaptability and multifunctionality, *Trichoderma* spp. are promising candidates for bioremediation in pesticide-impacted environments, especially in tropical agricultural systems like those found in Indonesia.

Although *Trichoderma* spp. are primarily aerobic fungi, they are frequently isolated from paddy fields (Saleh and El-Akshar 2020; Jena et al. 2023; Akbari et al. 2024), where soil conditions alternate between aerobic and anaerobic phases due to regular flooding and drainage cycles. Oxygen remains available in surface layers and near the roots, enabling their survival. However, data on the response of *Trichoderma* species to glyphosate and fipronil in paddy soil are still limited. Understanding their growth in pesticide-containing media under controlled conditions is essential for evaluating their tolerance and potential for bioremediation. Therefore, this study aimed to investigate the in vitro growth of *Trichoderma* spp. isolated from pesticide-exposed paddy rhizosphere and to evaluate their antagonistic activity against *Fusarium oxysporum.*

MATERIALS AND METHODS

Soil sampling

Soil samples were collected from the rhizosphere of 75-day-old lowland paddy plants. At the time of sampling, the soil was moderately moist but not waterlogged. Prior to sample collection, a structured field survey was conducted to document the history of pesticide use. The survey included direct interviews with local farmers using a standardized questionnaire designed to gather information on pesticide types, frequency, and duration of use. Based on the survey results, selected farmer reported consistent application of glyphosate and fipronil over the past three years. This information was used as a criterion for selecting the sampling site to study the interaction between long-term pesticide exposure and *Trichoderma* spp. in paddy soils. The sampling site was located in Oesao Village, East Kupang District, at coordinates (Lat: -10.104337°, Long: 123.825951°) and an elevation of 24 meters above sea level (Figure 1).

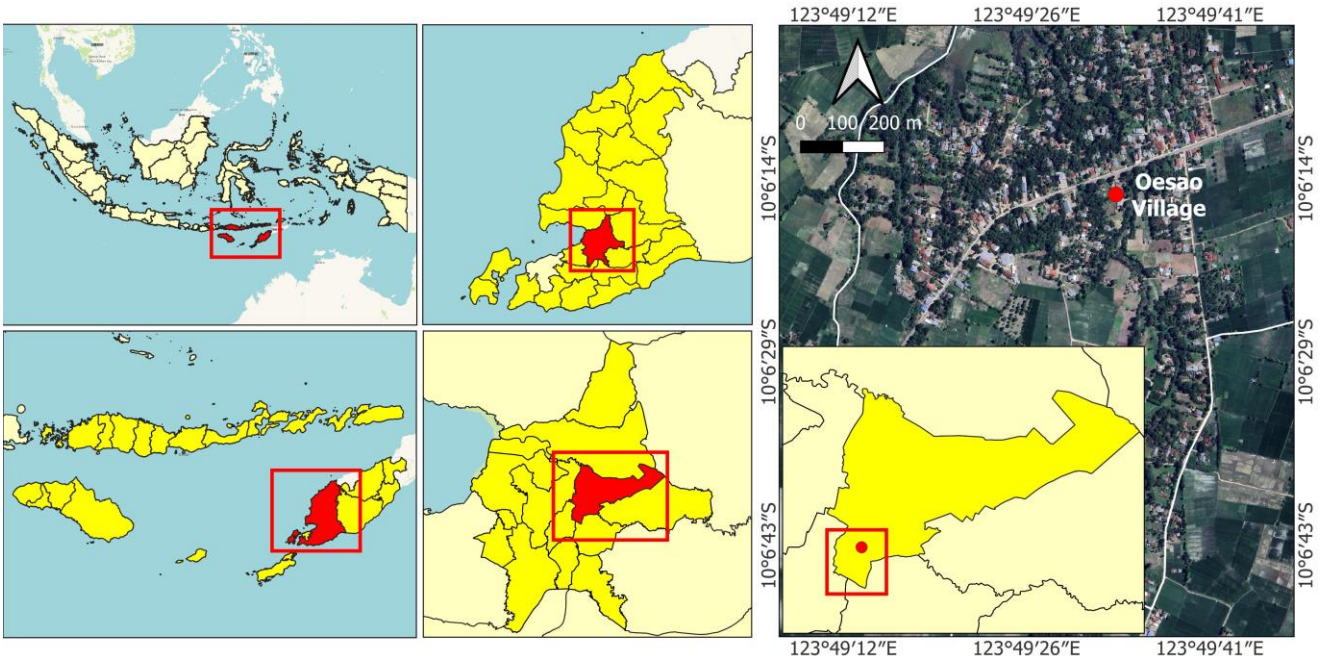

Figure 1. Map of sampling location. Red dot on the island showing the sampling site in Oesao Village, East Kupang Sub-district, Kupang, East Nusa Tenggara, Indonesia

A random sampling method was employed across an 8 acre (800 m²) lowland paddy field. Five sampling plots (5 × 5 m each) were selected—four near the corners of field and one at the center. Soil samples were collected at a depth of 0-15 cm near the paddy roots, with three replications per point. The collected soil was pooled into a basin, thoroughly homogenized, and placed in clean plastic bags for transport. Samples were stored in an icebox to preserve moisture and microbial viability and transported to the laboratory for further analysis.

Soil fertility analysis indicated moderate total nitrogen content (0.25-0.30%), high available phosphorus (80.22-92.13 ppm P_2O_5), and sufficient potassium (1.11-1.16 me/100 g). Organic carbon content was 2.89%, with a neutral pH (7.56), high Cation Exchange Capacity (CEC) of 41.20 me/100 g , and base saturation of 84.58%. These soil properties support a favorable environment for microbial activity, including the growth and function of *Trichoderma* spp.

Procedures

Isolation and morphological identification of Trichoderma *spp.*

Isolation of *Trichoderma* spp. from the rhizosphere of paddy was conducted using the direct plating method following serial dilution (Mishra et al. 2019). A 10 g soil sample was suspended in 100 mL of sterile distilled water and thoroughly homogenized. Before the soil settled, 1 mL of the suspension was aseptically transferred using a sterile pipette into 9 mL of distilled water. This serial dilution process was repeated three times to obtain dilution levels ranging from 10^{-1} to 10^{-5} g/mL. From each dilution, 100 µL of the suspension was transferred to Potato Dextrose Agar (PDA) using the pour plate technique. The plates were then incubated at 26°C until fungal colonies emerged. Developing colonies with morphological characteristics of *Trichoderma* were sub-cultured onto fresh PDA plates for purification. The isolates were further purified on PDA medium supplemented with chloramphenicol and incubated at room temperature for 7 to 14 days.

Following incubation, both macroscopic and microscopic characteristics of the isolates were examined for identification, adhering to the guidelines provided by Barnett and Hunter (1972) and Watanabe (2010). Macroscopic identification included observations of colony morphology, such as shape, color, and margin characteristics on PDA medium in Petri dishes

After seven days of incubation, fungal structures were examined under a compound microscope at 400× magnification to observe the morphology of conidiophores and conidia. For microscopic identification, a small portion of a single colony was transferred onto a glass slide using a sterile inoculation needle, along with a small amount of PDA medium. The sample was then stained with lactophenol cotton blue, covered with a cover slip, and gently pressed to ensure even spreading before observation under the microscope.

Molecular identification of Trichoderma *spp.*

Molecular identification of *Trichoderma* spp. was performed through a series of steps to ensure accurate species determination. DNA extraction was conducted using the Quick-DNA Magbead Plus Kit (Zymo Research, D4082). The ITS region was amplified using universal primers ITS1 and ITS4, following the method of White et al. (1990). DNA amplification was carried out using the MyTaq HS Red Mix, 2X kit (Bioline, BIO-25048). The amplified ITS gene products were subjected to electrophoresis. Bidirectional sequencing was performed using the Sanger DNA sequencing method with capillary electrophoresis (subcontracted to 1st BASE). Bioinformatics analysis of the sequencing results was then performed.

Preparation of test pesticides

The commercial herbicide Roundup 486 SL (active ingredient isopropylamine salt of glyphosate, 486 g/L) and the insecticide Penalty 50 SC (active ingredient: fipronil, 50 g/L) were procured from the local market and used throughout the study. The tested pesticides were applied at three recommended dosages, including a control, for laboratory experiments.

Pesticide application on PDA medium

For this, poisoned food technique was used to evaluate the potency of *Trichoderma* isolates treated with different concentrations of glyphosate and fipronil. The primary objective was to assess the sensitivity of *Trichoderma* sp. to these pesticides by measuring colony growth and calculating the Relative Inhibition Rate (RIR). Glyphosate was tested at three levels: 5.0 mL/L (recommended concentration), 12.5 mL/L, and a control (without glyphosate). Similarly, fipronil was tested at 2.0 mL/L (recommended concentration), 4.0 mL/L, and a control (without fipronil). Each treatment was replicated three times.

In vitro antagonism test of Trichoderma *spp. against* Fusarium oxysporum

Fusarium oxysporum used in this study was sourced from the Plant Disease Laboratory of Universitas Nusa Cendana. The isolate was originally obtained from infected rice plants, and was stored in PDA medium at room temperature for about a month. Before being used in the antagonism test, the culture was reactivated by sub-culturing it onto fresh PDA to ensure healthy and active growth. *Trichoderma* sp., which were tested for growth on glyphosate and fipronil, was then evaluated for its antagonistic ability to inhibit *F. oxysporum.* The ability of *Trichoderma* spp. to inhibit *F. oxysporum* was tested using the dual culture method on PDA medium. Inocula of *Trichoderma* spp. and *F. oxysporum* (each 0.5 cm in diameter and seven days old) were placed 3 cm from the edge of the Petri dish, with a 2 cm gap between the two inocula (Simamora et al. 2024). Each treatment was performed in triplicate, following a completely randomized design. Colony diameters of both *Trichoderma* sp. and *F. oxysporum* were measured, and the percentage of inhibition

of *Trichoderma* sp. against *F. oxysporum* was calculated. The percentage inhibition of *Fusarium oxysporum* by *Trichoderma* spp. was calculated using the following formula:

$IP=(r_1-r_2)\ r_1^{-1}\times 100\%$

Where:

r_1 : The radial growth of *F. oxysporum* from the point of inoculation toward the edge of Petri dish (in the absence of *Trichoderma*), and

r_2 : The radial growth of *F. oxysporum* from the point of inoculation to the point of interaction with *Trichoderma*.

Observation parameters

The observed parameters included colony diameter, growth inhibition rate, and mechanisms of inhibition. Sensitivity classification of *Trichoderma* sp. was based on its response to pesticide exposure, following the criteria set by Dalimunthe et al. (2015):

1. Highly sensitive (>90% inhibition)
2. Sensitive (>75-90% inhibition)
3. Moderately resistant (>60-75% inhibition)
4. Resistant (>40-60% inhibition)
5. Highly resistant (<40% inhibition)

Data analysis

Colony diameter and inhibition rate data were analyzed using Variance Analysis (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level.

RESULTS AND DISCUSSION

Isolation and morphological identification of *Trichoderma* spp.

Based on macroscopic and microscopic identification, a total of four *Trichoderma* spp. isolates were obtained from the rhizosphere of paddy plants (Table 1; Figure 2).

Molecular identification of *Trichoderma* spp.

Molecular identification based on ITS region analysis revealed that isolates *Trichoderma* 01 and *Trichoderma* 03 were closely related to *Trichoderma harzianum*, while *Trichoderma* 02 showed more similarity to *T. camerunense*. Notably, *Trichoderma* isolate 04 was clearly different from the other isolates. BLAST analysis of the ITS sequence from *Trichoderma* 01 showed 100% identity and query coverage with *T. harzianum* isolate M3951 (GenBank accession: MK738149.1), as well as with several other closely related sequences (e.g., MK870875.1, MH153633.1), confirming its identification as *T. harzianum*. Similarly, *Trichoderma* 03 displayed 100% identity with the same *T. harzianum* isolate and 99.8-100% identity with several additional *T. harzianum* and *Trichoderma* sp. sequences (e.g., MK120584.1, MK870785.1), further supporting its classification as *T. harzianum*. The electrophoresis image of the amplification products is shown in Figure 3, the sequence assembly results of the PCR products are presented in Table 2, and the phylogenetic trees are illustrated in Figure 4.

Growth of *Trichoderma* spp. on media containing glyphosate and fipronil

Colony diameter of Trichoderma *spp.*

The impact of glyphosate and fipronil on the colony diameter growth of different *Trichoderma* species after seven days of incubation is presented in Table 3. The test results also demonstrate that all four *Trichoderma* spp. isolates successfully persisted in media containing the pesticides glyphosate and fipronil (Figure 5).

Relative inhibition rate of Trichoderma *spp.*

Analysis of variance showed that different dosage of glyphosate and fipronil significantly affected the relative inhibition rate of *Trichoderma* spp. as shown in Table 4.

In vitro antagonism test of Trichoderma *spp. against* Fusarium oxysporum

The level of inhibition exerted by *Trichoderma* spp. against *F. oxysporum* exhibited significant differences. According to the 5% DMRT results, *T. harzianum* 01 showed greater efficacy, resulting in the highest percentage of inhibition against *F. oxysporum* (Table 5). The lowest inhibition percentage was obtained by *Trichoderma* 04, but in general, all *Trichoderma* species tested had very high antagonistic abilities (above 70%) against *F. oxysporum*. The macroscopic and microscopic visualization of *Fusarium oxysporum* inhibition by *Trichoderma* spp. is presented in Figures 5, 6, and 7.

Table 1. Macroscopic and microscopic characteristic of *Trichoderma* spp. isolated from the rhizosphere of paddy

Isolates code	Macroscopic characteristics	Microscopic characteristics	Conidia size (µm)
Trichoderma 01	White hyphae turning light green, circular colony with green rings, cotton-like texture, full Petri dish coverage by day 5	Hyaline conidiophores, oval conidia, short, thick phialides	1.64-3.40
Trichoderma 02	White to green mycelium; circular with 4 concentric rings; full coverage by day 5	Grape-like round conidia; hyaline, long conidiophores; short phialides	1.71-3.76
Trichoderma 03	White hyphae turning dark green with white edges; circular with central ring; cotton-like texture. Complete radial growth was observed by the fifth day of incubation.	Oval, slightly elongated conidia; thick hyaline conidiophores; short, thick phialides	0.89-1.87
Trichoderma 04	Cotton-like texture; white to green by day 3, then dark green; circular ring-like colony; full coverage by day 5	Short, branched thick conidiophores; oval conidia; short, erect phialides	0.55-0.88

Table 2. Sequence assembly results

***Trichoderma* 01**	
Sequence Assembly 600bp	
1	GAACCTGCGG AGGGATCATT ACCGAGTTTA CAACTCCCAA ACCCAATGTG AACGTTACCA
61	AACTGTTGCC TCGGCGGGAT CTCTGCCCCG GGTGCGTCGC AGCCCCGGAC CAAGGCGCCC
121	GCCGGAGGAC CAACCAAAAC TCTTATTGTA TACCCCCTCG CGGGTTTTTT TATAATCTGA
181	GCCTTCTCGG CGCCTCTCGT AGGCGTTTCG AAAATGAATC AAAACTTTCA ACAACGGATC
241	TCTTGGTTCT GGCATCGATG AAGAACGCAG CGAAATGCGA TAAGTAATGT GAATTGCAGA
301	ATTCAGTGAA TCATCGAATC TTTGAACGCA CATTGCGCCC GCCAGTATTC TGGCGGGCAT
361	GCCTGTCCGA GCGTCATTTC AACCCTCGAA CCCCTCCGGG GGGTCGGCGT TGGGGATCGG
421	CCCTCCCTTA GCGGGTGGCC GTCTCCGAAA TACAGTGGCG GTCTCGCCGC AGCCTCTCCT
481	GCGCAGTAGT TTGCACACTC GCATCGGGAG CGCGGCGCGT CCACAGCCGT TAAACACCCA
541	ACTTCTGAAA TGTTGACCTC GGATCAGGTA GGAATACCCG CTGAACTTAA GCATATCAAT
***Trichoderma* 02**	
Sequence Assembly 603bp	
1	GCGGAGGGAT CATTACCGAG TTTACAACTC CCAAACCCAA TGTGAACGTT ACCAAACTGT
61	TGCCTCGGCG GGATCTCTGC CCCGGGTGCG TCGCAGCCCC GGACCAAGGC GCCCGCCGGA
121	GGACCAACCA AAACTCTTTT TGTATACCCC CTCGCGGGTT TTTTATAATC TGAGCCTTCT
181	CGGCGCCTCT CGTAGGCGTT TCGAAAATGA ATCAAAACTT TCAACAACGG ATCTCTTGGT
241	TCTGGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA TGTGAATTGC AGAATTCAGT
301	GAATCATCGA ATCTTTGAAC GCACATTGCG CCCGCCAGTA TTCTGGCGGG CATGCCTGTC
361	CGAGCGTCAT TTCAACCCTC GAACCCCTCC GGGGGGTCGG CGTTGGGGAT CGGCCCTCCC
421	TTAGCGGGTG GCCGTCTCCG AAATACAGTG GCGGTCTCGC CGCAGCCTCT CCTGCGCAGT
481	AGTTTGCACA CTCGCATCGG GAGCGCGGCG CGTCCACAGC CGTTAAACAC CCAACTTCTG
541	AAATGTTGAC CTCGGATCAG GTAGGAATAC CCGCTGAACT TAAGCATATC AATAAGCCGG
601	AGG
***Trichoderma* 03**	
Sequence assembly 605bp	
1	GAACCTGCGG AGGGATCATT ACCGAGTTTA CAACTCCCAA ACCCAATGTG AACGTTACCA
61	AACTGTTGCC TCGGCGGGAT CTCTGCCCCG GGTGCGTCGC AGCCCCGGAC CAAGGCGCCC
121	GCCGGAGGAC CAACCAAAAC TCTTATTGTA TACCCCCTCG CGGGTTTTTT TATAATCTGA
181	GCCTTCTCGG CGCCTCTCGT AGGCGTTTCG AAAATGAATC AAAACTTTCA ACAACGGATC
241	TCTTGGTTCT GGCATCGATG AAGAACGCAG CGAAATGCGA TAAGTAATGT GAATTGCAGA
301	ATTCAGTGAA TCATCGAATC TTTGAACGCA CATTGCGCCC GCCAGTATTC TGGCGGGCAT
361	GCCTGTCCGA GCGTCATTTC AACCCTCGAA CCCCTCCGGG GGGTCGGCGT TGGGGATCGG
421	CCCTCCCTTA GCGGGTGGCC GTCTCCGAAA TACAGTGGCG GTCTCGCCGC AGCCTCTCCT
481	GCGCAGTAGT TTGCACACTC GCATCGGGAG CGCGGCGCGT CCACAGCCGT TAAACACCCA
541	ACTTCTGAAA TGTTGACCTC GGATCAGGTA GGAATACCCG CTGAACTTAA GCATATCAAT
601	AGGCC
***Trichoderma* 04**	
Sequence assembly 613bp	
1	AAACTCGGTA ATGATCCTTC CGTAGGGGGA CCTGCGGAGG GATCATTACC GAGTTTACAA
61	CTCCCAAACC CAATGTGAAC GTTACCAAAC TGTTGCCTCG GCGGGGTCAC GCCCCGGGTG
121	CTCCCAAACC CAATGTGAAC GTTACCAAAC TGTTGCCTCG GCGGGGTCAC GCCCCGGGTG
181	CCTCGCGGAC GTATTTCTTA CAGCTCTGAG CAAAAATTCA AAATGAATCA AAACTTTCAA
241	CAACGGATCT CTTGGTTCTG GCATCGATGA AGAACGCAGC GAAATGCGAT AAGTAATGTG
301	AATTGCAGAA TTCAGTGAAT CATCGAATCT TTGAACGCAC ATTGCGCCCG CCAGTATTCT
361	GGCGGGCATG CCTGTCCGAG CGTCATTTCA ACCCTCGAAC CCCTCCGGGG GATCGGCGTT
421	GGGGATCGGG ACCCCTCACA CGGGTGCCGG CCCCGAAATA CAGTGGCGGT CTCGCCGCAG
481	CCTCTCCTGC GCAGTAGTTT GCACAACTCG CACCGGGAGC GCGGCGCGTC CACGTCCGTA
541	AAACACCCAA CTTTCTGAAA TGTTGACCTC GGATCAGGTA GGAATACCCG CTGAACTTAA
601	GCATATCAAT AGG

Table 3. Effects of pesticides on the colony diameter growth of *Trichoderma* spp. (cm) on seventh day post-inoculation in PDA media containing glyphosate and fipronil

Treatments	Glyphosate dosage (mL/L)			Fipronil dosage (mL/L)		
	0	**5.0**	**12.5**	**0**	**2.0**	**4.0**
T. harzianum 01	9^{b}	9^{b}	6.40^{c}	9^{a}	9^{a}	9^{a}
T. camerunense 02	9^{b}	7.17^{a}	2.30^{a}	9^{b}	6.97^{a}	8.23^{b}
T. harzianum 03	9^{b}	6.60^{a}	3.27^{a}	9^{a}	8.30^{a}	8.13^{a}
Trichoderma 04	9^{b}	7.03^{a}	6.20^{a}	9^{b}	6.60^{a}	5.77^{a}

Note: Values in the same row followed by the same letter are not significantly different according to the 5% DMRT test

Table 4. Relative inhibition rate of *Trichoderma* spp. under glyphosate and fipronil treatments

Treatments	Glyphosate dossage (mL/L)		Fipronil dosage (mL/L)	
	5.0	**12.5**	**2.0**	**4.0**
T. harzianum 01	0.00^{a}	28.89^{a}	0.00^{a}	0.00^{a}
T. camerunense 02	20.37^{ab}	74.44^{b}	22.59^{bc}	8.52^{ab}
T. harzianum 03	26.67^{b}	63.70^{b}	7.78^{ab}	9.63^{ab}
Trichoderma 04	21.85^{ab}	31.11^{a}	26.67^{c}	35.93^{b}

Note: Values in the same row followed by the same letter are not significantly different according to the 5% DMRT test

Discussion

Trichoderma species are widely recognized for their ecological versatility and beneficial roles in a variety of ecosystems. In agriculture, they are particularly valued for their ability to suppress soil-borne pathogens, promote plant growth, and support soil health. In this study, four *Trichoderma* isolates were obtained from the rhizosphere of paddy plants in Kupang, East Nusa Tenggara: *T. harzianum* 01, *T. camerunense* 02, *T. harzianum* 03, and an unidentified isolate referred to as *Trichoderma* 04. These findings are especially relevant in the context of tropical agriculture, where *Trichoderma* spp. are commonly applied as biocontrol agents.

Among the identified isolates, *T. harzianum* is one of the most widely studied and commonly reported species within the genus. It is known for its strong antifungal activity and effectiveness in suppressing a broad range of soil-borne plant pathogens (Chaverri et al. 2015; Bunbury-Blanchette and Walker 2019; Patkowska et al. 2020). *Trichoderma harzianum* has been isolated from various substrates, including soil, plant tissues, and mushrooms, highlighting its cosmopolitan distribution (Chaverri et al. 2015; Jaklitsch and Voglmayr 2015; Innocenti et al. 2019). In addition to its biocontrol potential, this species may also aid in the degradation of agrochemicals. Szpyrka et al. (2020) demonstrated that *T. harzianum* Rifai T-22 accelerated the breakdown of herbicides such as clomazone, fluazifop-P-butyl, and metribuzin in soils rich in organic matter, indicating its potential for reducing pesticide residues.

The detection of both *T. harzianum* and *T. camerunense* in paddy soils further highlights the ecological adaptability of *Trichoderma* species to tropical environments. The frequent occurrence of *T. harzianum* in rice fields aligns with its well-established role in rice-based agroecosystems, where it contributes to disease suppression and improved soil health (Chowdhury et al. 2024). In contrast, *T. camerunense* is less frequently reported, making its presence in this study particularly noteworthy. Martinho et al. (2019) isolated *T. camerunense* from mangrove forests in southeastern Brazil and reported its ability to produce hydrolytic enzymes and biosurfactants. In addition, this species showed emulsifying and surface-active (tensiometric) properties, as well as strong cellulolytic and xylanolytic enzyme activity—traits that enhance its survival in diverse or stressful environments and suggest its potential for biotechnological applications. Its occurrence in Indonesian paddy soils suggests a possible role in maintaining microbial balance or suppressing soil-borne pathogens, although its specific ecological function remains to be further explored.

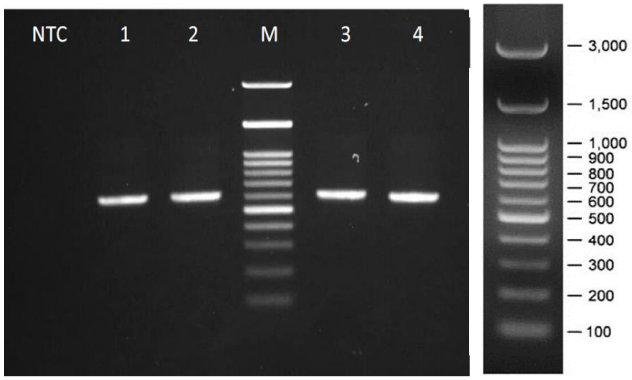

Figure 3. Electrophoresis result of amplification products. Note: 1 µL of the amplification product was visualized on a 1% agarose gel in TBE buffer. M: 100 bp DNA ladder (in 2.5 µL); NTC: Negative Amplification Control. The sample code corresponds to the serial number in the DNA Quantification Results table

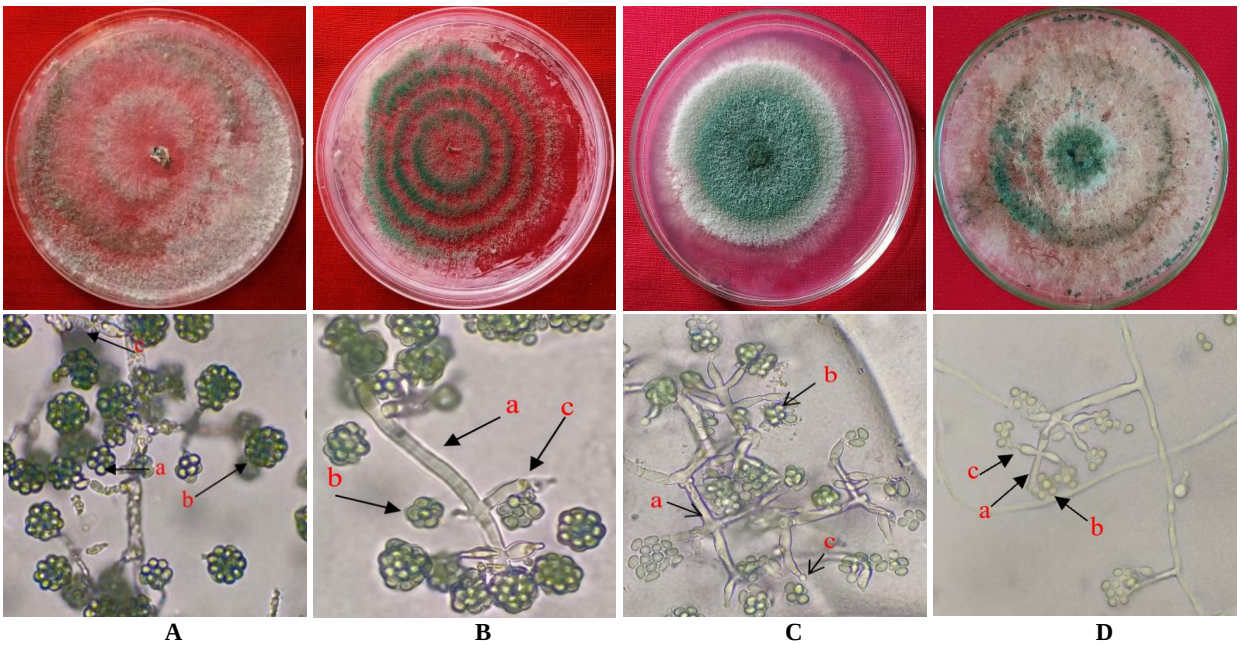

Figure 2. Macroscopic and microscopic appearance of *Trichoderma* spp. A. *Trichoderma* 01; B. *Trichoderma* 02; C. *Trichoderma* 03; D. *Trichoderma* 04. The first row displays seven-day-old pure cultures of *Trichoderma* spp. on PDA media, while the second row illustrates microscopic characteristics: a. Conidiophore; b. Conidia; and c. Phialide

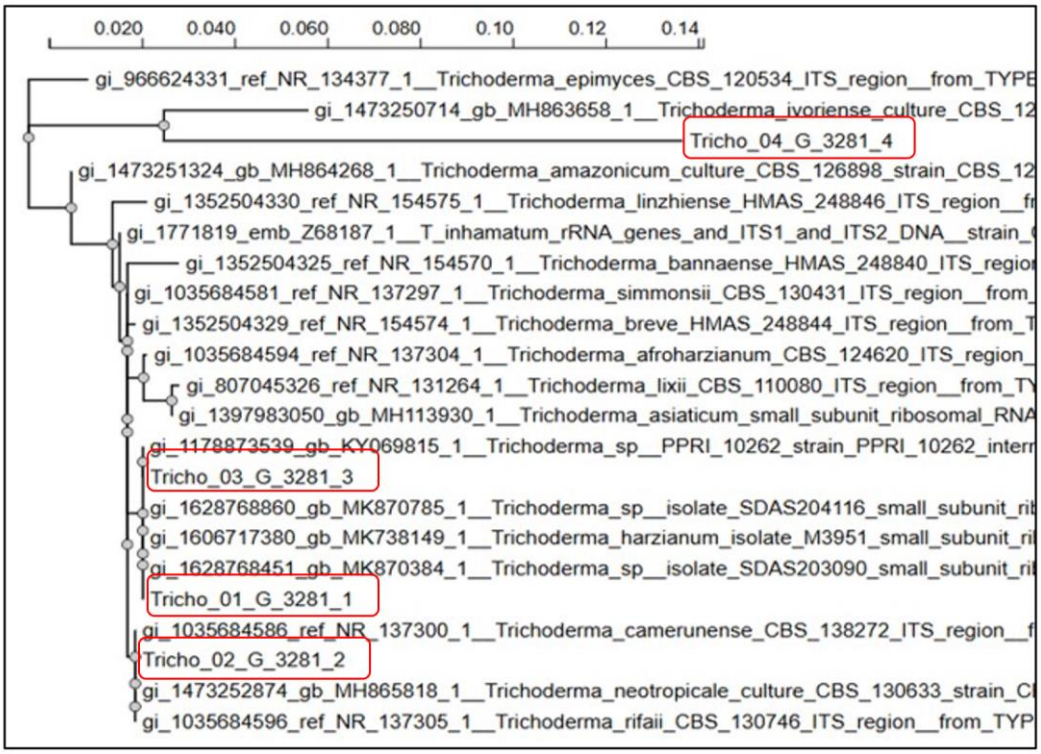

Figure 4. The phylogenetic tree of four *Trichoderma* isolates, designated as Tricho_01 to Tricho_04. The tree was generated and visualized using the PhyML Newick Tree format, employing the PhyML/One Click workflow mode available on NGPPhylogeny (https://ngphylogeny.fr/workflows/oneclick)

Taxonomically, *T. camerunense* belongs to the *T. harzianum* species complex and was first described from soil samples in Cameroon. It is characterized by relatively small phialides and the absence of sexual reproductive structures such as stromata (Bissett et al. 2015; Chaverri et al. 2015; Jambhulkar et al. 2024). Although it has not previously been reported in Indonesia, its presence here is plausible given the country's rich microbial biodiversity and the widespread distribution of *Trichoderma* species in tropical agricultural systems.

In addition to the identified species, *Trichoderma* 04 exhibited distinct morphological and cultural characteristics, suggesting it may represent a novel or uncharacterized species within the genus. To confirm its identity, advanced molecular analysis, such as multilocus phylogenetic analysis or whole-genome sequencing, are recommended. Further investigation into its biological activity including biocontrol potential, enzymatic profiles, and secondary metabolite production—could reveal novel traits of agricultural relevance. The isolate's ability to grow on pesticide-containing media also suggests potential for use in mycoremediation or the degradation of agrochemical residues in contaminated soils.

Under control conditions (0 mL/L glyphosate and fipronil), all *Trichoderma* isolates exhibited maximum colony growth (9 cm). However, increasing pesticide concentrations led to varied responses among species. *Trichoderma harzianum* 01 showed a significant reduction in growth at 12.5 mL/L glyphosate (6.40 cm), while *T. camerunense* 02 was more sensitive, with colony diameter decreasing to 2.30 cm at the same concentration. Similarly, *T. harzianum* 03 and *Trichoderma* 04 displayed reduced growth at higher glyphosate levels, shrinking to 3.27 cm and 6.20 cm, respectively. These results suggest that *T. camerunense* is the most sensitive to glyphosate exposure among the isolates.

In contrast, fipronil had a less pronounced effect on colony diameter. *Trichoderma harzianum* 01 maintained full growth across all fipronil treatments, indicating strong tolerance. *Trichoderma camerunense* 02 showed a decrease in growth at 4.0 mL/L fipronil (8.23 cm), while *Trichoderma* 04 exhibited the greatest reduction (5.77 cm). Overall, glyphosate exerted a stronger inhibitory effect than fipronil, with *T. camerunense* being the most sensitive.

Previous studies have demonstrated that *Trichoderma* spp. are capable of tolerating and growing in pesticide-contaminated environments, largely due to their metabolic flexibility and detoxification mechanisms. These fungi produce a range of hydrolytic enzymes, such as oxidoreductases, peroxidases, and dehydrogenases that help break down pesticide molecules into less toxic forms. Additionally, *Trichoderma* spp. synthesize secondary metabolites that can neutralize harmful substances, enhancing their survival and bioremediation potential (Spinelli et al. 2021; Zhang et al. 2021; Bharti et al. 2023; Firincă et al. 2024). These traits make *Trichoderma* spp. valuable candidates for sustainable soil management, especially in areas with prolonged pesticide use.

Macroscopic and microscopic observations revealed that although *Trichoderma* spp. could grow on pesticide-containing media, their mycelia appeared thinner and exhibited a whitish coloration, indicating reduced conidia production. This finding aligns with previous studies by da Silva et al. (2018) and Mendarte-Alquisira et al. (2024), which showed that such changes reflect fungal responses to environmental stress, often resulting in reduced growth and sporulation.

The Relative Inhibition Rate (RIR), calculated based on the reduction in colony growth, served as an indicator of each isolate's sensitivity to pesticide exposure. Higher RIR values reflected greater sensitivity. *Trichoderma harzianum* 01 showed no inhibition at the recommended glyphosate concentration (5.0 mL/L), indicating full resistance, while *T. camerunense* 02 exhibited a 20.37% inhibition, suggesting moderate resistance. At the higher concentration (12.5 mL/L), *T. camerunense* 02 experienced 74.44% inhibition, indicating a transition from moderate resistance to sensitivity. In contrast, fipronil caused only mild inhibition across all isolates, confirming a generally higher tolerance to fipronil.

Table 5. Inhibition percentage (%) and mechanism of *F. oxysporum* suppression by *Trichoderma* spp.

***Trichoderma* isolates**	**Inhibition percentage [1)]**	**Inhibition mechanism**
T. harzianum 01	83.96 d	mycoparasitism
T. camerunense 02	72.38 ab	mycoparasitism
T. harzianum 03	76.92 bc	mycoparasitism
Trichoderma 04	70.59 a	mycoparasitism

Note: [1)]Numbers marked by distinct letters differ significantly ($P<0.05$) according to the DMRT test

Figure 5. Macroscopic appearance of 7-day-old *Trichoderma* spp. colonies grown on media containing glyphosate and fipronil. A. Control; B. Glyphosate 5.0 mL/L; C. Glyphosate 12.5 mL/L; D. Fipronil 2.0 mL/L; E. Fipronil 4.0 mL/L. Rows are arranged from top to bottom as follows: 1st row: *T. harzianum* 01, 2nd row: *T. camerunense* 02, 3rd row: *T. harzianum* 03, 4th row: *Trichoderma* 04

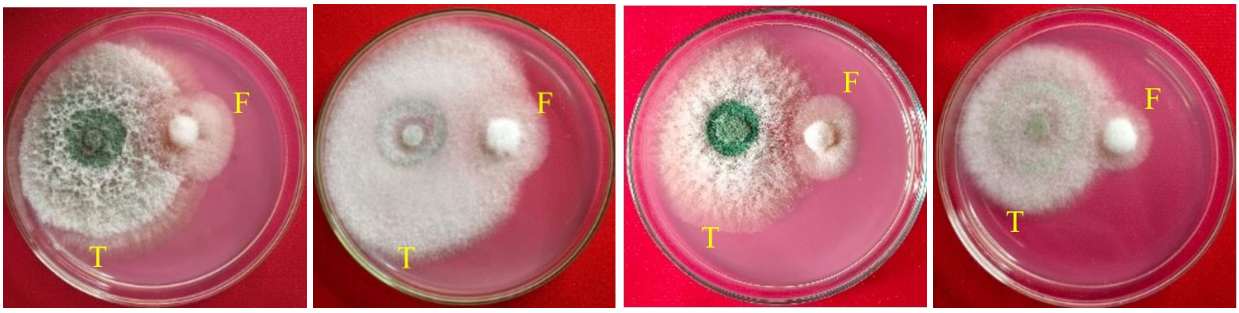

Figure 6. In vitro inhibition of F. oxysporum by *Trichoderma* spp. A. *T. harzianum* 01; B. *T. camerunense* 02; C. *T. harzianum* 03; D. *Trichoderma* 04. T: *Trichoderma*, F: *F. oxysporum*

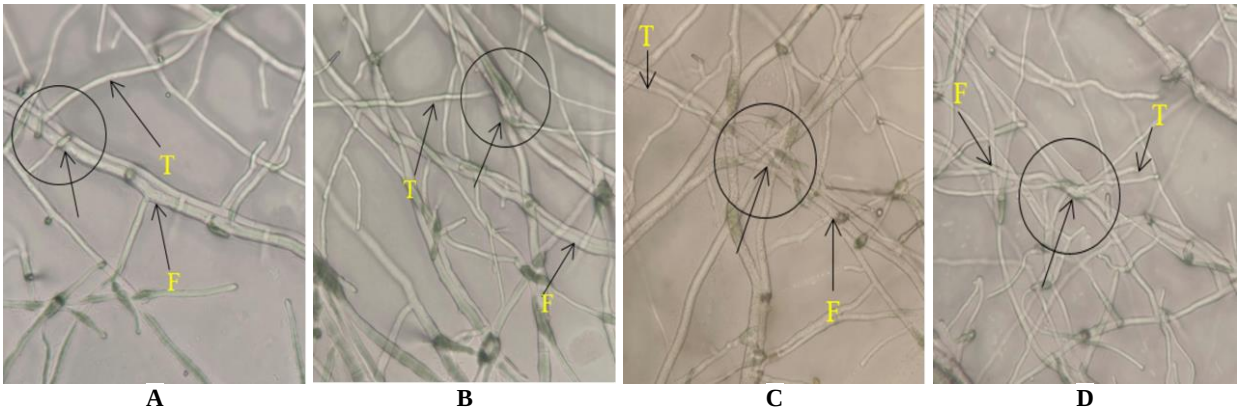

Figure 7. The interaction between *Trichoderma* spp. and *F. oxysporum* in dual culture confrontation: Attachment and coiling of *Trichoderma* spp. around *F. oxysporum* hyphae (indicated by black circle). A. *T. harzianum* 01; B. *T. camerunense* 02; C. *T. harzianum* 03; D. *Trichoderma* 04. T: *Trichoderma*; F: *F. oxysporum*

The in vitro antagonism test assessed the biocontrol potential of *Trichoderma* spp. against *Fusarium oxysporum* using the dual culture method. All isolates exhibited strong antagonistic activity, with inhibition rates exceeding 70%. *Trichoderma harzianum* 01 showed the highest inhibition rate (83.96%), indicating superior efficacy in suppressing the pathogen. *Trichoderma* 04 showed the lowest, substantial inhibition rate of 70.59%. The other isolates, *T. harzianum* 03 and *T. camerunense* 02, recorded inhibition rates of 76.92 and 72.38%, respectively.

This high level of inhibition was attributed to mycoparasitism—a mechanism in which *Trichoderma* attacks and degrades the mycelia of *F. oxysporum*. This involves secretion of hydrolytic enzymes such as chitinases, glucanases, and proteases, which break down the pathogen's cell wall and allow *Trichoderma* to penetrate and destroy its hyphae. Additionally, the production of antifungal metabolites contributes to suppression, reinforcing the fungus's role as an effective biocontrol agent (Conte et al. 2025).

Microscopic analysis confirmed these interactions, showing all four *Trichoderma* isolates coiling around, penetrating, and damaging the hyphae of *F. oxysporum*. According to Yao et al. (2023), antifungal compounds and enzymes produced by *Trichoderma* spp. inhibit pathogen development. These fungi typically produce both volatile and non-volatile metabolites, as well as cell wall-degrading enzymes, such as β-1,3-glucanase, chitinase, and cellulase, targeting fungal cell walls composed mainly of β-1,3-glucan and chitin (Manzar et al. 2022; Tyśkiewicz et al. 2022).

In conclusion, this study demonstrated that *Trichoderma* spp., particularly *T. harzianum* 01, were able to tolerate glyphosate and fipronil exposure and exhibited strong antagonistic activity against *Fusarium oxysporum*. Growth responses varied among isolates, and glyphosate generally exhibited a stronger inhibitory effect than fipronil. All isolates remained viable on pesticide-amended media and were capable of suppressing the pathogen in vitro. These findings highlight the potential of *Trichoderma* spp. as biocontrol agents in pesticide-affected soils. Future research should focus on validating these results under field conditions, investigating the mechanisms underlying pesticide tolerance, and exploring their broader impacts on plant health and soil quality. Their integration into sustainable crop management practices could reduce chemical inputs and support long-term agricultural resilience.

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