

# Exploration and Quality Assessment of Local *Trichoderma* spp. from the Rhizosphere of Bamboo (*Gigantochloa apus*) Against *Fusarium* Wilt Disease in Tomato Plants

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## ABSTRACT

This study aims to explore and test the quality of *Trichoderma* spp. local from the rhizosphere of apus bamboo (*Gigantochloa apus*) against *Fusarium* spp. wilt disease both *in vitro* and *in vivo* in tomato. This research was conducted in the Keerom area, Jayapura, which is one of the centers of food crop production and horticulture in Papua. The data obtained from this study were analyzed by comparing the inhibition of three *Trichoderma* spp. isolates (*Trichoderma* sp. 1 isolate, *Trichoderma* sp. 2 isolate, and *Trichoderma* sp.3 isolate) against *Fusarium* spp. The results of the antagonist test showed that the isolate of *Trichoderma* sp.3 had the highest inhibition (80.56%) against *Fusarium* spp., followed by *Trichoderma* sp.2 (66.84%) and *Trichoderma* sp.1 (27.62%). The implication of this study is that *Trichoderma* sp. 3 has high potential as an APH in the control of *Fusarium* spp. wilt disease in tomato plants. The use of *Trichoderma* spp. based APH can be an effective and environmentally friendly alternative in reducing the use of chemical pesticides and maintaining the balance of the agro-environmental ecosystem.

**Key words:** biological control agent; exploration; *Fusarium* spp.; *Trichoderma* spp.

## INTRODUCTION

*Fusarium oxysporum* is one of the major pathogens affecting tomato plants (Syam, 2014). This fungus causes sudden plant death, starting from young to mature leaves, with symptoms including the yellowing or paling of leaf veins. When cut open, the stems and root tissues appear brown in color and odorless. Infected plants typically wilt, dry out, and eventually die (Suryanti *et al.*, 2015).

The common practice among communities to manage plant diseases often involves environ-

mental manipulation (Sukmadjaja, 2001), which can lead to environmental pollution and pose risks to human health (Jibril *et al.*, 2016). Therefore, an effective and environmentally friendly control strategy that is also safe for human health is urgently needed. One such approach is the use of biological control agents (BCAs) (Erwin, 2000). A well-known fungal BCA is *Trichoderma* spp., which has been widely applied for biological control (Bukhari & Safridar, 2018).

*Trichoderma* spp. is a fungus with great potential as a biological agent capable of suppressing the growth of pathogenic fungi and enhancing crop yields (Bukhari & Safridar, 2018). *Trichoderma* spp. is easy to obtain and propagate, making it widely used both as a pathogen control agent (Karim *et al.*, 2021) and as a soil fertility enhancer (Karim *et al.*, 2021; Thuy & Trai, 2025).

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The diversity of naturally occurring *Trichoderma* species is high, offering promising potential as a source of disease control.

In light of this issue, it is necessary to conduct an exploration study of *Trichoderma* spp. fungi from the bamboo rhizosphere, considering the unique biological characteristics of the bamboo rhizosphere. This study aims to obtain local isolates and evaluate their antagonistic potential against *Fusarium* spp.—the primary causal agent of Fusarium wilt disease in tomato plants—through both *in vitro* and *in vivo* assays.

## MATERIALS AND METHODS

### Time and place of the research

The research was conducted at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Cenderawasih University, and at the Laboratory of the Plant Protection and Horticulture Agency (BPTPH) of Papua Province over a period of six months, from December 2023 to May 2024. The population of this study consisted of *Trichoderma* spp. present in the bamboo rhizosphere, while the samples were *Trichoderma* spp. isolates identified from the bamboo rhizosphere.

### Tools and Materials

The tools used in this study included: an autoclave, laminar air flow cabinet, hemocytometer, pipettes, analytical balance, petri dishes, forceps, culture racks, cover glass, microscope slides, test tubes, beakers, large brown envelopes, camera, Bunsen burner, microscope, ruler, hot plate, vortex mixer, caliper, toothpicks, cotton, label paper, tissue paper, inoculating needle, syringe, hand trowel, soil pH meter, thermometer, hand counter, and writing instruments.

The materials used included: local isolates of *Trichoderma* spp. obtained from bamboo rhizosphere exploration, isolates of the pathogenic *Fusarium* sp. obtained from infected tomato plants, sterile water, *Sabouraud Dextrose Agar* (SDA), Rose Bengal, and 70% alcohol.

### Isolation of *Trichoderma* spp. from the bamboo rhizosphere

Soil samples from the rhizosphere of *Gigantochloa apus* (Bambu Apus) were collected from three different locations: Arso Kota, Koya Koso, and Entrop. Soil samples were taken at a depth of 5–15 cm. The collected soil samples were placed in large brown envelopes, labeled with the date and plant origin, and then brought to the laboratory.

One gram of each soil sample was weighed and placed into a test tube, followed by the addition of 10 ml of sterile water. The suspension was then homogenized using a vortex mixer. Serial dilutions were performed up to 10<sup>6</sup>. From the final dilution, 0.1 ml was taken and inoculated onto SDA and Rose Bengal media. Colonies exhibiting the morphological characteristics of *Trichoderma* spp. were selected and subcultured on SDA media until pure isolates were obtained (Tuminem, 2021).

### Isolation of *Fusarium* spp.

*Fusarium* spp. was isolated from the roots and stems of tomato plants showing symptoms of wilting. Diseased root tissues were cut and surface-sterilized using 70% alcohol for 1 minute, then rinsed three times with sterile distilled water, and air-dried using sterile cotton or tissue paper. The stem segments were cultured on SDA and Rose Bengal media, followed by incubation. Fungal colonies identified as *Fusarium* spp. were then subcultured on SDA media to obtain pure isolates.

### Identification of *Trichoderma* and *Fusarium* isolates

Fungal identification was conducted based on both macroscopic and microscopic morphological characteristics. Macroscopic identification included observation of colony color and surface, colony margin characteristics, and the presence of concentric rings (Gandjar *et al.*, 1999). Microscopic characteristics observed included the hyphae or mycelium, spore color and shape, hyphal structure, and the presence or absence of septa. The identification of fungi referred to the identification

manuals *Compendium of Soil Fungi* (Domsch *et al.*, 1980) and *The Diversity of Trichoderma spp. in South Africa* (du Plessis, 2015).

**Quality assessment of biological control agent products in the laboratory- Spore density measurement**

This activity was carried out by calculating the spore density of *Trichoderma* spp. The measurement was conducted using a 10<sup>6</sup> serial

dilution. One drop of the diluted suspension was placed into the counting chamber of a hemocytometer, covered with a cover glass, and observed under a microscope. Spore density was then calculated using the following formula:

$$S = \frac{t \times d}{N \times 0.0025} \times 10^6$$

where :

- t : number of spores counted in the hemocytometer grid
- d : dilution factor
- N : number of hemocytometer grid squares counted
- 0.0025: volume of the hemocytometer chamber (in milliliters)
- 10<sup>6</sup> : constant to convert the result to spores/ml

**Conidial viability test**

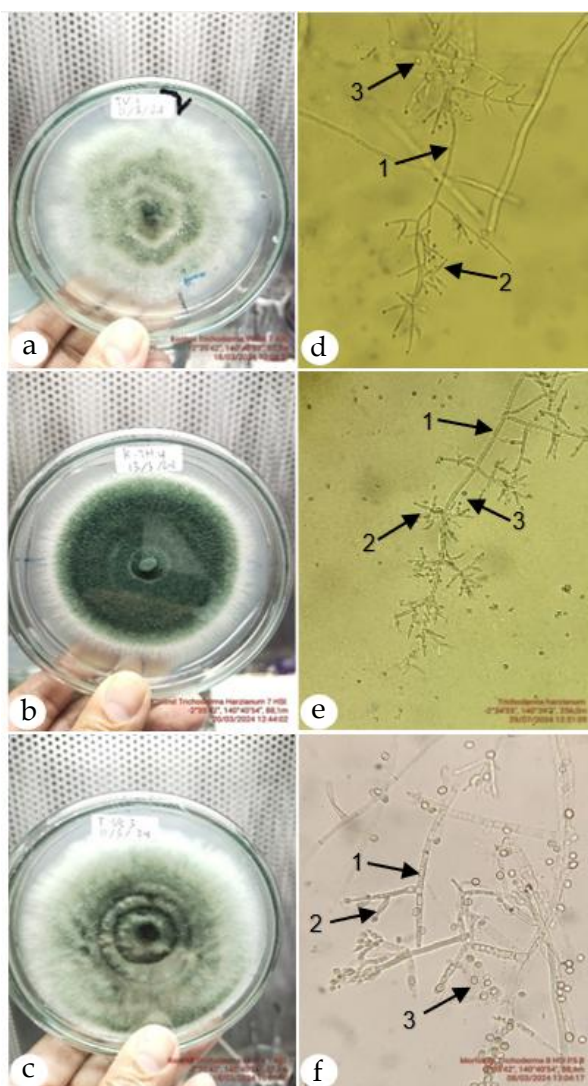
SDA media in petridishes was cut using a sterile 0.5 mm straw and placed on microscope slides, with three replicates per slide. One drop of a 10<sup>3</sup> spore suspension was applied to each medium slice using a syringe. Each SDA medium slice was then covered with a cover glass. A 9 cm petridish was prepared by placing moistened cotton with sterile water at the base. Sterile toothpicks were positioned on both the left and right sides of the petridish to support the slide glass, which was placed on top. The setup was incubated for 24 hours.

Conidial germination rate was calculated using the following formula:

$$\text{Viabilitas} = \frac{\text{jumlah spora berkecambah}}{\text{total sporayang diamati}} \times 100\%$$

**Antagonistic test of *Trichoderma* spp. against *Fusarium* spp.**

The antagonistic test was conducted using the dual culture method. SDA medium was prepared in petri dishes. *Trichoderma* and *Fusarium* isolates were taken using a sterile 0.5 mm diameter straw from the edge of each fungal colony. The *Trichoderma* isolate was placed on the SDA medium at a distance of 3 cm from the right edge of the petri dish and marked with a "T," while the *Fusarium* isolate was placed 3 cm from the left edge and marked with an "F." The plates were



**Figure 1.** *Trichoderma* spp. isolates: (a) *Trichoderma* sp.2 and (b-c) *Trichoderma* sp.3. The microscopic morphology is shown in images (d-f), highlighting the structures of conidiophores, phialides, and conidia. These features confirm the identity of the isolates as members of the *Trichoderma* genus.

then incubated at room temperature. After incubation, the colony radius was measured, and the percentage of growth inhibition was calculated using the following formula (BSN, 2014):

$$P = \frac{(r1 - r2)}{r1} \times 100\%$$

where:

- P : percentage of inhibition
- r1 : radius of the pathogen colony without antagonist (control)
- r2 : radius of the pathogen colony with antagonist (treatment)

This study employed a Completely Randomized Design (CRD), consisting of 7 treatments and 5 replications.

### Preparation of rice media

Rice media was prepared as a propagation substrate for *Trichoderma* and *Fusarium* isolates. The rice was washed and soaked for 1 hour. After soaking, it was drained for 1–2 hours, then steamed and stirred (flipped) for 5–15 minutes until half-cooked. The steamed rice was then cooled.

Next, 100 grams of the rice were placed into heat-resistant plastic bags. A filter or a small PVC pipe (paralon) was inserted into each bag, which was then sealed using rubber bands. The bags were sterilized using an autoclave at 121°C and 1 atm pressure for 15 minutes. After cooling, the medium was ready for use in propagating *Trichoderma* and *Fusarium* isolates.

### Inoculation of *Trichoderma* spp. and *Fusarium* spp. onto rice media

Inoculation was carried out by preparing *Trichoderma* and *Fusarium* isolates, which were then aseptically inoculated onto the rice media inside a Laminar Air Flow (LAF) cabinet. The inoculated media were subsequently incubated for two weeks.

### Spore harvesting

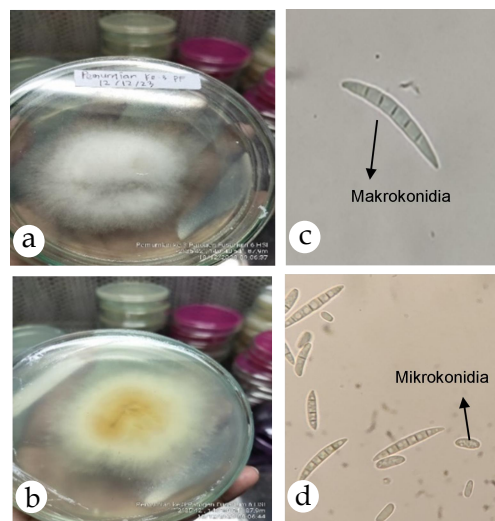
Spore harvesting was carried out on the 14th day, when sporulation had fully occurred and spores were evenly distributed across the rice

medium. The rice was removed from the plastic bags, crushed, and dried in a closed room for 2–3 days to reduce the moisture content. The dried medium was then blended to separate the spores from the rice substrate. The resulting material was sieved to obtain spore powder or spore flour. The spore powder was then ready to be used for *in vivo* testing.

### In vivo antagonistic test

The planting medium used was a mixture of soil and compost in a 2:1 ratio. The soil was sieved and then sterilized using an autoclave. After cooling, the soil was allowed to rest for approximately 1–2 days before being mixed with compost.

Tomato seeds were sown in seedling trays for 7 days. After 7 days, the seedlings were transferred to small polybags with a diameter of 5 cm and maintained for 1 month. After 1 month, the seedlings were transplanted into larger polybags measuring 32 × 40 cm. Application of *Trichoderma* spp. was carried out simultaneously



**Figure 2.** The morphological characteristics of the *Fusarium* sp. isolate. Image (a) shows the top view of the colony, while image (b) presents the bottom view, revealing a creamy to yellowish pigmentation. Image (c) illustrates the macronidia, which are crescent-shaped with 3–5 septa, and image (d) shows the micronidia, which are oval to elliptical in shape and possess 1–3 septa. These features confirm the identity of the isolate as *Fusarium* sp.

with *Fusarium* spp. into polybags filled with the soil-compost mixture, using a dose of 5 grams per polybag/treatment. Observations were conducted during the vegetative growth phase, continuing until the tomato plants treated with *Fusarium* spp. showed signs of death.

The parameters observed included the number of plants that died due to *Fusarium* spp. infection and the number of healthy plants.

The percentage of plant mortality was observed weekly starting from the time the treatment was applied until the plants were 4 weeks old. The mortality percentage was calculated at the end of the observation period using the following formula:

$$\int \text{mortality percentage} = \frac{\text{number of dead plants}}{\text{total number of plants observed}} \times 100\%$$

#### Data analysis

The data were analyzed descriptively, including the macroscopic and microscopic characteristics of *Trichoderma* spp. and *Fusarium* spp., spore density, spore viability, and *in vivo* test results, which were presented in tables, graphs, or images. Quantitative data analysis was performed on the results of the antagonistic test of *Trichoderma* spp. isolates against *Fusarium* spp.

The results of the antagonistic test were analyzed using analysis of variance (ANOVA) with the SPSS software, followed by Duncan's multiple range test (DMRT) for post-hoc comparisons.

## RESULTS AND DISCUSSION

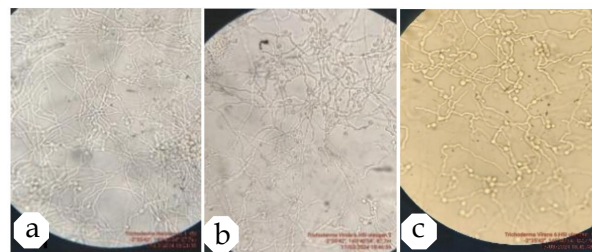
### Characteristics of *Trichoderma* spp. from the bamboo rhizosphere

Based on the exploration of the bamboo (*Gigantochloa apus*) rhizosphere, three *Trichoderma* spp. isolates were obtained from three different locations: Koya Koso (coded as *Trichoderma* sp.1 or T1), Arso Kota (*Trichoderma* sp.2 or T2), and Entrop (*Trichoderma* sp.3 or T3) (Figure 1).

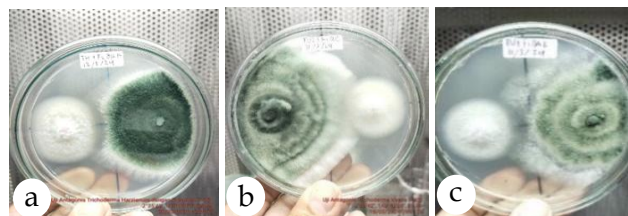
In addition to *Trichoderma* sp., other fungi were also found at each sampling site: *Aspergillus*

sp. was detected at all three locations, and *Penicillium* sp. was found at Arso Kota and Entrop. The purified *Trichoderma* sp. isolates from the bamboo rhizosphere displayed colony morphological characteristics similar to those typically observed in *Trichoderma* species. The three different isolates, which resembled *Trichoderma* sp., were labeled as follows: *Trichoderma* sp.1 (T1) from Arso Kota, *Trichoderma* sp.2 (T2) from Koya Koso, and *Trichoderma* sp.3 (T3) from Entrop.

*Trichoderma* sp.1 isolate exhibited fine, cotton-like white mycelium. The center of the colony was green and surrounded by white mycelium. The colony margin was lobed, spreading toward the edge of the petri dish, and had a smooth surface. By the fifth day after isolation, the mycelium



**Figure 3.** Shows the spore germination of *Trichoderma* spp. isolates. Image (a) represents isolate T1, (b) shows isolate T2, and (c) displays isolate T3. All three isolates exhibited successful germination, indicating high spore viability, which supports their potential as effective biological control agents.



**Figure 4.** The interaction between *Trichoderma* spp. and *Fusarium* spp. at 7 days after inoculation (DAI). Image (a) shows the dual culture of *Trichoderma* sp.1 and *Fusarium* sp., (b) displays *Trichoderma* sp.2 and *Fusarium* sp., and (c) presents *Trichoderma* sp.3 and *Fusarium* sp. The antagonistic response of each *Trichoderma* isolate against *Fusarium* can be observed from the inhibition zones formed in the culture plates.

gradually turned green.

Microscopically, the conidiophores were septate, with often irregular and highly branched structures. The conidia were round to oval in shape, light green in color, and possessed slender phialides.

*Trichoderma* sp.2 initially appeared white



**Figure 5** presents the rice-based spore powder and solid rice media for fungal propagation. Images (a, a') show the spore powder and rice medium of *Trichoderma* isolate T1, (b, b') correspond to isolate T3, (c, c') to isolate T2, and (d, d') display the medium prepared with the *Fusarium* isolate. Each isolate demonstrates successful colonization and sporulation on rice substrate, highlighting its suitability for large-scale propagation.

during the early stages of growth, with the central part of the colony turning green on the second and third days. It then developed into a dark green circular shape with a distinct boundary, while the edges remained cotton-like white. Eventually, the entire upper surface of the colony turned dark green.

Microscopically, the conidiophores were septate, erect, and vertically branched. The conidia were round to oval, light green in color, and the phialides were flask-shaped. *Trichoderma* sp.3, based on macroscopic observation, initially formed white colonies on the third day. By the fourth day, the color had changed to a dark greenish white, which persisted until the seventh day. The colony surface was thick, round, and dense, resembling cotton, with ring-like concentric circles and smooth colony margins.

Microscopically, *Trichoderma* sp.3 had irregularly branched, septate hyphae. The conidia were round to elliptical, and the phialides were short, round, clustered at each conidiophore, and present in groups of 3 to 5. According to Watanabe (2002) in *Morphologies of Cultured Fungi and Key to Species*, *Trichoderma* colonies grown on PDA media show a range of colors depending on the species. This was confirmed by Suanda (2019), who reported that the colony color of *Trichoderma* fungi varies from white to greenish white to dark green. Additionally, *Trichoderma* species exhibit diverse appearances, which correspond to their species differences—some have dark greenish white colonies, while others have light greenish white ones (Molebila *et al.*, 2020).

#### Isolation of *Fusarium* spp. from tomato plants

Based on the results of the study, the pathogenic fungus identified was *Fusarium* spp., which causes wilt disease in tomato plants. The isolation results showed that *Fusarium* spp. had whitish-yellow mycelium, septate hyphae, and crescent-shaped macroconidia with 3–5 septa (Figure 2).

The *Fusarium* isolate was obtained from purified samples of tomato plant tissues exhibiting symptoms of *Fusarium* wilt. The colony structure resembled that of fungi belonging to the *Fusarium*

genus. Based on macroscopic characteristics, the colony appeared white with a cotton-like texture and exhibited a concentric growth pattern. The reverse side of the colony was cream to yellowish in color.

Microscopically, *Fusarium* spp. was found to produce two types of conidia: macroconidia and microconidia. The macroconidia were crescent-shaped with pointed ends and had 1–5 septa, while the microconidia were oval to elliptical and contained 1–3 septa.

#### **Biological control agent quality - Spore density of *Trichoderma* spp.**

Based on the spore density data presented in Table 8, the isolate with the highest spore density was *Trichoderma* sp.1. According to the Center for Seed and Plantation Crop Protection (*Balai Besar Perbenihan dan Proteksi Tanaman Perkebunan*, BBPPTP, 2014), the required spore density to meet the standard for biocontrol agents—particularly *Trichoderma* sp. —must be equal to or greater than  $1 \times 10^6$  spores/mL.

Therefore, the spore density data obtained in this study indicate that the number of spores produced by *Trichoderma* spp. meets the quality criteria for biocontrol agents. *Trichoderma* sp.1 had the highest spore density at  $1.3 \times 10^{11}$  spores/mL, followed by *Trichoderma* sp.3 with  $1.2 \times 10^{10}$  spores/mL, and *Trichoderma* sp.2 with  $1.1 \times 10^9$  spores/mL. These results comply with the Indonesian National Standard (SNI 8027.3: 2014) on the Quality Testing Requirements for Biological Control Agents (*Agens Pengendali Hayati*, APH) of *Trichoderma* spp., which require a minimum of  $10^6$  spores/mL. According to Syahnen *et al.* (2014), a high spore density or compliance with regulatory standards indicates the potential of a biological control agent to effectively inhibit pathogen infection.

#### **Spore viability of *Trichoderma* spp.**

The viability test of spore isolates—*Trichoderma* sp.1, *Trichoderma* sp.2, and *Trichoderma* sp.3—used for controlling *Fusarium* sp. wilt disease in tomato plants showed that all isolates had 100% spore viability. The higher the

germination rate or viability of the spores, the better their suitability as a component in biopesticide formulations (BBPPTP, 2014).

The viability of *Trichoderma* spp. spores derived from bamboo rhizosphere exploration demonstrated strong potential for application as biological control agents. Overall, the viability test results indicated that all three *Trichoderma* species possess promising potential for use in sustainable agriculture, particularly in biological control and plant growth enhancement.

The viability test of *Trichoderma* spp. conformed to the Indonesian National Standard (SNI 8027.3:2014) for quality requirements of biological control agents (*Agens Pengendali Hayati*, APH), which specifies a minimum conidial viability of  $\geq 60\%$ . According to Ramli (2004), spore viability is considered good if it ranges from 80–100%, moderate at 70–85%, and poor at 55–70%.

#### **The *in vitro* antagonistic test of *Trichoderma* spp. against *Fusarium* spp.**

The statistical analysis of the *in vitro* antagonistic test showed that treatments sharing the same letter notation were not significantly different from each other, while those with different letter notations indicated significant differences.

The average antagonistic activity of *Fusarium* (F) was 12.46%, which was the lowest among all treatments. *Trichoderma* sp.1 (T1) had an average antagonistic activity of 46.81%, showing improved antagonistic ability compared to *Fusarium*, but not significantly different from F. *Trichoderma* sp.2 (T2) had an average antagonistic activity of 38.09%, higher than F but not significantly different from F or T1. *Trichoderma* sp.3 (T3) had an average antagonistic activity of 46.30%, similar to T1 and T2, and not significantly different from F.

The combination of *Trichoderma* sp.1 and *Fusarium* (T1F) showed an average antagonistic activity of 27.62%, which was lower than T1 alone but still higher than F. The combination of *Trichoderma* sp.2 and *Fusarium* (T2F) had an average antagonistic activity of 66.84%, showing a significant increase compared to F. The combination of *Trichoderma* sp.3 and *Fusarium* (T3F)

showed the highest antagonistic activity at 80.56%, which was significantly different from F.

These results indicate that *Trichoderma* spp. possess strong potential as effective biocontrol agents in inhibiting the growth of *Fusarium* spp. The antagonistic test results in this study comply with the Indonesian National Standard (SNI 8027.3:2014) for quality testing of *Trichoderma* spp. biological control agents, which requires an inhibition rate of  $\geq 50\%$  against *Fusarium* spp.

**Preparation of rice media, inoculation and preparation of spore meal of *Trichoderma* spp. and *Fusarium* spp.**

The preparation of rice media in this study aimed to serve as a propagation substrate for *Trichoderma* spp. and *Fusarium* spp. Rice is considered one of the most effective media for the mass production of *Trichoderma* spp. due to its nutritional content, which supports fungal growth (Uraial *et al.*, 2012). These materials contain carbohydrates, fiber, nitrogen, phosphate, and potassium – nutrients essential for the growth and development of *Trichoderma* spp.

Materials with high carbohydrate concentrations are particularly effective in promoting fungal growth. Higher growth rates lead to greater conidial production, whereas limited growth results in fewer conidia being produced (Rizal *et al.*, 2018).

*Trichoderma* spp. and *Fusarium* spp. were propagated on rice media for 14 days until *Trichoderma* mycelium fully colonized the substrate. The production of *Trichoderma* spore powder involved cultivating the fungus on a suitable medium, followed by harvesting and drying of the spores. The dried spores were then ground into a fine powder, resulting in a product that is easy to store and apply (Figure 5).

Recent studies have demonstrated the effectiveness of rice media for the propagation of *Trichoderma* sp. A study by Rahmawati *et al.* (2021) reported that rice media effectively supported the optimal growth of *T. harzianum*, producing high spore counts and robust mycelial development. These findings highlight that the use of rice as a

growth medium is not only practical but also economically viable for large-scale production.

**In vivo test on tomato plants**

The results presented in Table 4 show varying percentages of tomato plant mortality from 7 to 28 days after transplanting (DAT). At 7 DAT, the mortality rate across all treatments remained at 0%. By 14 DAT, disease incidence began to increase, with mortality rates of 40% in the control group, 20% in the *Fusarium* treatment, and 0% in all *Trichoderma* treatments (T1, T2, T3) as well as the

**Table 1.** Spore density of *Trichoderma* spp. isolates.

No	Isolates	Spore density (ml)
1.	Isolate <i>Trichoderma</i> sp.2 T2	$1,1 \times 10^9$
2.	Isolate <i>Trichoderma</i> sp.1 T1	$1,3 \times 10^{11}$
3.	Isolate <i>Trichoderma</i> sp.3 T3	$1,2 \times 10^{10}$

**Table 2.** Spore viability of *Trichoderma* spp. isolates.

No	Isolates	Spore visibility (%)
1.	Isolate <i>Trichoderma</i> sp.2 T2	100
2.	Isolate <i>Trichoderma</i> sp.1 T1	100
4.	Isolate <i>Trichoderma</i> sp.3 T3	100

**Table 3.** Percentage of antagonistic activity of *Trichoderma* spp. against *Fusarium* spp.

No	Treatment	Mean antagonistic effect (%)
1.	F	12,46 a
2.	T1	46,81 ab
3.	T2	38,09 ab
4.	T3	46,30 ab
5.	T1F	27,62 ab
6.	T2F	66,84 b
7.	T3F	80,56 b

Note: Means followed by the same letter are not significantly different according to DMRT at the 5% significance level.



combined *Trichoderma* + *Fusarium* treatments (T1F, T2F, T3F).

At 21 DAT, the mortality rate in the control group remained at 40%, while the *Fusarium* treatment increased to 40%. All *Trichoderma* and combination treatments still showed 0% mortality. By 28 DAT, the mortality in the control group remained at 40%, and the *Fusarium* treatment reached 100% mortality in tomato plants. In

contrast, both the individual *Trichoderma* treatments and the *Trichoderma* + *Fusarium* treatments still showed 0% mortality.

These findings indicate that all three *Trichoderma* spp. isolates were effective in suppressing *Fusarium* spp. in the tested plants, as evidenced by the absence of plant death in the *Trichoderma* + *Fusarium* treatments, in stark contrast to the 100% mortality observed in the



**Figure 6.** The growth of tomato plants 28 days after inoculation (DAI) with *Fusarium* sp. and the application of various *Trichoderma* isolates. Figure 6a depicts plants inoculated solely with *Fusarium* sp., showing clear symptoms of disease. Figure 6b represents the untreated control group. Figure 6c, 6d, and 6e illustrate treatments with *Trichoderma* isolates T1, T2, and T3, respectively, all of which show healthy plant growth. Figure 6f, 6g, and 6h show combined treatments of *Trichoderma* and *Fusarium* for isolates T1F, T2F, and T3F, respectively, also demonstrating healthy plant development and effective disease suppression.

*Fusarium*-only treatment.

Mortality in the *Fusarium* treatment increased progressively at 7, 14, 21, and 28 DAT in each polybag. According to Antara *et al.* (2015), infections caused by the pathogenic fungus *Fusarium oxysporum* typically increase between weeks 4 to 7 after planting and tend to decrease from weeks 8 to 10. This trend suggests that up to the seventh week, *F. oxysporum* infection is not yet strongly suppressed by antagonistic *Trichoderma* spp., whereas starting from the eighth to tenth weeks, disease suppression by *Trichoderma* spp. becomes more evident.

All three *Trichoderma* species demonstrated similar effectiveness in controlling *Fusarium* spp. This aligns with findings by Hermosa *et al.* (2012), who reported that various *Trichoderma* species exhibit effective antagonistic mechanisms against soil-borne pathogens, including *Fusarium*.

## CONCLUSION

Based on the results of this study, it can be concluded that three isolates of *Trichoderma* were identified: *Trichoderma* sp.1, *Trichoderma* sp.2, and *Trichoderma* sp.3. The quality assessment of biological control agents (*Trichoderma* spp.) isolated from the bamboo rhizosphere demonstrated their effectiveness in controlling *Fusarium* sp. in tomato plants, both *in vitro* and *in vivo*. Among them, *Trichoderma* sp.3, isolated from the bamboo rhizosphere, exhibited the highest antagonistic potential against *Fusarium* sp.

The *in vitro* and *in vivo* antagonistic tests confirmed that all three *Trichoderma* spp. isolates possess strong biocontrol capabilities against *Fusarium* sp., indicating their potential use in managing *Fusarium* wilt in tomato cultivation.

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