
**Proceeding of International**
Conference on Science and Technology
Lembaga Penelitian Pengabdian kepada Masyarakat dan Dakwah Islamiyah,
Universitas Islam Kuantan Singingi, Indonesia, Agustus 7th 2025
DOI : https://doi.org/110.36378/internationalconferenceuniks.v3i1

ISSN 2985-8739

**Page: 35-46**

# Identification of Fungi Causing Rubber Leaf Fall and Testing the Inhibitory Effect of *Trichoderma* spp. Consortium against These Fungi *in vitro*

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***Abstract***

*Rubber (Hevea brasiliensis Muell. Arg) is a major commodity in Riau Province, but its production has declined due to various factors, including diseases. Rubber Leaf Fall Disease (RLFD), caused by Pestalotiopsis sp., is a significant disease affecting rubber, which is a relatively new concern in Indonesia. As an environmentally friendly control alternative, this study aimed to identify the causal fungus of RLFD and to test the inhibitory effect of a consortium of five Trichoderma spp. isolates against Pestalotiopsis sp. in vitro. The research employed observational methods for morphological identification of Pestalotiopsis sp. and an experimental method with a completely randomized design (CRD) for inhibitory testing. The experimental design consisted of 24 treatments with 3 replications. Data from the inhibitory activity were analyzed using analysis of variance and Duncan's multiple range test at 5%. The results confirmed that the fungus causing RLFD is indeed Pestalotiopsis sp., and showed that the consortium of T. pseudokoningii + T. koningii and the consortium of T. koningii + T. harzianum were the most effective in inhibiting its growth in vitro, with inhibition percentages of 35.00% and 34.00%, respectively. This finding suggests that these specific Trichoderma consortia have strong potential as biological control agents against RLFD.*

*Keywords: Inhibitory power, Leaf fall, Identification, Rubber, Consortium, Trichoderma*

## 1. Introduction

Rubber (*Hevea brasiliensis* Muell. Arg.) is a latex-producing plantation commodity that plays an important role in the Indonesian economy. In addition to oil and gas, rubber is also one of the main export commodities that contribute to the country's foreign exchange earnings [1]. With the increasing demand for exports, the government and the community continue to expand rubber plantation areas, including in Kampar District, Riau Province. However, data [2] indicates that rubber production in Kampar District decreased from 74,952 tons in 2020 to 62,665 tons in 2021, accompanied by a decline in productivity from 0.83 tons/ha to 0.81 tons/ha. This decline was attributed to various factors, including pest and disease outbreaks [3].

One of the main diseases affecting rubber plants is leaf fall disease [4]. Leaf fall disease caused by *Pestalotiopsis* sp. can result in a reduction in rubber production of over 30% in Indonesia [5]. Leaf drop disease caused by *Pestalotiopsis* is a new disease, first reported to attack rubber plantations in North Sumatra in 2016, then spreading to South Sumatra in 2017. By 2018, the disease had spread to various regions, with a total affected area of 22,804 hectares. Currently, the affected area continues to increase and has reached 382,000 hectares. The disease has been found in several regions such as North Sumatra, West Sumatra, Jambi, South Sumatra, Bangka Belitung, Bengkulu, Lampung, West Java, Central Java, South Kalimantan, West Kalimantan, Central Sulawesi, and Riau [6].

The characteristic symptoms of *Pestalotiopsis* sp. infection appear as light to dark brown spots with dark edges in irregular circular shapes on the leaf surface. Each leaf typically shows more than one spot, and the spots are accompanied by black aservules. These symptoms appear on green to dark green leaves that are more than one month old. Infected leaves turn reddish-yellow, and severe attacks cause leaf drop [7].

The most common control method used by farmers is the application of synthetic fungicides containing active ingredients such as methyl thiophanate, propiconazole, or hexaconazole at high doses to control this disease. Therefore, the development of more environmentally friendly control alternatives is necessary. The use of biological agents is one of the control approaches supporting sustainable agriculture [8]. *Trichoderma* spp. is one of the potential biological agents due to its ability to inhibit plant pathogens and promote plant growth [9].

This study utilized five *Trichoderma* isolates, namely *T. harzianum*, *T. viride*, *T. koningii*, *T. pseudokoningii*, and *Trichoderma* sp., as biological agents to control leaf fall disease caused by in rubber plants. Research [10] showed that biofungicides in pellet form containing isolates of T. *harzianum,* T. *pseudokoningii,* T. *koningii,* and T. *viride* were able to inhibit the growth of *Ganoderma boninense* Pat. in vitro, with the highest inhibitory activity shown by T. *harzianum* (58.84%) and T. *pseudokoningii* (52.57%), compared to T. *koningii* (35.06%) and T. *viride* (26.83%). A study [11] also reported that *Trichoderma* sp. isolated from the rhizosphere exhibited inhibitory activity up to 81.21%.

The five *Trichoderma* isolates used are known as antagonistic fungi and aggressive mycoparasites, with five modes of action: parasitism, antibiosis, competition, lysis, and induction of resistance. Therefore, all five have potential as biological agents in the control of leaf drop disease, especially when used in consortium form. According to [12], a consortium is a combination of several microorganisms that work synergistically and cooperatively. The use of antagonistic fungal consortia is considered more effective than single isolates because they can complement each other in survival and nutrient utilization from the growth medium.

Research [13] supports the effectiveness of consortia, showing that the combination of four endophytic fungal isolates from sago plants, such as treatment K5 (Trichoderma sp. from leaves + *Thielaviopsis* sp.), K10 (from roots + stems + *Thielaviopsis* sp.), K3 (from roots + *Thielaviopsis* sp.), K6 (from stems + *Thielaviopsis* sp.), and K11 (from roots + fronds + stems + *Thielaviopsis* sp.), was able to suppress the growth *of Colletotrichum capsici* on PDA medium. Treatment K6 proved to be the most effective, reducing C. *capsici* infection on red chili peppers by 70.33%.

## 2. Research Method

This study was conducted at the Plant Pathology Laboratory (0°28'53.7"N 101°22'45.4"E), and the Plant Ecophysiology Laboratory (0°28'48.1"N 101°22'39.7"E) at the Faculty of Agriculture, University of Riau, Binawidya Campus km 12.5, Simpang Baru Village, Binawidya District, Pekanbaru. Sampling of rubber plants showing symptoms was conducted in Rimbo Panjang Village, km 22 (0°25'00.0"N 101°17'00.0"E – 0°31'00.0"N 101°40'42.0"E), Tambang District, Kampar Regency. This study was conducted over a period of four months, from October 2023 to January 2024.

### 2.1 Experimental Design

The research was conducted through observation and experimentation. The observational study involved the isolation and morphological identification of *Pestalotiopsis* sp., the fungus causing leaf fall in rubber trees, and compatibility testing between *Trichoderma* spp. isolates. The experimental study was conducted to test the inhibitory effect of *Trichoderma* spp. consortia against *Pestalotiopsis* sp.

The experimental study was conducted using a completely randomized design (CRD) with 24 treatments and 3 replicates, resulting in 72 experimental units. Each experimental unit consisted of 2 Petri dishes. The treatments tested were the Trichoderma spp. consortium
*Trichoderma* spp. (K) consisting of *Trichoderma pseudokoningii* (TP), *Trichoderma koningii* (TK), *Trichoderma harzianum* (TH), *Trichoderma viride* (TV), and *Trichoderma* sp. (TS) as follows: K1 = TP + TK, K2 = TP + TH, K3 = TP + TV, K4 = TP + TS, K5 = TK + TH, K6 = TK + TV, K7 = TK + TS, K8 = TH + TV, K9 = TH + TS, K10 = TV + TS, K11 = TP + TK + TH, K12 = TP + TK + TV, K13 = TP + TK + TS, K14 = TP + TH + TV, K15 = TP + TH + TS, K16 = TP + TV + TS, K17 = TK + TH + TV, K18 = TK + TV + TS, K19 = TH + TV + TS, K20 = TP + TK + TH + TV, K21 = TP + TK + TH + TS K22 = TP + TH + TV + TSK23 = TK + TH + TV + TS K24 = TP + TK + TH + TV + TS

### 2.2 Research Implementation

#### 2.2.1 Sampling of diseased plants

*Sampling* points were determined *purposively* by selecting one rubber tree showing symptoms of leaf fall disease. Symptomatic leaf samples were obtained from a community-owned rubber plantation located in Rimbo Panjang Village, km 22, Tambang Subdistrict, Kampar District, with a land area of approximately 500 m². This location was chosen because more than 50% of the rubber trees showed symptoms of leaf fall. The rubber plantation is approximately 15 years old, and most of the plants exhibit characteristic symptoms of leaf drop disease caused by *Pestalotiopsis* sp. The symptoms appear as spots on the leaves measuring 0.5 to 2 cm in size, which continue to spread, causing necrosis in the tissue around the center of the leaf, resulting in yellowing and eventual leaf drop. The collected leaves were placed in plastic bags and transported to the laboratory for isolation.

#### 2.2.2 Isolation of fungi causing leaf fall disease

The isolation process was carried out by cutting rubber leaves infected with leaf fall disease at the border between healthy and diseased tissue, measuring 0.5 cm × 0.5 cm. The leaf pieces were then sterilized using a 1% NaOCl solution for 30 seconds, followed by 70% alcohol for 30 seconds, and rinsed three times with sterile distilled water. The leaf pieces were then inoculated into PDA medium and incubated at room temperature. When the mycelium began to grow around the leaf pieces, the researchers collected the fungus using *a cork borer* and transferred it to a new medium until a homogeneous isolate was obtained. The isolate suspected to be *Pestalotiopsis* sp. was then propagated and observed for morphological identification, both through macroscopic and microscopic characteristics.

#### 2.2.3 Identification of the fungus causing leaf drop disease

After the isolation of the fungal pathogen causing leaf drop disease was completed, the next step was to perform morphological identification of the fungus. The researcher observed the macroscopic morphology of the mycelium growing on PDA medium at 3, 5, and 7 days after incubation (dai). At 30 dai, the fungi were observed microscopically, noting the shape and direction of mycelium spread, the shape and structure of conidiophores, conidia, and hyphal color.

### 2.2.4 Revitalization of *Trichoderma* spp. and *Pestalotiopsis* sp. isolates

*Trichoderma* spp. and *Pestalotiopsis* sp. isolates were rejuvenated by transferring the growing hyphae from the parent culture using *a* sterile *cork borer* into new Petri dishes containing 20 ml of sterile PDA medium. The isolates were then incubated at room temperature for 7 days until homogeneous growth was obtained.

### 2.2.5 Measurement of diameter and growth rate of *Trichoderma* spp.

The diameter and growth rate of *Trichoderma* spp. were measured by growing the fungi on PDA medium. A piece of the fungal culture was placed exactly in the center of the Petri dish and incubated at room temperature for 7 days. Observations were stopped when one of the fungal colonies reached the edge of the Petri dish.

### 2.2.6 Compatibility test of *Trichoderma* spp. fungi

The compatibility test was performed by placing two *Trichoderma* spp. isolates in a single Petri dish containing sterile PDA medium, with a distance of 3 cm between isolates (Figure 1). The compatibility test was observed by checking whether the isolates showed compatibility, indicated by the presence or absence of a clear zone on the medium. The purpose of this test is to determine whether the *Trichoderma* spp. isolates used do not inhibit each other's growth.

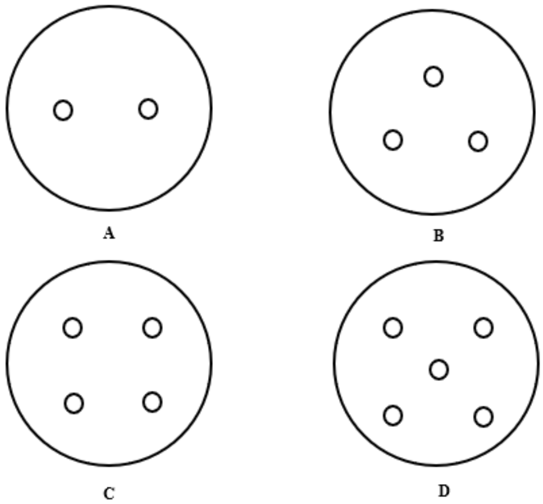



Figure 1. Schematic diagram of *Trichoderma* spp. placement in the compatibility test, (A) Testing of 2 *Trichoderma* spp. species, (B) Testing of 3 *Trichoderma* spp. species, (C) Testing of 4 Trichoderma spp. species *Trichoderma* spp. (D) Testing of 5 species of *Trichoderma* spp.

### 2.2.7 Propagation and activation of *Trichoderma* spp. consortium growth

The propagation of *Trichoderma* spp. isolates was carried out on PDA medium and incubated for 5 days at room temperature. After that, the isolates were activated according to method [14] by growing *Trichoderma* spp. in liquid medium as a starter (inoculum). The starter was prepared using potato extract, soluble starch, and calcium carbonate weighed according to requirements. Activation was performed in 250 ml jam jars by mixing 7.5 ml of potato extract, 6.25 ml of rice starch, 0.25 grams of calcium carbonate, and *Trichoderma* spp. isolates according to treatment, then adding distilled water until the jar was full. This mixture is then incubated for 5 days at room temperature while being homogenized using *a rotary shaker.*

### 2.2.8 Fermentation

Fermentation of *Trichoderma* spp. isolate was carried out following the method [15] referenced in [14]. A total of 100 ml of activated starter was cultured in a liquid medium consisting of 7.5 ml of corn soaking water, 7.5 grams of sucrose, 1.25 grams of calcium carbonate, and 0.25 grams of ferrous sulfate. The culture was fermented in 250 ml jam jars and incubated using *a rotary shaker* at 180 rpm for 3 days at room temperature. The filtrate was obtained by filtering the culture using a modified Buchner funnel.

### 2.2.9 Measurement of diameter and growth rate of *Trichoderma* spp. consortium

The diameter and growth rate of the *Trichoderma* spp. consortium were measured by drilling a hole in the center of the PDA medium in a Petri dish using *a cork borer*. The consortium suspension was dropped into the hole using a dropper, and the dish was incubated at room temperature for 7 days. Observations were stopped when one of the fungal colonies reached the edge of the Petri dish.

### 2.210 Inhibitory activity test of *Trichoderma* spp. consortium against *Pestalotiopsis* sp.

The inhibitory activity of *Trichoderma* spp. consortium against *Pestalotiopsis* sp. was tested using a modified double culture method. PDA medium was perforated using *a* 5 mm diameter *cork borer*, then *Trichoderma* spp. consortium suspension was added to the holes. The distance between the inoculation points of the consortium and *Pestalotiopsis* sp. was set at 4 cm, and the plates were incubated at room temperature for 3 days. The arrangement of fungi in this test can be seen in Figure 2

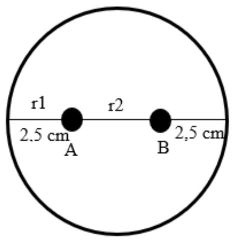



Figure 2. Scheme of fungal placement in the inhibitory activity test of *Trichoderma* spp. consortium against *Pestalotiopsis* sp. Legend: A = *Pestalotiopsis* sp. fungus, B = *Trichoderma* spp. fungus, r1 = distance

between colonies of Pestalotiopsis sp. that moved away from the Trichoderma spp. consortium, r2 = distance between colonies of Pestalotiopsis sp. that remained near the Trichoderma spp. consortium,

### 2.3 Observations

#### 2.3.1 Characteristics of the fungus causing leaf drop disease isolated from rubber plants

Macroscopic observations were conducted on the fungal pathogens causing leaf drop disease in rubber plants on days 3, 5, and 7 after incubation (hsi). These observations were performed visually by examining the color of the mycelium, its growth direction (upward or lateral), and the texture of the mycelium (smooth or rough). Meanwhile, microscopic observations were conducted on day 30 hsi using a binocular microscope with a magnification of 10 x 40. Microscopic identification was based on the descriptions in the book "*Introduction to Tropical Fungi*" [16] and the results of the study [7].

#### 2.3.2 Compatibility test of *Trichoderma* spp.

The compatibility test *for Trichoderma* spp. was observed daily for 7 days after the fungi were grown simultaneously on PDA medium. Observations were stopped when a clear zone appeared on the medium, indicating incompatibility between isolates. The results of these observations are presented in the form of tables and figures.

#### 2.3.3 Colony diameter of *Trichoderma* spp. and *Trichoderma* spp. consortium (mm)

Measurements of the diameter of *Trichoderma* spp. colonies and consortia were taken daily during the incubation period on PDA medium. Observations were stopped once one of the colonies in the treatment had covered the entire surface of the Petri dish. To facilitate measurement, the researcher used millimeter paper and drew vertical and horizontal lines intersecting at the center of the colony on the bottom of the Petri dish. The diameter of *Trichoderma* spp. and consortium colonies was calculated using the following formula:

$$D = \frac{d1 + d2}{2}$$

Notes:
D = diameter of *Trichoderma* spp. consortium colony
d1 = vertical diameter of the Trichoderma spp. consortium colony *Trichoderma* spp., d2 = horizontal diameter of the *Trichoderma* spp. consortium colony.

#### 2.3.4 Growth rate of colonies *Trichoderma* spp. and *Trichoderma* spp. fungal consortium (mm/day)

The growth rate of *Trichoderma* spp. colonies and *Trichoderma* spp. consortiums was determined by measuring the daily growth rate of the fungal colonies, then calculating the average growth rate until one of the Trichoderma spp. colonies or *Trichoderma* spp. consortiums filled the Petri dish. The calculation of the growth rate of *Trichoderma* spp. fungal colonies and *Trichoderma* spp. consortia was based on the following formula:

$$V = D(n+1) - Dn$$

Notes: V= , growth rate of the *Trichoderma* spp. consortium; Dn = diameter of the fungus on day n; Dn+1 = diameter of the fungus on day n+1.

#### 2.3.5 Inhibition test of *Trichoderma* spp. consortium against *Pestalotiopsis* sp. (%)

The inhibitory ability of the Trichoderma spp. consortium *Trichoderma* spp. against *Pestalotiopsis* sp. was measured daily after the pathogen and *Trichoderma* spp. consortium were co-cultured for 3 days, with observations ceased 30 days after co-cultivation. The percentage of inhibition of the *Trichoderma* spp. consortium against the growth of *Pestalotiopsis* sp. fungus was calculated using the formula:

$$P = (r1 - r2) / r1 \times 100\%$$

Notes: P = Inhibition rate, r1 = Radius of the pathogen colony moving away from the *Trichoderma* spp. consortium, r2 = Radius of the pathogen colony moving toward the *Trichoderma* spp. consortium.

### 2.4 Data Analysis

Data on the macroscopic and microscopic characteristics of the fungus causing rubber leaf drop and the compatibility test between *Trichoderma* spp. isolates were analyzed descriptively and presented in the form of tables and figures. Data on the diameter of *Trichoderma* spp. fungal colonies, growth rate of *Trichoderma* spp. fungal colonies, diameter of *Trichoderma* spp. consortium colonies, growth rate of *Trichoderma* spp. consortium, and inhibitory activity of *Trichoderma* spp. consortium against *Pestalotiopsis* sp. were statistically analyzed using analysis of variance at the 5% level using SPSS software. The linear model used in the analysis of variance is as follows:

$$Y_{ij} = \mu + K_i + €_{ij}$$

Notes:
$Y_{ij}$ = Observation results on one experimental unit with several concentrations of chitosan treatment i and replicate j
$\mu$ = General mean value
$K_i$ = Effect of several concentrations of chitosan i
$€_{ij}$ = Random effect of the concentration of chitosan i and repetition j

## 3. Results and Discussions

### 3.1 Macroscopic and microscopic characteristics of the fungus causing rubber leaf fall

The identification of the fungus causing rubber leaf drop disease includes macroscopic and microscopic characteristics, conducted in reference to [16] and the study [7] . The observation results can be seen in Table 1, Figure 10, and Figure 11.

Table 1. Macroscopic and microscopic characteristics of fungi causing leaf drop disease in rubber plants.

| Characteristic | Research Findings | Gandjar *et al.* (1999) and Kusdiana *et al.* (2020) |
|---|---|---|
| **Macroscopic:** | | |
| Colony color | White | White |
| Growth direction | Grows laterally | Grows laterally |
| Mycelium texture | Smooth | Smooth |
| **Microscopic:** | | |
| Hyphal structure | Hyaline and branched hyphae | Hyaline and branched hyphae |
| Conidia | Elongated to oval and hyaline in shape | Fusiform to oval-shaped conidia |

Macroscopic observations showed that the fungal colonies causing leaf drop disease on rubber plants had white mycelium, a smooth texture, and formed concentric circular patterns. On day 3 after incubation, the mycelium began to spread sideways with a smooth texture and white color (Figure 3A). On day 5 hsi, the colonies began to form concentric patterns (Figure 3B). On day 7, these patterns developed into several concentric circles resembling a flower shape (Figure 3C). Based on macroscopic and microscopic characteristics, the fungus was identified as *Pestalotiopsis* sp., supported by descriptions from [16] and [7] as shown in Table 1.


A B C

Figure 3. Macroscopic characteristics of the fungus causing rubber leaf drop disease on PDA medium (A) 3 hsi, (B) 5 hsi, (C) 7 hsi.

Observations also showed that the growth direction of the mycelium tended to spread sideways, with a smooth mycelium structure (Figure 3). This is consistent with the findings of [7], which stated that *Pestalotiopsis* sp. has a smooth colony texture and forms a flower-like pattern. Additionally, [17] reported that the colony of *Pestalotiopsis* sp. have smooth to wavy margins and white mycelium resembling cotton.

Further microscopic observations revealed that *Pestalotiopsis* sp. has branched hyphae and a hyaline color (Figure 4.1A). The conidia are elongated to oval in shape (Figure 4.1B). Research [18] also described that the microscopic characteristics of the *Pestalotiopsis* sp. fungus are that it has a fruiting body in the form of aservules, which contain conidia with 2–5 septa, thick walls, an elongated shape, and tapered at both ends. One end of the conidia has 3–5 whip-like hairs.

1

2


Figure 4. Microscopic characteristics of the fungus causing rubber leaf drop disease (1A and 1B) based on microscopic observations of *Pestalotiopsis* sp. (2A) according to [5] and (2B) according to [7] (A) branched and hyaline hyphae, (B) conidia.

## 3.2 Colony diameter of *Trichoderma* spp. fungi

Results of observations on the diameter of *Trichoderma* spp. fungal colonies *Trichoderma* spp. grown on PDA medium after statistical analysis using analysis of variance showed no significant effect. The average diameter of *Trichoderma* spp. fungal colonies can be seen in Table 2.

Table 2. Average diameter of Trichoderma spp. fungal colonies *Trichoderma* spp. on PDA medium at 3 hsi

| *Trichoderma* spp. | Average colony diameter (mm) |
|---|---|
| *Trichoderma* sp. | 90.0 |
| *T. koningii* | 89.00 |
| *T. pseudokonigii* | 87.5 |
| *T. viride* | 86.83 |
| *T. harzianum* | 86.66 |

The colony diameter results indicate that the four *Trichoderma* spp. fungal isolates, namely *T. koningii*, *T. pseudokoningii*, *T. viride*, and *T. harzianum,* have relatively similar average colony diameters. The respective averages are 90.00 mm, 89.00 mm, 87.50 mm, 86.83 mm, and 86.66 mm. The insignificant differences in average colony diameter indicate that *Trichoderma* spp. fungi have uniform growth capabilities under various conditions.

This uniformity of growth is thought to be due to the high adaptive ability of *Trichoderma* spp. to various environmental conditions. This is in line with [19], which states that *Trichoderma* spp. is a type of mold that can grow in various environmental conditions, has a fast growth rate, high spore productivity, and produces strong antibiotics that enable it to compete with other microbes in its surroundings. Additionally, *Trichoderma* spp. can adapt well to specific media and temperatures. The results of study [20] indicate that *Trichoderma* spp. can grow optimally at temperatures ranging from 15–20 °C. The similarity in average colony diameter can also be attributed to the uniformity of nutrients in the growth medium used, namely PDA medium. This medium contains carbohydrates, water, and protein from potato substrate, glucose, and agar. Research [21] states that carbon compounds in PDA serve as the primary energy source for fungal growth. Additionally, according to [22], carbon is the primary essential nutrient because fungi require carbon in larger quantities than other nutrients.

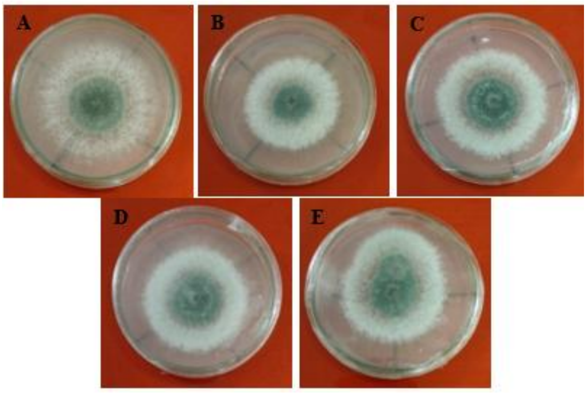
### 3.3 Growth rate of *Trichoderma* spp. fungal colonies

Observations of the growth rate of *Trichoderma* spp. fungal colonies over 3 days after inoculation on PDA medium showed that there were no statistically significant differences between isolates based on analysis of variance. The average growth rates of the colonies for each isolate were as follows: *Trichoderma* sp. (30.00 mm/day), T. *koningii* (29.66 mm/day), T. *pseudokoningii* (29.16 mm/day), T. *viride* (28.94 mm/day), and T. *harzianum* (28.88 mm/day) as shown in Table 3.

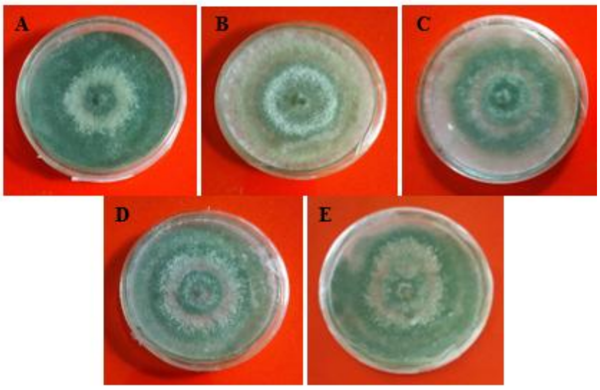
Table 3. Average growth rates of *Trichoderma* spp. fungi on PDA 3 hsi medium.

| *Trichoderma* spp. | Average growth rate (mm/day) |
|---|---|
| *Trichoderma* sp. | 30.00 |
| *T. koningii* | 29.66 |
| *T. pseudokonigii* | 29.16 |
| *T. viride* | 28.94 |
| *T. harzianum* | 28.88 |

The relatively small difference in growth rates is thought to be closely related to the availability of nutrients in the PDA growth medium. Nutrient factors such as sugar, polysaccharides, organic acids, and lipids as carbon sources greatly influence the growth and development of fungi, as stated by [23]. Additionally, according to [24], carbon plays a crucial role in cell growth and division, while nitrogen is essential for cell synthesis and providing a food source for fungi. The growth of *Trichoderma* spp. fungal colonies during the observation period can be observed in Figure 5.




3 HSI




7 HSI

Figure 5. Growth of *Trichoderma* spp. fungi on PDA medium. (A) *Trichoderma* sp, (B) *T. koningii,* (C) *T. pseudokoningii,* (D) T. *viride,* (E) T. *harzianum.*

### 3.4 Compatibility between *Trichoderma* spp. isolates

The results of the compatibility test of five *Trichoderma* spp. isolates showed that all five *Trichoderma* spp. isolates tested simultaneously on PDA medium exhibited compatible interactions between one isolate and another, as shown in Table 4 and Figure 6.

Table 4. Compatibility among *Trichoderma* spp. isolates on PDA medium at 7 hsi.

| Isolates tested | Compatibility |
|---|---|
| *T. pseudokoningii* + *T. koningii* | + |
| *T. pseudokoningii* + *T. harzianum* | + |
| *T. pseudokoningii* + *T. viride* | + |
| *T. pseudokoningii* + *Trichoderma* sp | + |
| *T. koningii* + *T. harzianum* | + |
| *T. koningii* + *T. viride* | + |
| *T. koningii* + *Trichoderma* sp | + |
| *T. harzianum* + *T. viride* | + |
| *T. harzianum* + *Trichoderma* sp | + |
| *T. viride* + *Trichoderma* sp | + |
| *T. pseudokoningii* + *T. koningii* + *T. harzianum* | + |
| *T. pseudokoningii* + *T. koningii* + *T. viride* | + |
| *T. pseudokoningii* + *T. koningii* + *Trichoderma* sp | + |
| *T. pseudokoningii* + *T. harzianum* + *T. viride* | + |
| *T. pseudokoningii* + *T. harzianum*+ *Trichoderma* sp | + |
| *T. pseudokoningii* + *T. viride* + *Trichoderma* sp | + |
| *T. koningii* + *T. harzianum* + *T. viride* | + |
| *T. koningii* + *T. viride* + *Trichoderma* sp | + |
| *T. harzianum* + *T. viride* + *Trichoderma* sp | + |
| *T. pseudokoningii* + *T. koningii* + *T. harzianum* + *T. viride* | + |
| *T. pseudokoningii* + *T. koningii* + *T. harzianum* + *Trichoderma* sp | + |
| *T. pseudokoningii* + *T. harzianum* + *T. viride*+ *Trochoderma* sp | + |
| *T. koningii* + *T. harzianum* + *T. viride* + *Trichoderma* sp | + |
| *T. pseudokoningii* + *T. koningii*+ *T. harzianum* + *T. viride* + *Trichoderma* sp | + |

Notes: (-) = incompatible, (+) = compatible

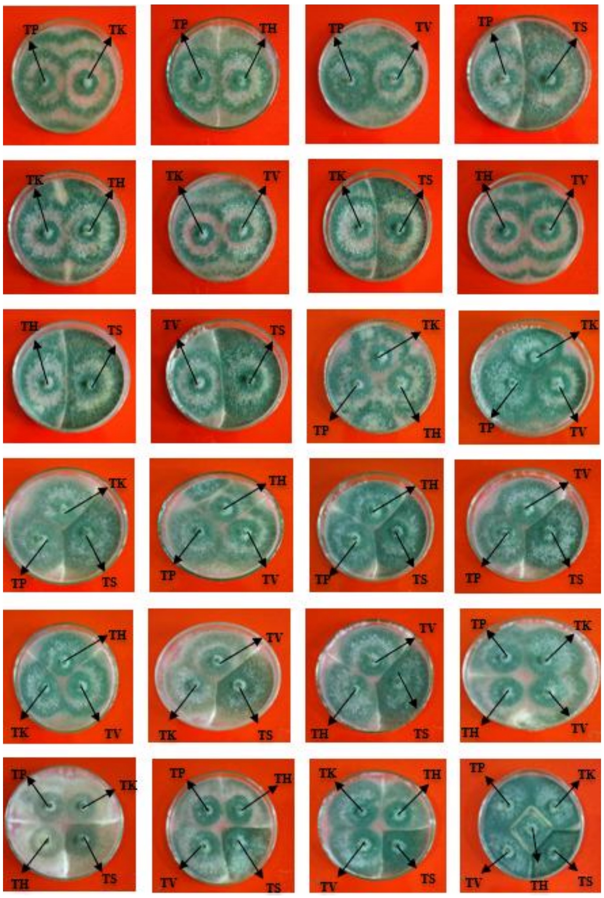


Figure 6. Compatibility test of five *Trichoderma* spp. fungal isolates on PDA medium at 7 hsi. TP = *T. pseudokoningii,* TK = *T. koningii,* TH = *T. harzianum,* TV = *T. viride,* and TS = *Trichoderma* sp.

The compatibility test results showed that the five *Trichoderma* spp. isolates grown simultaneously on PDA medium exhibited mutually supportive interactions. No inhibition zones were observed at the hyphae contact areas, and colony growth appeared to merge without mutual dominance. This phenomenon indicates that the isolates possess similar or highly similar physiological and morphological characteristics.

Generally, *Trichoderma* spp. colonies are green in color, although variations such as light green or yellowish hues have been observed in some cases [25]. Compatible interactions between isolates are indicated by normal growth, green spore color, no *overgrowth*, and the formation of distinct boundary lines between colonies [26].

These boundary lines indicate that there is no significant competition for space or nutrients. Spores and mycelium from each isolate can grow side by side in the same medium. Conversely, if incompatibility occurs between isolates, a clear zone or " " may appear, with one colony becoming more dominant than the others [27].

### 3.5 Diameter of *Trichoderma* spp. consortium

The results of observations on the diameter of *Trichoderma* spp. consortium colonies grown on PDA medium and analyzed statistically using analysis of variance showed significant effects. The results of the DNMRT test at the 5% level are presented in Table 5 .

Table 5. Average diameter of *Trichoderma* spp. consortium on PDA medium at 3 hsi.

| *Trichoderma* spp. Consortium | Average Colony Diameter (mm) |
|---|---|
| *T. pseudokoningii* + *T. koningii* | 90.00 a |
| *T. koningii* + *T. harzianum* | 90.00 a |
| *T. harzianum* + *T. viride* | 90.00 a |
| *T. pseudokoningii*+ *T. koningii*+ *T. harzianum*+ *Trichoderma* sp | 90.00 a |
| *T. pseudokoningii* + *Trichoderma* sp | 90.00 a |
| *T. koningii*+ *T. viride*+ *Trichoderma* sp | 90.00 |
| *T. koningii* + *Trichoderma* sp | 90.00 a |
| *T. pseudokoningii* + *T. koningii*+ *T. harzianum*+ *T. viride* | 90.00 a |
| *T. koningii* + *T. harzianum* + *T. viride*+ *Trichoderma* sp | 90.00 a |
| *T. pseudokoningii* + *T. koningii*+ *T. harzianum* + *T. viride* + *Trichoderma* sp | 89.83 a |
| *T. pseudokoningii* + *T. harzianum* | 89.83 a |
| + *T. pseudokoningii* + *T. harzianum89.83* a | 89.83 a |
| *T. pseudokoningii* + *T. harzianum* + *Trichoderma* sp | 89.83 a |
| *T. pseudokoningii* + *T. koningii*+ *Trichoderma* sp | 89.83 a |
| *T. pseudokoningii* + *T. koningii*+ *Trichoderma sp* | 89.83 a |
| *T. koningii*+ *T. harzianum*+ *T. viride* | 89.66 a |
| *T. koningii* + *T. viride* | 89.50 |
| *T. pseudokoningii* + *T. viride* | 89.50 a |
| *T. pseudokoningii* + *T. harzianum* + *T. viride* + *Trichoderma* sp | 89.16 a |
| *T. pseudokoningii* + *T. koningii*+ *T. viride* | 89.00 a |
| *T. viride* + *Trichoderma* sp | 88.50 a |
| *T. pseudokoningii* + *T. harzianum* + *T. viride* | 88.16 ab |
| *T. harzianum* + *Trichoderma* sp | 86.33 b |
| *T. harzianum* + *T. viride* + *Trichoderma* sp | 86.33 b |

Numbers followed by different lowercase letters indicate significant differences according to the DNMRT test results at the 5% level after transformation with $\sqrt{y + 0,5}$

The average colony diameter of the *T. harzianum* + *Trichoderma* sp. consortium and the *T. harzianum* + *T. viride* + *Trichoderma* sp. consortium was 86.33 mm. Both consortia showed no significant differences from each other and were not significantly different from the consortium of T. *pseudokoningii* + , *T. harzianum* + , and *T. viride*, which had an average diameter of 88.16 mm. However, all three consortia showed significant differences from other *Trichoderma* spp. consortia.

Differences in average colony diameter are likely related to the compatible interactions between *Trichoderma* species after being combined into a consortium. Harmonious combinations between isolates allow colony growth to proceed simultaneously without mutual inhibition. This condition is consistent with reports stating that compatible *Trichoderma* spp. isolates exhibit similarities in growth rate, spore formation patterns, and spore color [28].
Additionally, variations in colony diameter among consortia may be linked to nutrient utilization. The volume of PDA medium used for each consortium was 20 ml, while each consortium consisted of more than one

species. Therefore, nutrient requirements become more complex and demand balanced availability to maintain optimal growth. This aligns with the statement that fungal growth is highly influenced by nutrient availability; both deficiencies and excesses of nutrients can inhibit colony development [29]. The appearance of *Trichoderma* spp. consortium isolates on PDA medium at 7 hsi can be seen in Figure 7.

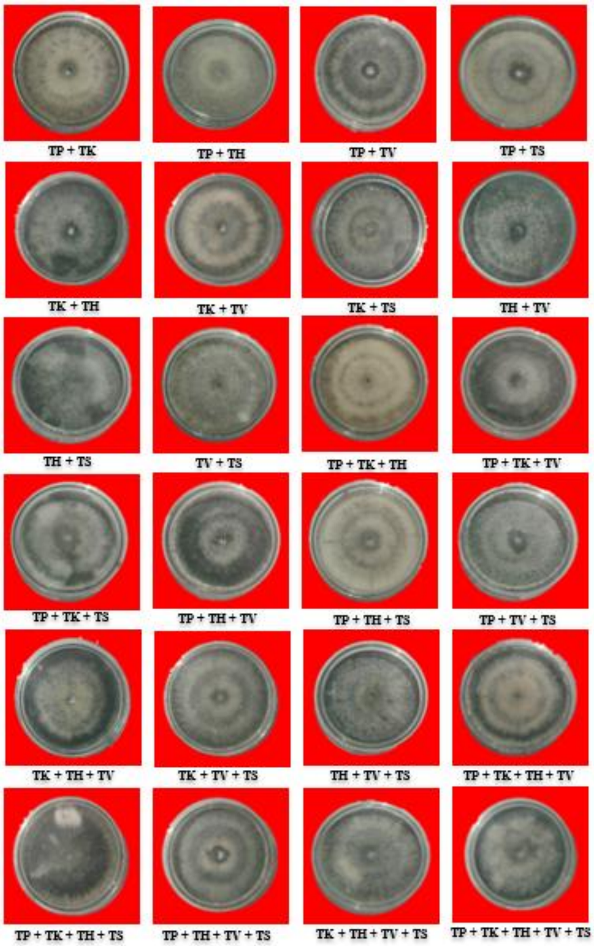



Figure 7. Appearance of *Trichoderma* spp. consortium isolates on PDA medium at 7 hsi. Legend: TP = *T. pseudokoningii,* TK = *T. koningii,* TH = *T. harzianum,* TV = *T. viride,* and TS = *Trichoderma* sp.

### 3.6 Growth rate of *Trichoderma* spp. consortium

The results of growth rate observations of *Trichoderma* spp. consortium colonies re-cultured on PDA medium, analyzed statistically using analysis of variance, showed significant effects. The results of the post-hoc DNMRT test at the 5% level are presented in Table 6.

Table 6. Average growth rate of *Trichoderma* spp. consortium on PDA medium at 3 hsi.

| *Trichoderma* spp. consortium | Average Growth Rate (mm/day) |
|---|---|
| *T. pseudokoningii* + *T. koningii* | 30.00 a |
| *T. koningii* + *T. harzianum* | 30.00 a |
| *T. harzianum* + *T. viride* | 30.00 a |
| *T. pseudokoningii*+ *T. koningii*+ *T. harzianum*+ *Trichoderma* sp | 30.00 a |
| *T. pseudokoningii* + *Trichoderma* sp | 30.00 a |
| *T. koningii*+ *T. viride*+ *Trichoderma* sp | 30.00 |
| *T. koningii* + *Trichoderma* sp | 30.00 |
| + *T. pseudokoningii* + *T. koningii*+ *Trichoderma sp* | 30.00 a |
| *T. koningii* + *T. harzianum* + *T. viride*+ *Trichoderma* sp | 30.00 a |
| *T. pseudokoningii* + *T. koningii*+ *T. harzianum* + *T. viride* + *Trichoderma* sp | 29.94 a |
| *T. pseudokoningii* + *T. harzianum* | 29.94 a |
| *T. pseudokoningii* + *T. harzianum*+ *Trichoderma* sp | 29.94 a |
| *T. pseudokoningii* + *T. harzianum* + *Trichoderma* sp | 29.94 a |
| *T. pseudokoningii* + *T. koningii*+ *Trichoderma* sp | 29.94 a |
| *T. pseudokoningii* + *T. koningii*+ *Trichoderma sp* | 29.94 a |
| *T. koningii*+ *T. harzianum*+ *T. viride* | 29.90 a |
| *T. koningii* + *T. viride* | 29.84 a |
| *T. pseudokoningii* + *T. viride* | 29.83 a |
| *T. pseudokoningii* + *T. harzianum* + *T. viride* + *Trichoderma* sp | 29.73 |
| *T. pseudokoningii* + *T. koningii*+ *T. viride* | 29.67 a |
| *T. viride* + *Trichoderma* sp | 29.50 a |
| *T. pseudokoningii* + *T. harzianum* + *T. viride* | 29.40 ab |
| *T. harzianum* + *Trichoderma* sp | 28.77 b |
| *T. harzianum* + *T. viride* + *Trichoderma* sp | 28.77 b |

Numbers followed by different lowercase letters indicate significant differences according to the DNMRT test results at the 5% level after transformation with $\sqrt{y+0,5}$

The consortium of *T. harzianum ( + ) Trichoderma* sp. and *T. harzianum ( + ) T. viride ( + ) Trichoderma* sp. showed the same average growth rate of 28.77 mm/day. This value is not significantly different between the two or compared to the consortium of T. *pseudokoningii* + *T. harzianum* + *T. viride,* which has an average growth rate of 29.40 mm/day. However, all three consortia show significant differences when compared to other *Trichoderma* spp. consortia.

The difference in growth rates is thought to be related to the level of compatibility between isolates within a consortium. Compatible combinations allow fungi to grow harmoniously without inhibiting each other, and exhibit similar growth and sporulation patterns [28].

In addition to species interactions, nutrient availability also influences growth rate. In all treatments, the volume of growth medium (PDA) used was the same, namely 20 ml, while each consortium consisted of several species requiring different nutrient intakes. An imbalance between the amount of nutrients available and the total requirements of each consortium can affect growth effectiveness. In line with this, fungal growth will be optimal if nutrients are available in sufficient and balanced quantities; both nutrient deficiencies and excesses can disrupt the growth process [29].

### 3.7 Inhibitory effect of *Trichoderma* spp. consortium on the growth of *Pestalotiopsis* sp.

The results of the inhibitory effect of *Trichoderma* spp. consortium on the growth of *Pestalotiopsis* sp. fungus, analyzed statistically using analysis of variance, showed significant results. The results of the DNMRT test at the 5% level can be seen in Table 7.

Table 7. Average inhibitory activity of the *Trichoderma* spp. consortium against the growth of *Pestalotiopsis* sp. fungus on PDA medium at 3 hsi.

| *Trichoderma* spp. consortium | Average Inhibitory Activity (%) |
|---|---|
| *T. pseudokoningii* + *T. koningii* | 35.00 a |
| *T. koningii* + *T. harzianum* | 34.00 a |
| *T. pseudokoningii* + *T. harzianum* + *T. viride*+ *Trichoderma* sp | 27.33 ab |
| *T.* pseudokoningii + *T. viride*+ *Trichoderma* sp | 27.33 ab |
| *T. pseudokoningii* + *T. koningii* + *T. harzianum* | 26.66 ab |
| *T. viride* + *Trichoderma* sp | 25.33 abcd |
| *T. koningii* + *T. viride*+ *Trichoderma* sp | 25.00 abcd |
| *T. koningii* + *T. harzianum* + *T. viride* | 25.00 abcd |
| *T. pseudokoningii* + *T. koningii* + *T. harzianum* + *Trichoderma* sp | 25.00 abcd |
| *T. pseudokoningii* + *Trichoderma* sp | 24.33 abcd |
| *T. pseudokoningii* + *T. harzianum*+ *Trichoderma* sp | 23.33 abcd |
| *T. koningii*+ *T. viride* | 20.33 abcde |
| *T. pseudokoningii* + *T. koningii* + *T. viride* | 20.33 abcde |
| *T. pseudokoningii* + *T. viride* | 17.66 bcde |
| *T. pseudokoningii* + *T. harzianum*+ *T. viride* | 17.00 bcde |
| *T. koningii* + *Trichoderma* sp | 16.66 bcde |
| *T. pseudokoningii* + *T. koningii*+ *T. harzianum* + *T. viride* | 16.66 bcde |
| *T. pseudokoningii* + *T. koningii*+ *T. harzianum* + *T. viride*+ *Trichoderma* sp | 16.00 bcde |
| *T.* pseudokoningii + *T.* koningii+ *Trichoderma* sp | 15.33 bcde |
| *T. koningii*+ *T. harzianum* + *T. viride*+ *Trichoderma* sp | 14.33 bcde |
| *T. harzianum*+ *Trichoderma sp* | 13.66 cde |
| *T. pseudokoningii* + *T. harzianum* | 12.66 de |
| *T. harzianum*+ *T. viride* | 10.66 e |
| *T. harzianum* + *T. viride*+ *Trichoderma* sp | 9.33 |

The numbers followed by different lowercase letters are significantly different according to the results of the DNMRT test at the 5% level after transformation with the arc *sine*√

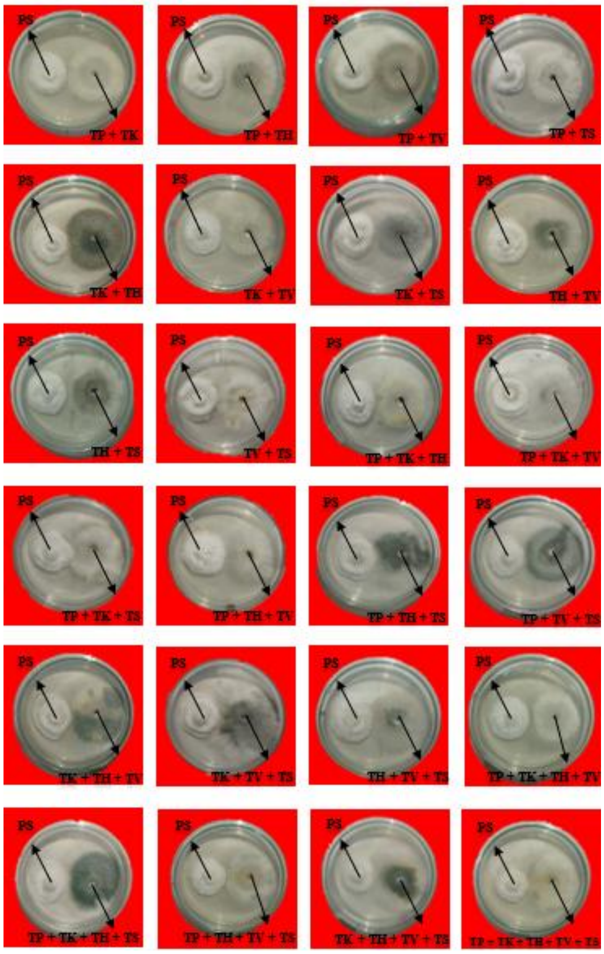



Figure 8. Inhibitory activity of *Trichoderma* spp. consortium against *Pestalotiopsis* sp. on PDA medium at 3 hsi. Legend: PS = *Pestalotiopsis* sp., TP = *T. pseudokoningii,* TK = *T. koningii,* T H = *T. harzianum,* TV = *T. viride,* and TS = *Trichoderma* sp.


Figure 9. Inhibitory activity of *Trichoderma* spp. consortium against *Pestalotiopsis* sp. on PDA medium at 30 hsi. Notes: PS = *Pestalotiopsis* sp, TP = *T. pseudokoningii,* TK = *T. koningii,* TH = T. *harzianum,* TV = T. *viride,* and TS = *Trichoderma* sp.

The percentage of inhibitory activity of the consortium *T. pseudokoningii* + *T. koningii* against the fungus *Pestalotiopsis* sp. was significantly higher at 35.00% and not significantly different from the consortium *T. koningii* + *T. harzianum, T. pseudokoningii* + *T. harzianum* + *T. viride* + *Trichoderma* sp, *T. pseudokoningii* + *T. viride* + *Trichoderma* sp, T. *pseudokoningii* + *T. koningii* + *T. harzianum, T. viride* + *Trichoderma* sp, T. *koningii* + *T. viride* + *Trichoderma* sp, T. *koningii* + *T. harzianum* + *T. viride, T. pseudokoningii* + *T. koningii* + *T. harzianum* + *Trichoderma* sp, T. *pseudokoningii* + *Trichoderma* sp, T. *pseudokoningii* + *T. harzianum* +*Trichoderma* sp, T. *koningii* + *T. viride, T. pseudokoningii* + *T. koningii* + *T. viride,* and significantly different from other *Trichoderma* spp. consortia. The consortium of T. *harzianum* + *T. viride*+ *Trichoderma* sp. had a lower inhibition percentage of 9.33%, which was not significantly different from the consortium of T. *harzianum* + *T. viride*, which had an inhibition percentage of 10.66%, as shown in Table 7.

The consortium *of T. pseudokoningii* + *T.* koningii , and the consortium *of T. koningii* + *T. harzianum*, when compared to the consortium of *Trichoderma* spp., demonstrated better inhibitory activity in suppressing the growth of *Pestalotiopsis* sp., the fungus causing leaf drop disease in rubber. This is because the consortium treatment had a larger average diameter of 90.00 mm and a faster growth rate of 30.00 mm/day, enabling it to enhance spatial competition with *Pestalotiopsis* sp. This aligns with the study by[30] , which states that antagonistic fungi should have faster growth rates to outcompete pathogenic fungi in terms of space dominance and inhibit their growth. High growth rates determine the activity of antagonistic microorganisms against target pathogens.

The percentage of inhibitory activity of the *Trichoderma* spp. consortium against the growth of *Pestalotiopsis* sp. ranged from 9.33% to 35.00%. These values are categorized as weak or moderate inhibition. This aligns with the criteria outlined by [31], where inhibition rates below 30% are classified as weak, above 40% as strong, and 0% indicates no inhibitory activity.

This relatively low level of inhibition is thought to be related to the fermentation stage carried out prior to testing. This process allows the formation of secondary metabolites produced by the *Trichoderma* spp. consortium. As explained by [32], secondary metabolites are organic compounds that do not play a direct role in growth or reproduction but are produced when the growth phase enters a metabolically stable condition. The characteristics and effectiveness of the antifungal compounds produced are highly dependent on the genetic factors of the species and strain, which influence the quantity and type of metabolites produced [32].

Another factor that also influences the results is the testing method used. Observations were conducted using the well method, a modification of the *dual culture* method, because the *Trichoderma* spp. consortium was used in the form of an activated and fermented suspension. Generally, the conventional *dual culture* method involves the direct inoculation of antagonistic fungi and pathogens into a single petri dish. The well method is rarely used in such studies because it is more complex in terms of handling [33].

Macroscopic observations on the third day after inoculation (hsi) showed that the mycelium of the *Trichoderma* spp. consortium began to grow toward the *Pestalotiopsis* sp. colonies. This phenomenon indicates a mechanism of mycoparasitism, characterized by the formation of hyphal branches toward the target. As described by [34], this growth is triggered by a response to the presence of α-lectin proteins bound to chitin in the pathogen's cell wall.

The percentage of inhibitory activity of the *Trichoderma* spp. consortium on day 3 hsi was classified as weak, while development on day 30 showed different results. The *Trichoderma* spp. consortium appeared more dominant and was able to significantly suppress the growth *of Pestalotiopsis* sp. At this stage, a mechanism of competition for space and nutrients occurred, characterized by the dominance of the consortium's mycelium in filling the Petri dish, covering the pathogenic fungal colonies. This condition was caused by the limited availability of nutrients in the medium used. In line with [35], competition occurs when two microorganisms require the same nutrient source directly. The PDA medium used in this study contains carbohydrates, amino acids, proteins, as well as minerals and micronutrients required by both types of fungi [36].

In addition to competition for nutrients and space, the mechanism of mycoparasitism is also becoming increasingly apparent. The *Trichoderma* spp. consortium successfully covered the colonies and hyphae *of Pestalotiopsis* sp., even growing on top of them, causing the pathogenic hyphae to become increasingly thinner. This activity is associated with the production of enzymes and antibiotic compounds such as trichodermin and gliotoxin, which are known to be effective in suppressing the growth of pathogenic fungi [37].

## 4. Conclusion

The cause of leaf drop disease in rubber plants in Rimbo Panjang Village, km 22, Tambang Subdistrict, Kampar District is *Pestalotiopsis* sp. The use of a *Trichoderma* spp. consortium is effective and capable of inhibiting the growth of *Pestalotiopsis* sp. The consortium of T. *pseudokoningii* + *T. koningii* and the consortium of T. *koningii* + *T. harzianum* were more effective in inhibiting the growth of *Pestalotiopsis* sp., the causative agent of leaf drop disease in rubber plants, *in vitro*, with inhibition percentages of 35.00% and 34%.

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