In-Vitro antagonistic study of indigenous *Trichoderma* sp. from Temanggung, Indonesia against tomato leaf mold pathogen

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Abstract. Leaf mold disease in tomato (Lycopersicum esculentum), caused by Passalora fulva, is a destructive pathogen that leads to leaf wilting, defoliation, and reduced crop yield. In Indonesia, this disease is increasingly problematic, and current control methods rely on chemical fungicides that harm soil and the environment. This study investigates the antagonistic potential of indigenous Trichoderma sp. isolated from the rhizosphere of bamboo, mahogany, and coffee trees in Temanggung, Indonesia. Fungal isolates were cultured on Potato Dextrose Agar (PDA) and identified macroscopically and microscopically. Using a Completely Randomized Design (RAL), dual culture and culture filtrate inhibition tests were conducted on one control and 3 treatments with 3 replications. Data were analyzed via ANOVA and further tested using the 5% Least Significant Difference (LSD) method. Results showed significant inhibition of P. fulva by Trichoderma sp., with dual culture inhibition rates of T1 (85.4%), T2 (83.4%), and T3 (80.6%), and culture filtrate inhibition rates of T1 (46.7%), T2 (27.1%), and T3 (33.2%). The bamboo rhizosphere isolate (T1) demonstrated the highest antagonistic activity in both methods. These findings suggest that Trichoderma sp. from bamboo rhizosphere serves as a natural biocontrol agent and act as an eco-friendly alternative to chemical fungicides.

1 Introduction

Tomato plants (*Lycopersicum esculentum*) are common agricultural crops with a unique flavor, characterized by a blend of sweetness and acidity, claimed that the high levels of lycopene, citric acid, and malic acid found in tomato fruits have anticancer qualities as well as the ability to lower the risk of hypertension and other cardiovascular disorders [1]. Tomatoes with high source of vitamins and minerals have numerous benefits, including serving as a vegetable addition, a beverage, an appetite stimulant due to their mineral content, and they can even be used as a cosmetic ingredient [2]. In Indonesia, the market need for tomatoes is still growing. In terms of Indonesia's vegetable export volume and value in 2023,

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tomatoes are ranked fifth. It appears that the lack of variety, quantity, quality, and supply continuity are the primary problems with Indonesian tomato production. [3].

Furthermore, many challenges are still faced by tomato farmers, one of which is the presence of pests and diseases. These attacks caused by insects or pathogens lead to various diseases such as fruit rot, leaf mold disease, and fusarium wilt disease. *Passalora fulva* is one of the organisms responsible for tomato leaf mold, and this fungal disease was first described by Cooke in 1883. The initial symptoms of the disease appear as early as 7-10 days after the initial infection, manifesting as pale green or yellowish spots on the upper leaf surface, which then enlarge and turn into yellowish spots. Initially, this disease attacks the lower leaves and then spreads to the upper parts, ultimately affecting the entire plant and causing it to die [4].

The management of tomato leaf mold disease often involves the use of chemical fungicides, which can lead to environmental degradation and the development of resistance in the disease against commonly used fungicides, making them more potent [5]. One environmentally friendly approach is to use antagonistic microorganisms to suppress plant diseases, as an alternative to synthetic fungicides, commonly known as biofungicides. One of the biofungicides that can be employed is the fungus *Trichoderma* sp. The utilization of *Trichoderma* sp. as a biofungicide is reported to have several advantages, including safety of application, the production of limited and controlled toxins, and chitinase enzymes that are effective in inhibiting the growth of various plant pathogenic fungi [6]. *Trichoderma* sp. is a genus of worldwide filamentous fungi found in all soil type especially rhizosphere and climate zones. They are saprophytic organisms, meaning they can colonize and decompose decaying organic substances. They also have parasitic properties toward other fungus, as well as symbiotic and endophytic capacities toward plants which help in resistance towards pathogens [7].

The aim of this research is to determine the ability of the antagonistic fungus Trichoderma sp., isolated from the Temanggung region in Indonesia from the rhizosphere soil to inhibit the growth of the pathogenic fungus *Passalora fulva*, the causative agent of leaf mold disease in tomato plants (*Lycopersicum esculentum*), through in vitro experiments evaluating the antagonistic potential.

2 Methods

The research was conducted at the Pest and Disease Observation Laboratory/LPHP in Temanggung as well as a total of three *Trichoderma* sp. isolates were collected from the area. These three isolates were obtained from soil isolations in the rhizosphere of bamboo, mahogany, and coffee plants. The antagonistic test used the dual culture and culture filtrate methods as well as samples were tested and collected using the Completely Randomized Design (CRD) of three replications for each sample. The equipment used in this study includes Laminar Air Flow (LAF), incubator, autoclave, petri dish, inoculation needles, Bunsen burner, aluminium foil, measuring glass; scissors; knife, cork borer, scalpel, Erlenmeyer flask, centrifuge, Whatman filter paper, plastic wrap, microscope, tissues, and toothpicks. The materials used in this research are local potatoes, potato dextrose agar (PDA), 70% alcohol, distilled water, NaOCl 3%, ethanol, chloramphenicol antibiotics for inhibiting bacterial growth, *Trichoderma* sp. fungus isolate, and *Passalora fulva* fungus identified from the laboratory.

2.1 Isolation of *Trichoderma* sp. and *Passalora fulva* Fungus

Three isolates of *Trichoderma* sp. obtained from LPHP Temanggung were recultured on PDA media inoculated a colony of *Trichoderma* sp. and then incubated for 7 days [8]. There

were three *Trichoderma* sp. isolates originated from different areas and Rhizosphere soils in Temanggung listed in Table 1.

Table 1. Origin of *Trichoderma* sp. Isolates

Isolate Code	Origin of Isolate	Plant Type
T1	Kandangan	Coffee (*Cofea* sp.)
T2	Ngadirejo	Mahogany (*Swietenia* sp.)
T3	Mbulu	Bamboo (*Gigantochloa* sp.)

The observation of the morphology of the obtained isolates was conducted both macroscopically and microscopically, involving the examination of colony shape, color, fungal growth direction, and surface characteristics. Morphological features of *Trichoderma* sp. including conidiophore shape, phialide, and conidia were examined. The identification of *Trichoderma* sp. morphology was based on the observational identification [9].

Microscopic observations were conducted using slide cultures. The inside of a Petri dish was lined with a circular tissue, and sterile distilled water was added to provide optimum humidity for fungal growth. On top of the tissue, two sterile toothpicks were placed, and above them, a microscope slide containing 1 cm^2 pieces of PDA was positioned. *Trichoderma* sp. was gently scraped onto the PDA pieces using an inoculation needle and then incubated for 24 hours. Observations were made using a microscope, and a coverslip was placed for identification purposes [10].

Furthermore, the isolation process for the Plant samples affected by *Passalora fulva* fungus were collected from rice fields and tomato plantations owned by farmers in the Kedu area, Temanggung, which showed symptoms of leaf mold disease. Isolation was carried out by immersing the leaves infected with *Passalora fulva* in 70% alcohol and 3% NaOCL for 2 minutes, followed by rinsing with sterile distilled water. The infected parts of the leaves, approximately 1×1 cm in size, were then cut and placed on the surface of PDA (Potato Dextrose Agar) and incubated at room temperature until the fungus grew on the Petri dish [11]. The fungus species was then identified and kept in the laboratory.

2.2 Dual culture method for in-vitro antagonistic test

The test was conducted by taking each isolate of *Trichoderma* sp. and *Passalora fulva* using a cork borer with a diameter of 0.5 cm. The *Trichoderma* sp. isolates were placed on PDA in a Petri dish with a distance of 3 cm from the edge of the Petri dish, and the *Passalora fulva* isolates were placed 3 cm from the opposite edge of the Petri dish, facing the *Trichoderma* sp. As a control, *Passalora fulva* isolates were placed on PDA media in a Petri dish without *Trichoderma* sp. The cultures were incubated at room temperature for 7 days. The parameter observed for calculation in this study was the percentage of growth inhibition (PGI) [12], is determined based on the equation:

$$P\ (\%) = ((R1-R2))/R1 \times 100\%$$

Agenda:
P : Percentage Growth Inhibition (%)
R1 : Radius of the *Passalora fulva* colony growing opposite to the antagonistic microorganism
R2 : Radius of the *Passalora fulva* colony growing towards the antagonistic microorganism (*Trichoderma* sp.)

2.3 Culture filtrate method for in-vitro antagonistic test

Trichoderma sp. was grown on PDB media for 7 days. The culture was then filtered using Whatman filter paper to separate the fungal structures from the liquid. The filtrate was centrifuged for 20 minutes at 4000 rpm and then filtered again using Whatman filter paper. The *Trichoderma* sp. filtrate was mixed with liquid PDA media at a ratio of 1:9. Once it solidified, *Passalora fulva* was placed in the center of a Petri dish by cutting a 0.5 cm diameter piece of *Passalora fulva* on PDA and placed it in the middle of the Petri dish. The culture was then incubated at room temperature for 7 days. For the control treatment, only PDA media without the addition of *Trichoderma* sp. filtrate was used.

The parameters observed in the culture filtrate test are the diameter of the *Passalora fulva* fungus compared to the diameter of the control fungus, as well as the percentage inhibition of *Trichoderma* sp. on the growth of the pathogen colony using the following formula [12]:

$$P\ (\%) = ((DK-DP))/DK \times 100\%$$

Agenda:
P : Percentage Growth Inhibition (%)
DK : Diameter of the *Passalora fulva* control colony:
DP : Diameter of the *Passalora fulva* colony in the control group.

2.4 Data Analysis

Data processing was conducted using a one-way ANOVA test at a 5% significance level. The one-way ANOVA test aimed to determine the differences in antagonistic activity among each treatment. If the data analysis results show a significant effect of the treatment, further tests were carried out using the Least Significant Difference (BNT) test of 5% significance level.

3 Results and discussions

Based on the analysis of three *Trichoderma* sp. species isolated from the roots of bamboo, coffee, and mahogany plants in the Temanggung district, differences in colony color were observed, but the colony shapes exhibited similarities. They all appeared as circular colonies that filled the culture medium, had thread-like hyphae, and unevenly scattered spores on the upper part of the hyphae as shown in Figure 1. Colony colors varied after 7 days of inoculation, ranging from dark green, greenish yellow to bluish green. White color in the colonies represented hyphae, while the green color indicated spores/conidia. Microscopic characteristics showed no significant differences among the three isolates. In the microscopic observation of isolate T1, the isolate had upright branched conidiophores arranged vertically, short phialides, and oval-shaped conidia. In isolate T2, the conidiophores had a branched form, and masses of spores (conidia) were present on each phialid. The phialides were vertical, short, and thick, and the conidia were also round. In isolate T3, the conidiophores were upright and branched, arranged vertically, phialides paired and narrowing at their ends, and the conidia were oval-shaped.

Based on the macroscopic and microscopic observations, in accordance with the identification, the results indicate that the isolate from bamboo roots is *Trichoderma harzianum*, the isolate from mahogany roots is *Trichoderma aureoviride*, and the isolate from coffee roots is *Trichoderma koningii* [13].

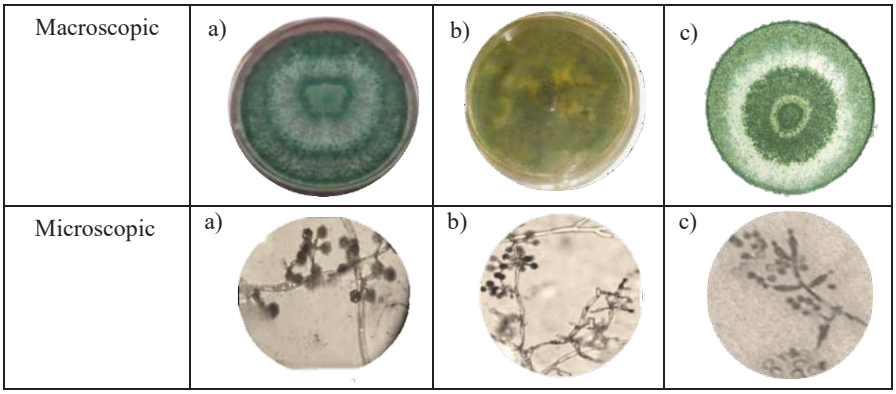

Figure 1. The three *Trichoderma* species isolated; a) *Trichoderma* from bamboo roots (T1), b) *Trichoderma* from mahogany roots (T2), c) *Trichoderma* from coffee roots (T3).

3.1 In-vitro antagonism test with dual culture method

Observations of pathogen inhibition percentage were conducted on the 7th day after inoculation. Antagonistic activity testing at 7×24 hours showed that *Trichoderma* sp. fungi have the potential to inhibit the growth of *Passalora fulva*. In this study, the diameter of *Passalora fulva* tested with *Trichoderma* sp. on the 5th and 7th day exhibited a smaller diameter compared to the control *Passalora fulva* as indicated in Table 2.

Table 2. Diameter of *Passalora fulva* Colony

	Colony Diameter (cm) based on days		
Treatment	**3**	**5**	7
Control (*Passalora fulva*)	2,57	4,83	6,10
Passalora fulva + T1	1,52	1,20	1,24
Passalora fulva + T2	1,03	1,27	1,30
Passalora fulva + T3	0,98	1,19	1,24

Based on the results of the 5% LSD test, it is evident that the antagonistic abilities, expressed as percentages, differ among the three *Trichoderma* sp. fungi as shown in Table 3. This indicates that *Trichoderma* sp. fungi have the potential to inhibit the growth of *Passalora fulva* as mentioned in Table 2.

Table 3. The percentage of inhibition of the antagonistic test.

Treatment	**Percentage of Inhibition (%)**
Control (*Passalora fulva*)	0*a*
Passalora fulva + T1	85,4*cd*
Passalora fulva + T2	80,61*b*
Passalora fulva + T3	83,4b*c*

Notes: Numbers followed by different letters are significantly different in the post-hoc LSD test at a 5% significance level.

Based on statistical analysis, the treatments *Trichoderma* T1×*Trichoderma* T3 and *Trichoderma* T2×*Trichoderma* T3 against *Passalora fulva* have the same notation, indicating that these treatments are not significant or do not show a significant difference. However, when compared to the treatment *Trichoderma* T0 and *Trichoderma* T2, they have different

notations, meaning that there is a significant difference between them. The percentage of *Passalora fulva* pathogen inhibition by *Trichoderma* sp. fungi that showed the highest result was the Trichoderma T1 treatment with a result of 85.4%, followed by *Trichoderma* T3 and *Trichoderma* T2 with percentages of 83.4% and 80.6%, respectively.

The results of this test indicate varying percentages of inhibition, with all three isolates having inhibition percentages above 60%. This means that all three isolates possess a high antagonistic capability to inhibit the growth of *Passalora fulva*. Inhibition percentages reaching 30% represent minimal inhibitory activity, and when inhibition percentages exceed 60%, it can be said that the antagonistic fungus has a high effectiveness against the pathogen [13,14]. This is because the faster the growth of the fungal colony, the more antibiotic substances are produced to degrade the cell walls of the pathogenic fungus, resulting in a higher antagonistic percentage.

3.2 In-Vitro Antagonism test with culture filtrate method

It was observed that the growth diameter of the control treatment (without *Trichoderma* sp.) was higher compared to the diameter of *Passalora fulva* treated with *Trichoderma* sp. The data of the control diameter and the mean diameter treated with *Trichoderma* sp. is presented in Table 4.

Table 4. Diameter of *Passalora fulva* Colony culture filtrate in cm.

Treatment (day)	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Control (*Passalora fulva*)	0,65	1,04	2,57	3,98	4,83	5,20	6,10
Passalora fulva + T1	0,45	0,84	1,48	1,96	2,20	2,95	3,24
Passalora fulva + T2	0,70	1,03	1,98	2,82	3,23	3,90	4,54
Passalora fulva + T3	0,63	0,92	1,30	1,84	2,43	3,19	4,07

In the growth of the *Passalora fulva* fungus in the culture filtrate test, when observed in terms of the daily growth rate, it can be seen that the pathogen treated with *Trichoderma* sp. filtrate exhibited inhibited growth rates. In the testing using Trichoderma sp. filtrate, it is suspected that the inhibitory ability is due to the content of enzymes and toxins. Enzymes and toxins produced by Trichoderma can inhibit the pathogen by damaging the cell walls of the pathogen [14].

The results indicated that all tested filtrates of *Trichoderma* sp. isolates were able to inhibit the growth of *Passalora fulva*. The filtrate of *Trichoderma* sp. isolate with the highest inhibition percentage after 7 days of inoculation was from *Trichoderma* T1 isolate, which was 45.67%, followed by *Trichoderma* T3 with 33.23%, and *Trichoderma* T2 with 27.16%. The results of the percentage inhibition analysis for culture filtrate can be seen in Table 5.

Table 5. Percentage of Inhibition in Culture Filtrate Test

Treatment	Percentage of inhibition (%)
Control (T0)	0*a*
P. fulva T1	46,67*d*
P. fulva T2	27,16*b*

Notes: Numbers followed by different letters are significantly different in the post-hoc LSD test at a 5% significance level.

The statistical results of the *Trichoderma* sp. filtrate test on the growth of Passalora fulva shows that all tested filtrates of *Trichoderma* sp. isolates inhibited *Passalora fulva*. The results indicate that different *Trichoderma* sp. isolates yield different outcomes. Based on the statistical analysis, treatments with T2 and T3 against *Passalora fulva* have the same notation, meaning these treatments are not significant or do not exhibit a significant difference. However, when compared to the treatment with T1, it has a different notation, signifying a significant and distinct treatment effect. The filtrate of *Trichoderma* sp. isolate with the highest inhibition percentage was from *Trichoderma* T1 isolate at 46.67%, followed by T3 at 33.23%, and T2 at 27.16%. *Trichoderma* sp. filtrate exhibited better capabilities in inhibiting the growth of pathogenic fungi compared to other antagonistic fungi. This is due to the fact that different *Trichoderma* sp. species or isolates within the same species can produce various types of secondary metabolites, resulting in differences in their ability to inhibit the growth of pathogenic fungi [15].

4 Conclusion

The results of antagonistic inhibition tests indicate that in both dual culture and culture filtrate tests, *Trichoderma* sp. can inhibit the growth of *Passalora fulva*. The highest inhibitory capability against *Passalora fulva* was observed in *Trichoderma* T1 isolate obtained from bamboo root isolation, with an inhibition rate of 85.4% in dual culture and 45.67% in culture filtrate.

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