J. Trop. Plant Pests Dis.
Vol. 24, No. 1, March 2024
Pages: 128–138

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.124128-138

RESEARCH PAPER

Molecular identification of *Trichoderma* sp. Margodadi isolate and its potential against *Phytophthora capsici* causing foot rot of black pepper

Joko Prasetyo[1], Nur Baitullah Juniar[2], Rusdi Evizal[2], Tri Maryono[1], & Cipta Ginting[1]

Manuscript received: 11 August 2023. Revision accepted: 12 December 2023. Available online: 15 March 2024.

ABSTRACT

Trichoderma has the potential to suppress fungal pathogens and thus control plant diseases, including Phytophthora foot rot, which is the most devastating disease of black pepper in Lampung. Identification of a microorganism can not only rely on its morphological characteristics, but it is also necessary to identify it molecularly at the species level. This research was aimed at identifying the fungus *Trichoderma* sp. Margodadi isolates at the species level and to know the potential of *Trichoderma* sp. Margodadi isolates and their secondary metabolites to control *P. capsici*. This research was conducted from March to November 2021 at the Laboratory of Plant Disease, Department of Plant Protection, and the Laboratory of Agricultural Biotechnology, Faculty of Agriculture, University of Lampung. Identification of *Trichoderma* was done by morphological characteristics and molecular methods. The ability of *Trichoderma* to suppress *P. capsici* was tested by dual culture. The effect of secondary metabolites on the growth of *P. capsici* was determined in vitro at concentrations of 0% (control), 10%, 20%, 30%, and 40%. The experimental design used was a completely randomized design consisting of five treatments repeated five times. The data obtained from the test were analyzed using ANOVA, followed by the LSD test at 5%. The results of this study showed that *Trichoderma* sp. Margodadi isolate had a close relationship with *Trichoderma asperellum* and had the ability as an antagonist to inhibit the growth of *P. capsici* up to 47.23%, and the secondary metabolites produced could inhibit the growth of *P. capsici* up to 72.53% with the best concentration of 40%.

Key words: Phytophthora foot rot, *P. capsici*, *Trichoderma asperellum*

INTRODUCTION

Pepper plants (*Piper nigrum* L.) are important spice plants in Indonesia including Lampung province. These plants produce black pepper, white pepper, and powdered pepper commodities that have high economic value as an export commodities and as a trade commodities in the country. Total pepper export from Indonesia varied from 62,605 ton in 2012 to 39,691 ton in 2019 (Ichsan & Permata, 2022). In 2022, the value of black pepper exports from Indonesia was approximately 50.49 million U.S. dollars (Statista, 2023). In addition, these commodities are also important in Indonesian domestic markets.

In cultivating pepper plants, there are various problems, one of which is foot rot. Foot rot disease is caused by *Phytophthora capsici* Leonian (synonym: *P. palmivora* var. *piperis*), a soil-borne fungus. This disease could cause huge losses as it damages plants starting from the nursery and young pepper plants to mature plants that bear fruit. Affected plants could wither and die if the disease occurs in the roots or the base of the stem (Ginting & Maryono, 2012). The pathogen could attack all parts of the plants, causing losses of around 25–30%. Foot rot incidence could be as high as 50% or 65% (Thomas & Naik, 2017).

This disease should be controlled with an integrated control approach including cultural and chemical methods. So far, however, chemical means are still the main method used to control pepper stem rot disease, with fungicides used including metalaxyl, phosphonates, and copper-based fungicides (Jibat & Asfaw, 2023). In addition, Rini & Remya (2020) reported that fenamidone + mancozeb was effective in controlling the disease. Nysanth et al. (2022) suggested that the disease should be controlled by using beneficial and antagonistic rhizospheric microorganisms, important among them being *Trichoderma* spp. and *Pseudomonas fluorescens*. This biological control should be combined with phytosanitation and other good agricultural practices.

Corresponding author:
Cipta Ginting (cginting2011@gmail.com)

[1]Department of Plant Protection, Faculty of Agriculture, University of Lampung. Jl. Prof. Soemantri Brodjonegoro No. 1, Lampung, Indonesia 35145
[2]Department of Agrotechnology, Faculty of Agriculture, University of Lampung. Jl. Prof. Soemantri Brodjonegoro No. 1, Lampung, Indonesia 35145

Trichoderma is a ubiquitous hypomycetes fungus found in various soil and root habitats, particularly those rich in organic material. *Trichoderma* is considered a superior biocontrol agent due to its high reproductive capacity, ability to survive in challenging environments, efficient utilization of nutrients, aggressive behavior against phytopathogenic fungi, promotion of plant growth, and induction of defense mechanisms (Manzar et al., 2022). *Trichoderma* employs various biocontrol mechanisms, including mycoparasitism, competition, antibiosis, and the enhancement of plant defense systems (Manzar et al., 2022; Guzmán-Guzmán et al., 2023).

Trichoderma sp. has antagonistic properties or the ability to inhibit the growth of soil-borne pathogenic fungi (Ivayani et al., 2018; Ginting et al., 2017; Gusnawaty et al., 2017). *Trichoderma* sp. can produce secondary metabolites that have antifungal properties (Song et al., 2018; Tyśkiewicz et al., 2022). *Trichoderma* also produces secondary metabolites that can induce plant resistance to various pathogens (Mukhopadhyay and Kumar, 2020; Guzmán-Guzmán et al. 2023). Chet et al. (1998) stated that antibiosis is an antagonism mechanism that involves the metabolites, enzymes, volatile and nonvolatile compounds, or toxins produced by a microorganism that cause lysis to cell. Control using *Trichoderma* sp. could vary in effectiveness depending on species. This is due to differences in morphology and physiology (Widyastuti et al., 2006). Thus, *Trichoderma* has the potential to suppress the pathogen and thus control the disease, including Phytophthora foot rot which is the most devastating disease of black pepper in Lampung.

Morphological identification of fungi is considered ineffective because these microorganisms are cryptic, so it is very difficult to distinguish species from one another if only using a morphological approach (Sibero et al., 2018). Identification of species is expected to be accurate and precise because it should predict the properties that are required for application or that are relevant in pathology (Cai & Drushinina, 2021). Therefore, a molecular identification process is needed to determine the species of a fungus. Molecular identification is carried out to know fungi to the species level because identification based on the morphology of fungi can only provide matches up to the genus level (Susilowati et al., 2020). Molecular identification of fungi has high sensitivity and accuracy. The ITS region can be used as a genetic marker because it has fairly high sequence variation even within the same species, and all fungi have this area.

The objectives of this research were to identify molecularly the fungus *Trichoderma* sp. Margodadi isolates to the species level and to determine the potential of *Trichoderma* sp. Margodadi isolates against *P. capsici.*

MATERIALS AND METHODS

Research Site. This research was conducted at the Laboratory of Plant Disease, Department of Plant Protection, and the Laboratory of Agricultural Biotechnology, Faculty of Agriculture, University of Lampung from March to November 2021.

Fungal Isolates Used in this Study. The *P. capsici* fungus isolate used was the result of isolation from pepper seedling planted in polybags containing contaminated soil. The soil used was taken from the soil around the roots of pepper plants infected with foot rot disease at the Cahaya Negeri Agricultural Experimental Station, North Lampung (Figure 1A). Pepper leaves showing symptoms of *P. capsici* infection were taken, and the pathogen was isolated at the Laboratory of Agricultural Biotechnology, Faculty of Agriculture, the University of Lampung using CTA media (400 g of carrots, 400 g of tomatoes, 20 g of agar block, 3 g of $CaCo_3$, and 200 mL of aquadest) that had been added with lactic acid (Figure 1B). Identification of the fungus *P. capsici* was carried out macroscopically and microscopically (Figure 1C). The *Trichoderma* fungus used in this study is *Trichoderma* sp. Margodadi isolate collection of Plant Clinic, Department of Plant Protection, Faculty of Agriculture, University of Lampung.

Identification of *Trichoderma* sp.

Morphological Identification. The stock culture of *Trichoderma* sp. Margodadi isolate, which was stored for 2 months in test tubes containing potato sucrose agar (PSA) media (200 g potatoes, 20 g succrose, 20 g agar rods, 1000 mL distilled water, and 1.4 mL lactic acid), was rejuvenated in 9 cm glass petri dishes with PSA media, then incubated for 7 days. The slide culture technique (Gusnawaty et al., 2014) was used for microscopic examination of the fungus *Trichoderma* sp. Margodadi isolate identified using indentification book (Barnett & Hunter, 1998).

Molecular Identification. Total DNA of *Trichoderma* sp. Margodadi isolate was extracted using the cetyl trimethyl ammonium bromide (CTAB) method (Inglis et al., 2018). PCR (Sensoquest Thermalcycler, Sensoquest GmbH, Germany) was used to amplify the nuclear ribosomal ITS region using primer pairs ITS1 (5'-TCC

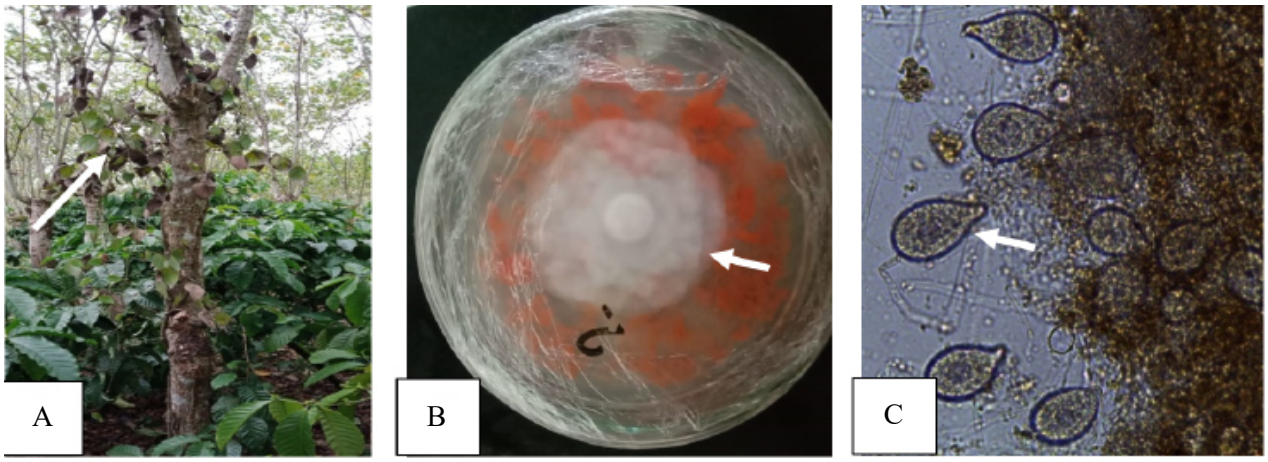

Figure 1. Foot rot symptom and morphological characteristics *P. capsici.* A. Pepper plants with symptoms of pepper foot rot disease (arrow) at the Cahaya Negeri Agricultural Experimental Station, North Lampung; B. *P. capsici* fungal colonies on CTA media (arrow); C. Microscopy examination of *P. capsici* (400× magnification) showing sporangium (arrow).

GTA GGT GAA CCT GCG G-3') and ITS4 (5'- TCC TCC GCT TAT TGA TC GC-3') (White et al., 1990) using a mixture of roughly 2 ng genomic DNA. PCR was performed with a total volume of 25 µL consisting of 12.5 µL Master Mix (Red Mix) (Bioline, USA), 1 µL ITS primer 1 and 1 µL ITS primer 4,1 µL template DNA (approximately 1 µg/µL), and 9.5 µL sterile water. DNA amplification included initial denaturation at 94 ºC for 3 min, followed by 30 cycles of denaturation at 94 ºC for 30 sec, annealing at 55 ºC for 45 sec, extension at 72 ºC for 1 min, and a final extension at 72 ºC for 10 min.

The PCR products were confirmed using agarose gel electrophoresis and sent to Genetika Science Indonesia Co., Ltd. for sequencing. MEGA XI was used to assemble and align the sequencing results, which were subsequently validated using BLAST (http://www.blast.ncbi.nlm.nih.gov). Clustal W was used to align sequenced ITS regions in MEGA XI (Tamura et al., 2021). MEGA XI was used to generate phylogenetic analyses using the Maximum Likelihood method (Felsenstein, 1981) with nucleotide substitutions using the Tamura-Nei model (Tamura & Nei, 1993). The relative robustness of branches was statistically assessed using bootstrap analysis with 1000 replications (Felsenstein, 1985). Relevant sequences from other species were retrieved from GenBank using reference sequences from previously published studies (Filizola et al., 2019). Outgroup taxa were *Protocrea pallida* (NR111329) (Brito et al., 2023).

Antagonistic Capability of *Trichoderma* sp. Against *P. capsici* in Vitro. The test was carried out using the dual culture method (Figure 2). *Trichoderma* sp. Margodadi isolate and *P. capsici* were planted opposite each other at a distance of 3 cm in sterile petri dishes containing PSA media and incubated for 7 days. The percentage of inhibition of the fungus *Trichoderma* sp. Margodadi isolate against *P. capsici* was compared with the petri dishes control treatment. In the control treatment, only *P. capsici* was grown without *Trichoderma*. The percentage of inhibition was calculated using the formula (Soenartiningsih et al., 2014):

$$PI = \frac{(D_1 - D_2)}{D_1} 100\%$$

PI = Percentage of inhibitation (%);
D_1 = Diameter of *P. capsici* colonies in the absence of antagonistic fungi (control);
D_2 = Diameter of *P. capsici* colonies against *Trichoderma* sp. Margodadi isolate.

Capability of Secondary Metabolites of *Trichoderma* sp. to Inhibit Growth of *P. capsici.*

Production of Secondary Metabolites of* Trichoderma *sp. The culture of *Trichoderma* sp. Margodadi isolate was rejuvenated and incubated for 7 days. Then, two petri dishes containing the isolates were taken and put into an erlenmeyer containing potato sucrose broth (PSB) media (200 g of potatoes, 20 g of succrose, and 1000 mL of distilled water that had been added with 1.4 mL lactic acid after cooling to + 45 ºC). The media containing the culture of *Trichoderma* sp. Margodadi isolate was homogenized using a shaker at a speed of 150 rpm at room temperature. Incubation was carried out for 7 days according to the stationary period in the

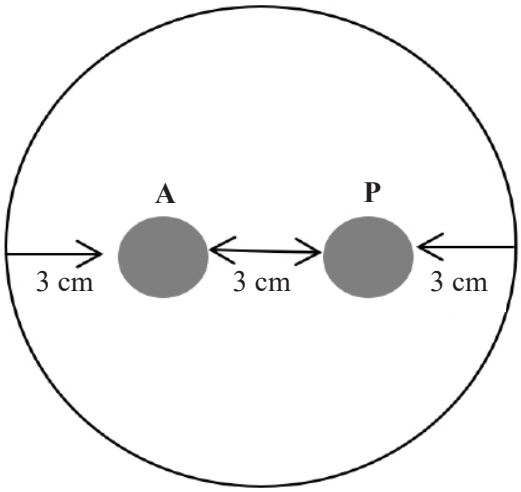

Figure 2. Placement of *Trichoderma* and *P. capsici* by dual culture method. A= *Trichoderma* sp. Margodadi isolate; P= *P. capsici.*

growth phase of the fungus *Trichoderma* sp. The results of the fermentation were filtered using Whatman 40 filter paper. The suspension that had been obtained was then centrifuged (HITACHI RX II Series, Hitachi Koki Co., Ltd, Japan) a speed of 4000 rpm for 15 min (Harni et al., 2017). The results were obtained in the form of supernatant and pellet. The supernatant was taken and stored in -20 ºC until it was used later.

Assessment of *Trichoderma* sp. Secondary Metabolites Againts *P. capsici.* The experiment was done using poissoned food method. The experimental design used was a completely randomized design consisting of five treatments and five replications. The data obtained from the test were analyzed using anova followed by LSD test at 5%. The supernatant was put into a PSA medium which was still liquid (temperature 45 ºC) in concentrations of 0%, 10%, 20% 30%, and 40%. After the media solidified, a 0.5-mm mycelium plugs of the pathogenic fungi were inoculated at the center of the PSA medium and incubated for 7 DAI at 28 ºC. Percentage of inhibition of growth of pathogenic fungi by secondary metabolites of antagonistic fungi with filtrate culture was calculated using the formula (Shentu et al., 2014):

$$PI = \frac{(DK - DP)}{DK} \times 100\%$$

PI = Percentage of inhibitation of *P. capsici* (%);
DK = Diameter of *P. capsici* fungal colonies in the control treatment;
DP = Diameter of *P. capsici* fungal colonies on treatment.

RESULTS AND DISCUSSION

Macroscopic observations of *Trichoderma* sp. Margodadi isolate showed that the colony was fibrous and round-shape that formed a circle with a flat surface. The initial growth of the colony has a white color, then became light green, and finally dark green on the upper surface (Figure 3A). Most saprophytic fungi begin with white mycelium and and change color as the mycelium matures (Stamets, 2000). Microscopic observation of *Trichoderma* sp. Margodadi isolate showed that the conidia were round with a greenish color, growing at the tip of the phialides (Figure 3B).

Based on the results of the ITS DNA sequence analysis in the BLAST program database, it showed that Margodadi isolate had a genetic relationship with *T. asperellum* with a 100% similarity level. The phylogenetic tree showed that Margodadi isolate was nested within *T. asperellum* with a bootstrap value of 100 (Figure 4). The phylogenetic tree in this work exhibits a resemblance to the phylogenetic tree constructed by Sutarman et al. (2021); Wu et al. (2017); Rai et al., (2016), wherein *T. asperellum* is grouped together with *T. harzianum* and *T. longibrachiatum* in a single clade. According to this information, it may be concluded that the *Trichoderma* Margodadi isolate is *T. asperellum*. Wu et al. (2017) state that accurate taxonomic understanding of *Trichoderma* isolates is crucial for the identification and characterization of prospective biocontrol species, as well as for preventing the entry of unrecognized fungal species into an ecosystem.

Phylogenetic analysis is an analysis that aims to compile phylogenetic relationships, which are generally described in a branching line like a tree, also called a phylogenetic tree. A phylogenetic tree is a form of

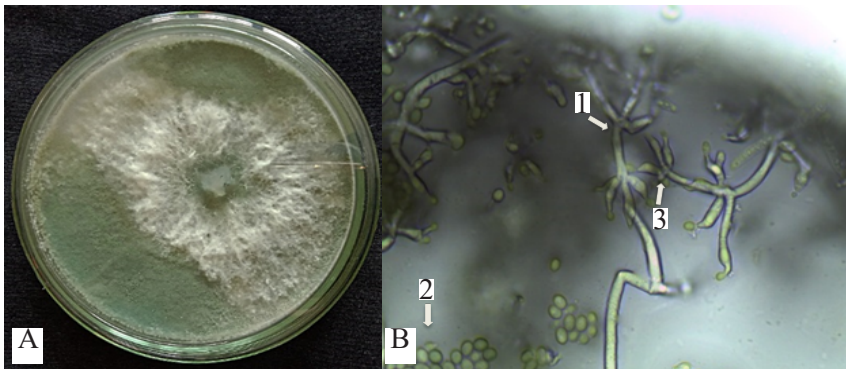

Figure 3. *Trichoderma* sp. Margodadi isolate. A. The colony culture at 7 days after incubation on PDA; B. Microscopic morphology (400×). 1. Conidiophores; 2. Conidia; 3. Fialid.

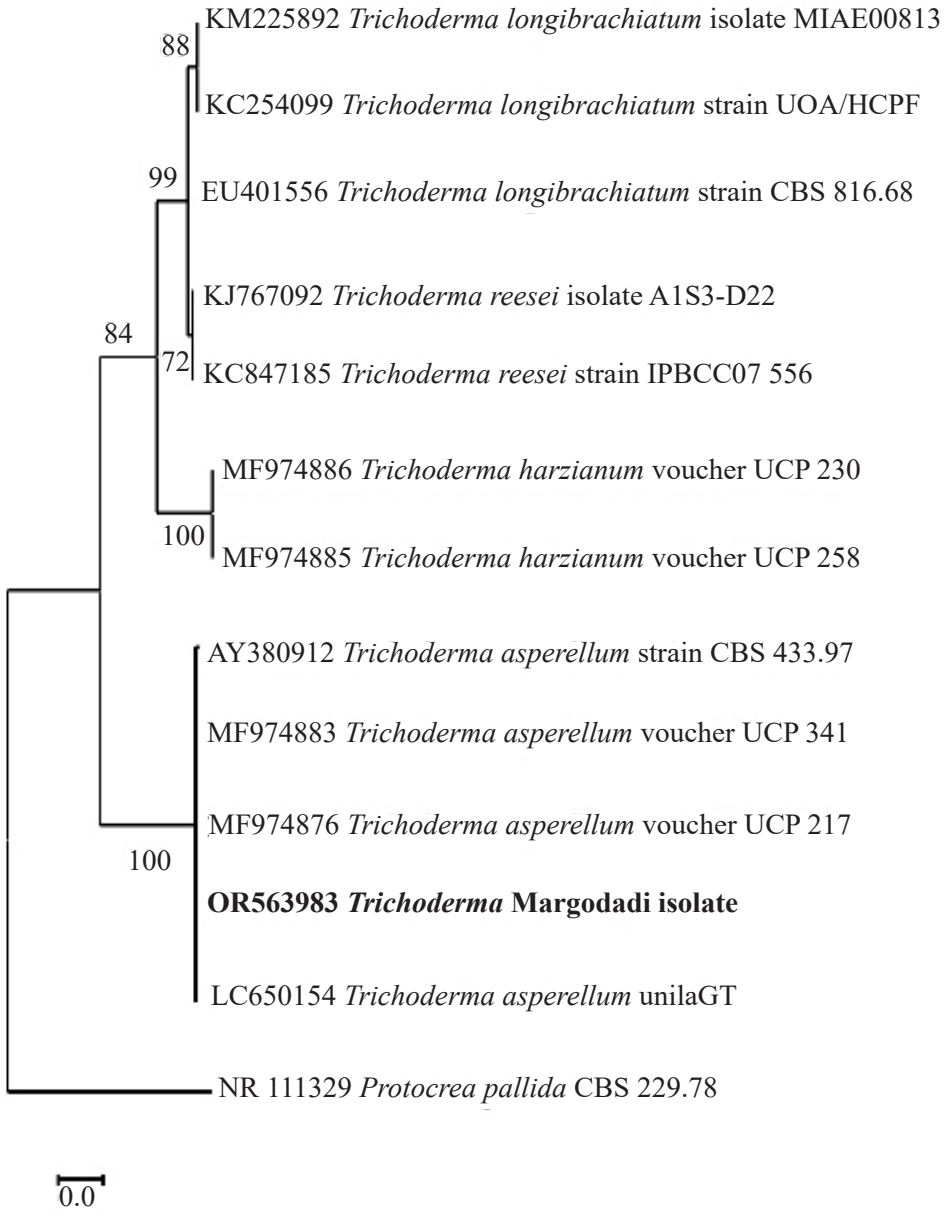

Figure 4. Phylogenetic tree of *Trichoderma* sp. Margodadi isolate (Acc. No. OR563983) inferred from ITS region (ITS1–5.8S–ITS4) sequences using Maximum Likelihood method with Tamura-Nei model (1000× bootstrap) as nucleotide substitutions model. The tree was rooted to *Protocrea pallida* CBS 299.78 (Acc. No. NR 111329).

description of the genealogy of living things, including animals and plants, that have branches resembling trees (Hall, 2013). As described by Ardiana et al. (2021), phylogenetic is a method that can be used to analyze the phylogenetic relationship of a living being. In phylogenetic, a group of organisms that share similarities in character or characteristics is considered to have a close genetic relationship. These similarities are considered descendants of a parent, which will later form a monophyletic group. In the phylogenetic analysis, there are in-group and out-group groups. Out group groups are needed because they can cause polarization of characters or traits, namely, apomorphic characters found in the in-group and plesiomorphic characters found in the out-group.

Based on in vitro tests, the ability of *T. asperellum* Margodadi isolate as an antagonist against the fungus *P. capsici* reached 47.23% (Figure 5). The inhibition of growth in diameter of *P. capsici* colonies was caused by the faster growth of *T. asperellum* Margodadi isolate, which could compete for space and higher nutrients compared to the growth of *P. capsici* colonies and antibiosis. The role of *T. asperellum* in suppressing the growth of *P. capsici* fungal colonies was based on the growth rate of the antagonist fungus. The faster the growth of the antagonist fungus, the more effective it is in suppressing the growth of pathogenic fungal colonies due to the mechanism of competition for space and nutrients (Widi et al., 2015).

The mechanism of antibiosis is indicated by the formation of a clear zone between the mycelium of antagonist and pathogenic fungi, due to the presence of antibiotic compounds naturally produced by several types of fungi in their metabolic processes (Fety et al., 2015). Organisms such as *T. asperellum* have a maximum and minimum inhibition value as a biocontrol agents against pathogenic fungi. The biocontrol ability of *Trichoderma* varies depending on the pathogen and culture conditions. The fungus *Trichoderma* is very important for suppressing the development of soil-borne pathogenic fungi (Hewedy et al., 2020). *T. asperellum* has potential as a biological agent because the growth of this fungal colony is very fast. It can be cultured easily in culture media or natural conditions.

The fungus *T. asperellum*, with antagonistic activity against *P. capsici* hyphae, can also infect oospores (Jiang et al., 2016). This suggests the potential for new applications of *T. asperellum* to control *Phytophthora* blight in fields colonized with oospores. *T. asperellum* can inhibit the growth of mycelia and the production of *P. capsici* sporangia, so this species has the potential to reduce primary inoculum and suppress infection (Ramirez-Delgado et al., 2018). Secondary metabolite application of *T. asperellum* Margodadi isolate on *P. capsici* isolates decreased sporangia production (Figure 6).

The results show that the growth of pathogenic fungi was slowed down more when a higher dose was used. At the dose of 40%, secondary metabolites had the best inhibitory value (72.53%) against the growth of *P. capsici* (Table 1). Meena et al. (2017) reported that the nonvolatile secondary metabolite of *Trichoderma* can inhibit the growth of *Alternaria alternata* up to 62.5%. The secondary metabolites produced by *T. asperellum* have the ability to inhibit the growth of pathogens. This is attributed to their production of both volatile and nonvolatile compounds, which effectively impede the growth of mycelia from various fungi (Howell, 2003). Wu et al. (2017) reported that *T. asperellum* secretes chitinase, glucanase, and protease enzymes that break down the cell walls of fungi and contribute to mycoparasitism. Furthermore, it produces xylanase,

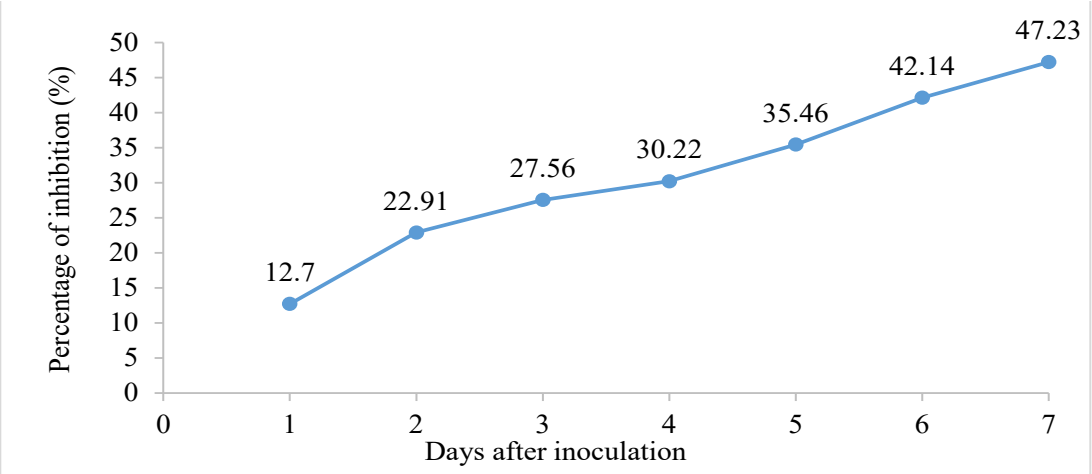

Figure 5. The antagonistic effect of *T. asperellum* Margodadi isolate against the fungus *P. capsici*

which contributes to the stimulation of plant defense mechanisms and boosts plant resistance. *T. asperellum* has the ability to synthesize nine primary metabolites that serve as precursors for antimicrobial chemicals. Additionally, it produces a diverse range of antimicrobial secondary metabolites, such as polyketides, alkanes, and other compounds. In addition to its ability to secrete antimicrobial compounds, *T. asperellum* also has the capacity to create an amino acid polypeptide that is encoded by the TabZIP15 gene. This polypeptide plays a role in enhancing the ability to tolerate stress caused by pathogen toxins (Yu et al., 2020).

The mechanism by which secondary metabolites inhibit the development of pathogens is through protein denaturation, both structurally and functionally, in pathogenic cells. Secondary metabolites have active compounds that can break the disulfide bonds connecting the cell wall protein polypeptides and cell membranes (Tyśkiewicz et al., 2022). Denaturation of structural proteins in the cell wall weakens the cell's protection, making it vulnerable. Meanwhile, denaturation of the pathogenic cell membrane leads to a loss of permeability properties, preventing the selective passage of substances into and out of the cell.

CONCLUSIONS

Based on the results of this study, *Trichoderma* sp. Margodadi isolate had a genetic relationship with *Trichoderma asperellum* reaching 100% which is supported by morphological characteristics and molecular identification. *T. asperellum* Margodadi isolate had the potential to inhibit the growth of *P. capsici*, as an antagonist agent with an inhibitory value of 47.23% and also the secondary metabolites produced could inhibit the growth of *P. capsici* by 72.53% with the best concentration of 40%.

ACKNOWLEDGMENTS

We would like thank to Research and Public Service Institute of University of Lampung that funded

Table 1. Effect of secondary metabolites concentration of *T. asperellum* Margodadi isolate against the fungus *P. capsici*

Concentration Treatment (%)	Inhibition of secondary metabolites on the observation (%) 1–7 DAI						
	1	2	3	4	5	6	7
0 (Control)	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
10	3.63 b	6.75 b	7.88 b	15.02 b	25.78 b	33.49 b	41.34 b
20	6.67 c	8.34 b	13.34 c	25.14 c	33.90 c	42.09 c	48.85 c
30	10.76 d	15.24 c	21.24 d	36.30 d	48.63 d	55.29 d	60.82 d
40	13.39 e	29.87 d	42.22 e	54.95 e	63.35 e	68.52 e	72.53 e

The numbers followed by the same letter in one column show no significant difference in the 5% LSD test. DAI= days after inoculation.

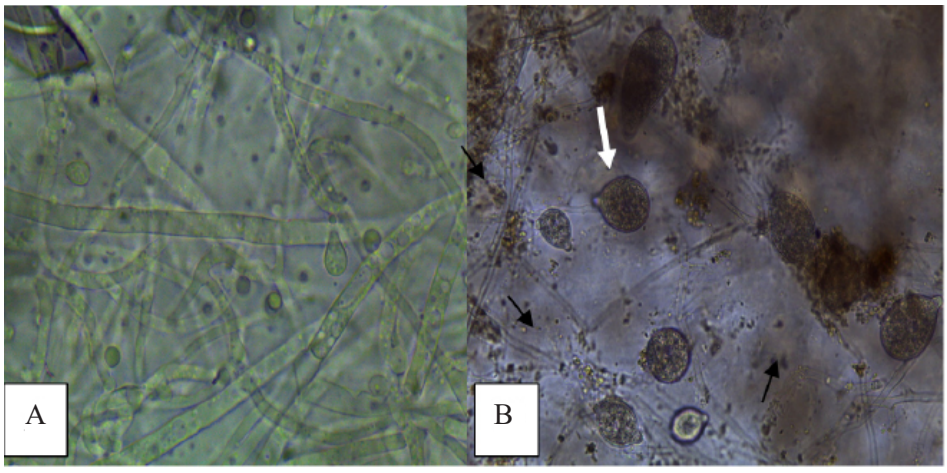

Figure 6. Microscopic photo of *P. capisici* (400×). A. *P. capsici* treated with secondary metabolites of *T. asperellum* Margodadi isolate, no sporangia production; B. *P. capsici* without treatment of secondary metabolites of *T. asperellum*, many sporangia produced (arrow).

part of this research and Plant Clinic, Department of Plant Protection, Faculty of Agriculture at University of Lampung for providing some materials used in this research.

FUNDING

Part of fund for this research was provided by Research and Public Service Institute of University of Lampung and Plant Clinic, Department of Plant Protection, Faculty of Agriculture at University of Lampung.

AUTHORS' CONTRIBUTIONS

JP and CG considered and planned the experiment. JP coordinated in doing the experiment including the molecular and morphological work and in writing the manuscript. CG participated in advising part of the experiment and in writing the manuscript. RE participated in advising about black pepper plant and field. NBJ participated in doing part of the experiment and in writing part of manuscript. TM participated in molecular aspect and in analyzing some of the data. All the authors have read and approved the final manuscript.

COMPETING INTEREST

We declare herewith that there is no competing interest such as financial or non-financial interests, professional or personal relationships that are directly or indirectly connected to the work submitted for publication.

REFERENCES

Ardiana SA, Astarini IA, Putra ING, Pertiwi PD, Sembiring A, Yusmalinda A, & Al Malik D. 2021. Keragaman genetik dan filogenetik longtail tuna (*Thunnus tonggol*) yang didaratkan di Pasar Ikan Pabean, Surabaya [Genetic diversity and phylogenetic of longtail tuna (*Thunnus tonggol*) landed in Pabean Fish Market, Surabaya]. *Musamus Fisheries and Marine Journal*. 3(2): 107–115. https://doi.org/10.35724/mfmj.v3i2.3375

Barnett HL & Hunter BB. 1998. *Illustrated Genera of Imperfect Fungi*. 4th ed. APS Press, St. Paul, Minnesota.

Brito VN, Alves JL, Araújo KS, de Souza Leite T, de Queiroz CB, Pereira OL, & de Queiroz MV. 2023. Endophytic Trichoderma species from rubber trees native to the Brazilian Amazon, including four new species. *Front. Microbiol*. 14: 1095199. https://doi.org/10.3389/fmicb.2023.1095199

Cai F & Druzhinina IS. 2021. In honor of John Bissett: authoritative guidelines on molecular identification of Trichoderma. *Fungal Divers*. 107: 1–69. https://doi.org/10.1007/s13225-020-00464-4

Chet I, Benhamou N, & Haran S. 1998. Mycoparasitism and lytic enzymes. In: Harman GE & Kubicek CP (Eds.). *Trichoderma and Gliocladium*. Vol 2. *Enzymes, Biological Control and Commercial Applications*. pp 153–171. Taylor and Francis. London.

Felsenstein J. 1981. Evolutionary trees from dna sequences: A maximum likelihood approach. *J Mol. Evol.* 17: 368–376. https://doi.org/10.1007/BF01734359

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*. 39(4): 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Fety, Khotimah S, & Mukarlina. 2015. Uji antagonis jamur rizosfer isolat lokal terhadap *Phytophthora* sp. yang diisolasi dari batang langsat (*Lansium domesticum* Corr.) [Antagonist test of rhizospheric fungal local isolate against *Phytophthora* sp. isolated from langsat stem (*Lansium domesticum* Corr.)]. *Protobiont*. 4(1): 218–225.

Filizola PRB, Luna MAC, de Souza AF, Coelho IL, Laranjeira D, & Campos-Takaki GM. 2019. Biodiversity and phylogeny of novel *Trichoderma* isolates from mangrove sediments and potential of biocontrol against *Fusarium* strains. *Micro. Cell Fact*. 18(1): 89. https://doi.org/10.1186/s12934-019-1108-y

Ginting C & Maryono T. 2012. Penurunan keparahan penyakit busuk pangkal batang pada lada akibat aplikasi bahan organik dan *Trichoderma harzianum* [Decrease of disease severity of foot rot of black pepper due to application of organic matter and *Trichoderma harzianum*]. *J Trop. Plant Pests Dis*. 12(2): 162–168. https://doi.org/10.23960/j.hptt.212162-168

Ginting C, Prasetyo J, Nurhidayat A, & Maryono T. 2017. Efikasi isolat *Trichoderma* terpilih dengan bahan organik untuk mengendalikan penyakit

busuk pangkal batang pada lada di lapangan [Efficacy of selected *Trichoderma* isolates with organic materials to control foot rot disease on black pepper in the field]. *J Trop. Plant Pests Dis.* 17(1): 77–83. https://doi.org/10.23960/j.hptt.11777-83

Guzmán-Guzmán P, Kumar A, de los Santos-Villalobos S, Parra-Cota FI, Orozco-Mosqueda MdC, Fadiji AE, Hyder S, Babalola OO, & Santoyo G. 2023. Trichoderma species: Our best fungal allies in the biocontrol of plant diseases-A review. *Plants*. 12(3): 432. https://doi.org/10.3390/plants12030432

Gusnawaty HS, Taufik M, Triana L, & Asniah. 2014. Karakterisasi morfologis *Trichoderma* spp. indigenus Sulawesi Tenggara [Morphological characterization *Trichoderma* spp. indigenous Southeast of Sulawesi]. *J. Agroteknos.* 4(2): 88–94.

Gusnawaty HS, Taufik M, Bande LSS, & Asis A. 2017. Efektivitas beberapa media untuk perbanyakan agens hayati *Trichoderma* sp. [Effectiveness test of several media for the propagation of biological agents *Trichoderma* sp.]. *J. Trop. Plant Pests Dis*. 17(1): 70–76. https://doi.org/10.23960/j.hptt.11770-76

Hall BG. 2013. Building phylogenetic trees from molecular data with MEGA. *Mol. Biol. Evol.* 30(5): 1229–1235. https://doi.org/10.1093/molbev/mst012

Harni R, Amaria W, Syafaruddin, & Mahsunah AH. 2017. Potensi metabolit sekunder *Trichoderma* spp. untuk mengendalikan penyakit vascular streak dieback (VSD) pada bibit kakao [Potential of *Trichoderma* spp. secondary metabolite in controlling vascular streak dieback (VSD) disease on cocoa seedlings]. *J TIDP.* 4(2): 57–66.

Hewedy OA, Lateif KSA, Seleiman MF, Shami A, Albarakaty FM, & El-Meihy RM. 2020. Phylogenetic diversity of *Trichoderma* strains and their antagonistic potential against soil-borne pathogens under stress conditions. *Biology.* 9(8): 189. https://doi.org/10.3390/biology9080189

Howell CR. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant deseases: The history and evolution of current concepts. *Plant Dis*. 87(1): 1–10. https://doi.org/10.1094/PDIS.2003.87.1.4

Ichsan & Permata R. 2022. An analysis of pepper exports and imports on economic growth in Indonesia. *J. Maliksussaleh Public Economics.* 5(2): 26–34. https://doi.org/10.29103/jmpe.v5i2.10469

Inglis PW, Pappas MdeCR, Resende LV, & Grattapaglia D. 2018. Fast and inexpensive protocols for consistent extraction of high-quality DNA and RNA from challenging plant and fungal samples for high throughput SNP genotyping and sequencing applications. *PLoS One*. 13(10): e0206085. https://doi.org/10.1371/journal.pone.0206085

Ivayani I, Ginting C, Yusnita Y, & Dirmawati SR. 2018. Effectiveness of the application of organic matter and *Trichoderma viride* from suppresive soil to control fusarium wilt on banana plant. *J Trop. Plant Pests Dis.* 18(2): 119–126. https://doi.org/10.23960/j.hptt.218119-126

Jiang H, Zhang L, Zhang J, Ojaghian MR, & Hyde KD. 2016. Antagonistic interaction between *Trichoderma asperellum* and *Phytophthora capsici* in vitro. *J. Zhejiang Univ. Sci*. 17(4): 271–281. https://doi.org/10.1631/jzus.B1500243

Jibat M & Asfaw M. 2023. Management of foot rot (*Phytophthora capsici*) disease of black pepper (*Piper nigrum* L.) through fungicides and cultural practices in Southwestern Ethiopia. *Int. J. Agril. Res. Innov. Tech*. 13(1): 48–50. https://doi.org/10.3329/ijarit.v13i1.67973

Manzar N, Kashyap AS, Goutam RS, Rajawat MVS, Sharma PK, Sharma SK, & Singh HV. 2022. *Trichoderma*: Advent of versatile biocontrol agent, its secrets and insights into mechanism of biocontrol potential. *Sustainability.* 14(19): 12786. https://doi.org/10.3390/su141912786

Meena M, Swapnil P, Zehra A, Dubey MK, & Upadhyay RS. 2017. Antagonistic assessment of *Trichoderma* spp. by producing volatile and non-volatile compounds against different fungal pathogens. *Arch. Phytopathol. Plant Prot*. 50(13,14): 629–648. https://doi.org/10.1080/03235408.2017.1357360

Mukhopadhyay R & Kumar D. 2020. *Trichoderma*: a beneficial antifungal agent and insights into its mechanism of biocontrol potential. *Egypt. J. Biol. Pest Control*. 30: 133. https://doi.org/10.1186/s41938-020-00333-x

Nysanth NS, Divya S, Nair CB, Anju AB, Praveena R, Anith KN. 2022. Biological control of foot rot (*Phytophthora capsici* Leonian) disease in black pepper (*Piper nigrum* L.) with rhizospheric microorganisms. *Rhizosphere*. 23: 100578. https://doi.org/10.1016/j.rhisph.2022.100578

Rai S, Kashyap PL, Kumar S, Srivastava AK, & Ramteke PW. 2016. Identification, characterization and phylogenetic analysis of antifungal *Trichoderma* from tomato rhizosphere. *SpringerPlus*. 5: 1939. https://doi.org/10.1186/s40064-016-3657-4

Ramirez-Delgado ER, Luna-Ruiz JdJ, Morino-Rico O, Quiroz-Velasquesz JDC, & Hernandez-Mendoza JL. 2018. Effect of *Trichoderma* on growth and sporangia production of *Phytophthora capsici. J Agric. Sci.* 10(6): 8–15. https://doi.org/10.5539/jas.v10n6p8

Rini CR & Remya J. 2020. Management of *Phytophthora capsici* infection in black pepper (*Piper nigrum* L.) using new generation fungicides and biopesticide. *IJAEB*. 13(1): 71–74. https://doi.org/10.30954/0974-1712.1.2020.9

Shentu X, Zhan X, Ma Z, Yu X, & Zhang C. 2014. Antifungal activity of metabolites of the endophytic fungus *Trichoderma brevicompactum* from garlic. *Braz. J. Microbiol*. 45(1): 248–254. https://doi.org/10.1590/S1517-83822014005000036

Sibero MT, Tarman K, Radjasa OK, Sabdono A, Trianto A, & Bachtiarini TU. 2018. Produksi pigmen dan identifikasi kapang penghasilnya menggunakan pendekatan DNA Barcoding [Pigment production and identification of the producing mold using a DNA barcode approach]. *Jurnal Pengolahan Hasil Perikanan Indonesia.* 21(1): 99–108. https://doi.org/10.17844/jphpi.v21i1.21454

Soenartiningsih, Djaenuddin N, & Saenong MS. 2014. Efektivitas *Trichoderma* sp. dan *Gliocladium* sp. sebagai agen biokontrol hayati penyakit busuk pelepah daun pada jagung [The effectiveness of *Trichoderma* sp. and *Gliocladium* sp. as a biocontrol agent for midrib rot disease in maize]. *JPPTP.* 33(2): 129–135.

Song YP, Miao FP, Fang ST, Yin XL, & Ji NY. 2018. Halogenated and nonhalogenated metabolites from the marine-alga-endophytic fungus *Trichoderma asperellum* cf44-2. *Mar. Drugs.* 16(8): 266. https://doi.org/10.3390/md16080266

Stamets P. 2000. *Growing Gourmet and Medicinal Mushrooms*. 3rd Revised edition. Ten Speed Press. California.

Statista. 2023. *Total Value of Black Pepper Exports from Indonesia from 2012 to 2022*. https://www.statista.com/statistics/1228472/indonesia-value-of-black-pepper-exports. Accessed 5 December 2023.

Susilowati DN, Sofiana I, Atmini KD, & Yuniarti E. 2020. Penapisan kapang asal lahan sulfat masam Kalimantan Selatan sebagai penghasil enzim ekstraseluler [Screening of acid sulphate soils fungi from south kalimantan as source of extracelular enzymes]. *Agric*. 32(1): 65–82. https://doi.org/10.24246/agric.2020.v32.i1.p65-82

Sutarman , Jalaluddin AK, Li'aini AS, & Prihatiningrum AE. 2021. Characterizations of *Trichoderma* sp. and its effect on *Ralstonia solanacearum* of tobacco seedlings. *J. Trop. Plant Pests Dis.* 21(1): 8–19. https://doi.org/10.23960/jhptt.1218-19

Tamura K & Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10(3): 512–26. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Tamura K, Stecher G, & Kumar S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol*. 38(7): 3022–3027. https://doi.org/10.1093/molbev/msab120

Thomas LM & Naik BG. 2017. Survey for the Incidence of Foot Rot of Black Pepper Caused by *Phytophthora capsici* Leonian in Shivamogga and Chickmagaluru Districts of Karnataka State. *Int. J. Pure App. Biosci.* 5(1): 293–298.

Tyśkiewicz R, Nowak A, Ozimek E, & Jaroszuk-Ściseł J. 2022. *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. *Int. J. Mol. Sci*. 23(4): 2329. https://doi.org/10.3390/ijms23042329

White TJ, Bruns T, Lee S, & Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, & White TJ (Eds.). *PCR Protocols: A Guide to Methods and Applications*. pp. 315–322. Academic Press. Florida.

Widi A, Rita H, & Samsudin. 2015. Evaluasi jamur antagonis dalam menghambat pertumbuhan *Rigidoporus microporus* penyebab penyakit jamur akar putih pada tanaman karet [Evaluation of antagonistic fungi in inhibiting the growth of *Rigidoporus microporus* causing white root disease in rubber plants]. *J TIDP.* 2(1): 51–60.

Widyastuti SM, Sumardi, Irfa'i, & Nurjanto HH. 2006. Aktivitas penghambatan *Trichoderma* spp. formulasi terhadap jamur patogen tular tanah secara in vitro [In vitro inhibitation activity of formulated *Trichoderma* spp. against soil-borne pathogenic fungi. Indones. *Jurnal Perlindungan Tanaman Indonesia.* 8(1): 27–34.

Wu Q, Sun R, Ni M, Yu J, Li Y, Yu C, Dou K, Ren J, & Chen J. 2017. Identification of a novel fungus, *Trichoderma asperellum* GDFS1009, and comprehensive evaluation of its biocontrol efficacy. *PLoS ONE.* 12(6): e0179957. https://doi.org/10.1371/journal.pone.0179957

Yu Z, Wang Z, Zhang Y, Wang Y, & Liu Z. 2020. Analysis of TabZIP15 transcription factor from *Trichoderma asperellum* ACCC30536 and its function under pathogenic toxin stress. *Sci. rep.* 10: 15084. https://doi.org/10.1038/s41598-020-72226-w