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Morphological characterization of *Trichoderma* spp. isolated from the oil palm rhizosphere in peat soils and its potential as a biological control for *Ganoderma* sp. in vitro

Karakterisasi morfologi *Trichoderma* spp. yang diisolasi dari rizosfer kelapa sawit di tanah gambut dan potensinya sebagai pengendali hayati *Ganoderma* sp. secara in vitro

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ABSTRACT

Trichoderma sp. is a saprophytic fungus found in various environments, one of which is in the rhizosphere of oil palm plants which can be used to control *Ganoderma* and increase the resistance of oil palm plants from stem rot disease. This study aimed to characterize the morphology of *Trichoderma* spp. origin of peat land in oil palm plantations in Kampar Regency and screening the potential in controlling *Ganoderma* sp. *Trichoderma* spp. from oil palm rhizosphere was collected from the smallholder oil palm plantations in Deli Makmur Village, Kampar, Indonesia. This research was conducted by several observations, such as the hypovirulence test; identification of the morphology of *Trichoderma* spp. fungus; growth and diameter test of *Trichoderma* spp. fungus; test of the inhibitory ability of *Trichoderma* spp. fungus against *Ganoderma* sp. LPTUNRI-Gan002 isolate; test for hyperparasitism of the fungus *Trichoderma* spp., which has high antagonistic power against *Ganoderma* sp. isolate. Six *Trichoderma* spp. isolates had morphological characteristics similar to two species, i.e., *Trichoderma harzianum* (LPTUNRI-Trc001, Trc004, Trc005, and Trc006 isolates) and *Trichoderma asperellum* (LPTUNRI-Trc002 and Trc003). LPTUNRI-Trc003 had the highest diameter (90 mm), growth rate (32.66 mm/day), and the highest ability to suppress *Ganoderma* sp. LPTUNRI-Gan002 (91.03%) compared to the other five isolates.

ABSTRAK

Trichoderma sp. merupakan jamur saprofit yang dapat ditemukan di berbagai lingkungan, salah satunya pada rizosfer kelapa sawit yang dapat digunakan untuk mengendalikan *Ganoderma* dan meningkatkan ketahanan tanaman kelapa sawit dari penyakit busuk pangkal batang. Penelitian ini bertujuan untuk mengkarakterisasi morfologi *Trichoderma* spp. asal lahan gambut di perkebunan kelapa sawit di Kabupaten Kampar dan penapisan terhadap jamur yang potensial dalam mengendalikan *Ganoderma* sp. *Trichoderma* spp. diperoleh dari rizosfer tanaman kelapa sawit pada perkebunan kelapa sawit rakyat di Desa Deli Makmur, Kampar, Indonesia. Penelitian ini dilakukan melalui beberapa tahapan yaitu uji hipovirulensi, identifikasi morfologi jamur *Trichoderma* spp., uji pertumbuhan dan diameter jamur *Trichoderma* spp., uji daya hambat jamur *Trichoderma* spp. terhadap *Ganoderma* sp. isolat LPTUNRI-Gan002, uji hiperparasitisme jamur *Trichoderma* spp. yang memiliki daya antagonis tinggi terhadap isolate *Ganoderma* sp. Enam isolate *Trichoderma* spp. memiliki karakteristik morfologi yang mirip dengan dua spesies, yaitu isolat *Trichoderma harzianum* (LPTUNRI-Trc001, Trc004, Trc005 dan Trc006) dan *Trichoderma asperellum* (LPTUNRI-Trc002 dan Trc003). LPTUNRI-Trc003 memiliki diameter (90 mm) dan laju pertumbuhan (32,66 mm.hari-1), serta kemampuan m enekan *Ganoderma* sp. LPTUNRI-Gan002 (91,03%) tertinggi dibandingkan dengan lima isolat lainnya.

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INTRODUCTION

Oil palm is the most widely cultivated and essential commodity in the plantation sector in Riau province. Based on data from the Central Agency on Statistics of Riau Province (2020), the area of oil palm plantations in Riau was 2,489,957 ha, with a production of 7,683,535 tonnes and a productivity of 3.08 tonnes/ha in 2018. Meanwhile, in 2019, the plantation area increased to 2,537,375 ha with a decrease in palm oil production to 7,466,260 tonnes and productivity to 2.94 tonnes/ha. The decrease in oil palm productivity in Riau Province was caused by several factors, including poor plantation management, lack of knowledge and technical farming capabilities (Suharno et al., 2015), and *Ganoderma* pathogenic fungus infection, which causes stem rot disease as the primary pathogen (Hushiarian et al., 2013).

Basal stem rot disease incidence by *Ganoderma* up to 50% can reduce yields by 50-80% (Lisnawita et al., 2016; Corley & Tinker, 2016). In addition, the Riau Province Plantation Service (2014) reported that *Ganoderma boninense* had caused damage to a community-owned oil palm plantation in Riau, covering an area of 533.8 ha. This pathogen must be controlled because it can attack young and old oil palm plants. Efforts to control stem rot disease in oil palm plants have been carried out, such as cultural control techniques, synthetic fungicides with carboxylic active ingredients, and biological control. Cultural control techniques can be performed through sanitation of the remains of oil palm trunks and roots infected with *Ganoderma* sp. Sanitation of this inoculum source can minimize contact between healthy roots and remnants of oil palm roots infected with *Ganoderma* sp. in the field (Naher et al., 2013). Chemical control by synthetic fungicides with the active ingredient carboxylic acid is commonly used. However, using synthetic fungicides in the long term will cause side effects such as the death of beneficial organisms around plants and the occurrence of resistance to pathogens. Also, fungicide overuse threatens human health and causes ecological problems (Lamichhane et al., 2017). On the other hand, biological control has a high potency to be developed as an environmentally friendly control method for plant disease.

Biological control using antagonistic fungi can inhibit the growth and development of pathogens by various mechanisms. The antagonist fungi can produce antibiotics and grow faster to compete for space and nutrients (Muhibbudin et al., 2021). The fungal rhizosphere with potential biological agents could control soil-borne pathogens by suppressing inoculum, preventing colonization, and protecting seed germination and plant roots from pathogen infection. The mechanisms of antagonistic fungi in inhibiting the development of plant diseases, in general, include antibiosis, competition, and parasitism (Sudarma et al., 2015). The fungal rhizosphere also has a role as a plant growth-promoting fungi (PGPF) (Murali et al., 2021). Priwiratama and Susanto (2014) stated that biological control was carried out using antagonistic fungi derived from the rhizosphere, such as *Trichoderma* spp.

Trichoderma spp. was an excellent biological control because it is easy to isolate and culture, proliferates on various substrates, affects plant pathogens, acts as a microparasite, can compete well in terms of food and space, and produces antibiotics (Nusaibah & Musa, 2019; Khan et al., 2020). Mukhlis et al. (2017) reported that *Trichoderma citrinoviridae* had an inhibitory power of (79.21%), *Trichoderma harzianum* had an inhibitory power of (78.78%), *Trichoderma virens* had an inhibitory power of (77.05%) and *Trichoderma atroviridae* had an inhibitory power of (76.18%). Therefore, place the fungus *Trichoderma* spp. isolate, which is applied in vitro and has antagonistic potential in inhibiting the growth of *Ganoderma* sp. Various *Trichoderma* spp. isolated from the rhizosphere of oil palm plants have the potential to control *Ganoderma* sp. and increase the resistance of oil palm plants from stem rot disease. This study aims to characterize the morphology of *Trichoderma* spp. origin of peat land in oil palm plantations in Kampar Regency and screening the potential in controlling *Ganoderma* sp.

METHODS AND MATERIALS

Material research

Peat soil collected from oil palm rhizosphere from the smallholder oil palm plantations in Deli Makmur Village, Kampar Kiri District, Kampar Regency, located on the coordinate of geographical information system 0°30′18″N - 101°11′09″E, 0°30′16″N - 101°11′05″E and 0°30′23″N - 101°11′04″E. Peat soil samples were taken with soil drill (5 cm diameter) at a depth of 20-40 cm as many as five points on the sample plants. Soil samples were put into sterile plastic clips and taken to the Plant Disease Laboratory.

Isolation of Trichoderma spp from peat soil

Isolation of the *Trichoderma* spp from peat soil rhizosphere of oil palm plants was carried out by dilution. First, 10 g of soil was dissolved with 90 ml of distilled water in an Erlenmeyer to obtain 100 ml of soil suspension (10^{-1} dilution) and homogenized using an orbital shaker for 2 minutes at a speed of 150 rpm. Then 1 ml of soil suspension from rhizosphere was put into 9 ml of distilled water in a test tube (10^{-2} dilution), then homogenized with an automatic shaker for 2 minutes and diluted to 10^{-6} dilution. Each 10^{-2}-10^{-6} dilution was taken using a dropper of as much as 1 ml, then poured into semi-solid PDA media using the pour plate method. The solid media was incubated at room temperature for five days. After the fungus grows, each fungal colony is taken with a sterile ose needle, then grown on sterile PDA media to obtain pure cultures (Sharma & Singh, 2014).

Hypovirulence assay

Hypovirulence assay for the fungal group refers to the technique used by Juan-Abgona (1996). This assay was used to determine whether a fungus was beneficial or a pathogenic fungus using cucumber seeds as indicator plants. Cucumber showed wide variation in disease susceptibility to different isolates, but hypovirulent isolates exhibited a consistent reaction on five different host cultivars. Pathogenicity tests using cucumber grown in the soil also showed consistent reactions with isolates selected either for hypovirulence or virulence (Juan-Abgona, 1996).

Cucumber seeds were disinfected with 70% alcohol for 1 minute and rinsed with distilled water twice. Seeds germinated in Petri dishes lined with filter paper, moistened with distilled water, and incubated in an incubator for two days at room temperature. Then, cucumber seeds were transferred into test tubes containing 2% water agar medium and ground for three days at room temperature. *Trichoderma* spp. isolates to be tested were incubated for three days on sterile PDA media after transferring seedlings into a 2 % water agar medium. The isolate of *Trichoderma* spp. cut with a 5 mm cork borer aged three days, placed in the middle of the hypocotyl of cucumber seedlings, and incubated at room temperature. Each treatment in the hypovirulence test consisted of 3 replications, and observations were carried out until 14 days after inoculation by looking at the symptoms of spotting from cucumber seedlings. The isolate of *Trichoderma* spp. from the oil palm rhizosphere in peat soil origin was categorized as hypovirulence isolate (non-pathogenic fungus) if the disease severity index (DSI) <2. The DSI was determined by the following formula DSI = $(\Sigma N) / Z$, where N was the disease severity scale of each seed and Z was the number of seeds used. The disease severity scale was measured based on the scale in Table 1.

Table 1. Disease severity scale in cucumber seedlings in hypovirulence assay

Scale	Description
0	Healthy and no disease infection
1	One or two light brown spots < 0.25 cm
2	Light brown spots (0.25 cm - 0.5 cm in size) and <10% wetness area in hypocotyl
3	Light brown to dark spots >1.0 cm and then joins with other spots and 10%<X<100% wetness area on hypocotyl (leaves still firm and white)
4	Hypocotyl leaves wilt and die

Source: Puspita et al. (2019)

The morphological character of Trichoderma spp

Trichoderma spp. morphological character was carried out by observing the macroscopic and microscopic characters. Macroscopic observations included morphological characteristics of a fungal colony: colony surface color, colony diameter, colony edge shape, colony surface texture, and colony shape. Microscopic observations included conidiophore, phialide shape, and conidial shape (Sharma & Singh, 2014). Identification of isolated fungi refers to the book on identifying fungi (Gusnawaty et al., 2014).

Potential of Trichoderma spp. as a biological control agent of Ganoderma sp.

Diameter and growth rate of Trichoderma spp. isolates

The growth of Trichoderma spp. from the rhizosphere of oil palm plants was observed by measuring the diameter of the colonies and the growth rate of fungal isolates grown on a PDA medium. The colony diameter of *Trichoderma* spp. was measured every day until one of the fungal mycelia filled the PDA medium in the Petri dish by making vertical and horizontal lines that intersect at the midpoint below the petri dish. Making a line under the petri dish aims to simplify the measurement and calculation. The diameter of the fungal colony was calculated based on the formula $D = (d_1 + d_2)/2$, where D was the diameter of the fungus, d_1 was the horizontal diameter of the mushroom, and d_2 was the vertical diameter. Growth speed was measured by measuring the daily growth rate of *Trichoderma* spp. rhizosphere origin. Then the average growth rate was calculated. The calculation of the growth rate of *Trichoderma* spp. origin of the rhizosphere was carried out based on the following formula $V = D(n + 1) - Dn$, based on the diameter (D) of the colony up to 7 days after inoculation (dai).

The antagonistic ability of Trichoderma spp. isolates

The antagonistic activity was measured by measuring the radius of the colony of *Ganoderma* sp. away from and near the fungus *Trichoderma* spp. using millimeter paper. The fungus with an inhibitory power of >50% could potentially be used as a biological agent (Otten et al., 2004). The percentage of inhibition could be calculated by the following formula $P = ((r1 – r2)/ r1) \times 100\%$, where P was the percentage of inhibition (%), r1 was the distance between *Ganoderma* sp. away from the antagonist fungus (mm) (calculated from the center of the growing point), and r2 was the distance between *Ganoderma* sp. which approaches the antagonist fungus (mm).

Inhibition mechanism of Trichoderma spp. isolates (hyperparasites phenomena)

The mechanism of inhibition by *Trichoderma* spp. could be observed by observing the hyperparasites phenomena. Observation of the fungus's hyperparasites phenomena of the fungus *Trichoderma* spp was carried out when the fungus hyphae of the fungus grew above (overgrown) the hyphae of the fungus *Ganoderma* sp. based on research (Yusnawan et al., 2019). First, observations were made by taking a glass object overgrown with fungus from the Petri dish. Then the object-glass was observed using a microscope to determine the interactions that occurred, including sticking, twisting, or lysis.

Data Analysis

The morphological diversity, diameter, speed growth, and inhibition by *Trichoderma* spp. isolates data obtained were analyzed descriptively and presented in the form of tables and figures.

RESULT AND DISCUSSIONS

Hypovirulence assay based on disease severity index (DSI)

A total of six isolates after pure isolation, namely: *Trichoderma* sp. isolate LPTUNRI-Trc001, *Trichoderma* sp. isolate LPTUNRI-Trc002, *Trichoderma* sp. isolate LPTUNRI-Trc003, *Trichoderma* sp. isolate LPTUNRI-Trc004, *Trichoderma* sp. isolate LPTUNRI-Trc005 and *Trichoderma* sp. isolate LPTUNRI-Trc 006 isolate. All isolates were tested for hypovirulence by observing the disease severity index of the fungus *Trichoderma* spp. to determine whether a fungus was beneficial or pathogenic. The hypovirulence assay result can be seen in Table 2 and Figure 1. All isolates of *Trichoderma* spp tested had a DSI value of < 2, categorized as a hypovirulent fungus. The hypovirulence properties indicated that the fungal isolates of *Trichoderma* spp tested were not classified as plant pathogenic fungi.

Table 2. Disease severity index of fungal isolates of *Trichoderma* spp. origin of oil palm rhizosphere

Isolat *Trichoderma* spp.	Disease severity index	Description
LPTUNRI-Trc001	0	Hipovirulen (non-pathogenic fungus)
LPTUNRI-Trc002	0.25	Hipovirulen (non-pathogenic fungus)
LPTUNRI-Trc003	0	Hipovirulen (non-pathogenic fungus)
LPTUNRI-Trc004	0	Hipovirulen (non-pathogenic fungus)
LPTUNRI-Trc005	0	Hipovirulen (non-pathogenic fungus)
LPTUNRI-Trc006	0.25	Hipovirulen (non-pathogenic fungus)

Figure 1. Hypovirulence test results of *Trichoderma* spp. origin of oil palm rhizosphere in cucumber seedlings. T1-T6 were *Trichoderma* spp. isolate LPTUNRI-Trc001-006. a) The reaction was virulent (Widya, 2019).

Puspita et al. (2019) stated that hypovirulent fungi had DSI values < 2 in indicator plants and did not cause disease symptoms or only caused symptoms of light brown spots < 0.5 cm, wetness area < 10% in hypocotyl cucumber seedlings. Elsharkawy et al. (2014) stated that fungi with a DSI value < 2 were hypovirulent fungi with very low infectivity and did not cause disease symptoms in plants can be considered a non-pathogenic reaction. However, based on Figure 1, the results of Widya's research (2019), the cucumber seeds had a DSI value > 2, indicating that the fungus was virulent. Elsharkawy et al. (2005) added that if the DSI value was > 2, the fungus was virulent and could infect indicator plants, causing disease in the host plant and could even cause death in the host plant.

The morphological character of Trichoderma spp mycelium

The six fungal isolates macroscopic and microscopic characteristics of *Trichoderma* spp. the origin of the oil palm plant's rhizosphere can be seen in Table 3 based on their morphology. Colony color development begins with white, greenish-white, light green, green and dark green after seven days of age on PDA media. Colonies formed from all isolates were spherical that found in LPTUNRI-Trc001, Trc004, Trc005, Trc006, and some formed concentric rings were found in LPTUNRI-Trc002 and Trc003 (Figure 2). In addition, morphological characteristics showed different forms of conidiophores and phialides, while the conidia of all isolates showed subglobose to ovoidal forms (Figure 3). In detail, the morphological characteristics of the six Trichoderma spp. isolates are presented in Table 3.

The two isolates of *Trichoderma* spp. (LPTUNRI Trc003 and 002) showed the macroscopic and microscopic characteristics, including the shape of conidiophores, phialides, and conidia has similarity to *Trichoderma asperellum*, forming one to two concentric rings near the inoculum with a dense conidial production and white conidia toward the green center, no pigmentation is observed and conidiophores symmetrically arranged, the main branches of conidiophores appearing below are often paired with equal in length and projecting nearly 90 degrees to the principal axis (Siddiquee, 2017)

Trichoderma spp. LPTUNRI Trc001, Trc004, Trc005, and Trc006 character were similar to *T. harzianum* character was, initially formed white mycelia which then changed into yellowish-green or dark green on the progression of its maturation and spreading, the conidiation has predominantly effused with pustules typically merged into large irregular masses with powdery surface appearance green conidial production in mature colonies (Siddiquee, 2017). The conidiophores branching patterns were broad, verticillate, and frequent branching, with one branching verticillate usually having three to four phialides. Phialides were characteristically elongated and lageniform in shape.

Table 3. Morphological characteristic of *Trichoderma* spp. isolates from the oil palm rhizosphere

Morphological characteristic	*Trichoderma* spp. isolates					
	LPT UNRI-Trc001	LPT UNRI-Trc002	LPT UNRI-Trc 003	LPT UNRI-Trc004	LPT UNRI-Trc005	LPT UNRI-Trc006
Macroscopic						
Colony color	Green to dark green	Dark green	Dark green	Green to dark green	Yellow to dark green	Green to dark green
Reverse colony color	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
Colony edge	Smooth	Wavy	Wavy	Smooth	Smooth	Smooth
Mycelial form	Floccose to arachnoid	Floccose to arachnoid	Floccose to arachnoid	Floccose to arachnoid	Floccose to arachnoid	Floccose to arachnoid
Mycelial color	Watery white	White	White	Watery white	Watery white	Watery white
Conidiation	spreading in several concentric rings	spreading along the colony margin, sometimes concentric rings	spreading along the colony margin, sometimes concentric rings	spreading in several concentric rings	spreading in several concentric rings	spreading in several concentric rings
Microscopic						
Conidiophore	spread to the top in pyramidal fashion and highly branches	highly branches and arrangemen t in symmetric order	highly branches and arrangement in symmetric order	long, straight, solitary	spread to the top in pyramidal fashion and highly branches	spread to the top in pyramidal fashion and highly branches
Phialid shape	flask-shaped	Ampuliform	Ampuliform	flask-shaped	flask-shaped	flask-shaped
Conidial shape	Subglobose	Ovoidal	Ovoidal	Subglobose	Subglobose	Subglobose

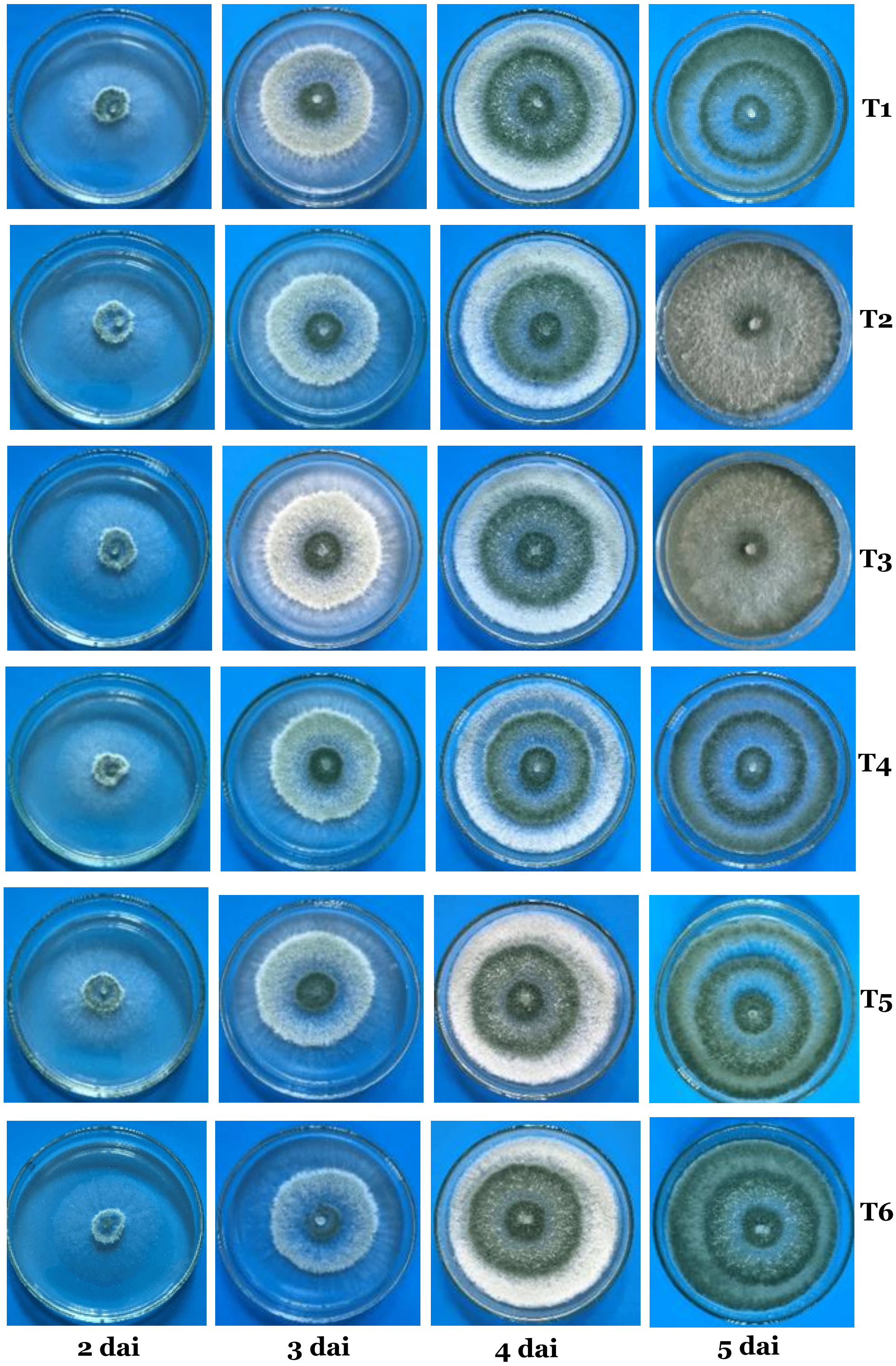

Figure 2. Microscopic characteristic of *Trichoderma* spp. origin of oil palm rhizosphere 5 days after incubation (dai) in PDA medium. T1-T6 were LPTUNRI-Trc001-006 isolate

Potential of Trichoderma spp. *as a biological control agent of Ganoderma* sp.

Diameter and growth rate of Trichoderma spp. *isolates*

The isolate of *Trichoderma* spp. the origin of the rhizosphere of oil palm plants showed that the diameter and growth rate were not significantly different after analysis of variance. The results of the DNMRT further test at the 5% level can be seen in Table 4. Table 4 shows that *Trichoderma* spp. LPTUNRI-Trc003 isolates had higher diameters (90.00 mm) and growth rates (32.66 mm/day), which were not significantly different from other isolates. Growth of *Trichoderma* spp. the LPTUNRI-Trc003 isolate was very fast so that it could fill the growing space on the third day of observation. *Trichoderma* spp. had good growth, a high growth rate, and a high antagonistic ability. Figure 2 shows the growth of *Trichoderma* sp. LPTUNRI-Trc003 isolate was very fast, so it was able to fill the growing space on the third day after incubation.

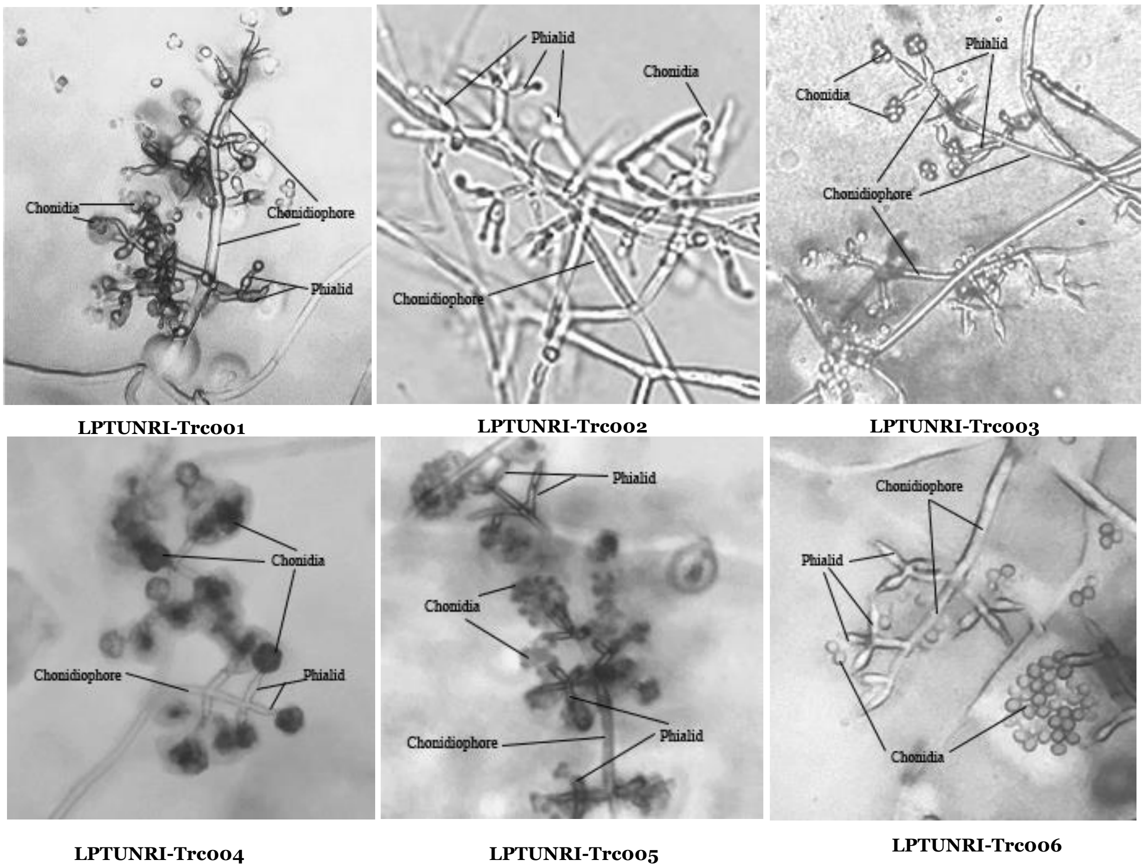

Figure 3. Microscopic characteristic of *Trichoderma* spp. conidiophore

Gusnawaty et al. (2013) stated that the ability of high growth speed was one of the main factors that determine the ability of an antagonist fungus to control diseases that attack plants so that with this ability, antagonistic fungi could compete in the control of space and nutrients so that they could inhibit the growth of pathogenic fungi. It was also supported by Nuraini (2017) that the competition of antagonistic fungi indicated by the domination growth in the Petri dishes disrupted the nutritional needs of pathogen and caused the growth of pathogenic fungi to be inhibited. Nurbaya et al. (2014) added that fungal colonies usually continue to grow until they encounter obstacles such as the tip of a Petri dish or other colonies.

Table 4. Diameter (mm) and growth speed (mm/day) 6 isolates of *Trichoderma* spp. origin of oil palm rhizosphere.

Trichoderma spp. isolates	Diameter (mm)	Growth speed (mm/day)
LPTUNRI-Trc001	80.00	32.50
LPTUNRI-Trc002	76.66	30.05
LPTUNRI-Trc003	90.00	32.66
LPTUNRI-Trc004	80.00	31.41
LPTUNRI-Trc005	80.00	31.41
LPTUNRI-Trc006	76.66	31.83

Antagonistic ability of Trichoderma spp. isolates

Table 5 shows that *Trichoderma* sp. LPTUNRI-Trc003 isolate has a high antagonistic ability to inhibit the growth of *Ganoderma* sp. LPTUNRI-Gan002 isolate was 91.03% but not significantly different from the other isolates. The ability of each isolate of *Trichoderma* spp. showed different inhibition on the growth of *Ganoderma* sp. LPTUNRI-Gan002 isolate. It was presumably due to differences in growth speed and ability to compete for nutrients from the growing media shown in Table 4 and Figure 2. Figure 4 shows that all *Trichoderma* spp. derived from the rhizosphere of oil palm plants have a high competition mechanism in filling the petri dish and using nutrients from PDA, which causes the *Ganoderma* sp. to be unable to compete and stunted growth.

Hutabalian et al. (2015) stated that biological agents that had fast growth would have the ability to inhibit the growth of pathogens, which is higher with competition for space and nutrients. Based on Figure 5, isolates of *Trichoderma* spp. the origin of the rhizosphere of the oil palm plant had an inhibitory power of >50%, which had the potential as a biological agent consisting of *Trichoderma* spp. isolates LPTUNRI-Trc001 (88.38%), Trc002 (89.34%), Trc003 (91.03%), Trc004 (88.05%), Trc005 (88.46%) and Trc006 (87.57%) . It was in line with the statement of Otten et al. (2004), which stated that fungi that had an inhibitory power of >50% had the potential to be used as biological agents. Each isolate had a different antagonistic power in inhibiting *Ganoderma* sp. The high antagonist ability could be caused by several factors, including growth rate and antagonistic mechanism. According to Amaria et al. (2013), fungal isolates with a high growth rate had a high Inhibition activity.

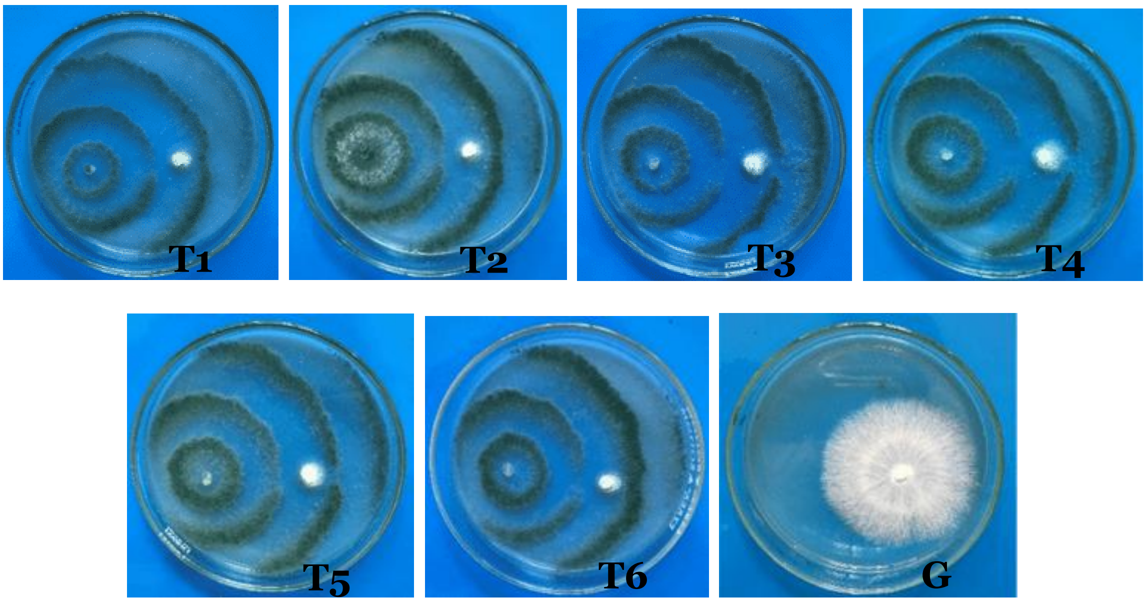

Figure 4. Inhibition activity of 6 isolates of *Trichoderma* spp. origin of oil palm rhizosphere against *Ganoderma* sp. five days after incubation on PDA medium. (T1-T6 = LPTUNRI-Trc001-006; G = *Ganoderma* sp. LPTUNRI-Gan002 isolate.

Inhibition mechanism of Trichoderma spp. isolates (hyperparasites phenomena)

The hyperparasitic type of the fungus *Trichoderma* spp. origin of the rhizosphere of oil palm plants with higher inhibition ability against the fungus *Ganoderma* sp. LPTUNRI-Gan002 isolate had hyperparasitic phenomena based on hyperparasitism test results. The types of hyperparasites can be seen in Table 5. Table 5 showed that the isolates of *Trichoderma* spp. The origin of the rhizosphere of oil palm plants has a hyperparasitic mechanism on *Ganoderma* sp., so growth was stunted. There were two types of hyperparasite mechanism: the entanglement type found in *Trichoderma* spp. isolates LPTUNRI-Trc001, Trc002, Trc004, Trc005, Trc006, and lysis of *Trichoderma* spp. LPTUNRI-Trc003 isolate. The results of the hyperparasitism test of the fungus *Trichoderma* spp. had high antagonistic power against *Ganoderma* sp. LPTUNRI-Gan002 isolates can be seen in Figure 4 and Figure 5.

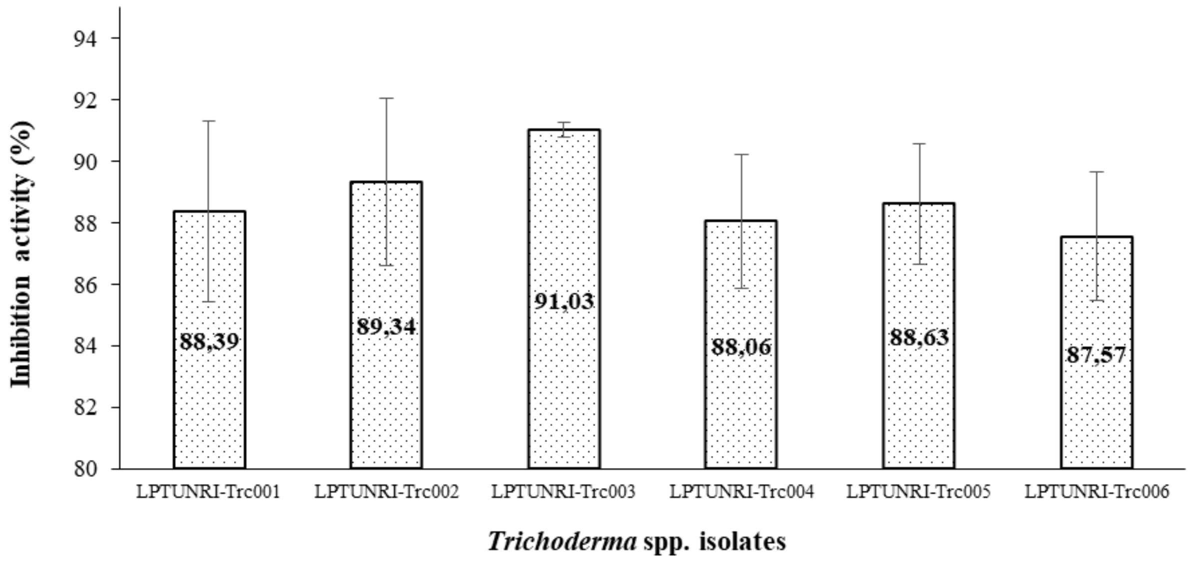

Figure 5. Inhibition of *Trichoderma* spp. origin of oil palm rhizosphere against *Ganoderma* sp.

Trichoderma spp. isolates LPTUNRI-Trc001, Trc002, Trc004, Trc005 and Trc006 showed a type of hyperparasitism interaction in the form of entanglement (Figure 6), showing the growth of mycelia of the fungus *Trichoderma* spp. wrapped around the hyphae of *Ganoderma* sp. Twisting is the stage of hyperparasitic interaction between antagonistic fungal hyphae and pathogenic hyphae. Twisting could disrupt the growth of *Ganoderma* sp. LPTUNRI-Gan002 isolate. Isolate Trc003 showed the type of hyperparasitism interaction in the form of lysis (Figure 6), which caused the hyphae of *Ganoderma* sp. intermittent and visible in some parts of the hyphae to become clear and destroyed. This type of lysis was an advanced phase of the coiling process by LPTUNRI-Trc003 isolate, which was thought to be due to the -1,3-glucanase enzyme, which can degrade the cell wall of *Ganoderma* sp.

Table 5. Types of hyperparasitic isolates of *Trichoderma* spp. origin of the rhizosphere of oil palm plants with higher inhibitory power against *Ganoderma* sp.

Trichoderma spp. isolates	Hyperparasitic
LPTUNRI-Trc001	Twist
LPTUNRI-Trc002	Twist
LPTUNRI-Trc003	Lysis
LPTUNRI-Trc004	Twist
LPTUNRI-Trc005	Twist
LPTUNRI-Trc006	Twist

Melysa et al. (2013) added that the antagonistic ability would appear because of the competition between two types of fungi that grow simultaneously, so there was competition for space and media used to grow each fungus. The growth speed of antagonist fungus indicated a competition mechanism in space and nutrition with pathogens. The faster growth of antagonistic fungi, the more effectively suppress the growth of pathogens (Amaria et al., 2015)

The entanglement and lysis mechanism were other mechanisms shown by six isolates of *Trichoderma* spp. known as mycoparasites. Marlina and Susanti (2013) stated that mycoparasites antagonistic fungi; when they reach their host, their hyphae wrap around or squeeze their host hyphae by forming a hook-like structure; after further twisting, the Trichoderma could penetrate the host hyphae by degrading some of its cell walls. Mycoparasite mechanism by *Trichoderma* spp. often begins with their growth along the host hyphae and continues with the formation of papilla-like structures. Cell wall degradation and lumen penetration occur at points where papilla-like structures form during the hyphal growth of *Trichoderma* spp. takes place along with the host (Sarma et al., 2014).

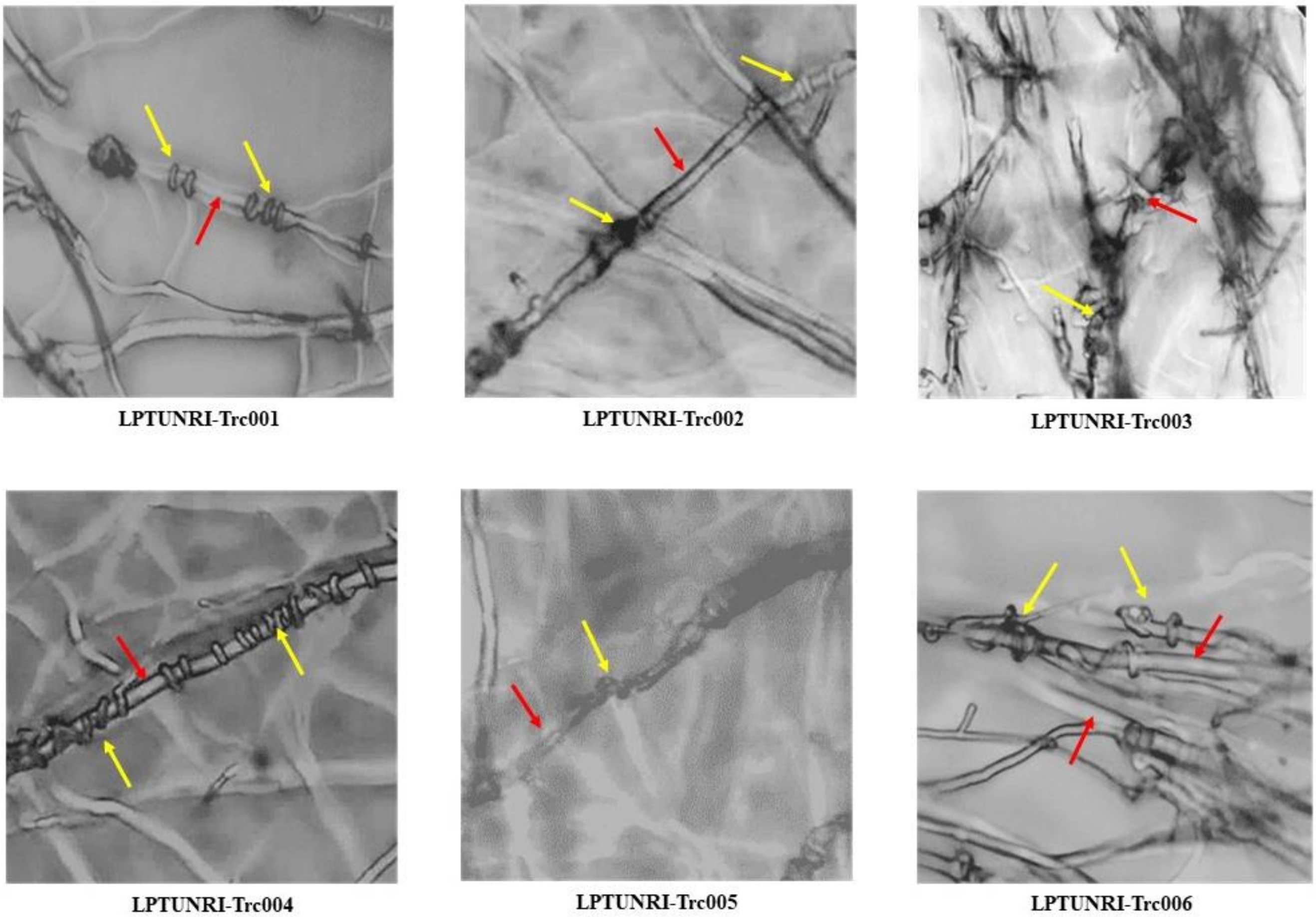

Figure 6. The form of hyperparasitic interaction of fungal isolates *Trichoderma* spp. origin of the rhizosphere of oil palm (yellow arrow) and *Ganoderma* sp. LPTUNRI-Gan002 (red arrow)

The potential of *Trichoderma* spp. as a biological control agent is known along with the critical mechanism of this fungus to parasitize, suppress, or even kill other plant pathogenic fungi (Mukherjee et al., 2012). Furthermore, Nusaibah and Musa (2019) stated that *Trichoderma*-based biocontrol mechanisms, especially on mycoparasitism production of antibiotics, hydrolytic enzymes, the diversity of secondary metabolites produced, competition, and induction of plant resistance by *Trichoderma* species, can directly inhibit the growth of some plant pathogens that can act either directly or indirectly against pathogens.

CONCLUSIONS

Six *Trichoderma* spp. isolates had morphological characteristics similar to two species, namely *Trichoderma harzianum* (LPTUNRI-Trc001, Trc004, Trc005 and Trc006 isolates) and *Trichoderma asperellum* (LPTUNRI-Trc002 and Trc003). *Trichoderma* sp. LPTUNRI-Trc003 has the highest diameter (90 mm), growth rate (32.66 mm/day), and the highest ability to suppress *Ganoderma* sp. LPTUNRI-Gan002 (91.03%) compared to the other five isolates. In addition, there were two types of hyperparasite phenomena, namely the twisted type (found in *Trichoderma* sp. isolates LPTUNRI-Trc001, Trc001, Trc002, Trc004, Trc005, Trc006) and the lysis type (*Trichoderma* sp. isolates LPTUNRI-Trc003).

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