

The Potential of *Trichoderma* spp. Endophytes as Biological Control Agents Against *Ceratocystis* sp. Causes of Acacia Stem Rot Disease *in vitro*

Fifi Puspita¹ Rachmad Saputra^{1,*} Nur Hasanah¹

¹Agrotechnology Department, Agriculture Faculty, Universitas Riau, Pekanbaru, 28293, Riau, Indonesia

*Corresponding author. rachmadsaputra@lecturer.unri.ac.id

ABSTRACT

Ceratocystis sp. is an important pathogen in acacia plants. This pathogenic fungus causes stem rot disease which can cause the death of mature plants within 4 - 7 years with a disease attack percentage of nearly 80%. The common controlling technique is carried out by using chemical pesticides, for that we need another alternative to reduce its negative impact, namely the use of *Trichoderma* spp. endophyte. This research aimed to determine the potential of the *Trichoderma* spp. endophytes in suppressing the development of the *Ceratocystis* sp. The study was conducted experimentally using a completely randomized design (CRD) consisting of 7 treatments with three replications, namely without *Trichoderma* sp. endophytic (E0), two isolates of *T. virens* endophytic of oil palm plants (E1-E2), one isolate of *T. harzianum* endophytic of rubber plants (E4), three isolates of *Trichoderma* sp. endophytic of acacia plants (E5-E7), and one isolate *Trichoderma* sp. endophytic of eucalyptus plant (E14). The parameters observed were the antagonistic ability, colony diameter, growth rate of *Trichoderma* spp. colonies, and its inhibition mechanism against *Ceratocystis* sp. Based on the results of this study, *Trichoderma* sp. endophytic E074 isolated from 4 years old *Acacia crasicaarpa* had the highest antagonistic ability (30.76%), diameter (90 mm), and growth rate (33.33 mm.day⁻¹) compared to other isolates with hyperparasitic entrapment type.

Keywords: acacia, biological control, endophytes, *Ceratocystis*, *Trichoderma*

1. INTRODUCTION

Acacia plantations (*Acacia crasicaarpa*) are industrial plantation forest commodities that have high economic value because they are widely used, especially as a source of raw material for the paper (pulp). One of the important diseases found in acacia is stem rot disease caused by *Ceratocystis* sp. *Ceratocystis* sp. is a pathogen that attacks forestry plants or industrial plants such as acacia plants with an attack percentage of nearly 80%. *Ceratocystis* sp. can cause death in mature plants within a period of 4–7 years which is marked by yellowing of the leaves, drying up and the tree finally dying, the bark of the trunk becomes blackish-brown and cracked [1].

According to Accordi, *Ceratocystis* sp. is a pathogen that does not have an incubation period, so that when there is a fungal wound it will immediately colonize and infect, therefore fast and precise control is needed [2]. Control efforts have been made to overcome attacks by *Ceratocystis* sp. namely by controlling the technical culture, the use of resistant varieties and using synthetic chemical fungicides, but the use of synthetic chemical fungicides excessively can cause poisoning in humans, environmental pollution, death of non-target organisms and resistance to pathogens. Pathogen control techniques

that are safer and more environmentally friendly are biological control by utilizing biological agents. Biological agents can be utilized, one of which is endophytic fungi. Endophytic fungi are fungi originating from plant tissue and do not harm their host plants [3].

One of the endophytic fungi that are often found and capable of acting as biological control agents is *Trichoderma* sp. This fungus can suppress disease development in plants, especially soil-borne pathogens through processes of mycoparasitism, competition, and antibiosis, which indirectly stimulate plant growth and induce resistance to disease [4].

The endophytic *Trichoderma harzianum* AK51 isolate from rubber plants has 70.6% inhibition power to control white root fungal disease in rubber plants [5]. *Trichoderma virens* endophytic fungus originating from oil palm roots is better, compared to the *Trichoderma virens* endophytic fungus originating from stems and midribs shown by the best antagonistic ability of 71.11% and the best antifungal compound of 25.74%. and the inhibitory mechanism that occurs is antibiosis in inhibiting the growth of the fungus *Rigidoporus microporus* [6]. *Trichoderma* sp. local isolates can inhibit *Ganoderma* sp. in *Acacia mangium* plants with inhibition power ranging from 38.09% -58.06% [7]. Control of *Ceratocystis* sp. by

using *Trichoderma* sp. endophytes have not been widely reported. The purpose of this study was to determine the effect of several *Trichoderma* sp. isolates endophytic and obtained the best isolates *Trichoderma* sp. endophyte to control *Ceratocystis* sp. in vitro.

2. MATERIALS AND METHODS

The research was conducted at the Laboratory of the Plant Protection Department of PT. Arara Abadi, Pinang Sebatang Village, Tualang District, Siak Regency, Riau. The research was conducted for 4 months from March to June 2019.

The materials used in this study were *Trichoderma* sp. endophytic origin of acacia and eucalyptus plants collected by the Laboratory of PT Arara Abadi R&D Perawang, isolate *Trichoderma* sp. endophytic origin of oil palm, the collection of Fifi Puspita and isolates from *Trichoderma* sp. endophytic origin of rubber plants from the collection of Wilda Andini, a pathogenic isolate of *Ceratocystis* sp. (013C-R1) PT Arara Abadi R&D Perawang laboratory collection, potato sucrose agar (PSA), streptomycin, 70% alcohol, 96% alcohol, distilled water, cotton, tissue paper, plastic wrap, aluminum foil and label paper.

The tools used in this study were stationery, ruler, camera, 9 cm diameter petri dish, bunsen lamp, cork borer (agar cutter), knife, binocular microscope, object glass, cover glass, measuring cup, Laminar Air Flow Cabinet (L AFC), oven, micropipette, spatula, scalpel, tweezers, loop needles, test tubes, Erlenmeyer, test tube, incubators and analytical scales.

This research was conducted experimentally using a completely randomized design (CRD) consisting of 7 treatments and 3 replications to obtain 21 experimental units. The treatments in this study were 7 isolates of *Trichoderma* sp. endophytic with high antagonistic ability (T) with high inhibition (> 50%) as follows: T1 (*Trichoderma virens* isolate endophytic oil palm plant roots); T2 (*Trichoderma virens* endophytic isolate from stems of oil palm plants); T4 (*Trichoderma harzianum* isolate endophytic rubber plant roots); T5 (*Trichoderma* sp. endophytic E074 *Acacia crasicarpa* isolate 4 years); T6 (*Trichoderma* sp. endophyte E075 *Acacia crasicarpa* isolate 4 years); T7 (*Trichoderma* sp. endophytic E080 *Acacia crasicarpa* isolate 3 years) and T14 (*Trichoderma* sp. E022 endophytic *Eucalyptus* plant).

The observation parameter of this study was the antagonistic ability of several *Trichoderma* sp. Isolates. endophytes against *Ceratocystis* sp., colony diameter (mm) and growth rate (mm.day⁻¹) isolates of *Trichoderma* sp. endophytic and hyperparasitic interaction type isolates *Trichoderma* sp. endophyte with high antagonistic ability against *Ceratocystis* sp.

The data obtained from the observations were analyzed descriptively and by variance. Descriptive data analysis includes the results of observations of the types of hyperparasitism. Data on the fungus *Trichoderma* sp. endophyte with high antagonistic ability against

Ceratocystis sp. and data is presented in tables and figures. Analysis of variety includes inhibitory data of the fungus *Trichoderma* sp. endophyte, diameter and growth rate of the *Trichoderma* sp. endophyte which has high antagonistic ability against *Ceratocystis* sp. To compare the average between *Trichoderma* sp. Isolates. The endophyte was followed by further tests with Duncan's new multiple range test (DNMRT) at the 5% level.

3. RESULTS AND DISCUSSION

3.1. Antagonist Ability of *Trichoderma* sp. Endophyte Against *Ceratocystis* sp. In Vitro

Antagonistic ability of 19 isolates of *Trichoderma* sp. endophytes against *Ceratocystis* sp. significant effect after analysis of variance. The results of the DNMRT follow-up test at the 5% level can be seen in Table 1.

Table 1 Antagonistic ability of *Trichoderma* sp. endophytes against *Ceratocystis* sp. 5 days after incubation in PSA medium.

Isolat	Daya antagonis (%)
T ₁	21,61 bc
T ₂	20,83 bc
T ₄	21,33 bc
T ₅	30,76 a
T ₆	24,00 ab
T ₇	21,88 bc
T ₁₄	22,66 bc

The numbers followed by the same lowercase letter are not significantly different after being transformed by $\sqrt{y + 0.5}$ according to the DNMRT test results at the 5% level.

Table 1 shows that the T5 isolate has a high antagonistic ability to inhibit the growth of the fungus *Ceratocystis* sp. namely 30.76% and significantly different from other isolates and without *Trichoderma* sp. isolates. endophytic but not significantly different from isolate E6 because isolate T5 has a faster growth and can be seen in the measurement parameters of diameter and growth speed (Table 2) so that it can inhibit the pathogen *Ceratocystis* sp. because of competition for space and nutrition.

According to Harman *et al.* fast growing fungi can outperform in control of space and nutrition and the end can suppress the growth of their opponent's fungi, the competition is in obtaining nitrogen and carbon [8]. This is following the results of research by Hutabalian *et al.* stated that biological agents that have fast growth will have the ability to inhibit the growth of pathogens with higher competition for space and nutrients [9].

According to Sunarwati and Yoza, antagonistic fungi that have an inhibitory ability of 26-50% are included in the group of fungi that have the low antagonistic ability because the inhibitory ability of antagonistic fungi is influenced by several factors, one of which is the growth of the fungus itself [10]. Amaria states that fungal isolates that have high inhibitory ability are antagonistic fungi isolates whose colony growth is faster than pathogenic

colonies [11]. This is in following what was stated by Octrina that antagonistic fungi can be categorized as having high inhibitory activity against pathogen growth if the percentage of inhibition reaches more than 60%, but if the percentage of inhibition only reaches 30% then the antagonistic fungi can be categorized as having an inhibitory effect [12].

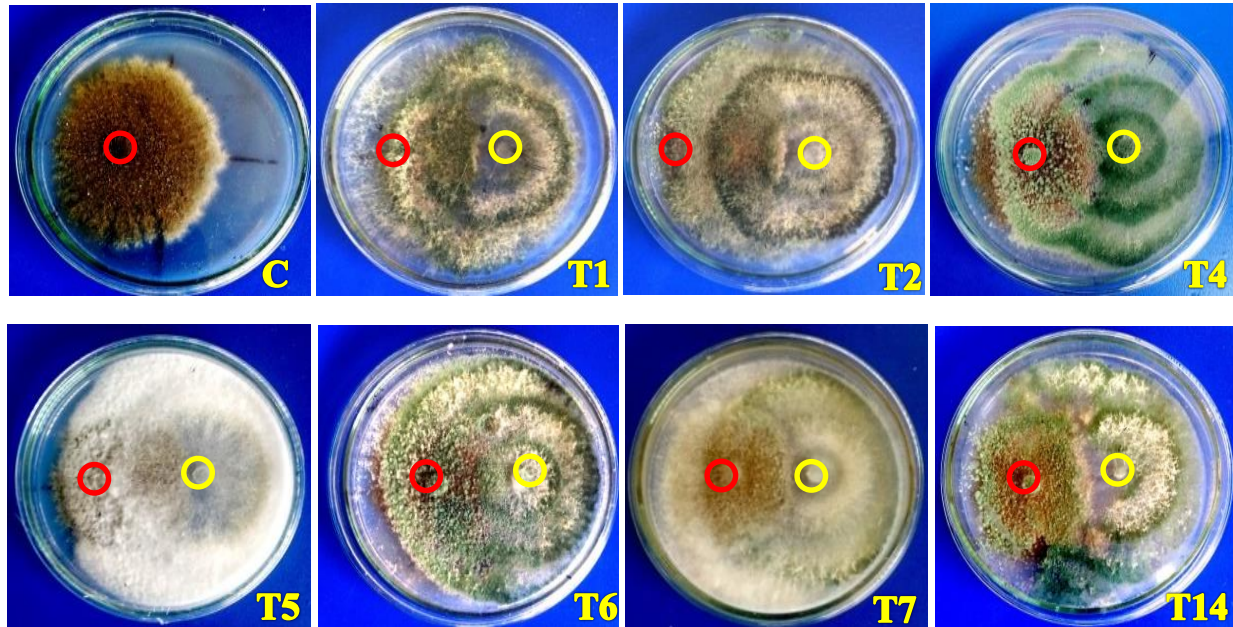


Figure 1 Antagonistic ability of *Trichoderma* sp. (yellow circle) against *Ceratocystis* sp. (red circle) on PSA medium. (C = control; T1, T2, T4, T5, T6, T7 and T14 = *Trichoderma* sp. isolates)

Based on Figure 4, it can be seen that 7 isolates (T1, T2, T4, T5, T6, T7 and T14) with moderate antagonistic ability did not form an inhibition zone but isolates of *Trichoderma* sp. endophytes have a mechanism of competition for space and nutrients in inhibiting the growth of the pathogenic fungus *Ceratocystis* sp. on PSA medium. Competition between *Trichoderma* sp. endophytes and *Ceratocystis* sp. occurs because of competition for nutrients from the PSA medium so that the fungus *Trichoderma* sp. Endophytes are superior in control of space and nutrition and can grow to meet PSA medium. This causes the growth of *Ceratocystis* sp. become obstructed. According to Melysa *et al.* antagonistic properties arise due to the competition that occurs between two types of fungi that are grown side by side [13]. This competition occurs due to the same needs of each mushroom, namely the need for a place to grow and nutrients from the media used to grow. Purwantisari and Hastuti reported that the faster the growth of antagonistic fungi, the more suppressed the growth of pathogenic fungi would be due to running out of growth space [14].

3.2. The diameter and growth rate of *Trichoderma* sp. high antagonistic ability (mm.day^{-1}).

The results of observing the diameter and growth rate of *Trichoderma* sp. endophytes with moderate antagonistic ability had a significant effect after analysis for variance. The results of the DNMRT follow-up test at the 5% level can be seen in Table 2.

Table 2 shows that the T5 isolate has a diameter and growth rate that tends to be high, namely 90.00 mm and $33.33 \text{ mm.day}^{-1}$ and is significantly different from isolate T7 (84.00 mm and $31.00 \text{ mm.day}^{-1}$) but is different not real with other isolates. The growth of isolate T5 was very fast so that it was able to fill the growing space on the third day of observation. This is related to the results of the antagonistic ability of the fungus *Trichoderma* sp. endophytes that can compete in the struggle for space and nutrition with *Ceratocystis* sp. Hutabalian *et al.* also reported that the faster the growth of a biological agent, the higher its ability to suppress pathogen growth due to competition for nutrients and living space [9].

Based on Figure 2, it can be seen that the growth of isolates T5 was faster than isolates T4, T14, T1, T2, T6, and T7. This is following the opinion of Djafarudin that the growth rate of fungal colonies is an important factor in determining its potential as a biological agent against pathogens [15]. According to Gusnawathy *et al.* the ability of high growth rates is one of the main factors that determines the ability of an antagonistic organism to control diseases that attack plants, so that with this ability microorganisms can compete in control of space and nutrients [16].

Table 2 Diameter (mm) and growth rate (mm.day⁻¹) of 7 isolates of *Trichoderma* sp. moderately inhibiting endophyte

<i>Trichoderma</i> sp. isolates	Diameter (mm)	Growth rate (mm.day ⁻¹)
T ₁	85,66 abc	32,50 ab
T ₂	85,00 bc	31,83 ab
T ₄	89,33 ab	32,83 ab
T ₅	90,00 a	33,33 a
T ₆	84,66 bc	31,83 ab
T ₇	84,00 c	31,00 b
T ₁₄	88,66 abc	32,83 ab

The numbers followed by the same lowercase letter are not significantly different according to the results of the Duncan New Multiple Range Test (DNMRT) at the 5% level.

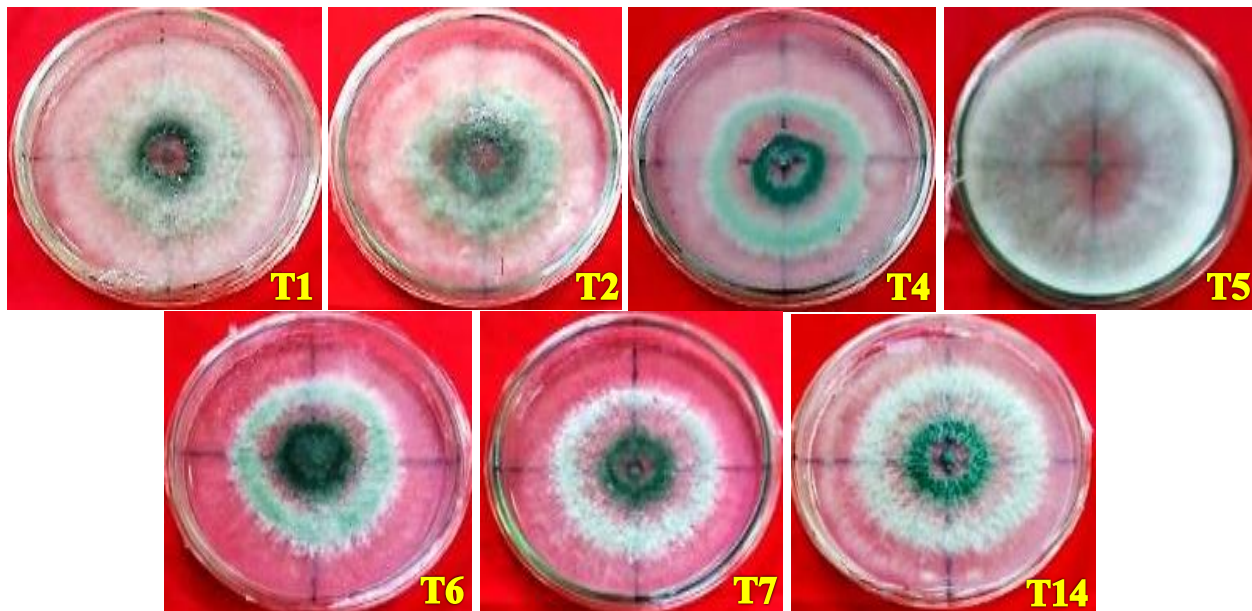


Figure 2 The diameter of the *Trichoderma* sp. endophytes 3 days after incubation in PSA medium. a) Isolate T1, b) Isolate T2, c) Isolate T4, d) Isolate T5, e) Isolate T6, f) Isolate T7, g) Isolate T14

3.3. *Trichoderma* sp. Hyperparasitic Type. Endophyte with High Antagonistic Ability Against *Ceratocystis* sp.

Trichoderma sp. Hyperparasitic type. endophyte with moderate antagonistic ability against the fungus *Ceratocystis* sp. has a hyperparasitic type based on the results of the hyperparasitic test. Hyperparasitic types can be seen in Table 3 and Figure 3.

Table 3 and Figure 3 shows that there are three types of hyphae interactions in *Trichoderma* sp. isolates. T1, T2, T4 and T6 isolates have interaction in the form of hyphal coiling and make the sticking and widening type that cause damage to the hyphae of *Ceratocystis* sp. This is

suspected because the antagonistic fungi produce various chemical compounds that are toxic to *Ceratocystis* sp. Sunarwati and Yoza stated that the genus *Trichoderma* was able to inhibit the growth of pathogenic hyphae by producing gliotoxin and viridin antibiotics [10].

The interaction between T4 isolates and *Ceratocystis* sp. Attachment is an initial process in inhibiting pathogenic fungi from the hyperparasitic activity which results in the cessation of pathogenic growth. The results of Alamsyah's research state that the process of attaching hyphae have shown an initial interaction between antagonistic fungi, which then the hyphae will pierce and suck the contents of pathogenic hyphae cells, resulting in the cessation of the growth of pathogenic fungal hyphae [17].

The isolates of T5 and T7 show the entrapment type that causes the hyphae of *Ceratocystis* sp. does not develop and stop to growth. This is supported by the opinion of Kurnia *et al.* stated that the antagonistic hyphae that trap pathogenic hyphae will cause the pathogenic hyphae to not develop so that their growth stops [3].

The T14 isolate causes the growth of pathogenic hyphae break off and it can be seen that in some parts of the hyphae becomes clear. It is suspected that there is a lysis mechanism so that the growth of pathogenic fungal hyphae becomes abnormal. This is supported by the opinion of Sunarwati and Yoza which states that another way biological agents can inhibit pathogens is by lysis. Lysis, namely the mycelium of biological agents, is capable of destroying or cutting the mycelium from pathogens, which in turn causes the pathogen's death [10].

The lysis mechanism is characterized by changing the color of the pathogenic fungal hyphae to become clear and empty, then some break off and eventually destroy [18].

Table 3 *Trichoderma* sp. Types of hyperparasitic against *Ceratocystis* sp.

<i>Trichoderma</i> sp. isolates	Types of hyperparasitic
T ₁ , T ₂ , T ₄ and T ₆	Winding and Sticking
T ₅ and T ₇	Entrapment
T ₁₄	Lysis

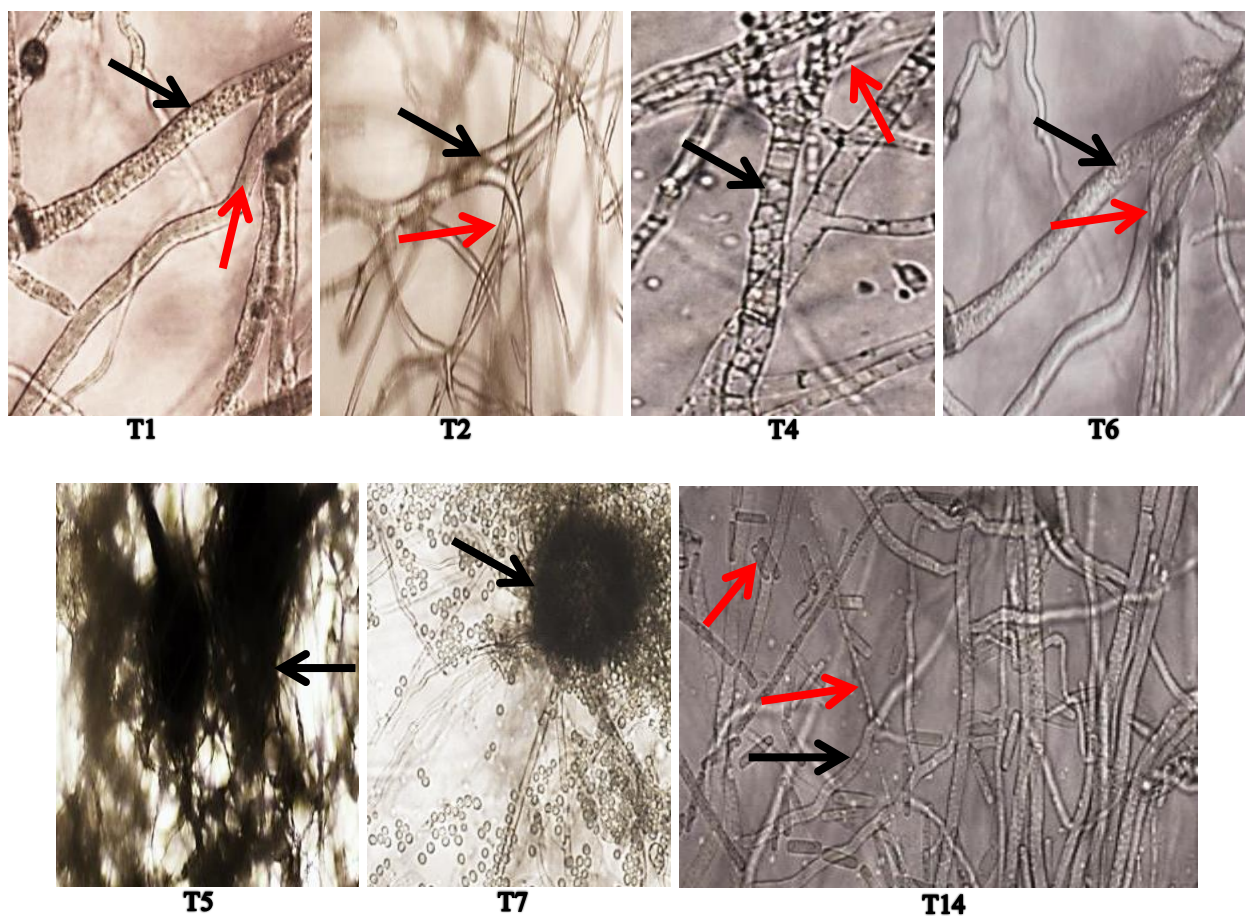


Figure 3 The type of hyperparasitic interaction of the *Trichoderma* sp. (black arrow) and *Ceratocystis* sp. (red arrow).

4. CONCLUSION

The isolates of *T. virens* endophytic roots of oil palm plants (T1), *T. virens* endophytes of oil palm stems (T2), *T. harzianum* endophytes of rubber plant roots (T4), *Trichoderma* sp. endophytic E074 from 4 years *Acacia crasicarpa* (T5), *Trichoderma* sp. endophytic E075 from 4 years *Acacia crasicarpa* (T6), *Trichoderma* sp. endophytic

E080 from 3 years *Acacia crasicarpa* (T7), and *Trichoderma* sp. endophytic E022 *Eucalyptus* plants (T14) are seven isolates that have moderate inhibitory ability to control stem rot disease caused by *Ceratocystis* sp. in vitro.

Isolate *Trichoderma* sp. endophytic E074 *Acacia crasicarpa* 4 years (T5 isolate) is the best treatment in inhibiting the pathogen *Ceratocystis* sp. with the highest

percentage of inhibitory ability (30.76%), and the largest colony diameter (90.00 mm), and the highest growth rate (33.33 mm.day⁻¹).

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