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Diversity and Mechanism of Inhibition of *Trichoderma* sp. Against *Fusarium* sp. Durian Plant Pathogen

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Abstract

Durians are plants that are widely cultivated in Indonesia because they have many benefits and delicious taste. It was reported that there was a decrease in durian fruit production due to leaf blight caused by the fungus Fusarium sp. Environmentally friendly control efforts use antagonistic agents such as the fungus Trichoderma sp. be one of the right solutions in controlling the disease. The purpose of this study were to explore the diversity of Trichoderma sp. in the rhizosphere of durian plants and evaluating the inhibition mechanism of Trichoderma sp. against Fusarium sp. The research was carried out in December 2021 - May 2022 at the PT. Pamorganda North Bengkulu and Laboratory of Plant Protection, Faculty of Agriculture, University of Bengkulu. The results showed that the main disease in durian plants was leaf blight caused by the fungus Fusarium sp. From the rhizosphere of healthy durian plants, 16 isolates of Trichoderma sp. had been isolated, which belongs to the species T. atroviridae, T. aurioviridae, T. harzianum, T. koningii, and T. viridae. Percentage of inhibition of Trichoderma sp against Fusarium sp. were ranging from 93.3 – 97.7% with the mechanism of inhibition were competition for space, nutrients and oxygen; antibiosis, lysis and parasitism.

Keywords: Antagonism; durian; Fusarium sp.; Trichoderma sp.

Introduction

Durian is known as a seasonal tropical fruit in Southeast Asia: Malaysia, Thailand, the Philippines and Indonesia (Leontowicz *et al*., 2011) and is cultivated in various regions of Indonesia including Bengkulu Province. In addition to having a delicious taste, durian fruit can also be processed into various kinds of processed foods, such as durian lempok and durian chips (Angelus, 2019; Dhamura *et al*., 2021).

Durian fruit production in Indonesia from 2018-2020 has decreased: 1,142,102 tons decreased to 1,133,195 tons. Durian production in Bengkulu province from 2019-2020 also decreased: 11,395 tons/ha to 9,260 tons/ha (Badan Pusat Statistik, 2020). Land expansion has been carried out, but the increase in durian production in Indonesia is still very low at only 5% per year and has not been able to meet the needs of durian in Indonesia (Santoso, 2012). Many factors cause the low production of durian fruits in Indonesia. among them are leaf blight (*Fusarium* sp.) cancer (*Phytophthora palmivora*), root rot (*Pythium complectens*), seedling disease (*P. palmivora*), dead end (*P. palmivora*), and upas fungus (*Upasia salmonicolor*) (Kwee, 1990; Semangun, 2007; Andy, 2014). *Fusarium* sp. can attack durian plants with symptoms of large patches on the leaves and eventually dead leaves, even in the nursery phase can cause stunted growth so that the seedlings cannot be used. The attack of this pathogen on durian plants causes losses of 40% (Semangun, 2007).

To overcome these problems, it is necessary to carry out appropriate control efforts. The use of antagonistic microbes that live around plant roots, such as *Bacillus* spp., *Pseudomonas* sp., *Gliocladium* sp., and *Trichoderma* spp. is one of the effective ways of controlling various plant diseases (Elhamshary *et al*., 2008; Soenartiningsih *et al*., 2011).

Purwantisari *et al*. (2009) reported that *Trichoderma* sp. has high antagonism properties to pathogens of cultivated plants and has a control mechanism that is able to inhibit the growth of pathogens and increase plant production. These fungi also have a wide range of mycoparasitism and are not pathogenic to plants. In addition, *Trichoderma* sp. become a good competitor in fighting for nutrients so that it is able to suppress the activity of soil-borne pathogens (Sudantha *et al*., 2011). It was further reported that *T. harzianum* and *T. virens* were able to inhibit the growth of *P. palmivora* and *P. capsici*, which causes stem base rot in pepper plants (Nisa, 2010). Umrah *et al*. (2009) reported that *Trichoderma* sp. has an inhibition of 82.7% against *P. palmivora* which causes cocoa fruit rot. *T. harzianum* effectively inhibits the growth of colonies of *Ganoderma* sp. in-vitro by 74% (Dendang, 2015).

The purpose of the study was to evaluate the diversity and antagonism mechanisms of *Trichoderma* sp. against *Fusarium* sp, the main pathogen of durian plants in Bengkulu durian plantations.

Research Method

The research was carried out in December 2021 - May 2022 at the durian plantation of PT. Famorganda, North Bengkulu and at the Plant Protection Laboratory, Faculty of Agriculture, University of Bengkulu. Rhizosphere soil samples of healthy durian plants were taken from 10% of the durian plant population as a source of *Trichoderma* sp. Rhizosphere soil samples of diseased durian plants were taken from 5 diseased durian plants by purposive sampling. *Trichoderma* sp. And *Fusarium* sp. isolated by the dilution method.

Characterization of *Trichoderma* sp. and *Fusarium* sp. is carried out macroscopically and microscopically, including: color, growth of fungi diameter, and the presence or absence of air mycelium, hyphae form, conidia form, conidiophores, chlamydospores, and phialide shapes. Furthermore, all fungi were identified using the *Trichoderma* species determination key book (Widyaastuti, 2007).

Antagonistic testing was carried out by the dual culture method (Dharmaputra, 1999), namely by growing *Trichoderma* sp. and *Fusarium* sp. diameter 7 mm. face-to-face in the PDA medium (Figure 1).

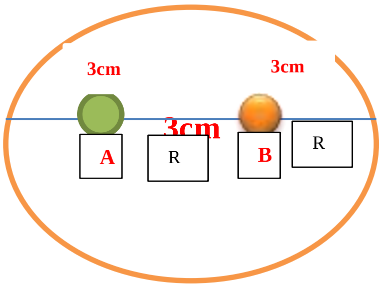


Figure 1. Dual culture method
A=colony of Trichodermsp.
B=colony of pathogen
R1=the radius of the pathogenic colony stays away from Trichodema sp.
R2: the radius of the pathogenic colony approaches Trichoderma sp

The observed variable is the percentage of inhibition calculated on the 8th day after incubation with formula (Dharmaputra, 1999) :
Inhibition (%) : (R1-R2)/R1 x 100%

The observed mechanism of antagonism (Farida, 1992), namely: competition of space, nutrition, and oxygen; antibiotics; as well as lysis and parasitism.

Results and Discussion

Durian Plant Disease Symptoms

Early symptoms of the disease can be seen with yellow spotted leaves and then yellow spots widen and become brownish. Further symptoms of the disease cause the leaves to fall off (Figure 2).

Figure 2. Symptoms of leaf blight on the upper and lower surfaces of the leaves

Durian plants affected by leaf blight show symptoms of large spots, blackish-brown, dry blight (such as exposure to hot water), and dead leaves. Symptoms of blight are seen at the tips of the leaves and the middle of the leaves, then widen to become like a blackish-brown burn and develop into dry and the leaves fall off. The symptoms of blight not only attack the leaves but also attack the stems to the roots (Semangun, 2007; Handoko, 2014).

Characterization of Durian Plant Pathogens

At the beginning of the growth, the pathogen colony was concentric spherical form that get larger with age, the colonies spread in all directions with hyphae growth structures pointing sideways and upwards. At 6-7 days after isolation (dai), the pathogenic mycelium grows rapidly and the aging of the isolates is grayish-purple, then brownish-gray with a white lower surface of the colony. Microscopically, the fungus has 2 types of conidia, namely crescent-shaped macro conidia, hyaline with 4 - 6 partitions and oval-shaped, curved microconidia with 1-3 septate. The hyphae are branched and have an oval spherical chlamydospore (Figure 3). Based on the above characteristics the pathogen is the fungus *Fusarium* sp. which causes leaf blight.

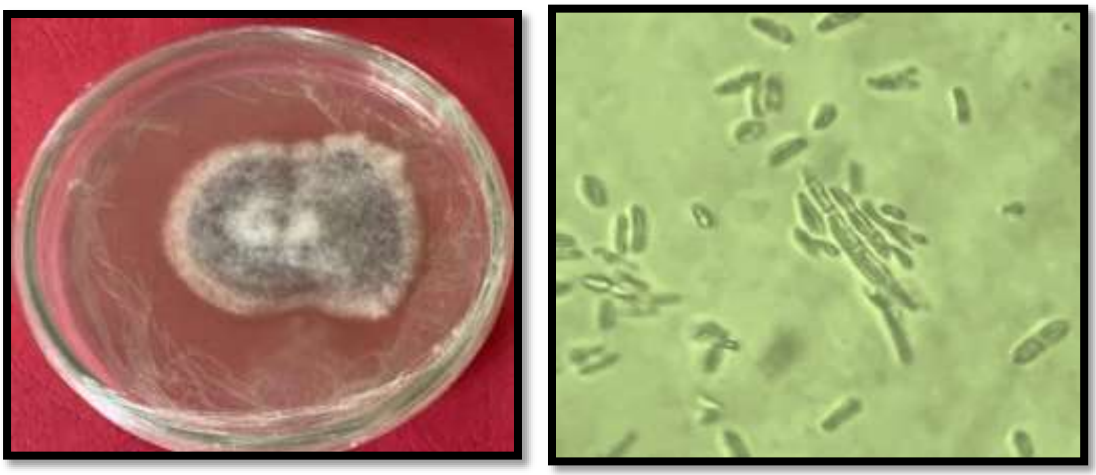


Figure 3. Colony and conidia of *Fusarium* sp. from durian plants

Sutejo et al. (2008) stated that Fusarium sp. has a slow growth rate and colonies fill the entire surface of the petri dish at the age of 20 days. The colony is white with purple or pink at the center of the colony. In isolates that form a large number of sporodochium, colonies will change from white to orange. Conidium of Fusarium sp. is formed on a mono phialide, long, and branchless conidiophore. Conidia of Fusarium sp. is hyalin-colored, elongated elliptical, pointed at both ends, crescent-shaped with odd or even in number, and in the hyphae there are chlamydospores (Handoko, 2014).

Characterization of Trichoderma sp. of the Rhizosphere of Durian Plants

From the soil in the rhizosphere of durian plants, 16 Trichoderma isolates were obtained, but after microscopic observation, the 16 Trichoderma isolates were classified into 5 species, these are *T. aurioviridae*, *T. viridae*, *T. harzianum. T. atroviridae*, and *T. koningii.*

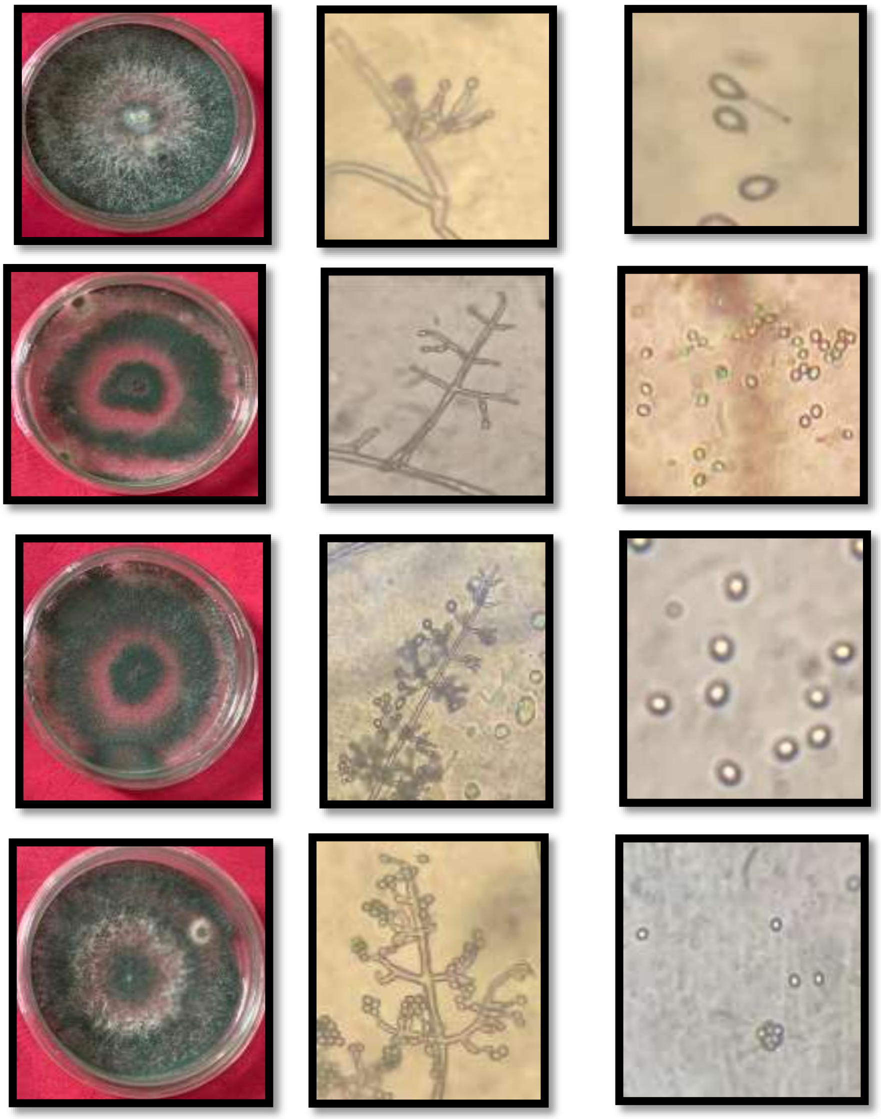


Figure 4. The macroscopic and microscopic characterization of *T. aurioviridae*, *T. viridae*, *T. harzianum. T. atroviridae,* and *T. koningii*

The first row of Figure 4 shows that the isolates of *T. aurioviridae* have branched, slender, long, and upward-curved conidiophores, conidia oblong-shaped and greenish-yellow in color, hyphae hyaline-colored and septate. In PDA media, the isolates are whitish-green, rounded in shape without forming a concentrate. This is in accordance with the Trichoderma type determination book (Widyaastuti, 2007) light green conidia, obovoid-shaped conidia, elongated gourd-shaped phialide curved upwards, and branched conidiophores. The isolate is in accordance with the characteristics of Gusnawaty (2014) that the isolate has a branched conidiophor shape, the conidia period is in each phialide, the phialide is vertical, long and curved. Conidia is green and oval-shaped. The colonies on PDA media are dark green, the surface is soft and rounded

The second row of Figure 4 showed that the isolate of *T. viridae* has septate hyphae, light green, rounded conidia, taper, thick, and small phialide, and branched conidiophores. In PDA media, colony is green by forming a curved concentric form. Yusmar (2020) stated that the *T. viridae* type has hyalin hyphae, semi-round or oval conidia and branched conidiophores with hyaline hyphae. Widyaastuti (2007) stated that the *T. viridae* type has horizontally branched conidiophore with rounded and oval conidia.

The third row of Figure 4 show that the isolates of *T. harziaum* show oval-shaped conidia and green in color, branched upright conidiophore, short and thick phialide, hyaline-colored septate hyphae and branched up to 2-3 phialides. On PDA media, colony is dark green and forms a curved concentric circle. This is in line with Gusnawaty (2014) reporting that the isolate has an upright, branched conidiophores shape arranged vertically. Phialide is short and thick. Conidia is green and oval-shaped. The colonies on PDA media are dark green and round. The diameter of the colony reaches more than 9 cm within 5 days. Widyaastuti (2007) stated that characterization of the type of *T. harziaum* has green oval conidia, erect, branched, and thick and short phialies.

The fourth row of Figure 4 shows that *T. atroviridae* has irregular curved, pointed, and thick phialide, as well as green, rounded conidia and branched conidiophores of 1-3 phialides. On the PDA media, the isolate is whitish-green and round in shape. Widyaastuti (2007) states that *T. atroviridae* has a tapered and thick phialide, branched, erect conidiophore, rounded and light green conidia with septate hyaline hyphae.

The last row of Figure 4 shows that the isolate of *T. koningii* is seen to have a cylindrical conidia that are light green, small phialide, hyaline septate hyphae, and branched upright conidiophores. The isolates on PDA media are green in color and form concentric circles. Gusnawaty (2014) showed that the fungi have an upright conidiophore, branched vertically. The phialide is pointed towards the apex and conidia are smooth-walled and rough-walled in oval-shaped green. The colonies in PDA media reach more than 5 cm within 5 days and the colonies are green and round. *T. koningii* has a cylindrical conidia in green, an erect conidiophore with 3-4 phialides, a small, tapered, and thick phialide with hyaline septate hyphae (Widyaastuti, 2007).

Antagonistic Mechanism of *Trichoderma* sp and *Fusarium* sp.

The effects of Trichoderma in the soil on the root system are not noticeable. However, the direct effects of these fungi can be observed in in vitro studies (Narrasawati et al., 2017). Therefore, a double culture test was carried out to see the inhibition percentage of *Trichoderma* sp. and *Fusarium* sp. Based on the results of the double culture test of 5 species of Trichoderma, it varies greatly. Each species has different inhibition capabilities, this has to do with the influence of environmental factors such as temperature and the virulence level of the fungi. Numerically, the inhibition percentage for 7 dai of each species has a noticeable difference, however, it is not statistically significant different as can be seen in Table 1.

. The ability of each species of *Trichoderma* sp. in controlling pathogenic fungi is different. This is due to their different morphology and physiology (Anwar, 2018). Of the 5 species tested, *T. aurioviridae* and *T. harziaum* had the highest percentage of inhibition at 97.7% and *T. koningii* had the lowest percentage of inhibition at 93.3%. Husdiani dual culturing method showed that *T. harzianum* had the highest inhibition percentage of 60.0% followed by *T. viridae* of 52.0% and the lowest one is *T. koningii* with an inhibition percentage of 43.3%. The ability differs from each species of *Trichoderma* sp. can be caused by the growth rate, levels and types of chemical compounds, as well as enzymes produced by each species (Amari et al., 2013). The high growth rate can determine the activity of antagonistic fungi against pathogenic fungi.

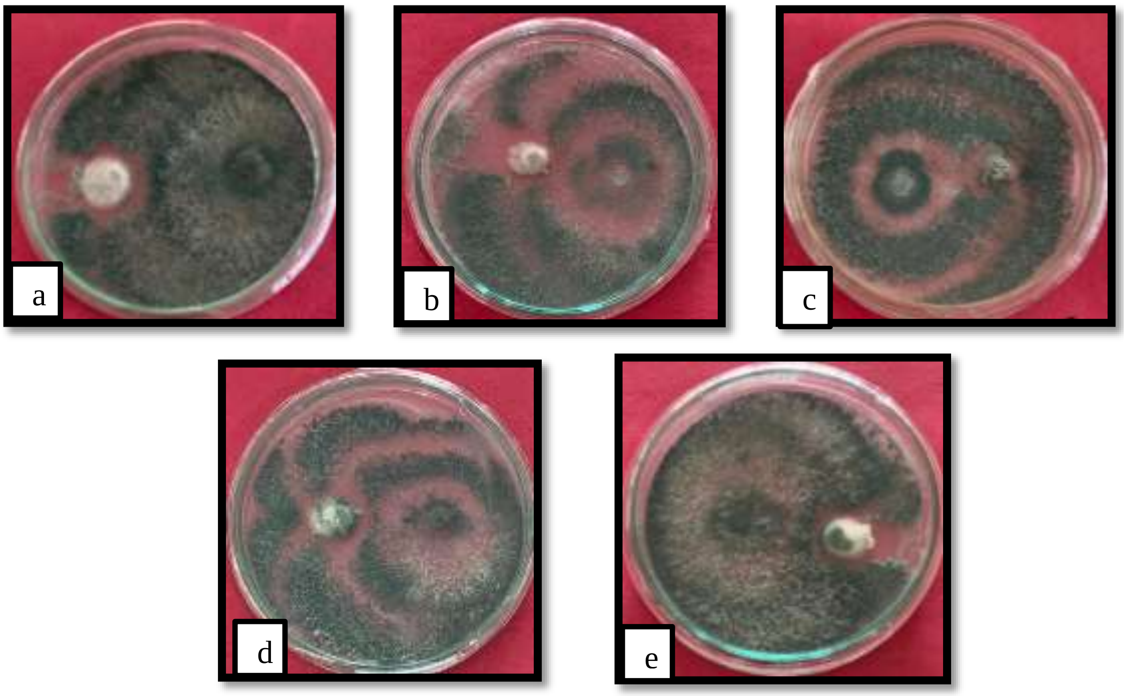
Table 1. Percentage of inhibition of *Trichoderma* sp. against *Fusarium* sp

Isolate	Percentage of inhibition (%) on day						
	1	2	3	4	5	6	7
T. aurioviridae	4,4	46,6	63,8	81,5	86,7	95,6	97,7
T. viridae	6,6	71,6	79,8	87,0	89,1	94,5	94,5
T. harziaum	7,7	71,3	78,6	81,3	81,3	97,7	97,7
T. atroviridae	6,6	38,5	67,9	68,3	73,8	78,6	94,3

Table 2. Antagonistic mechanism of *Trichoderma* sp. against *Fusarium* sp.

Isolate	Space, nutrition, and oxygen competition	Antibiosis	Lysis dan Parasitism
T. aurioviridae	+	+	+
T. viridae	+	+	+
T.harziaum	+	+	+
T.atroviridae	+	+	+
T. koningii	+	+	+

Mechanism of *Trichoderma* sp. in controlling *Fusarium* sp. in general is divided into three, namely competition for nutrients and oxygen, antibiosis, lysis and parasitism. The antagonistic properties of *Trichoderma* will appear when growing side



by side with *Fusarium* sp. on the medium of PDA (Table 2, Figure 5).

Figure 5. Antagonistic mechanism of *Trichoderma* sp. against *Fusarium* sp.
Note : a = *T.aurioviridae* vs *Fusarium* sp., b = *T.viridae* vs *Fusarium* sp., c= *T.harziaum* vs *Fusarium* sp., d = *T.atroviridae* vs *Fusarium* sp., e = *T.koningii* vs *Fusarium* sp

Based on the image above, it can be seen that the five species of Trichoderma isolates have the same antagonistic mechanism. First, the competition of space, nutrient and oxygen, it can be seen that the mycelium of each Trichoderma species grows faster compared to Fusarium sp. This is in accordance with the literature (Berlian et al., 2013) that nutritional needs are needed to maintain the germination rate of spores, The germination rate will decrease if there is nutritional competition with other microorganisms. Trichoderma also has a mechanism in the form of secondary metabolites in inhibiting pathogen development through protein denaturation, both structural and functional in pathogenic cells. The active compounds in secondary metabolites are also able to break down disulfide bonds that connect between cell wall protein polypeptides and cell membranes (Dewi, 2009).

Furthermore, each species of Trichoderma secretes an antibiotic, which is a clear zone formed between two fungal colonies due to the presence of secondary metabolite compounds produced by the antagonistic fungal colony so that the pathogenic fungi cannot grow close to the antagonistic fungi (Srinon et al., 2006). Antibiotics are characterized by inhibition of fungal growth which is characterized by the presence of an inhibition zone (Mejia et al., 2008). Trichoderma secretes antibiotics that are inhibitory compounds that make Fusarium sp. cannot grow beyond the clear zone (Figure 5). Berlian et al. (2013) stated that the compound produced by Trichoderma, namely siderophores, functions to clot iron and stop the growth of other fungi.

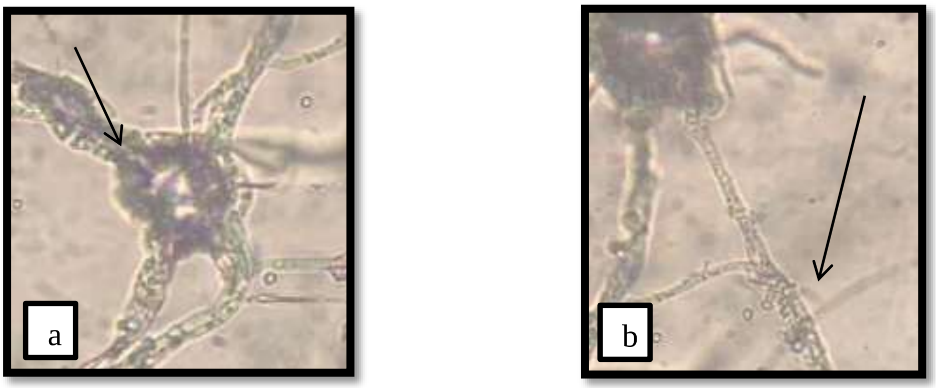


Figure 6. Lysis and hyper parasitism of *Trichoderma* sp. against *Fusarium* sp.
Note : a. Hyphae of *Trichoderma* is coil around hyphae of *Fusarium* sp. , b = Lysis of hyphae of *Fusarium* sp.

Figure 6 show that the hyphae of *Trichoderma* were coil around the hyphae of *Fusarium* sp. So that there is destruction of the hyphae *Fusarium* sp. It is because of Trichoderma sp. produces volatile and unvolatile compounds or toxins. Berlian *et al*. (2013) stated that if hyphae of *Trichoderma* sp. attached and coil around the host fungal hyphae, the host hyphae undergo vacuolation, lysis and finally disintegrate. *Trichoderma* sp. penetrates the host cell wall with the help of cell wall degrading enzymes such as chitinase, glucanase, and protease, and uses the contents of the host hyphae as a food source. Secondary metabolites released by *Trichoderma* sp. also causes a loss of permeability so that it cannot select substances that come out of the cell membrane This condition causes secondary metabolite compounds to enter the cell causing the cell to become lysis and die (Dewi, 2009).

Trichoderma sp. has secondary metabolites in the form of antibiotic compounds, enzymes, toxins, and hormones. Each species that produces antibiotics, namely: species *T. harzianum*, *T. viride, T. koningii* produce volatile compounds alkyl pyrones which are antifungal that can inhibit the germination and growth of mycelia; *T. harzianum, T. koningii*, and *T. viride* produce antibiotic compounds that include isonitrin A-D. Isonitrin A is effective as an antibacterial and antifungal, while isonitrin D is only effective as an anti-fungal by inhibiting fungal growth (Howell, 2005); *T. viride, T. atroviride, T. harzianum*, and *T. koningii* produce compounds 6-pentyl-α pyrone, which functions that can inhibit the growth of fungal hyphae (Harni, 2017).

Trichoderma sp. also has several benefits for durian plants, such as being able to fertilize the soil so that production can increase. This research confirm that *Trichoderma* can inhibit the growth of *Fusarium* sp. in vitro with a high percentage of inhibitiothn, therefore, it is recommended to apply *Trichoderma* sp. It should be done from the durian plant planted to the vegetative phase when the plant has not been attacked by pathogens.

Conclusion

The conclusion of this study are 1. Diseases that infected durian plants in PT. Famorganda is leaf blight caused by Fusarium sp. with symptoms of dry leaf shoots like burning, 2. Of the 16 *Trichoderma* isolates found, there are identified as *T. aurioviridae*, *T. viridae*, *T. harziaum, T. atroviridae* and *T. koningii*, 3. The percentage of inhibition of *T. aurioviridae* and *T. harziaum* were 97.7%, *T. viridae* was 94.5%, *T. atroviridae*: was 94.3%, and *T. koningii* was 93.3%, and 4. The mechanism of antagonism tbetween *Trichoderma* sp. and *Fusarium* sp. were spece, nutrition and oxygen competition, antibiosis, lysis and parasitism. It is recommended to use *Trichoderma* sp. for controlling *Fusarium* sp. in durian plants at PT. Famorganda, North Bengkulu.

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