POTENTIAL OF ENDOPHYTIC FUNGI OF DRAGON FRUIT PLANT (*Hylocereus polyrhizus*) AS A BIOLOGICAL CONTROL OF PATHOGEN of *Colletotrichum* sp.

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Abstract. Dragon fruit (*Hylocereus polyrhizus*) is one of the most widely cultivated fruit plants in Indonesia. Along with the development of dragon fruit cultivation, farmers face the problem of increasing pest and disease attacks. Environmentally friendly disease control efforts can use natural enemies of plant pathogens or biological control. This research was conducted at the IHPT Laboratory (Plant Pest Disease Science), Faculty of Agriculture, Mulawarman University from August to October 2023. Factorial experiment in a completely randomized design with 5 treatments and 10 replications was used in this study. The Single Factor in this study was the test of inhibition of antagonistic fungi against the growth of Colletotrichum sp. the data obtained were analyzed by using variance analysis (ANOVA).The data obtained were analyzed using variance analysis (ANOVA) and further tested using the Least Significant Difference (BNT) test at the 5% level. This study shows that in the stem of dragon fruit plants there are endophytic fungi *Trichoderma* sp., *Aspergillus flavus*, *Aspergillus niger, Gliocladium* sp. which are able to provide inhibitory activity against pathogenic fungal colonies. The endophytic fungus that has the highest inhibitory power is *Trichoderma* sp. by 5.99%. This shows that the compound compounds such as gliotoxin and glioviridin are able to inhibit the growth of pathogens.

Keywords: Dragon fruit (Hylocereus polyrhizus), endophytic fungi, Colletotrichum sp.

INTRODUCTION

Dragon fruit (*Hylocereus polyrhizus*) is one of the horticultural commodities that has a high economic value and has a variety of types that can be a source of income for the community. Dragon fruit is one of the fruit plants that are widely cultivated in Indonesia after being introduced for the first time in the 2000s (Jaya, I. K. D 2010). Dragon fruit is a tropical plant that belongs to the genus *Hylocereus* and *Selenicereus*, so this plant is one type of plant that can grow well in Indonesia. This plant also has nutritianol content that is very beneficial for human health. In 2016, dragon fruit was the center of production in East Kalimantan bears fruit throughout that it is included in one of the fruit production centers in Indonesia (Jumjunidang., *et al.* 2019). Currently, dragon fruit plants have been widely cultivated in Indonesia, such as Riau Province, Riau Islands, West Sumatra, Java Island, NTB and several other provinces, even dragon fruit plantings in East Kalimantan Province have reached 1500 ha (Faidah, Fkriatul.,*et al.* 2017). The types of plants cultivated in Indonesia are dominate by the type of superredmeat dragonfruit (*H.costaricensis*) or *Super red*. This type is classified as the sweetest among other types. Seeing the favourable prospects and opportunities, East Kalimantan has great potential to cultivate dragon fruit on a wider scale and added value and can improve the welfare of famers.

Diseases on dragon fruit plants have also been reported to reduce production in several countries such as Malaysia, Vietnam, Taiwan, and Brazil (Research Center, Fruit Plant 2014). The Indonesian Dragon Fruit Associations mentionedthatin 2016, there was a disease taht destroyed more than 50% of dragon fruit plants in East Kalimantan (Jumjunidang., *et. al.* 2019). Based on previous research, efforts to control the disease anthracnose currently still use synthetic chemical. The use of synthetic chemical pesticides is considered the main choice because it is considered to be able to control the disease quickly and practically. However, excessive use of chemical pesticides can result in negative impacts on the environment and humans. Therefore, to minimize the negative effects of using synthetic chemical in plant disease control, alternative methods that are environmentally friendly are needed. One method is the utilization of natural enemies of plant pathogens or biological control mthods or biocontrol (Hayati, A.H., *et al*. 2016). Plants disease control taht is widely studied to day is the utilization of microorganisms associated with plants, one of which fungi are found in plant tissue systems, such as leaves, flower, twigs or plant roots (Manurung, I,R., *et al.* 2014). Therefore, this study was conducted to determine the endophytic fungi found in dragon fruit plants as a control of *Colletotrichum* sp. disease.

MATERIALS AND METHODS

The research was conducted from August to October 2023 from the time of sampling until the antagonist tes process. The research was conducted at the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Mulawarman Uniersity, Samarinda. The sampling locations was in Batuah Village, Loa Janan District, Kutai Kartanegera Regency, East Kalimantan Provience. The materials used in this study were distilled water, *potato dextrose agar* (PDA) media, chloramphenicol, 70% alcohol, *methylene blue,* and healthy dragon fruit used in this study are *entkas, autoclave, laminar air flow,* Bunsen, petri dish, ose needle, *Erlenmeyer,* tweezers, scissors, microscope, *object glass, cover glass,* beaker

glss, micropipette, handsprayer, hemocytometer, cutter, label paper, aluminium foil, cotton, identification book, plastic wrap, tissue, spritus, ruler and stationery.

The design used in this study is a completely randomized design (CRD) with 5 treatments and 10 replicates so that 50 research units are obtained. The treatments and 10 replicates so taht 50 research units are obtained. The treatments carried out are:

P0 = *Colletrotrichum* sp. Without treatment (control)
P1 = *Colletrotrichum* sp. with *Trichoderma* sp.
P2 = *Colletrotrichum* sp. with *Aspergilus flavus*
P3 = *Colletrotrichum* sp. with *Aspergilus niger*
P4 = *Colletrotrichum* sp. with *Glicoclodium* sp.

Activities carried out in this study include: Isolation of endophytic and pathogenic fungi, identification of pathogen *Colletotrichum* sp., measurement of colony diameter of endophytic fungi and pathogenic fungi *Colletotrichum* sp., calculating spore density, and pathigenicity test using Koch's Postulates. Data from the study were analyzed by variance analysis uring a completely randomized design (CRD). Comparison of treatment means using the Least Significant Difference (BNT) test at the 5% level.

RESULTS AND DISCUSSION

Based on observations of the growth of plant material on PDA media and after isolations there is a pathogenic fungus *Colletotrichum* sp. Furthermore, the characteristics for the pathogenic fungus are described as follows:

Morphological Characteristies of Pathogenic Fungi

Tabel 1. Macroscopic and microscopic morphological characteristics of endophytic fungi

Morphological Characteristics	Observation Results	
	Macroscopic	Microscopic
Colony surface color	The coloris white, then becomes blackish after an incubation period of more than 7 days after after inoculation.	
Growth direction	Sideways until it fills the cup (9 cm) and up wards	
Spores		Hyaline, cylindrical/blunt tip

Description: Identification using a microscope with 400x magnification.

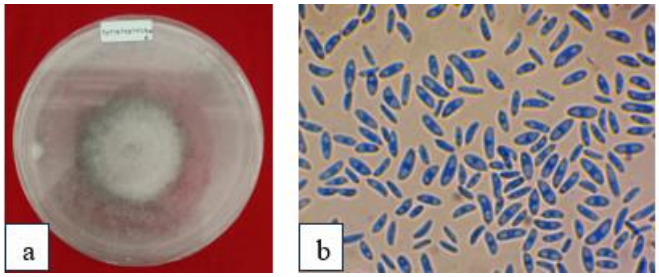

Figure 1. Fungus *Colletotrichum* sp.
(a) Macroscopic on PDA media.
(b) 400x magnification microscopy.

Morphological observations of *Collototrichum* sp. fungi macroscopically by aplplying *methylen blue* with 400x magnifications using optilab assistance are seen in Figure (1b). Microscopis characteristics of fungi include transparaent hyphae with blunt ends, the genus *Colletotrrichum* has the general characteristics of transparent and elongated conidia with rounded or long tapered ends with blac conidia (Jumjunidang., *et al.* 2019). The characteristics on macroscopic observation show the characteristics of the fungus *Colletotrichum gloesporiodes.*

Based on Koch's Postulate test shows that the disease on the stem of dragon fruit plants with symtoms of brownish spots and dry look is caused by the pathogen *Colletotrichum* sp. (Dwiastuti, M.E., & Melysa. N.F. 2014), so that in the test treatment that has been done it is proven that the fungus *Colletotrichum* sp. causes anthracnose disease on dragon fruit plants can be seen in Figure 2.

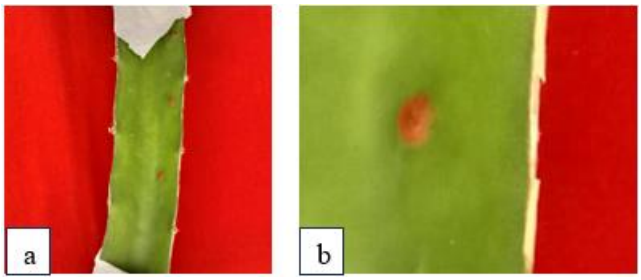

Figure 2. Koch's Postulate Test
(a) Stems of healthy dragon fruit plants treated with
(b) Symptoms of stem disease in dragon fruit plants

Morphological Characteristics of Endophytic Fungi

Based on observations of the growth of plant material on PDA media and after isolation there are several endophytic fungi on dragon fruit plants (*Hylocereus polyrhizus)* namely *Trichoderma* sp., *Aspergillus flavus, Aspergillus niger, Gliocladium* sp. then compare with the identification book *Introductory Mycology*(Alexopoulus C. J., *et al.* 1996).

Furthermore, the characteristics for each of these mushrooms are described as follows:

Tablel 2. Macroscopic and microscopic characteristics of endophytic fungi

Characteristics Morphology	*Trichoderma* sp	*Aspergillus flavus*	*Aspergillus niger*	*Gliocladium* sp.
Macroscopic				
Colony color	White to green in color old	Bright green or yellow-brown in color	Black	White to green in color
Growth Direction	Sideways to fil the cup (9cm) (roumded circle)	It spreads in all directions and clsters until it becomes granular.	Spread out in all directions	Spread in all directions
Colony texture	Smooth	Coarse like sand grains	Coarse likes and grains	Smooth
Microscopic				
Conidia shape	Round	Round	Round	Round
Brancing conidiophores	Branching	Branching	Unbranched	Branching
Hyphal shape	Insulated	Insulated	Not Insulated	Insulated
Hyphal color	Hialin	Hialin	Hialin	Hialin

***Trichoderma* sp.**

Based on the results of observations of the development of *Trichoderma* sp. fungi macroscopically showed that on the seventh day of observations after inoculation, the macroscopic characteristics of the fungus include round circular colonies, dark green in color with a circular shape with the direction of growth spreading on petri dishes, can be seen in Figure 3 below.

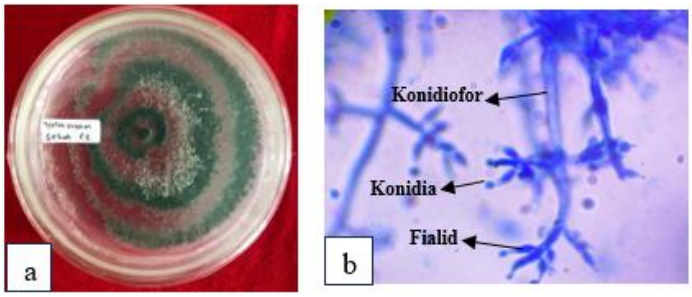

Figure 3. (a) colonies of *Trichoderma* sp. tampak macroscopically.
(b) conidiophores, conidia and fialids microscopically.

Observations of *Trichoderma* sp. macroscopically showedround, dark green colories with a circular shape with the direction of growth spreading on petri dishes. In accordance with the opinion of Sopialena (2020) which states that *Trichoderma* sp. on PDA media has a green color with a shape like a circle and the direction of growth spreads in all directions. Mi a opic appearance of *Trichoderma* sp. with the administration of methylen blue with a magnification of

400x using optilab assistance has concentratedhyphae, many branched conidiophores. *Phialide* is formed more 2-3 at the end of branching, and at each end of the *phialide a phialospore is* formed (Berlian, *et al.* 2013).

Trichoderma sp. is atype of fungus that does not cause disease in plants, in other studies many mention that this fungus includes antagonistic fungi for pathogenic fungi. In addition, *Trichoderma* sp. can survive and from chlamydospores in unfavourable conditions and is quite resistant to fungicides and herbicides (Berlian *et al.* 2013). Macroscopic observations showed that this fungus had an initial white colony color and after entering the third day the colony turned green with rounded colony growth and filled the petri dish quckly. Microscopic observation have the characteristics of having conidia that are slightly oval, have conidiaphores that are branched and septate mycelium.these macroscopic and microscopic observations are in accordance with the characteristics of *Trichoderma* sp. proposed by other researchers (Gandjar., I., Samson. *Et* al. 1999). The cell arrangement of this fungus is many-celled inrows forming fine threads called hyphae. This fungus has a flat hyphae shape insulated and branched to form woven and thickened called mycelium. *Trichoderma*sp. mycelium can produce various enzymes including urease, cellulase, glucanase and chitinase. *Trichoderma* sp. also produces metabolites such as citiric acid ang ethanol (Wangge, E.S.A., dkk. 2012).

Aspergillus flavus

Based on the results of observations of the development of *Aspergillus flavus* fungi macroscopically, it shows that on the seventh day after onoculation, the macroscopis characteristics og the fungus include bright green or brownish yellow colonies and the shape of the colonies clustered to become granular, for more details, it can be seen in Figure 4.

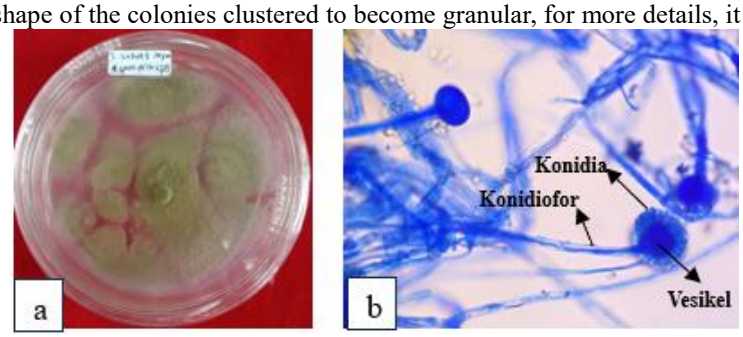

Figure 4. (a) macroscopic view of *Aspergillus flavus* fungal colonies.
(b) conidiophores, conidia and vesicles microscopically.

Macroscopic observations of *Aspergillus flavus* show bright green or brownish yellow colonies and clustered to granular colony shapes. Microscopic observations have concentrated hyphae, round conidia, conidiophores hav rough stems, round vesicles with sprea ding spore production.

Aspergillus flavus is a fungus that can have a negative impact on plants. *A. flavus* is udually know as the cause of rot on plant stems and can produce harmful a flatoxin compounds. However, *A. flavus* juga dapat memiliki potensi sebagai agensia hayati yang bersaing dengan patogen dan menghasilkan senyawa inhibiscan also have potential as a biological a gent that competes with pathogens and produces inhibitory compounds. Macroscopically, *Aspergillus flavus* has cylindrical to oval egg-dhaped conidia, long, erect and branched conidiophores and a transparent mycelium. *Aspergillus flavus* generally produces colonies that are yellow-brown in color. These colonies usually look like sand, the conidiophores are colorless, rough, the top is slightly rounded and the hyphal vessels spread very quickly, have a size of more than 1 mm under the vesicls with a rough texture (Ahmad, R.Z., dkk., 1999).

Aspergillus niger

Based on the results of observations of fungal develop[ment on the seventh day after inoculation, the macroscopic characteristics of the fungus include black colonies with granular-like mycelium and a shape that spreads in all directions, for more details can be seen in Figure 5.

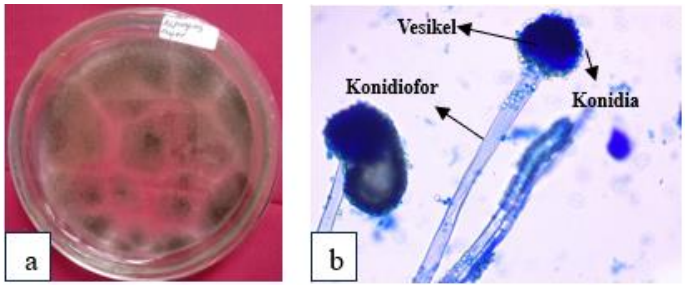

Figure 5. (a) Macroscopic view of *Aspergillus niger* colonies.
(b) Conidiophores, conida, and vesicles microscopically.

Macroscopic observation of *Aspergillus niger* showed black colonies with granular mycelium and a shape taht spread in all directions (Figure 5a). This is in accordance with the statement of Sopialena (2019) that the colonies are

black and the shape of the colonies spreads irregularly. Microscopic observatioms have large black spore heads, black and round conidia, thick-walled and transparent conidiophores, large round vesicles (Figure 5b). *Aspergillus* has white or yellow basic hairs with a thick dark brown to black conidiospore layer. Conidia heads are black. Rounded, tending to separate into looser parts with age. Coonidiospores have smooth, hyaline walls but are also brown. On the stem of dragon fruit plants, the endophytic fungus *A. niger* was obtained with the initial coony shape in the form of white color granules on a light black surface. The older the color changes to black. *A. niger* has hyaline hyphae and unbranched elongated hyphal structure, unconcentrated conidiophores, round conidia and blackish brown in color. Hyphae grow within two days with a fast growth time (Lindawati, S. and Rini, C.S.2019).

The mycelium of *Aspergillus niger* has unbrunched conidospore baffles, coarse or fine textured, with foot cells at the base supporting the vesicles located at the tip. Vesical taht from one or teo rows, will then support vialids taht are shaped like a pumpkin, the rows of vesicles will produce chains of filaloconidia that are smooth or rough. All *Aspergillus* species are characterized by conidiophores, which develope into large vesicles at the tip and are enclosed by phialids taht produce long chains of conidia. Fialids may arise directly from the vesicle (u*niseriate*) and from metulae, which are attached to the vesicle (*biseriate*). *Aspergillus* species can be identified mainly based on the structure of conidia, color, and shape of conidiosphores, conidia and phialids (Jokklik W.K., *et al*. 1988).

A. niger in its growt is directly related to the food substrate, simple molecules found around the hyphae can be directly absorbed, while more complex nolecules must be broken down first before being absorbed into the cell, by producing several extra-cellular enzymes. Organic matter from the substrate is used by *A. niger* for molecular transport activities to maintain cell structure and cell mobility.

***Gliocladium* sp**.

Based of the results of observations of fungal development on the seventh day after inoculation, the macroscopic characteristics of the fungus include white colonies and green surfaces. The direction of fungal growth is sideways until it fils the petri dish (9 cm) and upwards, for more details can be seen in Figure 6.

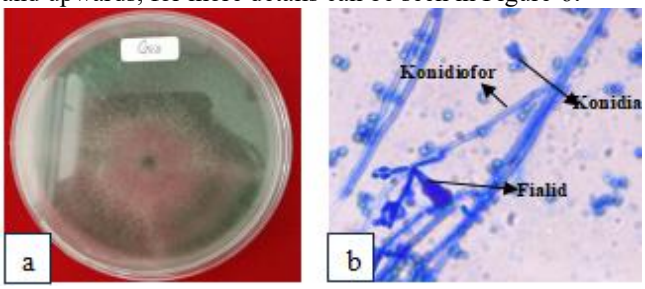

Figure 6. (a) Colonies of *Gliocladium* sp. macroscopically.
(b) conidiohores, phialids, and spores microscopically.

Macroscopic observation *Gliocladium* sp. showed white colonies and green surface. The direction of fungal growth is sideways until it fills the petri dish (9cm) and upwards. Microscopic observation have concentrated hyphae with erect conidiophores, phialids form groups at the ends of conidiophores and spores are round.

Gliocladium sp. is a soil fungus that lives saprophytically. The sexual reproductive organs of *Gliocladium* sp. are not yet know, while the asexual reproductive organs are conidiophores (spores) in the form of upright hyphae and the shape of the upper position is like *Penicillium* sp. forming branches as phialides (Bamett, IIL. 1958). On the stem of dragon fruit plants, one of the endophtic fungi, *Gliocladium* sp. colony characteristics with a yellowish white color, smooth texture with symmetrical round growt. The older the age of *Gliocladium* sp. the surface of the spores will turn light green. *Gliocladium* sp. and its close relative *Trichoderma* sp. are know to have the ability to kill several other fungal species using lytic enzymes such as chitinase. However, chitinase is not the only inhibitor of fungi by *Gliocladium* sp. Another mechanism carried out by *Gliocladium* sp. is the production of anti-fungal compounds and antibacterial compounds (Sopialena., dkk. 2019).

Growth rate of endophytic and pathogenic fungi

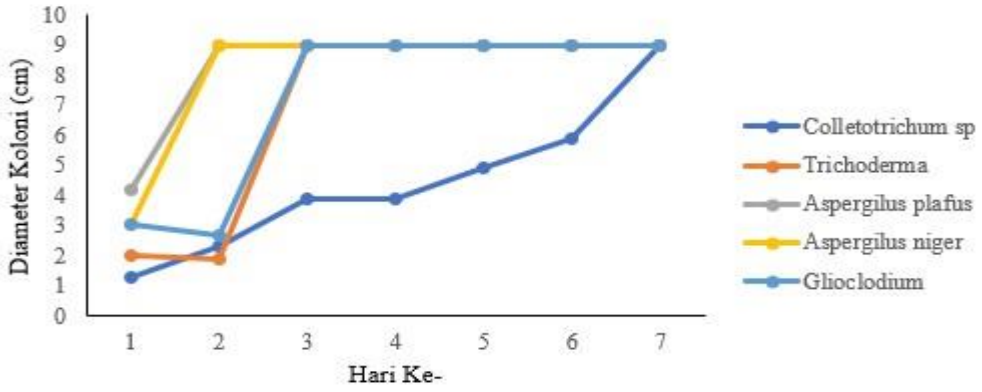

Figure 7. Colony growth rate of endophytic fungi and pathogenic fungi

Spore Density

Table 3. Spore density calculation

Mushroom Name	Spore Density
Trichoderma sp.	6,43 x 10^{-5}
Aspergillus flavus	5,16 x 10^{-5}
Aspergillus niger	6,28 x 10^{-5}
Gliocladium sp.	3,90 x 10^{-5}

Percentage of inhition test

Table 4. Percentage inhibition test of antagonistic fungi against *Colletotrichum* sp. seven days after inoculation (7 hsi).

Treatment	Repeat										Total	Average
	1	2	3	4	5	6	7	8	9	10		
P0	30,1	24.1	30.1	18.2	22.5	30.1	24.8	22.7	25.1	24.7	252.3	25.2 **a**
P1	10,0	84.5	37.9	31.7	77.5	70.7	44.8	11.4	84.5	55.5	598.6	59.9 **b**
P2	22.0	37.9	70.7	35.4	57.8	50.0	44.8	22.2	57.8	57.8	416.7	41.7 **b**
P3	70.77	81.7	50.0	22.2	84.0	57.8	22.2	39.3	57.8	22.2	448.1	44.8 **b**
P4	55.5	44.8	50.0	22.2	22.2	53.5	65.5	79.1	77.5	18.4	448.8	44.9 **b**
Total											2164.4	43.3

Notes: Numbers followed by different letters indicate significantly different values in the 5% BNT test (27.8)

Based on the results of the 5% BNT test, the percentage of inhibition of antagonistic fungi against *Colletotrichum* sp. seven days after inoculation showed that it was significantly different from the control, not significantly different from each endophytic fungal antagonist test treatment P1 (*Trichoderma* sp), P2 (*Aspergillus flavus*), P3 (*Aspergillus niger*), P4 (*Gliocladium* sp.).

The results of observations of endophytic fungal antagonist pathogens can be determined by the highest percentage of inhibition to the lowest percentage of inhibition. The highest percentage of inhibition in the endophytic fungal antagonist test was *Trichoderma* sp. with an average of 5,99% followed by *Gliocladium* sp. with an average of 4,49%, and *Aspergillus niger* with an average of 4,48% and the lowest percentage of inhibition was with an average of 4,17%. *Trichoderma* sp. is an isolate that has the highest percentage because ths fungus is able to antagonize other fungi with colony growth in 3 days has filed petri dishes and has the highest spore density compared to pathogenic fungi.

Antagonistic Mechanism

Table 5. Inhibitory mechanism of endophytic fungi against *Colletotrichum* sp.

Perlakuan	Jenis mekanisme		
	Kompetisi	Parasitisme	Antibiosis
Trichoderma sp.	-	-	+
Aspergillus flavus	+	-	-
Aspergillus niger	+	-	-
Gliocladium sp.	+	-	-

Description: Antagonistic Meechanism occurs (+). No Antagonistic Mevhanism(-)

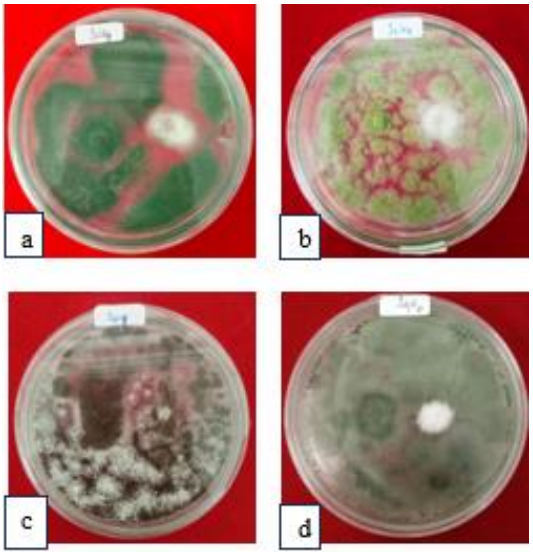

Figure 7. Endophytic fungi undergo antibiosis and competition mechanism
(a) *Trichoderma* sp., (b) *Aspergillus flavus,*
(c) *Aspergillus niger,* (d) *Gliocladium* sp

Observation of the antagonistic mechanism of endophytic fungi against *Colletotrichum* sp. causing disease in dragon fruit plants was carried out for seven days after incubation. Observations were made every day starting from the first day after incubation until the seventh day. Antagonistic properties arise because of the competition that occurs between two types of fungi that are grown side by side. This competition occurs because of the same needs of each fungus, namely the need for a place to grow and nutrients from the media used to grow. All endophytic fungal treatments have different inhibitory mechanism, namely competition and antagonism mechanisms. Competition is a mechanism for fighting for space and nutrients so that pathogenic fungi do not have the opportunity to obtain space and nutrients so that their development is limited. While antibiosis is a mechanism in inhibiting the growth of pathogenic fungi, basically these microorganisms are able to produce effective antibiotic compounds, so there will be an inhibition zone around the pathogenic fungal colonies on the growth medium. The fastest type of competition antagonist is obtained from the observation of *Colletorichum* vs *Aspergillus niger* followed by *Aspergillus flavus, Gliocladium* sp. and the type of antibiosis mechanism only occurs in the fungus *Trichoderma* sp.

CONCLUSIONS

Based on the results of the research conducrted, it can be concluded that on the stem of dragon fruit plants there are endophytic fungi *Trichoderma* sp, *Aspergillus flavus, Aspergillus niger* and *Gliocadium* sp., these endophytic fungi can suppress the growth of phatogenic fungi *Colletotrichum* sp. with antagonistic mechanism iin the form of competition and antibiosis mechanism. Endophytic fungi that have the highest inhibitory power with an average percentage value of 5,99% and a spore density of 6,43 x 10^{-5} and growth 3 days after inoculation has filed the petri dish is teh fungus *Trichoderma* sp., while the fastest growth rate 2 days after inoculation has filled the petri dish is *Aspergillus flavus* and *Aspergillus niger*.

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