Potential of Endophytic Fungi of Dragon Fruit Plant (*Hylocereus polyrhizus*) as a Biological Control Against *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 analysed by using varience analysis (ANOVA). The data obtained were analysed 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**.,** biological control.

1. 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 [1]. Dragon fruit is a tropical plant that belongs to the genus *Hylocereus* and *Selenicereus*, so this plant is one type of plant that can growing up verry well in Indonesia. This plant also has nutritianol content that is very beneficial for human health. In 2016, dragon fruit was the centre production in East Kalimantan bears fruit throughout that it is included in one of the fruit production

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centre in Indonesia [2]. 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 [3]. The types of plants cultivated in Indonesia are dominate by the type of super red meat dragon fruit (*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. The Indonesian Dragon Fruit Associations Mention in 2016, there was a disease that destroyed more than 50% of dragon fruit plants in East Kalimantan [2]. 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, because its 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 methods or biocontrol [4]. Plants disease control that is widely studied to that 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 [5]. Therefore, this study was conducted to determine the endophytic fungi found in dragon fruit plants as a control of *Colletotrichum* sp. disease.

2. Materials and Methods

The research was conducted from August to October 2023 from the time of sampling until the antagonist test 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 glass, micropipette, hand sprayer, 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 with total of 50 research treatments are obtained. The treatments carried out are control (*Colletrotrichum* sp. without endophytic fungi), *Colletrotrichum* sp. with *Trichoderma* sp., *Colletrotrichum* sp. with *Aspergilus flavus*, *Colletrotrichum* sp. with *Aspergilus niger*, and *Colletrotrichum* sp. with *Glicoclodium* sp.

Activities carried out in this study including isolation of endophytic fungi and pathogen *Colletotrichum* sp., measurement of colony growth and spore density, and pathogenicity test based on Koch's Postulates. Data from the study were analysed by variance analysis continued by comparison of treatment means using the Least Significant Difference (LSD) test at the 5% level.

3. Results and Discussion

3.1. Morphological Characteristies of Pathogenic Fungi in Dragon Fruit

Fig. 1a shows the healthy dragon fruit condition, whereas the Fig. 1b shows the dragon fruit plants treated with the studied disease, which then sjowed a symptoms of brownish spots and looks dry caused by anthracnose disease. A Koch's Postulate test treatment has been carried out showed that the disease is caused by *Colletotrichum* sp. pathogen [6].

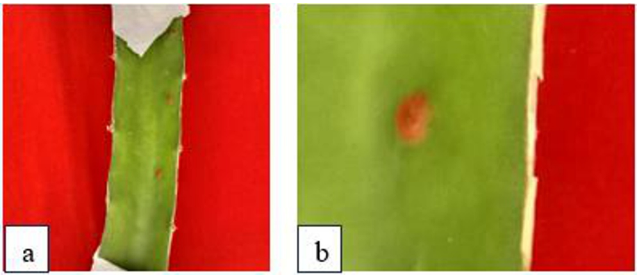

Fig. 1. Koch's Postulate Test. Stems of healthy dragon fruit plants treated with (a), Symptoms of stem disease in dragon fruit plants (b)

Morphological observations of the *Colletotrichum* sp. fungi is presented in Fig. 2a, whereas microscopic morphology was by applying *methylene blue* with 400x magnifications using optilab assistance is presented in Fig. 2b. Microscopic characteristics of the fungi include transparent hyphae with blunt ends meets the general characteristics of transparent and elongated conidia with rounded or long tapered ends with black conidia of the genus *Colletotrrichum* [2]. The characteristics on macroscopic observation show that the characteristics of the pathogenic fungus belongs to *Colletotrichum gloesporiodes* (Table 1.).

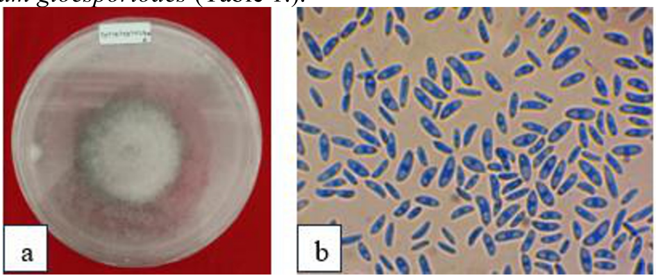

Fig. 2. Fungus Colletotrichum sp. Macroscopic on PDA media (a), 400x magnification microscopy (b)

Table 1. Macroscopic and microscopic morphological characteristics of pathogenic fungi

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

3.2. Morphological Characteristics of Endophytic Fungi in Dragon Fruit

Four endophytic fungi on dragon fruit plants (*Hylocereus polyrhizus*) were observed by growing the plant material on PDA media. Macroscopic and microscopic identification of the four fungi using a handbook of *Introductory Mycology* [7] showed that the four fungi were *Trichoderma* sp., *Aspergillus flavus, Aspergillus niger,* and *Gliocladium* sp. (Table 2).

Table 2. Macroscopic and microscopic characteristics of endophytic fungi

Characteristics	*Trichoderma* sp.	*Aspergillus flavus*	*Aspergillus niger*	*Gliocladium* sp.
Macroscopic				
Colony colour	White to green in colour old	Bright green or yellow-brown in colour	Black	White to green in colour
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 colour	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 coluor with a circular shape with the direction of growth spreading on petri dishes, can be seen in Fig. 3.

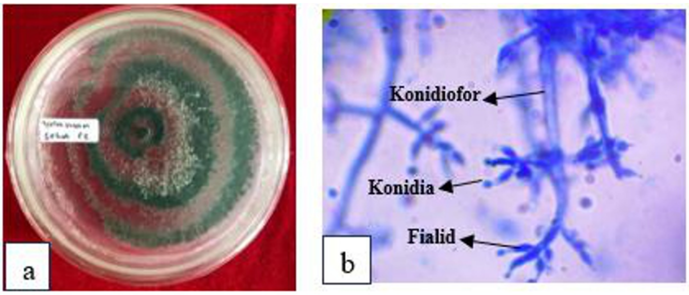

Fig. 3.. Colony and microscopic morphology of *Trichoderma* sp. Macroscopic view of colonies (a), Conidiophores, conidia and fialids (b).

Observations of *Trichoderma* sp. macroscopically shows, dark green colonies with a circular shape with the direction of growth spreading on petri dishes. In accordance with the opinion of Sopialena [8]. Which states that *Trichoderma* sp. on PDA media has a green colour with a shape like a circle and the direction of growth spreads in all directions. Microscopic appearance of *Trichoderma* sp. with the administration of methylene blue with a magnification of 400x using optilab assistance has condensed hyphae, 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 [9].

Trichoderma sp. is a type 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 [9]. Macroscopic observations showed that this fungus had an initial white colony colour and after entering the third day the colony turned green with rounded colony growth and filled the petri dish quickly. Microscopic observation has 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 [10]. The cell arrangement of this fungus is many-celled in rows 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 citric acid and ethanol [11].

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

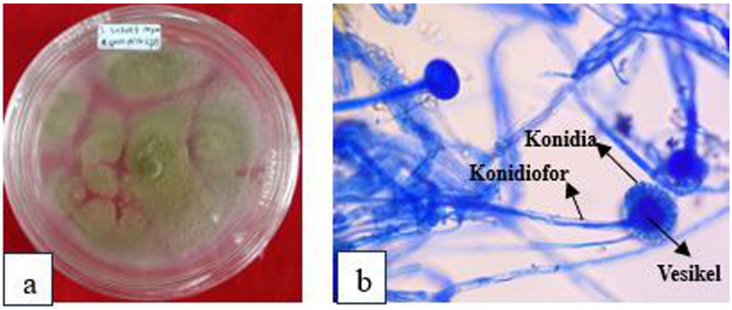

Fig. 4. Colony and microscopic morphology of *Aspergillus flavus*. Macroscopic view of colonies (a), Microscopic view of conidiophores, conidia and vesicles.

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 usually known as the cause of rot on plant stems and can produce harmful a flatoxin compounds. However, *A. flavus* can also have potential as a biological agent that competes with pathogens and produces inhibitory compounds. Macroscopically, *Aspergillus flavus* has oval-cylindrical to oblong conidia, long, peaked, and branched conidiophores and a transparent mycelium. *Aspergillus flavus* generally produces colonies that are yellow-brown in colour. These colonies usually look like sand, the conidiophores are colourless, rough, the top is slightly rounded and the hyphal vessels spread very quickly, have a size of more than 1 mm below the vesicles with a rough texture [12].

Aspergillus niger. Based on the results of observations of fungal development 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 Fig. 5.

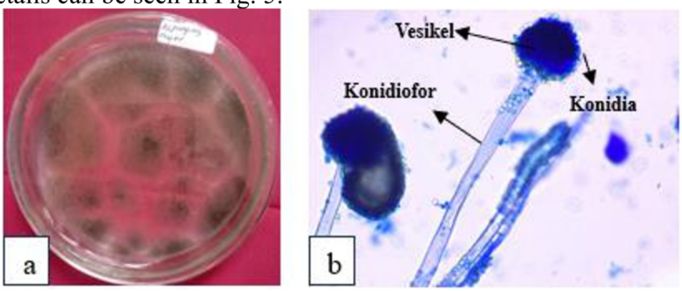

Fig. 5. Colony and microscopic morphology of *Aspergillus niger*. Macroscopic view of colonies (a), Microscopic view of conidiophores, conidia and vesicles (b).

Macroscopic observation of *Aspergillus niger* showed black colonies with granular mycelium and a shape that 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 colony's spreads irregularly. Microscopic observations 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 colony shape in the form of white colour granules on a light black surface. The older the colour changes to black. *A. niger* has hyaline hyphae and unbranched elongated hyphal structure, unconcentrated conidiophores, round conidia and blackish brown in colour. Hyphae grow within two days with a fast growth time [13].

The mycelium of *Aspergillus niger* has unbranched conidospore, coarse or fine textured, with foot cells at the base supporting the vesicles located at the tip. The vesicles, wich form one or two rown, will then support the vialid which is shaped like a flask, the rows of vesicles will produce chains of filaloconidia that are smooth or rough. All *Aspergillus* species are characterised by conidiophores, which develop into large vesicles at the tip and are enclosed by phialids that produce long chains of conidia. Phialids 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, colour, and shape of conidiosphores, conidia and phialids [14].

A. niger in its growth is directly related to the food substrate, simple molecules found around the hyphae can be directly absorbed, while more complex molecules 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 on 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 to fill the petri dish (9 cm) and upwards, for more details can be seen in Fig. 6.

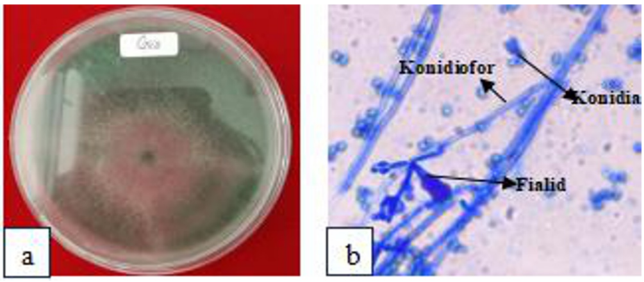

Fig. 6. Colony and microscopic morphology of *Gliocladium* sp. Macroscopic view of colonies (a), Microscopic view of Conidiohores, phialids, and spores.

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 has 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 [14]. On the stem of dragon fruit plants, one of the endophytes fungi, *Gliocladium* sp. colony characteristics with a yellowish white colour, smooth texture with symmetrical round growth. 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 known 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 [8].

3.3. Growth rate of endophytic and pathogenic fungi

The growth rate of pathogenic fungi (*Colletroticum* sp.) and the four endophytic fungi in dragon fruit plant is presented in Fig. 7. After three days of incubation the endophytic fungi have different spore density (x 10^5 per cm^2), i.e., 6.43, 5.16, 6.28, and 3.90 for *Trichoderma* sp., *A. flavus*, *A. niger*, and *Gliocladium* sp., respectively.

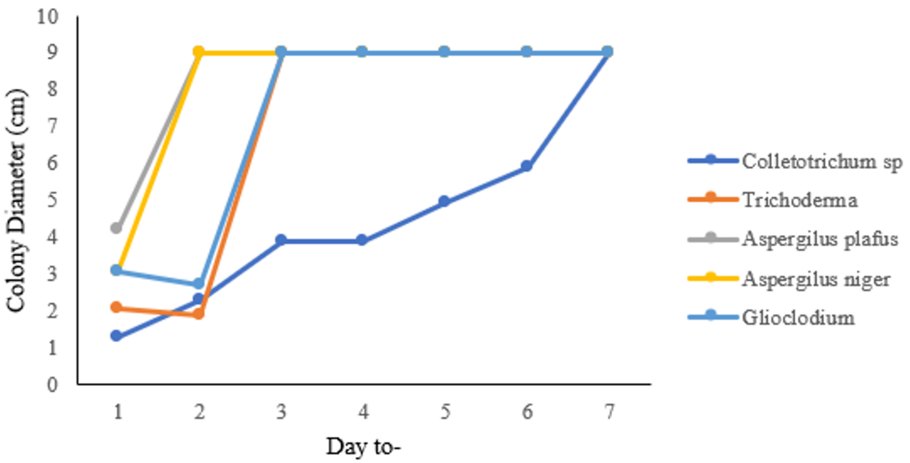

Fig. 7. Colony growth rate of endophytic and pathogenic fungi

3.4. Inhibitory Mechanism of Endophytic Fungi Against Colletotrichum sp.

After seven days of inoculation of each treatment, all the four endophytic fungi (*Trichoderma* sp., *A. flavus*, *A. niger* and *Gliocladium* sp.) showed significantly higher inhibition against *Colletroticum* sp. than control, however the inhibition of four endophytic fungi against the *Colletroticum* sp. were insignificantly different (Table 3).

Among the four endophytic fungi, descriptively *Trichoderma* sp. showed the highest inhibition (5.99%), followed by *Gliocladium* sp., *A. niger*, and *A. flavus* of 4.49, 4.48, and 4.17%, respectively. The *Trichoderma* sp. descriptively showed the highest inhibition because the fungi has the highest growth rate, i.e. 6.43 x 105 spores per cm2 compare to the others three fungi, which showed the growth rate in ranged of 3.90 – 5.16 x 10^5 spores per cm^2 after three days incubation.

Table 3. Inhibitory mechanism of endophytic fungi against *Colletotrichum* sp.

Colletotrichum sp.	Inhibition (%)*	Antagonistic mechanism type		
		Competition	Parasitism	Antibiosis
Without treatment (Control, p_0)	2.52±0.12 a			
With *Trichoderma* sp. (p_1)	5.99±0.89 b	-	-	+
With *Aspergillus flavus* (p_2)	4.17±0.74 b	+	-	-
With *Aspergillus niger* (p_3)	4.48±1.02 b	+	-	-
With *Gliocladium* sp. (p_4)	4.49±0.90 b	+	-	-

Note: Antagonistic mechanism occurs (+). No antagonistic mechanism(-). *) Data (mean±SE) were observed at seven days after inoculation. Data were analysed by ANOVA. The data in the column followed by different letters showed significantly different (LSD test, $p<0.05$).

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 (Fig. 8). 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.

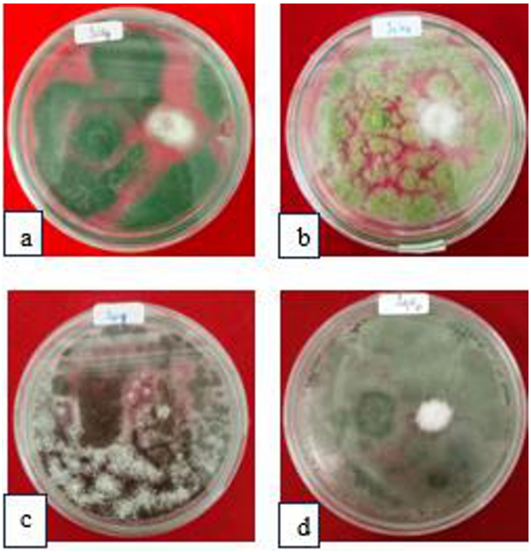

Fig. 8. Endophytic fungi undergo antibiosis and competition mechanism. *Trichoderma* sp. (a), *A. flavus* (b), *A. niger* (c), and *Gliocladium* sp. (d).

4. Conclusions

There are four endophytic fungi found from the stem of dragon fruit plants, i.e., *Trichoderma* sp, *Aspergillus flavus, Aspergillus niger* and *Gliocadium* sp.. These endophytic fungi can suppress the growth of pathogenic fungi *Colletotrichum* sp. with antagonistic mechanism in the form of competition and antibiosis mechanism. Descriptively, the *Trichoderma* sp. showed the highest inhibitory power of 5.99% (measured 7 days after inoculation) because the fungi showed the highest growth rate compare with the others three fungi.

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