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Research article

Control of Antracnose Disease in Tomato (Solanum Lycopersicum) Using Endophytic Fungi

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Abstract.
Anthracnose is a disease that affects tomato plants and causes farmers to lose money. The fungus *Colletotrichum coccodes* causes this disease, which manifests as small blackish spots on the tomato. The fruit then becomes shrivelled and dry, and it rots. One of the safest and most environmentally friendly methods of biological control is the use of endophytic fungi. These fungi are found in living plant tissue and can form colonies in the tissue without harming the host or causing disease symptoms. The goal of this study was to identify the different types of endophytic fungi found in tomatoes, as well as the role of endophytic fungi in the control of anthracnose disease. Plant samples were collected in Lempake Village in North Samarinda District, and antagonists between the endophytic fungi found in tomatoes and pathogens that cause anthracnose disease were tested. In the tomato plants, the researchers discovered four genera of endophytic fungi: *Aspergillus flavus, Aspergillus niger, Trichoderma* sp., and *Rhizopus* sp. Antagonist tests revealed that *Aspergillus flavus*, *Aspergillus niger*, and *Rhizopus* sp. fungi suppressed pathogenic fungi that cause anthracnose disease by 33.17%, 36.43%, and 38.37%, respectively. The fungus *Trichoderma* sp. had the best ability to control anthracnose.

Keywords: tomatoes, endophytic fungi, anthracnose, *Colletotrichum coccodes* Wallr. S. Hughes

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1. Introduction

Tomato (Solanum lycopersicum L.) is a horticultural commodity which fruit is popular and developed in Indonesia. Apart from being a vegetable, tomatoes are also used as raw material for medicines, cosmetics, as well as raw materials for food processing such as sauces and fruit juices. Therefore, tomatoes have a high economic value [1]. Anthracnose is a disease in tomato plants that can cause direct economic losses for farmers, because this disease is a disease caused by the fungus Colletotrichum coccodes Wallr. S. Hughes. This fungal infection in tomatoes is characterized by small blackish spots. Further attacks are the fruit shriveled, dry and rot [2]

The use of chemical pesticides is still a reliable way for farmers to maintain plant health, although not all farmers manage it wisely. Many research results show that the unwise use of synthetic pesticides has been detrimental to humans and agro-ecosystems [3]. Biological control of pests and plant diseases by utilizing natural enemies, such as predators, parasitoids, pathogens, and antagonists has long been declared as one of the components of integrated pest and disease control [4]. Biological control is able to reduce the dependence of plant production processes on fertilizers, pesticides, and chemical hormones. This control is becoming popular with increasing public attention to health and environmental sustainability. Safe and environmentally friendly control, one of which is biological control using endophytic fungi [5]. The aim of this study was to explore the endophytic fungi found in tomato plants, which were then used to control the pathogens that cause anthracnose Colletotrichum coccodes Wallr. S. Hughes in tomato plants.

2. Methodology

Figure 1: Tomato Plant Sampling Site.

This research has been conducted for 6 months, starting from May to October 2020. The location for sampling healthy plants and plants that experience symptoms of anthracnose disease is in Lempake Village. Geographically, Lempake Village is located at 0°25'26 South Latitude and 117°12'06 East Latitude, with an altitude of 21.427 masl, a slope of 44.99°, and with rainfall of 1,936 mm/year. This area is about 15 km from the provincial capital of East Kalimantan. Lempake Village, North Samarinda District, Samarinda City is an area with an area of 3,224 Ha [6].

The laboratory works was carried out at the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Mulawarman University, Samarinda.

The materials used in this study were Potato Sucrose Agar (PSA) media, samples of healthy tomato plants, sick tomato plants, 70% alcohol, water, distilled water, methilene blue, chloramphenicol, and spirits, potatoes, sucrose, chloramphenicol, agar and distilled water.

The activities carried out in the research include determination of location and sampling, sterilization of tools and materials, preparation of Potato Sucrose Agar (PSA) media, isolation of endophytic and pathogenic fungi, identification of endophytic fungi and pathogenic fungi, fungi purification, propagation of endophytic fungi and pathogenic fungi, in vitro antagonist tests, data retrieval.

Identification is done by looking at the morphological characteristics of the fungus using lab optics and a microscope. Purification of the fungus *Colletotrichum coccodes* and endophytic fungi was carried out from the results of fungal isolation that had been carried out previously. After doing the isolation, on PSA media the hyphae and fungi will grow, then take part of the spores using an ose needle and place them in a petri dish containing the new PSA media. Petri dishes are wrapped in cling wrap.

In Vitro Antagonist Test

In Vitro testing of endophytic fungi antagonists against disease fungi was carried out using the dual culture method by taking each pure culture of the characterization results belonging to the test endophytic fungi and pathogenic fungi using a ose needle, then the inoculum was placed in a 9-diameter petri dish. cm. For each test a center line is drawn and two points are given. The distance between them from the edge of the cup is 3 cm [7].

How to lay:

2.1. Data retrieval

1. Endophytic Fungal Morphology and Pathogens

The data taken is the result of isolation and identification from research that has been carried out by observing the special characteristics possessed by endophytic fungi and pathogenic fungi Colletotrichum coccodes, such as color and shape of the colony, type of hyphae (insulated or not insulated) and hyphae shape. macroscopic observations by using a mushroom identification key book. Microscopic observations of endophytic fungi and pathogenic fungi Colletotrichum coccodes include observation

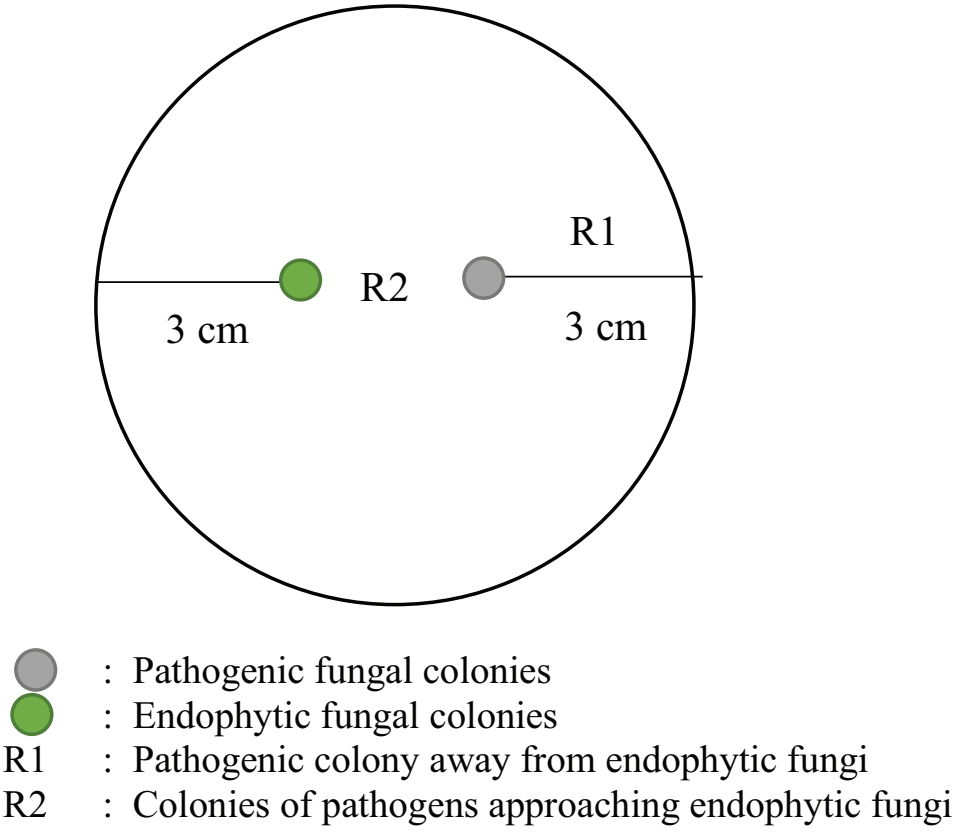

Figure 2: Dual culture method.

of conidiophores and phyalids under a microscope using a fungal identification key book [8].

1. Growth of Endophytic Fungi and Pathogens

To determine the growth of fungi (cm) was carried out by measuring the diameter of the colonies of each fungus every day after inoculation, measurements were carried out using a ruler.

1. Percentage Barriers %

The percentage of inhibition was calculated on the 8th day after inoculation, based on the formula proposed by [7], namely: $l = \frac{r1-r2}{r1}\ x\ 100\%$

Information :

l : Pathogenic fungal colonies

r1 : Pathogenic colonies away from endophytic fungal colonies (cm)

r2 : Pathogenic colonies approaching endophytic fungal colonies (cm)

1. Endophytic Fungus Antagonist Mechanism

Observations on the mechanism of inhibition were competition as indicated by the slow growth of *C. coccodes* fingers towards endophytic fungi, antibiosis as indicated by the clear zone between *C. coccodes* fungi and endophytic fungi, and parasitism as indicated by the growth of endophytic fungi that covered the entire medium including fungus *C. coccodes*. Determination of the selected antagonist isolate was taken from the results of the percentage of endophytic fungal inhibition which was more than 40% [9].

The research design used in this study was A Completely Randomized Design (CRD). If there is a significantly different F test, it is followed by the Least Significant Difference Test at the 5% confidence level.

3. Results and Discussion

Based on the results of the isolation of the sample health plant material in the field, the endophytic fungi in tomato plants were found as follows:

3.1. Aspergillus flavus

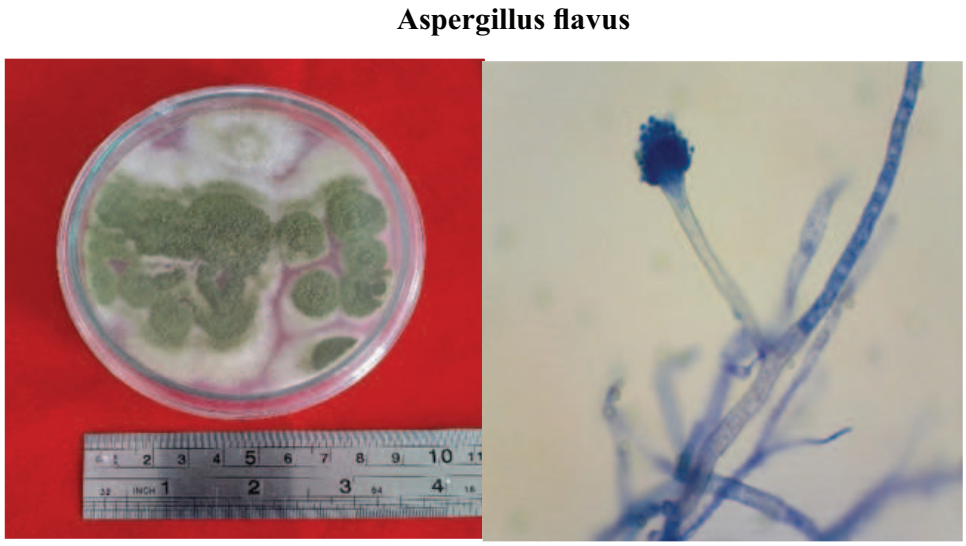

Figure 3: Colony of *Aspergillus flavus*.

When young, *Aspergillus flavus* colonies are white, and will change to a yellowish green color after forming conidia with a rough top surface of mycellium, then mycellium spreads in all directions to fill the petri dish after 7 days. This is in accordance with the statement [10] which states that the *Aspergillus flavus* fungus produces colonies that

are green or yellow gray to black in color. The conidiophores are colorless, rough, the tops are slightly rounded and the conidia are rough.

Microscopic observation showed a short smooth greenish stalk of conidia (conidiophores), conidia heads (vesicles) shaped like a club (clavate) and round, and became oval (columnar) with increasing age of the colony. Sterigmata appear to cover the upper half of the vesicle. Spores / conidia are round, greenish in color, and have a serrated surface (echinulate). Conidiophores are clear, non-pigmented, coarse, less than 1 mm long. When the age of the colony is old, the colony turns dark green and the top surface of mycellium forms rough. The results of these observations are in accordance with the statement [11] which states that the conidiophores of *Aspergillus flavus* are clear, non-pigmented, coarse, microscopically less than 1 mm in length.

The fungus *A. flavus* seen visually on PSA media has colonies that are bright green with a spreading round shape, the mycellium that is formed appears to grow upward with a peak filled with spores [12]. When viewed microscopically, *A. flavus* fungi have similar characteristics to *A.niger* fungi, namely conidiophores with smooth walls, hyaline hyphae and round conidia, but these fungi have a lighter color than *A.niger* fungi. This is in accordance with Iskandar's statement which states that *A. flavus* fungi produce colonies that are greenish yellow or yellow-gray to blackish in color. The conidiophores are colorless, rough, the upper part is slightly rounded and the conidia are rough with various colors. This fungus is very easy to find, as well as the genus *Aspergillus* sp.

3.2. Aspergillus niger

The growth of the *Aspergillus niger* fungus (Figure 4) begins with having white colonies then turning dark brown to black, circular but irregular and the mycelium is like grains of sand. In accordance with the statement [13] which states that, macroscopically the *A. niger* species shows compact white and yellow colonies on the lower surface of the colony which will turn dark brown to black after conidiospores are formed.

Observation of the *Aspergillus niger* fungus using a microscope found that microscopically characterized by conidial color, phialids filled the entire surface of the vesicles and large round vesicles. *Aspergillus niger* has a black colony color and the underside of the colony is yellowish white. The results of this observation are in accordance with the statement [13] which states that microscopically vesicles are round to semi-spherical. Conidia round to semi round and brown in color.

Aspergillus niger the colony is black with a white border and the underside of the colony is yellowish to brown. Microscopically characterized by conidial color, phialids

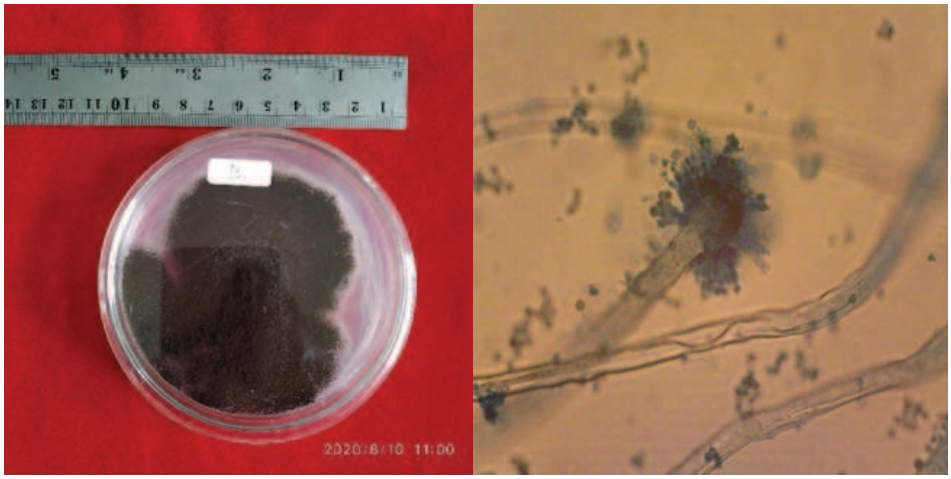

Figure 4: *Aspergillus niger* colony.

fill the entire surface of the vesicles and large round vesicles, spherical (radiate). The conidia stalk (conidiophores) has smooth walls, hyaline, but often brown [14]. According to [15], the characteristics of the fungus colony are black. The conidia was very clearly visible and the mycelia were very thick and white. *Aspergilllus* sp. is a fungus that is often found in nature in various mediums such as the root area (rhizosphere), plant philosphere, plants, food and drink. Fungis of the genus *Aspergillus* sp. This can produce antibitic compounds so that it is included in one of the biological control fungi

3.3. Rhizopus sp.

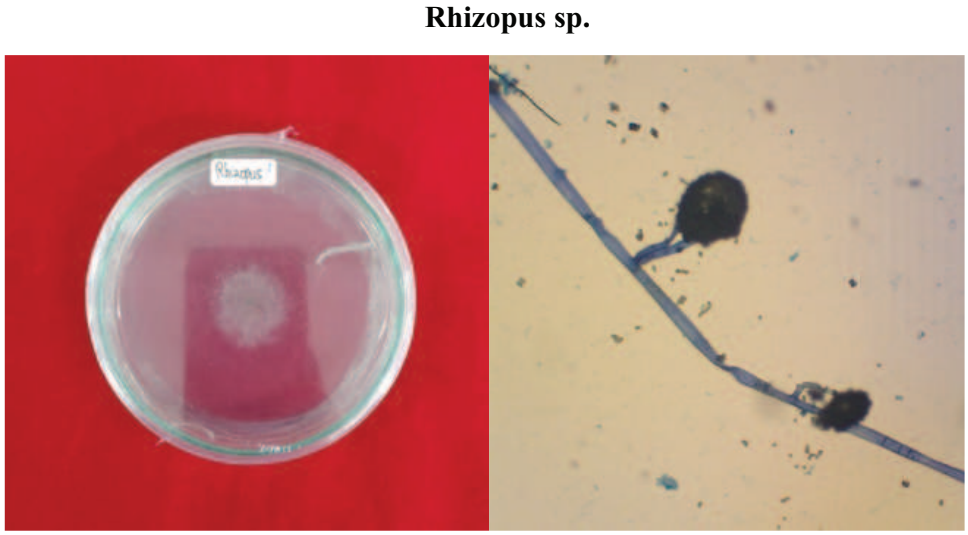

Figure 5: Colony of *Rhizopus* sp.

Observations on the petri dishes (Figure 5) show that *Rhizopus* sp. colony has a grayish-white color, the mycelium looks like a bunch of cotton (hyphae) and the initial colony which is grayish white over time will turn black, due to the large number of spores. This is in accordance with the statement [16] which states that the macroscopic observations of the Fungi *Rhizopus* sp. produces a white mold colony with black spores on PDA media

Observation using a microscope with a magnification of 400x (Figure 5) shows the characteristics of the fungus *Rhizopus* sp. that is, it has insulated hyphae and sporangium (where spores are formed) which are slightly rounded at the tip of the hypha. This is in accordance with the statement of [17] which states that the observations of *Rhizopus* sp. Microscopically, the hyphae appear insulated, and produce sporangium with a round shape at the tip of the hypha.

Rhizopus sp has white colonies at the beginning of growth, then grayish brown. Colonies are shaped like cotton that produce large amounts of sporangia, have long hyphae, are not seeped, have rhizoids, there are stolons that connect a series of sporangia consisting of 2-5 sporangiophores [15]. This is in accordance with the statement of [18] that *Rhizopus* sp. Has physical characteristics, namely having a multicellular body, its habitat on land as non-insulated saprophytes, the mycelium looks like a bunch of cotton (hyphae) and its initial colony which is grayish white over time will turns black, because of the large number of spores.

3.4. Trichoderma sp

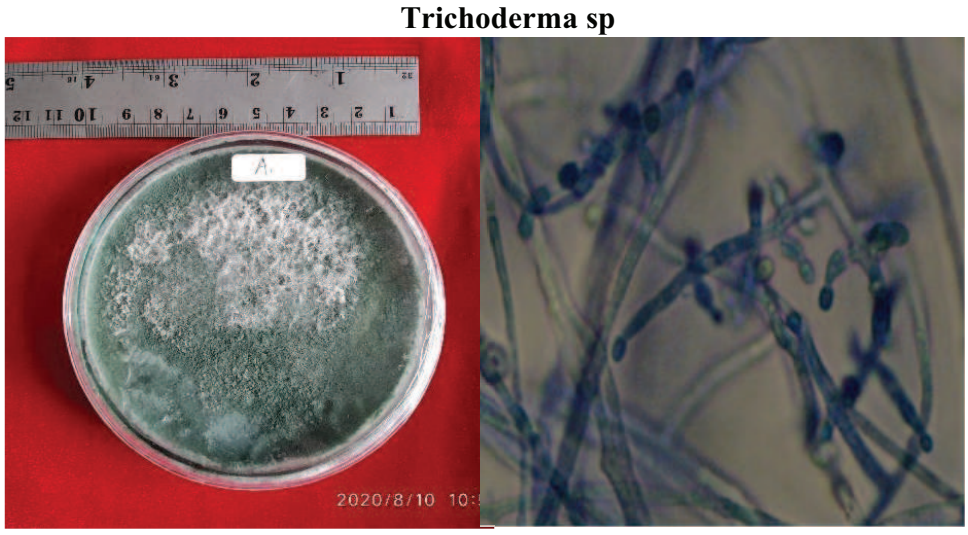

Figure 6: Colony of *Trichoderma* sp.

Direct observations that have been made on the growth of the *Trichoderma* sp. found in the stem (Figure 6). It can be seen that this Fungi colony initially has a white color but after a few days turns green. The spore growth quickly spread to all parts of the petri plate and was round in 7 days. The growth of the fungal colonies had filled the 9 cm petri dish. This is in accordance with the statement of [19] which states that the observations of *Trichoderma* sp. Macroscopically, fungal colonies at the beginning of growth are in the form of soft white mycelium which then at the age of 3 days turns dark green to form irregular circles

Identification of *Trichoderma* sp. Using a microscope with a magnification of 400x (Figure 6), the isolates of *Trichoderma* sp. which is found that has conidia that are round to oval like eggs, have conidiophores that are upright and branched and mycelium with septa. This is in accordance with the statement of [20] which states that the observations of *Trichoderma* sp. microscopically it shows the branched conidiophores type. The branches are short and smooth-walled with a few nodules. Conidia are nearly round / oval with a diameter of 2µm and dark green in color. Conidia accumulate in one pialid / stem pialid.

This Fungi colony initially has a white color but after a few days turns green. The spore growth quickly spread to all parts of the petri plate and was round in 7 days. The growth of the fungal colonies had filled the 9 cm petri dish. Microscopically, it has conidia that are round to oval like eggs, have conidiophores that are upright and branched and mycelium has septa. This is in accordance with the statement of [21] which states that the observations of *Trichoderma* sp. Macroscopically, fungal colonies at the beginning of growth are in the form of soft white mycelium which then at the age of 3 days turns dark green to form irregular circles. Microscopically shows the branched conidiophores type. The branches are short and smooth-walled with a few nodules. Conidia are nearly round / oval with a diameter of 2µm and dark green in color. Conidia accumulate in one pialid / stem pialid.

3.5. Colletotrichum coccodes Wallr. S. Hughes

Observations made on this pathogenic fungus (Figure 7) show that the isolates that grow on the petri dishes are grayish-white, round in shape and thickened with a texture like cotton. This is in accordance with [22] statement which states that macroscopically *Colletotrichum* has a grayish-white colony color, colony-like characteristics of cotton, there are insulation on hyphae, and hyaline conidiophores.

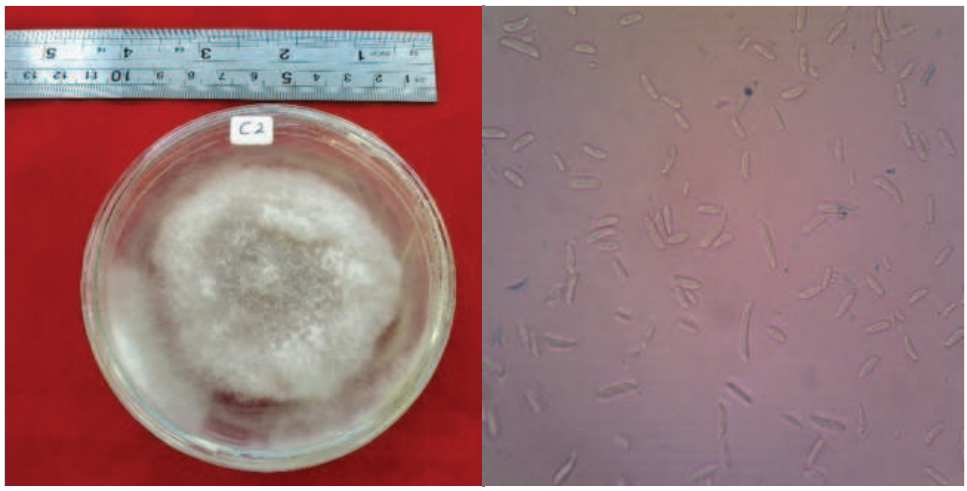

Figure 7: *Colletotrichum coccodes* Wallr. S. Hughes colony.

On observation using a microscope with a magnification of 400x (Figure 7), the characteristics of the *Colletotrichum coccodes* Wallr. S. Hughes fungus can be seen, namely having spores in the form of capsules with a tapered tip and spreading spores. This is in accordance with [23] statement which states that *Colletotrichum coccodes* Wallr. S. Hughes have spores that are cylindrical, spore ends that are tapered and spore size is 14.9 x 4.2 µm.

Fungus *Colletotrichum coccodes* Wallr. S. Hughes is one of the fungi that causes disease in tomato plants. Infection of the fruit usually occurs in the phase of young fruit until the fruit is ripe with initial symptoms marked by the appearance of blackish brown spots on the surface of the tomato fruit, where the spots turn soft [24] In the middle of the spot there are black dots which are groups of spores. The cycle of blight is caused by fungi *Colletotrichum* sp., which the fungus belongs to the category of fungi. Morphological characteristics of *Colletotrichum coccodes* Wallr. S. Hughes are macrocopically white in color with round growths and thick mycelium like cotton and thickened. This is consistent with the statement of [25] that fungi belonging to the genus *Colletotrichum* have macromorphological characteristics of white colonies and smooth colony textures like cotton.

3.6. Growth rate

The percentage of inhibition from the results of in vitro antagonism of endophytic fungi against *Colletotrichum coccodes* on tomato plants is presented in table 2. The percentage of inhibition observed was the growth of pathogenic fungi away from endophytic fungi (r1) and growth of pathogenic fungi that approached endophytic fungi

Table 1: Observation of the colony diameter of endophytic fungi and pathogenic fungi (cm).

Fungi	Growth Day-						
	1	2	3	4	5	6	7
A. flavus	1,2	2,6	4,4	6.2	7.5	8.6	9
A. niger	1	2.8	4,2	6	7,7	8.5	9
C. cocodes	0.6	1.8	3,4	5.7	6.8	8	8.5
Rhizopus sp.	1,1	2,4	4,3	6	7.5	8,4	9
Trichoderma sp.	1,2	2.7	4.5	6.3	7,8	8.7	9

(r2) on the 7th day. The results of the variance of the antagonist power test showed very significant different results. The results of the average percentage inhibition of each endophytic fungal isolate against the fungus *Colletotrichum coccodes* are presented in (Table 2).

Table 2: Endophytic fungal antagonist test against pathogenic fungi (%).

Treatment	Repetition					Average
	1	2	3	4	5	
A.flavus	39.46	27.87	30.40	37.53	30.58	33.17 a
A.niger	28.18	43.67	31.16	37.67	41.49	36.43 a
Rhizopus sp.	43.97	32.43	38.36	37.44	39.64	38.37 a
Trichoderma sp.	44.73	43.26	40.94	45.37	42.38	43.34 b

Note: Numbers followed by the same lowercase letter in the same column, means that they are not significantly different in the 5% LSD test (LSD = 6.59)

The results of the 5% LSD test of endophytic fungus inhibition against *C.coccodes* fungi that cause anthracnose disease in tomato plants, it can be seen that the treatment of *C.coccodes* Vs *Trichoderma* sp. significantly different to the treatment of *C.coccodes* Vs *A. flavus*, *C.coccodes* Vs *A. niger* and *C.coccodes* Vs *Rhizopus* sp ..

The results of the observation of the endophytic fungal antagonist test against pathogenic fungi in rice can be determined the highest percentage of inhibition to the lowest percentage of inhibition Tables 3 and 4. The highest percentage of inhibitory power, namely, *C.coccodes* Vs *Trichoderma* sp. with an average of 43.34%; followed by the fungus *C.coccodes* Vs *Rhizopus* sp. with an average of 38.37%; then the fungus *C.coccodes* Vs *A. niger* with an average of 36.43%; and the lowest percentage of inhibition of *C.coccodes* vs *A. flavus* with an average of 33.17%. This is in accordance with the statement of Loekas (2013) who conducted research on the inhibition of *Trichoderma* sp. namely in the range of 33.33 - 62.46%. The fungus *Trichoderma* sp. is an isolate that has the highest percentage in this test because this fungus is able to

antagonize other fungi with faster growth and has a high spore density compared to pathogenic fungi.

This happens because of the nature of the fungus *Trichoderma* sp. which is able to antagonize other fungi with faster growth. *Trichoderma* sp. damage the host hyphae by constricting, hooking, or appressorium-like structures and penetrating the host cell wall by secreting lytic enzymes. Namely proteinase, -1.3-glucanase, and chitinase. In addition, the purification/purification of chitinolytic enzymes produced by *Trichoderma* can be used for plant disease control [24].

3.7. Antagonist Mechanism

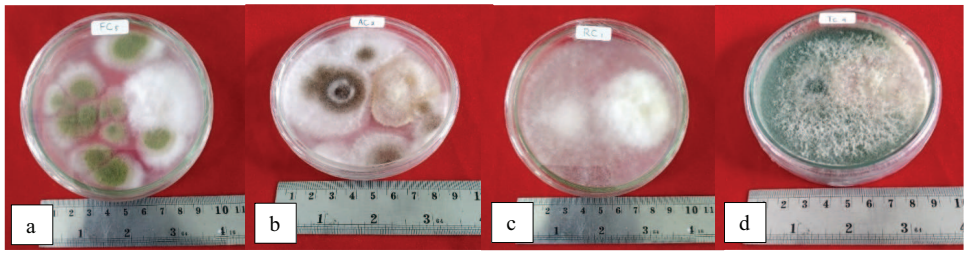

Figure 8: Mechanism of antagonist of endophytic fungi against pathogenic fungi. (a) *Aspergillus flavus*, (b) *Aspergillus niger*, (c) *Rhizopus* sp., (d) *Trichoderma* sp.

Observation of the antagonist mechanism of *C. coccodes* against endophytic fungi was carried out for 7 days after incubation. The results of the observations are presented in Figures 9 – 12. The antagonist mechanism is seen when the two fungi tested inhibit each other's growth. In this observation there are three mechanisms that occur, namely competition, antibiosis and parasitism. For more details, it can be seen in the image below:

Observation of the mechanism of inhibition of endophytic fungi against *C.coccodes* fungi was carried out every day until the 7th day after incubation. In vitro antagonist test results showed that 4 endophytic fungi inhibited the growth of *C.coccodes* through an antagonistic mechanism of growth space competition. These endophytic fungi controlled the growth space more than pathogenic fungi. This is in accordance with the statement of [26], which states that *Aspergillus niger* has a growth space competition mechanism because the *Aspergillus niger* fungus that grows towards pathogens can inhibit the growth of pathogens. [27] stated that the fungus *Aspergillus* sp. has an antifungal compound in the form of white crystals identified as phenolic. Apart from that *Aspergillus* sp. also capable of removing aspulvivone compounds, asterik acids, asteriquinone and others to inhibit the growth of pathogenic fungi. [28] stated in his research results, that *Rhizopus* sp. has the ability to compete for growth space which

is quite high, because it is able to cover pathogenic colonies so that the pathogens are not able to develop anymore. The faster the growth of endophytic fungi, then the growth of pathogenic fungi will be increasingly pressed because they run out of growth space.

Observations on the fungus *C.coccodes* Vs *Trichoderma* sp. showed the growth of the fungus *Trichoderma* sp. can control the growing space, so that pathogenic fungi become difficult or even unable to grow in a petri dish. This is in accordance with the statement of Alfizar et al, that *Trichoderma* sp. able to remove antibiotic compounds such as glyotoxin and glioviridin. These antibiotic compounds affect and inhibit many functional systems and make pathogens susceptible. Initial interactions of *Trichoderma* sp. that is, by means of its hyphae turning towards the host fungus it attacks. This shows the phenomenon of chemo-tropic response in *Trichoderma* sp. due to stimulation from the host hyphae or chemical compounds released by the host fungus. When the mycoparasites reach their host, the hyphae then coalesce or squeeze the host hyphae by forming a hook-like structure, these mycoparasites also sometimes penetrate the host mycelium by degrading part of the host cell wall. *Trichoderma* sp. produce enzymes and antibiosis compounds that can inhibit and even kill pathogens. These antibiotic compounds, namely gliotoxin, glyoviridin and Trichodermin, severely inhibit the growth of pathogens.

4. Conclusion

In the research on the potential of endophytic fungi to suppress the pathogen *Colletotrichum coccodes* Wallr. S. Hughes which causes anthracnose disease in tomato plants in Lempake Village. Several conclusions were obtained that endophytic fungi identified in tomato plant tissue, namely *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus* sp. and *Trichoderma* sp. and the four endophytic fungi colonies have different antagonistic power against the pathogenic fungus *Colletotrichum coccodes* Wallr. S. Hughes, the highest antagonistic power of endophytic fungi is *Trichoderma* sp. 43.34%. The antagonistic mechanism that occurs between endophytic fungi and the pathogenic fungus *Colletotrichum coccodes* Wallr. S. Hughes is competition for growth space.

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