Antagonist Test of Endophytic Fungi Against Hiyung Chili Leaves Growth of Fusarium oxysporum Causes Fusarium Wilt

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Abstract

F. oxysporum, which causes fusarium wilt, is a pathogen that spreads through the soil and can survive in the soil as mycelium or spores without a host. F. oxysporum usually attacks chili planting areas and can cause disease losses of up to 80%. Farmers usually control disease using chemicals or pesticides as the main control. Continuous use of pesticides can result in the death of natural enemies and cause pathogen resistance. One effort to overcome fusarium wilt without using pesticides is to use antagonistic agents, one of which is endophytic fungi. Endophytic fungi are fungi that are found in plant tissue systems such as leaves, flowers, twigs or plant roots. This research used a non-factorial Completely Randomized Design (CRD) with 5 treatments and 4 replications, so that 20 experimental units were obtained. Based on the research results, it shows that the endophytic fungus of hiyung chili leaves has a significant effect on the growth of F. oxysporum by inhibiting mutually intermingling growth mechanisms or competition mechanisms by isolate P1 (Acremonium sp.) and isolate P2 (Cladosporium sp.), mutually slight inhibition or antibiosis mechanisms by isolate P3 (Rhizoctonia sp.) and isolate P5 (Alternaria sp.), while overgrowth by antagonism or parasitism mechanism by isolate P4 (Sclerotium sp.). The fungus isolate with the highest inhibitory power was isolate P5 (Alternaria sp.), reaching 34.72% at 7 DAP. Meanwhile, the fungus isolate with the lowest inhibitory power was shown by isolate P2 (Cladosporium sp.), which only reached 17.46% at 7 DAP.

Keywords : Endophytic Fungi, Chili Leaves, Fusarium Oxysporum, Fusarium Wilt

Introduction

In 2017, cayenne pepper production was recorded at 1.15 million tons. Then production continued to increase until 2020. In 2021, the highest production of cayenne pepper occurred in July, reaching 134.4 thousand tons. Meanwhile, the lowest occurred in February, around 94.54 thousand tons. The Central Statistics Agency (BPS) noted that this number decreased by 8.09% from 2020 which amounted to 1.5 million tons. The decline in cayenne pepper production in 2021 is the first in the last five years. Therefore, it is known that the chili production in Indonesia does not fulfil the national chili needs, so it is necessary to import chilies reaching 16,000 tons/year (DJBPH, 2009).

In cultivating chili plants, there are several factors that can reduce production yields, one of which is attacks by Plant Pest Organisms (OPT) in the form of pests and pathogens which can cause plants to be attacked by disease, resulting in losses in crop yields (Nurjasmi & Suryani, 2020). A disease that is often found in chili plants is fusarium wilt caused by the pathogen *Fusarium oxysporum* which can cause significant losses to chili plants because it attacks plants from germination to maturity. *F. oxysporum*, which causes fusarium wilt, is a pathogen that spreads through the soil and can survive in the soil as mycelium or spores without a host (Huda, 2010). *F. oxysporum* usually attacks chili planting areas which can cause disease losses of up to 80% (Djafarudin, 2003).

Farmers usually control disease using chemicals or pesticides as the main control. Continuous use of pesticides in large quantities can result in the death of natural enemies and cause pathogen resistance (Soesanto, 2008). One effort to overcome fusarium wilt without using pesticides is to use antagonistic agents, one of which is endophytic fungi. Endophytic fungi are fungi that are found in plant tissue systems such as leaves, flowers, twigs or plant roots. Endophytic fungi infect healthy plants in certain tissues and are able to produce mycotoxins, enzymes and antibiotics (Sinaga, 2009)

In research conducted by Nurzannah et al. (2014) it is known that all endophytes from chili leaves have potential as biological agents. Penicillium sp. has the best ability to inhibit the growth of *F. oxysporum* with a disease severity percentage of 2.78% and a plant height of 29.40 cm when applied together with *F. oxysporum* in the screen house. In this research, we tested the ability of endophytic fungi in hiyung chili leaves to suppress the growth of *F. oxysporum* carried out in vitro.

Materials and Methods

Material needed in this research are: *F.oxysporum*, chili leaves, water, Potato dextrose agar (PDA), filter paper, spiritus, alcohol 70%, and Naocl 3%. While the tools used are petri dish, analytical balance, *hot plate*, jarumose, pinset, *object glass*, *slide glass*, *cover glass*, *autoklaf*, *laminar air flow*, oven, scales, hand sprayer, filter paper, blender, glass bottles, petridish, plastic, rotary evaporator, knife, jars, beakers, sieves, hoes, lid, name tag, cage, and a camera.

Statistical Analysis

Statistical analysis was carried out in four replicates for the one control and experimental samples. The data has been analyzed by one-way analysis of variance (ANOVA) followed by Turkey's test, Duncan's multiple range test for the average value of parameter among the five treatments and used to compare the means values between each treatments.

There are 5 levels of statistical used in this treatment :

1. endophytic fungi A (p_1),
2. endophytic fungi B (p_2),
3. endophytic fungi C (p_3),
4. endophytic fungi D (p_4),
5. endophytic fungi E (P_5).

Results and Discussion

The growth of hiyung Chili Leaf Endophytic Fungi

The result of the hiyung chili endophytic fungus.

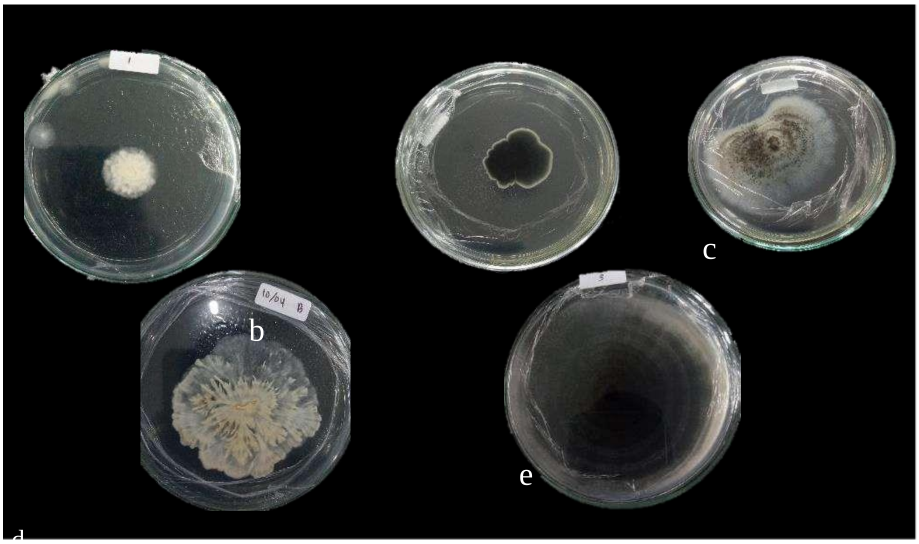

Figure 1. Hiyung chili leaf endophytic fungus isolates: (a) P1, (b) P2, (c) P3, (d) P4, and (e) P5.

The research results show that the leaves of chili plants contain various kinds of endophytic fungi. Based on the research results of Istikorini (2008), it was stated that chili plants in Indonesia contain an abundance of 111 isolates of endophytic fungi. In addition, endophytic fungi associated with plants increased plant height by 28.58%, root length by 34.57%, and fruit weight per plant by 22.22% compared to the control.

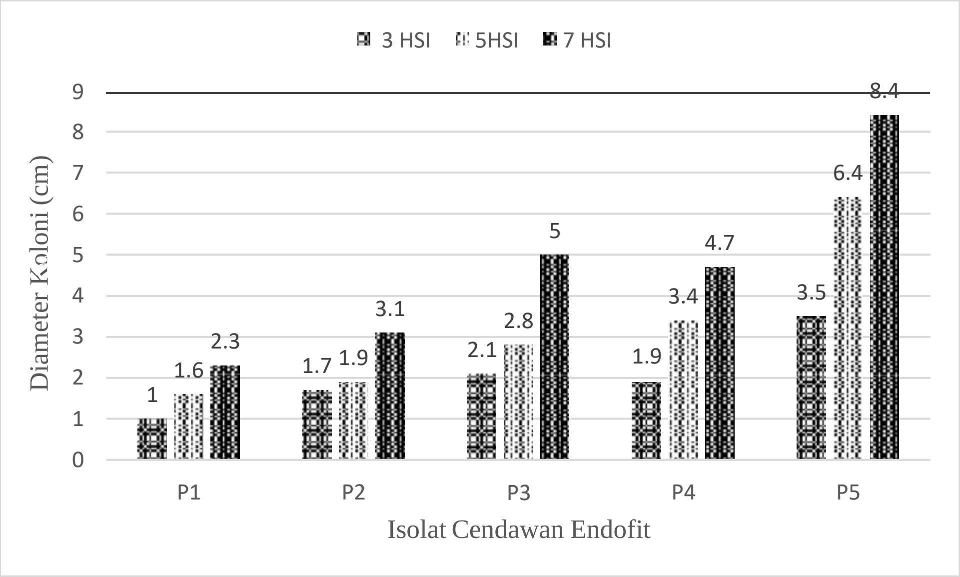

Figure 2. Colony growth diameter (cm) of endophytic isolates of hiyung fungus at 3, 5, and 7 days after inoculation (HSI).

Various types of endophytic fungi obtained from chili plant leaves have different colony diameters. This can show that each isolate has a different growth rate from each other. Observations on the growth rate of endophytic fungi were carried out on the 3rd, 5th and 7th days after isolation so that data on the colony diameter of the hiyung chili leaf endophyte fungus was obtained in Figure 2. Isolate P5 is an endophytic fungus which has the highest colony diameter, namely 8.4 cm among five endophytic fungi isolated for 7 days. The endophytic fungus isolate with the lowest colony diameter, namely isolate P1, only reached 2.3 cm 7 days after isolation. In isolates P2, P3, and P4, it can be seen that they had a total colony diameter ranging from 3-5 cm for 7 days after isolation.

The five isolates of the hiyung chili leaf endophytic fungus that were observed were used as endophytic fungi in the antagonism test with the pathogenic fungus *F. oxysporum*. The fungus isolates obtained had different colors and colony diameters, this shows that the isolates came from different genera with different growth rates. One of the characteristics of antagonistic microbes is characterized by the speed of mycelial growth of the endophytic microbes themselves (Shehata & El-Borollosy, 2008). In addition, endophytic fungal colonies are a source of secondary metabolites that are useful in biotechnology, agriculture and pharmaceuticals (Ye et al., 2020).

Isolate P1, on the 7th day after isolation had a colony diameter of 2.3 cm with a white colony color and getting older in the middle of the colony producing a blackish gray color which can be seen in Figure 1a. Spread of this isolate laterally on surfaces such as cotton. At 7 DAP, isolate P2 had a higher diameter than P1, reaching 3.1 cm. This isolate grew with blackish green colonies and a rough, velvety, mountainous surface that can be seen in Figure 1b. Isolate P1 is the isolate that has the lowest diameter among the other four isolates. Meanwhile, in Figure 1c, the P3 isolate is white and the middle part is blackish gray. This isolate spread laterally and had a colony diameter reaching 5 cm on the 7th day after isolation, which means this isolate had a higher diameter than isolates P2 and P4. The fungus isolate which has a diameter of 4.7 cm is a P4 isolate with white colonies and as they get older, these colonies will produce black sclerotia as a form of survival. While, the fungus isolate which had the highest diameter reached 8.4 cm with black colonies surrounded by white, the surface of this isolate was rough. This fungus is a P5 isolate.

Based on the results of observations of the P1 fungus isolate, macroscopically the colony was white, but over time it became blackish gray in the middle of the isolate with a cotton-like surface shape. Apart from that, microscopically it can be seen that this isolate has septate hyphae, branched conidiophores, and contains chlamydospores. This is in accordance with the observations of Samson et al. (2010), namely fungi belonging to the genus Acremonium sp. has yellowish green, white and pink or orange colored characters, branched conidiophores, hyaline conidia which are elliptical and short and sometimes contain chlamydospores. Macroscopic characters of Acremonium sp. has the characteristic white to brown color of the colony, the surface of the colony in the middle looks like cotton, the branched conidiosphorus is generally coated with chromaphyll, has phialids. Single-celled conidia appear somewhat clustered to form one head, the shape of the conidia is elongated to round, hyphae and chlamydospores can also be formed (Akmalasari et al., 2013)

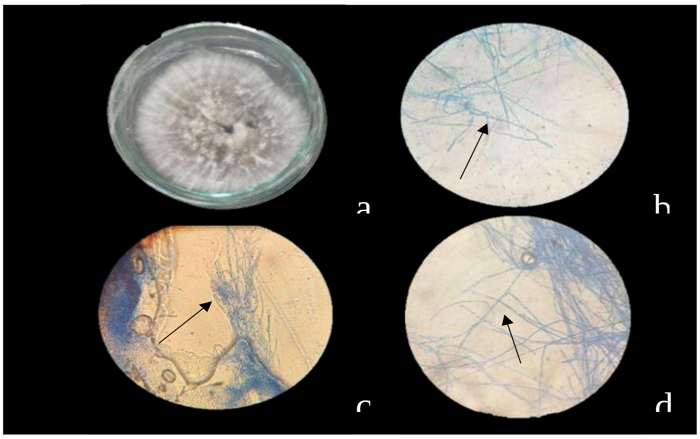

Figure 3. Isolate P1 (Acremonium sp.) on PDA media: (a) macroscopic (colony), (b) Chlamydospores, (c) Conodiophores, (d) hyphae.

Based on the results of observations of the P2 fungus isolate, it shows that macroscopically the fungus isolate has blackish green colonies surrounded by white. The surface of the colony is mountainous with a rough, velvet-like texture. The microscopic characteristics of this fungus isolate are that it has multinucleate insulated hyphae, conidia in the form of 1 to 2 septate chains, cylindrical in shape and smooth walls found in branching chains with an elliptical shape. Meanwhile, conidiophores are lateral or terminal on the hyphae. This is in accordance with observations made by Watanabe (2002), Barnett & Barry (1972), and Samson et al. (2010), namely fungi belonging to the Genus Cladosporium have conidiophores that are branched, erect and hyaline in color. Conidia form chains of brown or hyaline, round, ovate, ellipsoidal, subglobose and cylindrical.

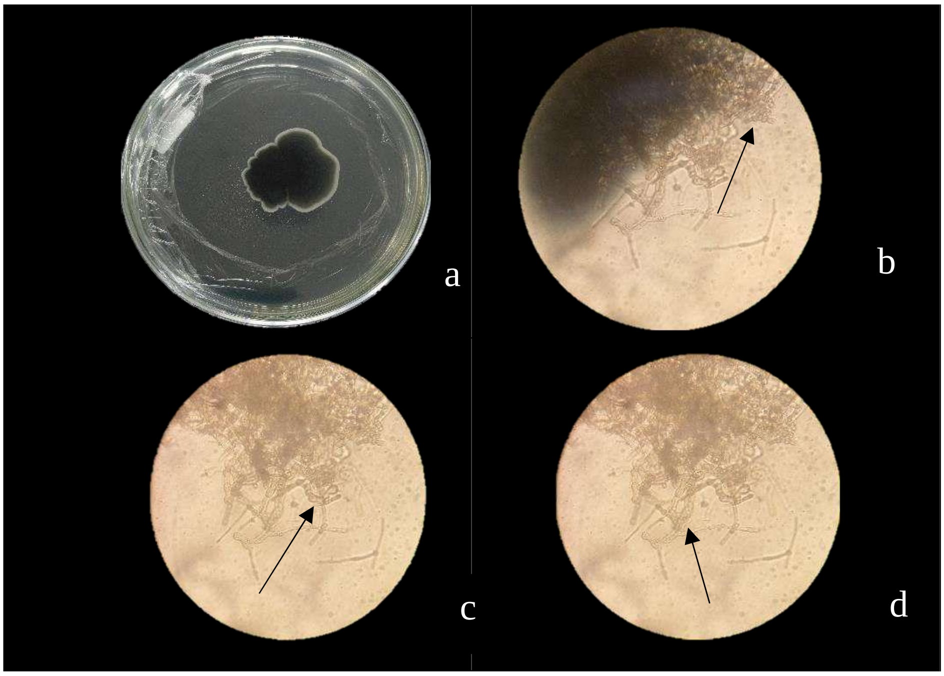

Figure 4. Isolate P2 (Cladosporium sp.) on PDA media: (a) macroscopic (colony), (b) conidia, (c) conodiophores, (d) hyphae.

Based on the observation results, isolate P3 could be included in the genus Rhizoctonia, because macroscopically the colonies were white to blackish gray. Apart from that, microscopically this fungus does not produce spores and has insulated hyphae. This is in accordance with the opinion of Barnet (1972), that the fungus Rhizoctonia sp. has morphological characteristics, namely that the hyphae branching has an acute angle at the branching point, there is a curve and there are partitions. According to Semangun (2008), Rhizoctonia sp. in the tropics it never produces spores. Streets (1972), stated that the mycelium consists of long cells and produces branches that are approximately perpendicular to the main hyphae and have partitions. Under certain conditions Rhizoctonia sp. produces lump-like sclerotia that expand and are triangular in shape.

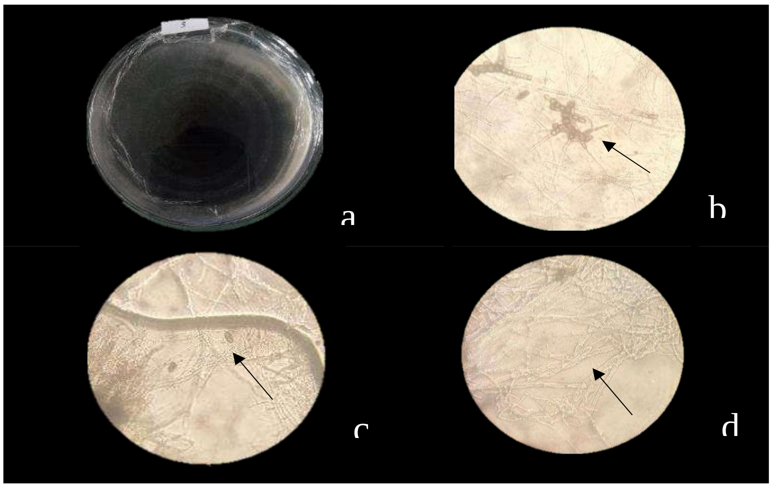

Figure 7. Isolate P5 (Alternaria sp.) at PDA median: (a) macroscofic (colony), (b) conidia, (c) conidiofor, (d) Hyphae.

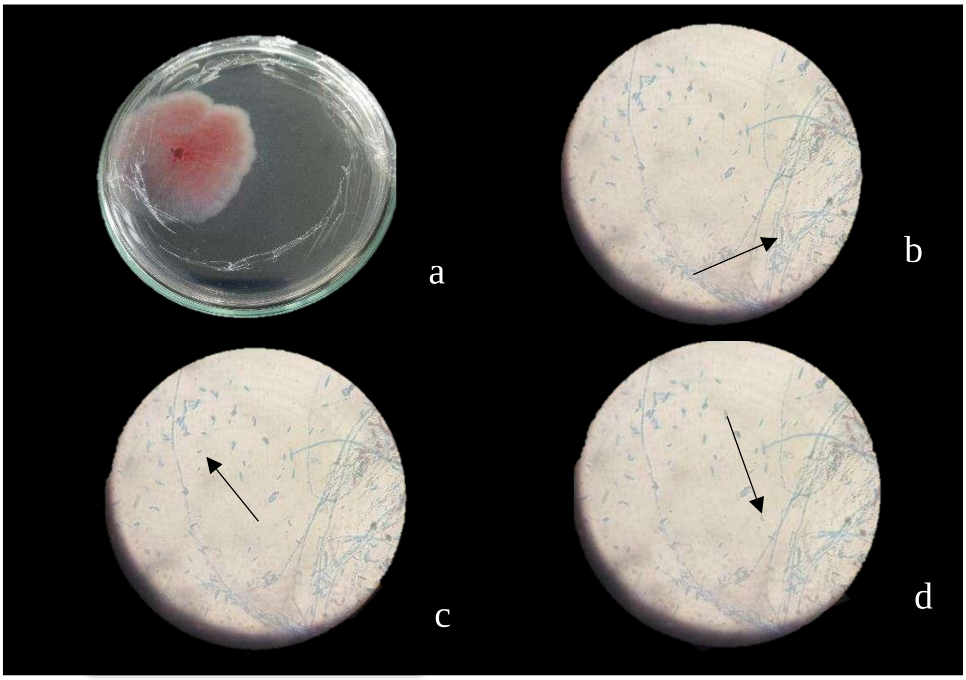

Figure 8. Isolate *F. oxysporum* at PDA media: (a) macroscofis (colony), (b) macroconidia, (c) microconidia, (d) hyphae.

The identification results showed that the colonies were pink to purple on PDA media. According to Kalman et al. (2020), changes in the color of the fungal colony *F. oxysporum* f.sp. cepae depends on the age of the culture. Young cultures have white colonies. When the culture has matured, the color is typical of each Fusarium spp. will appear and change color to purple, white, gray, or sometimes light brown.

sMicroscopically, this fungus has insulated hyphae, crescent-shaped macroconidia and oval-shaped microconidia. Fusarium spp species produce three types of vegetative (asexual) spores, namely macroconidia, microconidia and chlamydospores, the proportions of which vary between species (Kalman et al., 2020). F. oxysporum which has very many microconidium formations, is generally single-celled, oval to kidney shaped and formed in a false head. Macroconidia are very abundant, slender sickle shaped.

After identified macroscopically and microscopically, this pathogen is tested with a pathogenicity test based on Koch's postulate theory to ensure that the pathogen is the cause of fusarium wilt disease. The pathogenicity test procedure was adapted from Syukur et al. (2007). The pathogenicity test was carried out in vitro on the fruit and leaves of chili plants by injuring healthy tissue and then placing *F. oxysporum* inoculum on these parts.

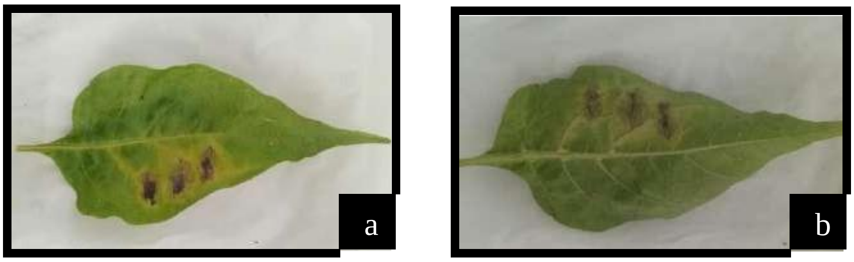

Figure 9. Pathogenesis test on chili plant leaves; a) front look, b

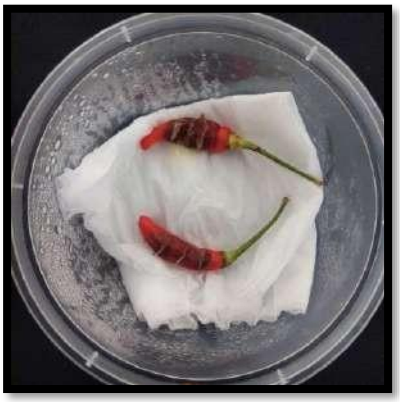

Figure 10. Pathogenesis test on chili plants.

Observations on chili plant leaves showed that the plant leaves experienced chlorosis (yellowing leaves) on the part inoculated with the *F. oxysporum* pathogen. Chlorosis is a condition of plant tissue, especially leaves, which is damaged or fails to form chlorophyll so that it is not green, but yellow or pale, almost white (Salisbury et al., 2019). Visual symptoms of Fusarium wilt on infected plants show that the lower edges of the leaves turn dark yellow, spreading rapidly to the inside so that the entire surface of the leaf turns yellow. These symptoms are caused by the pathogen *F. oxysporum* which continues to penetrate plant tissue. Observations on the fruit of the chili plant showed that the fruit of the plant experienced necrosis on the part inoculated with *F. oxysporum*. Necrosis or death of plant parts, a limited collection of cells in certain tissues die and black spots or spots are visible on the plant organs (Fahmi, 2012). Necrosis can occur on fruit, stems, roots, edges and leaves of plants. Based on the results of these observations, it can be confirmed that the pathogen *F. oxysporum* is the pathogen that causes fusarium wilt disease by causing symptoms of chlorosis on the leaves and necrosis on the fruit.

Inhibing the growth of the pathogen *F. oxysporum*

Based on the results of observations at 3, 5, and 7 days after isolation by measuring the area of pathogen colonies on PDA media in petri dishes. The results of observations and measurements show that five isolates of the hiyung chili leaf endophytic fungus have the ability to inhibit the growth of the mycelial pathogen *F. oxysporum* which can be seen in the following figure.

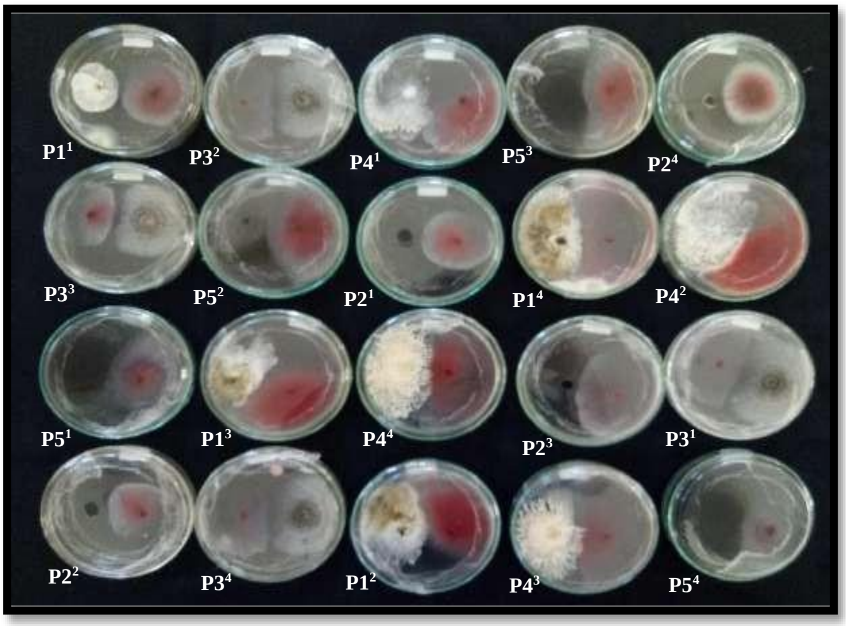

Figure 11. Antagonism test of the endophytic fungus hiyung chili leaves against the *F. Oxysporum* pathogen.

Five isolates of hiyung chili leaf endophytic fungi came from various genera, such as P1 from the genus Acremonium sp., P2 from the genus Cladosporium sp., P3 from the genus Rhizoctonia sp., P4 from the genus Sclerotium sp., and P5 from the genus Alternaria sp. Based on the results of observations on the seventh day, it showed that the highest percentage of inhibitory power came from the genus Alternaria sp. Meanwhile, the lowest comes from the genus Cladosporium sp. Fungus isolates with high inhibitory criteria are antagonistic isolates with faster colony growth compared to pathogen colonies, causing the growth of pathogen colonies to be inhibited. By having fast growth, antagonistic fungi can outperform pathogens in controlling nutrients and space so that pathogen growth is hampered (Sari & Setiawanto, 2015).

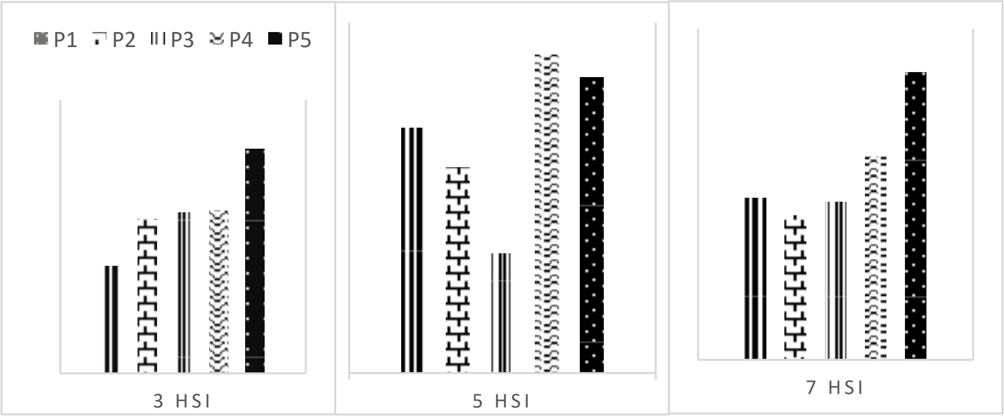

Figure 12. Percentage of inhibitory of endophytic fungi in hiyung chili leaves against *F. oxysporum* pathogen at 3, 5, 7 HIS.

The endophytic fungus P1 (Acremonium sp.) has slower colony growth than pathogenic fungi. Morphologically, the Acremonium and Fusarium species are very similar and may be confused with each other except that Fusarium usually grows faster and has colonies with a characteristic hairy appearance. Based on percentage categories, the strong inhibitory power is >40%, whereas<30% are weak, and not having the ability is 0% (Prastya et al., 2014). Therefore, it is known that the fungus Acremonium sp. has an inhibitory power of 19.57% at 7 HSI so it is included in the category of fungi with weak inhibitory power against the pathogenic fungus *F. oxysporum*.

The fungus isolate P2 (Cladosporium sp.) had slower colony growth compared to other isolates or the pathogen F. oxysporum. This fungus has a colony diameter of 3.1 cm at 7 DAP. Apart from that, this fungus is also included in the category of fungi that have weak inhibitory power because the percentage of inhibitory power is <30%, which only reaches 17.46% at 7 HSI. Several Cladosporium species are known to obtain nutrition by becoming parasites (mycoparasites) on various types of rust fungi such as Puccinia horiana, P. striiformis f. sp. tritici, P. recondita, and Cronartium quercuum (Zhan et al. 2007; Baiswar, Chandra & Kumar, 2008; Dolińska, Bartkowska & Schollenberger, 2011).

In fungal isolates P1 (Acremonium sp.) and P2 (Cladosporium sp.) it is thought that the inhibitory mechanism is carried out by means of competition or mutually intermingling growth (Skidmore & Dickinson, 1976). This is due to the slow growth of pathogen colonies towards endophytic fungus colonies. Meanwhile, in the P3 fungus isolate (Rhizoctonia sp.), colony growth was faster compared to the pathogenic fungus, namely F. oxysporum. However, this fungus isolate is included in the low inhibitory category, namely only reaching 19.05% at 7 HSI. This means that the inhibitory power at 5 and 7 HSI is not significantly different, but is significantly different at 3 HSI, namely the inhibitory power reaches 35.29%, which is in the high inhibitory category. The mechanism of inhibition of the P3 fungus isolate (Rhizoctonia sp.) which was seen from the third to the seventh day was mutually slight inhibition or an antibiosis mechanism (Skidmore & Dickinson, 1976). The antibiosis mechanism is characterized by the presence of a clear zone or zone of inhibition (clear zone) between endophytic fungi and pathogenic fungi.

In the fungus isolate P4 (Sclerotium sp.) with the pathogenic fungus *F. oxysporum*, it was seen that the fungus isolate grew faster. The percentage of inhibitory power on the third day was 35.66%, this percentage was significantly different from the fifth day, namely the inhibitory power reached 40.88%, which means that the percentage had increased by 5.22%. Meanwhile, on the seventh day, this isolate experienced a drastic decrease compared to other fungus isolates, so that the percentage of inhibitory power became 24.60%. Differences in resistance can be caused by production secondary metabolite compounds produced by endophytic fungi (Vinale et al., 2009). Apart from that, another factor is the ability to compete for nutrients, oxygen and space (Yulianto, 2014). The mechanism of inhibition of P4 fungus isolates or the genus Sclerotium sp. is an overgrowth by antagonist mechanism or a parasitism mechanism (Skidmore & Dickinson, 1976). On the seventh day, it was seen that the growth of the mycelia of the endophytic fungus grew above the mycelia of the pathogen. Endophytic fungal hyphae that grow on the surface of pathogenic hyphae include an inhibitory mechanism that utilizes niches or hyperpredation (Gao et al., 2010).

The fungus isolate P5 (Alternaria sp.) had faster colony growth compared to the pathogen *F. oxysporum*. This fungus isolate is a fungus that has the highest inhibitory power compared to other isolates, reaching 34.72% on the seventh day. Fungal isolates that have high inhibitory power are antagonist isolates that have faster colony growth compared to pathogen colonies and the development of antagonist colonies can also suppress the development of pathogen colonies. The mechanism of inhibition of P5 fungus isolates from the genus Alternaria sp. is a mechanism of antibiosis or mutually slight inhibition (Skidmore &

Dickinson, 1976). This antibiosis mechanism process occurs when there is sufficient nutrition for the antagonist, which is indicated by the separation of the inhibition zone, namely visible clear boundaries that do not mix between endophytic fungi and pathogens. The factor for the formation of this clear zone is due to the production of secondary metabolites from endophytic fungi and the presence of concentrations of antibiotic compounds (Zuhria et al., 2017).

Conclusion

Hiyung chili leaf endophytic fungi can inhibit the growth of *F. oxysporum* by mutually intermingling growth inhibition mechanism or competition mechanism by isolate P1 (Acremonium sp.) and isolate P2 (Cladosporium sp.), mutually slight inhibition or antibiosis mechanism by isolate P3 (Rhizoctonia sp.) and isolate P5 (Alternaria sp.), while overgrowth by antagonism or parasitism mechanism by isolate P4 (Sclerotium sp.). The best hiyung chili leaf endophytic fungus in inhibiting the growth of *F. oxysporum* is isolate P5 (Alternaria sp.) with the highest inhibitory power among other isolates, reaching 34.72%. Apart from that, this fungus is an isolate of an endophytic fungus which has the highest colony diameter reaching 8.4 cm on the seventh day.

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