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The inhibition of Fusarium wilt in Chili by Endophytic Fungi isolated from Green Betel (*Piper betle* L.) Leaf

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Abstract

Green Betel (*Piper betle* L.) leaves are often used as medicine by local people because they contain antibacterial and antifungal substances. These substances can be produced by plant metabolites and are also the results of metabolites produced by endophytic fungi. Endophytic fungus is an organism that is associated with healthy host tissues without causing disease symptoms. Endophytic fungi from betel leaf are used as biocontrol agents against *Fusarium oxysporum* causes wilt disease that attacks Chili (*Capsicum annuum* L.), which is one of the main agricultural commodities in Indonesia. Fusarium wilt disease is a disease that significantly reduces crop yields based on crop yields. This research was conducted in a chili farm in Cangkringan, Sleman, Yogyakarta. This study aimed to test the antagonism of endophytic fungi with the dual culture technique. Three types of green betel leaf endophytic fungi were used for dual culture test against *Fusarium oxysporum*. The observation result showed the highest inhibition ability of 73.37% dual culture method. The inhibitory mechanisms of green betel leaf endophytic fungal isolates include parasitism, antibiosis, and competition. Endophytic fungi of green betel leaf are effective as biocontrol agents of wilt *Fusarium chili* disease.

Keywords: Biocontrol; *Capsicum annuum* L; *Fusarium oxysporum*; parasitism; antibiosis; competition

1. Introduction

Chili (*C. annuum* L.) is a horticultural commodity with crucial economic value in Indonesia. Chili contains nutrients needed for human health, such as protein, fat, calcium, phosphorus, iron, vitamins, and alkaloid compounds such as capsaicin and flavonoids. The use of superior seeds is a condition for obtaining economically profitable crop production. However, chili cultivation has various problems, one of the diseases that often attacks chilies is Fusarium wilt. The fungus *Fusarium oxysporum* can attack chili plants from germination to maturity. Fusarium wilt can attack 30-40% of an area and cause crop failure [1].

Control of Fusarium wilt in chili plants is often done with a synthetic fungicide because it is considered more effective. Excessive use of synthetic pesticides is detrimental to humans and agro-ecosystems, so an alternative that is more environmentally friendly and effective to stop Fusarium wilt is sought. One of the biological control of plant diseases is the use of non-pathogenic endophytic fungi biological agents. Endophytic fungi are fungi that live and infect plant tissues without causing disease symptoms[2].

Previous research showed that green betel leaf extract was able to inhibit the growth of the fungus *F. oxyporum* with an inhibition of 76.11% [3]. Nevertheless, studies on the endophytic fungi from green betel leaf against the pathogen *F. oxyporum* in vitro have never been carried out. The aims of this study were to determine the effectiveness of the endophytic fungus in betel leaf greenery which has the most potential to inhibit the fungus that causes Fusarium wilt and to determine the mechanism of inhibition against the cause of Fusarium wilt in chili (*C. annuum* L.) plants by measuring peroxidase activity.

Endophytic fungi are fungi that live in plant tissues without causing disease symptoms in their host plants. Endophytic fungi are able to produce bioactive compounds such as antibacterial and antifungal compounds [4]. Endophytic fungi that grow on medicinal plant tissues can also produce compounds that have the same properties as their host plants [5]. Compounds produced by endophytic fungi often have greater activity than the activity of compounds from their host plants [6]. Mechanisms for controlling pathogens using biological control agents include [5].

1. Mycoparasitism

The interaction of plant pathogenic fungal hyphae and antagonists can be in the form of entanglement of antagonistic hyphae around the pathogenic fungal hyphae. This antagonistic hyphae entanglement occurs due to the contact stimulus required for entanglement. After the entanglement of the hyphae occurs, it will usually be followed by the lysis of the pathogenic hyphae. Based on research in *F. sambucinum*, it optimizes the thickening of the cell wall so that it becomes more compact. Thick hyphae cells are a defense when facing *T. harzianum,* so it takes longer to kill the host during mycoparasitism[2], [3].

2. Competition

Nutrient competition occurs when biocontrol agents reduce the availability of certain substances, thereby limiting the growth of pathogens. Competition between biological control agents and pathogens leads to biological control in addition to nutrition as well as space competition [4]. Based on research on the results of antagonism tests, the hyphae of pathogens *F. oxysporum* and *A. solani* became curled. This is due to the faster growth of endophytic fungi so that they can provide changes to pathogens to inhibit their growth or produce antibiotic compounds that can inhibit the growth of pathogens [7].

3. Antibiosis

Fungi produce alkaloids and mycotoxins that can increase plant resistance to disease. Endophytic fungi produce antibiotic compounds that can damage and inhibit the growth of pathogens. Symptoms of antibiosis are characterized by the presence of empty zones between the pathogenic fungi and antagonist fungi and changes in the shape of the pathogenic hyphae due to the production of compounds

with antibiotic properties. In certain cases, the antibiosis compounds are volatile, which evaporates and inhibits the growth of the pathogen.

The parts of the betel plant (*P. betle* L.), such as roots, stems, and leaves, have the potential for treatment. The active components of betel leaf are affected by age, type of leaf, and sunlight. Betel leaf contains essential oils consisting of phenolic compounds and some of their derivatives, eugenol, and chavicol. Phenol compounds and their derivatives can denature bacterial cell proteins. The eugenol compound is bactericidal by increasing the permeability of the bacterial membrane. Apart from giving a distinctive odor to betel nut, the chavicol compound also has five times the bactericidal properties of other phenolic compounds. Various studies have proven that green betel leaf extract (*P. betle* L.) has antibacterial activity [8].

2. Experiments Procedure

Isolation of Green Betel Leaf Endophytic Fungi and Fusarium oxysporum

Chili plants were sampled from farmers' rice fields in 3 different places, namely Wukirsari, Cangkringan, and Pakembinangun, Pakem, Sleman Regency, Yogyakarta. The selection of chili plants attacked by fusarium wilt has the characteristics of a yellow-green leaf with leaf strands still attached to the stems or has experienced leaf loss, the base of the stem is brownish, and there are deep lesions. Chili plants affected by wilt disease are removed from the ground, cut horizontally, and split into two parts at the base of the stem to observe visible symptoms. The prepared stems of chili plants were then cultured on a PDA growth medium to isolate *Fusarium* sp.

After the fungi grew, a subculture was then carried out to obtain pure isolates of *Fusarium* sp. Fungi were sampled from PDA media and then transferred to new PDA media. Efforts were made to take samples with different morphological characteristics to obtain species diversity for screening *Fusarium* sp isolates [9].

Isolation of Endophytic Fungi from Green Betel Plants

Samples of betel plants were obtained from Pakem, Sleman Regency, Special Region of Yogyakarta. The isolation stage of endophytic fungi on betel leaf followed the isolation method on chili stems. The selected leaf are healthy, have no disease spots, are not too young, and are still fresh green in color. The leaf samples were then isolated with a PDA medium, and then colonies of endophytic fungi were obtained, which were then subcultured to obtain pure cultures. After isolating betel leaf on a PDA medium, the isolate purification process was then carried out to obtain a single colony of endophytic fungi. Purification was performed by selecting endophytic fungi in a petri dish with different morphological types; then, they were sampled and transferred to a new petri dish containing a PDA medium [10].

Endophytic Fungi Antagonist Test Against Pathogenic Fungi In Vitro

The antagonist test was carried out based on the dual culture method with modification [2], namely pieces of mycelium isolated from the fungus *Fusarium* spp. with a size of 0.5 x 0.5 cm2 and pieces of mycelium isolated from endophytic fungi from betel leaf green with a size of 0.5 x 0.5 cm2 were placed on PDA media in a petri dish with a diameter of 9 cm. The distance between the two isolates is 5 cm. Each treatment has three replications. As a control, pieces of mycelium isolated from pure endophytic fungi were grown on PDA media in Petri dishes. According to Figure 1, the mycelium area of endophytic fungi was observed from day 0 to day 10 when the control isolates had reached the edge of the petri dish. The following is a schematic picture of the dual culture [11].

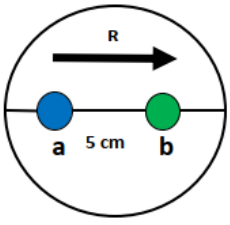

Figure 1. (a) Pathogenic isolate; (b) Endophytic Fungi

Calculating inhibition:

$$\% \text{ inhibition} = \frac{(R1-R2)}{R1} \text{ X } 100\%$$

Notes:
R1 = the distance of the growth of pathogenic fungal colonies close to the edge of the Petri dish
R2 = the growth distance of pathogenic fungi approaching endophytic fungi

3. Result and Discussion

Isolation of endophytic fungi from betel leaf and Fusarium oxysporum

Chili stems with symptoms of brown lesions at the base of the stems were collected from chili farming centers in 4 different agricultural fields in Cangkringan, Sleman, and Yogyakarta. The base of the stem of diseased plants was cut with a size of 0.5 x 0.5 cm2, dipped in 1% sodium hypochlorite for 1 minute, then dipped in 70% ethanol for 1 minute, then rinsed with sterile distilled water three times, with the aim of growing pathogens that cause disease that in diseased plant tissues [12]. Stem cuttings were grown on PDA medium (39 g/L), then incubated at room temperature for seven days. Each hypha of different fungi was separated and grown on a new slanting PDA medium for the purification of the fungal species. Endophytic fungi on each petri dish are coded. The last rinse of distilled water was used as a control by streaking it on a petri dish containing PDA [12].

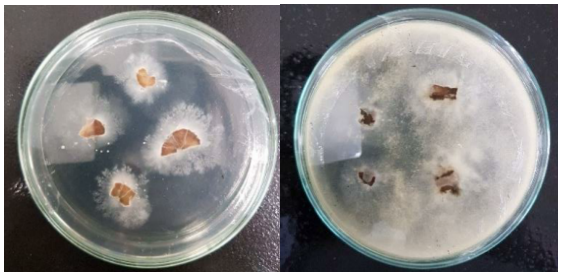

Figure 2. Colony morphology isolated from the stem base of chili infected with wilt

Macroscopic identification was observed based on the physical characteristics of the colonies seen at the age of 7 days isolated [13]. By Figure 2, the Hypha microscopic observation shows the characteristics possessed by *Fusarium* sp. With the shape of Crescent-like macroconidia [14]. Characteristics of the colonies, like the upper surface of the colony is white, the brownish surface underside of the colonies, no aerial hyphae, margins spread, macroconidia absent, microconidia: 7.69-12 µm and no false heads [15]. Figure 3 is one of the microscopic identification photos showing the presence of macroconidia, a characteristic marker of the fungi *Fusarium* sp [16]. because it has a spiky and curved structure like the moon sickles and scattered microconidia are small, consisting of a single cell [17].

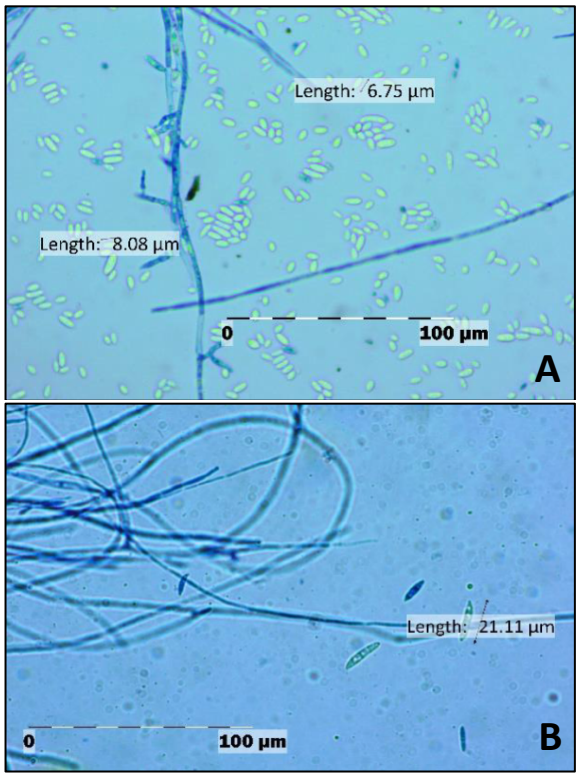

Figure 3. Photo of microscopic preparation of *Fusarium* sp (A; Isolate FU3 (*Fusarium* sp.) showed the presence of crescent-shaped macroconidia, B Isolate FU3 (*Fusarium* sp.) shows microconidia at 400X magnification)

Isolation of endophytic fungi from betel leaf

Green betel leaf samples were isolated with a PDA medium, and then colonies of endophytic fungi were obtained, which were then subcultured to obtain pure cultures [18]. The following is a picture of the betel leaf isolation results [19].

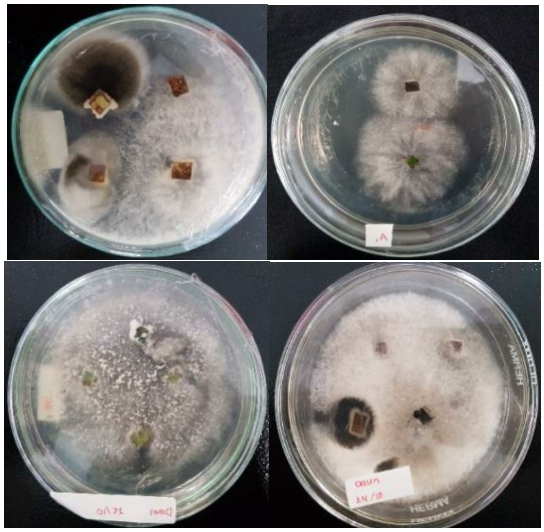

Figure 4. Fungi isolated from betel leaf on PDA medium showing different colony forms (A: Black and white colonies of aerial hyphae, B: White colonies like aerial hyphae threads, C: Gray colonies without aerial hyphae, D: Colonies that are colored thick white with the very high growth rate).

Endophytic Fungi Antagonist Test Against Pathogenic Fungi In Vitro

An antagonist test in vitro was conducted to determine the antagonistic activity of selected betel endophytic fungi against Fusarium pathogenic fungi. The antagonistic in vitro test was observed on the 10th day when the pathogen control hyphae had reached the edge of the petri dish [20]. The fungi used for the antagonist test were fungi that had passed the pathogenicity test, namely endophytic fungi with codes ES3, ES7, ES9, ES23, ES26, ES27, and ES28 [21]. The following is a Table of the percentage of inhibition of betel endophytic fungi against Fusarium pathogenic fungi [22][9].

Table 1. Percentage of Antagonism Test (Dual Culture)

No	Treatment	r	Radius days – (Cm)			Inhibition (%)	mean (%)
			1	5	10		
1		1	0,6	3	5,6	-	
2	ES0 FU3	2	0,6	3	5,7	-	
3		3	0,7	3	5,6	-	
4		1	0,6	1,2	1,5	73,2	72,2
5	ES3 FU 3	2	0,7	1,4	1,6	71,9	
6		3	0,6	1,3	1,6	71,4	
7		1	0,6	2,5	3,2	42,9	43,8
8	ES7 FU3	2	0,7	2,5	3,2	43,9	
9		3	0,6	2	3,1	44,6	
10		1	0,6	1,6	2	64,3	65,1
11	ES9 FU3	2	0,6	1,5	2	64,9	
12		3	0,6	1,5	1,9	66,1	
13		1	0,9	2,5	3,1	44,6	45,0
14	ES23 FU3	2	0,8	2,6	3,2	43,9	
15		3	0,7	2,5	3	46,4	
16		1	0,6	2,3	2,9	48,2	46,2
17	ES26 FU3	2	0,6	1,7	3,1	45,6	
18		3	0,7	2,5	3,1	44,6	
19		1	0,6	1,4	1,5	73,2	73,4
20	ES27 FU 3	2	0,7	1,5	1,5	73,7	
21		3	0,6	1,5	1,5	73,2	
22		1	0,6	1,8	2,5	55,4	53,9
23	ES28 FU3	2	0,7	2	2,7	52,6	
24		3	0,6	2	2,6	53,6	

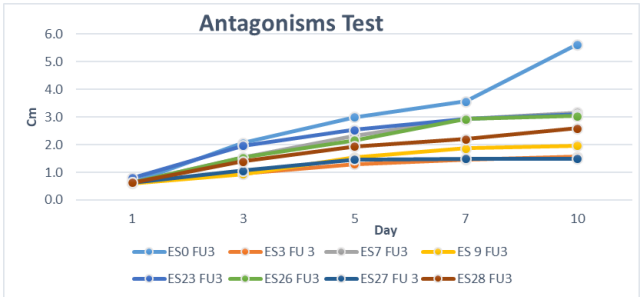

Figure 5. Colony Diameter of Fusarium Fungi for 10 Days on PDA Media

Table 1 shows that the diameter of the control pathogen is larger than the diameter of

the pathogen in the dual culture, due to competition for space and nutrients. Endophytic fungi grow faster than pathogenic fungi, thus enabling these endophytic fungi to obtain nutrients faster and more than pathogenic fungi. In addition to faster growth factors, it can also be influenced by substances resulting from secondary metabolites produced by endophytic fungi, which are capable of inhibiting the growth of pathogens [8][15].

In the dual culture test, the endophytic fungi isolates had the highest to lowest percentage of pathogen inhibition, namely ES27 of 73.4%, ES3 of 72.2%, ES9 of 65.1%, ES28 of 53.9%, ES26 of 46, 2%, ES23 45% and ES7 43.8%. The highest to lowest values are based on data on the average percentage of inhibition that has been statistically tested with the SPSS program. From Table 2, the ANOVA test results showed a significance <0.05 so that it could be stated that the inhibition of pathogens was significantly different from one another. The following sample Table is below.

Table 2. Oneway Anova test results of inhibition measurement (dual culture)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3031.278	6	505.213	366.602	.000
Within Groups	19.293	14	1.378		
Total	3050.571	20			

Table 1 shows that the diameter of the control pathogen is larger than the diameter of the pathogen in the dual culture, due to competition for space and nutrients. Endophytic fungi grow faster than pathogenic fungi, thus enabling these endophytic fungi to obtain nutrients faster and more than pathogenic fungi. In addition to faster growth factors, it can also be influenced by substances resulting from secondary metabolites produced by endophytic fungi, which are capable of inhibiting the growth of pathogens [17][23].

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According to research by Nazia Manzar et al. 2022, mycoparasitism can play a role in antagonism interactions by producing cell wall degrading enzymes to attack fungi specifically. Differences in growth rates cause inhibition due to competition for nutrients and space [3]. The highest to lowest values are based on data on the average percentage of inhibition that has been statistically tested with the SPSS program.

The results of the ANOVA test showed a significance <0.05 so that it could be stated that the inhibition of *Fusarium oxyporum* was significantly different from one another; however it turned out that isolate E3 and isolate E27 had the same level of significance, so they were not significantly different [25].

In general, the ability of endophytic fungi to suppress soil-borne pathogens usually involves one or several inhibiting mechanisms. The mechanism of antagonistic microbial inhibition against pathogens includes producing antibiotics, toxins, competition for space and nutrients, producing siderophores, and HCN.

The following are the results of the antagonism test

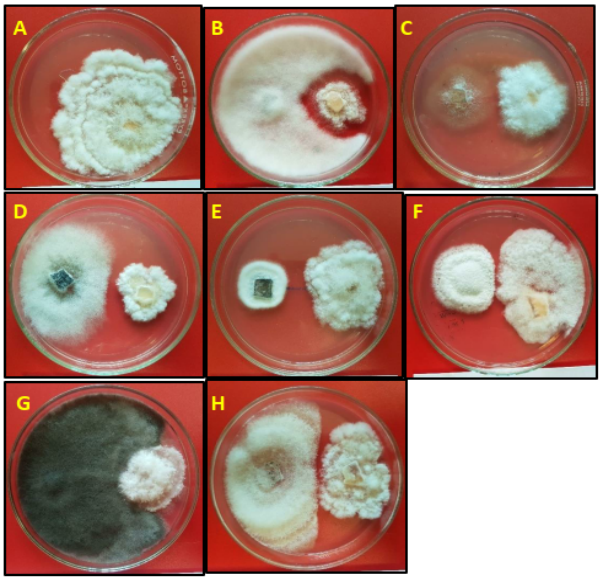

Figure 6. Results of the antagonism test (dual culture) on day 10 A; Control/FU3 (*Fusarium oxyporum*), B; ES3FU3, C; ES7FU3, D; ES9FU3, E; ES23FU3, F; ES26FU3, G; ES27FU3 And H; ES28FU3. The right side is *Fusarium oxyporum* isolate, while the left side is betel endophytic isolate

The results of macroscopic observations showed that almost all endophytic fungi green betel leaf isolates had a competition mechanism, except for isolates ES7, isolate ES23, isolate ES26 and isolate ES28 which had a wider colony diameter of *Fusarium oxyporum* than the colonies of endophytic isolates. So it can be said that this type of endophytic fungus has no potential to be used as a biocontrol agent because of its ability to not suppress the growth of the fungus *Fusarium oxyporum*. The inhibition evaluated has one or more inhibition mechanisms, namely competition, antibiosis, and parasitism. The form of microscopic interaction of endophytes and *F. oxysporum* can be seen microscopically in the following isolate preparations:

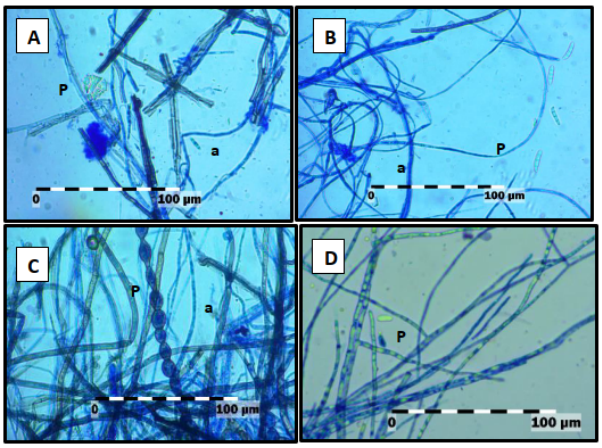

Figure 7. Microscopic Forms of Interaction of Endophytes and *F. oxysporum*, A; hyphae *Fusarium oxyporum* in the inhibition zone were damaged, broken, and more transparent in color, interaction with isolate ES3, B; hyphae *Fusarium oxyporum* experienced inhibition so that their sizes were smaller, found in the interaction of isolates ES9, C; Hyphae of isolate *Fusarium oxyporum* are wrapped around endophytic hyphae so that they form self-defense structures in the form of chlamydospores found in endophytic interactions E27, D; FU isolates, *Fusarium oxyporum* without treatment (400x).

Based on observations of dual culture interactions through preparations, it can be seen that *Fusarium oxyporum* isolates grown with ES3 endophytes caused growth disturbances in *Fusarium oxyporum* hyphae, the results of this study are in line with research by Hilda Karim et al. 2018 which was indicated by the presence of an inhibition zone as seen from the confluence of the two colonies in the dual test culture and *Fusarium oxyporum* hyphae experienced lysis which was marked by structural damage such as fractures, and the color became translucent. ES3 isolates have a mechanism of antibiosis inhibition; this is because endophytic fungi produce antibiotic compounds that can damage and inhibit the growth of *Fusarium oxyporum* [19].

Dual culture and *Fusarium oxyporum* hyphae experienced lysis which was marked by structural damage such as fractures, and the color became translucent. ES3 isolates have a mechanism of antibiosis inhibition; this is because endophytic fungi produce antibiotic compounds that can damage and inhibit the growth of *Fusarium oxyporum*. Previous studies

reported that endophytic fungi produce alkaloids and mycotoxins, making it possible to use them to increase plant resistance to disease, such as a pathogen that causes Fusarium wilt. The characteristics found in ES3 preparations match the characteristics of the antibiosis mechanism described by), which states that the type of antibiosis inhibition is characterized by the presence of an inhibition zone and lysis of the pathogenic hyphae of *Fusarium oxyporum* stated that the interaction of pathogenic hyphae and antagonists was marked by the change in color of the pathogenic hyphae to become clear and empty because the contents of the cells were utilized by biocontrol agents as nutrients [4][26].

The mechanism of ES9 endophytic isolates has an inhibition mechanism in the form of competition which is characterized by the shrinking of *Fusarium oxyporum* hypha due to encounters with endophytic fungi. The meeting can cause the hyphae to shrink because the nutrients obtained by *Fusarium oxyporum* are reduced or also because the ES9 fungus produces a compound that suppresses the growth of *Fusarium oxyporum* so that the growth of the hyphae is disrupted [23].

The endophytic isolate ES27 has the ability to compete and parasitize with the characteristics of a faster growth rate to fight for space and nutrients as well as the ability to parasitize, which is characterized by antagonistic fungal hyphae to *Fusarium oxyporum* hyphae which causes the hyphae to not develop so that the growth of *Fusarium oxyporum* stops. This inhibition causes a defensive reaction from the *Fusarium oxyporum* fungus in the form of enlarged hyphae cells as a form of self-defense from the hyphae coils of endophytic fungi [17].

In Figure 7, the hyphae of *Fusarium oxyporum* are malformed so that they enlarge to form a bubble-like structure. This is in accordance with the results of Indratmi's research (2008) in Amalia (2014), which states that hyphae damage is a form of interaction, namely in the form of changes in the shape/malformation of *Fusarium oxyporum* hyphae. The hyphae become spiral-shaped and curved irregularly and experience shortening. FU3 isolate is an isolate of *Fusarium oxyporum* without any other fungi or control [7]. Based on the mechanism of mycoparasitism between endophytic fungi and pathogenic fungi, it can be divided into three phases, namely: antagonistic interaction before each hyphae meet directly followed by the chemical attraction between the two organisms and then parasitism [13].

4. Conclusion

The observation result showed the highest inhibition ability of 73.37% dual culture. The inhibitory mechanisms of green leaf endophytic fungal isolate include parasitism, antibiosis, and competition. Endophytic fungi of green betel leaf are effective as biocontrol agents of wilt Fusarium chili disease. Green betel leaf endophytic fungi have the potential to inhibit the Fusarium wilt of chili plants.

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References

[1] A. P. Siti Ferniah, Budi Setiyadi Daryono, Rina Sri Kasiamdari, "Characterization and Pathogenicity of Fusarium oxysporum as the Causal Agent of Fusarium Wilt in Chili (*Capsicum annuum* L.)," *Microbiol. Indones.*, vol. 8, no. 3, pp. 121–126, 2014, doi: 10.5454/mi.8.3.5.

[2] Mukarlina, S. Khotimah, and R. Rianti, "Uji antagonis *Trichoderma harzianum* terhadap *Fusarium* spp. penyebab penyakit layu pada tanaman cabai (*Capsicum annum*) secara in vitro," *J. Fitomedika*, vol. 7, no. 2, pp. 80–85, 2010.

[3] N. Manzar *et al.*, "*Trichoderma*: Advent of Versatile Biocontrol Agent, Its Secrets and Insights into Mechanism of Biocontrol Potential," *Sustain.*, vol. 14, no. 19, pp. 1–32, 2022, doi: 10.3390/su141912786.

[4] C.-T. Lo, "Possible inhibition-processes of biocontrol agents to plant pathogens," *Plant Pathol. Bull.*, vol. 7, no. vi, pp. 155–

166, 1998, [Online]. Available: http://140.112.183.1/cpps/pdf/07-4/7-4-1.pdf

[5] A. Sehrawat and S. S. Sindhu, "Potential of biocontrol agents in plant disease control for improving food safety," *Def. Life Sci. J.*, vol. 4, no. 4, pp. 220–225, 2019, doi: 10.14429/dlsj.4.14966.

[6] M. A. C. Latz, B. Jensen, D. B. Collinge, and H. J. L. Jørgensen, "Endophytic fungi as biocontrol agents: elucidating mechanisms in disease suppression," *Plant Ecol. Divers.*, vol. 11, no. 5–6, pp. 555–567, 2018, doi: 10.1080/17550874.2018.1534146.

[7] A. T. Kurnia, M. I. Pinem, and S. Oemry, "102117-ID-penggunaan-jamur-endofit-untuk-mengendal," vol. 2, no. 2337, pp. 1596–1605, 2014.

[8] S. Fuadi, Efektivitas Ekstrak Daun Sirih Hijau (*Piper betle* L .) Terhadap Pertumbuhan Bakteri *Streptococcus pyogenes* In Vitro. 2014.

[9] U. Hasanah and dan Idramsa, "uji antijamur patogen ekstrak metabolit sekunder jamur endofit tumbuhan raru (*Cotylelobium melanoxylon*) test antifungal phatogen extract secondary metabolites of endophytic fungi raru plant (*Cotylelobium melanoxylon*)," *J. Biosains*, vol. 1, no. 2, 2015.

[10] M. A. Pandit *et al.*, "Major Biological Control Strategies for Plant Pathogens," *Pathogens*, vol. 11, no. 2, pp. 1–21, 2022, doi: 10.3390/pathogens11020273.

[11] I. Manurung, M. Pinem, and L. Lubis, "Uji Antagonisme Jamur Endofit Terhadap *Cercospora Oryzae Miyake* Dan *Culvularia Lunata* (Wakk) Boed. dari Tanaman Padi Di Laboratorium," *J. Agroekoteknologi Univ. Sumatera Utara*, vol. 2, no. 4, p. 101992, 2014.

[12] M. F. Imron, S. B. Kurniawan, and H. S. Titah, "Potential of bacteria isolated from diesel-contaminated seawater in diesel biodegradation," *Environ. Technol. Innov.*, vol. 14, p. 100368, 2019, doi: 10.1016/j.eti.2019.100368.

[13] P. L. Kunth, L. P. Rosa, and D. Wahyuni, "Isolasi dan Identifikasi Fungi Endofit Tanaman Suruhan (*Peperomia pellucida* L . Kunth) Isolation and Identification of Endophytic Fungi of Suruhan Plant Abstrak," *Bioma*, vol. 22, no. 1, pp. 26-45 p, 2020.

[14] A. Robles-Carrión, M. Leiva-Mora, L. Herrera-Isla, A. Sánchez-Rodríguez, and R. Torres-Gutiérrez, "Morphological and molecular identification of Fusarium species associated with vascular wilt of babaco (*Vasconcellea heilbornii* var. *pentagona Badillo*)," *Rev. Protección Veg.*, vol. 31, no. 3, pp. 184–193, 2016.

[15] G. K. Frimpong, A. A. Adekunle, O. T. Ogundipe, M. K. Solanki, S. Sadhasivam, and E. Sionov, "Identification and toxigenic potential of fungi isolated from capsicum peppers," *Microorganisms*, vol. 7, no. 9, pp. 1–10, 2019, doi: 10.3390/microorganisms7090303.

[16] R. Wikandari *et al.*, "Assessment of microbiological quality and mycotoxin in dried chili by morphological identification, molecular detection, and chromatography analysis," *Int. J. Environ. Res. Public Health*, vol. 17, no. 6, 2020, doi: 10.3390/ijerph17061847.

[17] A. V. Simamora, M. V. Hahuly, and J. B. Henuk, "Endophytic fungi as potential biocontrol agents of Phytophthora palmivora in the cocoa plant," *Biodiversitas*, vol. 22, no. 5, pp. 2601–2609, 2021, doi: 10.13057/biodiv/d220519.

[18] V. García-Gaytán, F. C. Gómez-Merino, L. I. Trejo-Téllez, G. A. Baca-Castillo, and S. García-Morales, "The Chilhuacle Chili (*Capsicum annuum* L.) in Mexico: Description of the Variety, Its Cultivation, and Uses," *Int. J. Agron.*, vol. 2017, 2017, doi: 10.1155/2017/5641680.

[19] H. Karim, L. Hamka, N. Kurnia, and M. Junda, "Effectivity of Anatagonistic Bacteria in Controlling of Fusarium Wilt Diseases of Banana (*Musa paradisiaca*) by in Vitro," *J. Phys. Conf. Ser.*, vol. 1028, no. 1, 2018, doi: 10.1088/1742-6596/1028/1/012014.

[20] T. J. An *et al.*, "Prevalence, Characterization, and Mycotoxin

Production Ability of Fusarium Species on Korean Adlay (*Coix lacrymal-jobi* L.) Seeds," *Toxins (Basel).*, vol. 8, no. 11, pp. 1–11, 2016, doi: 10.3390/toxins8110310.

[21] M. Joshi, R. Srivastava, A. K. Sharma, and A. Prakash, "for its pathogenic and non-pathogenic nature in tomato (*Solanum lycopersicum*)," *J. Appl. Nat. Sci.*, vol. 5, no. 1, pp. 108–117, 2013.

[22] B. a Summerell, R. Botanic, G. Sydney, N. S. Wales, and J. F. Leslie, "to Fusarium Identification," *Plant Dis.*, no. February, pp. 117–128, 2003.

[23] P. Chaisemsaeng, W. Mongkolthanaruk, and W. Bunyatratchata, "Screening and Potential for Biological Control of Anthracnose Disease (*Colletotrichum capsici*) on Chili Fruits by Yeast Isolates," *J. Life Sci. Technol.*, vol. 1, no. 4, pp. 201–204, 2013, doi: 10.12720/jolst.1.4.201-204.

[24] A. Jacobs, R. Govender, and S. W. Van Heerden, "*Fusarium oxysporum* f. sp. *lycopersici* race 3 causing tomato wilt in South Africa," *Australas. Plant Dis. Notes*, vol. 8, no. 1, pp. 145–147, 2013, doi: 10.1007/s13314-013-0118-6.

[25] S. E. Nurzannah, L. Dan, and D. Bakti, "Potency of Endophytic Fungi from Chilli as Biocontrol Agents to Control Fusarium Wilt (*Fusarium oxysporum*) on Chilli and Their Interaction," *J. Online Agroekoteknologi*, vol. 2, no. 3, pp. 1230–1238, 2014.

[26] N. Sari, "Review of Endophytic Fungi as Biocontrol Agents Against Plant Pathogen," *Gontor AGROTECH Sci. J.*, vol. 6, no. 1, p. 55, 2020, doi: 10.21111/agrotech.v6i1.3734.