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Mode of Actions and Pathogenicity of 11 Endophytic Fungi on *Fusarium oxysporum*

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ABSTRACT: *Fusarium oxysporum* is the pathogenic cause of the disease on chili plants that can reduce production in the cultivation process that needs to be done to control an environmentally friendly manner using endophytic fungi. This study aims to test the inhibition, the mechanism of antagonism, and pathogenicity of eleven endophytic fungi origin of pepper plants Bengkulu province against the pathogen *Fusarium oxysporum* in *vitro.* Research conducted in the Laboratory of Plant Protection, Faculty of Agriculture, University of Bengkulu from December 2020 to February 2021. The study consists of: the rejuvenation of the fungi isolates of endophytic and fungi pathogens *Fusarium oxysporum*, dual culture test, the mechanism of antagonism test, as well as pathogenesis endophytic fungi test. Endophytic fungi isolate obtained from the collection of the laboratory of Plant Protection, University of Bengkulu. The results of the research show from eleven endophytic fungi only 1 endophytic fungi which has a percentage of inhibition of 50% of the isolates of *Rhizoctonia* sp.2. The mechanism of antagonism occurs consisting of the competition between the space of nutrients and oxygen, hiperparasitisme, and antibiosis. patogenensitas test show only isolates of *Rhizoctonia* sp.2 that do not produce patches on the chili seeds.

Keywords: Endophytic fungi, mechanism of antagonism, pathogenicity.

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INTRODUCTION

Fusarium oxysporum is a fungi pathogen that causes fusarium wilt. Fusarium wilt is one of the obstacles in the process of cultivation in agriculture, especially in chili plants. In the process of cultivation, the plant is often impaired due to various diseases of plants causing the decrease of production results even death of the plant (Soesanto, 2019). However, at this time a lot of technology has been developed to control pests and plant diseases. Control efforts are performed with a variety of environmentally friendly technologies. One of them is the use of microorganisms endophytic as agents antagonistic to biologically control.

Endophytic fungi are fungi that reside or live in the recesses of the plant can give a positive response to the plant so that the plant to be healthy and protected from the pathogen. According to Harman (2011), endophytic fungi can help plants avoid the attack of pathogens, stress abiotic because of endophytic within the plant, colonize plant tissues, and help the plant in the process of growth to be better.

In the process of inhibition against pathogens, endophytic fungi have a mechanism of inhibition. Hallmann and Berg (2006) mention mechanism inhibition of endophytic fungi on pathogens are distinguished into two types, namely the mechanism of the direct and the mechanism is not direct. Mechanism

directly from fungi endophytic i.e., antibiosis, competition, and lysis. While mekanisme is not immediate is to induce plant resistance and increase growth for the plant. Bengkulu is an area with rich biodiversity. Previously, exploration and identification of endophytic fungi on chili plants have been done in Pekik Nyaring, BengkulHowever it is not known how the traffic endophytic fungi isolate against the pathogen *Fusarium oxysporum*. Therefore it is necessary to do further testing of the inhibition, the mechanism of antagonism, and pathogenesis of each isolate. Therefore study aims to test the inhibition, the mechanism of antagonism, and the pathogenicity of origin of chili plants Bengkulu province against the pathogen *Fusarium oxysporum* in *vitro.*

MATERIALS AND METHODS

The research was conducted in the Laboratory of Plant Protection, Faculty of Agriculture, the University of Bengkulu from December 2020 to February 2021. As for the stages of the research carried out beginning with the rejuvenation of 11 fungi endophytic collection of the laboratory of Plant Protection, University of Bengkulu on the media Potato Dextrose Agar (PDA). Endophyticfungi is the result of the exploration of the berof origin of chili plant in the village of Pekik Nyaring, Kelurahan Pondok Kelapa, Kabupaten Bengkulu Tengah.

Further, test the culture of a dual culture test on the media PDA. Endophytic fungi isolated and pathogenic *Fusarium oxysporum* took using a cork borer of diameter 7 mm, and then inoculated into Petri dishes containing media PDA is dealing with a distance of 30 mm. After that, the whole petri dish was incubated at room temperature. The observed variable is the inhibiting zone (%) calculated on the 8th day after incubation with the formula:

$$\text{Inhibiting zone (\%)} = \frac{R1 - R2}{R1} \times 100\%$$

Description: R1 = radius of the colony of the pathogen away from the colonies of endophytic fungi

R2 = radius of the colony of the pathogen approaching a colony of endophytic fungi

Further testing of the mechanism of antagonism based on the results of the dual culture test the mechanism of antagonism of each endophytic fungi. The mechanism of antagonism was identified which includes: competition between the space of nutrients and oxygen, antibiosis, lysis, and parasitism.

The last pathogenicity test eleven fungi isolates of endophytic on seedlings of chili plants by the method of bioassay. Patogenensitas test carried out on the seeds of chili peppers at the age of 7 days after planting (HST). The test is done with the use of 3 seedlings of chili plants healthy in a petri dish diameter 14 cm which has been given the filter paper moist before. Then the stem of the plant chili was wounded using a sterile needle and given the endophytic fungi taken using a cork borer from the culture medium. Do the same thing on the entire boletus endophytic and also the pathogen *Fusarium oxysporum*. Hereinafter all of the petri dish is incubated at a temperature of 30˚C for 7 days. The variables observed that the symptoms of which appear in the form of long patches on the trunk and were observed after 1 week.

RESULTS AND DISCUSSION

Test the Antagonism of Endophytic Fungi and Pathogenic *Fusarium oxysporum*

The Inhibition Percentage

The results of inhibition that occurs in eleven endophytic fungi against the pathogen *Fusarium oxysporum* varied. The following data are the results of inhibition percentage eleven endophytic fungi isolates. Based on the results of the inhibition percentage at 7 days of incubation only CE7 (*Rhizoctonia* sp) which the inhibition reached 50%. Then there is the CE10 (*Curvularia* sp.) reaching the percentage of inhibition of 47%, followed by the CE5 (*Rhizoctonia* sp) which has inhibition by 42,58%. Then their endophytic fungi represent the percentage above 30% i.e. CE9, CE6, CE11, and the last CE4, and endophytic fungi the remaining percentage of inhibition of below 30%.

Table 1. The inhibition percentage of the eleven isolates of endophytic fungi against the growth of *F. oxysporum* at 7 days of incubation

The origin of the Isolates/Location of the Garden	Isolate	the Inhibition
Pekik Nyaring 1	*Fusarium* sp.	0,31%
Pekik Nyaring 1	CE2	23,75%
Pekik Nyaring 1	CE3	5,13%
Pekik Nyaring 1	CE4	31,25%
Pekik Nyaring 1	*Rhizoctonia* sp.	42,58%
Pekik Nyaring 1	CE6	35,81%
Pekik Nyaring 1	*Rhizoctonia* sp.	50%
Pekik Nyaring 2	CE8	14,65%
Pekik Nyaring 2	*Curvularia* sp.	39,44%
Pekik Nyaring 2	*Curvularia* sp.	47,15%
Pekik Nyaring 2	*Curvularia* sp.	32,12%
Average		29.29%

The high low percentage of barriers that occur can be affected by environmental conditions and other factors that may affect the endophytic fungi. According to Pamekas *et al.* (2021), the inhibition of endophytic fungi against the pathogen *Pyricularia oryzae* is influenced by environmental conditions that vary across all isolates. Environment origin endophytic fungi in the field with laboratory environment have differences. So that affects the inhibition of each isolate. Furthermore, other factors that cause differences in the rate of inhibition of the eleventh isolates namely differences in the ability to grow each isolate tested. This is following the statement Liswarni *et al.* (2018), namely the difference in the rate of growth of each isolate and its ability to compete to get the nutrients from the growing medium affects the ability of the endophytic fungi in inhibiting fungal pathogens. The last factor is because of the biological compound that is produced each fungi isolates of endophytic different. Endophytic fungi can produce a variety of biological compounds, especially antibiotics that can suppress the growth of pathogens. However, a high-low compound that is produced would also affect the effectiveness of inhibition of the endophytic fungi against pathogens. Manurung (2014) explained that the antibiotic compounds produced by

endophytic fungi are less effective in inhibiting the growth of pathogens so that the diameter of the fungi pathogens is greater than the endophytic fungi, and the percentage of endophytic fungi is lower.

The Mechanism of Antagonism

The mechanism of antagonism which occurs in eleven endophytic fungi isolates varied, from the competition between the space of nutrients, and oxygen, antibiosis, and parasitism. The observation of the mechanism of antagonism is done on day 7 after incubation and the results are obtained as in table 2.

The type of mechanism of competition between space, nutrients, and oxygen occurs in the whole endophytic fungi against the pathogen *Fusarium oxysporum.* The nature of the antagonist will appear when fungi live side by side so that raises competition. On each of the isolates showed the ability to compete is different. It is influenced by the ability of each endophyte fungi in growing. Endophytic fungi able to urgent *Fusarium oxysporum* fungi causing *Fusarium oxysporum* is increasingly out of room to grow. According to Mukarlina (2010), the need for space of nutrients and oxygen endophytic fungi different causes competition against fungi pathogens.

The mechanism of antagonism is another place that antibiosis. There are eight ends of it fungi with mechanism antibiosis namely *Rhizoctonia* sp.(CE7), *Curvularia* sp. (CE9, CE10, CE11), CE3, CE4, CE6, CE8. Antibiosis is the inhibition of growth is characterized by the presence of a zone of inhibition (Mejia *et al.* 2008). This is under antibiosis that occurs is indicated by the presence of a clear zone on the media between the end of its fungi with the *F. Oxysporum* fungi. The clear zone occurs due to endophytic fungi that produce antifungal compounds so they can inhibit fungal pathogens.

Table 2. The mechanism of the antagonistic endophytic fungi

The origin of the Isolates/ Location of the Garden Pepper Plants	Isolate	the Competition space nutrients, and oxygen	Antibiosis	Lysis and Parasitism
Pekik Nyaring 1	*Fusarium* sp.	+	-	+
Pekik Nyaring 1	CE2	+	-	+
Pekik Nyaring 1	CE3	+	+	-
Pekik Nyaring 1	CE4	+	+	-
Pekik Nyaring 1	*Rhizoctonia* sp.	+	-	+
Pekik Nyaring 1	CE6	+	+	-
Pekik Nyaring 1	*Rhizoctonia* sp.	+	+	-
Pekik Nyaring 2	CE8	+	+	-
Pekik Nyaring 2	*Curvularia* sp.	+	+	+
Pekik Nyaring 2	*Curvularia* sp.	+	+	+
Pekik Nyaring 2	*Curvularia* sp.	+	+	-

Description : (+) occurs the mechanism of antagonism, (-) does not occur the mechanism of antagonism

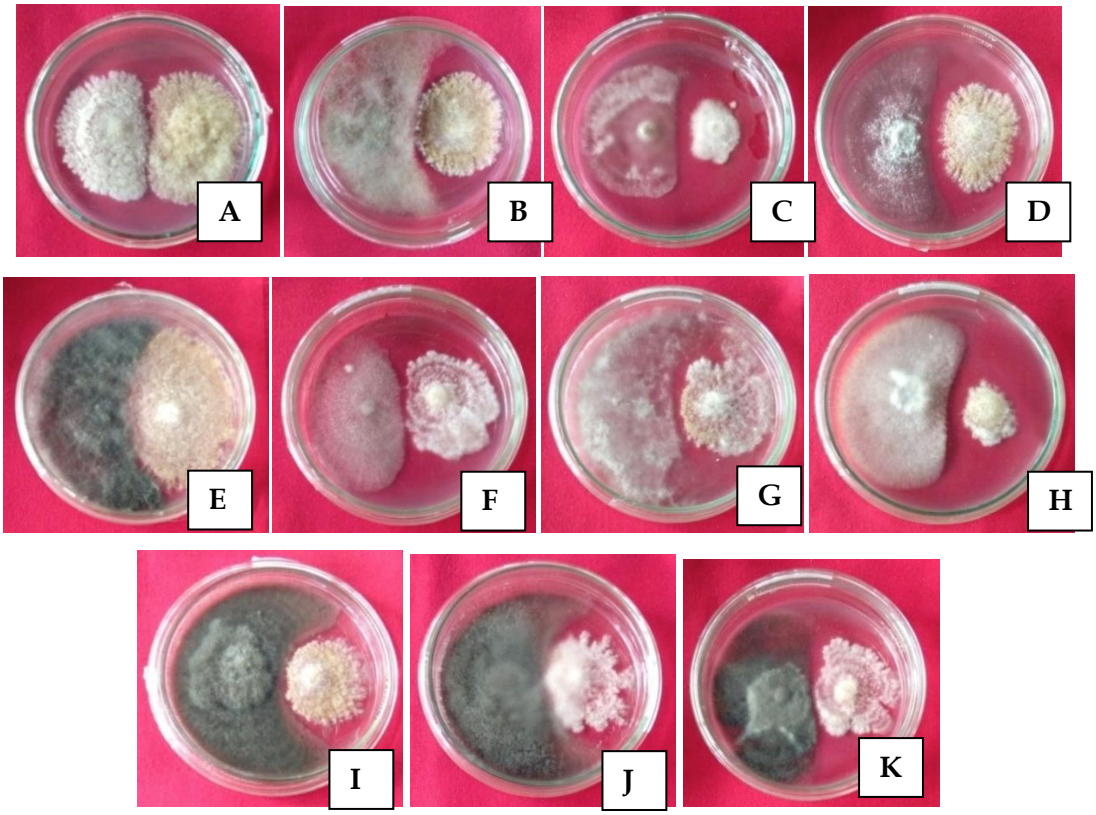

Figure 1. Dual culturetest endophytic fungi vs *Fusarium oxysporum*.
Description : (A) *Fusarium* vs CE1, (B) *Fusarium* vs CE2, (C) *Fusarium* vs CE3, (D) *Fusarium* vs CE4, (E) *Fusarium* vs CE5, (F) *Fusarium* vs CE6, (G) *Fusarium* vs CE7, (H) *Fusarium* vs CE8, (I) *Fusarium* vs CE9, (J) *Fusarium* vs CE10, (K) *Fusarium* vs CE11.

The mechanism of the latter is the mechanism hiperparasitisme. There are 3 fungi mechanisms hiperparasitisme, namely CE1, CE2, and *Rhizoctonia* sp (CE5). However, in this study, there have been no endophytic fungi mechanisms lysis. Sari (2020) states, that the endophytic fungi can crochet with the form loops on the hyphae of the fungi pathogen so that the hyphae of the pathogen become entangled.

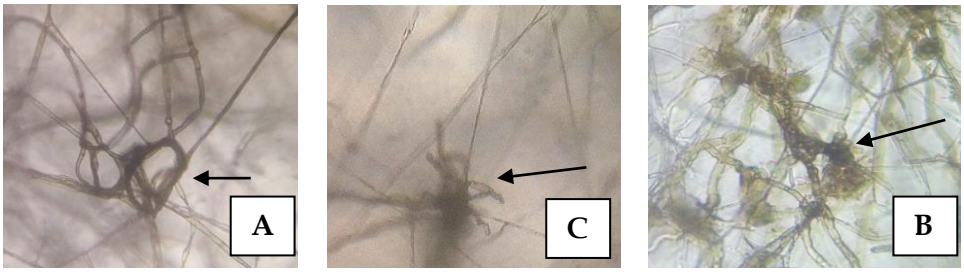

Figure 2. Hiperparasitisme endophytic fungi against *Fusarium oxysporum*.
Description : (A) CE5 ensnare *Fusarium oxysporum*, (B) CE2 ensnare *Fusarium oxysporum*, (C) CE10 ensnare *Fusarium.*

Pathogenicity Test of Endophytic Fungi

A pathogenicity test was conducted to evaluate the potential of endophytic fungi as fungi pathogens with a long look of spotting that occurs on the chili seeds.

Table 3. Pathogenicity test of endophytic fungi

Treatment	Length blots (mm)	Pathogenicity
Fusarium (pathogen)	31,6 a	+
Fusarium sp.	33,3 a	+
CE2	a 13.3 bcd	+
CE3	10bc	+
CE4 +	30a	+
Rhizoctonia sp.	20abc	+
CE6	3,3 d	+
Rhizoctonia sp.	0d	-
CE8	26,6 ab	+
Curvularia sp.	3,3 d	+
Curvularia sp.	The 13.3 bcd	+
Curvularia sp.	23,3 abc	+

On the observation, 7 days of incubation (HSI) conducted visible variations in the length of the patches produced by each endophytic fungi. Spotting with the average value of the lowest in the treatment CE7, then CE6 and CE9. Isolates CE7 is the isolates with the ability to inhibit the best on the test culture double the previous. This suggests that the isolates CE7 have potential as endophytic fungi the best of all isolates. The difference in the length of the spotting occurs because the compounds of metabolites or compounds of antibiotics produced by endophytic fungi are very low so that it is not able to suppress the long spotting that occurs. The content of the compounds of secondary metabolites produced by fungi endophytic is less effective in the suppression of spotting. According to Kasujtianingati (2004), this is caused by the low concentration of antibiotics produced by endophytic fungi and the breakdown of these antibiotics by microorganisms so that it becomes less effective.

CONCLUSION

The results of in vitro study showed that of eleven endophytic fungi only 1 endophytic fungi which has a percentage of inhibition of 50% of the isolates of *Rhizoctonia* sp.2. The mechanism of antagonism which occurs in the endophytic fungi test consists of a competition between the space of nutrients and oxygen, hiperparasitisme, and antibiosis. Test patogenensitas show only isolates of *Rhizoctonia* sp.2 that do not produce patches on the chili seeds.

SUGGESTION

Tests of the potential of obtained endophyte fungi need to be done for induction testing of the resistance of chili plants to the pathogen *Fusarium oxysporum* that causes fusarium wilt disease in vivo.

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