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The Ability of *Trichoderma* sp and *Pseoudomonas fluorencens* to Inhibit the Growth of Anthracnose Fungus *In Vitro* at BBPP Ketindan Lawang

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ABSTRACT

Anthracnose is an important disease in chili plants because yield losses can reach 80%. This disease is caused by the fungus *Colletotricum* spp. The control that is often used is with chemical pesticides which cause negative effects on the environment. The refore it is necessary to use alternative control using biological agents that are environmentally friendly and support sustainable agriculture. This activity aims to determine directly the causes of anthracnose disease and the inhibition percentage of *Tricoderma* sp and *Pseoudomonas fluorencens* against anthracnose fungi in vitro on chili plants at the Ketindan Agricultural Training Center (BBPP). The test used the pour plate method (combining media with biological agents) with 3 treatments, 3 replications and 1 control. The test results showed that *Trichoderma* sp had an inhibition percentage of 100% while *Pseudomonas fluorencens* had an inhibition percentage of 28.06% and 100%. *Trichoderma* sp can inhibit the growth of anthracnose fungi because it has a competition mechanism for nutrients and space, hyper-parasitism, and produces antibiotic compounds while *Pseoudomonas fluorencens* has a mechanism for producing antibiotic compounds. Based on the observations, it can be concluded that *Trichoderma* sp and *Pseoudomonas fluorencens* were able to inhibit the growth of the fungus *Colletotricum gloeospoioides* in vitro.

Keywords: Anthracnose, Biological agents, Drag

Introduction

Chili plants are one of the horticultural commodities which are a very important need for people in Indonesia. The chili commodity has a high economic value, this is supported by the demand for chili commodities which always increases every year, especially on certain days such as the celebration of religious holidays in Indonesia. The need for this commodity is also increasing in line with the growing variety of types and menus that use cayenne pepper as a flavor enhancer (Nawangsih et al., 2002; Fatahillah, 2017).

The productivity of cayenne pepper in Indonesia in 2019 according to data from the Ministry of Agriculture (2020) is still relatively low at 8.23 tons/ha, while its production potential can reach 20 tons/ha. One of the causes of the low productivity of chili plants is the presence of plant-disturbing organisms, both pests and pathogens. Pathogens that often attack plants are the fungus *Colletotrichum* spp. which causes anthracnose disease in chilies (Hasbi et al., 2021). Yield losses due to anthracnose disease can reach 80% if conditions are favorable for the development of the pathogen (Prihatiningsih et al., 2020).

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Control of anthracnose disease usually uses chemical pesticides which can have a negative impact on the environment, while controls that are environmentally friendly and can minimize the use of chemical pesticides are by using biological agents. Biological agents have different antagonistic mechanisms and have the potential to control several disease-causing bacteria and fungi (Harmaningrum et al., 2015). One way is to utilize biological agents such as the fungus *Trichoderma* spp. and the bacterium *Pseudomonas fluorencens*. The role of biological agents to control OPT will be more potential so that it can overcome the decrease in the productivity of chili plants. Therefore, this study aims to examine the ability of *Trichoderma* sp and *Pseoudomonas fluorencent* to inhibit the growth of anthracnose fungi in vitro at the Ketindan Agricultural Training Center (BBPP).

Material and Methods

This research was carried out for one month, from January to February 2023 at the Plant Protection Laboratory, Ketindan Lawang Agricultural Training Center. The materials used in this study were *Nutrient Agar* (NA), *Potato Dextrose Agar* (PDA), distilled water, 70% alcohol, *Trichoderma* sp and *P. fluorenens*. The research method was to explore symptomatic chili plants, isolate pathogenic fungi, purify isolates, identify pathogenic fungi, and test the inhibition of *Trichoderma* sp and *P. fluorencens* against pathogenic fungi using the pour plate method with 3 treatments, 3 replications and 1 control. Observations were made for 7 days and data were taken (the diameter of the colonies growing on the treatment medium). The inhibition of biological agents on the growth diameter of the fungal colonies in each treatment was determined using the formula:

$$\mathrm{DH} = \frac{(\mathrm{a}-\mathrm{b})}{\mathrm{a}}\ x\ 100\%$$

Where:
DH: inhibition of biological agents on the diameter of fungal colonies
a: colony diameter in control treatment
b: colony diameter in the treatment of biological agent concentration formulations

Results and Discussion

Exploration of diseased cayenne pepper plants was carried out on the land of the Ketindan Agricultural Training Center (BBPP) (Figure 1a). Cayenne pepper plants planted in BBPP land were 70 HST. Sampling and observation of diseased plant samples are plants infected with the pathogen that causes anthracnose disease which can be seen from the symptoms of the attack, in which there are symptoms in the form of brown spots on the surface of the chili fruit (Figure 1b). According to (Nurjasmi & Suryani, 2020) the symptoms of anthracnose disease are shown by the presence of small blackish brown spots on the surface of the fruit which gradually enlarge and form indentations and then the fruit becomes soft rot. Spots soon develop to reach the entire surface of the fruit. Further symptoms of anthracnose disease cause the fruit to become dry and wrinkled.

After the symptomatic abai fruit samples were obtained, the pathogenic fungi were isolated (Figure 2). Isolation aims to separate the desired microorganism from the natural substrate or from its host. Then an attempt is made to obtain the target microorganism in the form of culture. Cultures obtained from isolation are called isolates. Mushrooms that grow are then purified and transferred to new media and then identified macroscopically, and microscopically (Triasih et al., 2022).

The result of the identification of the fungus that has been carried out is the fungus *Colletotrichum gloeospoiroides* Macroscopic observation (Figure 3) has the characteristics of a white colony, and there is an orange spot in the middle of the colony. While microscopic observation (Figure 4) has the characteristics of insulated hyphae, cylindrical conidia with blunt ends. This is by the statement of Sari (2021) that the macroscopic characteristics of the color of the colony are white with an orange spot in the

center of the colony, the surface of the cobwebs is uneven and the microscopic characteristics of the eye conidia/aservuli are light to dark brown, conidia unicellular, hyaline, cylindrical (rounded at tip), hyaline conidiophores to dark. According to Sudirga (2016) that the fungus *C. gloeosporioides* has insulated hyphae, cylindrical spore shape, blunt spore tips, spore size 16.1 x 5.6 µm with a growth rate of 12.5 mm per day.

(a)

(b)

Figure 1. (a) Exploration of chili plants (b) Symptoms of anthracnose disease

Figure 2. Isolation results of pathogenic fungi

Table 1. Results of Microscopic and Macroscopic Observations

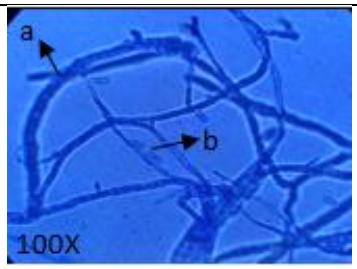
Patoghen	Macroscopic observation	Microscopic observation
Colletotrichum gloeospoiroides	 Figure 3. The fungus *C. gloeosporioides* macroscopically	 Figure 4. The fungus *C. gloeosporioides* microscopically: (a) Hyphae, (b) Conodia

Table 2. Test of inhibition of *Trichoderma* sp on the growth of *C. gloeosporioides* in vitro

Percentage of inhibition (%)					
Biological Agency Treatment	Test			Amount	Average
	I	II	III		
Control	0	-	-	0	0
2,5 ml	100	100	100	300	100
5 ml	100	100	100	300	100
7,5 ml	100	100	100	300	100
Amount				900	

Based on Table 2, the results of the *Trichoderma* sp inhibition test on the fungus *C. gloeosporioides* showed that the percentage of the 2.5 ml, 5 ml, and 7.5 ml treatment inhibition test was 100%. It can be ascertained that Trichoderma sp can inhibit the growth of anthracnose fungus (*C. gloeosporioides*) in vitro. This statement is by research (Khairul et al., 2017) showing that the fungus Trichoderma sp. capable of inhibiting the growth of the genus *Colletotrichum*. Figure 5 is a picture in which the fungus *C. gloeosporioides* does not grow hyphae in the inhibition test of *Trichoderma* sp.

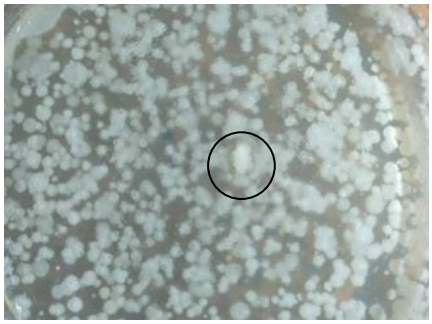


Figure 5. Sample images of the inhibition test of *Trichoderma* sp against the fungus *C. gloeosporioides* 2.5, 5 and 7.5 ml treatments

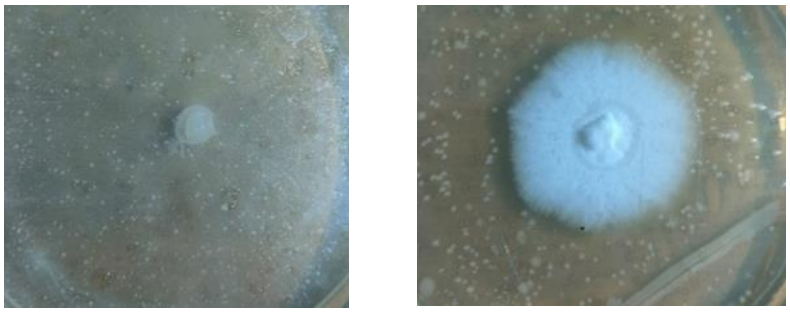
The inhibition process is because *Trichoderma* sp. has an antagonistic mechanism that can inhibit the growth of pathogenic fungi. This is probably due to the role of the antagonistic mechanism of *Trichoderma* sp. can occur through competition for space, nutrients, and oxygen, parasitism, and the ability to produce antibiotics or inhibitory compounds that are secreted into the growth media (Pasalo et al., 2022). According to Khairul et al. (2017), Nur'Aini et al. (2013) that *Trichoderma* hyphae are able to wrap around pathogenic hyphae so that the growth of pathogens is inhibited. *Trichoderma* sp. has the ability to secrete antibiotic compounds that function as antifungals in inhibiting growth and even becoming pathogenic fungal microparasites. *Trichoderma* sp. can produce chitinase and β-1,3 glucanase enzymes which are capable of breaking down pathogenic cell walls. In addition, Trichoderma is capable of producing the antibiotic 3-2-*hydroxyprophyl*-4-2-*hexadienyl*)-2-5*(5H)-furanon* which is capable of inhibiting the growth of pathogenic microbial spores and hyphae. The fungus *Trichoderma* sp. proven to be able to inhibit the growth of other pathogenic fungi such as *Phytophthora palmivora* in vitro.

Based on Table 3, the results of the inhibition test of *P. fluorescens* against the fungus *C. gloeosporioides* showed that the 2.5 ml treatment had a percentage of 28.06%, while the 5 ml and 7.5 ml treatments had a percentage of 100% inhibition. In the 5 ml and 7.5 ml treatments, it was found that

C. gloeosporioides did not grow at the same rate as men (Figure 6a), while in the 2.5 ml treatment *C. gloeosporioides* grew (Figure 6b).

Table 3. Test of inhibition of *P. fluorencenst* on the growth of *C. gloeosporioides in vitro*

Percentage of inhibition (%)					
Biological Agency Treatment	Test			Amount	Average
	I	II	III		
Control	0	-	-	0	0
2,5 ml	26,31	31,57	16,31	84,19	28,06
5 ml	100	100	100	300	100
7,5 ml	100	100	100	300	100
Amount				900	



(a) (b)

Figure 6. Sample images of *P. fluorenens* inhibition test against *C. gloeosporioides* (a) 5 and 7.5 ml treatment, (b) 2.5 ml treatment

P. fuorencens has one of the mechanisms in inhibiting the growth of pathogens, namely by releasing antibiotic compounds. This is in accordance with research (Haggag & El Soud, 2012) that antagonistic bacteria *P. fluorescens* are capable of producing antibiotics, such as antifungal, antibacterial, antitumor, and antiviral. Based on research (Nur'Aini et al., 2013) also said that the inhibition of the growth of *C. gloeosporioides* by *P. fluorescens* was caused by the presence of antibiotics such as agrocin, herbicolin, oomycin A, phenazine, pyoluteorin which are produced by *Pseudomonas*. Another mechanism suggested by *P. fluorescens* is by secreting a pseudobactin siderophore that is produced in a ferric ion-limited state. Nurmayulis et al. (2013) suggested that the application of *P. fluorescens* obtained test results that rhizo-bacteria from the *P. fluorescens* group had a higher inhibitory ability compared to bacteria from other bacterial groups. *P. fluorescens* was reported to be effective in controlling shoot wilt in rice caused by *Rhizoctonia solani*, fusarium wilt in tomatoes caused by *Fusarium oxysporum* f.sp. *lycopersici* (Ramamoorthy et al., 2002; Nurmayulis et al., 2013).

Conclusion

Based on the results of the study, it was concluded that anthracnose is an important disease of chili plants which results in a yield loss of 80%. The fungus that causes anthracnose disease in chili peppers at BBPP Ketindan is *C. gloeosporioides*. One of the environmentally friendly control efforts is

to use biological agents with inhibition tests of *Trichoderma* sp and *P. fluorescens* which are proven to inhibit the growth of the fungus *C. gloeosporioides in vitro*.

In connection with the research that has been carried out, the following suggestions are addressed, namely that it is necessary to carry out further research related to the in vivo test of the inhibition of *Trichoderma* sp and *P. fluorescens* in order to obtain effective results.

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References

Fatahillah, F. (2017). Uji penambahan berbagai dosis vermikompos cacing (*Lumbricus rubellus*) terhadap pertumbuhan vegetatif cabai rawit (*Capsicum frutescens* L.). *Jurnal Biotek*, *5*(2), 191-204.

Haggag, W. M., & El Soud, M. A. (2012). Production and optimization of *Pseudomonas fluorescens* biomass and metabolites for biocontrol of strawberry grey mould. *American J Plant Sci*., *3,* 836-845. doi: 10.4236/ajps.2012.37101.

Harmaningrum, N. W., Mihardjo, P. A., & Majid, A. (2015). Peningkatan potensi agen hayati untuk mengendalikan penyebab penyakit antraknosa (*Colletotrichum* sp.) pada tanaman cabe jawa (*Piper retrofractum* Vahl.) melalui penambahan bahan organik. *Berkala Ilmiah Pertanian, 1*(1), 1-4.

Hasbi, N. S. B., Rosa, H. O., & Liestiany, E. (2021). Intensitas serangan penyakit antraknosa yang disebabkan Oleh *Colletotrichum* sp. pada tanaman cabai rawit dan cabai besar di Desa Karya Maju Kecamatan Marabahan Kabupaten Barito Kuala. *JURNAL PROTEKSI TANAMAN TROPIKA*, *4*(3), 380-385.

Khairul, I., Montong, V. B., & Ratulangi, M. M. (2018). Uji antagonisme *Trichoderma* sp. Terhadap *Colletotrichum capsici* penyebab penyakit antraknosa pada cabai keriting secara In Vitro. *In Cocos*, 9(6), 1-8. https://doi.org/10.35791/cocos.v1i2.20109

Nawangsih, A., Imdada, H. P., & Wahyudi, A. (2002). *Cabai hot beauty Jakarta*. Penebar Swadaya.

Nur'Aini, F., Sukamto, S., Wahyuni, D., Suhesti, R. G., & Ayunin, Q. (2013). Penghambatan Pertumbuhan *Colletotrichum gloeosporioides* oleh *Trichoderma harzianum, Trichoderma koningii, Bacillus subtilis* dan *Pseudomonas fluorescens*. *Pelita Perkebunan, 29*(1), 2013, 44-52

Nurmayulis, Syabana, M. A., & Syafendra, Y. (2013). Pengendalian penyakit antraknosa (*Colletotrichum capsici*) pada cabai merah dengan beberapa bakteri sebagai agen biokontrol. *Jurnal Agroekoteknologi*, *5*(1), 33-44.

Nurjasmi, R., & Suryani, S. (2020). Uji antagonis actinomycetes terhadap patogen *Colletotrichum capsici* penyebab penyakit antraknosa pada buah cabai rawit. *Jurnal Ilmiah Respati*, *11*(1), 1-12.

Pasalo, N. M., Kandou, F. E. F., & Singkoh, M. F. O. (2022). Uji antagonisme jamur *Trichoderma* sp. terhadap patogen *Fusarium* sp. pada tanaman bawang merah *Allium cepa* isolat lokal tonsewer secara in vitro. *Jurnal Ilmu Alam dan Lingkungan*, *13*(2), 1-7.

Prihatiningsih, N., Djatmiko, H. A., & Erminawati, E. (2020). Komponen epidemi penyakit antraknosa pada tanaman cabai di kecamatan baturaden kabupaten Banyumas. *Jurnal Agro*, *7*(2), 203-212.

Ramamoorthy, V., Raguchander, T., Samiyappan, R. 2002. Induction of Defense-related Proteins in Tomato Roots Treated With Pseudomonas floerescens and Fusarium oxysporum f.sp. lycopersici. *Plant dan soil*, *239*, 55-68.

Sari, N. (2021). Identifikasi dan uji patogenisitas *Colletotrichum* spp. dari cabai merah (*Capsicum annuum*): Kasus di Kricaan, Magelang, Jawa Tengah. *Jurnal Ilmu Pertanian Indonesia* (JIPI), *26*(2), 243−250

Sudirga, S. K. (2016). Isolasi dan identifikasi jamur *Colletotrichum* spp. isolat PCS penyebab penyakit antraknosa pada buah cabai besar (*Capsicum annuum* L.) di Bali. *Jurnal Metamorfosa*, *3*(1), 23-30.

Triasih, U., Abadi, A. L., Muhibbudin, A., & Widyaningsih, S. (2022). Uji beberapa jamur antagonis terhadap *Colletotrichum gloeosporiodes* penyebab penyakit busuk buah apel manalagi (*Malus sylvestris*) secara in vitro. In *Agropross: National Conference Proceedings of Agriculture* (pp. 389-397).