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Antagonist Test of *Streptomyces* sp. AGAINST *Colletotrichum* sp. From Several Chili Varieties In Vitro

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Abstract

Chili plants are horticultural commodities that play an important role in Indonesia's economic development. Low chili plant production could be the consequents of anthracnose disease (Colletotrichum sp.) with symptoms of the dry shrinked and blackening of the fruits. Up to this date, chemical control with pestiside are the most widely used. Excessive use of chemical pesticides can led to environmetal pollution and ecosystem damage. Therefore, biological control that organic and safe for the environment were applied. The antagonistic agents in the form of Streptomyces sp. were employed as one of the way to suppress pathogen growth. The study aimed to determine the potential of Streptomyces sp. in inhibiting the growth of Colletotrichum sp. from several chili varieties. A Completely Randomized Design method was used, with 3 treatments (large chili, curly chili and cayenne pepper) and 6 replications. Chili fruit exhibited the anthracnose symptoms was isolated and Colletotrichum sp infection was validated. Furthermore, antagonistic tests were carried out against Streptomyces sp. against the pathogen, three isolates are antagonistic as indicated by the interaction of mechanism in the form of antibiosis produced by bacteria in inhibiting the pathogen that causes anthracnose disease. The result show that CR isolate has 50.13% inhibition. This is significantly different from the other two isolates, CB and CK, with 34.49% and 30.34% inhibition.

Keywords: *Anthracnose, Chili plants, Colletotrichum sp. , Streptomyces sp.*

Introduction

Chili is one of the horticultural commodities that has important economic value in Indonesia. In addition to being used as a vegetable or cooking spice, chili also has a high selling value, so it can increase farmers' income Tanjung *et al*., (2018). One of the triggers for low chili production is the attack of anthracnose disease caused by *Colletotrichum* sp. This disease is very detrimental to farmers because it attacks the fruit, so that the fruit is damaged by anthracnose attacks and can affect the quality of the fruit and reduce the quantity or quality of production (Duriat *et al.*, 2007). According to Widodo & Hidayat (2018), loss of chili harvest due to anthracnose disease in the rainy season can reach 50-100%. This disease has initial symptoms in the form of small blackish spots and grooves on green fruit and ripe fruit (Ainy *et al.*, 2015).

Control of anthracnose disease has so far relied on the use of chemical pesticides. According to Ona *et al*., *(*2021) in general, the continuous use of chemical pesticides with inappropriate doses can cause long-term damage to soil, air, living organisms, and the environment. Therefore, environmentally friendly control efforts are needed by utilizing antagonistic agents.

One of the antagonist agents comes from the genus Streptomyces. This bacteria is a group of antagonistic microorganisms that are used as biological control agents for pathogens that cause plant diseases. According to Zamoum *et al.*, (2017) *Streptomyces* sp. is able to produce secondary metabolites (antimicrobial compounds) that play a role in protecting plants against pathogens. Therefore, the purpose of this study was to determine the potential of *Streptomyces* sp. in inhibiting the growth of *Colletotrichum* sp. fungi from several varieties of chili in vitro.

Research Method

This research was conducted at the Phytopathology Laboratory, Department of Plant Pests and Diseases, Lambung Mangkurat University, Banjarbaru. The research scheme implemented was sterilization of equipment, *making Yeast Mealt Agar* and *Potato Dextrose Agar* media, purification of *Streptomyces* sp., sampling, isolation and purification of pathogens and finally antagonistic test of *Streptomyces* sp. against *Colletotrichum* sp. The method used for pathogen identification refers to the reference method. While the method used for antagonistic test is Completely Randomized Design (CRD) with three treatments (large chili varieties, curly chilies, and cayenne peppers) each of which was repeated six times.

Results and Discussion

Identification of Pathogens Causing Anthracnose Disease

Table 1. Symptoms and Morphology of Anthracnose Disease

Isolate	Symptom	Macroscopic			Microscopic	
		Color	Shape	Texture	Seta	Conidia
CB	The fruit shrivels, widens, and dries out with a brown to black color on the affected parts	Greyish white	Round with ring	Cotton	Dark brown	Cylinder
CK	The fruit is attacked in almost all parts until it dries out and turns blackish brown	Browni sh white	Round with ring	Cotton	Brown	Falcate
CR	More than half of the fruit is affected, the center is black and the edges are brown on the affected part	Cokelat keabua n	Round with ring	Cotton	Dark brown	Cylinder

Figure 1. Symptoms of anthracnose disease in the fields of a. Large chili, b. Curly chili and c. Bird's eye chili

Anthracnose disease caused by *Colletotrichum* sp. is one of the main obstacles in chili cultivation in Indonesia. This disease is known to be very detrimental because it attacks various parts of the plant, especially the fruit, which directly affects the harvest. According to Nasution *et al.*, (2020) *Colletotrichum* sp. attacks on chili plants not only cause major economic losses but also reduce the quality and storage life of the harvest. Anthracnose disease can cause damage to plant parts and reduce production by 20-90%.

The chilies selected from the field are chilies that show symptoms of anthracnose disease with characteristics of wrinkled fruit, blackish brown spots on the surface of the fruit, soft to rotten (Semangun, 2004). The chili samples that have been collected are then isolated and macroscopic and microscopic observations are carried out to validate whether the chili samples are infected with anthracnose disease. Anthracnose disease can be caused by *Colletotrichum* sp. which has been widely reported as a dominant disease in chili plantations. The spread of *Colletotrichum* sp. disease is greatly influenced by environmental factors such as high humidity and warm temperatures, these conditions greatly support the development of pathogenic conidia which are the main cause of infection (Waller *et al.*, 2002). This makes the rainy season a critical period in controlling anthracnose disease. Rainwater splashes or improper irrigation can spread pathogen spores from one plant to another, accelerating the spread of infection in agricultural land.

Control that is generally carried out by farmers and surrounding communities is with chemicals in the form of pesticides, this can have a negative impact on the ecosystem and environment. This is also in line with Herlinda *et al.*, (2015) who stated that control efforts with chemical pesticides are still the main choice for most farmers, but this approach has long-term negative impacts such as pathogen resistance, environmental pollution, and harm to non-target organisms. Therefore, a biological control approach using antagonistic agents such as *Streptomyces* sp. is an environmentally friendly alternative solution. These bacteria are known to be able to produce antibiotic compounds and enzymes that can inhibit the growth of plant pathogens, including *Colletotrichum* sp. (El-Tarabily & Sivasithamparam, 2006).

The results of research by Jog *et al.*, (2014) showed that several isolates of *Streptomyces* sp. have great potential in suppressing the development of *Colletotrichum* sp. colonies in vitro. In addition, the application of this biological agent also supports plant growth through the mechanism of increasing nutrient availability and stimulating root growth. Thus, the biological control approach not only functions as a method of disease control, but also provides overall agronomic benefits for chili plants.

Three different types of chilies from the field were infected with anthracnose disease as seen from their symptoms. This infection occurs in fruit that is approaching maturity with symptoms of shriveled and dry fruit accompanied by blackish brown spots in the shape of circles. This is in line with the research of Sari & Rina (2021) who took samples of chili fruit infected with anthracnose with symptoms that began with small black wounds that then widened, the flesh of the chili fruit became soft and shriveled in the affected part. In addition to the fruit, anthracnose disease can also attack other parts of the plant such as seeds, stems, and leaves.

Figure 2. Macroscopic morphology of isolates originating from
a. Large chili, b. Curly chili and c. Bird's eye chili.

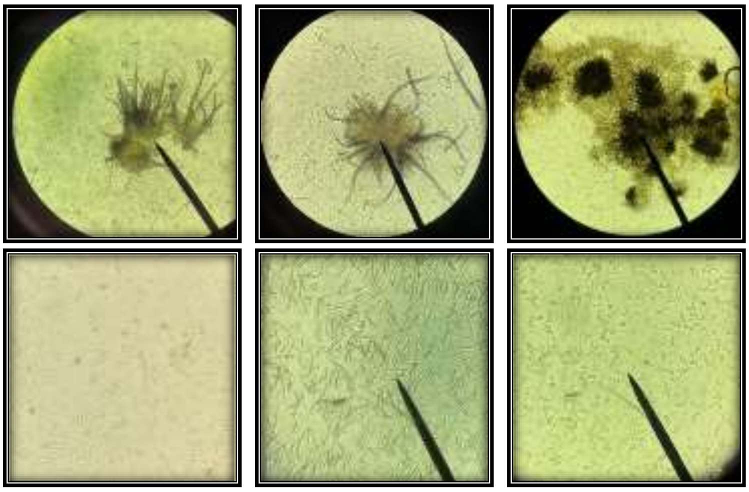

Figure 3. Microscopic morphology a. CB Seta, b. CK Seta, c. CR Seta, d. CB Conidia, e. CK Conidia and f. CR Conidia

The chili fruit with symptoms was then isolated and grown on *Potato Dextrose Agar* media until it filled the petri dish. Macroscopic morphology is a collection of mycelium that starts with a white color which gradually changes to blackish brown. Macroscopically, the colony refers to the genus Colletotrichum. This is in line with research by Benatar *et al.,* (2023) which states that *Colletotrichum* is generally dull white then grayish. Furthermore, according to Sudirga (2016), on PDA media, the isolate *Colletotrichum* sp. produces a lot of mycelium with grayish white colonies with the back of the cup being blackish brown. In addition, the colony also shows a ring-like pattern/shape. This is in line with Alvarez *et al.,* (2020) that *Colletotrichum* sp. colonies can form concentric rings.

The results of microscopic observations of isolates include observations of seta and conidia which are then compared with the literature. The results of observations of seta for isolates coded CB, CK and CR show that the seta are brown to dark brown in color with varying sizes. This is in line with the research of Mariana *et al.,* (2021) who identified *Colletotrichum* sp. with many seta and have cut tips with a brown to dark brown color. Silva *et al.,* (2019) also stated the results of their analysis of *Colletotrichum* sp. which showed many seta with a brown color. The results of the observation of conidia for the CK code looked like a crescent moon, pointed tip, slightly bent and not straight. After being compared with the literature, the isolate was *C. truncatum* which came from Indonesia, Malaysia, Thailand and Sri Lanka. This isolate dominates anthracnose disease in Asia up to 44%. Furthermore, for the observation of conidia of isolates CB and CR, there were similarities, namely cylindrical conidia with blunt tips.According to the literature, this isolate is *C. gloesporioides* which is in line with the research of Than *et al.*, (2008) who identified *C. gloeosporioides* with conidia with both ends rounded. Then according to Mariana *et al.*, (2021) conidia with rounded tips are a characteristic of the pathogen *C. gloeosporioides*. This is evidenced by the results of macroscopic and microscopic morphological observations that the three samples were attacked by anthracnose disease caused by *Colletotrichum* sp. from different species.

Antagonist Test of *Streptomyces* sp. against *Colletotrichum* sp.

Streptomyces sp. is one of the antagonist groups that has the potential for biological control. *Streptomyces* sp. is a gram-positive soil-dwelling bacteria which is the largest genus of Actinomycetes and is able to produce antibiotics. Therefore, an antagonist test was carried out against the pathogen that causes anthracnose disease in chili plants that has been identified, namely *Colletotrichum* sp. Previous research conducted by Sahriyanor (2023), namely the antagonist test of *Streptomyces* sp. against *Colletotrichum* sp. using the flanking method of pathogenic fungi showed successful inhibition with a percentage above 50% so this test aims to determine whether the dual culture method test is also able to suppress the growth of *Colletotrichum* sp. in vitro.

Figure 4. Results of antagonistic tests of test isolates against *Colletotrichum* sp.
a. CR, b. CB and c. CK

Table 2. Percentage of inhibitory power of *Streptomyces* sp. against *Colletotrichum* sp.

Isolate	Percentage of Inhibitory Power (%)	Antibiosis
CR	50.13[a]	+
CB	34.49[b]	+
CK	30.34[b]	+

Table 3. Quality requirements for antagonistic agents

Parameters	Unit	Mark
Conidium density	per ml	> 10^6
Conidium viability	%	> 60
Pathogenicity to plants	-	Negative
Antagonism		
-Antibiosis	-	Positive
-microparasitism	-	Positive
-Inhibition	%	>50%

Note: the condition is met if one of the antagonism parameters is met

The results of the antagonistic test of *Streptomyces* sp. bacteria against anthracnose pathogens (*Colletotrichum* sp.) vary. According to Sahriyanoor (2024), the difference is thought to be influenced by the quality and quantity of secondary metabolites produced by *Streptomyces* sp. and differences in the types of isolates available. The isolate that was able to inhibit >50% was the isolate with a CR code of 50.13%. Meanwhile, isolates CB and CK had an inhibition power of 34.49% and 30.34%. According to Hudi (2014), an isolate is said to be antagonistic if it meets one of the antagonism parameters. The three test isolates can be said to be antagonistic because the CR isolate has an inhibition percentage of >50%. Although the other two isolates (CB and CK) do not meet the inhibition requirements, the three isolates have an antibiosis mechanism which is characterized by the formation of a clear zone between the test isolate and the pathogen. According to Melysa *et al.*, (2013) antagonism is an interaction that occurs due to competition between antagonistic bacteria and pathogenic fungi that grow side by side in a petri dish. Furthermore, research by Chair *et al.*, (2023) showed that the antagonistic mechanism is a way of working to inhibit pathogens that cause disease infections.

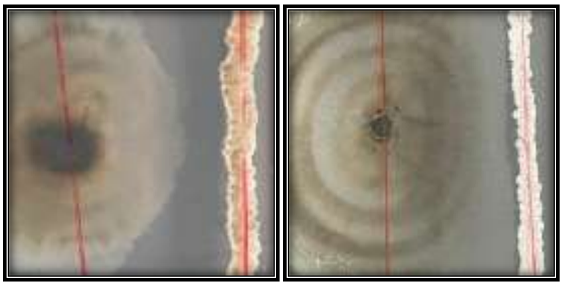

Figure 5. Mechanism of antibiotic interaction

In the test isolate, a clear zone can be observed in the interaction area between *Streptomyces* sp. and *Colletotrichum* sp. which indicates the presence of an antibiosis mechanism. This mechanism refers to the ability of a microorganism, in this case *Streptomyces* sp., to inhibit the growth of other microorganisms through the production of antimicrobial compounds such as antibiotics. According to Chair *et al.*, (2023) the antibiosis mechanism is seen by the formation of a clear zone between the antagonist agent and the pathogen in a space where neither grows. Then according to Strobel & Daisy (2003), the production of antibiotics by soil microbes such as *Streptomyces* sp. is part of their survival strategy. These antibiotics can inhibit the growth of plant pathogens in certain ways, such as damaging cell structure or disrupting important metabolic processes. In this context, the antibacterial activity produced by *Streptomyces* sp. can be an effective biological control approach against *Colletotrichum* sp. which is known as the pathogen that causes anthracnose disease in various horticultural plants. This is in line with Prepagdee *et al.*, (2008), that *Streptomyces* sp. produce hydrolytic compounds such as chitinase which can degrade the fungal cell wall so that the fungus cannot grow normally. In addition to these mechanisms, antibiotic activity can also inhibit cell wall formation, change the permeability of target cells, inhibit the work of enzymes that play a role in pathogen growth, interfere with protein and nucleic acid synthesis (Kong *et al.*, 2020).

Conclusion

The conclusion of this study is that pure isolates of fungi from chili fruit with anthracnose symptoms have been successfully isolated. The results of macroscopic and microscopic identification showed that the isolate was *Colletotrichum* sp. Furthermore, the results of the antagonistic test of *Streptomyces* sp. against *Colletotrichum* sp. namely there was one isolate with the CR code with an inhibitory power of >50%, namely 50.13% and two other isolates, namely CB and CK, had an inhibitory power of 34.49% and 30.34%. The mechanism of interaction of Streptomyces sp. against the three treatments was in the form of antibiosis which was marked by the presence of a clear zone produced by bacteria as an antibiotic against pathogens.

The suggestion from this study is that further research is needed on antagonistic bacterial isolates, both through in vivo testing and field-scale testing aimed at suppressing anthracnose disease caused by the pathogen *Colletotrichum* sp.

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