ISSN No:-2456-2165

Biological Control Techniques for Chili Plant Disease by Using Streptomyces Sp. and Trichoderma Sp.

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Abstract:- Research has been conducted in the Penan Village, District of Ampenan, Mataram City, Lombok Island, Indonesia. The study was conducted in a completely randomized design consisting of 4 treatments, namely treatment with Streptomyces sp. (S), treatment with Trichoderma sp. (T), treatment with a combination of Streptomyces sp. and Trichoderma sp. (ST), and without treatment biological agent (K). Applications of a biological agent were carried out before planting by soaking chili seeds and watering the rhizosphere of chili plants after two weeks of planting. Results showed that the compatibility test of the two biological agents were not inhibit each other. This showed that the two biological control agents were not influence each other so that they could be used together to control plant pathogens. Combination treatment (ST) showed the best results in delaying the incubation period of leaf spot disease of chili to 9.6 days and 74 days on anthracnose chilies disease. Combination treatment of biological control agents was able to reduce the intensity of leaf spot disease to 16.2% and 11.6% in anthracnose. The growth of the plants was the best by treatment of Streptomyces sp. and Trichoderma sp (ST) by proving the highest height of plants compared to other treatments.

Keywords:- *Biological Control Agent, Streptomyces sp., Trichoderma sp.*

I. INTRODUCTION

Chili is one of the favorite horticultural commodities that has received attention in Indonesia. This attention is due to the fact that most of Indonesian people like spicy and hot flavors. The production of chili plants often have experienced disturbance caused by plant pest organisms such as plant diseases.

The used of plant disease control on chili is by using synthetic chemical fungicides. The continued usage of synthetic chemical fungicides can cause various problems due to the difficulty of decompose or degrade naturally. Some problems are the resistance from pathogens and the presence of residues in the carcinogenic food ingredients (Pal et al., 2006). Referring to those problems, the need for safety environment control and food security for consumers especially for chili plants have been taken into consideration.

The best alternative approach is by using biological control agents due to the consideration of safetyness for humans and the environment. One potential of biological control agents use Streptomyces sp. The ability of Streptomyces sp. in controlling disease has been reported by several researchers. Muthahanas (2007) managed to isolate 45 Streptomyces sp. Isolates. from the rhizosphere of tomato plants, chili, and shallots. Results showed that among 45 isolates, the inhibition of pathogenic fungi varied from 20% to 70% in vitro tests in the Laboratory. Mutahanas (2009) also reported the success of the use of Streptomyces sp. to control wilt tomato caused by Fusarium on a plastic home scale. Alam M, et al (2012) reported that Streptomyces sp. CIMAP-A1 isolates were able to inhibit the growth of Stemphylium sp., Botrytis cinerea, Sclerotinia sclerotiorum, Colletotrichum spp., Curvularia spp., Corynespora cassicola and Thielavia basicola in vitro tests.

Trichoderma sp. is one of the antagonistic microorganisms and is a good biological controlling agent (BCA) to overcome chili plant diseases. Ningsih, Hastuti and Listyiorini (2016) reported that T. harzianum was able to inhibit the growth of Fusarium solani that caused the wilt chili plants by 60%, T. veridi by 52% and T. converting by 43.33%.

Some researchers have reported the ability of the biological control agent Streptomyces sp. and Trichoderma sp. in controlling plant diseases. However, the ability of the biological control agent is still limited to one type of biological control agent for plant disease. Increasing the ability of the biological control agent to control plant diseases can be done by combining several controlling agents. The possibility of the combination relates to the synergism between the usage of controlling agents. The research question relates to what is the ability of the biological control agent Streptomyces sp. and Trichoderma sp. in controlling plant pathogens when they are combined? The research question becomes the basic reason in conducting a study of "Combination of biological control agents Streptomyces sp. and Trichoderma sp. in controlling chili plant disease".

The purpose of this study was to determine and evaluate the ability of the biological control agent Streptomyces sp. and Trichoderma sp. as individually and to analyze the ability of combination between the biological control agent Streptomyces sp and Trichoderma sp. into a formula for controlling disease in chili plants.

II. MATERIALS & METHODS

A. Study Site and Design

The research was carried out in the Microbiology Laboratory of the Faculty of Agriculture, University of Mataram and in farmers' paddy fields in Penan Village, District of Ampenan, Mataram City, Lombok Island, Indonesia. The study was conducted from July to November 2018.

The implementation of research in the Laboratory includes a test of compatibility between Streptomyces sp. Isolates. with Trichoderma sp. Tests were carried out by growing together with Streptomyces sp. Isolates. and Trichoderma sp. on the petri dish. The growth of the two isolates was observed daily until the 14th day. Growth measurements were carried out by measuring the diameter of the two isolates. Other observations were also made on the possibility of antagonists between the two isolates.

Research activities in the field include planting chili as well as the application of biological control agents Streptomyces sp. and Trichoderma sp. Research in the field was arranged with a Completely Randomized Design consisting of 4 treatments and each treatment was repeated by 5 times to obtain 20 experimental units. The four treatments are:
S = Treatment of Biological Control Agents Streptomyces sp.
T = Treatment of Biological Control Agents Trichoderma sp.
ST = Treatment of Biological Control Agents Streptomyces sp. and Trichoderma sp.
K = Without Treatment Biological Control Agents

B. Implementation of the Experiment and Observation

Formulation of biological control agent Streptomyces sp. and Trichoderma sp. were used in liquid form. Streptomyces sp. BSi Isolate is a private collection from the results of previous studies, and Trichoderma isolates were the isolates of Mulat Isnaini's collection. Multiplication of biological control agents is carried out by growing each Streptomyces sp. and Trichoderma sp. by 5 mm into molasses media (sugar cane juice) 2%. Molasses media containing Streptomyces sp. is placed on several erlenmeyer containers. Erlenmeyer containing a mixture of molasses and culture pieces of Streptomyces sp. was grown by shaking for 6 hours every day using a shaker for 7 days.

The treatment of biological control agents was carried out by soaking chili plant seeds on each biological control agent solution for 15 minutes before the seeds were planted in the field. Seeds that have been soaked are planted with a spacing of 50 x 60 cm on a 1 x 4 m dam.

The next treatment of biological control agents is by splashing each biological control agent in the area around the base of the chili plant as much as 10 ml when the plant is 2 weeks after planting.

The development of the disease is observed by observing and examining all symptoms of diseases that arise in plants such as the occurrence of wilting or yellowing of leaves of plants caused by pathogens or other symptoms of chili plants. Observations were conducted on the incubation period of the disease since day one after pathogenic inoculation. At the end of the experiment, the intensity of the disease in each treatment will be calculated.

$$\text{Disease Intensity} = \frac{\text{Number of Sick Plants}}{\text{Total Plant}} \times 100\%$$

The observed of growth parameters include plant height and fruit weight.

C. Data Analysis

Data from observations will be analyzed by Analysis of Variance (ANOVA). The different test used the Honest Significant Difference (HSD) test at the level of 5% using the MINITAB program. Data from the analysis will be displayed in the form of tables and graphs as well as photographs of observations.

III. RESULTS AND DISCUSSION

A. Colony Growth and Symptoms

Based on the compatibility test, it was found that the growth of Streptomyces sp. colonies was slower than that of Trichoderma sp. colonies. It showed that the size of Trichoderma sp. colonies was almost the same size of Streptomyces sp. colonies on the second day of testing (Figure 1). Eventhough the growth of the Trichoderma sp. colonies was faster than that of Streptomyces sp. colonies, but it was not block out the growth of Streptomyces sp. colonies. However, the growth of Trichoderma sp. continues to develop rapidly and begins to cover Streptomyces sp. colonies on the third day of testing.

ISSN No:-2456-2165

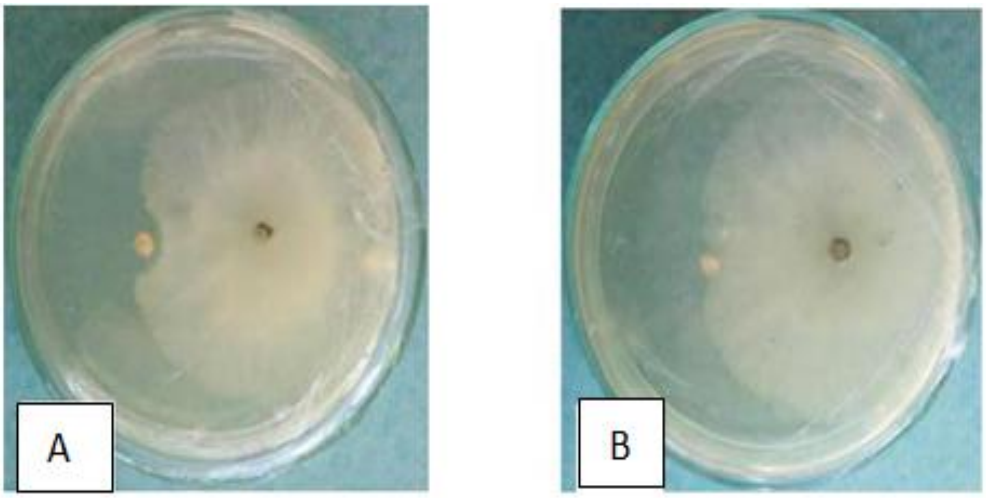

Fig 1:- Colony growth of Streptomyces sp. and Trichoderma sp. on the second day (A) and the third day (B) testing

The compatibility test results between BCA Streptomyces sp and Trichoderma sp. showed that the growth of the two BCA colonies did not inhibit each other (Figure 2). Although the colony of Streptomyces sp. covered by the colony of Trichoderma sp., but the growth of Streptomyces sp. colonies the did not stop. This showed that the BCA was not compete in terms of competing space or nutrition. Moreover, it can be said that the anti-microbial substances of the two colonies were not influence each other. The ability to grow together between the two colonies indicated that the two BCAs could be used together in controlling plant diseases.

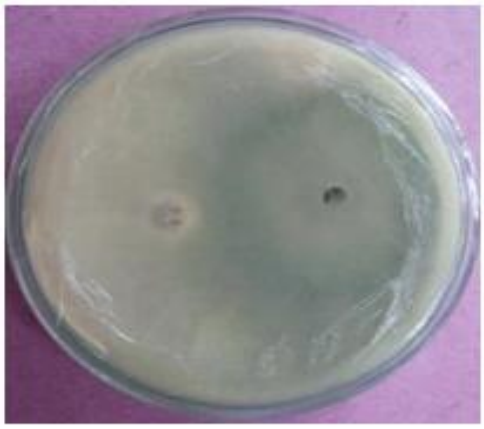

Fig 2:- Colony growth of Streptomyces sp. and Trichoderma sp. do not inhibit each other incompatibility testing

Experiments in the field area showed that diseases that occur in chili plants were spotting diseases on the leaves of chili plants (Figure 3) and anthracnose in chili fruits (Figure 4). Symptoms of the disease were spherical and dry leaf spots. Spots expanded to a diameter of about 5 mm. The center of the spot colored with pale to whit with darker color at edges.

Fig 3:- Symptoms of patches of Cercospora chili leaves

Fig 4:- Symptoms of chili fruit anthracnose

ISSN No:-2456-2165

B. Incubation Period

The incubation period of Cercospora leaf spot disease was different in each treatment. The incubation period of leaf spot disease in the BCA treatment of Streptomyces sp. and Trichoderma sp. (ST) was taken the longest period, with the average of 9.6 days, followed by the BCA treatment of Streptomyces sp. (S) with the average of 7.6 days and then followed by the BCA treatment of Trichoderma sp. (T) with the average of 7.2 days. The fastest incubation period in control treatment (K) was 6 days on average (Table 1).

Chili anthracnose disease in this study began to appear when age of plant was 61-day-old by control treatment (K) with an average incubation period of 64 days, followed by BCA treatment of Trichoderma sp. (T) with 66.4 days, then BCA treatment of Streptomyces sp. (S) with 69 days and BCA combination treatment of Streptomyces sp. and Trichoderma sp. (ST) with 74 days (Table 1).

Treatment	Incubation Period (days)			
	Leaf spots		Anthracnose	
ST	9.6	a	74.0	a
T	7.2	b	66.4	bc
S	7.6	b	69.0	b
K	6.0	b	64.0	c
HSD 5%	1.76		2.90	

Table 1:- Incubation period of Cercospora and Anthracnose leaf spot disease (Description: numbers followed by the same letters in the same column are not significantly different.

The difference in the incubation period showed that each BCA had a different ability to inhibit the occurrence of leaf spot and anthracnose disease in chili plants. The duration of the incubation period in the ST treatment was suspected that the two BCAs synergized in inhibiting the occurrence of disease in chili plants. The delayed in the incubation period in the BCA treatment was explained that BCA colonized the rhizosphere roots first rather than pathogens, so that it could be able to delay the infection process around the roots of plants. The ability of Trichoderma sp. in colonizing roots reported by Yedidia et al (1999) in Nurbailis and Martinus (2011), that some strains of Trichoderma sp. able to colonize and endophytic in the root tissue of cucumber plants and caused the increased activities of resistance compounds in the roots and leaves of plants. Vurukonda at al (2018) reported that Streptomyces sp. was able to colonize plant tissue from roots to other parts on the ground.

The delay in the incubation period with the combination treatment of two BCAs was reported by El-Sharkawi at al (2015). The results showed that the incubation period increased significantly compared to controls in both CVs. wheat (Morocco and Sids-1) as a result of spraying T. harzianum, Streptomyces viridosporus and a combination of both. This increased in incubation period was reached up the highest days by T. harzianum + S. viridosporus with 16 days, followed by S. viridosporus with 14 days, and by T. harzianum with 12 days as the lowest effect

C. Disease Intensity and Plant Height

Results found that all BCA treatments were able to reduce the intensity of leaf spot and anthracnose disease in chili fruits. The highest intensity of leaf spot disease occurred without treatment (K) which was 29.6%, followed by treatment T with 21.4%, treatment S with 24% and treatment ST with 16.2%. The highest intensity of anthracnose disease in K treatment was 18.4%, followed by treatment S with 16.6%, T treatment with 14.4% and in ST treatment with 11.6% (Table 2).

Treatment	Disease intensity (%)			
	Leaft Spot		Anthracnose	
ST	16.2	c	11.6	a
S	24.0	b	16.6	ab
T	21.4	b	14.4	a
K	29.6	a	18.4	c
HSD 5%	4.23		4.17	

Table 2:- Incubation period of Cercospora and Anthracnose leaf spot disease (Description: numbers followed by the same letters in the same column are not significantly different)

Based on data it was found that BCA combination treatment of Streptomyces sp. and Trichoderma sp. was able to reduce the intensity of the disease. It was suspected that the ability of BCA to colonize rhizosphere of the roots in chili plants had an influenced on the decreased number of initial potential inoculums so that the intensity of the disease decreased. The similar results were reported by Astiko et al. (2015) that biological control using mycorrhizae from several rhizosphere plants can reduce the intensity of the disease from Sclerotium. rolfsii in soybean plants, and reduce the intensity of sclerotium stem rot from the rhizosphere of cassava plants. The number and type of BCA used also affects the intensity of the disease. This happened because each BCA produced different types and quantities of secondary metabolites or antibiotics. Biles and Hill (1988) in El-Sharkawi at al (2015) explained that T. harzianum was effective in reducing the sporulation capacity of fungus Cochliobolus sativus in wheat germ. Besides inhibits pathogen development, Streptomyces sp. it also was able to induce resistance to the host so that it can control the disease caused by Phytophthora infestants (Bochow and Fritzche, 1991).

The BCA treatment affected plant height at all treatments. The highest height of crop in the BCA (ST) combination treatment was 76.46 cm and the lowest without treatment (K) was 64.90 cm at age of 10 week after planting (Table 3). BCA speed up growth of the plants because of the production of auxin and giberelin. Manulis at al, (1994) in Vurukonda at al, (2015) stated that Streptomyces sp. produced Indole-3-Acetic-Acid (IAA) which was classified as a growth regulator belongs to the group of auxin. Auxin played a major role in lengthen and enlarge of plant cells.

Treatment	Plant height (cm)									
	2 weeks		4 weeks		6 weeks		8 weeks		10 weeks	
ST	19.82	a	29.84	a	38.44	a	53.44	a	76.46	a
S	18.18	a	28.86	a	35.68	ab	50.78	a	72.78	ab
T	16.92	ab	26.76	ab	33.04	ab	48.04	ab	70.04	ab
K	13.60	b	21.90	b	27.90	b	42.90	b	64.90	b
HSD 5%	4.46		5.46		7.86		7.87		9.19	

Table 3:- Plant height at 2, 4, 6, 8, and 10 weeks (Description: numbers followed by the same letters in the same column are not significantly different)

IV. CONCLUSION

Combination between BCA Streptomyces sp. with Trichoderma sp. showed the best results in delaying the incubation period, suppress the intensity of leaf spot and anthracnose disease in chili fruit, and enhance plant growth.

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