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The Potential of *Bacillus* spp. in Suppressing *Colletotrichum capsici* that Causes Anthracnose in Red Chili

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Abstract

Red Chili *(Capsicum annum* L.*)* is a horticultural plant classified as an important commodity with high economic value. One of the main problems in the production of red chili is anthracnose disease caused by the *Colletotrichum capsici* pathogen. *Bacillus* spp. expected to have the potential to inhibit the growth of *C. capsici* and stimulate plant growth. This study aims to determine the potential of *Bacillus* spp. in suppressing the growth of the *C. capsici* fungus and stimulating the growth of red chili. This study used a Completely Randomized Design (CRD) with the treatment without *Bacillus* spp., chemical fungicides, *Bacillus* sp. (Ba-6), *Bacillus* sp. (Ba-9), *Bacillus* sp. (Ba-12), *Bacillus* sp. (Ba-15), and *Bacillus* sp. (Ba-17). The treatment was repeated four times, and the treatment unit contained five red chili/polybags. The results showed the highest suppression of *C. capsici* in treating *Bacillus* sp. Ba-15 was 6.25% compared to the negative control. Bacillus sp is the best bacteria that can stimulate red chili growth. Ba-9 has an average plant height of 20.10 cm and an average number of leaves of 10.49.

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Keyword
Bacillus spp;
Colletotricum capsica;
Anthracnose;
Red Chili.

Introduction

Red chili (*Capsicum annum* L.) is one of the important horticultural commodities in Indonesia, which has high economic value. The demand for chili products will continue to increase along with the increase in population. National chili demand in one year for big cities is around 800,000 tons/year and will increase by about 10-20% on religious holidays; therefore, this commodity has a good opportunity to be developed (Anonim, 2012). Chili cultivation is influenced by several factors that can decrease its production. These results caused that the productivity of red chili in Indonesia is still far from its potential, which can reach 12-20 tons/ha (Syukur *et al.,* 2010). One of the main factors causing the low productivity of chili in Indonesia because the pathogens that cause plant diseases.

The primary disease in red chili is Anthracnose, caused by the *Colletotrichum capsici* pathogenic fungi. Anthracnose can cause the chili shoots on mature plants to die, followed by infection in the fruit. This problem causes chili productivity to decrease (Prasetyo, 2017). Anthracnose disease in chili can cause yield losses of up to 90%, especially during the rainy season (Badan Pusat Statistik, 2018).

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Anthracnose is the most common disease and almost occurs in every chili-growing area. Pathogen infection can occur from the plant in the field until the plant is harvested because it can reduce production in quality and quantity. A severe level of attack can make the plant die. Attacks on fruit can result in fruit damage and a decrease in the quality of chili, so their economic value is also low (Nurhayati, 2011).

To control Anthracnose, we generally still use chemical fungicides because it is considered easy and effective. Using chemical fungicides in the long term will harm the environment and humans. An alternative to reduce the use of chemical fungicides is to use biological fungicides (Elfina *et al.,* 2015). Several studies have been conducted to control Anthracnose. One study uses biological fungicides that are more environmentally friendly and harmless to consumers, including *Bacillus spp.* (Putro *et.al.,* 2014).

Bacillus is a biological agent that has the potential to be developed as an antifungal. *Bacillus* has been widely used because it can produce antimicrobials that can suppress the growth of various plant pathogens and belong to the group of PGPR (*Plant Growth Promoting Rhizobacteria*) bacteria that can stimulate plant growth (Shafi *et al.,* 2017; Fira *et al.,* 2018). *Bacillus* has advantages compared to other bacteria and can produce endospores for surviving structures when environmental conditions are unfavorable (Prihatiningsih *et al.,* 2014). This study aims to determine the potential of *Bacillus* spp. to suppress the growth of *C. capsici* fungi and stimulate the growth of red chili.

Materials and Methods

This research was conducted from January to April 2021 in the Plant Health Laboratory and screen house of the Faculty of Agriculture, University of Pembangunan Nasional "Veteran" Jawa Timur.

The materials used in this study were *Bacillus* spp. from Kediri Ba-6, Ba-9, Ba-12, Ba-15, and Ba-17 isolates, a collection of Dra. Endang Triwahyu P., M.Si., *Colletotrichum capsici* isolates, NA media, PDA media, aquades, cotton, tissue, label paper, alvaboard, 70% alcohol, spirtus, aluminum foil, wrap, NB media, garden soil, compost, NPK fertilizer, 5% formalin, chemical fungicide active ingredient benomyl, and healthy chili seeds of Gada MK variety.

This study was arranged in a single, factor Completely Randomized Design (CRD) with seven treatments. There are Kn (control without treatment), Kp (control of chemical fungicides), S1 (*Bacillus* sp. Ba-6 isolate), S2 (*Bacillus* sp. Ba- 9 isolate), S3 *(Bacillus* sp. Ba-12 isolate), S4 (*Bacillus* sp. Ba-15 isolate), S5 *(Bacillus* sp. Ba-17 isolate). Each treatment was repeated four times, so the total number of experiments was 28 units. Each experimental unit consisted of five red chilies.

Preparation of Planting Media

The planting media used were soil and compost with a ratio of 1:1. The sterilization of the planting media refers to the method (Musafa *et al.,* 2015). The media was sterilized using 5% formalin at a dose of 2.5 ml/kg, and this sterilization process lasted for 15 days. Then, the sterile media was distributed into polybags.

Production of *Bacillus* spp. Suspension

Bacillus spp. cultures grown on slanted NA media were taken ten *ose* needles and put into an Erlenmeyer containing 60 ml of NB media. Then homogenize it using a vortex for 30 minutes and incubate it for 20 hours. The bacterial suspension density was 10^9 cfu/ml (Putro *et al.,* 2014).

Production of *C. capsici* Suspension

Fungi cultures grown on PDA media aged seven days were taken by adding 10 ml of sterile aquades and then rubbed with an *ose* needle. Then put it into an Erlenmeyer containing 40 ml of sterile aquades and homogenize it using a vortex for several minutes. The fungi suspension was taken using a pipette, and the amount of conidia density was calculated using a *Neubauer hemocytometer*. The appropriate density for fungal suspension was 10^6 conidia/ml (Putro *et al.,* 2014).

Testing In Vivo *Bacillus* spp. to The *C. capsici* fungi on Red Chili

This treatment was carried out by soaking the roots of chili seedlings aged 24 days in *Bacillus* spp. suspension in a 10^9 cfu/ml density for 30 minutes. Immersion of red chili seeds in the negative control treatment with sterile aquades, while in the positive control, the chili seeds were applied a chemical fungicide by the active ingredient benomyl using the dosage recommended on the label. Then, the seeds are planted in polybags containing sterile media. The chili plants were observed for five weeks after planting (WAP). Watering plants carried out the treatment of plants in the morning by paying attention to soil moisture and fertilizing with 2 grams of NPK fertilizer in each plant every two weeks (Purwanto, 2020).

Observation Variable

The observations were made to determine the growth and resistance of red chili.

1. Observations on the growth of red chili included plant height and number of leaves. Observations were made starting after inoculation of *Bacillus* spp. up to 35 days at seven-day intervals.
2. Observation of plant resistance included incubation period and intensity of disease attack. The disease attack was observed every week from the day after *C. capsici* inoculation until five weeks. The following formula calculates Disease Intensity (%):

$$I = \frac{\Sigma ni.vi}{N.Z} \times 100\%$$

Information:

I = Disease intensity

ni = The number of each symptom area class (each treatment five chilies)

vi = i-th damage category

N = number of fruits observed

V = highest value of attack category

The value of the attack category (score) to Anthracnose is based on the scale of plant damage affected by the disease (Jauhari and Majid, 2019). The value of the attack category (scores) is as follows:

0 = No damage
1 = Leaf spot 1%-25%
2 = Leaf spot 26%-50%
3 = Leaf spot 51%-75%
4 = Leaf spot 76%-100%

The criteria for red chili resistance to Anthracnose are as follows (Mufida, 2020):

0 % -10 % = Very Resistant
11 % -20 %= Resistant
21% - 40% = Moderate
41 % - 70 % = Susceptible
X > 70% = Very Susceptible

The data obtained is then analyzed by Analysis of Variance (ANOVA) to determine whether each treatment has an effect. If it is significantly different, it is continued with the 5% Least Significance Different (LSD) test.

Results and Discussion

The growth of Red Chili

Red chilies were damaged, so the data presented in plant growth only reached the third observation (3 WAP). The results showed that the bacteria *Bacillus* spp. potential to increase plant growth. The treatment of *Bacillus* spp. significantly differed in plant height at the ages of 2 WAP and 3 WAP (Table 1). The treatment of *Bacillus* spp. significantly differed in the number of leaves in red chili at the second observation (2 WAP). Meanwhile, in the third observation, the treatment of *Bacillus* spp. was not significantly different in the number of leaves in red chili. The average number of leaves in red chili is shown in Table 2.

Table 1. An Average of Plant Height in Red Chili

Treatments	Plant Height (cm)		
	1 WAP	**2 WAP**	**3 WAP**
Kn (control without treatment)	8,00	15,01 **d**	18,96 **abc**
Kp (control of chemical fungicides)	7,03	14,37 **cd**	18,08 **ab**
Bacillus sp. Ba-6 isolate	7,85	13,91 **bc**	18,60 **ab**
Bacillus sp. Ba-9 isolate	8,38	13,85 **bc**	20,10 **c**
Bacillus sp. Ba-12 isolate	7,92	13,02 **ab**	19,36 **bc**
Bacillus sp. Ba-15 isolate	7,86	13,17 **ab**	18,38 **ab**
Bacillus sp. Ba-17 isolate	8,23	12,57 **a**	18,01 **a**
LSD 5%	NSD	0,97	1,31

Note: The numbers followed by the same letter in the same column are not significantly different in the 5% LSD test. NSD = not significantly different

Table 2. An Average Number of Leaves in Red Chili

Treatments	Number of Leaves		
	1 WAP	2 WAP	3 WAP
Kn (control without treatment)	5.40	8.55 **c**	10.35
Kp (control of chemical fungicides)	5.35	8.25 **abc**	9.93
Bacillus sp. Ba-6 isolate	5.55	8.35 **bc**	10.28
Bacillus sp. Ba-9 isolate	5.50	8.65 **c**	10.49
Bacillus sp. Ba-12 isolate	5.40	7.80 **a**	10.31
Bacillus sp. Ba-15 isolate	5.40	8.20 **abc**	10.08
Bacillus sp. Ba-17 isolate	5.35	7.90 **ab**	9.98
LSD 5%	NSD	0.49	NSD

Note: The numbers followed by the same letter in the same column are not significantly different in the 5% LSD test. NSD = not significantly different

Based on the results obtained regarding the effect of applying *Bacillus* spp. on the growth of red chili, the *Bacillus* spp was shown. Isolate had different effects on plant height growth (Table 1) and the number of leaves (Table 2). It is suspected that this is due to *Bacillus* spp.'s ability to develop in different plant tissues. The average plant height and number of leaves in the third week showed that the best result is *Bacillus* sp. Ba-9 isolate with an average plant height of 20.10 cm and a number of leaves of 10.49. Besides being able to inhibit the growth of pathogens, antagonist bacteria are also widely known as PGPR (Plant Growth Promoting Rhizobacteria) (Jatnika *et al.,* 2013). This shows that *Bacillus* sp. Ba-9 isolate has the potential to stimulate plant growth. Previous studies have shown that *Bacillus subtilis* isolate is able to synthesize Indole Acetic Acid (IAA) and gibberellins (Yulistiani, 2015). IAA hormone is a hormone that plays a role in plant growth and development; therefore, certain synthesized bacteria cause increased plant growth (Herlina *et al.,* 2016). The Ba-9 isolate was suspected that this isolates able to synthesize the highest IAA and gibberellin hormones, so it was the best isolate for increasing the growth of red chili.

The Resistance of Red Chili to Anthracnose Disease

Bacillus spp. has the potential to increase plant resistance to pathogens because *Bacillus* spp. able to colonize plant tissue (Suwarno and Masnilah, 2020), so it can enter into plant tissue to infect and minimize pathogens. Colonization that occurs in the roots can trigger plants to produce jasmonic acid and plant ethylene to induce plant resistance to pathogens (Djaenudin, 2016).

Based on observations, the incubation period or the appearance of symptoms on the third day after treatment. Figure 1 shows a comparison between healthy red chili leaves and those infected with Anthracnose. The leaves that are infected by Anthracnose symptoms have round spots with a light gray to white center and dark brown edges.

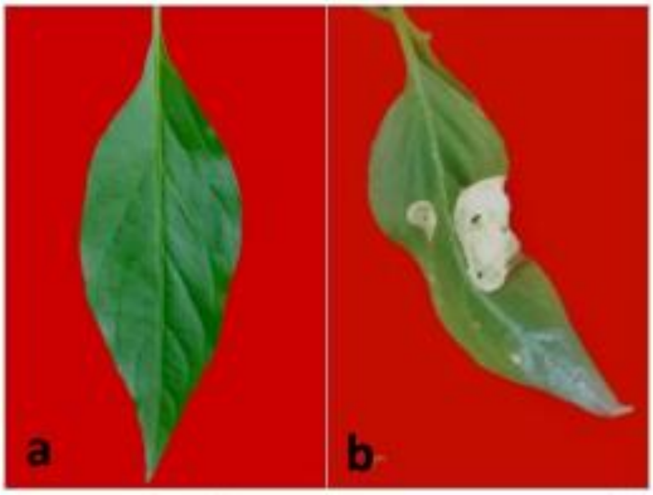

Figure 1. Comparison of Red Chili Leaves (a) Healthy Leaves (b) Anthracnose Infected Leaves

Based on observations, the incubation period of red chili is relatively fast. The results of previous studies stated that the fastest incubation period of *C. capsici* in chili was 12 days (Herwidyati *et al.,* 2013). It is suspected that this occurs because influenced by environmental conditions there are temperature and humidity that support the growth, reproduction, and development of the pathogen. The development of the pathogen and the severity of the disease are affected by high humidity. The environmental conditions during the treatment were suitable and supported the growth of *C. capsici*. There is an average temperature between 24^{o}C–30^{o}C with a high relative humidity of 80%–90%.

Symptoms of Anthracnose found in red chili can be seen in Figure 2. Anthracnose can cause death in chili shoots which are characterized by dried chili shoots and blackish-brown color (Figure 2a). Symptoms of Anthracnose on red chili leaves include yellowish white leaf spots on the edges, but the deeper the leaves get whiter and look like burning, in the middle of the spots, there are black dots which are the acervulus by *C. capsici* fungal (Figure 2b). Furthermore, the symptoms of Anthracnose on the stems of red chili are characterized by dry stems, and there is acervulus fungal that look like black bulges (Figure 2c).

Figure 2. Symptoms of Anthracnose on Red Chili (a) Symptoms on Shoots (b) Symptoms on Leaves (c) Symptoms on Stems

The observation of symptoms on stems was carried out further to ensure that symptoms in chili caused due to *C. capsici* infection. The results showed that on the stems, there were acervulus protrusions which, when observed using a microscope at 10x10 magnification, looks like blackthorns (Figure 3a). Microscopic observations were continued by making symptomatic preparations on the stems. The results of microscopic observations at 40x10 magnification showed that the conidia were crescent-shaped, not insulated, and there were acervuli which are characteristic of the *C. capsici* (Figure 3b). Red chili that shows symptoms of the disease is then re-isolated (Figure 3c).

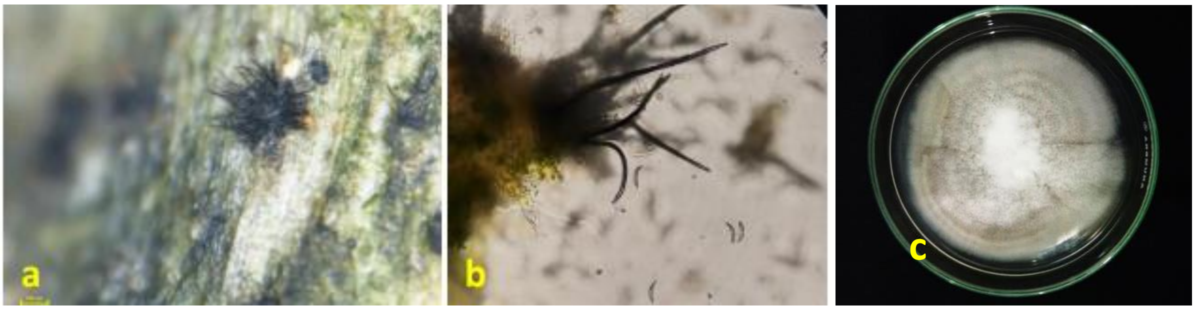

Figure 3. (a) Microscopic direct observations on the stem at 10x10 magnification (b) microscopic observations using a glass object at 40x10 magnification (c) The results of PDA media re-isolated in 7 days aged.

The next observation of plant resistance was carried out by observing the disease intensity of red chili. Observations were made five times every seven days. The results of observations showed that the development of disease intensity (%) of Anthracnose on plants of red chili with *Bacillus* spp. was not significantly different (Figure 4).

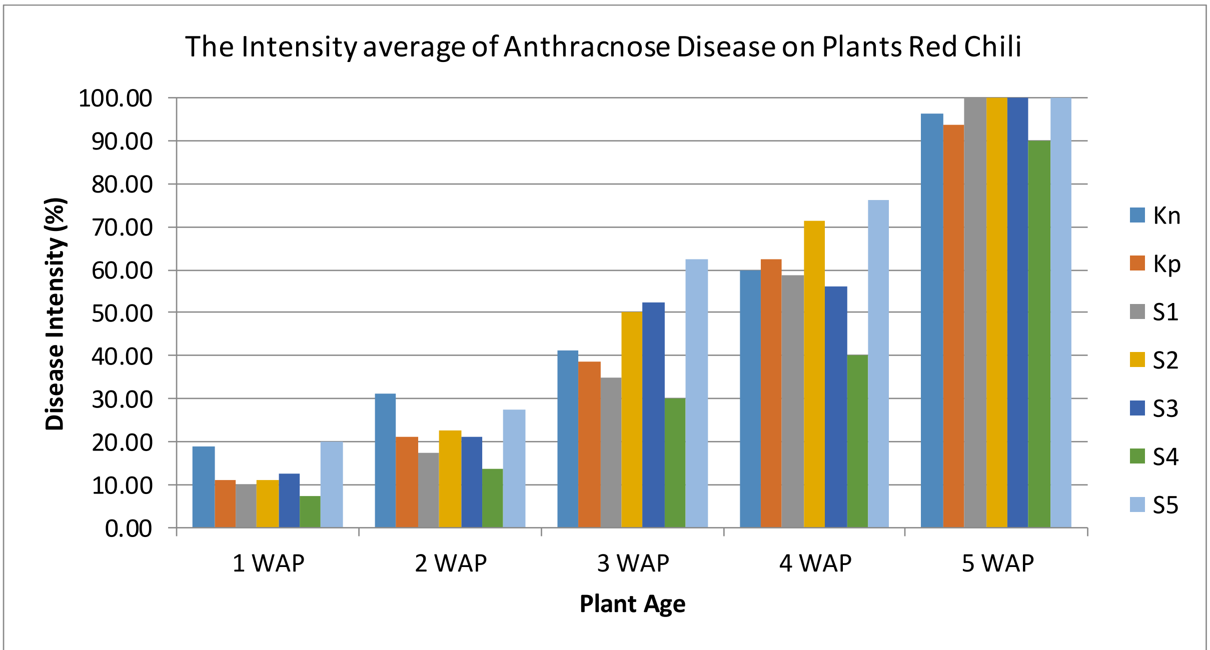

Figure 4. The Histogram of Treatment Effect on The Intensity Average of Anthracnose Disease on Plant Red Chili

Description: Kn=Control without treatment, Kp=Control of chemical fungicides, S1= *Bacillus* sp. Ba-6 isolate, S2= *Bacillus* sp. Ba-9 isolate, S3= *Bacillus* sp. Ba-12 isolate, S4= *Bacillus* sp. Ba-15 isolate, S5= *Bacillus* sp. Ba-17 isolate.

The lowest Anthracnose disease intensity was found in the *Bacillus* sp. Ba-15 isolate. Observations in the second week showed that the intensity of Anthracnose disease ranges from 21% -40%, which indicated that the plant resistance was moderate. Then in the third week, plant resistance to chemical fungicide control treatment, *Bacillus* sp. Ba-6 isolate and Ba-15 were moderate, with the intensity of Anthracnose disease ranging from 21-40%. While in the control without treatment, *Bacillus* sp. Ba-9, Ba-12, and Ba-17 isolates were classified as susceptible because the intensity of Anthracnose disease ranges from 41%-70%. The fourth-week observation showed that plant resistance was very susceptible because the intensity of Anthracnose disease ranges from >70%, except for the *Bacillus* sp. Ba-15 isolate, which has a disease intensity of 40%, is classified as moderate. The fifth week of observation showed that plant resistance in all treatments was very susceptible because the intensity of the disease was >70%.

The results of the observation of disease intensity are suspected to indicate that *Bacillus* spp. capable of stimulating the increased resistance of plants in red chili. The observations from the first week to the fifth week showed that the intensity of plant disease given *Bacillus* sp. isolate Ba15 treatment was lower than the Kn treatment (control without treatment). The mechanism of plant resistance that occurs in red chili is suspected to be resistance induction of ISR (Induced Systemic Resistance). According to (Prihatiningsih *et al.,* 2017), the tested *B. subtilis* was able to act as a siderophore producer qualitatively. The ability of bacteria to produce siderophores is an important component in PGPR because siderophores are able to bind iron (Fe^{3+}) into siderophores-iron bonds that become available to plants. The siderophores that are produced by microorganisms are beneficial for plants because they can inhibit the growth of pathogens. There is a lack of Fe^{3+} needed by pathogens because Fe^{3+} is already bound by siderophores (Sharma and Johri, 2003). Iron is an important element in disease development. Therefore with the binding of iron by siderophores, the pathogen is less able to infect, thus inhibiting the disease development.

The increase in disease intensity from the first week of observation to the fifth week of observation is suspected because of environmental conditions that support the growth of pathogens. In addition, the *Bacillus* spp. bacteria suspected that the colonization in plant tissue had not been maximized; therefore, plant resistance and the antagonistic mechanism of *Bacillus* spp. Not maximal. The observation of plant intensity in the third week showed that symptoms of Anthracnose began to appear on the stems. As a result, the intensity of Anthracnose disease in some red chili has reached 100%.

Conclusion

Ba-9 isolate of *Bacillus* spp. bacteria showed the best results in stimulating the growth of chili with the parameters of plant height and the highest number of leaves, with an average plant height of 20.10 cm and an average number of leaves of 10.49. The application of *Bacillus* spp. able to suppress the growth of *C. capsici* pathogenic fungi in red chili with the best results in the treatment of *Bacillus* sp. Ba-15 isolate which able to suppress the growth of *C. capsici* by 6.25% compared to the negative control.

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