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EFFECT OF GROWING MEDIA ON THE GROWTH OF *Streptomyces* sp. AND *Trichoderma harzianum* AS ANTHRACNOSE DISEASE BIOCONTROL

Pengaruh Media Tumbuh Terhadap Pertumbuhan *Streptomyces* sp. dan *Trichoderma harzianum* sebagai Biokontrol Penyakit Antraknosa

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ABSTRACT
Streptomyces sp. and *Trichoderma harzianum* is a biological agent that is effective in controlling *Colletotrichum capsici*, the cause of disease in chili plants. Different growing media can affect the growth and activity of these two microorganisms. This study aims to evaluate the growth of *Streptomyces* sp. and *Trichoderma harzianum* on various media, namely rice washing water, coconut water, and sugar potato extract. The research phase includes growing media test, effectiveness test of *Streptomyces* sp. and *Trichoderma harzianum* in chili peppers, and effectiveness test of *Streptomyces* sp. and *Trichoderma harzianum* on chili seeds. The results showed that the medium of rice washing water is more suitable for *Streptomyces* sp., coconut water media is more suitable for *Trichoderma harzianum*, and rice washing water media is more suitable for the combination of the two biological agents. Optimal growth in these media affect the effectiveness of biocontrol in suppressing the severity of anthracnose disease in chili.

Keywords: *Streptomyces* sp., *Trichoderma harzianum, Growing media, Colletototrichum capsici, Biocontrol*

ABSTRAK
Streptomyces sp. dan *Trichoderma harzianum* merupakan agens hayati yang efektif dalam mengendalikan *Colletotrichum capsici,* penyebab penyakit pada tanaman cabai. Media tumbuh yang berbeda dapat mempengaruhi pertumbuhan dan aktivitas kedua mikroorganisme ini. Penelitian ini bertujuan untuk mengevaluasi pertumbuhan *Streptomyces* sp. dan *Trichoderma harzianum* pada berbagai media, yaitu air cucian beras, air kelapa, dan ekstrak kentang gula. Tahap penelitian meliputi uji media tumbuh, uji efektifitas *Streptomyces* sp. dan *Trichoderma harzianum* pada buah cabai, dan uji efektifitas *Streptomyces* sp. dan *Trichoderma harzianum* pada benih cabai. Hasil penelitian menunjukkan bahwa media air cucian beras lebih sesuai untuk *Streptomyces* sp., media air kelapa lebih sesuai untuk *Trichoderma* harzianum, dan media air cucian beras lebih sesuai untuk kombinasi kedua agens hayati tersebut. Pertumbuhan yang optimal pada media ini berpengaruh terhadap efektifitas biokontrol dalam menekan keparahan penyakit antraknosa pada cabai.

Kata kunci: *Streptomyces* sp., *Trichoderma harzianum*, *Media tumbuh, Colletototrichum capsici, Biocontrol*

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INTRODUCTION

Anthracnose is one of the diseases in chili plants that has the potential to cause losses of 60%, even if proper control is not carried out, the loss of results reaches 100% (Nurjasmi and Suryani, 2020). Anthracnose disease in chili plant is generally caused by the fungus *Colletotrichum capsici*. This fungus attacks almost all parts of the chili plants. Some typical symptoms in anthracnose disease, among others, in infected fruits are characterized by concave, necrotic, circular to angular lesions with concentric rings of black acervuli (Jojy *et al*., 2024).

The use of biological agents such as *Streptomyces* sp. and *Trichoderma harzianum* is an environmentally friendly alternative in controlling anthracnose disease in chili plants. From the results of research by Shahfitri et al. (2018) showed that chitinolytic bacterial isolates that have been formulated have the potential to be used as biological control agents against *C. capsica*, the cause of anthracnose in chili plants. Research of Agnihotri *et al.,* (2024) proved that *Trichoderma harzianum* effectively suppresses the growth of *Colleotrichum capsici* in vitro.

The effectiveness of biological agents is also strongly influenced by the growing media used in propagation. According to Jumadi *et al.,* (2021), biological agents that will be applied to planting media should be mass propagated in alternative growing media that can support the growth and development of biological agents in order to have better adaptability to the new environment.

Growing media for biological agents has an important role in supporting their growth and development. Some natural ingredients that can be used as an alternative to growing media include rice washing water, coconut water and sugar potato extract. Considering that the nutrient content in these three media can support the growth of *Streptomyces* sp. and *Trichoderma harzianum*. Rice washing water contains organic compounds and minerals such as carbohydrates, nitrogen, phosphorus, potassium, magnesium, sulfur, iron, glutein, cellulose, hemi cellulose protein, and thiamin (Sudartini *et al*., 2020; Ali *et al*., 2023). While coconut water contains 94% water and 5% sugar (aldohexose, fructose, and disaccharides), 0.02% protein, and 0.01% lipid (Tuyekar *et al*., 2021). And according to Al Chalik *et al.,* (2021), potato extract is quite high in carbohydrates. Potatoes have a fairly high water content about 75%, a source of vitamins C, B1, B2, as well as several types of minerals such as phosphorus, iron and calcium.

This study aims to determine the effect of growing media on the growth of *Streptomyces* sp. and *Trichoderma harzianum*, as well as its effectiveness in controlling anthracnose disease in chili plants.

MATERIALS AND METHODS

Place and time of research

This research will be carried out from July 2024 to November 2024, at The Plant Health Laboratory of the Faculty of Agriculture, Pembangunan Nasional "Veteran" Jawa Timur University.

Material

The materials used were aquades, alcohol 70%, PDA media, GNA media, sugar potato extract media, the first rice washing water, young coconut water, and *Colletotrichum capsici* a pathogen isolates from exploration of chili plant land in Bangeran village, Mojokerto, East Java. *Streptomyces* sp. S6 isolates from the exploration of the soil rhizosphere of chili plants by Hakim, L. (2023) and *Trichoderma harzianum* isolate TM which is the result of exploration of red onion leaves by Ummah, R. (2023). Chili fruit used to test the effectiveness of the biocontrol agent is a local chili fruit measuring about 30-40 mm. And the chili seeds used to test the effectiveness of biocontrol agent are local chili seeds obtained from local farmers sampling locations.

The tools used are petri dish, ose needle, cork borer, pipette, tweezers, erlenmeyer, autoclave, scapel knife, microscope, digital scales, Laminar air flow, Shaker, Bunsen, test tube, micropipette, haemocytometer and stationery.

Method

1. Growing media Test

Preparing the culture of *Streptomyces* sp. (14 days) and *Trichoderma harzianum* (7 days). Preparing sugar potato extract media

and young coconut water media. Meanwhile, the rice washing water used is taken at the first rice washing. All alternative media materials are then sterilized in an autoclave. The next step, slice of *Streptomyces* sp. (15 plong) and *Trichoderma harzianum* (12 plong) were inoculated into each 100 ml of liquid sugar potato extract media, rice washing water media and young coconut water media. The suspension are incubated in a shaker at a speed of 150 rpm at room temperature for 6 weeks.

Observation of the microorganism population was conducted on the 1st week to the 6th week after inoculation, with the pour plate method, by taking 1 ml of suspension of biocontrol agents in each treatment and was invested in 10 ml of warm liquid PDA media (50°C), after which it was poured on a sterile petri dish. The number of colonies on PDA media on each treatment was observed (Fitriana et al., 2019).

This growing media test use 2 factors, namely the type of biological agents (*Streptomyces* sp., *Trichoderma harzianum* and the combination of them) and types of growing media (rice wash water, coconut water and sugar potato extract), this test were prepared using a complete randomized design, so there are 9 combinations of treatments with repeat 4 times. The observation parameter in this growing media test is the number of colonies of *Streptomyces* sp., and *Trichoderma harzianum* in each of the growing media.

2. Effectiveness Test against anthracnose disease in chili

The effectiveness of *Streptomyces* sp. and *Trichoderma harzianum* against anthracnose disease in this chili fruit using the best 2 treatments from the test results of growing media, namely a combination treatment *Streptomyces* sp. and *Trichoderma harzianum* with rice washing water media and sugar potato extract media.

This effectiveness test was conducted by spraying a suspension of biocontrol agents to the surface of the chili fruit. Chili fruit that has been treated was dried, and put into a petri dish, then closed for 1 day. Next, the chili fruit was injected with the suspension of 0.01 ml *C. capsici* (106 conidia/ml) on the surface of chili fruit and incubated for 10 days.

This effectiveness test use a complete randomized design with 3 treatments, namely control treatment (without suspension of biocontrol agents), the combination suspension of *Streptomyces* sp. + *Trichoderma harzianum* in rice washing water media, and the combination suspension of *Streptomyces* sp.+ *Trichoderma harzianum* in sugar potato extract media. Each treatment consists of 4 repetitions and each repetition consists of 5 chili. Observation was carried out on the 7th day after inoculation and the 14th day after inoculation. Observational parameters are symptoms score assessment using disease severity formula.

To calculate the severity of anthracnose disease in chili fruits using the following formula:

$$\mathrm{KP} = \sum \frac{n\ x\ v}{N\ X\ Z} \times 100\%$$

Note:
n = number of fruits per spotting class
v = score value of each spotting class
N = number of fruits observed
Z = the score value of the highest spotting area class.

To determine the severity of the disease using scoring values of (Wartono, 2021) with modifications, namely:
Score 0 = healthy chili fruit without disease symptoms;
Score 1 = chili fruit with symptoms in the form of 0-3mm wide black spots;
Score 2 = chili fruit lesions on one side of the fruit without acervulus area of 3-7. 5 mm;
Score 3 = chili with lesions, there is little acervulus and concentric area of 7.5- 15mm;
Score 4 = chili with lesions, there are many black acervules with concentric circles of 15-30mm;
Score 5 = chili with larger lesions and dry > 30mm.

In addition, to determine the criteria for chili damage based on the severity of the disease, a modified method was used according to Sarianti & Subandar (2022) in-Table 1.

Table 1. Criteria for the damage of the chili based on the severity of the disease

Score	% Severity	Criteria	Category
1	0-20	Very low	Resistance
2	21-40	Low	Somewhat resistant
3	41-60	Medium	Somewhat resistant
4	61-80	Height	Vulnerable
5	81-100	Very high	Very vulnerable

3. Effectiveness Test against anthracnose disease in chili seeds

This effectiveness test was conducted to determine the incidence of disease (IP) fallen sprouts on chili seeds given biocontrol agent treatment. The disease incidence (IP) of fallen sprouts is calculated using the following formula:

$$IS = (n/ N) \times 100\%$$

IS = attack intensity (%)
n = number of symptomatic plants
N = the number of plants in treatment

The surface of the chili seeds are sterilized with 1% NaOCl, then soaked in sterile warm water. The chosen seed is the sunken seed. The selected seeds are soaked in the suspension of *C. capsici* (10^6 conidia/ ml) for 30 minutes, then let it stand for some time before soaking with each biocontrol agent treatment for 6 hours. Seeds that have been treated are planted in polybags containing planting media (soil and compost, 2:1) that have been sterilized using an autoclave.

This study used a complete randomized design with 3 treatments, namely control treatment (without suspension of biological agents), the combination suspension of *Streptomyces* sp. + *Trichoderma harzianum* in rice washing water media, and the combination suspension of *Streptomyces* sp.+ *Trichoderma harzianum* in sugar potato extract media. Each treatment consisted of 4 Tests and each test consisted of 5 chili seeds. Observations were made at 7hst, 14hst and 21hst.

RESULTS AND DISCUSSION

1. Growth test results of *Streptomyces* sp. and *Trichoderma harzianum* on growing media

a. Results obeservations of the growth *Streptomyces* sp. in sugar potato extract media, rice washing water media and coconut water media are presented in the Figure 1.

In Figure 1. shows that the highest number of *Streptomyces* sp. colonies are in the medium of rice washing water (S ACB). Rice washing water contains various elements that can support the growth of *Streptomyces* sp., among them are carbohydrates, protein in small amounts, and mineral elements such as potassium, magnesium, and phosphorus. Carbohydrate content in the form of starch and sugar dissolved in rice washing water is thought to be used by *Streptomyces* sp. as the main source of energy. While the content of protein / amino acids that are soluble in rice washing water, is thought to be used by *Streptomyces* sp. for the synthesis of proteins and enzymes. Mineral elements such as potassium, magnesium and phosphorus that are also dissolved in rice washing water were thought to be able to support cellular metabolism of *Streptomyces* sp. According to Sudartini *et al*., (2020), rice wash water contains organic and mineral compounds such as carbohydrates, nitrogen, phosphorus, potassium, magnesium, sulfur, iron, glutein, cellulose, hemi cellulose protein, and thiamin.

Streptomyces sp. has the ability to produce extracellular enzymes that can digest starch into simple sugars, so the medium of rice washing water provides a good source of energy for its growth. The mineral content contained in rice washing water is also thought to support the activity of important enzymes for the synthesis of secondary metabolites, such as antibiotics that are characteristic of *Streptomyces* sp. *Streptomyces* sp. requires a neutral to slightly acidic pH (6-7), which generally corresponds to the pH of rice washing water. This statement is corroborated by the results of research Kumar *et al*.,

(2024) proved that *Streptomyces fradiae* isolated from soil can grow in the medium of rice washing water. Rice washing water is proven to be an alternative growing medium for commercial growth media, with good lipid and productivity results that produce 85.4% fatty acids and 60.3 g/L biomass.

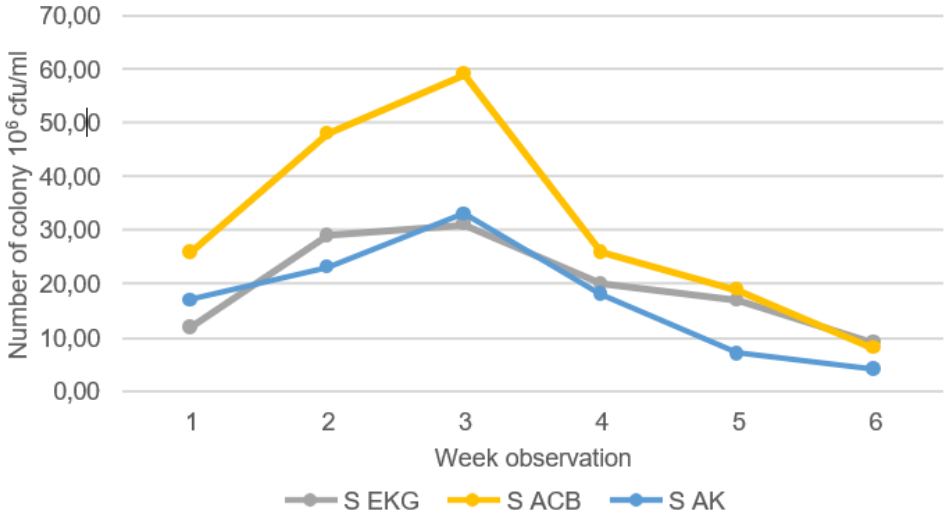

FIgure 1. Growth dynamics of Streptomyces sp. on alternative growing media
Note: S EKG =*Streptomyces* sp. in sugar potato extract media; S ACB = *Streptomyces* sp. in rice washing water media; S AK= *Streptomyces* sp. the coconut water media

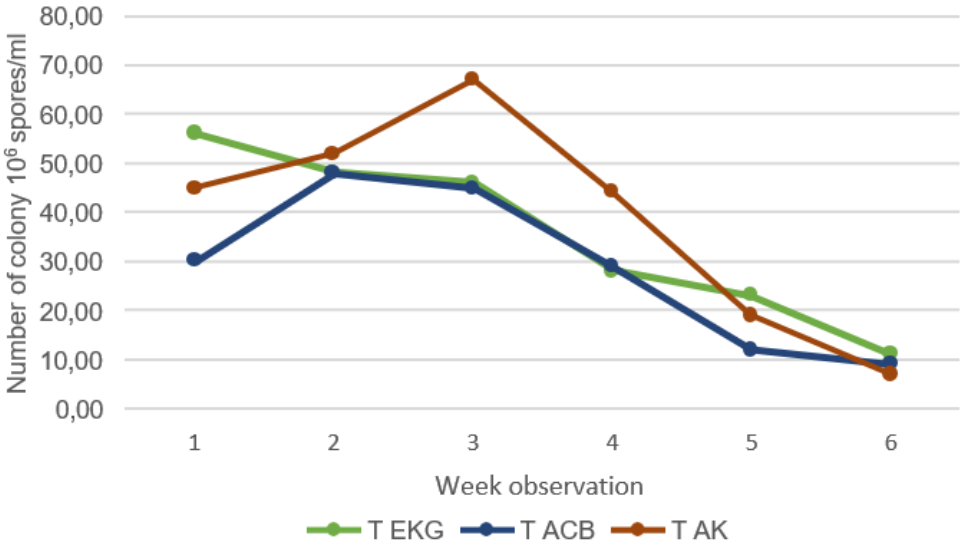

Figure 2. Growth dynamics of Trichoderma harzianum on alternative growing media
Note: T EKG = *Trichoderma harzianum* in sugar potato extract media; T ACB = *Trichoderma harzianum* in rice wash water media; T AK= *Trichoderma harzianum* in coconut water media.

b. The growth observation results of *Trichoderma harzianum* on sugar potato extract media, rice washing water media and coconut water media are presented in Figure 2.

Based on Figure 2. shows that the highest number of *Trichoderma harzianum* colonies are in coconut water media (T AK) compared to the other two growing media. In coconut water media there are nutrients such as glucose and fructose, minerals, B vitamins and amino acids. The content of glucose and fructose in coconut water is thought to be used by *Trichoderma harzianum* as the main source of energy, because it can

support the rapid growth of *Trichoderma harzianum*. The content of amino acids, vitamins, and minerals in coconut water is thought to increase metabolism and spore production of *Trichoderma harzianum*. Based on nutritional content coconut water contains 94% water and 5% sugar (aldohexose, fructose, and disaccharides), 0.02% protein, and 0.01% lipid (Tuyekar *et al.,* 2021). Research results of Boblina *et al*., (2020) proved that coconut water can increase the number of colonies of *Trichoderma* spp., because the water- soluble material is capable of being used by Trichoderma for growth or also called LSF (Liquid State Fermentation). And the research results of Triasih *et al*. (2021) states that the growth of *Trichoderma* sp. in the formulation of liquid media coconut water and rice washing water were increased between 2.53-9.19 times compared to control media that only use aquades.

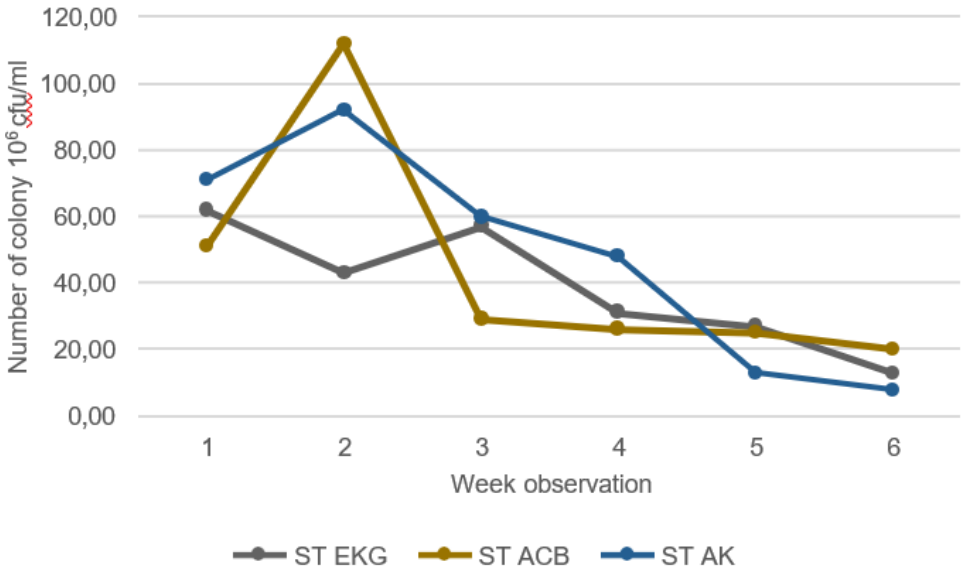

Figure 3. Combined growth dynamics of Streptomyces sp. and Trichoderma harzianum on alternative growing media

Note: ST EKG = Streptomyces sp. and Trichoderma harzianum in sugar potato extract media;
ST ACB = Streptomyces sp. and Trichoderma harzianum in rice wash water media;
ST AK= Streptomyces sp. and Trichoderma harzianum in coconut water media

c. The results observations of the growth of *Streptomyces* sp. and *Trichoderma harzianum* on sugar potato extract media, rice washing water media and coconut water media are presented in Figure 3.

Based on Figure 3 shows that the largest number of colonies are the combination treatment of *Streptomyces* sp. and *Trichoderma harzianum* is on the medium of rice washing water (ST ACB). *Streptomyces* sp. and *Trichoderma harzianum* has a different growth mechanism. *Streptomyces* sp. Requires complex carbons to form filamentous mycelium, while *Trichoderma harzianum* makes faster the use of simple sugars. In rice washing water complex carbohydrates are available, thus supporting the growth of both biological agents optimally. According to Hairuddin *et al*., (2018), the content possessed by rice washing water includes carbohydrates, nitrogen, phosphorus, potassium, magnesium, sulfur, iron, and vitamin B1.

Coconut Water media and sugar potato extract media are rich in simple sugars, so it was more suitable for *Trichoderma harzianum*, and *Streptomyces* sp. may be less optimal. *Streptomyces* sp. and *Trichoderma harzianum* is more optimal in rice washing water media, allegedly because the media provides gradual nutrition, supports the compatibility of the two and has a stable environment. Coconut water media and sugar potato extract media tend to be more

supportive of *Trichoderma harzianum* than *Streptomyces* sp., so that the growth of the combination of the two is less than optimal.

Based on the observation data, the treatment by the different types of media shows significantly on the number of colonies of the biocontrol cal agents (Table 2). In combination with *Streptomyces* sp. and *Trichoderma harzianum* showed that the average number of colonies tended to be more than the growth of colonies that were not combined. The test results of growing media are presented in Table 2.

Table 2. The colony growth of Streptomyces sp. and Trichoderma harzianum on alternative growing media for 6 weeks

Treatment	Average density of spores/colony x10^6					
	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**
S EKG	12a	29a	31a	20ab	17b	9a
S ACB	17ab	48ab	59bc	26b	19b	8a
S AK	26bc	23a	33a	18a	7a	4a
T EKG	56cd	48ab	46b	28b	23c	11ab
T ACB	30bc	48ab	45b	29b	12ab	9a
T AK	45cd	52b	67c	44c	19b	7a
ST EKG	62d	43a	57bc	31b	27c	13ab
ST ACB	51cd	112c	29a	26b	25c	20b
ST AK	71d	92bc	60bc	48c	13ab	8a

Note: S EKG = *Streptomyces* sp. in sugar potato extract media; S ACB = *Streptomyces* sp. in rice washing water media; S AK= *Streptomyces* sp. in the coconut water. T EKG = *Trichoderma harzianum* in sugar potato extract media; T ACB = *Trichoderma harzianum* in rice wash water media; T AK= *Trichoderma harzianum* in Coconut Water media. ST EKG = *Streptomyces* sp. and *Trichoderma harzianum* in sugar potato extract media; ST ACB = *Streptomyces* sp. and *Trichoderma harzianum* in rice wash water media; ST AK= *Streptomyces* sp. and *Trichoderma harzianum* in coconut water media.

At the 1st week showed that the *Streptomyces* sp. in sugar potato extract media treatment was significantly different from the *Streptomyces* sp. and *Trichoderma harzianum* in sugar potato extract media and *Streptomyces* sp. and *Trichoderma harzianum* in coconut water media treatment, while the other treatments were not significantly different. This is thought to be due to the growth of *Streptomyces* sp. tend to be slower than the growth of *Trichoderma harzianum*, as well as the possibility of adjustments to grow in alternative media (Table 2). At the 2nd week showed that the treatment of *Streptomyces* sp. and *Trichoderma harzianum* in rice wash water media and *Streptomyces* sp. and *Trichoderma harzianum* in coconut water media significantly different from other treatments (Table 2). This is indicated by the highest number of colonies. This is thought to be due to the nutritional content of rice washing water, which is a source of complex carbohydrates (starch from the rest of the rice) which provides a long-term energy source for *Streptomyces* sp. and *Trichoderma harzianum*. This complex carbohydrate is thought to support the growth of *Streptomyces* sp. and *Trichoderma harzianum* on an ongoing basis. According to Hairuddin *et al*., (2018) the content possessed by rice washing water includes carbohydrates, nitrogen, phosphorus, potassium, magnesium, sulfur, iron, and vitamin B1. According to Chatri *et al*., (2014), carbon is influential in the process of cell growth and multiplication, while nitrogen is influential in the process of protein synthesis. If the content of nitrogen and carbon elements obtained is balanced, the ability of sporulation will increase.

While the nutrient content in coconut water and sugar potato extract media tend to contain simple sugars (glucose and fructose) which are more easily absorbed but do not support long-term growth because they run out quickly. In addition, high sugar

concentration is also thought to cause osmotic pressure which is less than ideal for the combination growth of *Streptomyces* sp. And *Trichoderma harzianum.*

In the 4th to 6th week shows the declining number colonies of *Streptomyces* sp. and *Trichoderma harzianum* (Table 4). This happens because both biocontrol agents are in the stationary phase followed by the death phase. The decrease in the number of colonies is also thought to be due to the availability of nutrients in each media treatment is getting less. According Wahyuningsih and Zulaika (2019), the microbial growth phase starts from the lag phase (adaptation phase), exponential phase (log phase), stationary phase (stagnant phase) and death phase.

2. The results of *Streptomyces* sp. and *Trichoderma harzianum* against anthracnose disease in chili

The results of *Streptomyces* sp. and *Trichoderma harzianum* against anthracnose disease in chili pepper fruits are presented in the following of table 3:

Table 3. Severity of disease in chili fruits

Treatment	Severity of disease percentage (%)	
	Day 7	Day 14
ST EKG	44b	71b
ST ACB	32a	69a
K0	46b	89c

Note: ST EKG = *Streptomyces* sp. and *Trichoderma harzianum* in sugar potato extract media;
ST ACB = *Streptomyces* sp. and *Trichoderma harzianum* in rice washing water medium;
K0 = control (without *Streptomyces* sp. and *Trichoderma harzianum*)

Based on the data in Table 3 shows that the combined application of *Streptomyces* sp. and *Trichoderma harzianum* in the medium of rice washing water (ST ACB) was able to suppress the severity of the disease by 32% on 7hsi and by 69% on 14hsi. It is suspected that rice washing water provides more balanced nutrition, supporting the growth of *Streptomyces* sp. and *Trichoderma harzianum*, and increase the production of secondary metabolites that effectively suppress anthracnose attack. According to Hairuddin *et al*., (2018), the content possessed by rice washing water includes carbohydrates, nitrogen, phosphorus, potassium, magnesium, sulfur, iron, and vitamin B1. And according to Chatri *et al*., (2014), carbon is influential in the process of cell growth and multiplication, while nitrogen is influential in the process of protein synthesis. If the content of nitrogen and carbon elements obtained are balanced then the ability of sporulation will increase.

While in combination treatment of *Streptomyces* sp. and *Trichoderma harzianum* on sugar potato extract media (ST EKG) was able to suppress the severity of the disease by 44% on 7 days and by 71% on 14 days. This occurs allegedly due to EKG media that contain a lot of glucose nutrients but tend to trigger growth imbalances between *Streptomyces* sp. and *Trichoderma harzianum* so that its effectiveness in suppressing the disease tends to decrease.

From the results of the percentage of disease severity (Table 3), then followed by assessment of the damage categories chili to classify the level of damage into certain categories (Table 1). This is done as an effort to facilitate the evaluation of the effectiveness of the treatment on the disease.

Based on the data in Table 3 shows that with the combined treatment of *Streptomyces* sp. and *Trichoderma harzianum* on the medium of rice washing water on 7 days by 32% and in sugar potato extract on 7 days by 44% included in the category of moderately resistant. While the combination treatment of *Streptomyces* sp. and sugar potato extract on rice washing water media on 14 days by 69% and in sugar potato extract media on 14 days by 71% are included in the vulnerable category.

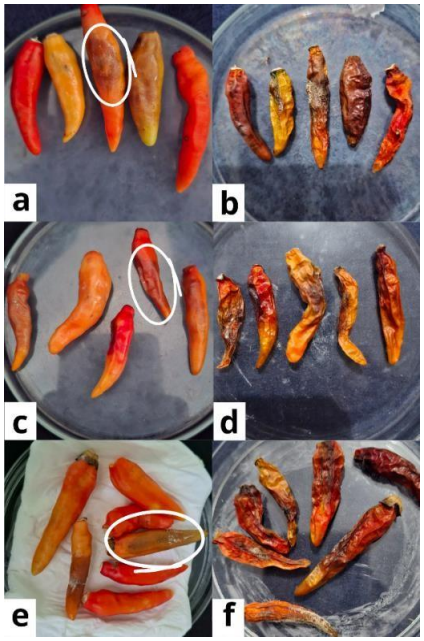

Figure 4. The effectiveness of *Streptomyces* sp. and *Trichoderma harzianum* against anthracnose disease in chili; (a) *Streptomyces* sp. and *Trichoderma harzianum* in sugar potato extract day 4; (b) *Streptomyces* sp. and *Trichoderma harzianum* in sugar potato extract day 10; (c) *Streptomyces* sp. and *Trichoderma harzianum* in rice wash water Day 4; (d) *Streptomyces* sp. and *Trichoderma harzianum* in rice wash water day 10; (e) Control Day 3; (f) Control day 10.

In Figure 4 shows that anthracnose symptoms appear about 2-3 days after inoculation in the control treatment (figure 4e), while for the combination treatment of *Streptomyces* sp. and *Trichoderma harzianum* in sugar potato extract media and in rice wash water, symptoms of anthracnose appeared after 4-5 days of inoculation (figures 4a and 4c). The difference in the time of appearance of these symptoms are thought to be due to the inhibition mechanism by biocontrol agents sprayed on the chili fruit. *Streptomyces* sp. and *Trichoderma harzianum* on chili is one of the efforts that can be done to suppress the severity of anthracnose disease during the fruiting period.

The effectiveness of *Streptomyces* sp. and *Trichoderma harzianum* is applied to the chili fruit is intended to provide a realistic picture of the effectiveness of biological agents in the field. Because *Streptomyces* sp. and *Trichoderma harzianum* has a synergistic mechanism that are effective in suppressing anthracnose disease, so it can be intended as an environmentally friendly and sustainable control efforts to reduce the economic impact of anthracnose disease.

3. The results of *Streptomyces* sp. and *Trichoderma harzianum* against anthracnose disease in chili seeds

The percentage results of the falling sprouts in chili seeds are presented in Table 4 below:

Table 4. The percentage of falling sprouts on chili seeds

Treatment	germination failure percentage (%)		
	7 days	14 days	21 days
ST EKG	5a	20b	45b
ST ACB	0a	10a	35a
Control	28b	57c	85c

Note: ST EKG =*Streptomyces* sp. and *Trichoderma harzianum* in sugar potato extract media; ST ACB = *Streptomyces* sp. and *Trichoderma harzianum* in rice washing water medium; K0 = control (without *Streptomyces* sp. and *Trichoderma harzianum*).

Based on Table 4, the percentage of falling sprouts in the control treatment showed a fairly high percentage of 28% on 7 days, 57% on 14 days and 85% on 21 days after planting. The percentage of falling sprouts in the control treatment tended to increase compared with the combination treatment of *Streptomyces* sp. and *Trichoderma harzianum*. This occurs due to the absence of an inhibitory mechanism against *Colletotrichum capsici* infection.

In the combination treatment of *Streptomyces* sp. and *Trichoderma harzianum* showed a lower percentage of falling sprouts. This happens due to the combination of *Streptomyces* sp. and *Trichoderma harzianum*, that can suppress *Colletotrichum capsici* infection through several mechanism, including the mechanism of competition, production of secondary metabolites and induction of plant resistance. So that there is a delay in the incidence of falling sprouts or reduce the incidence of falling sprouts. This statement is reinforced by Nasution's (2019) research, showing that soaking local chili seeds using *T. harzianum* suspension is able to control anthracnose disease in local chili seedlings caused by the fungus *Colletotrichum capsici*. And in the research of Suryaminarsih and Mujoko (2020), *Streptomyces* sp. and *Trichoderma harzianum* are able to suppress the severity of Fusarium wilt in tomato plants.

Based on Table 4, the combined treatment of *Streptomyces* sp. and *Trichoderma harzianum* in the medium of rice washing water (ST ACB) showed the incidence of falling sprouts began to occur on 14 days after planting with a percentage of 10%, then increased to 35% on 21 days after planting. This happens allegedly because the rice washing water media can support the synergistic activity of *Streptomyces* sp. and *Trichoderma harzianum*. In the medium of rice washing water, the combination of *Streptomyces* sp. and *Trichoderma harzianum* are able to produce secondary metabolites, so it can suppress the development of *Colletotrichum capsici*. While in the treatment of *Streptomyces* sp. and *Trichoderma harzianum* in sugar potato extract media (ST EKG) showed the incidence of falling sprouts occurred on 7 days by 5%, then increased to 20% on 14 days, and increased again to 45% on 21 days after planting. This is thought to be because the nutrient content of the sugar potato extract media, which is rich in simple carbohydrates, not only supports the growth of biological agents, but also provides an advantage for the pathogen *Colletotrichum capsici*. *Streptomyces* sp. and *Trichoderma harzianum* has to compete harder to inhibit *Colletotrichum capsici.* This causes the effectiveness of *Streptomyces* sp. and *Trichoderma harzianum* becomes lower in sugar potato extract media.

The difference in the percentage value of sprouts fall between the combination treatment of *Streptomyces* sp. and *Trichoderma harzianum* in rice washing water media with sugar potato extract media are thought to be caused by the differences in nutrient content in each media, so that it can affect the interaction between biological agents with pathogens *Colletotrichum capsici*. The growth of *Streptomyces* sp. and *Trichoderma harzianum* in rice washing water media are more supported, and it also support the production of secondary metabolites, making it more effective in suppressing the incidence of falling sprouts caused by *Colletotrichum capsici.*

Figure 5. The results of control treatment on chili seeds (without *Streptomyces* sp. and *Trichoderma harzianum*); a. on 7 days after planting; b. on 14 days after planting; c. on 21 days after planting.

Based on Figure 5 shows that in the treatment of control, the incidence of falling sprouts began to occur at the age of 7 days with the condition of sprouts do not grow optimally according to age. The infection process has actually started since the beginning of planting, but the real symptoms occur on 7 days (Figure 5a). Continued infection process continues to occur up to 14 days (Figure 5b), visible sprouts wet decay, dark brown to black and sprouts are not able to survive/ die before growing completely. At 21 days (Figure 5c) can be seen that the sprouts have died, the rest of the dead plant tissue is covered with mycelium and spores of *Colletotrichum capsici*, this can be a source of spread for the pathogenic spores. The incidence of falling sprouts occurs faster in this control treatment, because there is no inhibitory mechanism against pathogen infection, so that pathogen activity tends to be more aggressive.

Figure 6. The results of the combined treatment of *Streptomyces* sp. and *Trichoderma harzianum* in sugar potato extract media on chili seed; a. on 7 days after planting; b. on 14 days after planting; c. on 21 days after planting.

Based on Figure 6, the combination treatment of *Streptomyces* sp. and *Trichoderma harzianum* in sugar potato extract media applied to chili seeds showed symptoms of falling sprouts occurred from the age of 7 days, but can still be suppressed in the early stages by a combination of *Streptomyces* sp. and *Trichoderma harzianum*. The symptoms of falling sprouts that can be seen are the appearance of brown spots at the base of the stem on 7 days (Figure 6a). Then at the age of 14 days began to show symptoms of falling on sprouts significantly (Figure 6b). This happens because the infection continues to occur despite the combined treatment of *Streptomyces* sp. and *Trichoderma harzianum*. When the process of soaking chili seeds in a combination treatment of *Streptomyces* sp. and *Trichoderma harzianum* in sugar potato extract media, *Colletotrichum capsici* that have infected chili seeds also get access to nutrients from sugar potato extract media. This enables nutrient competition between biological agents and *Colletotrichum capsici*. In addition, *Streptomyces* sp. and *Trichoderma harzianum* produces suboptimal secondary metabolites in sugar potato extract media, so that the protection of chili seeds becomes suboptimal. And at the age of 21 days the sprouts is seen to die, the rest of the plant tissue looks brown, dry and there are mycelium of *Colletotrichum capsici*, white like thin cotton enveloped the rest of the dead tissue (Figure 6c).

Figure 7. Results of the combined treatment of *Streptomyces* sp. and *Trichoderma harzianum* in the medium of rice washing water on chili seeds; a. on 7 days after planting; b. on 14 days after planting; c. on 21 days after planting.

Based on Figure 7, *Streptomyces* sp. and *Trichoderma harzianum* in the medium of rice washing water applied to chili seeds showed symptoms of falling sprouts occurred from the age of 14 days. At the beginning growth of the sprouts (1-7 days), sprouts grow normally, healthy and no symptoms of infection with pathogens (Figure 7a). It is suspected that the combination of biological agents is still actively developing and competing with pathogens, as to suppress infection. But at the age of 14 days visible symptoms of falling sprouts can be seen (Figure 7b), it is suspected that the attack of infection *Colletotrichum capsici* increased faster than a combination of *Streptomyces* sp. and *Trichoderma harzianum*, so that the pathogen can be dominant and cause more severe infections. On rice washing water media, *Streptomyces* sp. and *Trichoderma harzianum* are thought to be able to produce secondary metabolite compounds, but over time the effectiveness of secondary metabolite compounds is also thought to decrease due to different environmental factors when applied to chili seeds. So that the defense against infection of *Colletotrichum capsici* becomes less effective at the age of 14 days. In addition, the nutrient content of rice washing water can also support the growth of pathogens if the environmental conditions are more favorable to the pathogens, so that the pathogens develop aggressively and cause the sprouts to collapse. At the age of 14 days, it is suspected that there may be a decrease in antagonistic activity of biological agents due to changes in environmental conditions. And at the age of 21 days can be seen that the sprouts die, the rest of the plant tissue is brown, dry and there are mycelium *Colletotrichum capsici* white like thin cotton enveloped the rest of the dead tissue (Figure 7c).

CONCLUSION

1. *Streptomyces* sp. isolates S6 can grow optimally on rice washing water media, *Trichoderma harzianum* TM isolates can grow optimally on coconut water media, and a combination of *Streptomyces* sp. S6 and *Trichoderma harzianum* isolates TM can grow optimally in rice washing water compared to coconut water and potato sugar extract.
2. *Streptomyces* sp. isolates S6 and *Trichoderma harzianum* isolates TM in rice washing water media can significantly suppress the severity of anthracnose disease in chili fruits and can suppress the incidence of falling sprouts on chili seeds.
3. *Streptomyces* sp. isolates S6 and *Trichoderma harzianum* isolates TM showed synergistic effects in the inhibition of *Colletotrichum capsici* growth, supporting its use as a biological control of anthracnose disease in chili plants.

REFERENCES

Agnihotri, P. K., Kumar, D. Y., & Singh, D. (2024). Antagonistic effect of Trichoderma isolates on Anthracnose Disease (Colletotrichum capsici) of Chilli (Capsicum annuum L.). *Annals of Plant and Soil Research*, *26*(2), 510-515. https://doi.org/10.47815/apsr.2024.10392

Al Chalik, F., Nopsagiarti, T., & Haitami, A. (2021). Uji Konsentrasi Ekstrak Kentang Terhadap Pertumbuhan Sub Kultur Pisang Roti Pada Media Ms Modifikasi. *Green Swarnadwipa: Jurnal Pengembangan Ilmu Pertanian*, *10*(3), 373-382.

Ali, F., Yeni, Rahhutami, R., Darma, A.W., dan Sesanti, N.R. (2023). Efektifitas Molase, Air Cucian Beras dan Air Kelapa pada media Baglog untuk Produksi Jamur Tiram Putih (Pleorotus ostreatus). Jurnal Agricultural Biosystem Engineering, 2(1).

Boblina, B., Beura, S. K., Panda, A. G., & Mishra, M. K. (2020). Evaluation and assessment of shelf life of liquid substrates and talc formulation for mass production of native Trichoderma spp. *Journal of Pharmacognosy and Phytochemistry*, *9*(3), 911-915.

Chatri, M., Handayani, D., & Septiani, J. (2018). Influence of media (mixture of rice and sugar cane) on Trichoderma harzianum growth and its resistance to Fusarium Oxysporum by in vitro.

Bioscience, *2*(1), 50-60. DOI: 10.24036/02018219984-0-00

Fitriana, I. N., Suryaminarsih, P., Mindari, W., & Wiyatiningsih, S. (2019). Studi pertumbuhan multiantagonis *Trichoderma* sp. dan *Streptomyces* sp. dalam suspensi akar, humat cair dan ekstrak kentang gula. Berkala Ilmiah Agroteknologi - PLUMULA, 7(1), 25–32.

Hairuddin, R., Yamin, M., & Riadi, A. (2018). Respon pertumbuhan tanaman anggrek (dendrobium sp.) Pada beberapa konsentrasi air cucian ikan bandeng dan air cucian beras secarainvivo. *Jurnal Pertanian Berkelanjutan*, *6*(2), 23-29.

Hakim, L. (2023). Uji Potensi *Streptomyces* spp. untuk menekan pertumbuhan Fusarium pada tanaman cabai rawit. Skripsi. Fakultas Pertanian UPN Veteran Jawa Timur.

Jojy, E. T., Amrutha, P., Johnson, J. M., Sajeena, A., Rafeekher, M., & Paul, A. (2024). Cultural, morphological and pathogenic variability in the Colletotrichum capsici isolates, inciting anthracnose and fruit rot of chilli (Capsicum annuum L.) in different agro-climatic zones of Kerala, India. *International Journal of Plant & Soil Science*, *36*(6), 304-316.

Jumadi, O., Juanda, M., Caronge, M. W., dan Syafruddin.(2021). Trichoderma dan Pemanfaatan. Biopress. Makassar.

Kumar, J. R., Anandan, D., & Kaliyaperumal, V. (2024). Biodiesel Production from Rice-Wash Water with Streptomyces fradiae Fermentation: Improved Free Fatty Acid Concentration. *Journal of Pure & Applied Microbiology*, *18*(2). DOI:10.22207/JPAM.18.2.33

Nasution, D. P. Y. (2019). Pengujian Lama Perendaman Bibit Cabai Lokal Dengan *Trichoderma harzianum* Dalam Mengendalikan *Colletotrichum capsici*. *GrahaTani*, *5*(3), 846-852.

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.

Sarianti, S., & Subandar, I. (2022). Insidensi Dan Severitas Penyakit Antraknosa Pada Tanaman Bawang Merah Di Kampong Tanah Bara Kecamatan Gunung Meriah Kabupaten Aceh Singkil. Jurnal Pertanian Agros, 24(1), 202–210.

Sudartini, T., Kurniati, F. dan Lisnawati, N.A. (2020). Efektivitas Air Cucian Beras dan Air rendaman Cangkang Telur pada Bibit Anggrek Dendrobium. Jurnal AGRO, 7(1).

Suryaminarsih, P. dan Mujoko, T. (2020). Kompetisi Agens Hayati *Streptomyces* sp., *Gliocladium* sp., dan *Trichoderma harzianum* terahadap *Fusarium oxysporum* pada Akar Tanaman Tomat. Jurnal Cropsaver 2020, 3(1), 17-21.

Syahfitri, D., Mubarik, N. R., & Manaf, L. A. (2018). Penggunaan bakteri kitinolitik sebagai pengendali hayati Colletotrichum capsici pada tanaman cabai. *Jurnal Fitopatologi Indonesia*, *14*(4), 120-120.

Triasih, U., Widyaningsih, S., dan D. Erti, M. (2021). Pengaruh Formulasi Media Cair terhadap Pertumbuhan Agen Hayati yang Berasal dari Jamur Antagonis *Trichoderma* sp. dan *Gliocladium* sp. serta Potensinya dalam Mengendalikan Penyakit Bercak Daun Alternaria sp. pada Tanaman Apel. *Gontor AGROTECH Science Journal*, 7(2), 163-182.

Tuyekar, S. N., Tawade, B. S., Singh, K. S., Wagh, V. S., Vidhate, P. K., Yevale, R. P., ... & Kale, M. (2021). An overview on coconut water: As a multipurpose nutrition. *Int. J. Pharm. Sci. Rev. Res*, *68*(2), 63-70. DOI: http://dx.doi.org/10.47583/ijpsrr.2021.v68i02.010

Ummah, R. (2023). Uji Kompatibilitas Dan Uji Fitokimia Bioaktivator *Trichoderma harzianum* Dari Lahan Bawang Merah Minim Dan Intensif Fungisida Terhadap Patogen *Alternaria* sp. Tesis. Fakultas Pertanian UPN Veteran Jawa Timur.

Wahyuningsih, N., & Zulaika, E. (2019). Perbandingan pertumbuhan bakteri

selulolitik pada media nutrient broth dan carboxy methyl cellulose. *Jurnal sains dan Seni ITS*, *7*(2), 36-38.

Wartono. (2021). Insidensi Dan Keparahan Penyakit Busuk Pada Tanaman Cabai: Studi Kasus di Lahan Petani Desa Ciampea Udik, Ciampea, Bogor.

Wati, M., Damhuri, Safilu. (2017). Pengaruh Pemberian Air Beras Terhadap Pertumbuhan dan Produktivitas Tanaman Tomat (Solanum lycoersicum L.). J.Ampibi, 2(1),49-56.