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Study of *Streptomyces* spp. to control purple blotch disease caused by *Alternaria porri* in shallot plant

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ABSTRACT

Alternaria porri is a fungal pathogen that causes purple blotch on shallots, this fungus can cause crop yield loss of 3 – 57%. *A. porri* was obtained from the Sumber Brantas onion farm and then isolated by planting infected tissue. *Streptomyces* spp. is a filamentous bacterium that is abundant in the soil and can be used as a biological agent, decomposer and plant growth promote. *Streptomyces* spp. was obtained one isolate from shallot land location in Pare, Kediri, East Java (BMP: Bawang Merah Pare) and three isolates from Sidera, Palu, Central Sulawesi (BMS: Bawang Merah Sidera) (BMS1, BMS2, BMS3). The purpose of this study was to determine the efficacy of *Streptomyces* spp. to control *A porri* consist of *in vitro* and *In vivo* antagonist test. Antagonist test was carried out by dual culture method and the *Streptomyces spp*. which can inhibit the development of *A porri* would be used antagonist test *in vivo.* The study was designed using a Factorial Complete Randomized Design with two factors, candidate isolates of biological control agents and concentrations of *Streptomyces* spp. be diluted into 5%, 10%, and 15%, it will applied on shallot plant Bauji variety. The results of the *In vitro* test shown the highest inhibition zone from BMP 17.75% while BMS1 13.75%, BMS2 8,75%, and 8.50%. *in vivo* test shown lowest disease severity value in BMP 15% concentration was 1.13% while diseases severity of BMS1 was 1.27%, and BMS2 was 1.80%. Therefore, *Streptomyces* spp. has potency as an alternative pesticide for sustainable agriculture.

Keywords: Biological control, Antagonist, diseases severity

Kajian *Streptomyces* spp. Pengendali Penyakit Bercak Ungu yang Disebabkan Oleh *Alternaria porri* pada Tanaman Bawang Merah

ABSTRAK

Alternaria porri merupakan jamur patogen penyebab bercak ungu pada tanaman bawang merah, jamur ini dapat menyebabkan kehilangan hasil panen sebesar 3 – 57%. *A. porri* diperoleh dari lahan bawang merah Sumber Brantas kemudian diisolasi dengan menanam jaringan tanaman terinfeksi. *Streptomyces* spp. adalah bakteri berfilamen yang melimpah di tanah dan dapat digunakan sebagai agens pengendali biologis, pengurai dan pemacu pertumbuhan tanaman. *Streptomyces* spp. diperoleh satu isolat dari lokasi lahan bawang merah Pare, Kediri, Jawa Timur (BMP: Bawang Merah Pare) dan tiga isolat dari Sidera, Palu, Sulawesi Tengah (BMS: Bawang Merah Sidera) (BMS1, BMS2, BMS3). Penelitian bertujuan untuk mengetahui manfaat *Streptomyces* spp. dalam mengendalikan *A porri* yang terdiri dari uji antagonis *in vitro* dan *in vivo*. Uji antagonis dilakukan dengan metode kultur ganda dan *Streptomyces* spp. yang dapat menghambat perkembangan *A porri* akan digunakan uji antagonis secara *in vivo*. Penelitian menggunakan Rancangan Acak Lengkap Faktorial dengan dua faktor yaitu jenis isolat agens pengendali hayati dan konsentrasi *Streptomyces* spp. yang diencerkan menjadi 5%, 10%, dan 15% akan diterapkan pada tanaman bawang merah varietas Bauji. Hasil uji *in vitro* menunjukkan zona hambat tertinggi dari BMP 17,75% sedangkan BMS1 13,75%, BMS2 8,75%, dan 8,50%. Uji *in vivo* menunjukkan nilai keparahan penyakit terendah pada konsentrasi BMP 15% sebesar 1,13% sedangkan keparahan penyakit pada BMS1 sebesar 1,27%, dan BMS2 sebesar 1,80%. Oleh karena itu, *Streptomyces* spp. memiliki potensi sebagai pestisida alternatif untuk pertanian berkelanjutan

Kata Kunci: Pengendalian hayati, Antagonis, Keparahan penyakit

INTRODUCTION

Shallot is one of the main produce that have a lot of beneficial quality, such as : anti oxidants, improve heart problem, and cancer prevention (Shahrajabian, 2020). Asian region specifically Indonesia have the one of the highest consumption of Shallot according to Central Statistics Agency 2020 of indonesia stated that the consumption rate of shallot

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reaches around 1,815,445 tons and an increase of 12% from 2019 which was only 1,580,247 tons (BPS, 2020). The increased production of shallots is inseparable from the attack of microorganisms that damage plants, one of which is purple blotch disease on shallot plants.

A. porri is a pathogenic fungi that can cause purple botch. It can cause yield loss reaching 3-57% if not treated well both in seeds and bulbs of shallots plants (Nurosid *et al.*, 2018). The attack of *A. porri* generally occurs during the rainy season which is characterized by the *presence* of whitish spots, gradually purple in the oval shape, grayish and black powdery (Mohsin *et al*., 2016). Control of *A. porri* is dominated by using chemical fungicides which can damage the environment so alternative controls are needed.

Streptomycetes are gram-positive bacteria belonging to the order Actinomycetales and In the *Streptomycetaceae* family, there are more than 570 different types of *Streptomycetes* bacteria (Vurukonda *et al.*, 2018). According to research Queendy and Roza, (2019) stated that *Streptomyces* sp. have a antifungal compound known as bafilomycins B1 and C1. Suyaminarsih *et al.*, (2019) reasearch showed that *S. griseurubens* can inhibit the growth of *Fusarium oxysporum* in Tomato rhizosphere. The results Setyawati *et al*., (2021) of also showed that the molecular identification and morpho-physiological characterization of *Streptomyces* sp. as a producer of antimicrobial compounds.

Research conducted by Suryaminarsih *et al*., (2020) stated that *Streptomyces* spp. can inhibit the fungus *Fusarium oxysporum* up to 90%. Research by Purnomo *et al*., (2017) added that *Streptomyces* spp. can inhibit the fungus *Sclerotium rolfsii* up to 84.10%. *Streptomyces* spp. which successfully live in exposed environments with high levels of pathogen attack and insecticide applications, are thought to have the potential to be used as biological agents and *Plant Growth Promoting Rhizobakter* (PGPR). In addition, the rhizosphere of plantations is known to be rich in diverse potential microbial populations. Based on this, it is necessary to conduct research related to the role of *Streptomyces* spp. in shallot fields as a biological agent for purple spot disease caused by the fungus *A. porri*.

MATERIALS AND METHODS

Streptomyces spp. Isolation and Identification

Streptomyces spp. was obtained one isolate from shallot land location in Pare, Kediri, East Java (BMP: Bawang Merah Pare) and three isolates from Sidera, Palu, Central Sulawesi (BMS: Bawang Merah Sidera) (BMS1, BMS2, BMS3).

Isolation was carried out by means of serial dilution . The dilutions used were serial dilutions 10^{-4}, 10^{-5} and 10^{-6}. The initial step was carried out by weighing 1 gram of soil and then placing it into a test tube containing 10 ml of distilled water, after that it was vortexed for 1 minute and 1 ml was taken to be poured into the second test tube. This dilution is carried out up to the sixth tube or 10^{-6} dilution. The next step is inoculating the 10^{-4}, 10^{-5}, 10^{-6} dilution sample on GNA media in a petri dish by pouring technique and leveling the suspension using a sprinkler then incubating for 14x 24 hours. Isolates that have grown will be identified on the macroscopic and microscopic shape of *Streptomyces* spp. (Putri *et al*., 2021).

Isolation and Identification of *Alternaria porri.*

Searching for *A.porri* on shallots fields in Sumber Brantas, Batu, Malang, East Java. Infected leaf samples were cut into 5 parts with a size of approximately 1 cm and sterilized by immersing them in 70% alcohol solution for 30 second then soaking them in distilled water for 1 minute and placing them on a sterile tissue to dry. Finally, the specimens were planted in a petri dish containing PDA media and incubated for 7 days. Isolate that have grown will be identified on the macroscopic and microscopic shape of *A. porri*.

Preparing Biological Control Agent (BCA)

Potato Sucrose Extract (PSE) will be use as a liquid growth medium for *Streptomyces* spp. Potato sucrose extract consist of 450 gram of peeled potato, 42 gram of sucrose, and 1 liter of aquadest and sterilized them using autoclave on 121°C for 15 minutes. Preparing suspension of BCAs by dissolve the bacteria in 10 pods isolate *Streptomyces* spp. (diameter 0,5 cm) in 250 ml of PSE It was incubated on a shaker (150 rpm) on room temperature for 14 days. Calculate the bacterial spore density by following Suryaminarsih and Mujoko, statement (2020) that the spore density of *Streptomyces* spp. 1.8 x 10^7 has a real impact.

in vitro test

In vitro test was carried out to determine *Streptomces* spp. effectiveness against *A. porri* using dual culture methods. The media used in the antagonist test was PDA media. *A. porri* culture was cut using a 5 mm diameter *cork borer* and placed across from the *Streptomyces* spp culture. Then it was incubated and observed for 7 days, according to Anjarsari *et al*., (2021) the data for measuring the percentage of Inhibition Zone (DH) was calculated using the following formula:

$$DH = \frac{r1 - r2}{r1} x100\% \quad \dots (1)$$

description :
DH = Percentage of inhibition (%)
r1 = Radius of pathogenic fungal colonies that move away from antagonist bacterial colonies
r2 = Radius of pathogenic fungal colonies approaching antagonist bacterial colonies

in vivo test

In vivo test was carried out using Beuji variety shallots, shallots were planted in polybags measuring 20×20 cm in Sidoarjo area, East Java. Suspension of

Streptomyces spp. on potato sucrose extract (PSE) medium will be poured directly to the soil around 200 ml after that it was left incubated in the soil for 14 days before planting the shallots. Treatment of biocontrol differentiates into three concentrations; there are 5, 10, and 15%. Biocontrol application for treatment in 5% concentration of *Streptomyces* spp. is made by dissolving 5 ml of a suspension of biological agents on 100 ml of potato sucrose extract, 10% concentration with dissolving 10 ml of suspension of biocontrol agents on 100 ml of potato sucrose extract, and 15% concentration with dissolving 15 ml of suspension of biocontrol agents on 100 ml of potato sucrose extract.

Inoculation of *A. porri* on shallot plants refers to Hersanti *et al*., (2019), namely spraying a suspension of 15 ml/plant at a spore density of 10^7 spores/ml on the leaves of shallot plants aged 14 days after planting The data for measuring the severity of disease based on damage left by *A. porri* in the leaves then it was calculated using the following formula :

$$I = \frac{\sum_{i=0}^{k} k.nk}{Z \times N} \times 100\% \qquad \dots(2)$$

Note :
I = Disease severity (%)
K = Scale
nk = Number of leaves showing symptoms from each attack category
N = Total number of plant
Z = Highest attack category

The determination of purple blotch disease severity was conducted based on the scoring system used below Arwiyanto (1995):
0 = no symptoms of attack
1 = Leaf damage area $0 < x \leq 10$ %
2 = Leaf damage area $10 < x \leq 25$ %
3 = Leaf damage area $25 < x \leq 50\%$
4 = Leaf damage area $50 < x \leq 75\%$
5 = Leaf damage area $75 < x \leq 100\%$

RESULT AND DISCUSSIONS

Identification of Streptomyces spp

Characteristics of *Streptomyces* sp. based on specific colony growth. Colonies of *Streptomyces* sp. is not accumulation of single and uniform clusters of cells like bacteria, however is a mass form of branching filaments of colonies forming branching hyphae complex where the hyphae will later form sporophores or chains aerial spores (Fardiyanti *et al*., 2021)

According to research by Fardiyanti *et al.*, (2021) that the color morphology of *Streptomyces* colonies is very various colors, namely white, gray (gray to brown), yellow, red, blue, green and violets. On the morphological results of these four isolates *Streptomyces* spp. rejuvenated on PDA media has macroscopic characteristics that is, it has a distinctive earthy odor in culture, has colonies dominated by white to white bone, small round shape. Microscopic observation of *Streptomyces* spp. looks like a fungi because it has hyphae and spores, hyphae are small in size and produce spores round and chain-shaped.

Identification of A. porri

Infected onion bulbs by the fungus *A. porri* can damage the physical properties of the tubers which affect the nutritional content, nutrients, and vitamins in shallots. Researchers conducted by Galande *et al.*, (2020) that the initial symptoms of disease attack purple spots caused by the fungus *A. porri* in the form of small irregular spots, the leaves look like a converging line, generally yellow then turns brown to dark brown. Further attacks enlarged spots with purple centers and cause tubers Rotten shallots are yellow then turn red-brown.

The results of the isolation were identified macroscopically as having the characteristics of colored colonies blackish gray, regular colony form without any concentric circles, and mycelium colonies spread evenly. Microscopic observation shows the characteristics – characteristic of having brown mycelium, conidium and conidiophores, The conidia are shaped like an inverted club and the conidiophores are erect in sections.

in vitro test

The results of the antagonism test of the four *Streptomyces* spp. isolates the rate of producing antibiosis with the greatest inhibitory percentage of 17.75% which can be seen in table 1.

Table 1. Percentage of inhibition of *Streptomyces* spp. (1.8 x 10^7 cfu/ml) against *A. porri*.

Treatment	colony inhibition of *A. porri* (%)				
Streptomyces spp.	…. Days After Incubation				
	3	4	5	6	7
BMP	45.75±10.21	26.5±20.50	16.00±3.67	17.75±4.60b	16.5±2.50
BMS1	44.25±12.81	24.25±6.65	14.25±4.60	13.75±3.35ab	14.25±4.38
BMS2	28.75±6.65	18.75±5.85	11.00±4.63	8.7±4.15ab	10.00±4.47
BMS3	30.25±7.29	19.75±4.82	13.00±6.28	8.5±3.20a	11.25±6.72
BNJ 5%	NS	NS	NS	8.42	NS

Note: Number followed by the same letter is not significant differences by BNJ test (α=0.05). NS= not significant. BMP: Isolate from Pare, BMS1: Isolate from Sidera1, BMS2 Isolate from Sidera2, BMS3 Isolate from Sidera3.

The difference in inhibition produced by *Streptomyces* spp. against *A. porri* due to the antibiotic compounds produced by *Streptomyces* spp. different, and from different regions. According to Raharini *et al*., (2012) stated that *Streptomyces* spp. was able to inhibit the growth of *A. porri* by destroying the cell walls of macroconidia and microconidia.

Activation of inhibition of *Streptomyces* spp. can produce intracellular hydrolytic enzymes such as chitinase to degrade pathogenic cell walls (Suryaminarsih *et al*., 2020). *Streptomyces* spp. produces chitinase which can degrade fungal cell walls, especially cell wall of *Alternaria porri* that caused purple blotch disease. This research also supported report from Syahrok *et al*., (2021) that *Streptomyces* spp. tested with pathogenic fungi caused purple blotch disease (*A. porri)* can inhibit through an antibiosis mechanism, bacteria *Streptomyces* spp. which forms an antibiosis mechanism because it has an antibiotic substance gliotoxin and toxic compounds that can inhibit the functional system of the fungus pathogens causing abnormal growth of hyphae (malformation). The mechanism of antibiosis is characterized by the formation of clear zonescan be seen in figure 1.

Figure 1 Antagonist test between *Streptomyces* spp. and *Alternaria porri*.
(a) BMP (b) BMS1 (c) BMS2 (d) BMS3

***in vivo* test**

In vivo test were conducted in Sidoarjo area, East Java with a temperature of 39ºC, according to Puspita *et al*., (2016), the growth and development of *A. porri* is the season with light rain, high humidity, and air temperature around 30-32ºC, therefore the condition doesnt support the growth of *A. porri*. The result were based on severity of the disease in shallots leaf then it was analyze using R – Studio.

The results showed that shallot plants affected by purple botch disease showed initial symptoms in the form of leaf spots, followed by brown elliptical spots, then progressed to dark brown (Figure 2). The lowest disease severity of shallots was obtained from the treatment of BMP 15% (1.13%) was then followed by BMS1 15% (1.27%) while the treatment that had a high level of damage was BMS2 5% and 10% (2.07%), 15% (1.87%) can be seen in Table 2. Treatment of BMS1 15% and BMP 15% can inhibit the pathogen *A. porri*, due to the level of concentration used and the type of *Streptomyces* spp. produce more antibiotic compounds than concentrations of 5% and 10%.

Figure 2 Symptoms of purple spots on shallot leaves infected by *Alternaria porri*

Table 2. Percentage of *A. porri* Infection Inhibition in Shallot Plants.

Treatment		Disease severity (%) ……. Days After Planting		
Streptomyces spp.	Consentration	14	21	30
BMS 2	5%	2.07±0.09a	1.87±0.25	1.67±0.25
BMS 2	10%	2.07±0.09a	1.67±0.25	1.53±0.34
BMS 2	15%	1.87±0.09ab	1.73±0.25	1.67±0.25
BMS 1	5%	1.80±0.16abc	1.60±0.28	1.47±0.25
BMS 1	10%	1.60±0.16abcd	1.47±0.25	1.40±0.16
BMP	5%	1.53±0.34abcd	1.53±0.25	1.47±0.25
BMP	10%	1.47±0.09bcd	1.40±0.16	1.33±0.18
BMS 1	15%	1.27±0.09cd	1.53±0.25	1.18±0.25
BMP	15%	1.13±0.09d	1.33±0.09	1.20±0.16
BNJ 5%	NS	0.38	NS	NS

Note: Number followed by the same letter is not significant differences by BNJ test (α=0.05). NS= not significant. BMP: Isolate from Pare, BMS1: Isolate from Sidera1, and BMS2 Isolate from Sidera2.

Percentage of the number of shallot leaves aged 30 days after application of BMP and BMS1, BMS2, and BMS3 showed a significant effect (α<0.05). The highest number of shallot leaf leaves was obtained from the treatment of BMP 15% (9.60) followed by BMS1 15% (8.40) and BMS2 15% (8.13), can be seen in Table 3. The high number of shallot leaves is caused by *Streptomyces* spp. has a role as a *Plant Growth Promoting Rhizobakter* (PGPR) that can enhance plant growth. This is following research conducted by Suryaminarsih *et al*., (2018) that *Streptomyces* sp. as PGPR is able to increase seed germination, hypocotyl length, and wet weight of shallot plants because it is able to produce *Indole Acetic Acid* (IAA) hormones and dissolve phosphate compounds.

Table 3. Number of shallot leaves aged 30 days after treatment.

Treatment		Average number of leaves
Streptomyces spp.	Consentration	
BMP	15%	9.60±0.43a
BMP	10%	9.13±0.34ab
BMS1	15%	8.40±0.28bc
BMS1	10%	8.33±0.25bc
BMS1	5%	8.27±0.25bc
BMS2	15%	8.13±0.34bc
BMP	5%	8.07±0.25bc
BMS2	10%	7.73±0.37c
BMS2	5%	7.47±0.25c
BNJ 5%		0.76

Note: Number followed by the same letter is not significant differences by BNJ test (α=0.05). NS= not significant. BMP: Isolate from Pare, BMS1: Isolate from Sidera1, and BMS2 Isolate from Sidera2.

High concentrations have a high number of colonies in form unit/ *colonies form unit* (cfu) so that the inhibition process is high, this is because the more number of CFU, the colonization process of an APH is faster and can produce a lot of antifungal compounds, based on research Putri *et al*., (2018) showed that the application of *Streptomyces* spp. a total of 10^8 cfu/ml was able to control jaundice in chilies by 57%, a study conducted by Handayani *et al.*, (2021) showed the concentration of *Streptomyces* sp. $1x10^5$ cfu/ml was able to control *F. oxysporum* and *S. rolfsii* by 60% and 58.89%, respectively.

Streptomyces spp. is an Actinomycetes bacterium that is widely found in soil, this bacterium has various types and the majority are able to control plant pathogenic fungi, research by Raharini *et al.,* (2012) showed 5 types of *Streptomyces* spp isolates. Those from the Bukit Jimbaran area were able to control *Fusarium oxysporum* disease with *Streptomyces* sp. which can control the growth of *F. oxysporum* by 84%, research by Handayani *et al*., (2021) used four *Streptomyces* spp isolates. (Di, BSi, BSc, and Imi) were able to control *F. oxysporum* and *R. rolfsii* disease, respectively.

CONCLUSIONS

The results of the *in vitro* antagonism test obtained the highest inhibition zone from *Streptomyces* spp. from Pare, Kediri, East Java (BMP: Bawang Merah Pare) 17.75%, *Streptomyces* spp. from Sidera (BMS: Bawang Merah Sidera), Palu, Central Sulawesi 1, 2, 3, each followed by (BMS1) 13.75%, (BMS2) 8.75%, and (BMS3) 8.50%. In the antagonism test on the shallot variety Bauji, the lowest disease severity value was obtained at a concentration of 15% is BMP 1.13% followed by *Streptomyces* spp. Sidera Palu Central Sulawesi BMS1 1.27% and BMS2 1.87%. Therefore, Streptomyces spp. has potency as an alternative pesticide and could increase good agricultural practices for sustainable agriculture.

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