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Use of Rhizobacteria as an Antagonistic Agent at Various Formulations to Control Sheath Rot Disease (*Sarocladium oryzae*) on Rice Crops

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ABSTRACT

The objective of this reserach were to determine the potency of antagonistic rhizobacteria and to find the best formulations of isolate to control sheat root disease (*Sarocladium oryzae*). The research was carried out from July to December 2019 in the Plant and Disease Laboratory and the Greean House of the Faculty of Agriculture Jember University. The experiment was conducted using a completely randomized design (CRD) 2 factors, namely isolates and formulation. The first factor was Rhizobacteria isolates, consisting of 4 factors levels; control, *Bacillus* sp. isolate, *Pseudomonas fluorescens* isolate, and *Bacillus* sp. + *Pseudomonas fluorescens* isolate. The second factor ws formulation isolates, consisting of 4 levels; glucose 10% + potato water 70% + bacterial starter 20%, Fructose 20% + coconut water 60% + bacteria; starter 20%, glucose 10% + CMC 2% + monmorilonite 68% + bacterial starter 20%, fructose 20% + CMC 2% + kaolin 58% + bacterial starter 20%. The result showed that the treatment of Bacillus sp. with formulation glucose 10% + CMC 2% + monmorilonite 68% + bacterial starter 20%, showed the best result, with a control effectiveness of 78.31%.

INTRODUCTION

Rice is a widely cultivated crop by Indonesin farmers since it is used for stapple food. In 2014, the national rice production in Indonesia falled short to 434 tons compared to that of the previous year (Biro Pusat Statistik, 2017), cuased by bad weather, pest infestation, and lack of suitable land. One of the most damaging pathogen are Fusarium and *Sarocladium oryzae* causing rice sheath rot diseases. It is an oval or oval-shaped patches with a gray or brown center, at a later stage, the spots will grow larger, unite and cover the entire leaf midrib. Sheat root attacks rice plants when the plant enters the generative phase, which causes disruption of panicle growth, where the flag leaf will still cover the rice panicle. Severe attacks of this disease are dark brown rice grains, even until no grain appears. Therefore, an alternative method is needed to control against the sheat rot disease, such as using rhizobcteria.

Rhizobacteria are bacteria that live around the roots of plants which are effective in controlling pathogens. Some types of rhizobacteria that can be used as biocontrol agents are *Bacillus* sp. and *Pseudomonas fluorescens*. *Bacillus* application is reported to be able to damage the patoen wall, thus causing the development of pathogens to be inhibited (Suriani, et al., 2018). The

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mechanism of suppression possessed by *P. fluorescens* is capable of producing antibiotic compounds that can inhibit the growth of pathogens. *Pseudomonas* sp. UB-PF45 is reported to be able to suppress downy mildew on maize by 50% (Jatnika et al., 2013). Both of these bacteria can also be categorized as PGPR (Plant Growth Promoting Rhizobacteria).

The use of rhizobacteria as biological control agents so far only limited to the laboratory scale and an experiment, considering the difficulty of application and traditional farmers' knowledge. Therefore, it was necessary to isolate Rhizobacteria and formualte them for antagonistic rhizobacteria.

The objective of this study was to determine the potency of some antagonistic rhizobacteria isolates and the types of formulations suitable for controlling sheath rot disease (*S. oryzae*)

MATERIALS AND METHODS

The study was conducted from July to December 2019 at the Laboratory of Pests and Plant Diseases and the green house of the Faculty of Agriculture, University of Jember. *S. oryzae* isolates used in the study were isolated from rice crops grown at Tanggul, Jember, showing symtoms of sheath rot disease. Rhizobacteria used in this research were collections of the Laboratory of Plant Pests and Diseases, the Faculty of Agriculture, University of Jember.

Isolation of pathogenic *S. oryzae* was done by taking symptomatic portions, then cut and sterilized using alcohol, continued by washing them by sterile water 3 times. Furthermore, the sterilized part were grown on the PDA media and incubated for 7 days. Rhizobacteria antagonist test with pathogen *S. oryzae*, carried out using PDA media. S. oryzae isolates (1 mm diameter) were placed at the center of the test media, and one antagonist rhizobacteria suspension (density $5x10^9$ CFU / ml) was etched in a circle with a diameter of 6cm, then incubated for 72 hours at 22°C temperature.

The experiment was carried out using a completely randomized design (CRD) consisting of 2 factors: isolates and formulations. Isolate factor (I) has 4 levels of factors, namely I0 = control, I1 = isolate *Pseudomonas fluorescens* sp. (PF 08) , I2 = *B*acillus sp (BS 45) . and I3 = *Bacillus* sp. + *Pseudomonas fluorescens* isolate 0(PF 08+BS45). The formulation factor (F) has 4 factor levels namely F1 = 10% glucose + 70% potato water + 20% starter bacteria, F2 = Fructose 20% + 60% coconut water + 20% starter bacteria, F3 = 10% glucose + CMC 2 % + 68% monmorillonite + starter bacteria, F4 = fructose 20% + CMC 2% + kaolin 58% + starter bacteria 20%. Then the two factors were combined to obtain 16 treatments, each of which was repeated 3 times. Pathogen application was done when the plant was 50 days old, by injecting a pathogen suspension into the leaf tissues. Rhizobacteria was applied when the crops was 7 days after planting (DAP), repeated twice every 7 days intervals, by spraying Rhizobacteria solution containing 30% of bacterial culture.

Data analysis were done by Analysis of variance, followed by mean separation analysis using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Isolates

On the sixth day after inoculation, *S. oryzae* isolate showed white-thin misellium having ascus. Microscopic observations showed that the fungus produced tube-like conidium

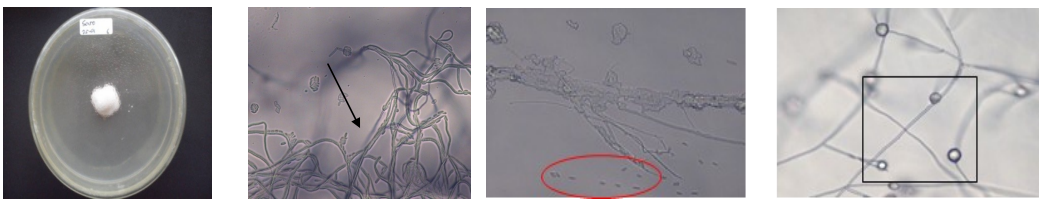

Figure 1. Macrosopic and microscopic image of *S. Oryzae* colony (A), Mycellium (B), conidium (C), and hyphae forming ascus (D).

(Figure 1).

The results of re-isolation of diseased plant tissue showed the same color as those of the first isolation, pale white isolates. The microscopic observation of mycelium and condias were also in accordance with the previous observations.

Rhizobacteria antagonist test with *S. oryzae* in vitro

Based on the rhizobacteria antagonist test with pathogen *S. oryzae*, it was known that all rhizobacteria isolates had the ability to inhibit the growth of pathogens with inhibitory levels of 66-77.6% (Table 1). These were consistent with the findigns reported by Arwiyanto *et al*., (1996), in which *Bacillus* spp. and *P. fluorescens* significantly suppresses the growth of pathogens. The highest inhibitory value wass shown by BS 45 with a percentage inhibition value of 77.3% (in pathogen isolate 1) and 77.6% (in pathogen isolate 2). *P. fluorescens* isolates which have the highest inhibitory percentage values were shown in PF 08 isolates with a value of 76.1 (in pathotene 1 isolates) and 75.3% (in pathogen isolates 2). Based on these results, isolates BS 45 and PF 08 were selected as bacterial starters in the formulation. Wakimoto (1986), have stated that the ability to inhibit the growth of pathogenic fungi is caused by the presence of antibiotic substances produced by bacteria. Besides the production of siderofor by antagonistic bacteria can also inhibit the iron ions needed by the pathogen, so that the availability in the medium is reduced or not available.

Table 1. Percentage inhibition of antagonistic rhizobacteria test results against *S. oryze* isolates in vitro

Rhizobacteria antagonistic isolates	Percentage inhibition	
	Pathogenic Isolates 1	Pathogen isolates 2
PF 08	76.1	75.3
PF 11	65.4	74.5
PF 01	65.7	72.3
PF 38	63.4	68.2
PF 08	73.4	67.7
PF 90	67.4	64.1
BS 45	77.3	77.6
BS 13	73.2	75.1
BS 03	67.5	74.8
BS 80	75.4	73.8
BS 05	77.1	76.2
BS 94	70.2	72.3
Control	0	0

Rhizobacteria antagonist test against S. oryzae *in vivo*

Based on research it is known that the average incubation period for S.oryzae pathogens occurs on the 9th day after inoculation. The fastest incubation period occurs in the control treatment (I0F1, I0F2, I0F3, I0F4) with an incubation period lasting for 7-8 days. The longest incubation period occurs in I2F3 treatment, where new symptoms appear after H + 14 inoculation of pathogens. This shows that the I2F3 treatment (PF 08 isolate with 10% glucose formulation + 2% CMC + 68% monmorillonite + starter bacteria) is able to reduce the level of infection, according to the results of research by Soesanto et al. (2010), which states that the use of P. fluorescens can reduce the infection rate up to 73.18-79.09%. Based on Table 2, it can be seen that the application of rhizobacteria does not significantly affect the incidence or incidence of the disease. Isolates and rhizobacteria formulations did not significantly affect the incidence of disease 84 HST. This happens because pathogens have infected rice plants, and cause symptoms in all treatments.

Table 2. $F_{calculated}$ of the disease incidence at 84 days after planting (DAP)

Source of variation	$F_{calculated}$	F_{table}
Isolate	1.90ns	2.90
Formulation	0.38ns	2.90
Isolate x Formulation	0.76ns	2.18

Remarks = *: significant; ns: not significant of the F test at 5%

The application of rhizobacteria in all treatments showed significant effects when compared to control treatments, where the control intensity of attacks was higher (Table 3). These showed that rhizobacteria weree able to suppress the intensity of pathogenic attacks (Bora and Ali, 2019). The inhibition of mycelium growth by bacteria was done by producing sideropores such as pseudobactin, pyochellin, pyoveradine, antibiotics, carboxylic acid, and chitinase. The treatment that showed

Table 3. Antagonism test of Rhizobacteria against S. oryzae *in vivo* (at 84 DAP)

Treatment	Severity of the disease
Control	61.5 d
PF 08 + glucose + potato water	53.7 c
PF 08 + fructose + coconut water	30.5 a
PF 08 + glucose + CMC + monmorillonite	32.4 a
PF 08 + fructose + CMC + kaolin	34.2 ab
BS 45 + glucose + potato water	34.2 ab
BS 45 + fructose + coconut water	32.4 a
BS 45 + glucose + CMC + monmorillonite	29.6 a
BS 45 + fructose + CMC + kaolin	33.3 ab
PF 08+ BS 45 + glucose + potato water	34.3 ab
PF 08+ BS 45 + fructose + coconut water	40.0 bc
PF 08+ BS 45 + glucose + CMC + monmorillonite	35.1 b
PF 08+ BS 45 + fructose + CMC + kaolin	37.1 b

Remarks = The number at of lanes followed by the

the lowest intensity value was I2F3 treatment (BS 45 isolate with 10% glucose formulation + 2% CMC + 68% monmorillonite + starter bacteria). This showed that the isolate Bacillus sp. had the ability to suppress the growth of pathogens better when compared with other treatments. Suriani et al., (2018) said *Bacillus* sp. has 2 kinds of pathogen suppression mechanisms namely directly and indirectly. Directly *Bacillus* sp. will produce poisons or toxins harmful to pathogeny, while the mechanism indirectly was that bacteria induce plant resistance by suppressing the number of pathogenic colonies in the roots.

According to Suwahyono (2013), the formulation of biological agents is expected to improve the stability of biological agents in various types of storage and facilitate the use or application. The best formulation in this study was shown by the 10% glucose formulation + 2% CMC + 68% monmorillonite + starter bacteria. (F3), with severity intensity values in the same formulation with different isolate factors showed the best results. This may be due to the media formulation in accordance with the growth of bacteria, so that bacteria multiply more quickly and colonize. In addition, the formulation is also related to the storage time, where the bacterial solid media tends to survive longer than the liquid media. This is according to the statement of Berleany *et al.* (2011), that momorilonite contains silica, iron, s and magnesium which can help the growth of bacteria.

CONCLUSION

Based on the results, it was concluded that *Bacillus* sp. (BS 45) isolate with 10% glucose formulation + 2% CMC + 68% monmorillonite + starter bacteria, showed the best results in suppressing disease severity with an effectiveness value of 78.31%. The best formula to suppress the diseases severity was found in *Bacillus* sp. (BS 45) isolate with 10% glucose formulation + 2% CMC + 68% monmorillonite + starter bacteria, supressing 78.31% of the disease severity.

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