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BIOCONTROL FOR RHIZOCTONIA STEM ROT DISEASE BY USING COMBINATION OF SPECIFIC ENDOPHYTE IN PADDY TIDAL SWAMP

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ABSTRACT

The use of combination of specific endophytic in tidal swamps to control stem root disease as biological control agents has not been done. It is expected that this combination is able to continuously protect plants from pathogen interference. The research was carried out in type C tidal swamp in Banjar regency of South Kalimantan, from March to November 2011, temperature 29-32^{o}C, and pH 4.0-5.5. The method used was Split Plot design. Biocontrol preparation for both types of endophytic was applied in seeds in 7 days after planting (DAP). Observation on high intensity and plant diseases of planting stage on tidal swamps (taradak, ampak and lacak) was conducted. The results showed that there was a reduction of disease ranging from 58.70 to 87.29%. The application of combination of two biocontrol agents (*T. viride* PS-2.1 + *P. fluorescent* PS-4.8), (Fusarium non-pathogenic PS-1.5 + *P. fluorescent* PS-4.8) and (*T. viride* PS-2.1+ FNP PS-1.5) isolate gave the best inhibition result, reduced disease intensity, and increased plant height. The result of soil analysis before and after application of endophytic showed that there was an increase in soil fertility with the element addition of N, P, K and pH.

Keywords: stem rot, endophytic combination, paddy, tidal swamp

INTRODUCTION

R. solani is one of the most important phytopathogen which attacks rice cultivated on tidal swamps of South Kalimantan, causing stem root disease. *R. solani* intensity constantly increases because it is difficult to control flood (Budi and Mariana, 2012). Therefore, effective and efficient controlling method safe for environment is required.

R. solani and other soil borne pathogens are controlled using synthetic, but the application is usually hampered by the coming tide which washes away the pesticide. On the other hand, planting local varieties in tidal swamps using three replacement stages can lead to inefficiency in pesticide use (Budi and Mariana, 2012). The use of specific biological agents should be done immediately because of consumers' demand for synthetic chemical free products.

According to Budi and Mariana (2009), in paddy antagonist isolation from three different types of tidal swamp (locally termed as: taradak, ampak and lacak) were founded 28 endophytic colonies consisting of 23 colonies of fungi and 4 colonies of bacteria. From the *in-vitro* test, there were 4 isolates having higher antagonistic potency, namely *Trichoderma viride* PS-2.1, Fusarium non-patogenic PS-1.5, *Pseudomonas fluorescent* PS-4.8, and *Bacillus sp* PS-3.14.

On biological control, *R. solani* can be parasitized by mycoparasites such as *Gliocladium* spp., *Trichoderma* spp. and *Verticillium biguttatum* Gams (Van den Boogert and Deacon, 1994). Fungus *V. biguttatum* is a mycoparasite with biological activity against the important plant pathogen *R. solani.* The biotrophic mycoparasite *V. biguttatum* was first isolated from sclerotia of *R. solani* on potato tubers in Netherlands (Jager and Velvis, 1988). This fungus is able to destroy sclerotia (Jager and Velvis, 1988) and hyphae (Van den Boogert and Deacon, 1994) of *R.solani*. When applied as a spore suspension, mycoparasite *V. biguttatum* has the ability to suppress stem canker and black scurf on potatoes (Jager and Velvis, 1988).

According to Howell and Stipanovic (1995),the growth of *Rhizoctonia solani* on the cotton plant can be controlled by seed treatment

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using *Gliocladium virens*. Antagonists of non-pathogenic Fusarium strains isolated from supressive soil have the capability of reducing the disruption caused by Fusarium wilt in some plant (Nel *et al*., 2006). While the bacterium *Pseudomonas capacia*, *P. fluorescens* and *P. gladio* were also able to control the growth of *P. solanacearum* causing wilt on tomatoes. Other bacteria such as *Bacillus mesentericus*, *B.megaterium*, *B. mycoides* and *Erwinia* sp. also act as biological control for wilt disease in several plants (Harman, 1999).

Combined antagonists might give a better result than a single antagonist beside its uncertain result due to influence from environmental condition and time of application (Yigit and Dikilitas, 2007). Use of *Trichoderma* spp. and *Gliocladium* sp. will provide optimal results in controlling soil borne and air borne pathogens when the application is done in low inoculum population (Cook and Baker, 1983).

The use of specific biological agents which have a coevolution will be able to stimulate the development of harmful rhizosphere micro-organisms, and more than one antagonist is usually isolated in the field (Budi and Mariana, 2009). Therefore, selecting the best combination of antagonists that can protect plants better from various pathogenic disorders is needed.

This research was conducted to determine the efficacy combination of specific antagonist isolate in planting stage in tidal swamps for biological control of stem rot disease in paddy. It is expected that by using the correct combination of antagonist will give a better result.

MATERIALS AND METHODS

Isolation and mass production of antagonist agents from plant samples were done by taking healthy plants as samples from the infested paddy planted areas in tidal swamp in Banjar regency of South Kalimantan; endophytic isolation was done on the root and the base of the plant (Budi dan Mariana, 2012). Isolation method was based on Homby methods (Fokkema *et al*., 1959) where dilution plate method (10^{-4} to 10^{-6}) was applied. Isolation of Pseudomonas fluorescens group using selective media (Sands and Rovira, 1970). Each Pseudomonas fluorescens group was isolated then tested according to Dhingra and Sinclair method (1995).

Test on *in vitro* inhibition potency through the combination of endophytes towards *R.solani* as well as the test measuring the suitability of antagonist combination were performed on potato dextrose agar (PDA) in a petri dish by growing isolates in pair. Then, measurements to figure out the inhibition potency were carried out by applying the formula of Fokkema (Fokkema *et al*., 1959):

$$I = (r_1 - r_2)\,(r_1)^{-1} \times 100$$

Remarks:
I is the percentage of inhibition;
r1 is the radius of A colony that grows in the opposite direction to B
r2 is the radius of A colony that grows in the direction of B

In vivo test mesuring the hampering ability of antagonist isolate towards stem rot disease pathogen was conducted via split plot design in a farming land and a greenhouse on sterilized soil. Pathogen inoculation was performed when it was three-week-old by springkling formulated pathogens (40 $kg.ha^{-1}$) in the soil around paddy stems. The application of antagonists was given one week before planting, and at the time of planting by soaking seeds for 24 hours into suspension with spore density of 10^{-4} per ml. Observations were carried out the next three weeks in every planting stage on tidal swamps (locally termed as: taradak, ampak and lacak) by counting the number of plants that showed wilt or stem rot symptoms and measuring the height of the plant.

All treatments are given according to randomized block design (RBD) with five replications. To know the effect of the differences among the treatments, the median value of Duncan's test (DMRT) at 5% was performed.

RESULTS AND DISCUSSION

The treatment performed could reduce the disease around 58.70 to 87.29% in comparison to control. The combination of T. viride PS - 2.1 + FNP PS - 1.5 represented the inhibition ability reaching up to 73.40%, and it reduced the disease intensity up to 79.67%. However, Combination of *T. viride* PS-2.1 + *P. fluorescens* PS-4.8 seemed to show better inhibition potency (78.00%) and disease intensity reduction (84.50%), while FNP PS-1.5 + *P. fluorescens* PS-4.8 managed to perform the best inhibition potency (82.00%) and disease intensity reduction potency (87.29%) (Table 1). But, the single isolate by Budi and Mariana

(2009) on inhibition ability pair test was known that all of the isolates had an ability to provide better inhibition towards *R. solani,* such as *Trichoderma viride* PS-2.1 (68.00%), Fusarium non-patogenic PS-1.5 (65.98%), *Pseudomonas fluorescent* PS-4.8 (55.50%) and *Bacillus sp* PS-3.14 (40.25%). Thus the combined use of several antagonists will be able to increase the inhibition on the development of stem rot disease of rice. Guetsky *et al.* (2001) stated that the appropriate combination would be able to enhance antagonistic potency. Olivain *et al*. (2006) proved that the use of either combined antagonists or antagonist applied singly was capable of inhibiting the development of pathogens causing tomato root disease.

The results also showed it was effective to give the application in one week before planting and soak the seeds for 24 hours before planting in the antagonist suspension with a density of 10^5 spores/ml, where the combined application of *T. viride* PS-2.1, *P. fluorescens* + PS-4.8 could reduce attack intensity by 84.50%, while the combined application of FNP PS-1.5 + *P. fluorescens* PS-4.8 amounted to 87.29%, and *T. viride* PS-2.1 + FNP PS-1.5 reduced the attack intesity by 79.67%. However, the combined antagonists precisely reduced the capability of inhibiting disease progression (Table 1 and 2).

The results of this study are in line with Yigit and Dikilitas (2007), implying that the use of FNP + *P. fluorescent* and *T. harzianum* T-22 + *P. fluorescent* in the laboratory test showed the better potency in inhibiting the development of *Fusarium oxysporum f. sp. lycopersici* Snyder and Hansen than singly applied antagonist. A combination of Fusarium isolates Fo47 + *P. fluorescent* strain C7 showed similary potency in inhibiting the development of Fusarium wilt on tomato. Combined application of *P. fluorescent* and *Bacillus subtilis* in sterilized soil test indicates the ability of the inhibition is much better than just one type of single application because the two bacteria proved to work synergistically. Biocontrol efficacy arising from combined use of two biocontrol agents did not depend greatly on biocontrol mechanisms. Combined use with at least one competitive biocontrol agents resulted in better control than combined use of two mycoparasitic (Xu and Jeger, 2013).

The potency of *Trichoderma* spp was allegedly taken from many studies indicated from the mechanism of action of enzymes such as β-1-3-glucanases and β 1-4 glucanases. Those enzimes were capable of producing substances, spurring the development of resistance (induced resistance) and competing for space and nutrients of pathogens (Olivain *et al*., 2006). Last but not least, this type of fungus was also capable of improving soil fertility, as proven by the growth of plant in the phase of taradak, ampak or lacak (Table 3). The presence of antagonistic fungi was able to suppress disease progression in addition their capability of supplying nutrients for plant growth (Altomare *et al.*, 1999; Hanson and Howell, 2004 and Harman, 2006).

Table 1. Percentage of inhibition in vitro test and Intensity of stem rot disease after application on sterilized soil in greenhouse

Treatments	In-vitro test Inhibition (%)	In-vivo test	
		Disease Intensity (%)	(Reduction) (%)
Control	00,00 [a]	78,7 [a]	00.00
T. viride PS-2.1	30,00 [c]	21,7 [d]	72.43
FNP PS-1.5	17,28 [b]	27,6 [c]	64.93
P. fluorescens PS-4.8	26,80 [c]	28,4 [c]	63.91
Bacillus sp PS-3.14	13.50 [b]	32,5 [c]	58.70
T. viride PS-2.1 + FNP PS-1.5	73.40 [e]	16,0 [de]	79.67
T. viride PS-2.1 + *P. fluorescens* PS-4.8	78,00 [e]	12,2 [e]	84.50
T. viride PS-2.1 + *Bacillus sp.* PS-3.14	25,00 [c]	24,8 [d]	68.49
FNP PS-1.5 + *P. fluorescenss* PS-4.8	82.00 [e]	10,0 [e]	87.29
FNP PS-1.5 + *Bacillus sp.* PS-3.14	42.75 [cd]	31,5 [c]	59.97
Bacillus sp. PS-3.14 + *P. fluorescens* PS-4.8	50.00 [d]	29,8 [c]	62.13

Remarks: **Within column, means followed by different letters are significantly different (P<0.01; LSD test).

Table 2. Stem rot disease intensity on some time treatments and antagonist combination in greenhouse

Antagonist		Plant attacked		Plant height (cm)
Formulation	Application time	Disease intensity (%)	Reduction	
	Control	85.14 a	0.00	28.20 a
T. viride PS-2.1 + *P. fluorescen* PS-4.8	7 d before planting	25.18 b	70.43	37.64 b
	seed soaking	20.44 c	75.99	39.12 b
	7 d before planting + seed soaking	10.47 d	87.71	49.29 c
T. viride PS-2.1 + *Bacillus sp* PS-3.14	7 d before planting	31.20 b	63.35	30.25 ab
	seed soaking	27.25 b	67.99	39.00 b
	7 d before planting + seed soaking	20.50 c	75.92	45.25 c
FNP PS-1.5 + *P. fluorescen* PS-4.8	7 d before planting	18.39 c	78.41	38.56 b
	seed soaking	13.78 cd	83.82	31.92 ab
	7 d before planting + seed soaking	10.00 d	88.26	52,20 c
FNP PS-1.5 + *Bacillus sp* PS-3.14	7 d before planting	34.40 b	59.60	37.64 b
	seed soaking	31.00 b	63.59	39.50 b
	7 d before planting + seed soaking	22.47 c	73.61	45.70 c

Remarks: **Within column, means followed by different letters are significantly different (P<0.01; LSD test).

FNP and *Trichoderma* sp. are similar in ability according to Benhamou *et al.* (2002) in a way that both of them could colonize the surface of plant roots quickly, protecting from any pathogenic attack. Almeida *et al.* (2007), however, agreed that those two were known for their role in producing chitinase, β-glucanase 1-3 and 1-4 glucosidase enzymes. Thomashow and Weller (1996) added that this ability was triggered by the presence of toxins, antibiotics and siderophores.

Various combination of antagonists proved to be able to improve fertility. This is in line with the test performed by Duijff *et al.* (1998) showing that there was synergism in the use of combination of *Pseudomonas fluorescent* WCS417 + FNP so as to inhibit the development of Fusarium wilt pathogen better than the antagonist given singly. The variability of control in different environmental conditions could be reduced by using two kinds of antagonist: F. oxysporum avirulen coupled with a group *Pseudomonas fluorescens* in controlling wilt disease in various crops. The combination of several bacterial isolates antagonistic to fungus *Trichoderma hamate* more effectively suppress disease caused by *R. solani* compared with any other antagonist isolates applied singly (Kwok *et al.,* 1987).

The intensity of disease which occurred during planting stage of cultivation (taradak, ampak and lacak) revealed that there was significant difference compared with control. All treatment combinations give the ability to suppress the disease intensity more than 50%, the best treatment was performed through the isolate combination of *T. viride* PS-2.1 + *P. fluorescen* PS-4.8 (Table 3).

The ability of the FNP in inhibiting progression of some diseases was pointed out by Weller (1988), in which plant defense mechanism was triggered by the availability of certain agents known as endophytic antagonists. Information previously presented by Pal and Gardener (2001) implies that endophyte as an induction agent of resistence (induced resistance) can stimulate plant resistence, which may be caused by the presence of certain chemicals, non-pathogenic microorganisms, or incompatible pathogenic or virulence.

The ability of endophytic fungus in inhibiting development of pathogen could be performed with the presence of antibiosis mechanism, competition and microparasit. Ozbay and Newman (2004) proved that the fungus *Trichoderma* spp was capable of accumulating carbon monoxide in the competition for space and nutrients. *T. harzianum* strain T24 was even capable of producing cellulose in the form of 1,3-glucanase enzyme one hour after inoculation,

whereas in strains SC164, SC 167 and SC 168 tested in a greenhouse, *Trichoderma* was found capable of inhibiting the *R. solani* attacks on tomatoes because of accumulation in chitinase and highest glucanase enzyme.

Thus, it is obvious that the combination of endophyte, once released to root zone of plants, plays a role in endophitic microbial conservation in rhizosphere environment which is expected to contribute to the conservation in biological environment. Similarly, augmented crop land in the growth of *rhizosphere T. viride* caused the continuous resistence due to a number of fungal antagonists which was capable of protecting the plants from pathogenic attack which may cause wilt disease. The application of *T. viride* on banana plant was also capable of preventing wilt disease caused by *Fusarium sp*.

Based on the results of soil chemical analysis, it proved the effect of antagonist application of chemical elements in soil. There was a significant increase when compared to the plant before given any application . Longer period of application had a tendency to to give more significant increase where the effect in taradak phase would be much lower than that of lacak.

The microbe echanced nutrion and pH soil as shown in Table 4. This is because the combination of fungi accelerated decomposition of organic material in soil. In addition, decomposition of compost may cause soil pH to become more alkalin, so the nutrient becomes available to plant. The increase of pH in soil could increase when added with glucose due to decarbolization and aminization of nitrogen compound in order to contribute to plant growth. According to Harman (2006), *Trichoderma* sp and plant symbionts are capable of enhancing the mechanism of resistence to root disease infected either directly or indirectly by changing the composition of microflora in roots. *Trichoderma* spp and *Gliocladium viren* produce chlamydospores, which actively grow as biocontrol fungus within 2 to 3 day period under no special aseptic condition. *G. virens* and *T. hamatum* with the weight of 1.5% (wt/wt) reduced damping-off desease on eggplant caused by *Rhizoctonia solani.* According to Harman (2006), antagonism involving cortex and epidermis of root can secrete bioactive molecules which lead to forming thallus cell wall of Trichoderma. Meandproteome plant transcript is able to spur plant resistance, increase nutrient absorption and plant growth.

Table 3. Disease intensity and plant height after application in each planting stage on type C tidal swamps in Banjar Regency of South Kalimantan

Treatments	Planting Stage on Tidal Swamps								
	Taradak			Ampak			Lacak		
	Symptom Intensity (%)	Reduction	Plant height (cm)	Symptom Intensity (%)	Reduction	Plant height (%)	Symptom Intensity (%)	Reduction	Plant height (%)
Control	33.50 c	0.00	15.25 a	42.00 c	0.00	47.20 a	55.25 c	0.00	93.50 a
T. viride PS-2.1 + *P. fluorescen* PS-4.8	12.50 a	62.69	36.00 c	10.40 a	75.24	69.50 c	16.00 a	71.04	145.50 c
T. viride PS-2.1 + *Bacillus sp* PS-3.14	20.25 b	39.50	20.25 b	25.42 b	39.48	56.25 b	20.18 b	63.48	130.75 b
FNP PS-1.5 + *P. fluorescen* PS-4.8	14.00 a	58.21	32.40 c	24.50 a	41.67	53.50 b	22.00 b	60.18	139.45 bc
FNP PS-1.5 + *Bacillus sp* PS-3.14	15.50 a	53.73	23.25 b	26.50 b	36.91	53.40 b	23.45 b	57.56	127.25 b

Remarks: **Within column, means values followed by different letters are significantly different (P<0.01; LSD test).

Table 4. The results of chemical analysis of soil before and after application of endophyte combination in tidal swamps

Treatments	Result of soil nutrient analysis					
	Before treatment			After treatment		
	N	P	K	N	P	K
Control	0.237	0.018	0.352	0.201	0.010	0.394
T, viride+ *P, fluorescens*	0.240	0.018	0.352	0.743	0.027	0.491
T, viride + *Bacillus sp,*	0.237	0.018	0.352	0.765	0.027	0.480
FNP + *P, fluorescenss*	0.239	0.018	0.352	0.740	0.028	0.594
FNP + *Bacillus sp,*	0.237	0.021	0.352	0.680	0.027	0.431

CONCLUSION

The combination of two specific endophytes in paddy tidal swamp showed that the inhibition ability is much better than singly applied endophyte. The best combination of two endophytes was *T. viride* PS-2.1 + *P. fluorescent* PS-4.8, Fusarium non-pathogenic PS-1.5 + *P. fluorescent* PS-4.8 and *T. viride* PS-2.1 + FNP PS-1.5 which reduced the intensity of stem root disease caused by *Rhizoctonia* sp, stimulated plant growth, soil pH, and soil fertility made of N, P and K which are available to plants.

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