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The Application of Endophytic *Trichoderma Viride* PS-3.7 Using Indigenous Tidal Swamp Aquatic Weeds Media to Control Paddy Wilt Disease

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ABSTRACT---- ***Characteristic of paddy at tidal swamp should passed taradak, ampak and lacak phase. Disease control by using synthetic pesticides sometimes constrained by land inundated. This research tries to antagonists and aquatic weeds utilizing site-specific swamps land. The result of the study giving evidence the best antagonist (Trichoderma viride PS-3.7) with aquatic weeds used as applicative media are considered to be able to increase the number of antagonistic spores, inhibiting, and antagonistic potential toward wilt disease in paddies caused by Fusarium oxysporum. Then, the number of spores produced in enceng gondok (Eichhornia crassipes),, kiambang (Salvinia molesta) and kayapu (Pistia stratatiotes) were (7.80×10^8 spora/g), (6.1×10^8 spora/g), (2.98×10^8 spora/g), respectively. The inhibiting capability of Trichoderma viride isolate PS-3.7 toward F. oxysporum was 80.44%, while it reached 90.22% toward Fusarium oxysporum. The results of the observation revealed that in taradak phase where the applicative medium of water hyacinth was employed, the intensity of disease was 15.71%, ampak phase, where kiambang was introduced, the disease intensity was 20.00%, while in lacak phase with the application of water hyacinth, the disease intensity was only as much as 10.70% in comparison with the control group which reached 45.60%.***

Keywords--- Trichoderma, aquatic weed, wilt disease, swamps land

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1. INTRODUCTION

The control on plant disease by applying synthetic pesticides in tidal swamp hampered by unpredictable tidal wave which washes away the pesticides. The absence of pesticides triggers diseases to come and to increase.

On the other hand, the nature has something to offer such as useful microbes which function to balance the nature but have not been maximally used. Endophytic microbes, for example, are quite potential in dealing with pathogenic disturbance commonly found in the land covered by tide.

During the research, some antagonists of four types of tide cover in South Kalimantan were identified. Twenty antagonists were found mostly from *Trichoderma harzianum*, *Gliocladium sp.*, *Pseudomonas fluorescent*, *Bacillus sp*. and *Trichoderma viride* [1]. Moreover, the proper applicative media were required in order that the antagonists could efficiently and effectively fit the natural application.

This research was aimed at investigating the effectiveness of antagonists after they were grown in the applicative media of enceng gondok (*Eichhornia crassipes*), kiambang (*Salvinia molesta*) and kayapu (*Pistia stratatiotes*) in order to control wilt disease in paddies and to find out the effect of endophytic applicative media on the growth of paddies grown in tidal swamps.

2. RESEARCH METHODS

Exploration of antagonist endophyte which taken from healthy plants are among infected wilt disease causes *Fusarium oxysporum* from several central location pairs rice cultivation swamps land in South Kalimantan Indonesia.

Propagation of pure isolates in PDA media for in-vitro tests in pairs to determine isolates that have the best inhibition [2]. The test measuring the inhibiting capability of endophyte with the direct opposition was done by employing the formula by Fokhema *et al*. (1959) [3]. Isolates of *Trichoderma spp* is the best based on inhibition propagated in 3 kinds of media applicative aquatic weeds water enceng gondok (*Eichhornia crassipes*), kiambang (*Salvinia molesta*) and kayapu (*Pistia stratatiotes*) to test in the field. Field study was conducted in tidal swamp of Barito Kuala regency in order to observe the intensity of wilt disease and the plant growth by applying Completely Randomised Design (CRD) with five replications. The difference between treatments was tested by applying Duncan's Multiple Range Test (DMRT) at level 5%.

3. RESULTS AND DISCUSSION

Base on the results of exploration on antagonistic endophytic from rice cultivations in tidal swamps at several locations founded 64 isolated. After being tested with in vitro dual test against with pathogen *Fusarium oxysporum* has been found that *Trichoderma viride* SP-3.7 has the best ability of inhibition with an average of 80.44%. *Trichoderma viride* SP-3.7 which obtained from tidal swamps type 3 rice plants 7^{th} location in Barito Kuala regency.

Abadi (1987) confirmed that antagonist test on *Trhichoderma spp* and *Gliocladium spp*. isolates toward pathogen isolate *Fusarium sp*. in oil palms indicated the presence of inhibition zone which might separate the growing ground of the tested microbes. This inhibition zone indicated that those fungi generated compounds which were diffused into a medium, so that it inhibited the growth of *Fusarium sp*. into antagonist isolate [4]. The antagonistic potential in isolate is related to the capability of isolate to hamper the growth of *R. solani*, as stated by Limberg, 1998 in Wakimoto (1986), due to the presence of antibiotic substance diffused, creating the bright zone on which pathogens cannot grow [5]. According to Geels *et al*. (1983), the antibiotic substance is proven to be able to be isolated from the bright zone 3 to 5 days after the antagonists are grown [6].

The research results revealed that there was an increase in the number of spores produced after the isolate of *Trichoderma viride* PS-3.7 was grown on applicative media of water hyacinth, kiambang and kayapu. In each gram of organic matter, water hyacinth could produce 7.80×10^8 spores, only 2.98×10^8 spores in kayapu, and 6.1×10^8 spores in kiambang. In comparison with the previous research [(1)], the use of rice hulls contributed to the production of approximately 12.8×10^8 spores, while only around 2.6×10^5 spores were produced without any addition of organic matters. The increase number of spores produced was closely related to the composition of organic matters in weeds rich in nutrition the fungi can rely on to keep producing. Suhaya (1991) agreed that every 100 gram of water hyacinth contained energy as much as 18 calories, 1 gram protein, 3.8 carbohydrates, 0.2 gram fat, 80 mg calcium, 45 mg phosphors, 4 mg irons, and vitamin contents such as 1000 IU vitamin A, 0.08 vitamin B1, and 50 mg vitamin C [7].

Therefore, a large number of spores produced in the applicative medium proved that the medium was capable of providing the space for growth and sporulation and playing a role in increasing the antagonist potential. According to Hjeljord *et al.* (2001), *Trichoderma spp* required nutrition from outside to germinate until the growth was stable. Lack of nutrition could lead to the low percentage of spore germination, the low prolongation of hyphae, and the decrease of sporulation. Easy access given to nutrition could enhance the capability of antagonistic agents [8].

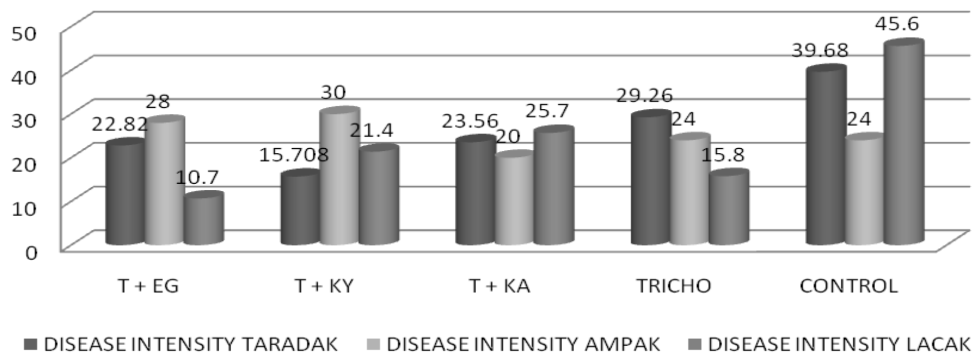

Figure 1. Effects of applicative media on endophytic capability of *T. viride* PS-3.7 to control the disease-transmitting stage in paddy

In taradak phase, the application of kayapu, water hyacinth, and kiambang to *T. viride* PS-3.7 isolate only hampered the disease intensity by 15.71%, 22.82% and 23.56%, respectively, while the intensity of disease was 39.68% in the control group. In the phase of ampak, the application of kiambang reduced the intensity of the disease by 20.00% compared with the control which still reached 24.00%. In the phase of lacak, the disease intensity in the control group increased to 45.6%. The application of isolate using organic matters of water enceng gondok, kiambang and kayapu gave different percentage of the disease intensity of 10.7%, 25.7%, and 21.4%, respectively (Figure 1). Paustian and Schnürer (1987) agreed that chemical compound resulting from decomposition of organic media required microbes as the source of nutrition taken externally for germination until the growth was stable. *Trichoderma sp.* antagonist possesses oligotrophic characteristic, and it still grows rapidly on the media lack of nutrient [9].

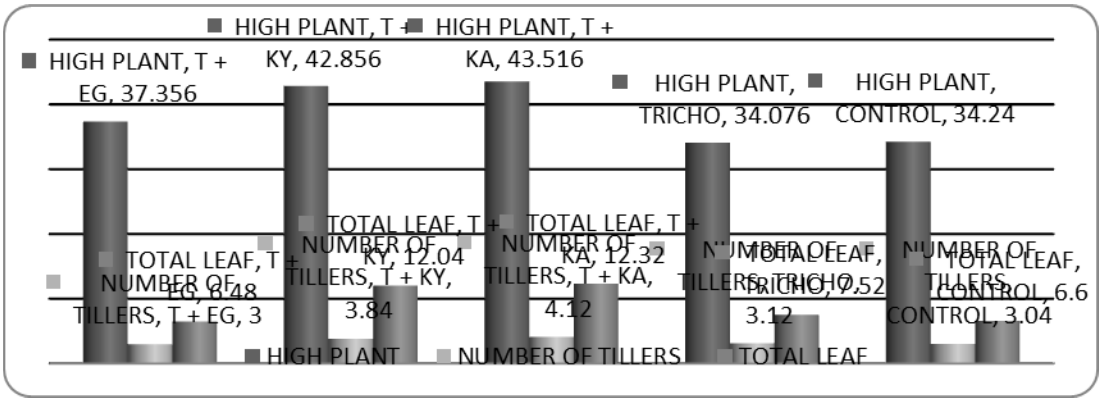

Figure 2: Effect of *T. viride* PS-3.7 formulation on plant height, number of tillers, and number of leaves

From the womb, kiambang simple protein 15-29%, leaf protein concentrates 48-60%, fiber 14-17%, 53330.46 IU beta carotene, calcium 6%, fat 4.1 to 5.8%, and minerals such as Fe, Mn, Mg, Na, Cu, Zn, and amino acids.
The different degree of potential after the media application was possibly caused by the presence of composition difference of nutrients, and toxins which may hamper the growth of microbes. Whipps (2001) stated that the inhibiting diseases could be enhanced by manipulating physical and chemical attributes of soil and microbiological environment through the application of soil organic matters. Microbes found in compost were to compete with pathogens for carbon, which can hamper the disease [10]. According to Simons *et al*. (1997), sugar content was mostly reported as the source of the primary carbon in root exudates. The synthesis of amino acid is required in the colonisation by *Pseudomonas Fluorescens* WCS365 in root in addition to the effect caused by phototrophic amino acid involved in rhizosphere competence [11]. In another case, the application of *Trichoderma hamatum* 382 to compost could boost plant resistance to *Botritys blight* on plant of Begonia [12]. The application of 5 tons/ha straw compost + 400 g/ha biofertilizer could inhibit the development of blight disease (*R. solani*) in paddies in 5 weeks before planting [13]. The addition of compost could inhibit the growth of *P. ulitimum* with the percentage of failure in germ tube higher in compost than that in sand.
The use of decomposed water weeds was proven to be able to provide high carbon and nitrogen source. According to Driesche and Bellows (1996), in order to enhance the effectiveness of *Trichoderma sp*., it is suggested that rhizosphere contain easily metabolised carbon and nitrogen source for amino acid, sugar, and root exudate production needed in the growth and development of antagonists [14].

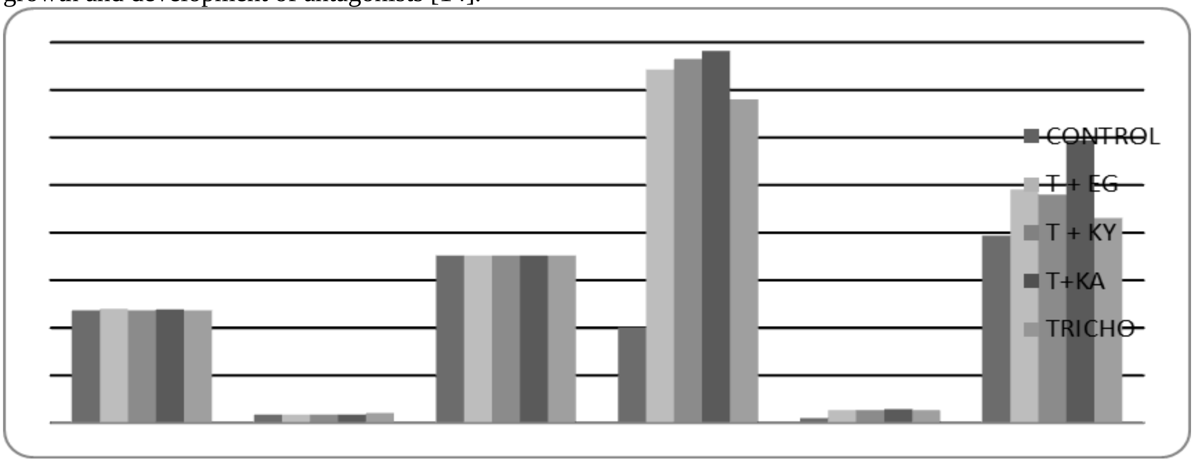

Figure 3. Chemical analysis result of organic contents

The betterment of inhibition capability in both *in-vitro* and *in-vivo* test was believed to be related to the result of organic matter decomposition in water hyacinth, kiamang and kayapu with high C/N ratio content, which may trigger theantibiotic production by *Trichoderma spp* [15]. Several types of antibiotics produced by *Trichoderma spp* comprise gliovirin, gliotoxin, viridin, pyrones, and peptaibols [16]. *Trichoderma koningii* SMF2 produces trichokonin which is capable of controlling several bacteria and fungi. Gliotoxin (epidithio diketopiperazine antibiotic) and viridian are categorised in antibiotics produced by *Trichoderma virens* [17].
Compost is able to enhance the antagonistic potential of some species of *Trichoderma sp* through the production of enzyme which can degrade pathogens' cell wall. Composted bark decreases cellulose and activates chitinase gen which could parasitise *R. solani* [13]. El-Katatny *et al*., (2000) agreed that the production of chitinases and glucanases by *T. harzianum*, was affected by carbon source and triggered by pH of the medium (pH 5.5 -6) [18].

In their study, Mew and Rosales (1984) reported that endophytic bacteria could also inhibit intensity of blight disease (*R. solani*) in paddies [19]. Hartaman *et al*. (1992) stated that *Pseudomonas capacia*, *P. fluorescens* and *P. gladio* had potential of controlling the growth of *P. solanacearum* which causes wilt disease in tomatoes. *Bacillus mesentericus*, *B. megaterium*, *B. mycoides* and *Erwinia sp*. could serve as biological control for wilt disease in certain plants [20].
The increasing antagonistic potential after treatment in applicative media indicated that the media had influence on endophytic antagonistic potential in soil. According to Mangenot and Diem (1979), Efficiency in biological control increases when the antagonists used is capable of growing on rhizosphere and has high persistence [21]. Weller *et al*. (2002) reported that the mechanism of plant resistance could be influenced by a certain cause such as the presence of antagonistic agents which are endophytic, so that they are potential to inhibit upcoming diseases [22].
Endophytic fungi are capable of inhibiting the development of pathogens due to antibiotic mechanism, competition and microparasite; Ozbay and Newman (2004) emphasised that *Trichoderma spp* was able to collect CO while competing for space and nutrition. In strain T24 of *T. harzianum*, the fungus was capable of producing cellulose enzyme as much as 1.3-glucanase an hour after inoculation, while in strain SC164, SC 167 and SC 168 in the test conducted in greenhouse, it was proven to be able to inhibit the attack *by Rhizoctonia solani* in tomatoes because of the chitinase enzyme and glucanase produced [23]. *T. viride* applied to banana could prevent wilt disease caused by *Fusarium* by systematically inducing resistance to the plant.

4. CONCLUSION

1. The best antagonist endophyte is *Trichoderma viride* PS-3.7, and applicative media of aquatic weeds could increase the number of spores produced, with the density of spores in every gram as follows: enceng gondok (7.80×10^8), Kiambang (6.1×10^8), and kayapu (2.98×10^8).
2. According to the *in-vitro* test results, applicative media of enceng gondok, kayapu and kiambang were capable of enhancing resistance of *T. viride* PS-3.7 to *F. osysporum* everage 80.44%.
3. According to *in-vivo* test, in the phase of taradak, the use of kiambang could lower the disease intensity up to 15.71% compared with the control (39.68%). In ampak phase, the application of kiambang could reduce the disease intensity by only 20.00%, while in lacak phase, the use of enceng gondok could reduce the disease intensity by only 10.70% compared with the control (45.60%).

5. ACKNOWLEDGEMENT

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