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Trichoderma spp. as Agent of Biological Control in Local Rice Diseases in Tidal Swamp Lands in South Kalimantan, Indonesia

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Abstract: *Trichoderma spp. has potential value to uses as biological control agent against Rhizoctonia solani, a potential diseases agent which are able to decrease rice productivity. The study of the effectiveness of Trichoderma spp. against Rhizoctonia solani in South Kalimantan is important to increase rice productivity, especially in tidal swamp field. The aim of the study is to evaluate the effectiveness of Trichoderma spp isolates from tidal swamp field against Rhizoctonia solani. Result of the study shows that Trichoderma spp. isolates from tidal swamp field was suspected as Trichoderma harzianum. The isolates are able to decrease plant disease intensity caused by Rhizoctonia solani about 7.4 %. Trichoderma isolates contributes to the plant growing which are represented by number of growing tiller per clump and plant height (about 12.2 cm). After application of Trichoderma isolates, the content of Nitrogen, Phosphor, Potassium in soils and soil pH increases. The uses of Trichoderma can be used as one of the methods in plant management and increase rice productivity in South Kalimantan.*

Keywords : *bio-pesticides, local rice management, Trichoderma, Rhizoctonia solani*

I. Introduction

Rice is an important food in the world. Rice productivity is the crucial issues among developing countries in Asia, including Indonesia. The rice crisis incidents contribute significantly to the future of community and lead to the numerous global crisis. Rice productivity facing numerous problem, mostly related to the environmental and safety food issues. The intensive uses of chemical pesticides has been identified contributes to the numerous recent environmental problems [1] [2].

Emerging awareness to the global environmental problems lead to the numerous actions to implement sustainable development [3]. Sustainable agriculture, including rice cultivation, recently received a lot of attention. Scholars argues that recent intensification of agriculture has been contributes to the decrease of environmental and biodiversity [4]. Extensive uses of pesticide to minimize pest has been reported caused soil and water pollution. Lake and river eutrophication has been identified related to the massive chemical fertilizer in the paddy field [5].

Integrated pest and disease management is crucial in sustainable agriculture. Integrated pest management aims to minimize risks and hazard to people and the environment. Principally, there are five principles of integrated pest management program, namely (1) pest recognition and identification, (2) pest monitoring and assessment, especially pest number and damage incident, (3) developing guideline for management action, (4) preventing pest, and (5) implementing combination biological, cultural, physical/mechanical and chemical management tools. Using natural enemy's organism has been practiced in the management of insect and pests. This tactics, called as bio-control, is one of the important activities in integrated pest management [6] [2].

Trichoderma species has been widely used as biological control agents in agricultural field. *Trichoderma* species has been reported effective for a range of crop diseases mitigation caused by pathogens, especially fungal plant pathogens. There are several mechanisms for *Trichoderma* to countermeasure pathogens, such as mycoparasitism, competition, and antibiosis. There is also possibility of these combinations in order to countermeasure plant diseases caused by pathogens. Taxonomically, *Trichoderma* belongs to the sub division of Deuteromycotina, classes Deuteromycetes, and familia Tuberculariaceae. The young colony of *Trichoderma* has white color, and change gradually become green to dark green. Colony color, however, depend on the colony strains and colony ages [8] [9].

Compared to the chemical control, there are numerous benefits in the usage of bio-control agents, namely (1) eco-friendly, (2)there are no negative impact to environment, (3) zero pollution and (4) able to protect agro-ecosystem biodiversity. Compared to the application of chemical pesticides, the application of

biological pest control often focus to the narrow pest target. Therefore, there are possibilities to maintain agricultural biodiversity. There are also no toxicity to environment and therefore increase healthy food [10].

In the perspective of sustainable agriculture practices, the application of *Trichoderma* as bio-control agent has been reported effective. The mitigation of plant disease using its antagonist species has been recognized environmentally friendly techniques. As far, there are numerous microorganism has been used as antagonist agent to mitigate plant diseases, encompasses *Trichoderma* sp., *Gliocladium* sp., and *Beauveria* sp. [10]. *Trichoderma* spp especially used as biocontrol agents to control *Clerotinia sclerotiorum* and *Sclerotinia minor* which lead to the crop reduction up to 50% [11] . It has been applied in many areas in the world.

Trichoderma viride and *T. harzianum* has been identified to countermeasure basal stem root diseases caused by *Rhizoctonia solani*. These species also reported able to countermeasure wilt disease caused by *Fusarium oxysporum*, and wilt disease caused by *Sclerotium* sp in tidal swamp lands in South Kalimantan about 60%-82% [12]. Tidal swamp lands in South Kalimantan especially have many potential number of *Trichoderma* which was less explored. The aims of the study is to evaluate the effectiveness of indigenous *Trichoderma* spp collected from tidal swamp lands in South Kalimantan against *Rhizoctonia solani*. This research is important for sustainable agriculture, especially in order to enhance and maintain rice productivity in South Kalimantan.

II. Methodology

Materials

Soil sample and *Trichoderma* isolates which were isolated from six regencies in South Kalimantan were used in this study. *Rhizoctonia solani* were used to test the bio-control abilities of the *Trichoderma* spp. isolates which were collected from tidal swamp lands from South Kalimantan.

III. Methods

***Trichoderma* spp. collection**

The collection and isolation of *Trichoderma* was following standard methods [12] [13] [14] *Trichoderma* isolates was collected from several sites of tidal swamp lands which are having no history of chemical fertilizer application. It is including Banjar, Tanah Laut, Barito Kuala, Kotabaru, Banjarmasin and Tanah Bumbu Regency in South Kalimantan. This sites selection also aims to covers numerous ecosystems from which the strains of *Trichoderma* from South Kalimantan may be isolated. About 100 gr soils sample was isolated from 15-25 cm depth from surface in rhizophere area. Soils was stored in plastic bag for further laboratory analysis.

Trichoderma spp. was isolated from collected soil. About 10 gr soils was collected and put at 100 ml 100 ml sterile water in Erlenmeyer flasks. Flask was incubated at temperature room to allow *Trichoderma* in soils suspended into water. About 1 ml suspension was then taken and mixed into 9 ml sterile water. Dilution was done until 10^3. In the next step, about 1 ml of suspension was plated onto PDA (Potato Dextrose Agar) medium to growing *Trichoderma*.

Purification

The *Trichoderma* isolates which are grows in PDA was purified by standard methods. The Trichoderma spp. isolates was identified in Laboratory of Plant Protection and Horticulture, Banjar, South Kalimantan. Focus of the purification was addressed to identify the species of *Trichoderma*.

***Rhizoctonia solani* isolation**

Rhizoctonia solani was isolated from rice plants in the field which are infected by *Rhizoctonia* pathogens using tissue plating techniques. Tissues plating techniques was done by selecting rice leaf with spot indicate plant infected by pathogens. About 2x4 mm of leaf was cut and wash using sterile water and following by Calcium hypochlorite $Ca(ClO)_2$ 5% wash in several second. These materials put in the Petri dish with agar water about 15 gr agar/liter water). Culture was incubated to grows *Rhizoctonia solani* colony [15].

***In vitro* test**

In vitro test was done in laboratory using standard methods. In vitro test was done by growing *Trichoderma* isolates and *Rhizoctonia solani* isolates in Petri dishes with PDA. In this experiment, Petri dishes plates without *Trichoderma* isolates were also utilized as controls.

***In vivo* test**

In vivo test was done in green houses using plant population which was infected by basal stem roots diseases that are isolated from tidal swamp lands. Observation was done three weeks after planting by calculating number of wilt disease or plant with basal stem roots diseases symptoms. These two treatments

were tested using completed randomized design. In order to identified the impact of treatment, the data was tested statistically using Duncan's Multiple range test Duncan (DMRT) $\alpha = 0.05$.

Antagonism mechanism

Antagonism mechanism includes antibiosis or lyses. It is an inhibition mechanism or destruction of cell or organism by particular compound. The observation of antagonism test was observed visually (Fig. 1).

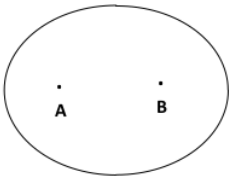

Fig 1. Antagonism test of *Trichoderma* and pathogens isolates

A = *Trichoderma* spp isolates
B = pathogen isolates

The degree of inhibition was calculated using Fokhema et al., (1959) formula as follow:

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

Where:

I = inhibition percentage
r_1 = Colony diameter of the *Trichoderma*
r_2 = Colony diameter of pathogens isolates

IV. Result and Discussion

***Trichoderma* spp. isolates from tidal swamp lands**

The morphological identification indicates that the species of *Trichoderma* spp which are isolated from field was suspected as *Trichoderma harzianum*. These species has smooth conidia. Morphological observation shows that the color of colony was dark green with grows diameter can reach 9 cm within five days at temperature 20˚C agar media. However, the molecular genetics assessment is needed to clarify the proper status of *Trichoderma* spp isolates from South Kalimantan. Molecular genetics is one of the powerful tools to identify and clarify the proper name of species.

On the surface of PDA plates, the colony of *Trichoderma* spp form cottony white mycelium and gradually color changes become with dark green towards the margins. The form of conidia was globose to sub-globose. The size of conidia was about 2.8-3.2 x 2.5-2.8 μm and smooth. This morphological characteristics similar with the *Tricodherma* isolates which are isolated and purified by previous authors who intensively observe Trichoderma [14] [16]. *Trichoderma* able to survive as saprophytes or parasites in other fungus species body.

The distribution of antagonist fungi *Trichoderma* spp found in numerous types of soil, including loam, sandy soil, forest soil and paddy field soil. This is also found in soil in the altitude 3.450 m asl. [17]. *Trichoderma* live in numerous types of soils and often found colonizing roots. *Trichoderma* was observed able to proliferate in healthy roots environments. In China, *Trichoderma* was reported found from north to south ecosystem of China, represent the wide range of *Trichoderma* distribution [18].

Antagonism test

In the laboratory, antagonism test of the ability of *Trichoderma* to inhibit *Rhizoctonia solani* shows the effectiveness of inhibition about 73.08 %. In the control, however, there a no antagonism evidence was identified, as shown by the value of inhibition percentage (Table 1and Fig.2). The antagonism of *Trichoderma* widely explored and concluded that this fungi provides benefits to protect roots from diseases and pathogens attack.

Table 1. The antagonism of Trichoderma spp to Rhizoctonia solani

No.	Treatments	Inhibition (%)
1.	*Trichoderma* spp.	73.08 a
2.	Control	0.00 b

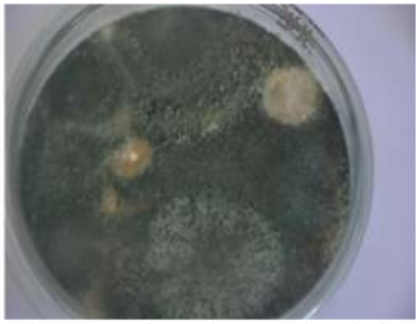

Fig 2. Antagonism test between *Rhizoctonia solani* pathogens and *Trichoderma* isolates collected from tidal swamp lands.

Trichoderma has ability to produce toxic substrate which is able to countermeasure other fungi or microbes in growth media. The substrate of *Trichoderma* has antagonism traits to plant pathogens, and has saprobes traits. A study confirms that pyrone which are produced by *Trichoderma harzianum* contributes to the antagonistic ability of this fungus. *Trichoderma harzianum* was widely uses as biological control in order to increase crops productivity [19] [20] [21].

Plant growing

The application of *Trichoderma* in paddy rice grows was significant (Table 2). This phenomena lead to the prospect of *Trichoderma* as an agent to increase rice and crops productivity, especially in South Kalimantan.

Table 2. Impact of Trichoderma treatment to the number of tiller and rice plant height.

No.	Treatment	Number of tiller	Plant height (cm)
1.	*Trichoderma* spp	42,0 a	135,2 a
2.	Control	38,0 b	114,0 b

Trichoderma isolates contributes to the plant growing which are represented by number of number of growing tiller per clump and plant height (about 12.2 cm). Scholar point out that one of the most important role of *Trichoderma* is their ability to support plants grows. It is also widely recognized that the symbiotic relationship between *Trichoderma* and plants can increase growth of the host plant [22] [23].

Disease intensity

Result of the experiment regarding intensity of Rice Sheath Blight Disease caused by *Rhizoctonia solani* shows that plant with *Trichoderma* application has relatively low disease. Trichoderma isolates able to decrease disease intensity caused by *Rhizoctonia solani* about 7.4 % (Table 3). Plant without application *Trichoderma* relatively attacked by disease easily.

Table 3. Impact of *Trichoderma* treatment to the intensity of Rice Sheath Blight disease (%)

No.	Treatment	Intensity of Rice Sheath Blight Disease (%)
1.	*Trichoderma* spp	0,1 a
2.	Control	7,6 b

The treatment of *Trichoderma* shows its ability to decrease plant diseases. It has been known that pathogen causing disease are able to survive in propagules or active saprophytes. Naturally, the survival of pathogens in the soil environments is suppressed by its antagonist. This is become the important key to control plant diseases caused by pathogens [19] [20].

In the control plant, the number of empty grain was higher than plant with *Trichoderma* treatment. High number of filled grain is related to the health status of plants, including effective photosynthesis and free metabolism disturbance caused by pest or disease. In the absence of *Rhizoctonia solani* leaf able to conduct photosynthesis properly to produce carbohydrates. This is become the key for the rice productivity. Scholar point out that disturbance in leaf, especially in photosynthesis process by numerous disease and pathogens are the major aspect to rice grains productivity [24].

Rice productivity

From the experiment, the application *Trichodema* spp contributes significantly in the number of tiller. Increase number of tiller potentially produce more grains, as indicated by impact of *Trichoderma* spp application in the grains productively as shown by weight of grains productivity (ton/Ha) and number of filled grains. Compared to the control, number of grains produced by rice with *Trichoderma* isolates application can be said double (Table 4). It is similar with the previous study that state *Trichoderma* able to increase crop productivity [25].

Table 4. The contribution of *Tricoderma* spp Application in rice productivity

No	Treatment	Number of tiller	Weight of grains	Ton/Ha of GRP	Number of grains	% unfiled grains
1.	*Trichoderma* spp	31,9 a	32,5 a	5,19	2770,9 a	2,9 a
2.	Control	22,4 b	16,5 b	2.63	1696,7 b	7,5 b

Note: GRP = grain rice productivity

Soil characteristics

The soils chemical before and after application of *Trichoderma* spp. show significant changes of several nutrients. After application of *Trichoderma* spp., the content of Nitrogen, Phosphor, Potassium and soil pH increases (Table 5). The availability of such nutrients is important for plant.

Table 5. Changes of soil performance after application *Trichoderma*.

pH		Nitrogen		Phosphor		Potassium	
Before	After	Before	After	Before	After	Before	After
6.31	7.59	0.08	0.46	9.67	29.8	0.12	12.4

Trichoderma contributes significantly in soil organic matter degradation, and therefore provides abundance nutrient in soil environments. *Trichoderma* able to grows in plant roots environment and able to protect root from numerous pathogens. These lead to the increase plant productivity, as shown by Table 4. This is similar with other research [17].

Based on ANOVA, treatments contributes significantly to plant grows as represented by the increasing number of tiller and plant height. Scholar point out that adding *Trichoderma* in soils environmental are able to increase nutrient availability which are needed by plants to grows. Plans grows are able to produce tiller. Scholars point out that the application of *Trichoderma* spp able to increase availability of Nitrogen, Phosphor and Potassiun which are needed by plants.

Besides able to countermeasure disease attack, *Trichoderma* spp treatment are important to increase plant grows and development. It is observed through the increasing number of tiller and plant height. Inhibition of disease agents lead to the normal functions of leaf in metabolism, including photosynthesis that are contributes to the plant grows. The application of *Trichoderma* spp. in swamp land contributes to the availability of nutrients which are needed by plant. Scholars point out that that there are increasing nitrogen content in soil with *Trichoderma* spp application [25] . The development of new tiller and therefore increasing number of tiller and productivity as a consequence of *Trichoderma* application was observed by scholars. *Trichoderma* contributes significantly in length of leaf, roots, number of tiller, steam diameters, and wet and dry weigh of plants [15].

Inhibition test

The inhibition test of *Trichoderma* spp to *Rhizoctonia solani* grows was 73.08 %. The development of inhibition zone represent the present of antibiosis compound which are produced by *Trichoderma* isolates that are able to inhibits grows of *Rhizoctonia solani*. This is similar with other previous study that report *Trichoderma* isolates produce antibiosis β-1,3 glucanase and Chitinase [26] [27].

V. Conclusion

This study shows that soil from tidal swamp lands contains *Trichoderma* spp. which are able to inhibits *Rhizoctonia solani* caused decreased of rice production in tidal swamp lands. The application *Trichoderma* able to increase plant resistance to disease, especially basal stem roots diseases and wilt disease. The antagonism test of *Trichoderma* spp. isolates to *Rhizoctonia solani* shows inhibition ability about 73,08 % through antibiosis and parasitism mechanism. *Trichoderma* plays an important role as decomposer which area able to provides plant nutrient.

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