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Exploration and Characterization of *Trichoderma* sp. in Conventional and Organic Rice Field in Bali

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ABSTRACT

Although *Trichoderma* spp has been widely used in the SRI technique of rice cultivation the sources of *Trichoderma* that can adapt well to the local environment have been an interesting issue for quite some time. It is commonly agreed that the local *Trichoderma* is much more preferred to be used for rice cultivation. In the present study, the *Trichoderma* from the soil of a rice field in Sukawati District, Bali where conventional fertilizer and pesticide has been applied for a very long time was isolated. After macroscopic observations (green color and sweet coconut smell) the single isolated fungi were very much resembled *Trichoderma Harzianum*. 2 mL of the *Trichoderma harzianum* solution was introduced in a plastic container containing 200 g half-boiled rice. After seven days the rice in the plastic appeared green. This sample will be used to produce more *T. Harzianum* for rice cultivation. One of the *T. Harzianum* treated rice hosts was coated with gold and subjected to SEM observation. Very highly populated *T. Harzianum* fungi were observed) dominated by hyphae. No conidia and conidiophores were spotted. Instead, quite a high density of spherical and oval shape spores was observed. Such a high density of *T. harzianum* will certainly meet the requirement for an efficient application in the cultivation field. It would also be interesting to see the interaction between such a highly populated *T. harzianum* with other fungi or pathogens in understanding the role of *Trichoderma* as an antipathogen.

1. INTRODUCTION

1.1. Research Background

Currently, the agricultural system in Indonesia tends to use conventional systems because it is more practical in controlling plant-disturbing organisms and increasing agricultural yields. Farmers' dependence on chemical pesticides in conventional systems should be urgently considered for reduction, and the unwise use of pesticides often causes health problems, environmental pollution, and disruption of ecological balance and results in increased residues in agricultural products. Therefore attention to more environmentally friendly control alternatives is growing to decrease the use of synthetic chemical pesticides.

One of the utilization of biological control agents in the form of fungi that have the potential as biological agents of antagonistic fungi includes *Trichoderma* sp. This fungus is preventive to the attack of plant disease pests as well as soil biological fertilizers and bio fungicides. *Trichoderma* sp. in

addition to being a decomposing organism can also function as a biological agent and stimulator of plant growth.

With the increasing demand for products in large quantities and of good quality, farmers are increasingly using pesticides to protect crops without paying attention to environmental and product health aspects. Meanwhile, fertilizer inputs are also given excessively so that excessive use of Nitrogen (N) and Phosphorus (P) fertilizers are found in Indonesian rice fields [1]. Meanwhile, it is suspected that the use of chemicals in agriculture can affect the development of *Trichoderma*. Based on the above conditions, it is necessary to conduct *Trichoderma* character research on organic and conventional agriculture.

1.2. Literature Review

Trichoderma is a saprophytic fungus that lives in the soil around plant roots [2]. *Trichoderma* obtains nutrients from dead plant residues by degrading nutrients from these plants. *Trichoderma* is the dominant component in the soil which is diverse and is a biological controller of pathogenic fungi in the soil [3]. *Trichoderma* is a fungus that belongs to the ascomycetes class which is useful as a controller for diseases caused by several types

of fungi. In nature, Trichoderma is found in forest lands and agricultural lands that have woody substrates. In addition to controlling disease, Trichoderma also acts as a decomposer of wood substrates. Trichoderma has many species currently found as many as 89 species [3]. There are 7 species commonly used as biological agents, namely *T. asperellum*, *T. harzianum*, *T. hamatum*, *T. koningii*, *T. longibrachiatum*, *T. pseudokoningii*, and *T. viridae*. Trichoderma was first recognized as a microbe that has antagonistic properties against other fungi. Trichoderma can kill other fungi by producing toxins and feed on other fungi by dissolving the lytic exudate enzymes. Trichoderma does not negatively affect rhizospheric beneficial fungi such as mycorrhizae [4]. Trichoderma can be found in forest land and agricultural land.

1.3. Research Objective

This study aimed to determine whether the fungus Trichoderma sp. on agricultural land with conventional, semi-organic, and organic farming systems. This study can also determine the impact on fungi of the genus Trichoderma

2. MATERIALS AND METHODS

2.1. Research Location

The study is located in Sukawati organic rice field at Gianyar Regency with an altitude of 75 meters above the sea level with a daily temperature of 29°C with a soil pH of 5.0. Rice fields in Dawan Village, Klungkung Regency, are 75 meters above sea level with a daily temperature of 28 °C and a soil pH of 5.5. Rice fields in Klungkung with a height of 50 meters above sea level. Rice fields in Renon with a height of 16 meters above sea level. Organic Rice fields in Jatiluwih with a height of 700 meters above the daily temperature of 24°C and soil pH of 6.5. Rice fields in Panjer with a height of 15 meters above the daily temperature of 24 °C and soil pH of 6.5. Rice fields in Batubulan with a height of 60 meters above the sea, a daily temperature of 24 °C, and soil pH of 6.5. Rice fields in Tegallalang with a height of 600 meters above the sea, the daily temperature of 24oC, and soil pH of 6.5. Rice fields in Payangan with a height of 750 meters above the sea have a daily temperature of 24 °C and a soil pH of 6,5. Rice fields in Singakerta with a height of 130 meters above the sea have a daily temperature of 24 °C and a soil pH of 6,5.

2.2. Sampling soil

A sampling of soil in conventional and organic rice fields was excavated 10 cm with an area of 100 cm² near the roots of rice plants. Taking soil samples at one location was taken 3 points each as much as 500 grams and then mixed the soil. After evenly mixed soil is put in a plastic container and labeled according to the name of the location where the soil is taken.

2.3. Growth Medium

The growth medium used is a medium of Potato Dextrose Agar (PDA) 200 grams of peeled potatoes, then cut into cubes. Boil potatoes with 1 liter of water until cooked and then remove the potatoes and add flour so that as much as 15 grams with 20 grams dextrose, finally add as much as 500 mg of Chloramphenicol every 1 liter. The media was sterilized by autoclaving at 121 °C for 15 minutes.

2.4. Preparation

Exploration of Trichoderma was carried out by diluting 10 grams of soil diluted in 90 ml of distilled water then labeled 10-1. Dilute again by taking 1 ml of lime with 9 ml of distilled water and then labeled 10-2. Continue to label 10-6 dilutions.

2.5. Inoculation

Take 1 ml of the soil solution diluted into the Petri dish containing PDA media then smooth it on the PDA. After that, incubate for 7 days at 25°C.

2.6. Purification

To see the existence of Trichoderma, purification of the fungus is carried out with growth infecting other fungi as one of the characteristics of Trichoderma which can infect and is green. After purification, Trichoderma species were identified by taking Trichoderma isolates and then bred them in a medium so that they were low in nutrients, then observed their growth. By identifying Trichoderma we can find out how many Trichoderma species are in each sample of paddy fields.

3. RESULT AND DISCUSSION

From the results of Trichoderma exploration in several places in the rice fields of Bali, Trichoderma colonies were found. When it appears after inoculation Trichoderma seen in petridish is very invasive because in addition to suppressing the growth of other fungi it can also grow on other fungi so that it can kill other fungi (Figure 1).



Figure 1. Invasion of *Trichoderma harzianum* in other fungi.

The time of emergence varies due to different cultivation methods in various locations. On land in Dawan Trichoderma growth is faster than Sukawati due to integrated farming and reduced use of synthetic fertilizers and pesticides. In Sukawati soil, it is slower because the use of synthetic fertilizers and synthetic pesticides is quite high so that Trichoderma has growth disorders because Trichoderma conidia are often exposed to pesticides residues. Trichoderma was not found in Klungkung soil due to excessive doses of pesticides and synthetic fertilizers and more frequent application. The appearance of Trichoderma in Sukawati and Dawan can be seen in Figure 2. In Jatiluwih land it is faster because Jatiluwih is a world cultural heritage so that it maintains an organic farming system. Characterization of Trichoderma spp. macroscopically including colony color and colony shape which can be seen in Table 1.



Figure 2. The emergence of *Trichoderma harzianum* in Sukawati (A) and the emergence of Trichoderma in Dawan (B))

Table 1. Observation colony in Petridish

	Observation (days)							Collony morph
	1	2	3	4	5	6	7	
Dawan	white	White	Greenish	Green	Green	Green	Green	Round
Sukawati	White	White	White	Greenish	Greenish	Green	Green	Round
Batubulan	-	-	-	-	-	-	-	-
Tegalalang	-	-	-	-	-	-	-	-
Payangan	-	-	-	-	-	-	-	-
Singakerta	-	-	-	-	-	-	-	-
Klungkung	-	-	-	-	-	-	-	-
Renon	-	-	-	-	-	-	-	-
Panjer	-	-	-	-	-	-	-	-
Jatiluwih	White	White	Greenish	Greenish	Greenish	Greenish	Greenish	Round and arise
Bedugul	White	Yellowish white	Yellowish green	Yellowish green	Yellowish green	Greenish Yellow	Greenish Yellow	Round and arise

Table 1 shows that of the 4 *Trichoderma* spp. Isolates, but *Trichoderma* was not found in rice fields in the villages of Batubulan, Tegalalang, Payangan, Singakerta, Klungkung, Renon, and Panjer due to the intensive use of chemical pesticides. The isolates, which were characterized by their morphology, developed different colony colors from day 1 to day 7. Colony

color development begins with white, slightly greenish-white, green, and dark after 7 days. In the research of Ref. [5], there was also a change in colony color in the identification of *Trichoderma*. The green color indicates that *Trichoderma* is ripe. The green color is usually *T. koningii*.



Figure 3. *Trichoderma* sp. colony on the first day (A) and seventh day (B) petridish. Morphology is seen on a microscope with a magnification of 1000x (C).

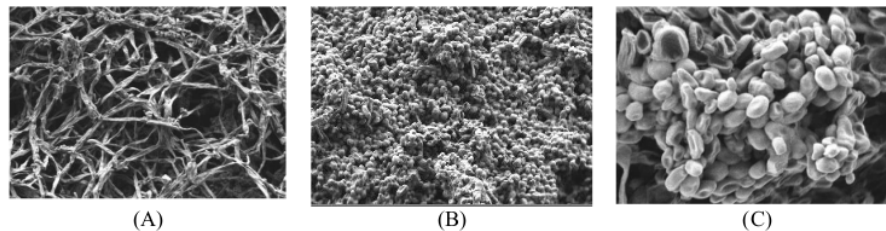


Figure 4. Scanning electron macroscopic image of Hyphae of *Trichoderma* at 500 magnification (A) and the spores at 500 (B) and 2.5K magnification respectively(C).

The results of the identification of the isolate can be seen in Table 1. From the identification of *Trichoderma* species in 4 rice fields, *Trichoderma* sp. Found in 4 locations. Because *Trichoderma* sp. It is the most adaptable to environmental pressures. This can be seen in Figure 1, where *Trichoderma* sp. strongest attacks other fungi [6]. *Trichoderma* sp. can control the space in the interaction of fungal pathogens. *Trichoderma* can produce some peptaibol antibiotics which are fungistatic and amino acids that can reduce the pathogenicity of pathogenic fungi [7].

Observations on Scanning Electron Microscopy (SEM) One of the rice hosts treated by *T. Harzianum* (Sugarwati isolate) was coated with gold and SEM observations were carried out. The fungus *T. Harzianum* was found which was very densely populated (Figure 1A) which was dominated by hyphae. No conidia and conidiophores are visible. On the other hand, relatively high densities of round and oval spores were observed. The high density of *T. harzianum* will certainly meet the requirements for efficient application in the field of cultivation. It is also interesting to see the interaction between *Trichoderma* sp. which is very dense with fungi or other pathogens in understanding the role of *Trichoderma* as an antipathogen.

2 4. CONCLUSION

Based on the results of this study, it can be concluded that the biodiversity of organic farming is higher than conventional agriculture. In conventional agriculture which is more intensive using inorganic fertilizers and synthetic pesticides, *Trichoderma* is not found. In organic farming, *Trichoderma* growth is faster than in conventional farming.

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