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INDIGENOUS *TRICHODERMA* SPP. COLLECTED FROM SHALLOT FIELDS SUPPRESS *FUSARIUM* ROT DISEASE

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ABSTRACT

Antagonist fungi have enormous potential to be a biological agent to control Fusarium basal rot (FBR) disease in shallot. This study aims to explore, isolated, and tested the antagonistic of *Trichoderma* sp. indigenous isolated from shallot fields against FBR disease. Exploration of isolates was carried out at the shallot planting center in Sigi Regency. The isolation characterization and inhibition tests were carried out at the Laboratory of Plant Pathology Tadulako University. The inhibition test was using two culture methods. The results showed that there were *Trichoderma* sp. obtained from shallot fields in the village of Sidera. In vitro test results showed *Trichoderma* sp., which was found to be able to inhibit the growth of the fungus *F. oxysforum*. The highest inhibition reached 69.18% within six days. As a comparison of inhibition of *Trichoderma harzianum,* a laboratory collection, within 6 days only reached 61.06%. *Trichoderma* sp. the origin of shallot fields was more effectively used as a biological agent in controlling FBR disease.

Keywords: Indigenous fungi, *Trichoderma* sp., shallot.

INTRODUCTION

Most shallot farmers in Palu and Central Sulawesi still use synthetic chemical pesticides to protect against pests. However, the use of pesticides has not been able to reduce yield losses due to pests. The main pests of shallot in Palu are leaf caterpillars (*Spodopteraexigua*), leafminers (*Liriomyzachinensis*), and Fusarium basal rot disease caused by *Fusarium oxysporum* (Rosmini and Nasir, 2013; Shahabuddin *et al*., 2013).

Excessive use of synthetic chemical pesticides have negative impact on the environment and humans (Mahmood et al., 2016). Besides, chemical pesticides not only harmful or pest targeted but also to the natural ecosystem is disturbed, emerge the resistant pests, threats to predators, and other biological agents, which are essential to support the crop production (Bommarco et al., 2011; Cloyd and Bethke, 2011). In some countries, the presence of pesticide residues in the soil also has a negative impact, can be carried up to the food chain, and in the end, can poison animals and humans (Jardim and Caldas, 2012; Wang et al., 2013).

The use of antagonistic fungi to control plant diseases lately has been developed to support the application of integrated pest and disease control (Knudsen and Dandurand, 2014; Shoresh et al., 2010). Soil fungi, such as *Trichoderma* sp., is a fungus that is widespread in the soil, functions as an antagonist that can suppress plant pathogens (Amin et al., 2010; Qualhato et al., 2013; Ru and Di, 2012).

The diversity of soil microorganisms is important in maintaining the balance of the soil ecosystem so that it is an indicator of soil health (Chaparro et al., 2012; Niemeyer et al., 2012). The fungal group has been recorded able to protect plants from pests and pathogens through antagonism (Monteiro et al., 2010). The antagonist fungi play a role in increasing

plant resistance through several mechanisms such as the production of hydrolytic enzymes, antibiotics, and can induce host plant resistance (Shoresh et al., 2010). The fungi can indirectly control pathogenic fungi by modifying environmental conditions, competing against nutrients and space, increasing plant growth and defense mechanisms, or directly by parasitic mechanisms (Kumar and Ashraf, 2017).

Although the study of the potential of this useful fungus has begun, its identification and characterization, especially in Central Sulawesi, have not yet been carried out, so it is important to explore, isolate and characterize and test *Trichoderma* spp. originated from Palu, Central Sulawesi. This study focused on isolating *Trichoderma* spp. indigenous on shallots plantation of Palu for biological control to FBR disease.

MATERIALS AND METHODS

Exploration and Collection of Indigenous *Trichoderma* spp. The sampling was carried out by observation of the fungus on the shallot field, which was suspected of having the *Trichoderma* sp. in Sigi Biromaru, Central Sulawesi, Indonesia. *Trichoderma* sp. obtained by taking ± 100 grams of soil (20 cm depth) from three randomly determined sampling points at each study location. Soil samples were stored in plastic bags and labeled accordingly, then transported to the Plant Pathology Laboratory of the Faculty of Agriculture, Tadulako University.

Isolation and Identification of *Trichoderma* spp. Soil samples were then homogenized and diluted up to series 10-4 (Junianto and Kukanto, 1995) and grown on potato sucrose agar (PSA) media till pure cultured of Trichoderma was found. Each *Trichoderma* sp. from different rhizosphere origins were labeled on the petri dish. The identification of *Trichoderma* sp. was done based on the morphological characters (Harman and Kubicek, 2002), includes the shape, edge, surface, and color of the colony. Microscopic observations of fungi included hyphae, conidiophores, conidia, which were recorded by using a microscope. A piece of cut PSA media (0.5 x 0.5 cm) placed on the center of object-glass and inoculated with pure cultured Trichoderma .then covered with covered glass. The object-glass then situated in a petri dish containing moist tissue paper that has been sterilized beforehand and incubated for two days (Syahputra et al., 2017).

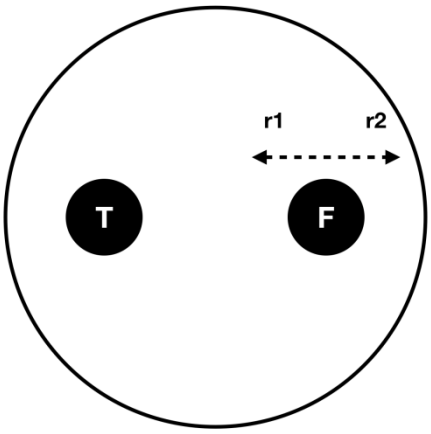

Figure 1. The illustration of the inhibition test of Trichoderma (T) against Fusarium (F). r1: radius of *F. oxysporum* fungi colony that grows in the opposite direction from the fungus of *Trichoderma* sp., r2: radius of *F. oxysporum* fungi colony growing towards the fungus of *Trichoderma* sp.

Propagation of *Fusarium oxysporum*. The isolate of *F. oxysporum* used in this study was the collection of the Plant Disease Laboratory, Faculty of Agriculture, Tadulako University. The fungus then regrowth on PSA for mass propagation.

Inhibition assay of *Trichoderma* sp. On *Fusarium oxysporum*. In vitro inhibition test of *F. oxysporum* was carried out using the two-culture method in a PSA media. The two fungi were placed separately on the PSA media with a distance of 2.5 cm and placed symmetrically. Subsequently, it incubated at room temperature for several days (Figure 1).

The pathogenic inhibition was calculated to determine the effect of antagonistic *Trichoderma* sp. to *F. oxysporum* (Naglot *et al.*, 2015) use the formula as follows.

$$\mathbf{R = \frac{r1 - r2}{r1} X\ 100\%}$$

Where r1: radius of *F. oxysporum* fungi colony that grows in the opposite direction from the fungus of *Trichoderma* sp., r2: radius of *F. oxysporum* fungi colony growing towards the fungus of *Trichoderma* sp.

RESULTS AND DISCUSSION

Indigenous *Trichoderma* spp. Collected. Two indigenous *Trichoderma* spp. collected from the shallot field. Further, *Trichoderma* spp. Collected subjected as *Trichoderma* sp. In-1 and *Trichoderma* sp. In-2. Morphological characteristics observation on PSA media found similar characteristics between two *Trichoderma* spp. collected. They were dark green, and hyphae spread rapidly on PSA (Figure 2a). Observation of the microscopic morphology of *Trichoderma* sp. (Figure 2b) was compared with *Trichoderma harzianum*, a collection of laboratories of plant pathology (Figure 2c).

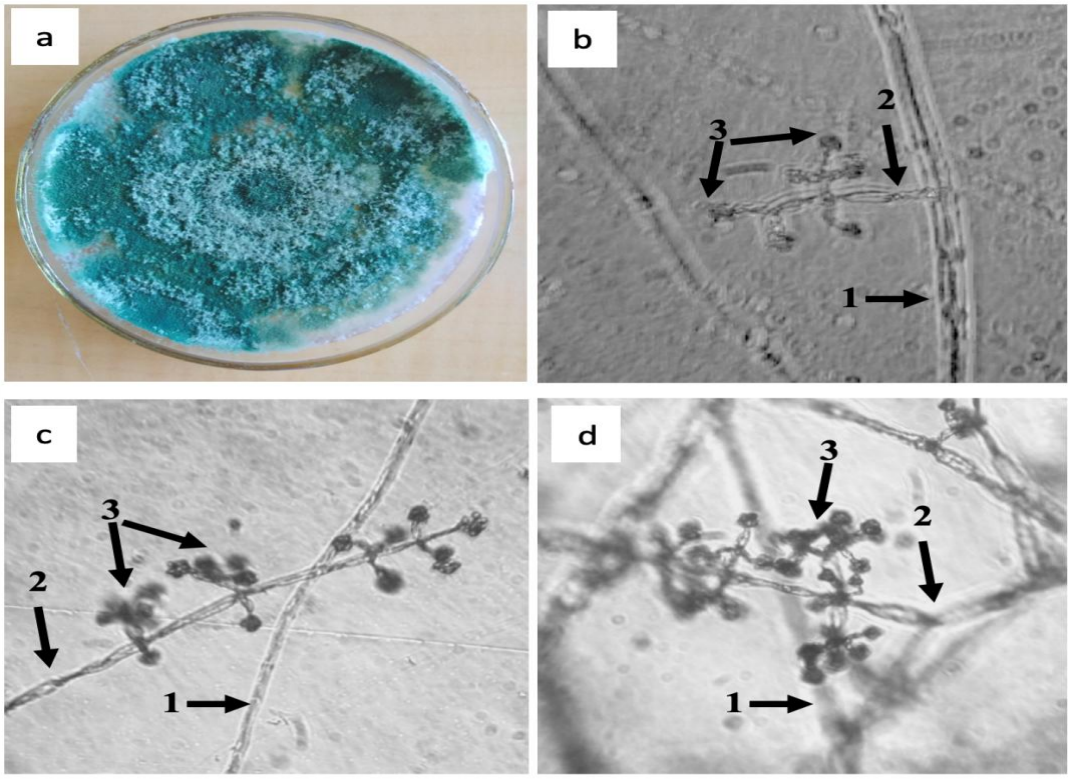

Figure 2. *Trichoderma* sp. on PSA (a), the microscopic fungal structure of *Trichoderma* sp. In-1 (b), *Trichoderma* sp. In-2 (c), and *Trichoderma* sp. In-3 (d). 1: hyphae; 2: conidiophore; 3: clustered branch of conidia. Magnification 400´.

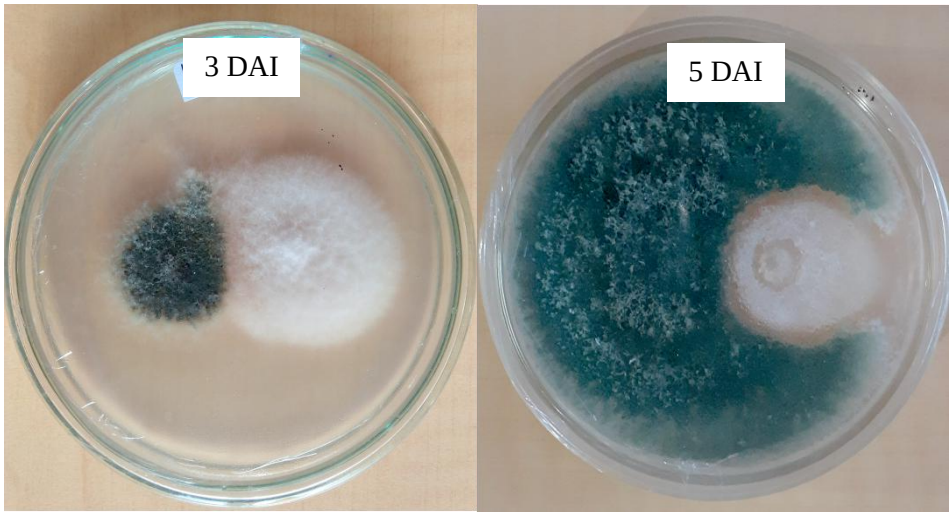

Figure 3. The inhibition of Trichoderma isolated from shallot field to Fusarium. DAI: the day after inoculation.

Table 1. Inhibition of *Trichoderma* spp. on *F. Oxysphorum.*

Trichoderma spp.	Inhibition (%)		
	3 DAI	4 DAI	5 DAI
T. harizianum	38.41 a	45.28 a	51.15 a
Trichoderma sp. In-1	44.93 b	50.00 b	56.87 b
Trichoderma sp. In-2	43.72 b	49.57 b	56.37 b

Data presented are mean (n=5). Mean value within each column followed by different letter differ significantly at p <0.05 according to Tukey's honestly significant difference. DAI: day after inoculation.

Inhibition of *Trichoderma* spp. The inhibition growth of *F. Oxysporum* by *Trichoderma* sp. observed from one day after inoculation on PSA until the petri fully covered by the fungi. Initially, *F. Oxysphorum* grew rapidly than, *Trichoderma* sp. (Figure 3a). However, until the seventh day, *Trichoderma* sp. occupied the PSA and suppressed *F. Oxysphorum* (Figure 3b). Both Trichoderma collected in this study were equally effective in inhibiting the growth of *F. oxysporum* (Table 1).

Trichoderma spp. Indigenous isolated from shallot fields showed effectively inhibited the growth of *F. oxysporum*. As an antagonist fungus, *Trichoderma* spp. able immediately to adapt to the new environment on PSA media. The inhibition of *Trichoderma* spp. indigenous required shorter time than *T. harizianum* in occupying the media. Within five days, the inhibitory of indigenous Trichoderma reached 56% and continues to increase until the sixth day. As a comparison, *T. harzianum* inhibits Fusarium about 51% on the 5th day after inoculation (Table 1). The inhibition of *Trichoderma* spp. isolated from the shallot fields significantly greater than *T. harzianum* after Tukey's HSD test.

Since firstly reported, in 1969 the identification of *Trichoderma* spp. was developed (Rifai, 1969). Subsequently, the number of *Trichoderma* spp. discovered. Currently, there has been a 104 list of *Trichoderma* spp. species recorded and deposited in the International Subcommission of Trichoderma database (http://www.isth.info/biodiversity/index.php). Morphologically, *Trichoderma* spp. identified by green conidia, fast-growth on media, and branched conidiophores (Figure 2) (Sharma and Singh, 2014).

Trichoderma spp. able to adapt rapidly in the new substrate and rapid colonizer since it has high sporulation capacity compared to the pathogens (Figure 3). This finding confirmed the previous study, which tested *T. harizianum* strains to suppress *Fusarium verticillioides* (Sobowale et al., 2009). *Trichoderma* spp. initiate the inhibition of the fungal pathogens in the same space. It begins by the contact of the hyphae of Trichoderma to hyphae of the fungal pathogen. Subsequently, *Trichoderma* occupied the space and nutrition in the substrate. A competition between two microorganisms in the same space might be a reason for suppression by *Trichoderma* sp. on Fusarium. Since *Trichoderma* sp. grows faster than *Fusarium*, it able to utilize the nutrients in the substrate than the competitor. Subsequently, *Fusarium* will lack nutrition and the growth depressed. As reported, Trichoderma is a great competitor to mobilize and utilize nutrients by produced inorganic acids (Promwee et al., 2014), it also survives in the situation where the ironless available by siderophores secretion (Nusaibah and Musa, 2019; Qi and Zhao, 2013; Vinale et al., 2013). Siderophore is needed by plant in solubilizing the iron, which basically unavailable in iron. Therefore, the iron competition will also suppress the pathogen due to a lack of Fe3+.

In addition, *Trichoderma* spp. have inhibition structure by forming a hook-like structure to suppress the growth of fungal pathogens (Lu et al., 2004). It can wrap around the hyphal pathogen and appear as a response to the presence of the fungal pathogen. In the end, the fungal pathogen suppressed. During the competition of space and nutrition, *Trichoderma* spp. produces lytic enzymes and antibiotics to suppress the growth of the fungal pathogens (Schuster and Schmoll, 2010). The common mechanism of the parasitism lead by *Trichoderma* spp. is degrading the cell wall of plant pathogens. Few lytic enzymes reported were chitinase, glucanase, and protease (Hirpara et al., 2017; Khare et al., 2018). On the other hand, *Trichoderma* spp. secrets secondary metabolites toxic to the plant pathogen during microparasitisms. The most significant toxic reported were pyrones, koninginins, viridins, nitrogen heterocyclic compounds, azaphilones, butenolides and hydroxy-lactones, isocyano metabolites, dopiopiperazines, and peptaibols (Vinale et al., 2014).

CONCLUSSIONS

Overall, biological control is intended to practice sustainable farming in the expectation of increasing agricultural production. This study provides primary data on the potential of Trichoderma spp. as a biological agent that can control Fusarium, the cause of shallot rot disease on an in-vitro test. This studyhas limitations since collected *Trichoderma* spp. indigenous of shallot field in Central Sulawesi has not been tested on a field scale. However, the results of the inhibition test to *Fusarium* BSR show promising results to be developed as biological control agents.

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