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Effectiveness Test of Several Media for Propagation Biological Agent *Trichoderma* sp.

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ABSTRACT

The ecology is negatively impacted by conventional farming methods that keep using more chemicals. Reducing the usage of chemicals is one strategy to combat these adverse effects. *Trichoderma* sp. is a type of organic fertilizer and pesticide that is beginning to be used extensively. A fungus called *Trichoderma* sp. is present in all types of soil. Technology is required to mass produce *Trichoderma* sp. on various types of media because its limited mass manufacturing makes its usage on a broad scale still needs to be completed. This study aims to evaluate the suitability of various mediums as propagation vehicles for *Trichoderma* species. The State Polytechnic of Lampung's Plant Laboratory conducted this study using a completely randomized design with 5 media treatments. Each treatment consisted of 4 replications. The propagation media used in this study were PDA media as a control, bran, sawdust, corn and rice. The results showed that the most effective growth media for *Trichoderma* sp. was bran media. The growth of *Trichoderma* sp. on bran media after 4 days of incubation was 100%. The average number of conidia of *Trichoderma* sp. the highest was found in bran media, which was 1.17×10^3/g of media.

1. Introduction

Adoption of conventional farming practices in the past actually increased agricultural productivity and yields significantly, but then production efficiency decreased due to the feedback effect of unwanted side effects. Conventional agricultural practices that use chemicals are not eco-friendly, have an impact on soil and environmental degradation, and reduce the quality of agricultural production (Rivai & Anugrah, 2016). Currently, sustainable agriculture continues to be developed. Sustainable agriculture systems are an alternative crop cultivation to achieve economically profitable and eco friendly. One of the efforts to achieve sustainable agriculture is to reduce the use of chemicals in plant cultivation activities. The use of inorganic fertilizers that have been going on for a long time continuously and excessively causes damage to soil structure and degradation of land quality. One effort to overcome this is to increase the use of organic fertilizers.

Trichoderma sp. is one of the organic fertilizers and insecticides. A fungus called *Trichoderma* sp. is present in all types of soil. *Trichoderma* sp. is a biological agent used to manage a variety of soil diseases that affect plants, and it has attracted much interest in recent years (Keliat, 2017). This fungi is known as an antagonistic fungi against many pathogenic fungi in growing media. However, it turns out that *Trichoderma* sp. also has other benefits as a growth-promoting fertilizer. As a fertilizer, *Trichoderma* sp. works to improve soil structure around plant roots by breaking down organic matter in the soil. As an organic pesticide, *Trichoderma* sp. is a biological agent with multiple inhibition mechanisms for pathogenic fungi and bacteria. *Trichoderma* sp. is a natural saprophytic soil fungus that can attack plant pathogens. This fungus is known to have a broad spectrum of control. It is known as a beneficial fungus because of its strong antagonistic properties in inhibiting the growth of pathogenic fungi. It has a directed control mechanism, which is believed to be able to increase crop yields (Muksin *et al*., 2013).

Based on the results of several studies, it is known that the mechanism of *Trichoderma* sp. as a biological control agent for Pryricularia grisae can interact directly or indirectly. Directly, *Trichoderma* sp. has a mycoparasitism mechanism against pathogenic fungi. The parasite mechanism begins with the mycelium of *Trichoderma* sp. attaches and grows to the pathogenic mycelium (P. grisae) by twisting and forming apresoria (penetration pins), which penetrate the pathogenic mycelium. After successful penetration, *Trichoderma* sp. begins to attack host cells by producing various enzymes that destroy fungal cell walls, such as glucanase, chitinase and protease (Harman *et al*., 2004). The indirect mechanism of *Trichoderma* sp. in inhibiting the development of rice blast disease is through the induction of plant resistance. One of the resistance reactions caused by *Trichoderma* sp. is an increase in chitinase enzymes in plant tissues. The chitinase enzyme is a pathogenesis-related protein known as PR-Protein. This enzyme has antifungal properties, can inhibit spore germination and induce fungal cell wall lysis (Oliveira *et al*., 2016).

The use of *Trichoderma* sp. still needs to be faster to develop due to limited mass production, so technology is needed to mass produce this fungus on several types of media. The media commonly used today for the propagation of *Trichoderma* sp. is a method of growing rice and corn, but this method of mass propagation requires a higher cost. For that, we need a new alternative media that can be used as a culture media with low economic value, adequate nutrition, efficiency, easy availability, abundant raw materials and *Trichoderma* sp. can be used to grow and develop.

Application biological fertilizers that have been socialized to the community needs to be increased in production. This is done in order to provide opportunities for farmers to use biofertilizers more widely. It would be even more appropriate to develop biological fertilizers based on the potential of microorganisms in Indonesia (Efendi, 2016). Based on this, this study aims to determine the effectiveness of *Trichoderma* sp. on various media so that the role of *Trichoderma* sp. as a biological fertilizer and organic pesticide can be optimally achieved.

2. Methodology

The research was conducted from May to October 2022 in the Plant Laboratory, State Polytechnic of Lampung. Five treatments and four replications made up the fully randomized design (CRD) used in this study. The analysis of variance (ANOVA) method was used to assess the research data, and then the least significant difference test (LSD) at the 5% level of significance. In this study, the propagation media used were PDA as a control, bran, sawdust, corn and rice.

The research implementation included making PDA media as a treatment media (control), *Trichoderma* sp. grazing as a treatment media, inoculation and observation. Corn and rice were prepared by soaking each media for 24 hours, then rinsing and steaming until soft. Bran and sawdust are prepared by soaking them for 24 hours and then pressing them to field capacity. Each media was weighed up to 25 g and placed in a glass bottle, which was then covered with aluminium foil. Bottles that containing media were sterilized in autoclave at 121°C for 15 minutes and ready to be used as propagation media. In each media, *Trichoderma* sp., which had been grown previously on PDA media, was inoculated for 2 weeks after incubation with a colony diameter of 5 mm, then incubated and ready for observation.

Observations were made from the inoculation of *Trichoderma* sp. on each media until *Trichoderma* sp. fills and fills the tube containing the media. Some of the variables observed in this study were the growth percentage of *Trichoderma* sp. and the number of conidia produced by *Trichoderma* sp. on each media. Growth of *Trichoderma* sp. on the propagation media according to the surface percentage of the media *Trichoderma* sp. visible visually. A number of conidia were calculated using a haemocytometer with the formula:

$$K = \frac{t \times d}{n \times 0.25} \times 10^6$$

K = number of spores/ml solvent;
T = number of spores in all sample boxes;
D = dilution factor;
n = sum of all calculated sample boxes and
0.25 = correction factor.

3. Results and Discussion

3.1 Growth Percentage *Trichoderma* sp.

The On each medium, *Trichoderma* sp. fungi were cultivated and kept at room temperature. Table 1 displays the findings of observations made each week for seven weeks.

Table 1. Growth Percentage of *Trichoderma* sp. on Various Propagation Media on Various Observation Times (%)

Treatment	Days after incubation (%)					
	2	3	4	5	6	7
PDA Media (Control)	25.00a	50.00bc	76.25c	100.00a	100.00a	100.00a
Rice media	25.00a	55.00b	84.50bc	100.00a	100.00a	100.00a
Bran media	10.00b	90.00a	100.00a	100.00a	100.00a	100.00a
Corn media	7.25c	40.00bc	87.50b	94.75b	100.00a	100.00a
Sawdust media	7.25c	35.00c	80.25bc	95.70b	100.00a	100.00a

Note: Numbers followed by different letters in the same column indicate significant differences according to LSD on α 0,05

Trichoderma sp. propagated on each media showed different growth rates. The longer the storage time, the higher the growth rate. The growth of *Trichoderma* sp. colonies is relatively fast. At 3 days after incubation, the mycelium grew to fill the Petri dish. Alfizar *et al*. (2013) explained that the growth of *Trichoderma* sp. on culture media can reach a diameter of 5–6 cm on the third day after inoculation. Visually, bran media showed the highest growth after 4 weeks of storage and was different from other media treatments. This is thought to occur because the content of bran media is the best for the growth of the fungi *Trichoderma* sp.

The nutrients in bran include 16.5% protein, 21.3% fat, 49.4% carbohydrates, and 11.4% crude fibre (Sari *et al*., 2019). Based on the results of the study by Uruilal *et al*. (2018), bran has a high carbohydrate content (27.01%), and the content of elements P and K are 0.69%, 1.92%, and low pH (6.16). This is supported by research results by Novianti (2018), stating that the growth of *Trichoderma* sp. is very dependent on the availability of carbohydrates, which are used as an energy source for its growth. Materials containing high concentrations of carbohydrates will encourage mould growth. In addition, the pH of the *Trichoderma* sp. propagation media also affects the growth of the fungi. Fungi can grow in a wide pH range. Usually, fungi grow high in a neutral pH environment namely pH 5 to pH 6.8 is suitable for mycelium growth and pH 5 at pH 8 is suitable for the growth of fungal spores (Febbiyanti *et al*., 2019). The level of acidity or pH of the media greatly affects the growth and production of enzymes in fungi. In general, fungi can grow and produce different enzymes at an acidic pH. Fungi can produce chitinase enzymes at a certain pH, which helps fungi grow better (Triasih *et al*., 2021).

The growth of the *Trichoderma* sp. on day 7 after incubation can be seen in Figure 1. Visually, fungi *Trichoderma* sp. that grows on bran media (A) is more numerous and has a greenish-white colour. The results of research by Gusnawaty *et al*. (2014) and Juliana *et al*. (2017) showed that the growth of *Trichoderma* sp. was marked by the development of colony colour from day 1 to day 7. Colony colour began to develop into white, bluish-white, light green, green and dark green after 7 days old. Kalay & Talahaturuson (2015) also show that *Trichoderma* sp. grows in a colonial form depending on the environment in which it grows. On nutrient-rich media, colonies are more numerous and whiter, and meiosis spores can form within a week. Whereas in low nutrient media, the colonies appear transparent, the colour of the colonies is green, yellow, or white.

Figure 1. Propagation media of *Trichoderma* sp; (A) Bran, (B) PDA, (C) Corn, (D) Sawdust, (E) Rice at 7 days after incubation

3.2 Number of Conidia of *Trichoderma* sp.

Table 2's observations show that the typical quantity of conidia produced by the fungus *Trichoderma* sp. Bran media had the highest score (1.17×10^3), which was significantly higher than that of the other treatments. The rice, corn, sawdust, and control medium differed significantly from each other. Sawdust media could not support the growth of conidia because there were only (4.25 x 10^3) conidia formed there. *Trichoderma* sp. conidia were used in media treatments, yielding considerably less conidia than other media treatments. The nutritional value of each media is directly related to the variation in the number of conidia *Trichoderma* sp. produced.

Table 2. Average Number of Conidia *Trichoderma* sp. on Various Propagation Media (per gram of media)

Treatment	The average number of conidia *Trichoderma* sp. on the propagation media
PDA Media (Control)	40.50×10^3 b
Rice media	25.60×10^3 c
Bran media	117.50×10^3 a
Corn media	12.75×10^3 d
Sawdust media	4.25×10^3 e

Note: Numbers followed by different letters in the same column indicate significant differences according to the LSD on α 0,05

Trichoderma sp. has smooth-walled conidia, and the initial colony is white, then turns bluish-white, then dark green, especially the part with lots of conidia. The results of the isolation and identification of *Trichoderma* sp. conidia are round and blue on the outside. Microscopic observation of the isolates made it possible to obtain the morphology of *Trichoderma* sp., which consists of branched and insulated hyphae (Wijaya *et al*., 2012). A high or low number of conidia in each media is thought to be influenced by the carbohydrate and protein content in the media as a food source. Gao *et al*. (2007), researchers who studied the impact of various nutrients on the development and sporulation of diverse biological agents, concluded that the properties of the isolate and the nutrient content of the artificial media influence mycelium growth and spore formation. The rate of growth and pathogenicity of fungi are thus significantly influenced by the nutritional content of solid and liquid substrates.

Growth of *Trichoderma* sp. is highly dependent on the availability of carbohydrates and protein (Uruilal *et al*., 2018). Carbohydrates and proteins are macronutrients for diffusion metabolism, which are transported into fungal cells with the help of carrier molecules (Juliana *et al*., 2017). Protein is used to stimulate the growth of *Trichoderma* sp. mycelium (Kurnia *et al*., 2012). In addition, it is thought that the elements C, H, and O are the three most significant elements present in organic composition as a source of sustenance. The primary role of nutrition is to supply active electron acceptors, building blocks for cells, and energy. (Kalay & Talahaturuson, 2015). This is in line with the opinion of Singh *et al*. (2014) that elements such as carbon and nitrogen play an important role in the cellular balance of *Trichoderma* sp. Wahyuni & Nst, (2019) also stated that microorganisms use carbon for growth and energy, while nitrogen is used for protein and reproduction

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4. Conclusion

Based on the observations that have been made, it can be concluded that bran media is the most effective media to be used as a media for the propagation of *Trichoderma* sp. because each observation variable shows the ability of *Trichoderma* sp. to grow and develop better than in other media.

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