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Utilization of Leaf Litter as Growth Media for Suren (*Toona sureni* Merr.) Rhizosphere Fungi Isolates

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Abstract. Leaf litter or organic matters can decompossed by microorganisms like fungi. This study aims to determine the ability of growth and the rate of decomposition of Suren (*Toona sureni* Merr.) rhizosphere fungi isolates on various organic media. The testing process was carried out at the Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Hasanuddin University, Makassar. The research method consisted of making fungi growing media, rejuvenating isolates and making organic media from teak leaf litter (*Tectona grandis*), Jabon (*Anthocephalus cadamba*), and Mahogany (*Swietenia macrophylla*). The highest growth of mycelium was found in *Fusarium* sp. The growth of the five best fungi isolates in the formulation of mahogany leaf litter and the highest average decomposition rate in the combination treatment of mahogany leaf litter and *Fusarium* sp.

Keyword: Decomposition, Rhizosphere Fungi Isolate, Leaf Litter

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1 Introduction

Organic matter waste, one of which is leaf litter can decomposed by microorganisms through the decomposition process. The fallen leaves of trees produce a lot of litter and will result in environmental pollution if it not handled properly. The waste of organic material has the potential to be used as compost through a decomposition process that can support plant growth.

Decomposition is a simple process of physical and chemical change by soil microorganisms called mineralization. The term decomposition is often used to describe a large number of processes experienced by organic materials, from decomposition and destruction process of

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organic matter into small particles so it is available for plant nutrients and can be reabsorbed by plants [1]. Naturally, organic material wastes can decompossed by microorganisms such as bacteria and fungi. The decomposition rate process is generally influenced by the environment condition that will affect decomposer growth and decomposed material.

Decomposer microorganisms play an important role in accelerating the decomposition of organic materials process which generally containing cellulose and lignin which is known as lignocelluloses. The decomposition process of organic matter is carried out by microorganisms including bacteria, actinomycetes, yeasts, and molds which act as bioactivation agents [2]. Organic decomposer fungi generally have a better ability than bacteria to breaking down plant debris. Fungi such as *Trichoderma* sp, *Fusarium* sp, *Pseudomonas*, *Phanerochaeta,* and *Thermospora* are organic decomposer fungi that break down the remaining plant, especially those containing hemicellulose, cellulose, and lignin.

Research conducted by [3] has obtained 33 fungi isolates from the Suren stand rhizosphere (*Toona sureni* Merr.). Five of them are superior isolates that able producing high IAA and cellulase, pectinase and chitinase enzymes [4]. This research is important to evaluate the ability of those isolates to decompose organic matter and can to evaluate the effectiveness of some organic media as ingredients or growth media of Suren (*Toona sureni*) rhizosphere fungi.

2 Research Methode

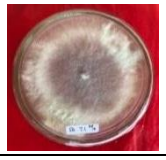
This research was conducted from November 2018 to February 2019 at Biotechnology and Tree Breeding Laboratory and Integrated Laboratory of the Faculty of Forestry, Hasanuddin University, Makassar, Indonesia.

The rhizosphere fungi isolate samples were obtained from Biotechnology and Tree Breeding Laboratory of the Faculty of Forestry, Hasanuddin University. The isolates were SB 4.2, SB 5.2, SB 6.1, SB 8.1, and SB 7.1 (Table 1).

2.1 Fungal Rejuvenation Process

The rejuvenation process is carried out by taking 1 piece of corkborrer isolates and placed in a petri dish containing PDA media. The surface of the petri dish is heated then covered with warp plastic. Furthermore, the fungus was observed for approximately 7 days.

Table 1 The Superior Rhizosphere Fungal Isolates

Isolate Code	Macroscopic Image	Genus
SB 4.2		*Trichoderma* sp. 1
SB 5.2		*Trichoderma* sp. 2
SB 6.1		*Trichoderma* sp. 3
SB 8.1		*Penicillium* sp.
SB 7.1		*Fusarium* sp.

2.2 Making Organic Media

The leaf litter around mahogany stands, teak and white jabon stand were collected. After the collection process, the litter brought to the laboratory then soaked for 7 days and sun-dried for 3 days. After the dried process, the litter is crushed using a blender with a medium texture. For organic media formulations, 3 containers containing a mixture of 500 g each leaf litter (mahogany, teak and jabon) 50 g mixture of rice and bran, corn flour 50 g, water 500 mL, and molasses 100 mL were prepared. All ingredients are mixed until homogeneous. The pH of each media measured on 7 for each type of organic media formulation. Empty sample bottles are weighed before being filled with media. Each organic media was put into a sample bottle of 40 g. Furthermore, the organic media formulation was sterilized using an autoclave at 121^0C for 2 hours. The sterilization process was carried out 2 times, then, the fungus isolates were inoculated into 5 corkborrer media bottles. Media that had been inoculated with fungus isolates were incubated for 40 days.

2.3 Observed Variable

Observing variables is conducted once every 5 days for 8 observations, which include variables:

1. Percentage of fungal mycelium growth is calculated based on scoring [5]:

0 = Nothing

1 = mycelium ≤ 25%

2 = mycelium 26-50%

3 = mycelium 51%-75%

4 = mycelium 76%-100%

2. Physical formulation of organic media such as texture, and color of organic media formulations [5]

3. The decomposition rate of organic media formulations was calculated from the reduced weight of the decomposed leaf litter weight carried out every 5 days for 40 days of observation. Estimation of the value of leaf litter decomposition rate is carried out according to the equation [6]:

$$W = \frac{W0-Wt}{W0} \text{ x } 100\ \% \quad (1)$$

$$\text{where: } D = \frac{W}{\text{Week/Day}} \quad (2)$$

Formula description:

Xt = Leaf litter weight after the t-observation period (g)

X0 = Initial leaf litter weight (g)

W = Weight loss (g)

D = Decomposition Rate

2.4 Research Design

The research designed using a completely randomized design (CRD) factorial with 2 factors. The first factor is the fungus isolates type consisting of 5 isolates and the second factor is leaf litter type. The number of treatment combinations was 18 combinations and each treatment combination was repeated 3 times so that the total treatment was 54 treatments.

2.5 Data analysis

The data obtained will be analyzed descriptively and presented in the decomposition rate value diagram and analyzed using Two Ways Analysis of Variance (ANOVA).

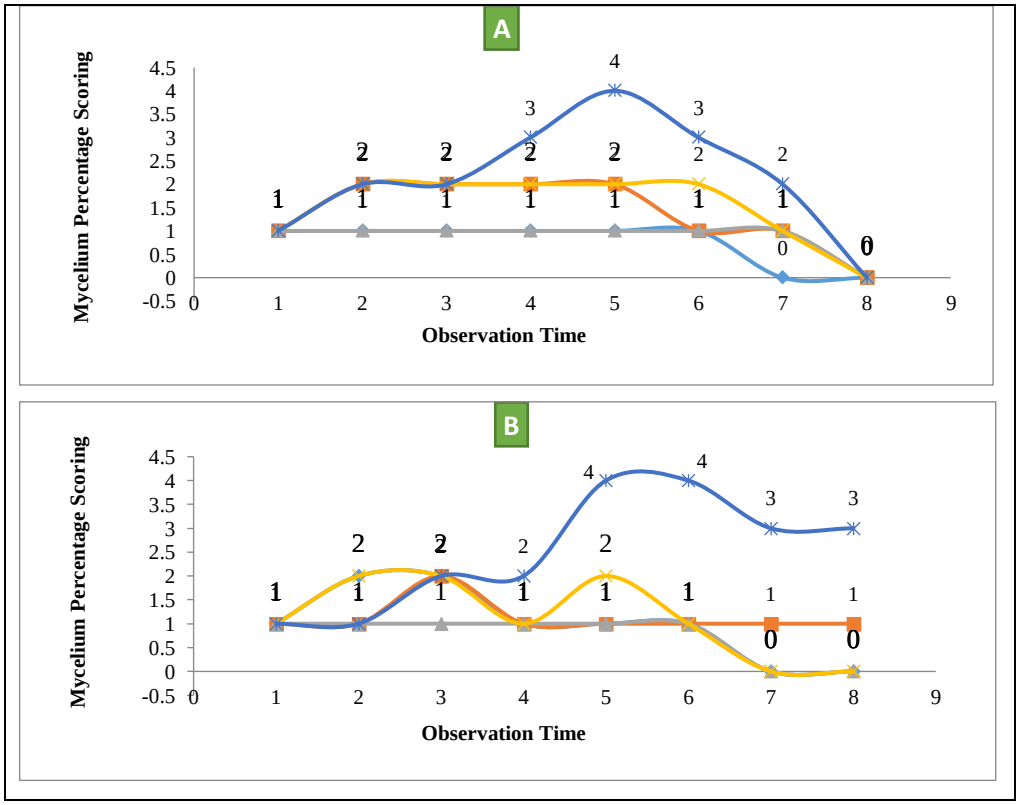
3 Result and Discussion

3.1 Percentage of Fungal Mycelium Growth

The fungus isolates which had been rejuvenated on potato dextrose agar (PDA) were tested for growth on three types of organic media formulations namely Jabon leaf litter (*Anthocephalus cadamba*), Teak (*Tectona grandis*) and Mahogany (*Swietenia macrophylla*). The addition of bran, cornflour, and glucose in each type of leaf litter is as the addition of nutrients needed by

the fungus growth. According to [7], the formula used in this study is food that contains a lot of carbohydrates such as rice, cassava, sweet potatoes, corn, molasses, and proteins such as peanuts and soybeans. Carbohydrates are the main source of energy found in the form of starch (starch) and sugars (mono and disaccharides). Sugar is used as food for fungi.

Fungal growth is characterized by the presence of mycelium which grows on organic media. Observation of fungus growth rate is done every five days for eight observations. Each media formulation has an acidity level (pH) between 6-7. The macroscopic observation showed white mycelium that appeared on the media formulation on the fifth day after inoculation. Mycelium is a collection of several hyphae that are threads that form a stretch of the plait. According to [8], mycelial growth requires nitrogen-containing material so that extracellular protein degradation occurs to meet the needs of the fungus during growth. Mycelium growth can be characterized by the appearance of white color like cotton that grows to spread on the media surface. According to [9] the range of pH needed during the growth of fungal mycelium is between pH 4-7 which will affect growth directly on the ability of fungus cell surface to provide nutrients. The results of the percentage of fungus mycelium growth in each type of organic media formulation are presented in Figure 1.



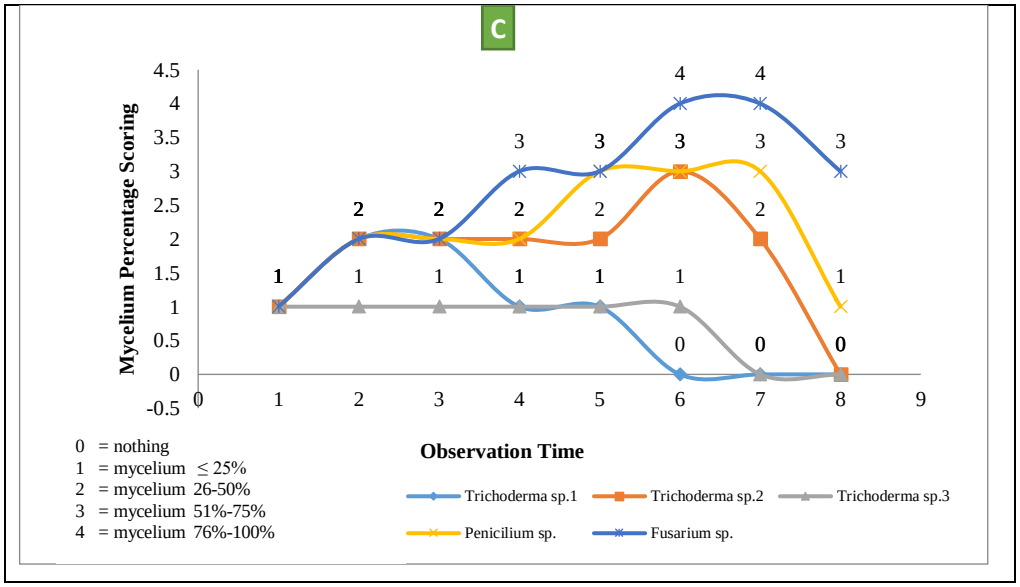


Figure 1 Growth curves percentage of superior mycelium isolates that have been inoculated in a leaf litter media formulation A: Jabon (*Anthocephalus cadamba*); B: Teak (*Tectona grandis*); C: Mahogany (*Swietenia macrophylla*)

Figure 1 shows that the percentage of mycelium growth of five fungi isolates at the beginning of observation was less than 25% but in the second observation until the last observation, the percentage of mycelium began to increase. Based on growth curve of mycelium fungi isolates which inoculated in Jabon leaf litter formulation (Figure 1A) showed that the highest mycelium growth produce in *Fusarium* sp. where the growth of mycelium continues to increase ie more than 75% or meet the Jabon leaf litter formulation, while on the sixth observation, mycelium growth begins to decline. While in Trichoderma sp.1 isolate mycelium growth remained less than 25% until the seventh observation where the mycelium showed no increase in growth. The mycelial growth rate also influences by the amount of water content in the media formulations besides the nutritional value. Generally, each type of leaf litter media formulation contains a large enough water needed for the growth of fungus mycelium. According to [10], mycelium growth in media is influenced by the nutrient's availability, and water activity influenced by the water content in media.

The fifth observation or 25 days after inoculation is the maximum growth of mycelium in each fungus isolate, it can be seen in Figure 1A that in the sixth observation is still visible, but until the eighth observation the mycelium is no longer visible or no growth. These results may be caused by the nutrient content of media for fungus isolates for growth were decreases. Growth and development of mycelium fungi isolates are influenced by nitrogen content in the substrate. The fungus mycelium cannot grow on nitrogen-deficient media, but excess nitrogen on the substrate can cause ammonia accumulation that can increase the pH thereby inhibiting mycelium growth [11].

Fusarium sp produces the highest mycelium growth percentage in Teak leaf litter formulation (Figure 1B). The growth of mycelium continues to increase by more than 75% but on the seventh observation, mycelium growth has decreased. The lowest growth of mycelium produces by Trichoderma sp.3 isolates which are less than 25% from the beginning of observation until seventh observation even tends to decrease and mycelium did not grow.

The results of the mycelium growth of fungus isolates in Mahogany leaf litter formulation (Figure 1C) obtained the same results with Teak leaf litter media where the highest mycelium found in *Fusarium* sp. Growth of mycelium isolates *Fusarium* sp. in mahogany leaf litter continued to increase by more than 75% fulfilling leaf litter formulation until the seventh observation but decreased in eighth observation. While Trichoderma sp.3 isolate showed the lowest growth of mycelium which is less than 25% from the beginning until seventh observation. Mycelium is not visible or does not experience growth. According to [12], the lack of nitrogen in media will cause the least growth of mycelium. Other factors such as water produced by microorganisms during the composting process will be lost due to air evaporation. Therefore, wetting the compost initially during the composting process was needed [13].

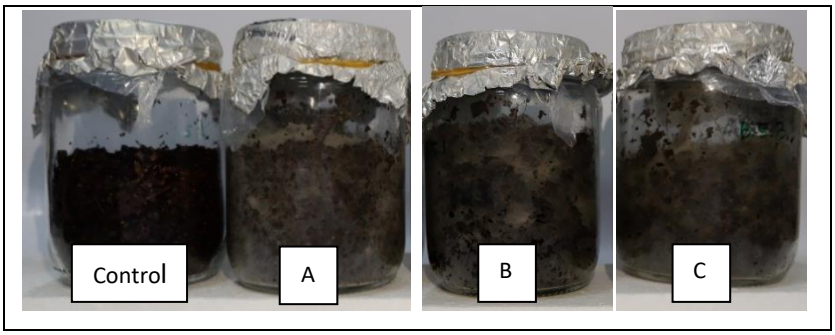


Figure 2 Jabon (A), Teak (B), and Mahogany (C) leaf litter media formulations inoculated by *Fusarium* sp.

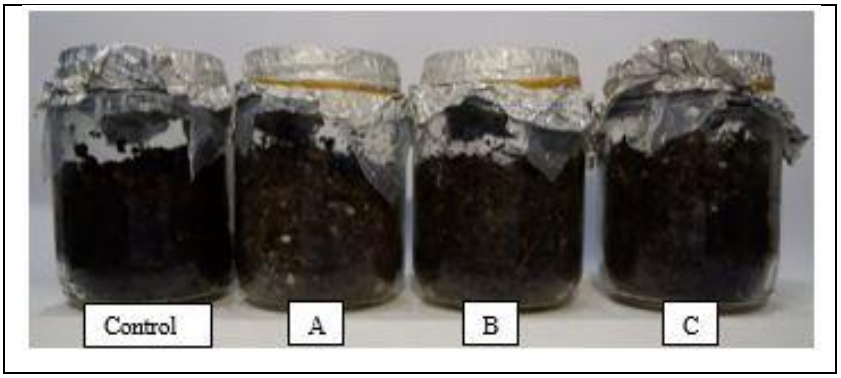


Figure 3 Jabon (A), Teak (B), and Mahogany (C) leaf litter media formulations inoculated by *Tricoderma* sp.3

3.2 Physical Observation of Organic Media Formulation

The results of physical observations showed the differences in color and texture of each organic media formulation type. At first observation, the color and texture of each formulation were the same which is brown and rough texture, but at the end of observation, the color and texture of each formulation had changed. This is influenced by fungus activity. The color of the organic media formulation varies between brown, yellowish, whitish, blackish, and greenish. The texture is also varied, including rough, rough sticky, and slightly crumb. The difference in characteristics can be presented in Table 2.

Table 2 Characteristics of Organic Material Formulations

Isolate	Characteristics of Material Formulations					
	Color			Texture		
	Jabon Leaf Litter	**Teak Leaf Litter**	**Mahogany Leaf Litter**	**Jabon Leaf Litter**	**Teak Leaf Litter**	**Mahogany Leaf Litter**
Control	Brownish	Brownish	Brownish	Rough	Rough	Rough
Trichoderma sp.1	yellowish	blackish	blackish	crumbs a little	Rough	Rough
Trichoderma sp.2	whitish	whitish	blackish	crumbs a little	Rough	crumbs a little
Trichoderma sp.3	yellowish	yellowish	blackish	crumbs a little	Rough	Rough
Penicillium sp.	whitish	yellowish	Greenish	crumbs a little	crumbs a little	Rough
Fusarium sp.	blackish	whitish	blackish	Rough	rough sticky	Rough

Based on last observations on media formulation in showed that mahogany formulations generally have the darkest color (black), except inoculation with isolates *Penicillium* sp. In jabon and teak litter showed variations in formulation color. The study conducted by [14] resulted that all treatments of bio activators in teak leaf litter showed a change from brownish yellow to blackish brown. The color changes occur due to the decomposition activity of composting organic material by fungus isolates. The color change is also influenced by mycelium color in the formulation. Generally, the formulation will turn blackish during the incubation period compared to the initial color, while the blackish color significantly affects the compost maturity. Good compost has a blackish brown color [15] or blackish, smelling like soil [16]. [17] also suggests that good compost is indicated by color characteristics that are different from constituent material, odorless, low moisture content, and have the same temperature as room temperature. Changes in compost physical properties can be seen by the color of compost from brownish yellow to blackish brown.

Leaf litter formulation texture testing carried out by squeezing used hand to find out the texture of each litter formulation. Jabon leaf litter that has been inoculated with fungi has a crumb

texture to almost all isolates compared to other leaf litter that has a generally coarse texture. The difference is not so significant because previously the leaf litter had been destroyed using a shredder with a size of approximately 1 mm. While the texture is somewhat crumbly influenced by fungi that can decompose or weathere organic's material.

3.3 Decomposition Rate of Organic Media Formulation

The ability of fungus isolates to decompose leaf litter can be calculating the weight of leaf litter at each observation, comparing between initial weight and final weight. Leaf litter weight slightly decreases in each observation. Because of the process of destroying organisms occurs gradually so that organic molecules are broken down into simpler forms such as carbon dioxide, water, and other mineral components [18].

Based on statistical analysis showed that isolate type had a significant influence on litter decomposition rate, it can be seen on Tukey's r tests (Table 3). Based on Table 3, *Fusarium* sp was significantly different from *Trichoderma* sp.1 and *Trichoderma* sp.3 but not significantly different from *Penicillium* sp. and *Trichoderma* sp.2. It shows that *Fusarium* sp. gives a better weight reduction effect compared to other isolates.

Table 3 Tukey Advanced Test Average Decomposition Rate of Several Types of Isolates

Type of Isolate	Average
Fusarium sp.	0,00132 [a]
Trichoderma sp.2	0,00111 [ab]
Penicillium sp.	0,00095 [ab]
Trichoderma sp.1	0,00019 [b]
Trichoderma sp.3	0,00093 [b]

Note: Numbers followed by the same letters show no significant difference based on the Tukey test

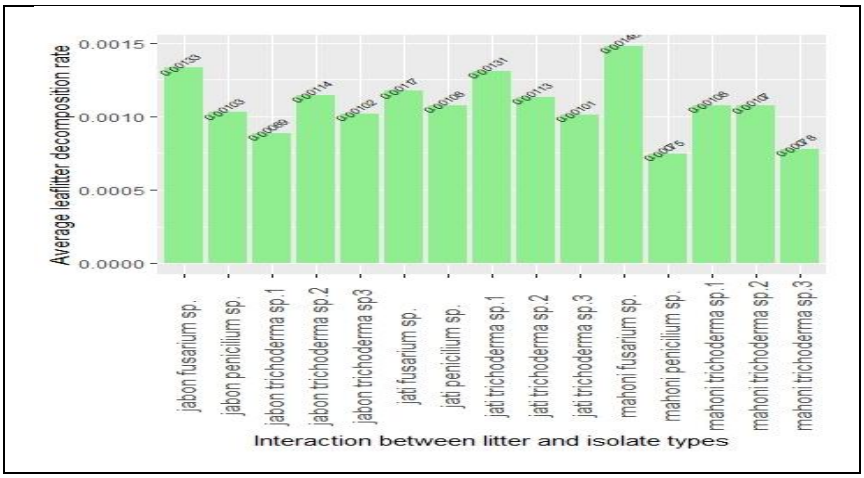


Figure 4 Average leaf litter Decomposition Rate of Interaction between Litter and Isolate Types

The average decomposition rate, a combination of mahogany leaf litter and *Fusarium* sp. showed the highest decomposition rate (0.00148) but, a combination of Mahogany leaf litter

with *Penicillium* sp produce the lowest (0.000726). The higher leaf litter decomposition means that higher fungus activity in decomposition. The decreasing of decomposition rate is caused by the organic's material that lower after fungus activities. At the beginning process, the leaf litter nutrient content and organic compounds were still quite high. Decomposition organisms can make these nutrients and organic compounds as substrates or food ingredients. According to [19], the decomposition process of material will naturally stop when limiting factors are not available or the material has been spent for the decomposition process itself.

When the nutrient content and organic compounds are decreases, the rate of decomposition also decreases. When easily decomposed organic compounds decrease and the decomposition process will also decrease. The leaf litter decomposition process involves various soil microorganisms that can recycle nutrients into the environment [20].

4 Conclusion

Our research found that the highest percentage of mycelium growth is found in *Fusarium* sp and *Fusarium* sp. gives a better weight reduction effect compared to other isolates. The highest average decomposition rate in the combination treatment of mahogany litter and *Fusarium* sp. Further research on the nutrient content of each leaf litter media formulation still needed to get a good quality biofertilizer.

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