Application of *Trichoderma* sp. to leaf litter decomposition (*Rhizophora mucronata*) on various salinity levels in Belawan

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Abstract. Mangroves can be defined as forest vegetation that grows between tidal lines or is affected by tides. *Trichoderma* sp. is one type of fungus that is found in almost all types of soil and in various habitats which is one type of fungus that can be used as biological agents controlling soil pathogens. The purpose of this research is to know the acceleration of the decomposition rate of *R. mucronata* leaf litter by *Trichoderma* sp. at various levels of salinity in Belawan and test the carbohydrate and protein content. The research was conducted in December 2019- March 2020. The technique of placing the sample in a litter bag is placed at 3 points of the observation station based on differences in salinity. Decomposition rate values obtained were 10.20 at station I, 8.50 at station II and 7.05 at station III from the initial weight of observation on the days -15 to the observation period on the 90 day. The highest carbohydrate content was at Station I on the days -60 which was 13% and the lowest carbohydrate yield on the station III on the days -15 was 2.42%. The highest percentage of protein is 9.7% on the days-15 at station II and the lowest percentage of protein is 4.83% on the days-15 at station I.

1. Introduction

Mangroves are one component of the ecosystem found in coastal areas, the resources contained in mangroves can be utilized by the community to meet their needs. Mangrove ecosystems have many benefits related to physical functions such as disaster mitigation that can reduce waves and hurricanes in the area behind it, can protect the coast from abrasion, tidal waves (rob), tsunamis, can hold mud and as a trap of sediment transported by surface water flow and can neutralize water pollution to a certain extent [1].

Mangrove forests can physically function as a barrier to coastal abrasion, biological mangrove forests function as a provider of food for the life of marine organisms and a source of energy for plankton, nekton and algae. There are 38 genus of mangrove in Indonesia. Ecological use of mangrove forests located in coastal areas can reduce the function of mangrove forests if not managed properly which will negatively impact the potential of the biota and the function of the forest ecosystem as a habitat [2].

Decomposition is a process that can destroy and decompose dead organic material which is assisted by biological and physical agents and then turns into mineral materials and organic colloidal humus. The decomposition of organic matter is also often called the mineralization process. This mineralization process is a microbial (decomposer) process in obtaining energy for its propagation. Several factors that can influence the process of decomposition of organic matter from the decomposer side include temperature, humidity, salinity, and pH. The role of this process is very large in the energy cycle and food chain in mangrove ecosystems [3].
Trichoderma sp. is one species that is often found in all species of soil and in various habitats which is one species of fungi that can be used as biological agents controlling soil pathogens and has become an important concern since the last few decades because of its ability as a biological controller of several plant pathogens [4].

Mangroves have one function, namely as a place for aquatic organisms to find food. That is because mangroves provide a source of nutrients derived from litter. In the mangrove ecosystem there are many fungi that affect the rate of leaf litter decomposition, so it is necessary to do research on the decomposition of R. mucronata leaf litter in Belawan to find out how much the influence of fungi to accelerate the rate of decomposition at various levels of salinity.

2. Materials and Method

2.1. Study site

This research was conducted in December 2019 - March 2020. The collection of R. mucronata leaf litter was carried out in Belawan. Determination of the dry weight of R. mucronata leaf litter was carried out at the Forest Cultivation Laboratory, Faculty of Forestry, University of North Sumatra. Determination of carbohydrate and protein content is carried out at the Medan Industrial Research and Standardization Center. Research location can be seen in the figure below. Figure 1 station 0-10, figure 2 station 11-20 and figure 3 station 21-30.

![Figure 1. Station 1 (0-10 ppt salinity)](image)

![Figure 2. Station 2 (11-20 ppt salinity)](image)

![Figure 3. Station 3 (21-30 ppt salinity)](image)
2.2. Materials
The tools used in this study are litter bags made of nylon with 1 mm mesh size, aluminum foil, permanent markers, hand refractometer, petri dishes, ovens, analytical scales, sewing needles, sewing thread, razor blades, ropes, gloves, cameras digital, Kjeldahl flasks, distillers, electric heaters / burners, Erlenmeyer flasks, upright coolers, measuring flasks, funnels, goiter pipettes, stop watch, measuring cups, burettes and drip pipettes.

The material used in this study was *R. mucronata* leaf litter, fungi isolates type Trichoderma sp. obtained from the previous year's experiment, 70% alcohol, label name, sample envelope, sterile water, selen mixture, conway indicator, boric acid solution 4% H$_3$BO$_3$, HCl 0.1 N, 40% NaOH, 3% HCl, 30% NaOH, Litmus paper, PP indicator, Luff-Schoorl solution, 20% KI, 25% H$_2$SO$_4$, 0.1 N Na$_2$S$_2$O$_7$ and 0.5% starch solution.

2.3. Sampling sites
The study was conducted in the Belawan mangrove area. Determination of the station point is measured using a refractometer based on the level of salinity. The lowest salinity level is 0-10 ppt is station I, the level of salinity 11-20 ppt is station II, and the highest salinity level is 21-30 ppt is station III. The technique of collecting data by using purposive sampling (taking data through consideration) is to determine 3 points of observation station based on differences in salinity.

2.4. Collection of Rhizophora mucronata leaf litters
As much as 50 g of *R. mucronata* leaf litter is put into a 40 x 30 cm litter bag and made of nylon with 1 x 1 mm mesh. The number of bags containing litter prepared was 54 bags (18 bags x 3 levels of salinity).

2.5. Fungal application
The fungus isolate that will be used is Trichoderma sp. This isolate was first rejuvenated by planting it on PDA media until it had sufficiently grown. Species of fungi that have grown on this PDA media, then cut to a size of 5 x 5 x 2 mm. The agar piece is then put into a test tube which has been filled with 2.5 ml of sterile water to be used as suspension. A total of 2.5 ml of suspension is placed evenly on the leaf litter found in the litter bag.

2.6. Monitoring of R. mucronata decomposition
Litter bags containing *R. mucronata* leaf litter are placed in a field that has various levels of salinity, according to treatment. Determination of the station point is done by measuring the salinity level using a refractometer. Salinity 0-10 ppt is station I, salinity 11-20 ppt is station II and salinity 21-30 ppt is station III. The tool used to determine the coordinates of stations is the Global Positioning System (GPS). A total of 54 litter bags were divided into 18 for each salinity, each containing 50 g of leaf litter *R. mucronata* is placed randomly by binding the four corners of the litter bag at the root or base of the nearest tree trunk. After litter has been placed in the field with varying degrees of salinity, data collection for the duration of the decomposition period is carried out during 15, 30, 45, 60, 75, and 90-day of observation periods.

2.7. Analysis of R. mucronata decomposition rate
Estimation of the average value of litter decomposition rates is carried out according to the following equation [5]:

\[
\frac{X_t}{X_0} = X_0. e^{-kt}
\]

\[
\ln \left( \frac{X_t}{X_0} \right) = -kt
\]

(1)
Where:
\[ X_t = \text{litter dry weight after the observation time (g)} \]
\[ X_0 = \text{initial litter weight (g)} \]
\[ e = \text{natural logarithm number (2.72)} \]
\[ k = \text{value of litter decomposition rate} \]
\[ t = \text{observation time (days)} \]

2.8. Carbohydrate content analysis
Carbohydrate content in \( R. \text{mucronata} \) leaf litter which decomposed can be determined by using the method of SNI 01-2891-1992. Hydrolysis of carbohydrates into monosaccharides which can reduce \( \text{Cu}^{2+} \) to \( \text{Cu}^{1+} \). The excess of \( \text{Cu}^{2+} \) can be controlled by Yodometry. The content of carbohydrate in the decomposition of \( R. \text{mucronata} \) leaves can be determined by first calculating the ash content in the following way:

Weigh carefully about 5 g of the sample into a 500 ml erlenmeyer, then add 200 ml of 3% HCl solution, simmer for 3 hours with an upright cooler. Cool and neutralize with 30% NaOH solution (with litmus and phenolphthalein) and add a little CH3COOH 3% so that the atmosphere of the solution is slightly acidic, then transfer the contents into a 500 ml measurement and squeeze until the line mark and then strain. Pipette 10 ml filter into a 500 ml erlenmeyer, add 25 ml Luff Schoorl solution (with a pipette) and some boiling stones and 15 ml distilled water. Heat the mixture with a steady flame, try so that the solution can boil within 3 minutes (use a stop watch), continue to simmer for exactly 10 minutes (counted when starting to boil) then quickly cool in a tub of ice. After chilling add 15 ml of 20% KI solution and 25 ml of 25% \( \text{H}_2\text{SO}_4 \) slowly, then pull the turret immediately with a 0.1 N tio solution (use a 0.5% starch solution pointer) also do the blank. Calculation: (Blank titers) x N tio x 10, equivalent to reduced irrigation. Then look in the list of Luff-Schoorl how many mg of sugar is contained for ml of tio used.

\[
\text{Glucose content} = \frac{W_1 \times fp}{W} \times 100\% \\
\text{Carbohydrate content} = 0.9 \times \text{glucose content} \tag{2}
\]

Where:
\[ W_1 = \text{sample weight} \]
\[ W = \text{glucose contained for ml used (mg)} \]
\[ fp = \text{dilution factor} \]

2.9. Protein content analysis
For protein analysis, it is carried out using the Semijkro Kjeldahl method SNI 01-2891-1992. The Kjeldahl method is a simple method for the determination of total nitrogen in amino acids, proteins and nitrogen-containing compounds. Protein analysis of the Kjeldahl method can basically be divided into three stages: (1) the destruction stage, (2) the distillation stage and (3) the titration stage. This method is suitable for semimicrobial use, because it only requires a small number of samples and reagents and a short analysis time.

The detailed analysis method is described as follows: weigh 2 g of the sample, put it in a 100 ml Kjeldahl flask, then add 1 g of the selen mixture and 25 ml concentrated \( \text{H}_2\text{SO}_4 \), then heat it on an electric heater or an incendiary flame until it boils and the solution becomes clear greenish (about 1 - 2 hours). Allow to cool, then dilute and put into 100 ml measuring cup, right up to the line mark.

Pipette 25 ml of solution and put it into a distiller, add 5 ml of 40% NaOH and a few drops of PP indicator. Flute for about 9 minutes, as a container to use 25 ml of 4% boric acid which has been mixed with the Conway indicator. Rinse the cooling end with distilled water. Then titer with 0.1N HCl solution. Work on blanking. Determination of protein content can be done by the formula:
Protein content = \[ \frac{(V_1 - V_2) \times N \times 0.14 \times f_k \times f_p}{W} \]  

Where:
- \( W \) = sample weight
- \( V_1 \) = HCl volume of 0.1 N used in the sample spin
- \( V_2 \) = volume of HCl used in blanking
- \( N \) = normality of HCl
- \( F_k \) = protein from food in general 6.25, milk and processed products 6.38, peanut oil 5.46
- \( F_p \) = dilution factor

3. Results and Discussion

3.1. Decomposition rate

The decomposition process of \( R. \) mucronata leaf litter was observed every 15 days for 90 days. Shrinkage of dry weight of \( R. \) mucronata leaf litter applied to fungi \( T \)richoderma sp. varies greatly. In (Figure 4) can be seen changes in the shrinkage of \( R. \) mucronata leaf litter.

![Figure 4](image)

**Figure 4.** Physical appearances of decomposed \( R. \) mucronata leaf litters. (A) Days-15, (B) Days-30, (C) Days-45, (D) Days-60, (E) Days-75 and (F) Days-90

Average residual litter decomposition has decreased very dramatically at the beginning of observation on the days-15 seen at station I which is 19.14 g, on station II 10.20 g and on station III equal 21.67 g. The longer the observation time of litter decomposition, the greater the percentage (%) of weight loss of leaf litter \( R. \) mucronata. The decrease in weight at the end of the observation on the days-90 was at Station I was 4.05 g, at Station II was 6.14 g and at Station III was 8.79 g. The results can be seen in the Figure 5.

The results of weighing carried out during the study found that the leaves of \( R. \) mucronata experienced rapid breakdown of leaves. The existence of fungi \( T \)richoderma sp. which affects the leaf litter so that \( R. \)mucronata leaves faster or is destroyed. The decomposition process carried out by the fungus will decompose organic particles by releasing enzymes so that they can decompose organic matter. This is consistent with the statement [5] which states that decomposition is followed by biological processes carried out by bacteria and fungi as decomposers to decompose organic particles by releasing enzymes so that they can break down organic matter into proteins. Apart from being used as a nutrient source for plants, decomposition can also be an important food source for fish and invertebrates. From the results of the decomposition rate can be seen in the Figure 6.
Figure 5. Average residual litter of *R. mucronata* leaves over the observation period of 90 days

![Graph showing average residual litter of R. mucronata leaves](image)

**Figure 6.** The average constant value of the decomposition rate (k) of leaf litter *R. mucronata*

Decomposition rate values obtained were 10.20 at station I, 8.50 at station II and 7.05 at station III from the initial weight of observation on the days-15 to the observation period on the days-90. Based on the results of the study, differences in location affect the value of the decomposition rate at each station. It is suspected that environmental factors, namely the level of salinity, which varies every day also causes the presence of organisms and decomposing microorganisms in the region also varies. According to [6] tides associated with salinity, the level of tidal frequencies greatly determines the change in salinity. The more frequent the tides occur, the level of salinity is increasing. The effect of salinity on macrobenthos (decomposer) density occurs indirectly, namely through the density of trees which results in an allowance for an increase in macrobenthos density.

3.2. **Macrozoobenthos**

Macrozoobenthos as an initial decomposer which chops the remains of the leaves and then re-emits them as dirt followed by fungi to break down organic matter into proteins and carbohydrates. Table 2 shows the species of macrobenthos found in *R. mucronata* leaf litter. The highest number of macrobenthos is at Station I.
Table 2. Macrozoobenthos species found in *R. mucronata* litter bags

| Class       | Order         | Genus       |
|-------------|---------------|-------------|
| Bivalvia    | Venoroida     | Polymesoda  |
| Crustaceae  | Decapoda      | Chiromantes |
| Gastropoda  | Mesogastropoda| Telescopium  |
| Malacostraca| Decapoda      | Litopenaeus  |
| Turbellaria | Macrostomida  | Microstonum  |

Figure 5. Macrozoobenthos (A) Gastropoda class, (B) Crustacea class, (C) Bivalvia class, (D) Turbellaria class, and (E) Malacostraca class.

The number of macrobenthos in litter bags at each station is different. Macrobenthos found in litter bags are mostly found at Station I. This is because the salinity level at Station I is relatively low. Macrobenthos found in litter bags include worms, crabs, snails, stews, and shrimp. According to [6] macrozoobenthos is a benthos group that has a size of more than 1 mm and adult growth has a size of 3 mm to 5 mm. Macrozoobenthos live sticking, reeling (sesile), meliang and immerse themselves both at the bottom of the water and at the bottom of the water surface. Macrozoobenthos that are commonly found in mangrove areas are macrozoobenthos of the *Crustacean*, *Polychaeta*, *Bivalvia* and *Gastropoda* classes.

3.3. Carbohydrate and protein content of decomposed *R. mucronata* leaf litters

*R. mucronata* leaf litter decomposed for 90 days decreased or decreased in weight. In the leaf litter of *R. mucronata* there is a carbohydrate content that can be seen in Figure 8. Carbohydrate content in the leaves of *R. mucronata* has decreased from the days-15 to the days-90. The results obtained on the days-15 were 7.46% at station I, 3.41% at station II and 2.42% at station III. The results obtained on the days-90 were 7.86% at station I, 3.45% at station II and 2.44% at station III. Based on the picture above the results obtained from the highest carbohydrate test were on Station I days-60 which was 13% and the lowest carbohydrate yield on station III days-15 was 2.42%.

Application of *Trichoderma* sp. become one of the factors decreasing content carbohydrate because fungi act as decomposers that can decompose organic matter. According to [7] The process of litter decomposition starts from the process of destruction carried out by small insects. Then process with the biological process carried out by bacteria and fungi as a decomposer assisted by enzymes that can decompose organic materials such as proteins, carbohydrates, and others. In addition to the carbohydrate content of the leaves of *R. mucronata* there is also a protein content that has increased from the days-15 to the days-90. The results of testing protein levels can be seen in Figure 9.
Figure 8. Carbohydrate content in leaf litter *R. mucronata* that has undergone decomposition.

![Carbohydrate content in leaf litter](image)

Protein content obtained on the days-15 was 4.83% at station I, 8.11% at station II and 9.7% at station III. Then the protein content obtained on the days-90 was 6.74% at station I, 7.08% at station II and 7.17% at station III. High or low content of organic matter is directly affected by differences in the volume of mangrove leaf litter which then falls into the sediment and eventually decomposes to become organic material.

4. Conclusion
Decomposition rate values obtained were 10.20 at station I, 8.50 at station II and 7.05 at station III from the initial weight of observation on the days-15 to the observation period on the days-90. The highest carbohydrate content was at Station I on the days-60 which was 13% and the lowest carbohydrate yield on the station III on the days-15 was 2.42%. The highest percentage of protein is 9.7% on the days-15 at station III and the lowest percentage of protein is 4.83% on the days-15 at station I.

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