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# SPECIES OF FUNGI INVOLVED IN THE DECOMPOSITION OF *Rhizophora apiculata* LEAF LITTER ON PULAU SEMBILAN

## *Jenis Fungi yang Berperan dalam Dekomposisi Serasah Daun Rhizophora apiculata di Pulau Sembilan*

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**ABSTRACT**

*Rhizophora apiculata* is one of the mangrove species that grows in coastal areas influenced by seawater. Decomposing leaf litter from *R. apiculata* is an organic material needed by microorganisms and organisms to grow and develop in the environment where they live. Microorganisms involved in accelerating the decomposition process are fungi. This research activity was carried out for six months, from June to December 2022. This research method uses litter bags to store leaf litter, which will be isolated and the rate of decomposition calculated. The results showed that there were 3 genera of fungi, namely *Aspergillus* sp., *Trichoderma* sp., and *Penicillium* sp. The decomposition rate of *R. apiculata* leaf litter was 0.13/day. The average carbohydrate content was 8,78%, and the average protein content was 5,45%.

Keywords: Decomposition, Fungus, Litter, *Rhizophora apiculata*

***ABSTRAK***

*Rhizophora apiculata merupakan satu diantara jenis mangrove yang tumbuh di daerah pesisir yang dipengaruhi oleh air laut. Serasahdaun R. apiculata yang membusuk merupakan bahan organik yang dibutuhkan oleh mikroorganisme danorganisme untuk tumbuh dan berkembang di lingkungan tempat tinggalnya. Mikroorganisme yang berperan penting dalam mempercepat proses dekomposisi adalah fungi. Kegiatan penelitian ini dilaksanakan selama 6 bulan dari bulan Juni sampai Desember 2022. Metode penelitian ini menggunakan Litter-bag untuk menyimpan serasah daun yang akan diisolasi dan dihitung laju dekomposisinya. Hasil penelitian menunjukkan bahwa terdapat 3 Genus fungi yaitu Aspergillus sp, Trichoderma sp, dan Penicillium sp. Laju dekomposisi serasah daun R. apiculata adalah 0,13/hari. Rata-rata kadar karbohidrat yaitu 8,78% dan rata-rata kadar protein yaitu 5,45%.*

*Kata kunci: Dekomposisi, Fungi, Rhizophora apiculata, Serasah*

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## INTRODUCTION

Mangrove forests are one of the unique ecosystems that can be found in Indonesia, an archipelago with a wide coastline. The diversity of mangrove forests in Indonesia is believed to be quite large, according to the INTAG Development Program Directorate, the area of mangrove forests in Indonesia reached 3.5 million hectares in 1996. However, based on 1999 data, the estimated area of Indonesian mangrove forests reached 8.6 million hectares, with condition of about 5.3 million hectares in damaged condition. Communities economically use mangrove forests as a source of fuel such as wood and charcoal, as well as building materials such as beams and boards. In addition, mangrove forests also provide benefits in the fields of textiles, food and medicine. The ecological function of mangrove forests is also very important in maintaining the stability of coastal conditions, protecting cliffs and river mouths from erosion and sea water intrusion. In addition, mangrove forests are also a habitat for fish, shrimp and crabs in their early stages of life (Rakhfid and Rochmady 2014; Raynaldo *et al.* 2021)

Mangroves, as highly productive ecosystems, play a pivotal role, particularly in the generation of litter, decomposition, and nutrient cycling. This heightened productivity is intricately linked to the food webs that rely on decomposed litter. The decomposition process, primarily stemming from mangrove leaves, holds significance due to its substantial contribution to the nutrient content in marine sediments. Although only a fraction of leaf decomposition is consumed by herbivores, the remaining organic matter serves as a potential source for estuarine food webs (Arfan *et al.* 2018).

Decomposition, defined as the breakdown of organic matter into nutrients, assumes a critical role in the production of dissolved organic matter (DOM) in the soil. The nutrient cycle initiates when leaves fall and undergo microbial degradation, a process vital to sustaining the ecosystem (Numbere and Gerardo 2016).

Most of the biomass production, especially leaf litter (about 40-85% of total litter), is eaten by fauna and decomposed by heterotrophic microbial communities living in intertidal sediments. Forest litter, especially in mangrove forests, has an important role in the ecosystem as a source of energy and nutrients for many decomposer organisms. This decomposition process produces significant organic matter and stores nutrients, which can then be exported to the marine and terrestrial environment through tidal changes and fresh water flows (Dhaour *et al.* 2022).

Marine fungi play an important role in the decomposition process and are the main link in the remineralization and transformation of decaying materials (Bunchan*et al.* 2003; Al-Nasrawi and Hughes 2012). Apart from fungi, groups of microorganisms and other organisms such as bacteria, worms, crabs and others, as well as environmental factors also take part in the litter decomposition process (Yunasfi and Suryanto 2008).

*Rhizophora apiculata* is one of the constituents of mangrove vegetation. This plant can grow up to 30 meters. These plants produce litter which then falls on the forest floor and will be decomposed. Litter decomposition process is a process of physical, biological and chemical changes. According to (Keuskamp *et al.* 2015). Physical changes are influenced by environmental factors such as temperature, salinity, and pH. This study aims to inventory the species of fungi in the decomposition process of *R. apiculata* leaf litter and to calculate the rate of decomposition of *R. apiculata* litter and to calculate the levels of carbohydrates and proteins contained in *R. apiculata* litter that undergo decomposition.

## RESEARCH METHOD

### Time and Location

This research was conducted for 6 months from June to December 2022. The research took place in the mangrove forest area of Pulau Sembilan Village and at the Forest Cultivation Laboratory, Faculty of Forestry, University of North Sumatra.

### Tools and Materials

The tools used in this research were hand refractometer, litter bag, autoclave, bunsen, 250 ml and 500 ml Erlenmeyer, Petri dish, spatula, beaker glass, test tube and rack, tweezers, ruler, pan, knife, microscope, laminar air flow, analytical balance, loop needle, matches, micro pipette, handspray, camera, stopwatch, stationery, container box, napkin, filter, mortal, plastic rope and stake. The materials used in this study were *R. apiculate* leaf litter, Potato Dextrose Agar (PDA) media, 96% alcohol, calmistine, seawater, tissue paper, cotton, masking tape, plastic wrap, name labels, aluminium foil, spirits and identification books. fungi.

### Procedure

#### *R. apiculata* Leaf Litter Samples

A total of 720 grams of *R. apiculata* leaf litter was carefully collected for this study. Each segment, weighing 40 grams, was meticulously placed into litter bags measuring 40 x 30 cm, constructed from 18 nylon nets (6 observations x 3 replications). The litter bags containing *R. apiculata* leaf litter were then positioned in

Figure 1 Research Location Map

the field using stakes. The placement of these litter bags in the field was done with precision to simulate natural conditions and ensure uniform exposure.

**Litter Data Collection *R. apiculata***

The collection of litter data was carried out at specific intervals following the placement of litter bags in the field. Data collection occurred in each experiment with measurements taken on Day 0 (Control), Day 15, Day 30, Day 45, Day 60, Day 75, and Day 90.

**Isolation of Fungi from *R. apiculata* Leaf *Litter***

The determination of the fungal population was carried out using the dilution method by making a dilution series of sample suspensions.

**Identification of Fungi Species Found in *R. apiculate* Litter**

Fungi that had grown on the media were observed for their macroscopic characteristics, namely colony characteristics such as the nature of hyphae growth, colony color and colony diameter. Fungi can also be grown on glass slides by cutting agar that has been overgrown with fungi and then placing it on a glass slide and covering it with a glass cover. This glass preparation culture is placed in a petri dish that has been moistened in the form of wet cotton. The glass culture was left for 1 week at room temperature. The developed fungi were observed using a microscope, their microscopic characteristics were the characteristics of the hyphae, the species of branching of the hyphae, and the characteristics of the conidia. The characteristics of each fungus were described and then matched with the mushroom identification key book.

**Determination of Fungal Species Diversity Index**

The diversity index of fungi species was calculated using Shannon's Index (Shannon and Weaver 1949; Ludwig and Reynold 1988) with the following formula:

$$H' = -\sum_{i=1}^{s} (Pi \ln Pi)$$

$$Pi = \left(\frac{ni}{N}\right)$$

Information:
H = Species diversity
S = Number of types
Pi = Proportion of total test sample

Table 1 Average Number of Colonies x $10^2$ (cfu/ml) of Each Fungi Species in *R. apiculata Leaf* Litter that have not Undergo the Process of Decomposition

| No. | Species Fungi | Average number of colonies x $10^2$ (cfu/ml) |
|---|---|---|
| 1. | *Aspergillus* sp. 1 | 0.4 |
| 2. | *Aspergillus* sp. 3 | 0.47 |
| 3. | *Trichodermaharzianum* | 2,4 |
| 4. | *Aspergillus* sp. 2 | 0.1 |
| Total Average Number of Colonies | | 3.37 |

**Analysis of Litter Decomposition Rate of *R. apiculata***

The wet weight and dry weight values that have been obtained are used to estimate the rate of litter decomposition. The rate of litter decomposition is obtained using the formula (Olson 1963):

$$Xt = X0 . e^{k t}$$

$$\ln (Xt/X0) = -kt$$

The formula for determining the length of litter remaining on the forest floor uses the formula:

1/k

Information:
Xt = litter dry weight after t observation time (g)
X0 = initial litter weight (g)
e = natural logarithmic number (2 .72)
k = litter decomposition rate
t = observation time (days)

**Carbohydrate and Protein Levels**

Carbohydrate and protein levels in the decomposed *R. apiculata* leaf litter can be determined using SNI 01-2891-1992.

## RESULTS AND DISCUSSION

**Species of Fungi Found in *R. apiculata* Litter That Have Not Experienced a Decomposition Process**

The results of the isolation of fungi found in *R. apiculata*leaf litterwhich had not undergo a decomposition process in the field (control) obtained 4 species of fungi namely *Aspergillus* sp.1, *Aspergillus* sp.3, *Trichoderma* sp and *Aspergillus* sp.2. In control, the fungus *Trichoderma harzianum* ranks first in terms of numbers, with an average number of colonies of 2.4 x $10^2$ (cfu/ml), followed by *Aspergillus* sp.3 of 0.47 x $10^2$ (cfu/ml), while the fungi *Aspergillus* sp.1 and *Aspergillus* sp.2 had the lowest average number of colonies. The average number of colonies of each species of fungus in *R. apiculata* leaf litter which have not undergo the decomposition process can be seen in Table 1. The differences in characteristics between the fungi found in *R. apiculata* leaf litter are presented in Table 2.

The four species of fungi that were successfully isolated from the controls were thought to be early decomposers already present in *R. apiculata* litter. According to Ramadanita (2012) early organisms that form colonies on a substrate are pioneer organisms. Fungi are a group of important decomposer microorganisms in the decomposition of mangrove litter. The diversity of fungi can affect the rate of decomposition of mangrove litter, mainly due to their role in breaking down complex organic compounds into simpler compounds that are accessible to other microorganisms.

**Species of Fungi Found in *R. apiculata* Litter Undergo Decomposition Process**

The results of the isolation of the fungi found in the leaf litter of *R. apiculata* which undergo a decomposition

process obtained various species of fungi, namely as many as 11 species. The largest average number of colonies isolated was *Aspergillus* sp. 3, namely 1.34 x $10^2$ (cfu/ml), while the lowest average number of colonies was occupied by *Aspergillus* sp. 5, *Aspergillus* sp. 6, *Aspergillus* sp. 7 that is equal to 0.01 x $10^2$ (cfu/ml). Of the 6 observations, the appearance of *Aspergillus* sp.1, *Aspergillus* sp.2, and *Aspergillus* sp.3 colonies appeared at each observation time with a colonization frequency of 100%. *Tricoderma* sp. appeared 4 times on the 15th, 30th, 45th, and 90th day of observation with a colonization frequency of 66.67%. 7 species occupy the lowest frequency including *Aspergillus* sp. 4, *Aspergillus* sp. 5, *Aspergillus* sp. 6, *Aspergillus* sp. 7, *Aspergillus* sp.8, *Aspergillus* sp. 9, and *Penicillium* sp. with a frequency of colonization of each species of 16.67%. The form of the colony and the microscopic characteristics of the dominant fungi, in colonizing *R. apiculata* before decomposition to decomposition within 15 days – 90 days are presented in Figure 2.

*Apergillus* sp. was the most common fungus found in this study, followed by the fungus *Thricoderma* sp. and *Penicillium* sp. *Aspergillus* sp belongs to the Ascomycetes class which is easy to find in nature, this is in accordance with the statement of Putra *et al.* (2020) that the *Aspergillus* sp. is one of the cosmopolitan fungi, because its distribution is very wide and can be found in various places. *Aspergillus* sp has good adaptability and protection against extreme environments, such as environments with high levels of salinity. The existence of *Aspergillus* sp can be found in such an environment. Some *Aspergillus* species also have the ability to produce enzymes that are able to break down complex organic compounds into simpler molecules. This allows *Aspergillus* to effectively optimize nutrient absorption and grow well in environments that have high salinity. In addition, the existence of an environment with high salinity can also reduce competition with other bacteria and fungi in terms of resources. This makes *Aspergillus* easier to grow and reproduce in these environments.

**Index of Fungal Diversity Found in *R. apiculata* Litter Undergo Decomposition Process**

The average value of the Shannon diversity index for the diversity of fungi in *R. apiculata leaf litter* that undergo decomposition was moderate with a value of 1.67. According to Jhingran *et al.* (1989); Saputra and Putri (2019) if H' >3 then diversity is high, if H' 1< H' >3 then diversity is moderate, and if H'<1 then diversity is low. A community is said to have high species diversity if there are many species with a relatively even number of individuals of each species. Meanwhile, if a community only consists of a few species with an unequal number of individuals, then the community has low diversity. The high or low diversity of a species depends on the interaction of two or more components, this is in accordance with the statement of Dimenta *et al.* (2020) which states that if the interaction of two abiotic and biotic components is disrupted, it will affect changes or disturbances that cause the ecosystem to be unbalanced so that it also affects the diversity of a species.

**Litter Decomposition Rate**

Litter decomposition was marked by a decrease in dry litter weight starting from the first observation day 15th to the sixth observation day 90th. During the decomposition process, the litter did not only experience

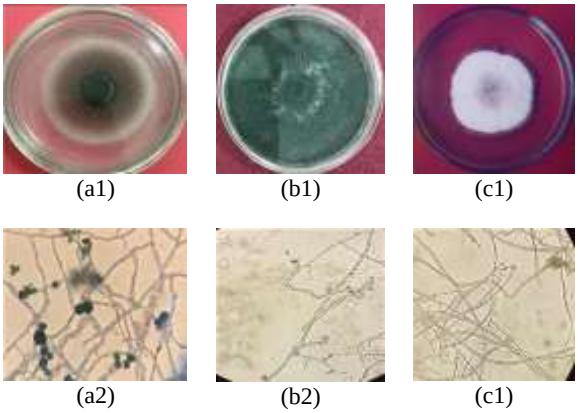


Figure 2 Colony shape and microscopic characteristics of the dominant fungi, in colonizing *R. apiculata* before decomposition to decomposition 15 days – 90 days. (a1) macroscopic form of *Aspergillus* sp, (a2) microscopic form of *Aspergillus* sp, (b1) macroscopic form of *Trichoderma* sp, (b2) microscopic form of *Trichoderma* sp, (c1) macroscopic form of *Penicillium* sp, (c2) microscopic form of *Penicillium* sp.

Table 1 Differences in the characteristics of various fungi found in *R. apiculata* leaf litter that undergo decomposition for 90 days on Pulau Sembilan.

| Fungal species | Colony color | Conidophore | Fialid | Conidia |
|---|---|---|---|---|
| *Apergillus*sp. 1 | Black in the middle and white on the edges, diameter 8.8 cm, aged 8 days | 132 – 98 µm | Lageniform, 14 – 16.8 x 1.4 – 2.8 µm | Globose 4.2 – 5.6 µm |
| *Trichoderma harzianum* | Grayish white at the beginning, whitish green middle at later stages, diameter 7-9 cm, 4 days | 5.0 – 7.0 µm 2.5 – 3.8 µm | Ampulliform and Lageniform, 3.5 – 7.5 x 2.5 – 3.8 µm | Globose, subglobose, ovoid 2.5 – 3.8 x 1.9 – 2.5 µm |
| *Penicillium*sp. 1 | White shaded by green, 5 cm aged 14 days | 132 – 231 µm | Ampulliform, 11.2 – 14 x 2.8 – 3.5 µm | Globose 1.4 – 4.2 µm |

a decrease in dry weight, the decomposed litter during each observation period also undergo a change in physical form, from intact to small fragments, which can be seen in Figure 3.

According to Ramadanita (2012); Pedro *et al.* (2019) that the speed of litter decomposition is affected by the speed at which the litter is fragmented. This solution is mostly carried out by many soil animals such as slugs, worms, insect larvae and others. Figure 4 shows that the fastest decrease in litter weight occurred on day 15 compared to after day 30, then slowed down to day 60 and quickly returned on day 75 to day 90. According to Sari *et al.* (2017) that the highest decomposition rate occurred in the first observation, this was due to the loss of soluble organic and inorganic materials and also the presence of microorganisms. The remaining weight of *R. apiculata* leaf litter during the decomposition period from day 0 to day 90 showed a decrease, according to Ramadanita (2012); Sari *et al.* (2017) the longer the litter decomposition time in the field, the faster the litter weight loss will be. The rate of weight loss of litter is influenced by the physical factors of the surrounding environment, the type of litter, the presence of litter-eating macrobenthos organisms and the frequency of microfauna colonization such as bacteria and fungi in the area. The average remaining leaf litter *of R. apiculata* can be seen in Figure 4.

*R. apiculata* leaf decomposition after 90 days of observation was on the 90th day with a value of 84.09%. This is influenced by several factors, one of which is the time factor. This is in accordance with the statement of Andrianto*et al.* (2015); Kanti*et al.* (2015) who stated that the time factor in measuring leaf litter decomposition has a very significant effect on the rate of litter destruction because the time factor is very closely related to environmental factors, it can be stated that environmental factors have a very significant effect on the rate of litter decomposition. The percentage of litter left behind can be seen in Figure 5.

Based on the observations, the percentage of litter left behind in Figure 5 shows that on the 90th day the percentage was 15.91%. The results of the data are influenced by the environment where the litter is immersed. According to Simbolon (2020); Alamsyah *et al.* (2018) that the rate of litter decomposition in water areas is higher compared to land areas because apart from

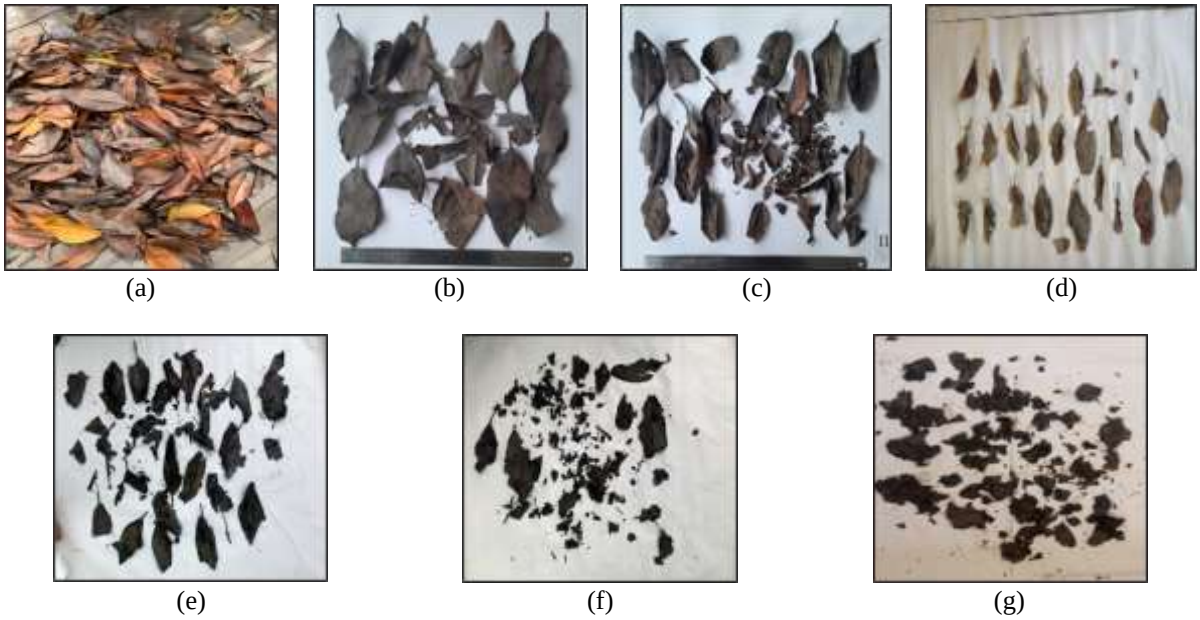


Figure 3 Residual litter that has been decomposed for 90 days. (a) 0 th day, (b) the 15th day, (c) the 30 day, (d) the 45th day, (e) the 60th day, (f) the 75th day and (g) the 90th day

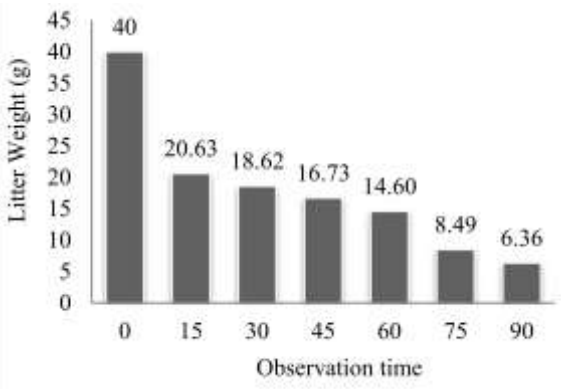


Figure 4 Average *R. apiculata* leaf litter *residue* during 90 days of observation

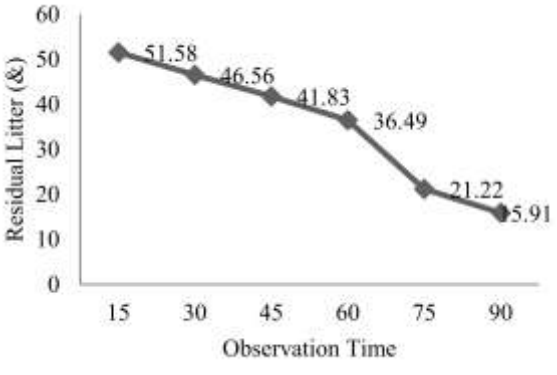


Figure 5 Percentage of Litter left behind

biological decomposition, in water areas the decomposition process is aided by a physical mechanism, namely the movement of the tides. Based on the data on weight reduction or remaining *R. apiculata* leaf litterin Figure 4, it can be seen that the average decomposition rate of *R. apiculata* leaf litter per year is 0.13/day. This figure indicates a relatively high rate of decomposition, with around 0.13 per day of initial litter decomposing each year. This indicates that the leaf litter in the mangrove ecosystem undergo a relatively fast transformation into simpler organic matter.

**Macrobenthos**

The macrobenthos identified in the litter bags belong to the Turbellaria class, the Crustaceae class, and the Gastropod class, as illustrated in Figure 6. These macrobenthic organisms play a crucial role in the decomposition process as decomposers of organic matter.

The presence of macrobenthos within the litter bags aligns with findings from Sari *et al.* (2017) and Wurst *et al.* (2018), suggesting that the presence of macrobenthos expedites the process of litter decomposition. Macrobenthos utilize litter as a primary food source, breaking it down into smaller particles. This breakdown facilitates subsequent decomposition by bacteria and fungi. The collaborative contributions of the Turbellaria, Crustaceae, and Gastropod classes within the macrobenthos community significantly aid in the decomposition of mangrove leaf litter.

By breaking down litter into smaller fragments, macrobenthos generate remnants and feces that serve as nutrition for other organisms, thereby accelerating the overall decomposition process. Their integral role in the decomposition of mangrove leaf litter is vital for maintaining the equilibrium of aquatic ecosystems and providing essential resources for other organisms within the mangrove ecosystem.

**Salinity**

Based on measurements taken at the study site, a salinity level ranging from 20 to 22 parts per thousand (ppt) was recorded. Salinity serves as a critical factor influencing the presence of microorganisms in the environment. Generally, the adaptability and survival of microorganisms decrease as salinity levels rise. High salinity conditions can lead to intolerance in microorganisms, resulting in their death (Thalib *et al.* 2021).

(a) (b) (c)

Figure 6 Macrobenthos of Turbellaria class (a), Crustaceae class (b), Gastropod class (c).

Salinity is recognized as a significant environmental factor that profoundly influences the growth of mangroves and the associated biota within their ecosystem (Matatulah 2019). The delicate balance of salinity in mangrove environments is crucial for sustaining the diverse life forms that inhabit these ecosystems. As salinity levels impact microorganisms, understanding and monitoring salinity becomes imperative for comprehending the ecological dynamics of mangrove ecosystems.

**Carbohydrate and Protein Levels Contained in *R. apiculata* Leaf Litter Undergo Decomposition Process**

The leaf litter from *R. apiculata* plays a significant role in contributing organic matter to the surrounding mangrove ecosystem. Microorganisms, such as bacteria and fungi, are capable of secreting enzymes that break down complex organic molecules, including proteins and carbohydrates. The carbohydrate and protein content in *R. apiculata* leaf litter is illustrated in Figure 7 and Figure 8.

On day 0, the total percentage of carbohydrate content was 11.5%, which increased to 15.8% on day 30. The most notable difference in the percentage of total carbohydrate content occurred between days 30 and 60, with an increase of 11.19%. The smallest percentage of total carbohydrate content was recorded on the 90th day, amounting to 3.19%. The analysis of carbohydrate content indicates that the duration of the decomposition

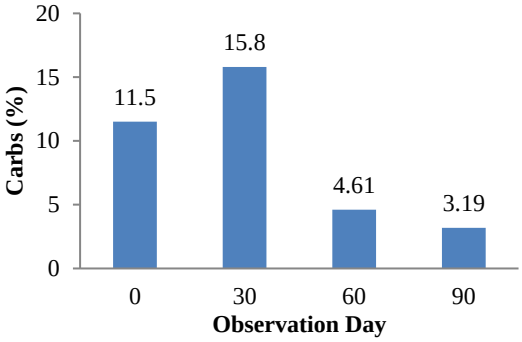


Figure 7 Average Total Carbohydrate Content of *R. apiculata Leaf* Litter that Has Experienced a Decomposition Process

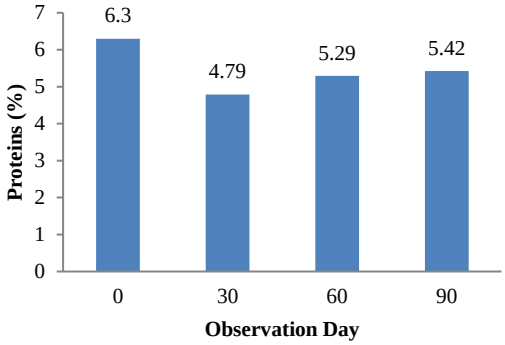


Figure 8 Total average protein content of *R. apiculata* leaf litter undergo decomposition process

period influences the carbohydrate content present in *R. apiculata* leaf litter. This aligns with the findings of Devianti and Tjahjaningrum (2017), stating that the rate of decomposition is influenced by various factors, including the chemical composition of the litter.

As depicted in Figure 8, an increase in protein levels is observed in litter undergoing decomposition from day 30 to day 90. The highest protein content, 6.3%, was recorded on day 0, while the lowest was observed on day 30, amounting to 4.79%. During the decomposition of mangrove leaf litter, the rise in protein levels is linked to the activities of microorganisms and macrobenthos involved in the process. Protein, a fundamental component of living organisms' tissues, including mangrove leaves, undergoes breakdown by microorganisms like bacteria and fungi. The protein content in *R. apiculata* leaf litter is advantageous for organisms, serving both in tissue formation and as an energy source. Being easily decomposed, protein, according to Sugiyarto and Setyaningsih (2007), significantly influences the rate of decomposition of plant residues, emphasizing the role of its quality and the organisms responsible for the breakdown process.

## CONCLUSIONS AND SUGGESTIONS

### Conclusion

There are 11 species of Fungi found in the leaf litter *of R. apiculata* which have undergo a decomposition process, namely *Aspergillus* sp.1, *Aspergillus* sp.2, *Aspergillus* sp.3, *Aspergillus* sp.4, *Aspergillus* sp.5, *Aspergillus* sp.6, *Aspergillus* sp.7, *Aspergillus* sp.8, *Aspergillus* sp.9, *Thricoderma* sp, and *Penicillium* sp. The value of the decomposition rate (k) of *R. apiculate* leaf litter was 0.13/day. The average carbohydrate content is 8.78% and the average protein content is 5.45%.

### Suggestion

It is advisable to carry out further research focusing on the role of each species of fungus found to increase the efficiency of litter decomposition. In addition, research with a longer period of time is needed so that the leaf litter of *R. apiculate* can be completely decomposed.

## ACKNOWLEDGMENT

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