Application of *Trichoderma* sp. 1 on *Rhizophora mucronata* leaf litter and its effect on milkfish feed in Belawan Sicanang, North Sumatra

Yunasfi[1,2*], *Budi* Utomo[1], *Afifuddin* Dalimunthe[1], *Ayu Salsa* Maida[1], *Amanatul* Fadhilah[3], and *Ipanna Enggar* Susetya[3]

[1]Forestry Study Program, Faculty of Forestry, University of North Sumatra Campus II Kuala Bekala Deli Serdang, 20353, North Sumatra, Indonesia
[2]Mangrove Center of Excelence, University of North Sumatra, Medan 20155, North Sumatra, Indonesia
[3]Water Resources Managemet Study Program, Faculty of Agriculture University of North Sumatra, Medan 20155, North Sumatra, Indonesia

Abstract. Leaf litter is is contains organic substances such as carbohydrates, proteins, and nutriens. Litter needs to decompose first so that organism and microorganisms can use it. Fungi help play break down litter in the environment. *Trichoderma* sp. 1 is a fungus that plays a role in the decomposition process. This study took place between June to September 2025 to determine the role of *Trichoderma* sp. 1, in breaking down *Rhizophora mucronata* leaf litter helps the growth of milkfish (*Chanos chanos*) seeds. 50 grams of the *R. mucornata* leaf litter was put into 20 litter bags. *Trichoderma* sp. 1 fungus was grown on PDA medium. Fungal agar cultures were cut into pieces and then mixed in a test tube with 2.5 ml of sterile water. Milkfish seeds were put into cages, with as many as 80 fish. Sampling was carried out every 15 days, using eight milkfish and one bag containing leaf litter. Measurement of the decomposition rate and the weight of the milkfish were carried out seven times. The *R. mucronata* leaf litter decomposed at a rate 16.54 grams/year, and the weight of the milkfish feed *Trichoderma* sp. 1 was 247.40 grams, while the control weighed 215.60 grams.

1 Introduction

Leaf litter is contains organic substances such as carbohydrates, proteins, and nutriens. Ecologically, mangroves release organic matter that supports aquatic life and provides habitat and food for wildlife, such as fish and crustaceans [1]. The organic material found within mangrove sediments originates from the decomposition of nearby materials like mangrove trees and microscopic organisms living on the sediment surface, along with with matter brought in by tidal action, such as free-floating algae, marine plants, and large algae. The amount each of these factors contributes differs based on the specific mangrove area and also between different mangrove areas. Starting with the decomposition process,

* Corresponding author: yunasfi@usu.ac.id

mangrove litter is an important element that the environment can use to form a food chain. According to Friesen *et al.* [2], macrobenthos are the first to break down mangrove litter, Bacteria and fungi work as decomposers, making enzymes that break apart organic matter and turn it into proteins. Litter is mostly made up of carbohydrates, proteins, and nutriens [3]. Litter needs to break down first so that organisms can use it. Fungi are very important in breaking down leaf litter [4].

2 Methods

2.1 Place and time

This research took place from June to September in the mangrove forest of Belawan Sicanang Village, located in Medan Belawan District, and also in the Forest Cultivation Laboratory at the Faculty of Forestry, University of North Sumatra.

2.2 Tools and materials

Tools used in this study were *a hand refractometer*, a 40 cm litter bag. x 30 cm, scales, rope raffia, sarong hand, analytical balance, glass cup, thread sew, razor blade, needle sew, , tube reactions, oven and cameras. The materials used were *R. mucronata* leaf litter, distilled water, types of fungi *Trichoderma* sp.1, and seeds milkfish.

2.3 Procedures

2.3.1 Sampling and placement of R. mucronata

R. mucronata leaf litter was collected as much as 1000 g of mangrove forest floor in Belawan Sicanang. Each 50 grams was put into 20 nylon litter bags that are 40 by 30 centimeters in size and have a 1 by 1 milimeter mesh.

2.3.2 Preparation of the fish cage and distribution of milkfish fry.

The fish cage measures 3 meters by 3 meters with a depth of 1 to 1.5 meters. The number of milkfish fry to be distributed in the cage is 200.

2.3.3. Application of fungi to litter.

The fungal isolate used is *Trichoderma.* sp.1. Fungi that have growth on media PDA is taken cut the agar into small pieces that are 5 millimeters long, 5 millimeters wide, and 2 millimeters high. The agar pieces that are already covered with fungi are next added to the test tube. Put 2.5 milliliters of sterile water into the bag to create a suspension smoothly evenly over the litter in the bag.

2.3.4. Placement of litter leaf R. mucronata which has been used for application Trichoderma fungi sp.1 in cages.

Placement of *R. mucronata* leaf litter that has been treated with a fungal application on pocket litter is carried out using the method of hanging the pocket containing the litter.

2.3.5. Salinity measurement.

Salinity measurements were carried out using a hand refractometer for each observation.

2.3.6. Measuring the development level of milkfish.

To check how much milkfish has grown, weight them every time observation.

2.3.7. Collection data.

Data collection happened after the litter was put into the field at these times: a. Day 15 b. Day 30 c. Day 45 d. Day 60 f. Day 75 g. Day 90. h. Day 105. For evvery observation, a single litter bag with 50 grams of litter was used.

2.3.8. Analysis of the rate at which the leaves of R. mucronata decompose.

The average value of the litter decomposition rate was estimated using this equuation (Olson, 1963):

$$X_t = X_0 . e^{-kt} \quad (1)$$

$$Ln\ (X_t/X_0) = -kt \quad (2)$$

Information:
X_t = dry weight of the litter measured after the time period t (g)
X_0 = initial dry weight of the litter
e = constant value of the natural logarithm logarithm (2.72)
k = decomposition rate constan of the litter
t = duration of observation time interval (days)

3 Results

3.1 Decomposition rate of *R. mucronata* leaf litter

Decomposition process, which includes the decomposition of litter, can produce organic matter that is important for the food chain. This is in accordance with Alongi [5], an important role in mangrove forest waters is nutriens contained in plant material are gradually released into the surrounding environment. The decomposition rate refers to how quickly litter breaks down being broken down by organisms into simpler and more easily decomposed structures. The decomposition process begins with the physical breakdown of leaves by organisms such as insects and earthworms [6]. Organic materials in the litter will break down into simple forms. Based on the calculation using the Olson [7], the decomposition rate constant (k) for *R. mucronata* leaf litter during the for 105 days observation period was estimated 16.54 g per year. Data on he dry weight of *R. mucronata* leaf litter are presented in Figure 1.

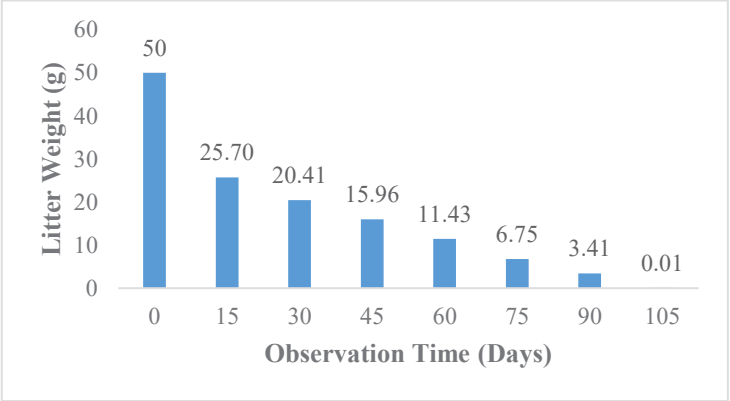

Fig. 1. Dry weight of *R. mucronata* leaf litter after 105 days

Based on the data obtained, it is clear that the mass of *R. mucronata* leaf litter went down by day 15, compared to day 30 and slowed down until day 105. This condition is thought to be caused by the new litter still containing more nutrients for microorganisms. The highest decomposition rate occurs in the early stages, as well as the presence of microorganisms fungi help break down different materials found in mangrove leaves [8].

3.2 Macrobenthos

Macrobenthos act as initial decomposers that tear litter into small pieces related to the food web in mangrove forests [9]. The four classes of macrobenthos found in *the R. mucronata* leaf litter bags, the data can be seen in Table 1.

Table 1 Number of macrobenthos found in litter bags over 105 days.

Class	Observation Time (Days)							Total	Average
	15	30	45	60	75	90	105		
Bivalves	8	9	7	5	4	4	3	37	6.16
Gastropoda	7	8	6	4	3	2	2	30	5
Polychaeta	7	6	4	4	3	2	2	26	4.33
Crustacea	8	6	5	3	3	2	2	25	3.5

Research result show that macrobenthos from class bivalves and oligochaetes play a role in the process of decomposition leaf litter *R. mucronata.* According to Raposeiro *et al.* [10], macrobenthos speed up decomposition litter at the stage end decomposition leaf with eat litter that has been conditioned in a way microbes. Macrobenthos breaks down the litter, making it easier for the fungus *Trichoderma* sp.1 to decompose the litter. This is consistent with the statement by Wang *et al.* [11] that macrobenthos plays an important role in accelerating litter decomposition..

3.3 Salinity

The concentration of salt in water is called salinity, and is usually expressed in parts per thousand (‰) or salt per thousand of water. Mangroves generally have a salinity between 11 and 25 ‰. Salinity can affect the diversity and number of macrobenthos in water areas, which can accelerate the rate of decomposition [12]. The salinity at the research site are presented in Table 2.

Table 2. Salinity at the research location

Observation Period (Days)	Salinity (‰)
15	18
30	18
45	20
60	20
75	18
90	18
105	18

Seawater typically has a salinity of 25-35 ppt, while brackish water typically has a salinity of 19-20 ppt [13]. Through tree density, which supports increased macrobenthos density, salinity indirectly affects macrobenthos density.

3.4 The role of fungi in litter decomposition

Trichoderma sp. 1 fungi are the primary decomposers responsible for breaking down the leaves from *Rhizophora mucronata* leaf litter, with high enzymatic ability to break down lignin, cellulose, and complex carbohydrates into simple compounds that support the ecosystem nutrient cycle. The application of *Trichoderma* sp. 1 fungi to *R. mucronata* leaf litter successfully increased the decomposition rate to 16.54 grams/year. This is in line with Numbere and Camilo [14], who stated that microbes like bacteria and fungi help break down mangrove materials and also play a role in changing and moving nutriens around.

3.5 Milkfish weight growth rate

The milkfish weight data during 105 days of observation is presented in Figure 2.

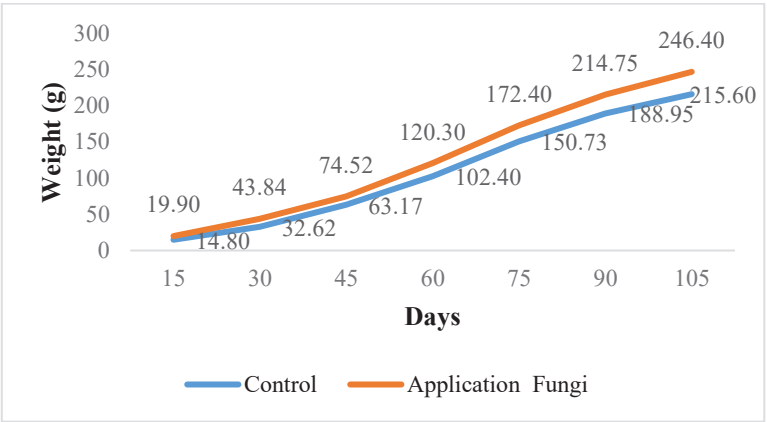

Fig. 2. Milkfish weight data for 105 days

Based on the results of observations of milkfish weight for 105 days of maintenance, it was found that the treatment with the application of fungi had a weight of 246.40 g which was much higher compared to the control weight which only had a weight of 215.60 g on the 105th day. Decomposed leaf litter from the *R. mucronata* process by fungi becomes a natural food source for milkfish that eat detritus. This is in line with the Hussain *et al.* [15] that the type of milkfish

food varies depending on the life stage and habitat, the main type of food consists of benthic and planktonic organisms.

4 Conclusion

The *Trichoderma* sp.1 fungi helped break down the leaves of to *R. mucronate* more quickly. The decomposition process happened because of the role played by macrobenthos from the classes bivalves, oligochaeta, crustaceans and gastropods that helped break down the litter. The application of *Trichoderma* sp.1 fungi to *R. mucronata* leaf litter successfully increased the decomposition rate to 16.54 grams/year, as well as increasing the weight growth of milkfish to 247.40 g, higher than the control which was only 215.60 g after 105 days.

References

1. N.A. Pradisty, A.A. Amir, M. Zimmer, Plant species-and stage-specific differences in microbial decay of mangrove leaf litter: the older the better?. Oecologia. **195**, 843-858 (2021). https://doi.org/10.1007/s00442-021-04865-3
2. S.D Friesen, C. Dunn, C. Freeman, Decomposition as a regulator of carbon accretion in mangroves: a review. Ecological Engineering. **114**, 173-17 (2018). https://doi.org/10.1016/j.ecoleng.2017.06.069
3. M.P. Krishna, M. Mohan, Litter decomposition in forest ecosystems: a review. Energy, Ecology and Environment. **2**, 236-249 (2017). https://doi.org/10.1007/s40974-017-0064-9
4. T. Osono, Functional diversity of ligninolytic fungi associated with leaf litter decomposition. Ecological Research. **35**, 30-43 (2020). https://doi.org/10.1111/1440-1703.12063
5. D.M. Alongi, Impact of global change on nutrient dynamics in mangrove forests. Forests. **9**, 10, 596 (2018). https://doi.org/10.3390/f9100596
6. M. Giweta, Role of litter production and its decomposition, and factors affecting the processes in a tropical forest ecosystem: a review. Journal of Ecology and Environment. **44**, 11 (2020). https://doi.org/10.1186/s41610-020-0151-2
7. J.S. Olson, Energy storage and the balance of producers and decomposers in ecological systems. Ecology. **44**, 322-331 (1963). https://doi.org/10.2307/1932179
8. M.R. Gmach, M.R. Cherubin, K. Kaiser, C.E.P. Cerri, Processes that influence dissolved organic matter in the soil: a review. Scientia Agricola. **77**, e20180164 (2019).
9. A.F. Bernardino, G.L.E. de Oliveira, H.L. Hadlich, R. Andrades, L.B. Correa, Mangrove clearing impacts on macrofaunal assemblages and benthic food webs in a tropical estuary. Marine Pollution Bulletin. **126**, 228-235 (2018). https://doi.org/10.1016/j.marpolbul.2017.11.008
10. P.M. Raposeiro, V. Ferreira, R. Guri, V. Gonçalves, G.M. Martins, Leaf litter decomposition on insular lentic systems: effects of macroinvertebrate presence, leaf species, and environmental conditions. Hydrobiologia. **784**, 65-79 (2017). https://doi.org/10.1007/s10750-016-2852-1
11. L. Wang, H. Liu, F. Carvalho, Y. Chen, L. Lai, L. Ge, Y. Luo, Top-Down effect of arthropod predator chinese mitten crab on freshwater nutrient cycling. Animals. **13**, 2342 (2023). https://doi.org/10.3390/ani13142342
12. N. Shadrin, E. Kolesnikova, T. Revkova, A. Latushkin, A. Chepyzhenko, N. Dyakov, E. Anufriieva, Macrostructure of benthos along a salinity gradient: The case of Sivash Bay (the Sea of Azov), the largest hypersaline lagoon worldwide. Journal of Sea Research. **154**, 101811 (2019). https://doi.org/10.1016/j.seares.2019.101811

13. J. Antony, K.P. Sandeep, R. Aravind, A. Panigrahi, C.P. Balasubramanian, Growth, survival, and osmoregulation of Indian white shrimp Penaeus indicus juveniles reared in low salinity amended inland saline groundwater and seawater. Journal of Coastal Research. **86**, 21-31 (2019). https://doi.org/10.2112/si86-004.1
14. A.O. Numbere, G.R. Camilo, Mangrove leaf litter decomposition under mangrove forest stands with different levels of pollution in the Niger River Delta, Nigeria. African journal of ecology. **55**, 162-167 (2017). https://doi.org/10.1111/aje.12335
15. M. Hussain, H.U. Hassan, M.A.M. Siddique, K. Mahmood, M.A. Abdel-Aziz, M.Y. Laghari, S. Rizwan, Effect of varying dietary protein levels on growth performance and survival of milkfish Chanos chanos fingerlings reared in brackish water pond ecosystem. Egyptian Journal of Aquatic Research. **47**, 329-334 (2021). https://doi.org/10.1016/j.ejar.2021.05.001