Rhizophora mucronata Leaf Litter Decomposition by Fungi on Various Level of Salinity in Belawan

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Abstract: *Rhizophora mucronata* is one mangrove species that is quite dominant found in Belawan. R. mucronata litter that falls to the forest floor will decomposed with soil microbial decomposers. Fungi are species that play an important role in the decomposition process and can assist the process of plant growth. The purpose of this study were to determine the frequency of colonization and the number of species and diversity index fungi in leaf litter R. mucronata at different levels of salinity. The research using purposive sampling method by determining the 3-point observation stations based on differences in salinity. The research showed that the highest fungi population found in station 1 with salinity 0-10 ppt worth 4.04×102 cfu / ml. The highest frequency of fungi colonization of litter decomposition process found in Trichoderma sp. The highest number of fungi species was found in the level of salinity 0-10 ppt as many as 13 species of fungi. The index species of fungi in Belawan waters show the same range that is currently illustrating that sufficient productivity, ecosystem conditions fairly balanced, ecologically balanced pressure.

1 INTRODUCTION

Mangrove known as ecological services both in the tropical and subtropical to provide niches (ecological niche) for a variety of flora, fauna and microbes. Endophytic fungus is one of the microbes that have been found in nearly all plant families, including mangroves. Community of endophytic fungi are important components of a forest ecosystem and contribute very real diversity and structure of the vegetation (Giordano et al, 2009).

The coastal area includes parts of the land and sea. Part mainland, both dry and submerged in water, still influenced by the properties of the ocean such as tidal, ocean breeze and salt water intrusion. Part of the ocean is affected by a natural process that occurs in the land, such as sedimentation and flow of fresh water, as well as by human activities such as deforestation and land pollution. The natural processes affect the difference flooding resulting in differences in salinity on growth and deployment zone of mangrove areas. Zone grows in mangrove areas reflect mangrove ecophysiological responses to environmental degradation. This will determine the adaptability of the species composition constituting a mangrove forest (Jumiati, 2008). Rhizosphere is an ideal area for the growth of soil microorganisms that generally didominansi by bacteria, aktinomicetes, and fungi. Rhizosphere rich exudate released by plants through the roots secretion process. The content of exudates include carbohydrates, amino acids, organic acids, enzymes, and other compounds. Microorganisms can take advantage of exudate through the decomposition process. Exudate decomposition by microorganisms produce energy and precursor compound. These precursor compounds can be utilized by microorganisms and plants (Widiastutik and Nur, 2014).

Litter is fallen leaves that fall to the forest floor. Litter decompose will donate organic material is a source of food for many species of fish and biota, as well as other organisms in the mangrove ecosystem. Litter decomposition process conducted by organisms such as crabs worms and microorganisms are bacteria and fungi (Yunasfi, 2006).

Utilization of various fungi species are expected to play a role in the decomposition of leaf litter mangove is one business that can be used to exploit the biological potential contained in the mangrove ecosystem. Fungi are the primary decomposers in decomposition of leaves of mangrove because it has

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the ability to degrade cellulose and lignin. Cellulose and lignin together constitute a major component of the cell wall constituent in the leaves (Yunasfi and Suryanto, 2008).

The diversity of fungi influence on the rate of leaf litter decomposition. Fungi are the main agents in the decomposition process so as to produce nutrients. Decomposition is closely related to bacteria and fungi which is the main agent in the decomposition process. Inhibition of this process will result in the accumulation of organic matter that can not be used directly by the manufacturer (Bako et al., 2016).

2 MATERIALS AND METHOD

We strongly encourage authors to use this document for the preparation of the camera-ready. Please follow the instructions closely in order to make the volume look as uniform as possible (Moore and Lopes, 1999).

The research was conducted in Belawan. The leaf litter *Rhizophora mucronata* obtained and observed in Belawan. Breeding and fungi identification carried out in the Laboratory of Plant Pests and Diseases Faculty of Agriculture, Universitas Sumatera Utara.



Figure 1: (a) Station 1 is at a salinity 0-10 ppt (b) Station 2 is at a salinity of 11-20 ppt (c) 3 stations namely at 21-30 ppt salinity.

The tools used in this study is a refractometer, Global Positioning System (GPS), bags of litter (litter bag) size 30×40 cm is made of nylon, needles, Erlenmeyer flask, glass beaker, burner, test tubes, Petri dishes, Autoclave, ose needles, glass objects, glass cover, oven, light microscopy, analytical balance, mortar, micropipette, 1 ml pipette tip, Bunsen, digital cameras, scissors, a ruler.

Materials used in this study was the leaf litter *Rhizophora mucronata*, Seawater, markers (stationery), rope, twine, alcohol, distilled water, tissue paper, cotton, potato, dextrose, agar, mask, cling wrap, stencil paper, aluminium foil, paper labels, and methylene blue.

3 METHOD

3.1 Data Collection

The data collection is done in situ and laboratory observations. Source data used are primary data. The primary data used is the result of the transect (sampling in the field) in the form of leaf litter R. mucronata and data about the identity, the population of each species, the diversity of species and frequency of each type of fungal colonization.

Techniques of data retrieval by means purposive sampling (Data retrieval through judgment) that determines the third point of observation stations based on differences in salinity. Station point determination conducted by measuring the level of salinity using a refractometer. Station 1 with salinity 0-10 ppt, station 2 with salinity 11-20 ppt, 3 stations with salinity 21-30 ppt. Determination of the coordinates of the station is done by using GPS (Global Positioning System).

The data collection is done after a long period of decomposition of litter placed on the ground with various levels of salinity, over time as follows:

- b. days 30
- c. days 45
- d. days 60
- e. days 75

For each time the survey was taken of the sample in the form of litter in bags of up to 75 days, and each time the survey is conducted three replications.

a. days - 15

3.2 Sampling

Mangrove leaves R. mucronata fallen collected and bagged litter (litter bag) made of nylon, measuring 40 \times 30 cm with a mesh of 1 \times 1 mm by 50 g. The number of bags that contain as many as 21 bags of litter prepared at each station. Once inserted leaves, litter bags sewn then provided with holes on both sides of the right and left pockets that can be connected with raffia. Then the litter bag tied tightly mangrove roots so that when the tide of litter bags can not be separated.

Identifikasian fungi was done by taking 3 bags containing litter taken for each level of salinity once in 15 days and taking the bag of litter do until the 90th days after the litter is placed in the field.

3.3 Sterilization Equipment and Materials

Sterilization is done by washing using soap cleaning tool. Tools that have been rinsed with clean water drained, to be wrapped in paper stencil. Sterilization of tools and materials is done with wet sterilization method, using *autoclave* at a pressure of 1.5 atm for 15 minutes. Then sterilized using oven dried at 121 ° C for 15 minutes to the farthest of unwanted microbes.

3.4 Making the Media PDA

Making the Media PDA (*Potato Dextrose Agar*) is done by boiling the potatoes that have been diced 250 grams using 1000 ml of distilled water. After boiling, potato juice filtered into a glass beaker and added dextrose and so each as much as 20 grams. The solution was homogeneous and still liquid was poured into 4 pieces of 250 ml Erlenmeyer flask, closed with a sterile cotton, aluminum foil and sealed with cling warp. Media put into an autoclave to be sterilized for 15 minutes at a pressure of 1.5 atm. Before doing the casting media, added 0.1 gram chlorompenicol. Chlorompenicol homogenized in liquid media and wa ready to be poured into the Petri dish.

3.5 Identification of Fungi

Rejuvenate fungi in pure culture on PDA and then incubated for 5-7 days at room temperature. Isolates of fungi have grown on the media, grossly identified by looking at the nature of the growing hyphae, colony colour and diameter of the colony. Fungal isolates were also grown on glass objects (objectglass), that is by putting the pieces in order of $4 \times 4 \times 4$ 2 mm on a glass slide, then stroked fungi loopful on the PDA media. Then covered with a glass cover (cover glass). Isolates on glass objects are placed in a petri dish which has been given in the form of wet cotton moisturizing. Isolates of fungi on a glass slide left for several days at room conditions to isolate the fungi grow sufficiently developed. When isolates of fungi have evolved removal of the cover glass that has been overgrown fungi carefully with the aim to dispose of the pieces in order. Next on the cut so that a few drops of 1 drop of methylene blue solution. Glass cover that has been overgrown with fungi subsequently placed on a solution of methylene blue on glass objects. Glass culture is observed using a light microscope to determine the characteristics of microscopic fungi that is characteristic of hyphae, hyphae whether there is a bulkhead conidiophores, as well as the characteristics of conidia or spores (form and sequence). The characteristics obtained later in the match with fungi identification key books according to Barnet and Barry (1987), Gandjar et al (1999), Watanabe (1937) This activity is carried on a litter every time retrieval from the field during the decomposition process.

3.6 Data Analysis

To analyse the data the diversity of fungi, used formula Shannon-Wiener diversity index (Krebs, 1985).

$$H' = -\sum_{i=1}^{s} [(\frac{ni}{N})\ln(\frac{ni}{N})]$$

- H' : Diversity Index
- s : The number of overall sample
- i : the data to-i
- ni : The number of i-th
- N : Total number of species

Diversity index has a range of values as follows: H' < 1 : lower Diversity

1 < H' < 3: Diversity is being

H '> 3 : High Diversity

4 RESULT AND DISCUSSION

4.1 Fungi That are Kind to the Leaf Litter Decomposition Process *R. mucronata* in Salinity 0-10 pp

The results showed there were 13 species of fungi decomposers that can be isolated from leaf litter decomposition process *R. mucronata* which in salinity 0-10 ppt. The average number of colony highest *Trichoderma* sp. with the average number of colonies of 0.99×10^2 cfu / ml. The average number of colonies can be seen in Table 1.

The level indicates that the emergence of a type of fungi *Trichoderma* able to compete with other fungi in the uptake of nutrients (nutrients) during decomposition. It accordance with the statement of Harman *et al.* (2004) which states that*Trichoderma* can be found in almost all soil types and in a variety of habitats, these fungi can multiply rapidly in the root zone.

In addition Trichoderma has the ability to compete with soil pathogens especially in getting Nitogen and Carbon. So this type in inoculation into the ground to suppress the disease that attacks the plant nursery, this is because in Trichoderma are toxins that can control the plant.

Table 1: The average number of colonies \times (10² cfu / ml) of each species of fungi within 15 days and the frequency of colonization in the process of leaf litter decomposition *R.mucronata* for 75 days at a salinity 0-10 ppt.

No.	Species of fungi	The	average nu	mber of co	olony (d	ays)	The average number of	CF (%) ^a	
		15	30	45	60	75	$colony \times (10^2 \text{ cfu/ml})$		
1	Aspergillus flavus	0,33	0,66	0	0	0	0,198	33,33	
2	Epicoccum nigrum	0,33	0	0	0	0	0,066	16,66	
3	Acremonium sp	0,66	0,33	0	0	0	0,198	33,33	
4	<i>Trichoderma</i> sp	0	1	1,66	1,33	1	0,998	66,66	
5	Aspergillus sp	0	0,33	0,33	2,33	0,33	0,664	66,66	
6	Trichoderma sp. 1	0	0	0,66	1	0,66	0,464	50	
7	Rhizopus stolonifer	0	0	0,33	0	0	0,066	16,66	
8	Trichoderma sp. 2	0	0	1,66	1,33	0,66	0,73	50	
9	Trichoderma sp. 3	0	0	0,66	0	0,33	0,198	33,33	
10	Rhizoctonia sp	0	0	0	0,66	0	0,132	16,66	
11	Curvularia sp	0	0	0	0,33	0,33	0,132	33,33	
12	Fusarium sp	0	0	- 0	0	0,33	0,066	16,66	
13	Penicilium sp	0	0	0	0	0,66	0,132	16,66	

4.2 Fungi that are Kind to the Leaf Litter Decomposition Process *R. mucronata* on Salinity 11-20 pp

The results showed there are 11 species of fungi decomposers that can be isolated from leaf litter decomposition process *R. mucronata* which in salinity 11-20 ppt. The average number of colony highest *Aspergillus sp.* and *Trichoderma sp.* 2. the average colony count of 0.66×102 cfu / ml. The average number of colonies can be seen in Table 2.

Aspergillus is one type of fungi that are cosmopolitan and easily isolated. This is consistent with the statement Mizanaet al. (2016) which states that the Aspergillus is a microorganism eukaryotes, is now recognized as one of the few living creatures that have spread area the most widespread and abundant in nature, than that of the mold is also a common contaminants on various substrates in tropical and subtropical regions.

Trichoderma is one type of fungi which has a role as decomposers of litter in the leaves of plants Mangrove called decomposers. This is consistent with the statement Susanto (2013) which states that Trichoderma sp. an antagonist fungus species commonly found in the soil, especially in organic soil and is often used in biological control. Species of Trichoderma sp. in addition as decomposers organisms, can also function as a biological agent. Biological control agents is one option of controlling plant pathogens promising because it is inexpensive, readily available, and safe for the environment.

No	Species of fungi	The	average n	umber of	colony (day	The average number of	CF	
		15	30	45	60	75	$colony \times (10^2 \text{ cfu/ml})$	% ^(a)
1	A <i>spergillus</i> sp	1,33	0,33	0	0,33	1,33	0,664	66,66
2	Cladosporium herbarum	0,33	0	0	0	0,33	0,132	33,33
3	Tidak Teridentifikasi	0,33	0	0	0	0	0,066	16,66
4	Aspergillus sp. 2	0	0,33	0	0,66	0,33	0,264	50
5	Rhizoctonia sp	0	0,33	0	0,33	0	0,132	33,33
6	S <i>cytalidium</i> sp	0	0	0,66	0	0,33	0,198	33,33
7	Rhizoctonia sp. 1	0	0	0,33	0,33	0	0,132	33,33
8	Trichoderma sp. 2	0	0	2,33	0,66	0,33	0,664	50
9	<i>Trichoderma</i> sp. 4	0	0	1,33	0,66	0	0,398	33,33
10	Trichoderma sp. 3	0	0	0	0,33	0	0,066	16,66
11	<i>Mucor</i> sp	0	0	0	0	0,66	0,132	16,66

Table 2: The average number of colonies \times (102 cfu / ml) of each species of fungi within 15 days and the frequency of colonization in the process of leaf litter decomposition R.mucronata for 75 days at a salinity of 11-20 ppt.

Table 3: The average number of colonies \times (102 cfu / ml) of each species of fungi within 15 days and the frequency of colonization in the process of leaf litter decomposition of *R. mucronata* for 75 days at a salinity of 21-30 ppt.

No.	Species of fungi	The a	average nu	umber of c	colony (da	The average number of	CF	
	species of fungi	15	30	45	60	75	$colony \times (10^2 \text{ cfu/ml})$	(%) ^a
1.	Trichoderma sp. 2	1,33	2,66	2,33	0,66	0,33	1,462	83,33
2.	Aspergillus fumigatus	1	0,66	0	0	1	0,532	50
3.	<i>Curvularia</i> sp	0,66	0,33	0	0,33	0,66	0,39	66,66
4.	Mucor sp	0	0,33	0	0	0,33	0,132	33,33
5.	Trichoderma sp. 4	0	0,66	0,33	0,66	1	0,53	66,66
6.	Mycocladus sp	0	0	0,33	0,33	0	0,132	33,33
7.	Humicola fuscoatra	0	0	0,66	0	0,33	0,198	33,33
8.	Scytalidium lignicola	0	0	0	0,66	0,33	0,198	33,33

4.3 Fungi that are Kind to the Leaf Litter Decomposition Process R. mucronata on Salinity 21-30 ppt

The results showed there were 8 species of fungi decomposers that can be isolated from leaf litter decomposition process R. mucronata which in salinity 21-30 ppt. The average number of colony highest Trichoderma sp. 2 with an average number of colonies of 1.46×102 cfu / ml. The average number of colonies can be seen in Table 3.

Besides being a very common type of fungus found in the soil and is a fungus that is antagonistic to other fungi. According to a statement from Herlina (2009) states that Trichodermasp. in promoting hormone / plant growth stimulators. In addition, application of Trichoderma sp. can optimize the core crop production.

However, Trichoderma sp. Having the ability to control fungal pathogens different. In accordance with the statement of Chet (1987) which states that the ability of each species of Trichoderma sp. in controlling different fungal pathogens, This is because the morphology and physiology are different. For example, Trichoderma harzianum and Trichodrma hamate produce glucanase and chitinase enzymes that can cause eksolisis host hyphae.

4.4 Comparison of type of Fungi in Different Levels of Salinity

The number of species of fungi found in leaf litter R. mucronata that which has undergone a process of decomposition at the level of salinity 0-10 ppt, 11-20 ppt, 21-30 are presented in Figure 2.

At the level of salinity found the number of different fungi. The higher the salinity level of the less number of fungi in it, it is consistent with the statement Yunasfi (2006) conditions were similar to those freshwater (brackish) is good enough for the growth and development of various types of fungi compared to conditions at higher salinity levels. This is in line with the statement of Silva and Fay (2012) which states that the fungus was reported more sensitive to osmotic stress than bacteria. There was a decrease in the total number of fungi in soil watered



Figure 2: Comparison of Total Type Fungi in Different Levels of Salinity.

with different concentrations of sodium Cloride. In the long-term decline in the genetic diversity of the fungus to the influence of hydrostatic and osmotic pressure rise that can alter the physiology of the fungus.

At each station there are differences in the number of species is found, it is due to several factors. Among them is the salinity, because of a higher level of saline in an area the less the population of fungi in it, this is in accordance with the statement Damanik (2010) that the microorganisms contained in water is influenced by physical factors and chemicals such as hydrostatic pressure, light, pH, salinity and temperature. One response to salinity microorganism is intolerant and will die in conditions of high salinity

4.5 Comparison Fungi Population in Different Levels of Salinity

Comparison population of fungi in leaf litter *R*. *mucronata* has undergone a process of decomposition at a rate of 0-10 ppt salinity, 11-20 ppt, 21-30 ppt presented in Figure 3.



Figure 3: Comparison of Population of the fungi at Different Levels of Salinity.

The highest average opulasi fungi found in leaf litter R. mucronata that the process of decomposition at 0-10 ppt salinity level is 4.04×102 cfu / ml (Figure 3). The number of fungal population showed that the nutritional needs of fungi at station 1 (0-10 ppt) is fulfilled, thus adding to the fungal population.

Differences in the number of population of fungi were obtained from each station depending on the resilience of the fungus can survive in the soil, nutrients for their life cycle, it is consistent with the statement of Hasyimi (2008) that the difference fungi to survive in different levels of salinity indicates that the need fungi are to fulfilled life on the salinity and able to withstand the salinity conditions. Fungi as other microorganisms, for life requires organic matter as an energy source.

4.6 Fungi Diversity Index at Different Levels of Salinity

Fungal diversity index contained in the leaf litter *R. mucronata* that which has undergone a process of decomposition at the level of salinity 0-10 ppt, 11-20 ppt, 21-30 are presented in Figure 4.



Figure 4: Graph Fungi Species Diversity Index at Different Levels of Salinity.

Fungal diversity index calculation results at a salinity 0-10 ppt, ppt 11-20, 21-30 ppt is 2.43; 2.3; 2.01 (Figure 4). The average value of Shannon index - Wiener's diversity of fungi in leaf litter*R. mucronata* which plays a role in the decomposition process with different salinity showed the same range that was.

Diversity of fungi can affect litter decomposition process, many fungi were found to indicate the high value of the rate of decomposition of organic matter from the litter. This is certainly good, especially for the plant because of the decomposition process will produce nutrient ready for use by the plant. The highest diversity index value that is at 0-10 ppt salinity, it is stated that salinity affects the diversity of fungi. The lower the salinity level then the higher the species diversity of fungi, while the higher the salinity level, the lower the diversity of fungi in it. This is consistent with the statement of Yunasfi and Suryanto (2008) stated below 10 ppt salinity level is more suitable environmental conditions for survival, growth and development of various types of fungi in litter higher in the salinity level influence can be seen by the number of species of fungi are present in the process of decomposition of litter.

5 CONCLUSIONS

The highest number of fungi populations found in station 1 with salinity 0-10 ppt worth 4.04×10^2 cfu / ml. And the frequency of fungal colonization of the highest in litter decomposition process at different levels of salinity that is *Trichoderma* sp. 2.

The index value multifaceted types of fungi in Belawan showed the same range that is moderate, illustrating the diversity of being, productivity sufficiently, fairly balanced ecosystem conditions, and the pressure of ecological balance.

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