

# Application of Various Species of Fungi on *Avicennia Marina* Growth that is 200 m Away from the Coastline in Belawan and Sembilan Island

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Abstract: Mangrove forest has many benefits, including helping to reduce the effects of storm surges, big waves, and winds. Mangrove forests will be disrupted if they are near industrial areas, this can result in a negative impact in the form of industrial waste either solid or liquid waste that can affect to surrounding environment. The purpose of this study was to determine the types of fungi that are expected to increase the growth of mangrove seedlings *A. marina*. This study uses several types of fungi, namely, the type of fungi *Aspergillus niger*, *Aspergillus sp. 2*, *Aspergillus sp. 1* and control. While the experimental design used in this research method was a Completely Randomized Design (CRD) with *A. marina* which was carried out at both river mouths for each location consisting of 2 plots (polybags and soil). The results showed that fungi had different abilities in increasing the growth rate of *A. marina* seeds which significantly affected the parameters of diameter, leaf width and total dry weight of plants. From the results of this study, it can be concluded that the *Aspergillus niger* fungi provides the best benefits for mangrove growth.

## 1 INTRODUCTION

Indonesia mangrove forest is one of the largest mangrove forest in the world. Conservation Indonesia in 2018 mention that Indonesia has 3.1 million hectares mangrove forest or 22.6 % of the total mangrove forest in the world. The mangrove forest distributed in the coastal eastern Sumatra, Kalimantan coast and Papua. The mangrove ecosystem has a very complex function, such as dampening ocean waves and storms, coastal protection from the process of abrasion and erosion. Mangrove also have ability for trappers of sediments, as a refuge and foraging, as well as a variety of species of aquatic biota brackish, as a place for recreation, and wood producer. As well as habitat for a variety of wildlife such as birds, crocodiles, monkeys and snakes (Ghuffran, 2012).

The word mangrove is a combination of the Portuguese language "mangue" and English "grove". In English, the word is used both for the community mangrove plant that grows in tidal range and also for the individual species of plants that make up that community. In addition, the mangrove forest for

most of Indonesian people are more likely known as mangrove forests. The use of the term mangrove forest is actually less precise and unambiguous, because the mangrove forests are forests composed and covered by many other plant genera and species. Therefore, Citation mangrove forest with mangrove forests should be avoided (Kusmana, 2005).

The mangrove forest has many benefits one of which can help reduce the impact of the storm, waves and wind. Mangrove forests provide an important defense against coastal erosion. As well as mangrove trees can also reduce the current wave of energy through mangrove forests, and a barrier between the waves and land. The real benefit of mangrove systems as well as social and cultural functions. Mangrove forests support the religious and spiritual values, in addition to aesthetic and recreational value for ecotourism (UNEP, 2014).

One of the pollutants in the water is heavy metal. Aquatic organism is a group of organisms that first experienced the direct impact of the effect of waste or heavy metal pollution in the waters. One of the aquatic organisms that receives the direct impact of heavy metal pollution is mangrove (Arisandy *et al*, 2012).

*Aspergillus niger* one of the most common species and is easily identified from the genus *Aspergillus*, *Moniliaceae* families, orders and classes *Monoliales* Fungi Imperfecti. *Aspergillus niger* can grow at a temperature of 35°C-37°C (optimum), 6°C-8°C (minimum), 45°C-47°C (maximum) and require adequate oxygen (aerobic). *Aspergillus niger* has a basic feathers are white with a thick layer of konidiospora dark brown to black. Conidial head is black, round, tends to separate into parts looser with age. Konidiospora have smooth walls, hyaline but also brown. *Aspergillus niger* in its growth directly related to the nutrients contained in the substrate, simple molecules that are around hyphae can be directly absorbed while more complex molecules must be broken down before absorbed into the cells, by generating some extra cellular enzymes. The organic material of the substrate used by *Aspergillus niger* for molecular transport activities, maintenance of cell structure and cell mobility (Eliza, 2014). The purpose of this study was to get information on the fungi species that can enhance the growth of mangrove *A.marina*.

## 2 RESEARCH METHOD

### 2.1 Time and Location

The research was conducted in estuary coastal areas Belawan Mangrove (Figure 1), Forest village of Kampung Nelayan (Lydia, 2011). The location was suspected contaminated with heavy metals because it very close to the industry and the station. Mangrove Forest and Sembilan Island area as the



A

area is not polluted (control) for much of the industry (Figure 1B).

### 2.2 Material and Method

The material for this research are Petri dishes, test tubes, spatulas, measuring cups, analytical balance, oven, kalifer, ruler, autoclave, paper labels, hoes, machetes, digital cameras, aluminum foil, scissors, polybags and plastic clingwrap. Field equipment materials used are different types of fungal isolates obtained in previous research by Sakila et. al., (2017). *A. marina* seedlings are ready for planting in the field, stakes, rope, wooden stakes which are made of bamboo, permanent markers, stationery and others.

### 2.3 Research Procedure

#### 2.3.1 Making the PDA

Making the Media Potato Dextrose Agar (PDA), potato peeled and weighed as much as 200 grams, then thinly sliced. Potatoes boiled with 1 liter of distilled water for 10-15 minutes, then filtered using a sieve. With a composition of 20 g to be incorporated into the filtrate the boiled potatoes, then cooked until boiling and stirring until there is no precipitate. Furthermore, the media sterilized by autoclaving at 121°C for 15 minutes. Lay (1994), the media is a must contain nutrients that are easily used by microorganisms.

#### 2.3.2 Rejuvenation Fungi

Media PDA heated to melt, sterile Petri dishes which have been prepared. Media PDA put in a Petri dish



B

Figure 1: (A) The Mangrove Forest village of Kampung Nelayan Belawan as areas suspected of heavy metal contaminated (B) Mangrove Sembilan Island area as the area is not polluted.

until all the bowls are filled. Fungi that have been isolated previously taken a bit that is 1 cm x 1 cm x 0.5 cm as the host and put into a Petri dish. Petri dishes containing fungi are then stored and waiting until the fungi grow and thrive. The fungus takes time to grow and develop is 3-4 days and the maximum growth will be seen after 1 week. Fell *et al.*, (1975), Fungi play an important role in mangrove ecosystems, especially in conjunction with the bacteria to accelerate the decomposition of leaf litter. The suspension of fungi can be seen in Figure 2.

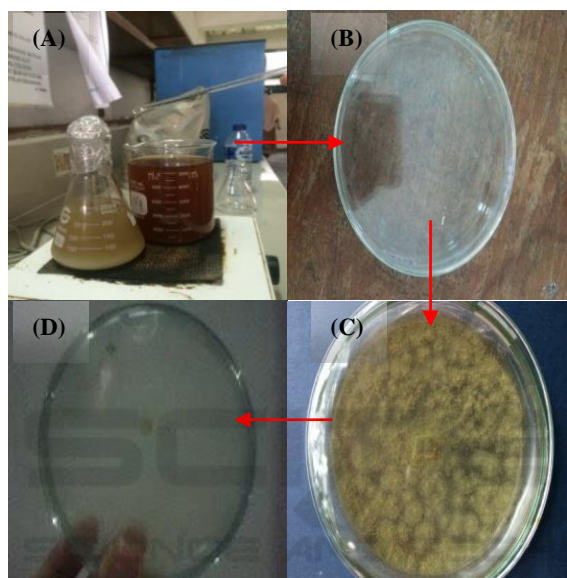


Figure 2: Preparation of the suspension to be used for the growth of the field (A) PDA (B) Petri dish (C) Fungi (D) Isolate Fungi.

### 2.3.3 Planting Seeds of *A. marina* in Field

*A. marina* seedlings to be planted is healthy seedlings was 15 days which has at least four leaves. Planting hole measuring 10 cm x 10 cm x 15 cm made as many as 24 for each plot. Planting be done by first tearing plastic bags or polybags with caution in order not to break his land so that it can damage the roots. *A. marina* seedlings are then put into a pit that had been prepared beforehand. Land located around the hole is used to cover the hole by pressing on the hole is backfilled so that the seedlings can stand firm. The seed is not broken due to wind and tide, in *A. marina* mounted side marker rod attached to the stems of seedlings. Suryono (2013), Planting should be done at low tide in order to facilitate the planting and spacing between plants.

### 2.3.4 Application Fungi of the Growing Media

Isolates of fungi that will be used are the fungi *Aspergillus niger*, *Aspergillus sp. 2* and *Aspergillus sp. 1*. These isolates advance rejuvenated by planting on PDA to sufficiently grow. For each type of fungi which has grown on PDA's, then taken by cutting the order of 5 x 5 x 2 mm. Pieces that have overgrown fungi which are then incorporated into a test tube which has been filled 2.5 ml of sterile water. A total of 2.5 ml suspension of the fungus is spread evenly around the roots of seedlings of *A. marina* (Figure 3a and Figure 3b). Tjandrawati (2003), the effectiveness of the bacterium is shown by how quickly litter is lost from the soil surface as soon as the fall of the litter from the plant.



Figure 3: Application of fungi in the growing media (A), Fungi (B) Fungi spread evenly around the roots of *A. marina*.

The parameters of *A. marina* seedlings were measured and analysed with statistical data is a trunk diameter, plant height, leaf area and the number and dry weight of plants.

#### a. Stem height (cm)

Seedling height measurements carried out once a fortnight for three months. The tool used is a ruler. To get a more accurate measurement marked approximately 1cm from the ground level as a starting point of measurement, so as to keep subsequent measurement using a fixed starting point.

#### b. Stem diameter (cm)

Stem diameter was measured by using a caliper. To get a more accurate measurement marked approximately 1 cm of the soil surface as a starting point of measurement, so as to keep subsequent measurement using a fixed starting point.

#### c. The number and leaf area

At the time of observation is calculated all the number of leaves of seedling. And the widest leaf area is calculated by using a caliper as leaf area size parameters.

#### d. Total dry matter

The sum total dry matter at the end of the research done by calculating the dry weight of the stalk with root dry weight.

### 2.3.5 Experimental Design

Planting seeds of *A. marina* performed on both the river mouth for each location consists of two plots (polybags and Soil). Placement within 200 m from the sea towards the land. This study uses a completely randomized design (CRD).

Linear model on a completely randomized design are:

$$Y_{ij} = \mu + \alpha_i + \sum_{ij}$$

Where,

$Y_{ij}$  = Observations on the treatment of fungal all replicates all  $i$  and  $j$

$\mu$  = General mean value of observation

$\alpha_i$  = Influence factor Fungi additions to- $i$ ;  $i = 1, 2, \dots, t$  and  $j = 1, 2, \dots, r$

$\sum_{ij}$  = Effect of treatment errors Fungi  $i$ , and  $j$  replicates all

Data collection was conducted after planting and the provision of fungi in the field, while the observation time is as follows:

- a. 15 days
- b. 30 days
- c. 45 days
- d. 60 days
- e. 75 days
- f. 90 days

The fungi species that can be used to enhance the growth of *A. marina* is derived from the previous year's trial:

- a. Control
- b. *Aspergillus niger*

c. *Aspergillus sp. 2*

d. *Aspergillus sp. 1*

## 3 RESULT AND DISCUSSION

### 3.1 Result

Observations and measurements were carried out on seeds of *A. marina* for 12 weeks showed a difference to as height, stem diameter, area and number of leaves and total dry weight. *A. marina* seedlings observational data can be seen in Table 1. Observations seed *A. marina* 12 Weeks After Planting in Belawan and Table 2. Observations seed *A. marina* 12 Weeks After Planting in Sembilan island.

#### 3.1.1 Stem Height

From measurements made during the 12 weeks, data showed stem height *A. marina* for Belawan area and Sembilan island regions. All the seeds of *A. marina* given treatment application of various types of fungi showed good growth. In areas of high Belawan larger increment contained in *A. marina* stem treated with *Aspergillus sp. 2* with an average height of 23.13 cm and the lowest for the treatment of stem with *A. niger* with an average height of only 22.81 cm. High added every week can be seen, where the growth rate of each treatment has increased. High accretion weekly chart can be seen in Figure 4.

Table 1: Results of the observation of seedlings *A. marina* 12 Weeks After Planting in Belawan.

Parameter Observation	Treatment				Unit
	K	<i>Aspergillus niger</i>	<i>Aspergillus sp.2</i>	<i>Aspergillus sp.1</i>	
Heigh average	22.90	22.81	23.13	23.10	cm
The average diameter	0.43	0.44 *	0.43*	0.42	cm
The width of the widest leaves	4.24	4.2	4.14	4.46	cm
Total dry matter	13.44*	13.49*	8.76*	12.31*	g
number of leaves	9	9	10	10	Sheet

Table 2: Results of the observation of seedlings *A. marina* 12 Weeks After Planting in Sembilan Island.

Parameter Observation	Treatment				Unit
	K	<i>Aspergillus niger</i>	<i>Aspergillus sp. 2</i>	<i>Aspergillus sp. 1</i>	
Heigh average	16.61	16.63	15.94	16.30	cm
The average diameter	0.33	0.34	0.34	0.35	cm
The width of the widest leaves	3.82 *	3.81	3.80	3.85 *	Cm
Total dry matter	12.35	12.99	12.17	12.46	G
number of leaves	7	8	8	8	Sheet

\*Description: significant at 95% confident level

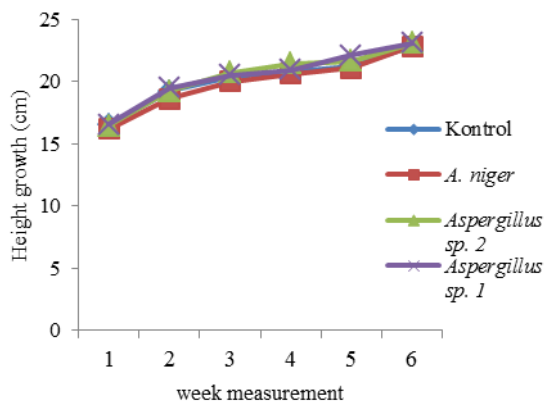


Figure 4: High Growth Stem of *A. marina* in Belawan.

*A. marina* stem with treatment fungi *A. niger* with an average height of 16.63 cm while the lowest for the treatment of seeds with fungi *Aspergillus sp. 2* ie.15.94 cm. height every week in the area of Sembilan Island illustrates that any given fungi affect the high growth of seedlings of *A. marina* albeit with growth rates vary. High accretion weekly chart can be seen in Figure 5.

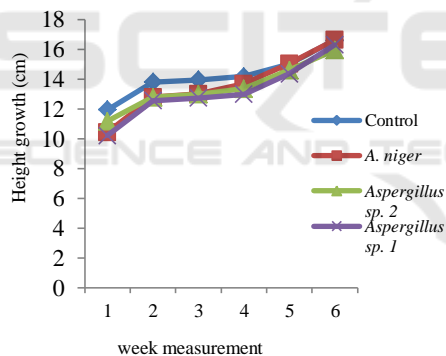


Figure 5: Height stem growth of *A. marina* in Sembilan Island.

### 3.1.2 Stem Diameter

Application of fungi affect the seed ling diameter *A. marina*. Diameter measurement results are shown in Table 1. In the area of diameter Belawan highest in *A. marina* stem treated with the fungi *A. niger* applications with an average diameter 0.43 cm. Diameter measurement chart *A. marina* stem to Belawan area can be seen in Figure 6.

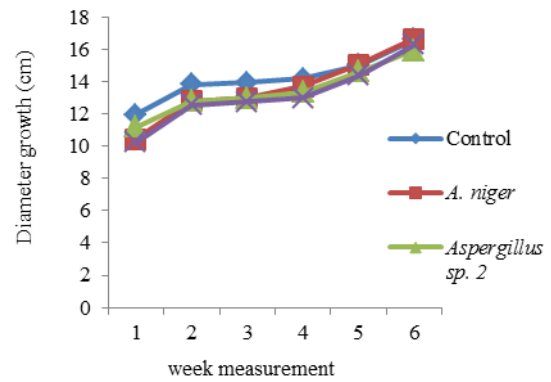


Figure 6: Growth of *A. marina* Stem diameter in Belawan.

As for the Sembilan island region Diameter highest in *A. marina* seedlings treated fungi application *Aspergillus sp. 1* with an average diameter of 0.35 cm. Diameter measurement chart *A. marina* seedlings in Sembilan Island can be seen in Figure 7.

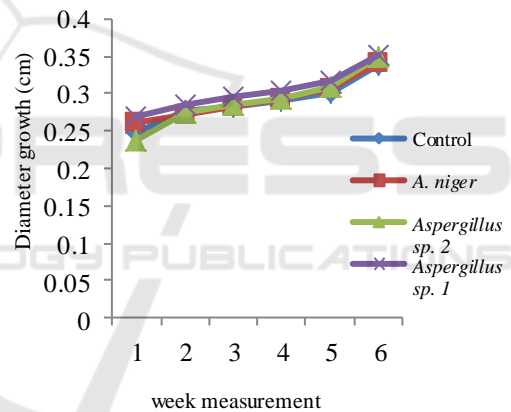


Figure 7: Diameter stem growth of *A. marina* in Sembilan Island.

### 3.1.3 Leaf Width

In the area of leaf surface width Belawan highest in *A. marina* leaf width with the treatment of *Aspergillus sp.1* ie.4.46 cm and the lowest was found in *A. marina* leaf width treated with *Aspergillus sp. 2* is 4.14 cm. Differences in leaf area can be seen in Figure 8.

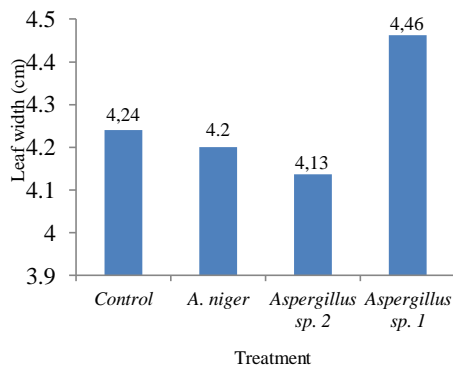


Figure 8: Comparison leaf width of *A. marina* in Belawan.

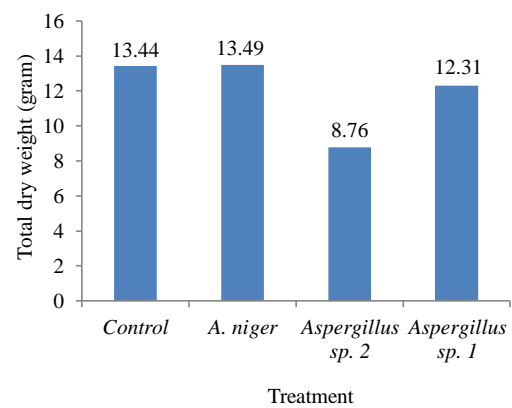


Figure 10: Total Dry Weight of *A. marina* in Belawan.

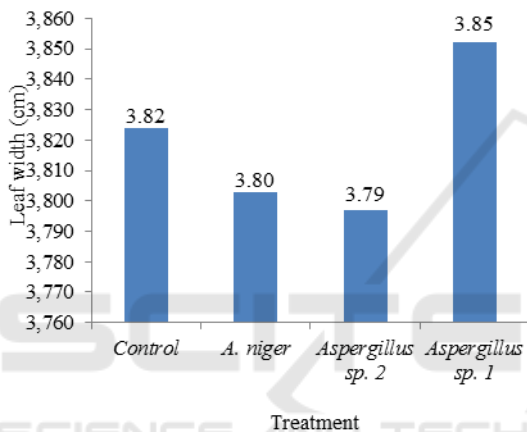


Figure 9: Comparison leaf width of *A. marina* in Sembilan Island.

The highest total dry matter on the location of Sembilan Island contained in the seed treatment *A. niger* as big as 12.99 g while the lowest for the treatment of seeds with fungi *Aspergillus sp. 2*, namely 12.17 total dry weight difference in each treatment is shown in Figure 11.

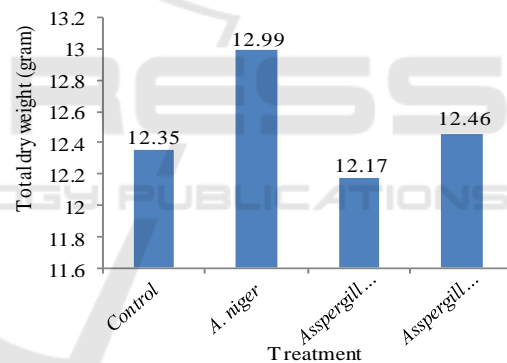


Figure 11: Total dry weight of *A. marina* in Sembilan Island.

### 3.1.4 Total of Dry Weight

Once the data is high and the next diameter, calculated the total dry weight of seedlings *A. marina* as listed in Table Variety Fingerprint Analysis Total Dry weight of seedlings of *A. marina*. Total dry weight of the sum of the dry weight of seedlings and root dry weight. The highest total dry matter in Belawan location contained in the seed treatment *A. niger* as big as 13.49 g and the lowest for the treatment of seeds with fungi *Aspergillus sp. 2*, namely 8.76 g. Differences in the total dry weight of each treatment can be seen in Figure 10.

### 3.1.5 Number of Leaves

The average number of the most well leaf growth rate in Belawan contained in the seedlings with fungi treatment *Aspergillus sp. 2* and *Aspergillus sp. 1* where the average of the two treatments is that 10 pieces while the lowest for the treatment of seeds with fungi *A. niger* and control where his average is 9 strands. Differences in the number of leaves on each treatment can be seen in Figure 12, the graph shows the number of leaves between treatment types of fungi *Aspergillus sp. 2* and *Aspergillus sp. 1* is the same and the number of leaves between treatment

types of fungi *A. niger* and without treatment or control is the same, it is influenced by the ability of species of fungi are different in increasing the growth rate of one growth rate of the number of leaves.

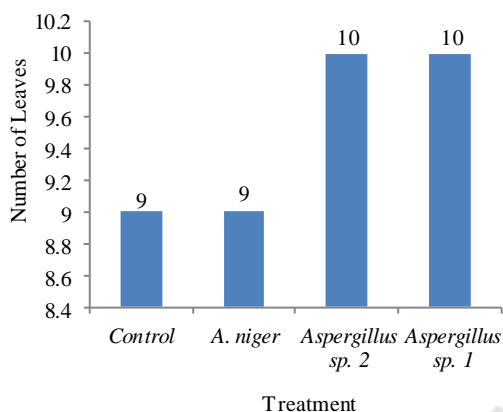


Figure 12: Number leaves of *A. marina* in Belawan.

The average highest number of leaves in the Sembilan Island contained in the seeds treated with the fungi *A. niger*, *Aspergillus sp. 2* and *Aspergillus sp. 1* where the average of these treatments are 8 pieces while the lowest was in control with his average is 7 leaves. Differences in the number of leaves on each treatment is shown in Figure 13.

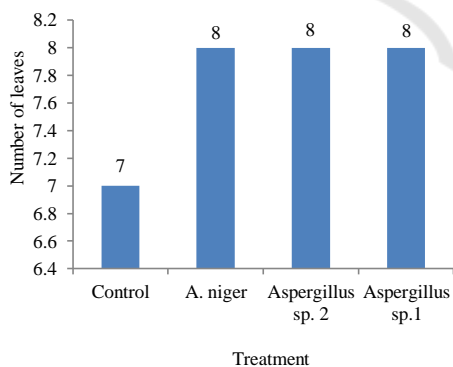


Figure 13: Graph Total leaf seedlings *A. marina* in Sembilan Island.

### 3.2 Discussion

The results of observations conducted on *A. marina* seedlings to all the parameters show that the fungus has a role in promoting the growth of seedlings,

either in addition seedling height, stem diameter increment, leaf width widest, number of leaves and total dry weight of the seeds.

#### 3.2.1 Stem Height

Based on observations of plant height were conducted in Belawan, fungi granting the application did not significantly affect plant height increment. High seed nicest *A. marina* seedlings *A. marina* growing amongst others provided treatment plants with a fungus is fungus treatment *Aspergillus sp. 2*, namely 23.13 cm and height growth of plants the lowest was in the treatment of fungi *A. niger* is 22.81 cm and without treatment or control that is 22.90 cm.

The results of the observations made in Sembilan Island, fungi delivery applications also did not significantly affect plant height increment. Stem height nicest *A. marina* seedlings *A. marina* growing amongst others provided treatment to the treatment plant fungi are fungi *A. niger*, namely 16.63 cm. and high growth lowest plant is treated with the fungi *Aspergillus sp. 2* is 15.94 cm. This is caused by the polluted environment whether it be household garbage and industrial wastes which are located around the area Belawan. The most high growth with low crop treatment fungi *Aspergillus sp. 2* allegedly caused by the carrying over of the pieces fungi, fungi which is applied to the plant by the high salinity of the water there and could also this is caused by the level wind speeds vary by region (Mukhlis, 2007), Given the good growth response is generally obtained at a low salinity. This is due to the mangrove plants are plants that are tolerant to salt (salt tolerance) no plants that require salt (salt demand).

The neighborhood has a very important meaning to the high growth rate of seedlings *A. marina* in helping the decomposition process either fungi, bacteria and other animals. according to a statement Fell *et. al.*, (1975) that the environment is taking part in litter decomposition process in which it was included into the process of decomposition of fungi, bacteria and other animals. As well as fungi also play an important role in the cycle, especially the carbon cycle, which plays a role for the survival of the whole organism. Trouve *et. al.* (2015) explained that the factors that influence plant growth rates are age, nutrition, and sunlight.

#### 3.2.2 Stem Diameter

Based on observations made in the field showed that the use of a type of fungi significant effect on stem

diameter at Belawan area. Stem diameter highest in *A. marina* seedlings treated *A. niger*, namely 0.44 cm and stem diameter growth was lowest for the *A. marina* seedlings treated with *Aspergillus* sp. 1, namely 0.42 cm.

The highest stem diameter in the area of Pulau Sembilan is on the seedlings of *A. marina* with the treatment of *Aspergillus* sp. 1, namely 0.35 cm and stem diameter growth was lowest for the *A. marina* seedlings without treatment or control, that is 0.34 cm. In observations made Sembilan Island region showed that the administration of fungi do not have a significant effect on stem diameter growth of seedlings of *A. marina* however, when compared to no treatment or control seeds, seedlings by stem diameter growth of fungi have a higher or better especially seed by fungi *Aspergillus* sp. 1.

Differences in diameter on each seedling of *A. marina* can be caused by the interaction between the type of mangrove seedlings that will provide a real impact on plant stem diameter (Munandar, 2009).

According Husnaeni (2013), one of the forms of interaction between the populations with other populations or between one individual with another individual is the nature of competition (competition). Competition occurs when two individuals have the same growth medium needs while the environment does not provide those needs in sufficient quantities. Factors affecting competition include water, nutrients, light, carbon dioxide, and space. These factors will affect the growth of stem height, stem diameter, chlorophyll, and the yield of the plants associated with physiological processes (photosynthesis and respiration) happens. Lempereur *et. al.* (2015) explained that differences in diameter growth in plants can also be affected by environmental conditions.

### 3.2.3 Leaf Width

Based on the results of observations made Belawan area. The highest leaf width is on *A. marina* seedlings with the treatment of *Aspergillus* sp. 1, which is 4.46 cm and width of leaves and lowest for the *A. marina* seedlings with the treatment of *Aspergillus* sp. 2, ie 4.14 cm, this is caused by a number of damaged leaves on the seedlings of *A. marina* to the treatment of fungi *Aspergillus* sp. 2.

While the results of the field observations made in the area of Sembilan Island, Award fungi affect the seed leaf width *A. marina*. The average yield measurements can be seen in Table 2 where the highest leaf width is on *A. marina* seedlings with the treatment of *Aspergillus* sp. 1, which is 3.85 cm and

width of leaves and lowest for the *A. marina* seedlings with the treatment of *Aspergillus* sp. 2, which is 3.80 cm.

Daniel *et. al.*, (1987) states that the success of seedling growth depends on three factors: the temperature of the soil, water availability, and the ability to produce root seedling. It describes the physiological readiness as the root system and leaves that are adaptive to the environment, is the dominant factor in the success of the growth of seedlings. Boyer (2013) leaves serves as a place to do photosynthesis and to regulate the transpiration process.

### 3.2.4 Total Dry Weight

The observation of the total dry weight in Belawan area shows that the use of a type of fungi provides a real impact on the total dry weight of the plant. Total dry weight of the highest in the treatment of seeds with fungi *A. niger*, namely 13.49 g.

While the observations for the total dry matter in the area of Sembilan Island showed that fungi administration did not significantly affect the total dry weight of the plant *A. marina*. Total dry weight of the highest in the treatment of seeds with fungi *A. niger*, namely 12.99 g.

Total dry weight is the result of overall plant growth includes indicates the ability of plants to absorb organic material. Roisatuz (2015) explains that, dry weight is the sum of the total dry weight of shoots with root dry weight. Dry weight shows the ability of plants to absorb organic materials used for the process of plant growth. Growth and development of plants need nutrients and water, absorption of water and nutrients were favorably affected by the growth of roots. Provision of fungi to plants provides a better root growth so that the absorption of nutrients and water can run well. Philippe and Deunff (2014) explains that, plant and air growth and development, air absorption and wells are good by root growth. Fungi for plants provide better growth. Nutrients and air can run well.

### 3.2.5 Number of Leaves

Based on observations made in the area of Belawan, fungi granting the application did not significantly affect the number of leaves of plants of *A. marina*. It can be seen from F. Calculate smaller than F. Table. For the average number of the highest leaf area Belawan contained in the seed treatment with fungi *Aspergillus* sp. 2 and *Aspergillus* sp. 1 where the average number of leaves of 10 strands and the



average number of leaves was lowest for the treatment of seeds with fungi *A. niger* and without treatment or control, by the average number of leaves 9 strands.

While the results of the observations made in the area of Sembilan Island, fungi delivery applications also did not significantly affect the number of plant leaves of *A. marina*. The highest number of leaves average value contained in seed treatment *A. marina* with 3 types of fungi that *A. niger*, *Aspergillus* sp. 2 and *Aspergillus* sp. 1 where three types of these fungi average value of the same, ie 8 strands. And the lowest number of leaves found in *A. marina* seedlings with no treatment or control ie 7 strands.

Many least number of leaves can also be caused by several factors, one of them is the temperature, Production of the new leaves of *Avicennia marina* occurs at a temperature of 18-20°C and if the temperature is higher than the production is reduced (Zikri, 2014).

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