The Effect of Sun Light Intensity on the Growth of *Azolla Nicrophylla* and its Symbiont *Anabeana Azollae* in Brackish Water

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Abstract. *Azolla microphylla* is a tropical and subtropical fern with a length of 1.5-2.5 cm that can be used as an organic fertilizer so that it can meet nutrient needs, especially for plants. The fern has a symbiont *Anabaena azollae* which can fix N2 freely in the air so that it can contribute to the need for N for plants in the soil. This research is aimed to study the effect of sunlight intensity on the growth of *A. microphylla* and its symbiont *An. azollae* in brackish water. A two factors (factor a; light intensity and factor b; duration of cultivation) completely randomized design was applied to the experiment. Sunlight intensity composed of 0, 30, 50, 70 and 100 %. Duration of cultivation consisted of 0, 7, 14, and 21 day. Every unit of experiment was set in a 100 x 100 x 20 cm plastic coated wood box, filled with brackish water of 3 ppt salinity up to 15 cm depth and 1 kg fermented cow dung, mixed gently and seeded with approximately 1 gram of fresh *A. microphylla*. Every box was covered with some pieces of wood based on its light intensity. Growth of the fern was measured by counting the number of the fern colony directly and weighted with analitical balance. While growth of *An. azollae* were counted by using sedwick rafter counting cell. It is concluded that the sunlight intensity significantly influences the growth of *A. microphylla* and its symbionts *An. azollae*. The best sunlight intensity was 70%. A dinamic growth recorded during the first week post inoculation in which dobling time took place. It is recommended to grow *A. microphylla* and its symbionts *An. azollae* under 70 % of the sunlight intensity.

1. Introduction

Macrophyte *Azolla microphylla* is a relatively small size of water-nailed plant [1], having a length of 1.5-2.5 cm. This plant grows in tropical and subtropical regions [2]. Some genera can grow in brackish waters, but these biota are referred to as freshwater plants. Taxonomists classify Azolla into 7-9 species. They are *Azolla caroliniana* Wild, *A. circinata* Oltz & Hall, *A. cristata* Kaulf, *A. filiculoides* Lam, *A. imbricata* Nakai, *A. japonica* Franch. & Sav, *A. Mexicana*, *A. microphylla* Kaulf, *A. nilotica* *A. pinnata* and *A. rubra* R.Br [1].

The photosynthetic conversion of sunlight energy into algal biomass in large-scale outdoor cultures is controlled by the availability of sunlight, the photosynthetic machinery of algae, nutrients, temperature and the design characteristics of the culture system. Sunlight is the main source of energy for the life of all living things. The influence of light is determined by its quality and intensity. Light intensity is the amount of energy received by a plant per unit area and per unit time (calories/cm²/day).
Thus the definition of intensity in question includes the duration of irradiation, i.e. the length of the sun shines in one day.

Azolla sp. is one type of algae plant that can be used as an organic fertilizer so that it can meet nutrient needs, especially for plants. This fern is symbiotic with cyanobacteria like An. baena and play a role in nitrogen fixation in the aquatic environment. This symbiont can fix N2 freely in the air so that it can contribute to the need for N for plants in the soil [3].

The fern generally used as biofertilizer and green manure in paddy rice fields. Nowadays Azolla sp. (both fresh or dried) are also used as feed ingredients for ruminants and non-ruminant types of livestock. In addition, its use as biofertilizer and animal feed, Azolla, ‘green gold’ is also used as medicine, cleaning water, human food and for biogas production [4].

In Indonesia of cultivation A. microphylla at this time still in very limited circles. Cultivation techniques that are applied are still conventional and household scale and meet their own needs. This biota is only stocked in columns of stagnant water on the ground like ponds, tanks and so on [5]. This kind of cultivation condition may lead to minimal growth so that the benefits provided are certainly not maximal.

Provision of fish food sources and coastal livestock (cows, goats, ducks, ducks and so on) in the form of green protein (green protein) is one alternative solution to the economic problems of coastal communities. Macrophyte A. microphylla with its symbiont An. azollae can be an agent which can be expected to answer this problem.

The nitrogen fixing symbiosis between cyanobacteria and the water fern A. microphylla is in contrast to other cyanobacteria–plant symbioses, the only one of a perpetual nature. Many think that Anabaena sp. this is a group of blue-green algae. However, from several readings it was found that Anabaena sp is a microorganism belonging to the filamentous cyanobacteria genus, found as plankton. Anabaena sp. is a bacterium that can photosynthesize and is widespread in freshwater and salt habitats.

Until recently the symbiosis between Azolla and An. azollae has received little attention. The symbiosis is apparently based on the fixation of nitrogen by the Anabaena; the fixation products apparently move to the Azolla frond as the dual organism can be grown independently of combined nitrogen. In agriculture A. microphylla can act as organic green fertilizer that can replace urea fertilizer with a better yield rate. This research is aimed to study the effect of sunlight intensity on the growth of A. microphylla and its symbionts An. azollae in brackish water.

2. Materials and methods

This research was conducted at the Laboratory of Marine Microbiology, Faculty of Fisheries and Marine Sciences, University of Riau, from May to June 2018. A two factors (factor a; light intensity and factor b; duration of cultivation) completely randomized design was applied to the experiment. Light intensity composed of 3 level; 0 % (a0), 30 % (a1), 50 % (a2) 70 % (a3) and 100 % (a4). Duration of cultivation consisted of day 0 (b1), day 7 (b2), day 14 (b3) and day 21 (b4). The parameters observed were the growth of A. microphylla and its symbiont An. azollae.

Every unit of experiment was set in a 100 x 100 x 20 cm plastic coated wood box. The units were filled with brackish water of 3 ppt salinity up to 15 cm depth and 1 kg fermented cow dung, mixed gently and seeded with approximately 1 gram of fresh A. microphylla. Every box was covered with some pieces of wood based on its light intensity. All unit are topped with transparent plastic to avoid rain disturbance. Growth of A. microphylla and its simbion An. azollae were examined at 1, 7, 14 and 21 days post inoculation. Growth of the fern was measured by counting the number of the colony directly and weighted with analitycal balance. While growth of An. azollae were counted by using sedwick rafter counting cell.

Effect of different intensities data were analysed with analysis of variance (ANOVA) at confidence level of α = 0.05. Water quality (pH, salinity, and temperature) was monitored daily in the morning, afternoon and evening time.
3. Results and Discussion

From the data collected it appears that the number of A. microphylla colonies continued to increase until the end of the experiment, especially in the first two weeks. But then growth rate began to decline by the third week. This phenomenon occurs for all four treatments, 30, 50, 70 and 100% light intensity. As for the 0% light intensity treatment, this fern is only seen alive in the early days of the first week. More detailed data is presented in Table 1. The increase in the number of these colonies and the slowing down of growth rates per unit time will be more clearly seen in Figure 1.

Table 1. Growth of A. microphylla (colonies).

| Time  | Intensity 30% | Intensity 30% | Intensity 50% | Intensity 70% | Intensity 100% |
|-------|---------------|---------------|---------------|---------------|---------------|
| Day 0 | 72            | 65            | 73            | 85            | 68            |
| Day 7 | -             | 150           | 167           | 229           | 106           |
| Day 14| -             | 272           | 224           | 381           | 249           |
| Day 21| -             | 315           | 268           | 442           | 287           |

Similar growth patterns can also be seen from the results of weighing the total weight of A. microphylla. Where the total weight of the colony continues to increase, especially in the first two weeks. Then this number continues to rise even though the rate of growth begins to decline by the third week. This phenomenon occurs for all four treatments, 30, 50, 70 and 100% light intensity. When compared with each other, the best growth of A. microphylla (weight and number of colonies) was treated with a light intensity of 70% (a3). Then followed by the treatment of light intensity 100%, 50% and 30%. When viewed from the treatment duration of cultivation, this fern is seen still growing, but the peak growth rate of this fern occurred at intervals of 14 days (b2). More detailed data are presented in Table 2. The pattern of weight gain and decrease in growth rate per unit time is clarified in Figure 2.
Table 2. Growth (g) of *A. michrophylla*.

| Time   | Intensity 0% | Intensity 30% | Intensity 50% | Intensity 70% | Intensity 100% |
|--------|--------------|---------------|---------------|---------------|----------------|
| Day 0  | 1.06         | 1.05          | 1.06          | 1.06          | 1.03           |
| Day 7  | -            | 9.30          | 9.63          | 10.07         | 9.56           |
| Day 14 | -            | 9.36          | 9.75          | 10.72         | 9.95           |
| Day 21 | -            | 9.68          | 10.02         | 11.08         | 9.76           |

![Graph showing growth of *A. michrophylla*](image)

Figure 2. Growth (g) of *A. microphylla*

The results showed that the weight-based doubling time of *A. microphylla* was achieved in less than 7 days to 14 days of age, and afterwards tended to be achieved more slowly because of the reduced availability of nutrient water in the container. This growth pattern has also been reported by [5], where in brackish water with a salinity of 5 ppt, this fern only grows normally under 14 days. The better the water nutrition the doubling time container is achieved the faster. The time to multiply the population of *A. microphylla* was initially only 7 days, and the subsequent doubling period was longer. At the age of 21 days the weight of *Azolla* sp. has reached 10 times. Based on the results of the study it can be seen that the highest weight growth was found in the treatment intensity of 70% with heavy weight (9.01, 0.65 and 0.27 grams). The intensity treatment was 50% (8.57, 0.12 and 0.36 grams), the intensity treatment was 30% (8.25, 0.06 and 0.32 grams) while the control was positive (8.53, 0.39 and 0, 19 grams) and negative controls have no growth (dead). The result is in accordance with [6].

*Azolla* sp. can breed vegetatively [7]. Vegetative propagation, side branches separating from the main branch or stem, followed by the formation of a wound covering due to separation. Side branches that separate grow into mature plants that can form new branches. This vegetative propagation is very fast with doubling time of biomass around 4-5 days. This separating plant to become *Azolla* sp, takes 10-15 days.

Growth of *An. azollae* was examined weekly from day of 0, 7, 14 and 21. Based on data of this study it can be noted that in general the number of *An. azollae* cells decreased dramatically for all sunlight intensity treatments. The number of cells is around 5,000 cells/liter on day 0, decreased dramatically on the 7th day after inoculation. Even at 0% intensity treatment, these Cyanobacteria...
cells are no longer found. More detailed data is presented in Table 2. The impact of this reduction will be more visible if illustrated in graphical form (Figure 3).

Table 2. Growth of An. azollae (cel/l).

| Time   | Intensity 0% | Intensity 30% | Intensity 30% | Intensity 70% | Intensity 100% |
|--------|--------------|---------------|---------------|---------------|----------------|
| Day 0  | 5.250        | 5.334         | 5.334         | 5.397         | 6.356          |
| Day 7  | 728          | 1.029         | 1.029         | 2.366         | 5.376          |
| Day 14 | -            | 288           | 288           | 1.506         | 1.086          |
| Day 21 | -            | 156           | 156           | 348           | 588            |

Figure 3. Growth of An. azollae

Growth patterns An. azollae which decrease over time can be understood. It is suspected that the decrease is related to nutrient content in the cultivating container. Similar data have been reported by some researchers [5], [8], and [9]. Water quality can also affect the growth of An. azollae where a decrease in acidity (pH) can drastically increase organic matter and reduce oxygen levels [10]. One of the main causes of decreasing water quality is an imbalance between bacteria and An. azollae. Temperature also affects the growth of An. azollae, the lower the incoming light, the lower the growth rate where the optimum temperature for growth of An. azollae is around 20-35°C. However, in this research the water temperature ranged from 32 - 35°C, pH 7-8 and salinity approximately 3 pp. Such water quality is considered as an ideal condition to A. microphylla [6].

4. Conclusions
Based on the results of the above research it can be concluded that the sunlight intensity significantly influences the growth of A. microphylla and its symbionts An. azollae. The best sunlight intensity was 70% intensity. A dynamic growth recorded during the first week post inoculation in which doubling time took place. It is recommended to grow A. microphylla and its symbionts An. azollae under 70% of the sunlight intensity.
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