Using of Fermentation Liquid from Mangrove Leaves "*Avicennia Marina*" in Combination of Temperature on Seaweed "*Kappaphycus Alvarezii*" in Controlled Media

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Abstract. The fermented liquid derived from mangrove leaves contains secondary metabolites in the form of lactic acid, bacteriocins, phytochemical compounds, and has primary metabolites in the form of macro and micro nutrients, as well as potential endophytic bacteria. This study aims to determine the performance of *A. marina* mangrove leaves ferment liquid combined with temperature on the growth and control of *K. alvarezii* ice-ice disease. The research method was that seaweed weighing 50-51g was soaked in fermented liquid for one hour, and cultured in a 33x23x19cm aquarium. Parameters evaluated included morphology, histopathology, and growth of seaweed. The results showed that the use of fermented liquid at maintenance temperatures of 27˚C and 30˚C did not show any Thallus bleaching. A temperature of 35˚C in the second week indicates bleaching. The condition of the seaweed Thallus tissue at 27˚C and 30˚C was still normal, at 35˚C the tissue was damaged and the Thallus weight decreased. The research results are very useful for seaweed farmers because they can increase growth and are able to control ice-ice disease caused by abiotic factors such as temperature.

Keywords: Avicennia marina, fermented liquids, endophytes, ice-ice, seaweed.

1 Introduction

Seaweed is currently Indonesia's mainstay commodity and has a high demand in the world market [1]. One of the seaweed commodities developed in cultivation is Kappaphycus alvaerezii. However, until now seaweed production has experienced problems, namely the emergence of ice-ice disease and decreased seaweed production [2]–[5]. This disease appears caused by abiotic factors such as temperature, salinity, and light intensity [6], and biotic factors including fungi [7] and bacteria, especially opportunistic bacteria [8].

Efforts to increase production and disease control have been carried out including the use of NPK fertilizers [9], the use of lysozyme enzymes [10], the superoxide dismutase enzymes [11], the use of local microorganisms from maja fruit [12], but the methods used have not shown maximum results. One strategy to increase production

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and control ice-ice disease is to use of fermentation liquid from mangrove leaves Avicennia marina.

The fermentation liquid from mangrove leaves A. marina is currently able to increase the growth of seaweed [12], [13] and is able to control ice-ice disease [2]–[4]. But it has not been tested against extreme environmental conditions such as increasing temperature. Based on this, it is necessary to use fermentation liquid from mangrove leaves A. marina with a combination of temperature on K. alvarezii seaweed in controlled media. This study aims to determine the performance of fermentation liquid from mangrove leaves A. marina combined with temperature on the growth of K. alvarezii seaweed. The use of this research is expected to be information material for farmers in increasing the production of K. alvarezii seaweed

2 Method

2.1 Time and location

The research was carried out from June to September 2022. Making fermented liquid, rearing and observing seaweed was carried out at the Integrated Laboratory of the Aquaculture Study Program, Faculty of Fisheries, University of Muhammadiyah Luwuk, and histology was carried out at the Fish Health Laboratory, Department of Aquaculture (BDP), Faculty Fisheries and Maritime Affairs (FPIK), IPB University Institute (IPB).

2.2 Test Seaweed and Fermentation Liquid

The test organism used was K. alvarezii seaweed with a size of 50 grams for each experiment. This seaweed was obtained from cultivators in Jaya Bakti Village, Pagimana District, Banggai Regency, Central Sulawesi Province. Making fermented liquid using the main raw material from mangrove leaves A. marina, the method for making fermented liquid using method [2], [4], [12], [13].

2.3 Experimental design

This study used a completely randomized design (CRD) consisting of four treatments and three replications, which can be seen in Table 1.

Table 1. Experimental design

Treatment Code	Water temperature (°C)	Combination with fermented liquid
M1	27	5 mL/L
M2	30	5 mL/L
M3	35	5 mL/L
K	26-28	-

2.4 Performance of fermentation liquid from mangrove leaves *A. marina*

The tested seaweed was adapted to the rearing medium for 3 days, then the seaweed weighing 50 grams was soaked in fermented liquid at a dose of 5 ml/liter for 30 minutes, then put in an aquarium with the size of each unit 33x23x19 cm, the seaweed was put in an aquarium based on respective treatment. Morphological and water quality observations were carried out every day, weight measurements every week.

2.5 Observed Parameters

Parameters observed included daily growth rate, absolute growth and water quality.

2.6 Daily Growth Rate

To determine the daily growth rate, the formula is used [14]:

$$DGR\ (\%) = \ln (Wf/W_0)/t \times 100$$

Description:
DGR = Daily growth rate (%)
Wf = The average weight of the tested seaweed at the end of the experiment (g)
W_0 = The average weight of the tested seaweed at the start of the experiment (g)
t = Length of treatment (days)

2.5.2 Absolute Weight Growth (W)

The absolute weight growth of seaweed is calculated using the formula [15]:

$$G = Wt - Wn$$

Description:
G = Absolute growth of test seaweed (g)
Wt = The average weight of the tested seaweed at the end of the experiment (g)
Wn = The average weight of the tested seaweed at the start of the experiment (g)

2.7 Thallus Morphology

Seaweed thallus morphology was observed every day, this observation was made visually, then observed by taking photos of the thallus morphology at the same distance [16].

2.8 Seaweed Histology

The histological process includes the preparation of seaweed by fixation of seaweed tissue using buffered neutral formalin (BNF) and dehydrating using graded alcohol solutions, namely 70, 80, 90, 95 and 100%. Furthermore, the sliced seaweed Thallus was clarified using xylol solution and impregnated in liquid paraffin to make solid blocks. The paraffin block containing the tissue was cut with a 6 μm microtome. The preparations were stained with hematoxylin and eosin. Furthermore, observations were made under a microscope with a magnification of 10 times [8].

2.9 Water quality

Water quality data includes measurements of temperature, salinity, pH, oxygen. Measurements of salinity, pH, oxygen and temperature were carried out every day, namely in the morning and evening.

2.10 Data analysis

To find out whether the fermented liquid combined with different temperatures has an effect on seaweed growth, an ANOVA test is carried out and if it is significantly different (significant) then it is continued with the Tukey test. While the morphological data of seaweed Thallus and histology were analyzed descriptively.

3 Result and Discussion

3.1 Specific Growth Rate

Daily specific growth rate and average weight of K. alvarezii during rearing can be seen in Fig. 1 and Fig. 2. The highest daily specific growth rate was obtained by treatment M1 (0.68 ± 0.01), followed by control treatment (0.43 ± 0.1), treatment M2 (0.50 ± 0.01), treatment M3 (0. 34 ± 0.1) (Fig. 1). The M1 treatment showed the highest average weight in the first week (4.04%) while the lowest was in the M3 treatment (3.92%). Then in the second week the M1 treatment increased by an average (4.06%) and the M3 treatment showed an average (3.98%). Then in the third week the M1 treatment experienced an increase with an average (4.07%) then in the M3 treatment it showed an average (3.99%). In the fourth week, the M1 treatment increased by an average (4.10%) and the M3 treatment showed an average (4.01%) (Fig. 2).

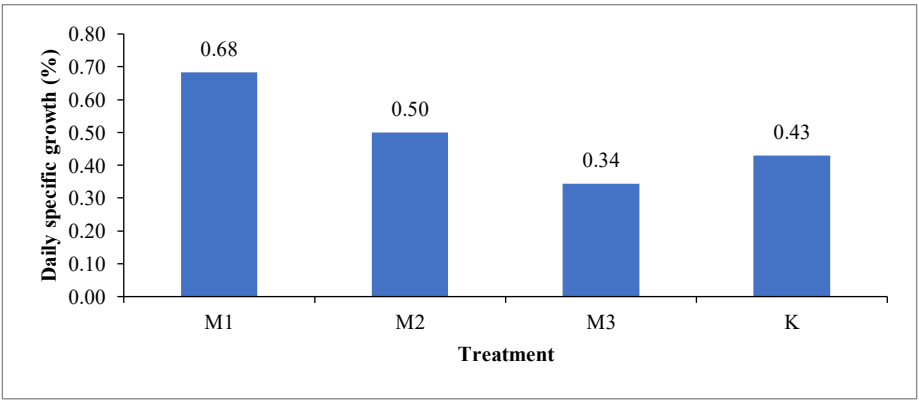

Fig. 1. Daily specific growth rate of seaweed

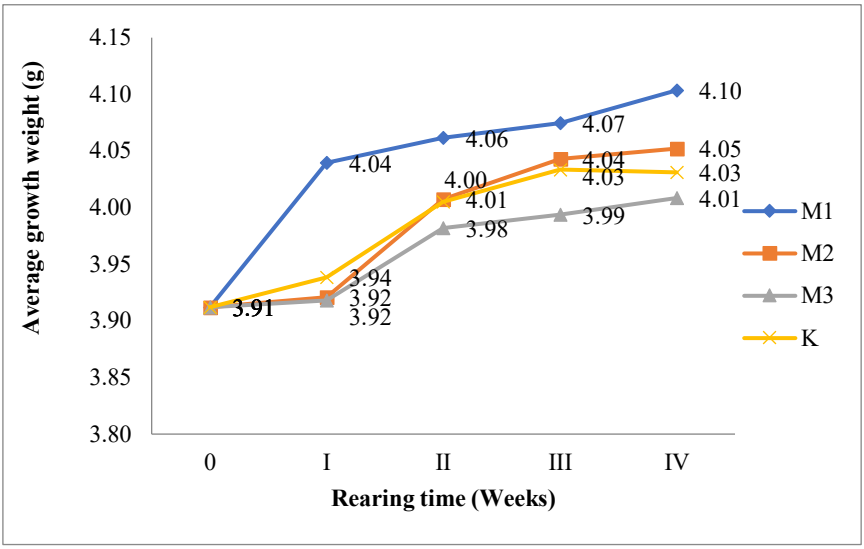

Fig. 2. average growth weight of seaweed

The high growth of seaweed in the early weeks shows that during this period, seaweed concentrated energy in absorbing and accumulating nutrients from the fermented liquid for its growth needs, especially at normal temperature (27°C) in the M1 treatment, although it was significantly different from M2, M3, and control, but did not affect its growth at the 4th week of age (28 days old), however, bleaching of the seaweed occurred in the M3 treatment, allegedly unable to control the effects of extreme temperatures (35°C) which was accompanied by a decrease in seaweed weight caused by temperature stress. prolonged extreme conditions, although the extreme temperature conditions at the beginning of rearing seaweed can adapt slowly. Seaweed enriched with nutrients and reared at 35°C resulted in slow growth and experienced overall talus bleaching at 28 days of age. [17] reported that high temperature affected the phytochemical content and antioxidant activity of seaweed. Nutrient enrichment proved to be efficient in increasing seaweed growth at extreme temperatures even though it only lasted 28 days of rearing.

3.2 Absolute Weight Growth

The average absolute growth rate of seaweed during rearing can be seen in Fig. 3. The highest average absolute weight growth results were obtained by treatment M1 (10.55 ± 0.06 g), then followed by control treatment (9.33 ± 0.13) treatment M2 (7.52 ± 0.10), treatment M3 (5.06 ± 0.10 g). The growth height of seaweed in the M1 treatment is suspected that the seaweed is in a normal environment (good temperature) and is able to absorb nutrients in the fermented liquid which contains nutrients for seaweed growth. Whereas the lowest growth was shown by the M3 treatment because under these conditions the seaweed was under prolonged extreme temperature pressure which resulted in stress for the seaweed and a decrease in growth. The increase in seaweed weight was

due to the intervention of endophytic compounds and bacteria present in the fermented liquid of mangrove leaves. Mangrove leaves fermented liquid contains endophytic bacteria that can associate with seaweed, secondary metabolites that are able to inhibit pathogens, while primary metabolite compounds in the form of macro and micro nutrients from the fermented liquid can increase the growth of seaweed [13], Furthermore [18] that the community bacteria associated with seaweed provide benefits by producing growth-promoting substances and seaweed morphology.

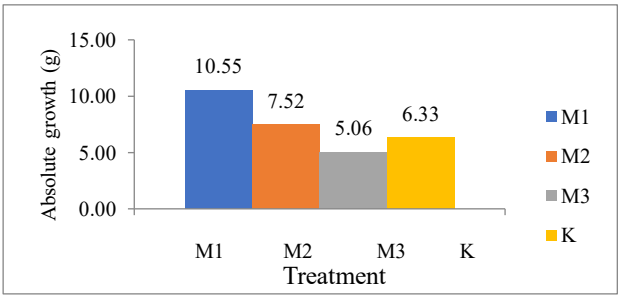

Fig. 3. Absolute weight growth of K. alvarezi seaweed during the research

3.3 Seaweed Thallus Morphology

Based on the picture above, it shows that the treatment of M1, M2, and controls in the first week to the last week of maintenance shows that the talus has changed in size to become bigger. Furthermore, in the M3 treatment it showed that in the first week the talus was still visible and the branches on the seaweed still looked normal but in the third week the color began to change accompanied by a decrease in weight, then it becomes pale reddish and in the fourth week it experiences bleaching or ice-ice disease which starts at the edge of the talus (young) and then goes to the base of the talus (old) and the talus begins to fall out (Fig. 4).

Changes in the morphology of seaweed are caused by ice-ice disease which is initiated by prolonged extreme temperatures and lack of nutrients which triggers the emergence of pathogenic bacteria. [19] stated that the occurrence of infectious diseases in seaweed is caused by an imbalance between the host, pathogen, and the seaweed media environment. According to [20] that unfavorable environmental conditions can reduce the resistance of seaweed. The temperature of 35°C seemed to be tolerated but only lasted a few days due to the high temperature and the lack of nutrients that the seaweed could not absorb properly resulting in a color change from brown to pink, then turned white and died. The morphology of seaweed changes due to environmental factors, namely temperature and lack of nutrients absorbed. The lack of nutrient distribution affects thallus metabolism. Thus, this condition reduces the growth of seaweed which affects the susceptibility to ice-ice disease [21]. The spread of ice-ice disease in seaweed causes discoloration and weight loss [22].

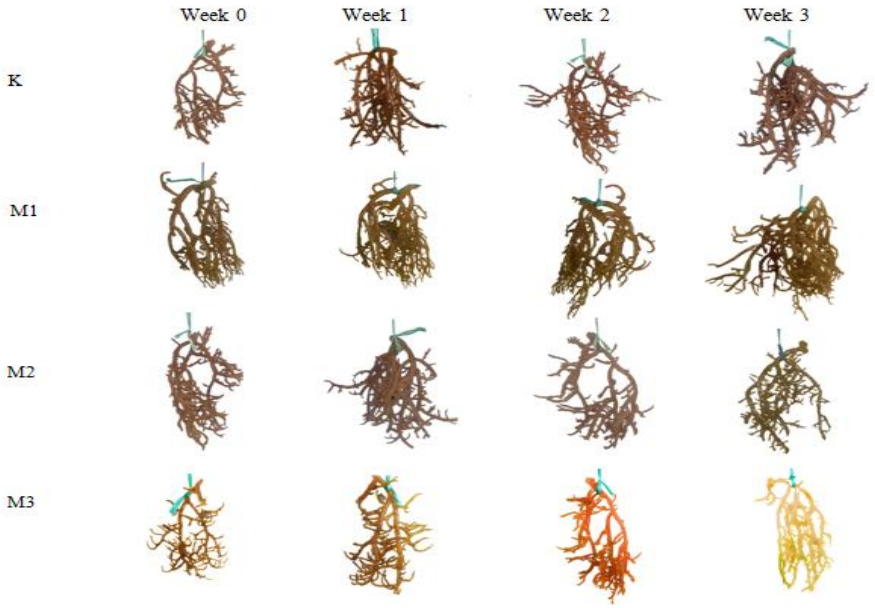

Fig. 4. Morphology of seaweed Thallus in each treatment

3.4 Seaweed Thallus Histology

Seaweed tissue in control treatment (K) without immersion in fermented liquid and treatment (M1) with soaking in fermented liquid combined with normal temperature did not show damaged tissue, but in treatment (M3) which was immersion in fermented liquid and combined with Extreme temperatures lead to tissue damage (Fig. 5).

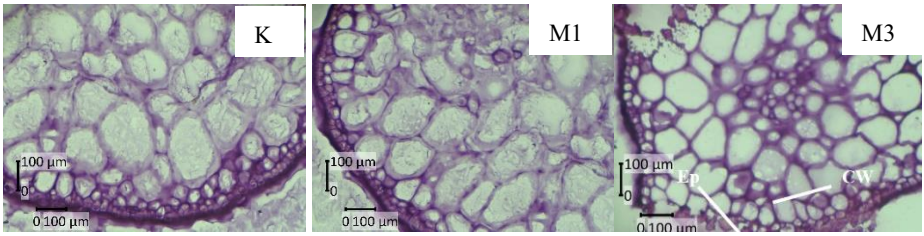

Fig. 5. Histology of seaweed Thallus. Normal seaweed Thallus tissue (K, M1), damaged seaweed tissue (lysis) (M3). The protoplasm (Ep) in the Thallus tissue that has undergone bleaching begins to disappear and there is a space between cells (CW). H&E stain, 10x magnification)

Seaweed thallus tissue in treatment K and treatment M1 was still under normal temperature pressure, so there was no effect caused by the given temperature combination. This can be seen in the condition of the talus tissue neatly arranged and tightly packed. Different things were shown by the M3 treatment, the combination of temperatures

given was outside the normal range for seaweed growth, so that the seaweed talus was damaged, the condition of the talus tissue was open and not neatly arranged. This condition initially occurred due to prolonged extreme temperature pressure. Increasing temperature triggers pressure on the seaweed [23], thus changing the physiology of the seaweed and weakening the external structure, resulting in the emergence of disease [24]. The occurrence of damage to the thallus undergoes a process of change in morphology, namely the color changes from brown to pink and then turns white and even causes destruction accompanied by mucus in the seaweed talus. Tissue damage is indicated by damage or loss of epidermal cells [25] and open spaces between cells so that they easily cause lysis to change tissue structure [26]. Damaged seaweed cell walls are caused by the degradation of carrageenan and cellulose and even lost by pathogenic bacteria [27] even good bacteria will become pathogens when environmental conditions are not normal [6] and cause an increase in pathogenic bacteria which are influenced by unfavorable environmental conditions, especially temperature, salinity, and nutrients [23].

3.5 Water Quality Parameters

The water temperature in all treatments was maintained at the desired temperature by using a heater. The temperature in the control treatment ranged from 27-28°C, the desired M1 was 27°C, and M2 was 30°C. The temperature range is still within the proper range for the growth of K. alvarezii seaweed. This is in accordance with [28] that K. alvarezii still develops well at temperatures ranging from 27-30°C. Furthermore, the M3 treatment showed a temperature of 35°C. According to [6] that the temperature of 33-35°C is an extreme temperature for seaweed, causing the seaweed to turn pale and even die.

Salinity measured in the control treatment was 25-30 ppt, M1 showed a range of 25-35 ppt, M2 treatment ranged from 30-55 ppt, while M3 treatment ranged from 30-65 ppt. [29] stated that the optimal salinity of the waters for cultivating K. alvarezii ranged from 22-33 ppt. [30] Seaweed can survive at salinities of 35 ppt and 55 ppt, decomposes within three days, and gradually turns white and becomes soft. The pH range measured during the study in the M1 treatment showed a range of 5.81-7.24, the M2 treatment showed a range of 6.11-7.66, the M3 treatment was 7.35-8.25 while the control 6.47-7.18. According to [31] that the optimal pH range that is good for the growth of K. alvarezii is in the range of 7-9.

4 Conclusion

Based on the research results, it can be concluded that the use of fermented liquid with a combination of temperatures has a significantly different effect on the growth of K. avarezii seaweed. The highest daily specific growth rate was in the M1 treatment (0.68%), while the highest absolute growth was in the M1 treatment (10.55 g). Water quality parameter, namely temperature affects other water quality parameters.

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