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ANALYSIS OF THE CYSTOCARP DEVELOPMENT OF SEAWEED (*Kappaphycus alvarezii*) ON MEDIA ENRICHED WITH A COMBINATION OF NITROGEN AND PHOSPHATE

Muhammad Sahir*, Rustam, Gunarto Latama, Isma Riskiani

Water Resources Management Study Program Agricultural Technology Institute, Campagaya Campus Tamasaju Village, North Galesong District, Takalar Regency, 92255.

*Corresponding author: muhammadsahir@itp-takalar.ac.id

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ABSTRACT

Seaweed (Kappaphycus alvarezii), in terms of providing seeds in cultivation activities, is still carried out vegetatively, namely cutting the thallus (cuttings), which is then cultivated until it is ready to be harvested (Rao & Reddy, 1997). However, such a method will experience problems, especially the provision of quality seeds on a large scale, and it does not depend on the season. The possible alternative to do is by generative seeding method. K. alvarezii can be developed for cultivation by utilizing the nature of generative reproduction through the development of carpospores, characterised by cystocarps on the surface of the thallus. However, the cystocarp that has been formed needs nutrients in the development process to produce spores of good quality and quantity. Nutrients that are needed to support cystocarp development include nitrogen and phosphate. The benefits of nitrogen and phosphate for seaweed growth cannot be replaced with other elements. This is due to the role of nitrogen as a constituent of protein and phosphate as a provider of energy. This study aimed to determine the optimum ratio of nitrogen and phosphate enrichment to cystocarp development. The research was carried out from November 2018 to February 2019 at the Seaweed Laboratory of the Takalar Brackish Aquaculture Fisheries Center (BPBAPT). The research location is Mappakalompo Village, Galesong District, Takalar Regency, South Sulawesi Province. They were analyzed at the water quality laboratory of Hasanuddin University to analyse the N and P content in the media. There were 6 treatments with 3 replications each, namely Treatment A: Without enrichment (SW), Treatment B: 1N=0.5 ppm: 1P=0.5 ppm, Treatment C: 2N=1 ppm: 1P= 0,5 ppm, Treatment D : 3N=1,5 ppm : 1P=0,5 ppm, Treatment E : 1N=0,5 ppm: 2P = 1 ppm, and Treatment F : 1N=0,5 ppm : 3P = 1.5 ppm. The results showed that the development of a shorter cystocarp phase was found in treatment D, which was 14 days. Meanwhile, treatment with a long time of 17 days occurred in treatment A (SW).

Keywords: Cystocarp, Kappaphycus alvarezii, Nitrogen, Phosphate.

INTRODUCTION

Seaweed (Kappaphycus alvarezii) is a polycellular macroalgae that lives in the ocean (Yunizal, 2004). Seaweed belongs to the Thallophyta group because it does not have true roots, stems and leaves. All parts of the plant are called thallus, so seaweed is classified as a low-level plant (Susanto dan Mucktianty, 2002). Seaweed is one of Indonesia's water commodities that has the potential to be developed because the demand for seaweed is

increasing both in the local market and in the international market (Rajagukguk, 2009).

High demand is an opportunity for seaweed cultivators to participate in increasing production to meet market needs. Intensive cultivation is not without obstacles, the availability of quality seeds, in the right quantity and on time is the most common obstacle experienced by cultivators. According to Rao and Reddy, (1997), So far, the provision of K. alvarezii seeds in cultivation in Indonesia has been carried out vegetatively, namely cutting the thallus (cuttings) which are then cultivated until they are ready to be harvested. However, such a method will experience problems, especially in the provision of seeds on a large scale. Another solution that can be done is the method of providing generative seeds. K. alvarezii can be developed for cultivation by utilizing the nature of generative reproduction through the development of carpospores which are characterized by the presence of cystocarps on the surface of the thallus. (Rini Pramesti dan Nirwani, 2007).

Seaweed (K. Alvarezii) as an algae that lives in waters, apart from being influenced by environmental factors, also requires several important nutrients in the optimal concentration for optimizing the growth. To meet the nutritional needs of K. alvarezii, additional nutrients can be added to support the development of the cystocarp to release spores. K. alvarezii in its growth really needs Nitrogen and Phosphate. The benefits of nitrogen and phosphate for seaweed growth cannot be replaced with other elements. This is due to the role of nitrogen as a constituent of protein and phosphate as a energy source (Lakitan, 2010).

These two elements are very limited in number and are said to be limiting factors. (Yunus, et al, 2010). This is what underlies the need for research on nutrient enrichment in appropriate and balanced amounts. The balance of nutrients (N and P) in the right amount is expected to have a positive influence on the development of the cystocarp of K. alvarezii.

Based on this background, this research was designed with the aim of determining the optimum ratio of nitrogen and phosphate enrichment for cystocarp development. The results of this study are expected to be useful as a source of information about the optimum ratio of N and P doses in the development process of Seaweed (K. alvarezii) cystocarp until it can release spores.

MATERIAL AND METHOD

Time and place

This research was conducted from November 2018 to February 2019, at the Seaweed Laboratory of the Takalar Brackish Water Aquaculture Center (BPBAPT). The research location is in Mappakalompo Village, Galesong District, Takalar Regency, South Sulawesi

Province. For the analysis of the N and P content in the media, they were analyzed at the water quality laboratory of Hasanuddin University.

Material Data

The tools used during the study are presented in table 1

Table 1. Tools and uses

No	Tool's name	Utility
1	Microscope	To observe the research sample
2	Hot plate	Heating stock media
3	Magnetic stirrer	Stirrer when heating
4	String of raffia	Spore substrate
5	Bottle capacity 100 ml	Research container
6	Erlenmeyer 500 ml	The container for washing the talus pieces
7	Fluorescent lamp (TL)	Light source
8	Bucket	Holds water
9	Razor blade	Cutting the thallus
10	Tweezers	Clamping the thallus when cutting
11	Micrometer pipette 1 ml	Taking test solution
12	100 ml measuring cup	Medium water meter
13	Analytical balance	Weighing the materials used
14	Lux Meter	Measuring light intensity
15	Handrefracto meter	Measuring salinity
16	Spektofotometer	Measuring nitrate and phosphate
17	Autoclove	Heating/sterilizing research materials
18	Timer	Setting the time for the lights to turn on
19	Rubber bracelet	Tie the plastic cover on the bottle container

The materials used are presented in table 2.

Table 2. Materials and their uses

No	Tool's name	Utility
1	Talus K. alvarezii (Sistokarp)	Test Organisms/Algae
2	Seawater 31 ppt	Water Research Media
3	N (NaH_2PO_4)	Nitrogen source material
4	P ($NaNO_3$)	Phosphate source material
5	Iodine	antiseptic
6	Aquabides	Media for making stock solutions
7	Alcohol	Research instrument sterilization
8	Tissue	Cleaning research tools
9	Freshwater	Tool washing and salinity dilution
10	Filter paper	Filter media water
11	Label	Labeling research containers

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Preparation of Research Containers and Tools

The experimental container used was a glass bottle with a 100 ml volume. The container is then cleaned using a tissue that has been given alcohol and sterilized by autoclaove at 121 ^{o}C for 1 hour. The container is then filled with 80 ml of medium water. Each container is inserted 1 piece of thallus that already has a cystocarp. Each container is affixed with a label according to treatment, and arranged according to the results of randomization and placed in a room with a temperature of ±30^{o}C.

Material Preparation

Seaweed thallus that has a cystocarp is cleaned beforehand of dirt and attached animals using flowing sea water. Furthermore, cut along 3 cm with an average diameter of 1.5 cm. The results of the pieces are then cleaned with sea water by spraying. To avoid contamination with micro-organisms, washing was carried out using 100 ml seawater which was given 1% iodine for 3 minutes by shaking in a glass container. The next step is to rinse the pieces again with sterile seawater 3 times. Each piece of seaweed is then put in a container that has been filled with treatment media at random. The salinity of the media used in this study was 31 ppt (Harwinda., et al, 2017). Each container is then covered with plastic to avoid contamination and reduce evaporation so that the salinity is relatively constant.

Production of Maintenance Media

The process of making N and P solution with a concentration of 1000 ppm each. Determination of stock dosage is then determined using the formula:

$$\text{ppm} = \frac{\text{Weight of solute}}{\text{Solution Weight}} \text{ x } 1.000.000$$

The water used at the stage of making the stock solution is using aquabidest. Each stock solution was made 1000 ml each. The element N (NaH2PO4) is used as much as 1 mg then put into the prepared aquabides. The stock media was heated using a hot plate stirred during the heating process. The heating process is carried out for ± 10 minutes (until the solution boils). The same method was done in the manufacture of element P (NaNO3). The stock solution was diluted according to the dose for each treatment. The desired dose is determined using the dilution formula:

$$M1 \text{ X } V1 = M2 \text{ X } V2$$

Is known:

M1 = Initial concentration of the substance

V1 = Initial volume

M2 = Concentration after dilution

V2 = Volume after dilution

The media were made with 3 concentrations of each, namely 0.5 ppm, 1 ppm,

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and 1.5 ppm for each element used (N and P). Seawater with a salinity of 31 ppt before use was sterilized using an autoclove. The water is then used to make a media solution of 1000 ml or 1 liter for each treatment. After all the doses are made, the next step is to combine the solution of N and P elements according to the treatment that has been determined.

Experimental design

Determination of the treatment dose in the study based on the need for Nitrogen in the form of Ammonium, Nitrate, and Phosphate for seaweed. The lowest nitrate range for algae growth is 0.3-0.9 mg/l, while for optimal growth is 0.9-3.5 mg/l (Sulistijo, 1996). According to Boyd (1990), the lowest nitrate tolerance limit for algae growth is 0.1 ppm, while the highest limit is 1 ppm. Hartomo & Widiatmoko (1994) stated that the appropriate ammonium level for seaweed growth was 0.5 ppm. According to Gusriana (2006), the acceptable phosphate range for seaweed growth is 0.9–1.8 ppm, while according to Effendi (2003), the good phosphate range is 0.02-1 ppm. The treatments used were 6 with 3 replications each:

Treatment A : No enrichment (SW)
Treatment B : 1N=0,5 ppm : 1P=0,5 ppm
Treatment C : 2N=1 ppm: 1P=0,5 ppm
Treatment D : 3N=1,5 ppm : 1P=0,5 ppm
Treatment E : 1N=0,5 ppm: 2P = 1 ppm
Treatment F : 1N=0,5 ppm : 3P = 1,5ppm.

Maintenance Stage

The thallus maintenance stage was carried out in a closed and controlled room at a temperature of 30 ^{o}C. The salinity used in the media is 31 ppt (Hariyati, 2014).

The lighting source uses 18watt fluorescent lamps with a light intensity of ±1000 lux (Syamsuddin, 2013). According to Prihatman (2000), fluorescent lamps produce more light with lower power than incandescent lamps. The white light produced is used by plants for photosynthesis with an intensity of 1000-3000 lux. The lighting settings (dark and light) are adjusted to the conditions in nature: 12 hours of light and 12 hours of darkness, using a timer. Media water changes are carried out every 7 days or when a change is required.

Cystocarp Observation

Observation of cystocarp was carried out every day by observing through a microscope with a magnification of 40x on cystocarp that had been marked previously. The marking aims to facilitate the process of observing the development of the same cystocarp. The observation process also uses a 13 MP camera specification, to show different changes in shape each treatment.

Data analysis

The process of cystocarp development was analyzed descriptively by looking at the time differences in morphological development.

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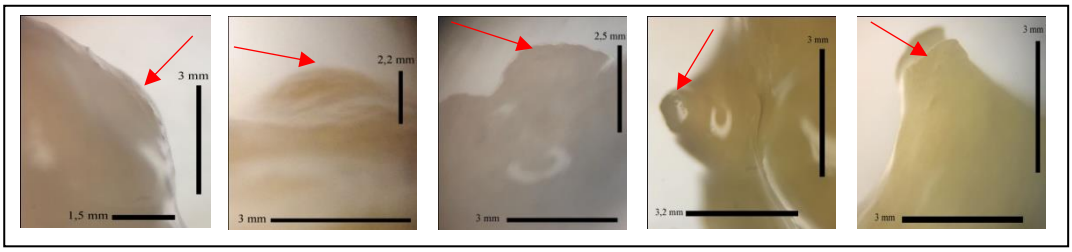
RESULTS AND DISCUSSION

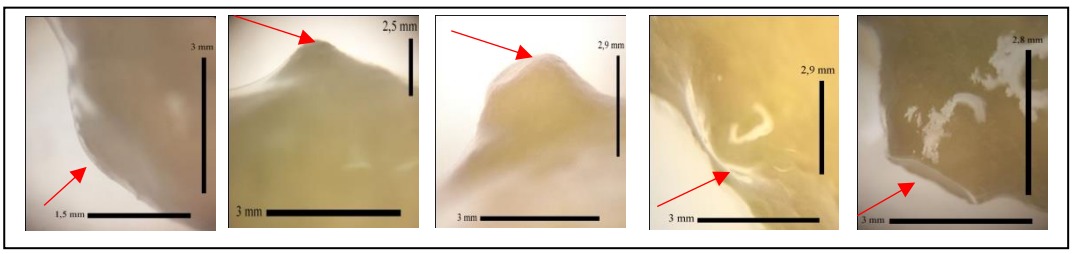
The results showed that the cystocarp underwent morphological changes during the study. Its shape continues to change from day to day observation. The development process is seen in the morphological changes of the cystocarp.

The process of cystocarp changes based on observation day for each treatment was presented in Fig 1.

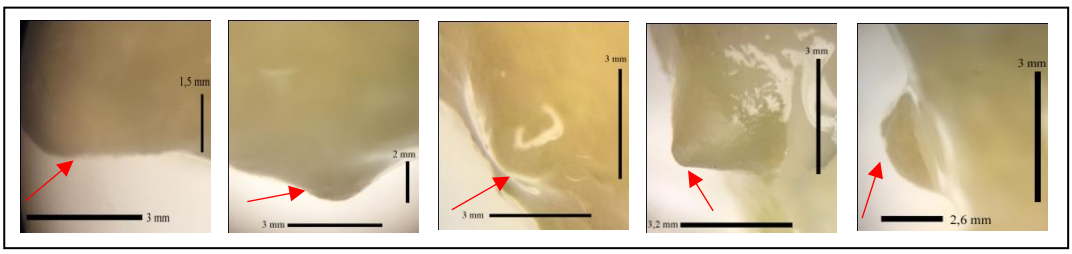
A. No Enrichment (SW)



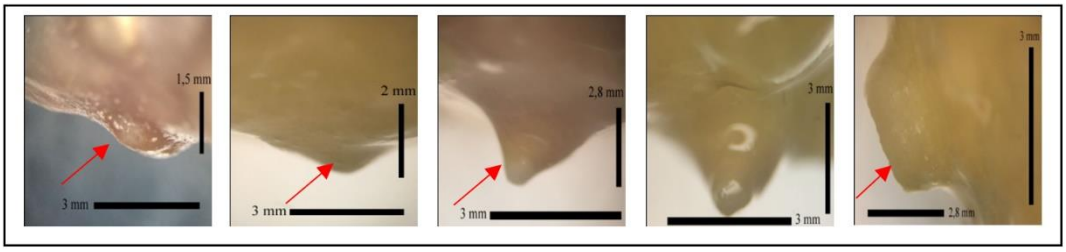
B. Dose 1N (0,5 ppm) : 1P (0,5 ppm)



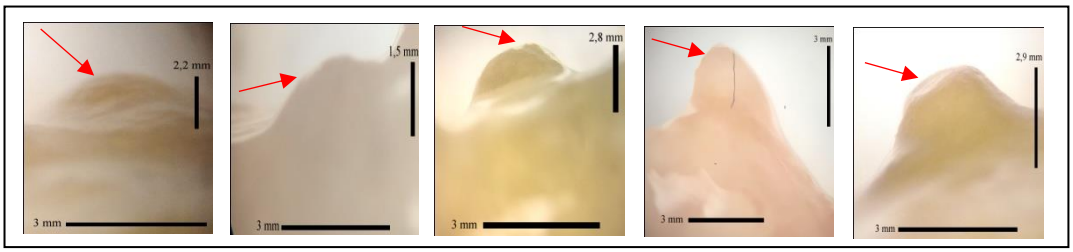
C. Dose 2N (1ppm) : 1P (0,5 ppm)



D. Dose 3N (1,5 ppm) : 1P (0,5 ppm)



E. Dose 1N (0.5 ppm) : 2P (1 ppm)



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F. Dose 1N (0,5 ppm) : 3P (1,5 ppm)

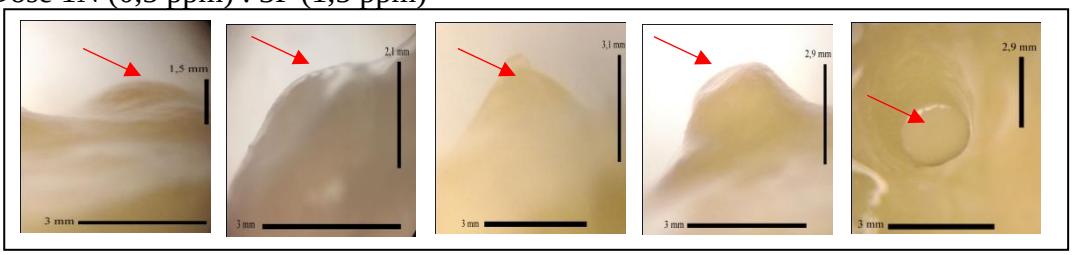

Figure 1. The process of cystocarp development in each treatment (A – F).

Based on Figure 1, it can be seen that there is a change in the shape of each phase of cystocarp development. At the beginning of the study, the cystocarp had a diameter of 3 mm and a height of 1.5 mm, then progressed every day of observation. The greater the diameter produced, indicating the cystocarp is growing and ready for the spore release stage. Observation results obtained data that the development of cystocarp, which takes a shorter time, is found in treatment D. The average growth of cystocarp based on the time it takes is also presented in Table 3.

Table 3. Average time (days) of observing cystocarp development from each treatment.

Treatment	Average days of cystocarp development
A	17±1,00[a]
B	16±1,00[a]
C	15±0,00[a]
D	14±1,00[a]
E	15±1,00[a]
F	15±1,00[a]

Note: The treatment had no significant effect (p>0.05) on the development time of the Cyctocarp *K. alvarezii* phase.

Table 3 shows that the average time required for each treatment varied, with a shorter cystocarp phase development time in treatment D, which was 14 days. Meanwhile, treatment with a long time of 17 days occurred in treatment A (SW). The higher N content compared to other therapies was thought to be tolerated and utilized by thallus. The dose ratio is one of the factors that can accelerate the development and maturity of the cystocarp. This result follows the statement of Wibowo., et al., (2009) that the appropriate ratio in waters is between N and P elements, where the N element is three times greater than the P element. Nitrogen is a macro element that is useful for stimulating the growth of a plant so that it can increase. N deficiency will inhibit the growth of seaweed because it is an element used in the photosynthesis process (Wibowo et al., 2009). Nitrogen and phosphate are essential

for seaweed in regulating metabolism and reproduction. Growth can be adequately achieved if the seaweed is supplied with nitrogen and phosphate. Seaweed can utilize nitrogen and phosphate through a diffusion process in all parts of its body (Djafar, 2011). The more often the seaweed absorbs nitrogen and phosphate in the maintenance medium, the faster the growth and maturation of the cystocarp increases. Nitrogen and phosphate are essential for seaweed in regulating metabolism and reproduction. Growth can be adequately achieved if the seaweed is supplied with nitrogen and phosphate.

CONCLUSIONS

Based on the research results, it can be concluded that treatment with a combination of N and P on cystocarp development occurred faster in treatment D with an average time of 14 days.

REFERENCES

Bayraktar, Hanefi, Sezer, Turalioglu 2005 A Boyd, C.E. 1990. Water Quality in Ponds for Aqua-culture. Birmingham Publishing Co. Birming-ham, Alabama.

Djafar, F. 2011. Study of nitrogen and phosphate retention of seaweed (Kappaphycus alvarezii) at various water flow velocities. Thesis. Postgraduate Program in Aquatic Sciences, Bogor Agricultural University, Pages 41 - 43. Bogor, West Java.

Effendi, H. 2003. Study of Water Quality for Management of Aquatic Resources and Environment. Canisius. Yogyakarta.

Gusriana. 2006. Seaweed Cultivation. Synergy Library, Bandung.

Hariyati, R. 2014. The Effect of Salinity Differences on the Number of Spores of Gracilaria sp. Department of Biology, Faculty of Science and Mathematics, Diponegoro University. Proceedings of Semnas Entrepreneurship Page: 248-254.

Hartomo & Widiatmoko. 1994. Membrane Technology. Andi Offset, Yogyakarta.

Harwinda, F K, Satyantini W H, and Masithah E W. 2017. The effects of salinity and temperature shock on Kappaphycus alvarezii seaweed spores release. Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine Airlangga University, Surabaya 60115.

Lakitan B. 2010. Fundamentals of Plant Physiology. King Grafindo Persada. Jakarta.

Prihatman, K. 2000. Light Manipulation to Stimulate the Growth of Aquatic Plants.

Office of the Deputy Minister of Research and Technology for the Utilization and Socialization of Science and Technology (BAPPENAS). Jakarta. 13 Pg.

Rajagukguk, Mark Magi. 2009. Analysis of Indonesian Seaweed Competitiveness in International Markets. [Thesis] Bogor: Faculty of Economics and Management, Bogor Agricultural University.

Rao, M.U. and Reddy B.S., 1997. Infuence of Dessication, Salinity and Temperature on the Liberation and Germination of Tetraspore of Dictyotadichotoma. Indian J. Marine Science 26 : 312 -314.

Rini Pramesti and Nirwani, 2007. Study of the Reproductive Organs of Gracilaria gigas Harvey in the Carposporophyte Phase. Journal of Marine Science Vol. 12 (2) : 93-96. Department of Marine Science, Faculty of Fisheries and Marine Sciences, Diponegoro University. Semarang.

Sulistijo. 1996. Development of Seaweed Cultivation in Indonesia. In: Introduction to the Types of Indonesian Seaweed. Oceanology Research and Development Center, Indonesian Institute of Sciences, Jakarta, pp. 45-50.

Susanto, A. B and Mucktiany. 2002. Seaweed Development Strategy in Vocational High School and Community Callege. Pros. National Marine Science and Technology Seminar.

Syamsuddin, R. 2013. Spore Discharge and Development, and Carrageenan Content Of Seaweed Kappaphycus alvarezii Illuminated With Different Light Colors. Faculty of Marine Sciences and Fisheries, Hasanuddin University. Makassar.

Wibowo, E K, Suryono, and Endah Setianingrum MR. 2009. Application of Differences in N, P and K Compositions in Cultivation of Eucheuma cottonii in Teluk Awur Waters, Jepara. Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Diponegoro University, Tembalang Campus, Semarang, Indonesia. Vol. 14(3):164-169.

Yunizal. 2004. Alginate Processing Technology. Marine and Fisheries Research Agency. Jakarta.

Yunus, Indah Wahyuni Abida, and Firman Farid Mushoni. 2010. Effect of Phosphate (TSP) on the Growth of Seaweed Echeuma cottonii. Department of Marine Sciences, Trunojoyo University, Bangkalan, Madura. Marine Journal, Volume 3, No 2.