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The effects of salinity and temperature shock on *Kappaphycus alvarezii* seaweed spores release

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Abstract. One of the reproductive aspects of development step that is considered as the solution of this issue is seaweed sporulation technique through which is induced through salinity and temperature shock. This study aims to determine the effect of combination and interaction of salinity and temperature shock on the release of *K. alvarezii* spores in order to produce superior seeds. This research was conducted using Complete Randomized Design Factorial which consists of nine combinations of treatments and three replications. The used treatment in this study is the combination of different environmental factors such as salinity shock and temperature shock. The data were analyzed using ANOVA (Analysis of Variance) followed by Duncan Multiple Range Test. The results showed that salinity (31 ppt, 33 ppt, and 35 ppt) and temperature (30°C, 32°C, and 34°C) shock affected the osmoregulation system and the release of *K. alvarezii* spores. The salinity shock and temperature shock had interaction with *K. alvarezii* spore release on the sixth and seventh day with the best treatment at 32°C temperature and 31 ppt salinity and released 5413 cells/ml spores on the seventh day.

1. Introduction

Seaweed as a fishery commodity that produces carrageenan has an important role in the industrial sector. It is much utilized in the food industry, cosmetics, pharmaceutical, and other industries such as the paper industry, textiles, photography, pasta, and fish canning [1]. The current market demand for *Kappaphycus alvarezii* has increased each year in line with the development of seaweed processing industry in Indonesia. The Ministry of Marine and Fisheries expects that the cultivation of seaweed and the processing industry sector can be developed to compete in the world market. [2] stated the efforts to increase the production of *K. alvarezii* require the availability of superior seaweed seed continuously.

Since 1970, the seed of *K. alvarezii* has always been obtained from vegetative reproduction. The repetitive clonal of vegetative development will result in a reduction in genetic variability, leading to the fall in the growth rate, levels of carrageenan, and gel strength [2]. Based on that reason, the development of reproductive aspects needs to be considered in order to increase productivity. One of the aspects of reproductive development step is the growing of seed through seaweed sporulation. In addition, [3] stated that the reproduction using spores is considered to be more efficient, fast-growing,



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and capable of producing superior seeds. It is also explained that there is a possibility of genetic engineering that could be done through spores reproduction [4]

However, reproduction by using spores depends on the season in which the release of spores is very rare. The reproductive cycle of *K. alvarezii* happens only once a year with the release of spore in August to September [5]. Therefore, research on the implementation of environmental manipulation in the provision of sustainable seaweed seeds throughout the year needs to be conducted.

It is explained that the release and germination of *K. alvarezii* seaweed spores are influenced by biotic and abiotic components [6]. Factors of the abiotic component are one of the factors that needs to be developed in the application. Some influential abiotic components in the reproduction of the seaweed are salinity, temperature, light, pH, and waves [7]. Temperature and salinity are important factors that determine the growth and reproduction of seaweed [2]. The rise or decline in the level of salinity could influence the turgor pressure that will induce seaweed to do an osmotic adjustment [8]. Meanwhile, temperatures have a fundamental effect on chemical reactions and metabolism level of seaweed. Both of these factors greatly affect the release of seaweed spores.

Thus, research on the effect of the application of salinity and temperature shock on spores release and germination is needed so that the optimal combination of temperature and salinity shock to produce *K. alvarezii* superior and sustainable seeds could be identified and understood.

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2. Methodology

2.1. Research time and place

This research was conducted from February to March 2017 at Seaweed Tissue Culture Laboratory, Marine Aquaculture Development Center Lombok.

2.2. Materials

The equipments used in this study were petridish, stopwatch, thermostat heater, aerator set, bottles with a diameter of 5 cm and height of 12 cm, an aquarium with a length of 60 cm, width of 30 cm and height of 30 cm, stereo microscopes, pipette, a beaker glass, hand counters, Sedgwick Rafter counting cell and thermometer. The research materials used were fresh water, sea water, *K. alvarezii* seaweed obtained from Lombok island.

2.3. Procedures

K. alvarezii seaweed was obtained from Gerupuk, Lombok. The first stage before doing the temperature and salinity shock towards *K. alvarezii* seaweed spores was the selection of seeds. Seaweed seedling selection was done to obtain eligible explant to be reproduced, cultivated and tissue cultured. *K. alvarezii* seaweed from nature were acclimatized in the aquarium for a week. Acclimatization in greenhouses was done by moving the seaweed on media containing sea water in aquarium.

The minimum size of the aquarium used was 90 cm x 30 cm x 35cm with a recirculating water system. A recirculation system was implemented using a multilevel aquarium. The arrangement of the aquarium water temperature allows the sea-floor to remain stable because the heat of the sun was covered by the aquariums placed above.

An increase of temperature of water in a beaker glass was done using a commercial thermostat to regulate the temperature [9]. Salinity shock treatment was done using three different salinity levels at each beaker glass. Certain salinity obtained by giving a certain appropriate weight of salts with the desired salinity. It is in accordance with Ask [10] that used this comparison:

$$\text{Salinity (ppt)} = \frac{\text{Salt Concentration (gr)}}{\text{Volume of Water (1 L)}} \quad (1)$$

Before the treatment was done in mini media (petridish), seaweed was washed with sea water, cleaned from other algae and attached dirt. Seaweed used as a sample in the shock process was the one

having sporophyte. The making of sterile seaweed explant was done by selecting the thallus and sterilizing the thallus. The sterilization process was conducted according to Harley [2] with some modifications. The steps are: (1) the young thallus was cut with the length of 5 cm using scalpel and tweezers on sterilized petridish, then rinsed with sterile sea water twice; (2) the thallus or explant were soaked for 8-10 minutes in a solution of liquid soap with a concentration of 2 drops per 200 ml, and then rinsed with sterile sea water 2 times; (3) explant were soaked for 2-3 minutes in 100 ml of seawater containing Povidone Iodine 1 % (10 ml of Betadine and 90 ml of sterile sea water), then rinsed two times with sterile seawater; (4) the explant were dried with paper towels and put into sterile media shock treatment temperature and salinity.

2.4. Environmental shock

The media used for the temperature and salinity shock treatment was sea water with different salinity levels and a certain temperature in a beaker glass. The salinity and temperature shock treatment were given with an increase in salinity i.e. 31 ppt, 33 and 35 ppt at different temperatures i.e. 30°C, 32°C, and 34°C.

The temperature and salinity shock treatment were done by putting the selected and cleaned thallus explant to beaker glasses, 6 explants for each beaker glass. Temperature and salinity shock was done for 30 minutes. After that, the seaweed was restored into a culture bottle of 100 ml with original water conditions on a rotary shaker for 7 days with a room temperature of 22-25°C and 1500 lux fluorescent light for 12 hours of light and 12 hours of dark.

Seaweed spores were counted using Sedgwick Rafter counting chamber [11]. The calculations were performed every day during the maintenance period after treatment to observe the number of spores released per day. The calculation was done based on the formula used by Bindu [12]:

$$\text{Cells/ml} = \frac{\text{Counted cells} \times 1000 \text{ mm}^3}{A \times D \times F} \quad (2)$$

Description:

A = Area of field

D = Depth of Sedgwick Counting Cell

F = Counted fields

2.5. Data analysis

The data of released spores yield obtained from the results of this research were analyzed using ANOVA (Analysis of Variance) to identify whether there was a difference of each treatment, according to the research experimental design used. The analysis of multiple distance trials was followed by Duncan with the SPSS program. Further tests using Test Duncan were needed to compare the treatments.

3. Result and Discussion

The results of the variant analysis show that there is no significant difference ($p > 0.05$) on the interaction of temperature and salinity shock treatment on the first day until the second day, while the third to seven days shows a significant difference ($p < 0.05$). The result of the *K. alvarezii* spores release is shown in table 1.

K. alvarezii spores had been released gradually every day. Seaweed thallus used as samples has different spore release responses. A3B2 treatment showed exponential phase on the second day, while other treatments experienced exponential phase on the fourth day. A1B2, A3B1, and A3B2 treatment showed declination phase on the sixth day, while the other treatments had not showed declination phase until the seventh day of observation.

The temperature and salinity shock treatment influenced spore releases significantly. Seaweed spores had also been released gradually during the 7 days of observation. According to Clesceri [6],

some abiotic components such as salinity, temperature, light, pH, current and waves are able to affect the reproductive system of seaweed. The results of the variant analysis showed that the given treatment had no significant difference ($p > 0.05$) on the interaction of temperature and salinity shock treatment on the first day to the fourth day, while on the fifth day to seven shows a significant difference ($p < 0.05$). This indicates that the spores of seaweed have a cell maturation phase before being released into the environment. The discharge of spores has a gradual releasing system which is divided into several processes such as spores formation, spores maturation, and spores release. These processes are strongly influenced by factors such as changes in temperature, salinity, and other abiotic components [2].

Spore release yield, shown in figure 1. and table 1., indicates that there are fluctuations and the phase of spores beginning to release. It also shows significant differences, indicating that the release of spores has a certain pattern, and does not directly occur after experiencing the stress on the environment. In addition, the data showed that the *K. alvarezii* seaweed needs a process for certain days to be released. It is in accordance with Ask [10] who mentioned that the release, growth and germination process of seaweed spores can be observed after five days. *K. alvarezii* seaweed spores experienced a maturation process before they were released. The transition process of spore filaments formation consists of thin layer formation, filament elongation, and formation of vegetative conchospore (spore) which is strongly influenced by the extreme change in temperature [13].

Table 1. *Kappaphycus alvarezii* seaweed spores release during 7 days

	16 Amount of Released <i>K. alvarezii</i> Spores (cell/ml) ± SD					
	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
A1B1	132,67 ^a ± 37,52	123,83 ^a ± 30,63	123,83 ^a ± 30,63	3078,03 ^c ± 1518,71	4484,39 ^{abc} ± 37,49	4953,15 ^{dc} ± 457,56
A1B2	141,52 ^{ab} ± 30,69	159,21 ^a ± 75,05	159,21 ^a ± 53,07	1362,06 ^{abc} ± 220,96	4970,89 ^c ± 353,35	1804,34 ^{abc} ± 1276,94
A1B3	238,81 ^{ab} ± 37,52	159,21 ^a ± 51,05	106,14 ^a ± 75,05	424,56 ^a ± 231,32	2998,45 ^{ab} ± 37,52	3856,33 ^{cde} ± 1649,73
A2B1	159,21 ^a ± 75,05	212,28 ^a ± 53,07	106,14 ^a ± 75,05	2618,06 ^{bc} ± 81,05	4033,29 ^{abc} ± 675,43	5413,06 ^c ± 414,49
A2B2	238,81 ^{ab} ± 37,52	300,73 ^a ± 30,63	132,67 ^a ± 37,52	618,98 ^{ab} ± 110,67	371,49 ^a ± 243,19	1450,55 ^{ab} ± 615,06
A2B3	159,21 ^{ab} ± 53,07	159,21 ^a ± 53,07	212,28 ^a ± 140,41	902,17 ^{ab} ± 424,53	689,91 ^{ab} ± 53,07	2794,96 ^{abcd} ± 425,65
A3B1	106,14 ^a ± 51,79	123,83 ^a ± 30,63	371,49 ^a ± 231,32	1892,77 ^{abc} ± 429,00	2282,01 ^{abc} ± 265,35	919,86 ^a ± 376,48
A3B2	283,04 ^b ± 82,39	972,9 ^b ± 391,14	1406,34 ^b ± 712,98	1432,83 ^{abc} ± 486,36	3060,37 ^{abc} ± 1648,87	1468,23 ^{ab} ± 1327,09
A3B3	212,28 ^{ab} ± 54,93	247,66 ^a ± 61,27	636,84 ^{ab} ± 231,32	3378,75 ^c ± 2869,85	3414,12 ^{bc} ± 963,07	3325,69 ^{bcde} ± 2575,01

A1B1 = Temperature 30°C (A1) dan Salinity 31 ppt (B1) A2B3 = Temperature 32°C (A2) dan Salinity 34 ppt (B3)
 A1B2 = Temperature 30°C (A1) dan Salinity 33 ppt (B2) A3B1 = Temperature 34°C (A3) dan Salinity 31 ppt (B1)
 A1B3 = Temperature 30°C (A1) dan Salinity 35 ppt (B3) A3B2 = Temperature 34°C (A3) dan Salinity 33 ppt (B2)
 A2B1 = Temperature 32°C (A2) dan Salinity 31 ppt (B1) A3B3 = Temperature 34°C (A3) dan Salinity 34 ppt (B3)
 A2B2 = Temperature 32°C (A2) dan Salinity 33 ppt (B2)

Temperature and salinity shock treatment simultaneously showed a significant difference on day three to seven. The results of Duncan Multiple Distance Test on day three to five showed that an increased temperature shock to 34°C could give a significant influence on the release of spores. In addition, the data of thallus weight decrease indicate that the higher the temperature, the higher the thallus lose its water from within. Seaweed thallus weight-loss can be seen in table 2. Temperature has a fundamental effect on the degree of chemical reactions, reproduction, and metabolism of seaweed [8]. The effect of

high-temperature shock will gradually affect photosynthesis, respiration, membrane stability and physiological changes [14]

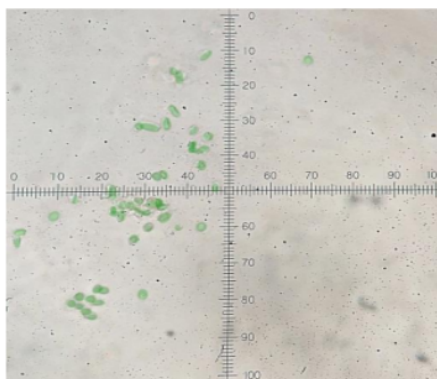


Figure 1. Released spores from seaweed *K. alvarezii* Thallus with 200x magnification.

Rising temperatures in the form of temperature shock treatment also elevated the cell membrane activity and water flow in plants. The rise in temperatures will increase water flow in plants that will further enhance the turgor pressure within the plant cell [15]. It urged the cell to increased pressure from within. Then, the fluid within the cell is pushed out. This term is called osmosis. In addition, a decrease in the weight of the thallus of *K. alvarezii* also caused the cell membrane to loose which will simplify the osmosis process. Meanwhile, the higher temperature could loose the pores on the surface of the plants [16]. When the pores which are considered as semipermeable membrane open up wider, the water-loss level will be higher [17]. This condition causes the plants to lose more fluids and pushes the spores to be discharged. The influence of force, pressure, and the weakening of the liquid on the layers of the skin pore could trigger algae cystocarp to force the seaweed spores to discharge through the venturi tube [18].

Salinity shock treatment has a significant difference and the best result was shown by 31 ppt salinity. The Duncan test showed that during the fifth day to the seventh day, 31 ppt salinity was very influential on the release of spores with the largest results. Seaweed spores can be released optimally on the salinity range of 30 ppt [19]. The development of good seaweed spores occurs in the range of salinity of 30 ppt [20]. The release of seaweed spores also has a narrow salinity range. It is also stated that the most suitable salinity for *K. alvarezii* spores to release is in the range of 29-34ppt. In addition, the thallus weight decrease data indicate that the higher the salinity, the higher thallus loses its water from within. The result of weight-loss of the thallus can be seen in table 2. This indicates that there is a presence of osmoregulation activity within seaweed cells. It is also considered as the most effective treatment in the weight-loss of *K. alvarezii* seaweed. Salinity shock directly affects the osmoregulation system on seaweed. The increase in salinity will directly increase the concentration of seawater correspondingly. So, the osmotic pressure will change differently. Increased salinity of sea water will result in the loss of water and ion absorption of the thallus. The entire process caused the change in osmotic pressure so that the seaweed will suffer osmosis. If the concentration of the environment outside the cell is higher than the concentration within the cell, this difference will cause the water to shrink out [8]. This progressive water-loss will be correspondingly loosening the space among the cells.

Table 2. Weight loss of *Kappaphycus alvarezii* thallus.

	Given Treatment		Thallus Weight Reduction (gr) ± SD
	Temperature Shock (°C)	Salinity Shock (ppt)	
A1B1	30	31	0 ^a ± 0
A1B2		33	0,016 ^b ± 0,005
A1B3		35	0,026 ^c ± 0,005
A2B1	32	31	0,013 ^b ± 0,005
A2B2		33	0,026 ^c ± 0,005
A2B3		35	0,036 ^d ± 0,005
A3B1	34	31	0,013 ^b ± 0,005
A3B2		33	0,026 ^c ± 0,005
A3B3		35	0,070 ^e ± 0,010

A1B1 = Temperature 30°C (A1) dan Salinity 31 ppt (B1) A2B3=Temperature 32°C (A2) dan Salinity 34 ppt (B3)
 A1B2 = Temperature 30°C (A1) dan Salinity 33 ppt (B2) A3B1 = Temperature 34°C (A3) dan Salinity 31 ppt (B1)
 A1B3 = Temperature 30°C (A1) dan Salinity 35 ppt (B3) A3B2 = Temperature 34°C (A3) dan Salinity 33 ppt (B2)
 A2B1 = Temperature 32°C (A2) dan Salinity 31 ppt (B1) A3B3 = Temperature 34°C (A3) dan Salinity 34 ppt (B3)
 A2B2 = Temperature 32°C (A2) dan Salinity 33 ppt (B2)

Disruption of the function of the plant cell wall is affected by the role of Golgi apparatus that are not optimal so that the cells will undergo osmosis. According to Davis [21], a different concentration of the liquid inside and outside the cell will push the Golgi apparatus to continue to strive balancing the nature until the environment condition gets isotonic. The cell will become increasingly dense, but the size will stay or become relatively smaller than the size of the previous condition [22].

The results of this research show that temperature and salinity affect the osmoregulation and release of spores. The results of the variant analysis showed that the interaction of temperature and salinity could obtain the biggest amount of spore cells on the seventh day with the A2B1 treatment. This treatment, with a temperature of 32°C and 31 ppt salinity, could obtain 5413.06 cells/ml. It shows the positive interaction between the two factors. Temperature and salinity are the factors that interact in giving the influence of seaweed physiology and reproduction. The temperature and salinity factors correspondingly interact with significant spores release. Seaweed reproductive system chain is affected by some combination of biotic and abiotic factors [6]. Temperature and salinity are the most important abiotic component factors which determine the growth and reproduction of seaweed [7].

K. alvarezii spores release is closely related to seaweed physiological system and environmental factors. The osmosis from seaweed cell, which is caused by the different concentration of the environment and spaces between cells that loosen, will carry spores out. The opening of the cystocarp pores will force seaweed spores to be released through the venturi tube with the influence of force, pressure, and the weakening of the adhesive liquid on the skin layer of algae [18]. Environmental factors such as temperature and salinity affect the connection between cells (the pit connection) to detach and release the reproduction cell [22].

4. Conclusion

Based on the discussion, it can be concluded that environmental manipulation using salinity and temperature shock have several effects on osmoregulation system and *K. alvarezii* spores release with 31 ppt salinity on the fifth day to the seventh day and 30°C temperature on the sixth day and seventh day. The addition of salinity and temperature shock also have an interaction with the release of *K. alvarezii* spores on the third day to the seventh day with A2B1 as the best treatment. A2B1 is the combination of 32°C temperature shock and 31 ppt salinity shock that could produce 5413.06 cells/ml

on the seventh day. The temperature and salinity shock can be used as the candidate of *K. alvarezii* seaweed reproductive method to obtain superior seeds which can be used for fishery development.

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