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Performance of Seaweed Seed *Kappaphycus alvarezii* Post Cold Transportation Method with Different Transportation Times

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ABSTRACT

This research was conducted in the waters of Oenaek, Kupang Regency, East Nusa Tenggara Province, Indonesia. The aim was to determine the growth and carrageenan content of seaweed seeds transported using the cold method. This research consisted of 4 treatments, namely treatment A (seed transportation for 2 days), treatment B (seed transportation for 4 days), treatment C (seed transportation for 6 days), and treatment K as a control without transportation (0 days). The results of the ANOVA analysis showed that there was no significant effect on absolute growth and carrageenan content on seaweed seeds that were transported for 2, 4, and 6 days compared to seeds that were not transported (0 days of transportation). Therefore, it can be concluded that seaweed seeds can be transported using the cold method at temperatures ranging from 22- 28°C for 6 days or 144 hours. The longer the transportation time, the greater the distance that can be traveled to send seeds so that it can meet the need for quality seeds for seaweed center areas that are constrained by seeds.

INTRODUCTION

The problem faced in seaweed cultivation is the provision of good and quality seeds. **Oedjoe *et al.* (2023)** stated that seaweed cultivators in several areas in East Nusa Tenggara province still use seeds repeatedly. Cultivators in East Sumba Regency have used seeds repeatedly for approximately 15 to 20 years, which can reduce the genetic quality of the seeds. According to **Yong *et al*. (2011)**, the decrease in growth and quality of carrageenan was caused by repeated use of seeds. In this context, **Sulistiani *et al*. (2012)** stated that the decrease in genetic variability could be caused by repeated use of seeds, thereby reducing growth rate, carrageenan content and gel strength. Genetic quality can be improved through breeding and propagation. However, breeding and propagation activities are still limited to certain areas because they require adequate supporting facilities and infrastructure. Therefore, one effort to bring quality seeds between regions is through seed transportation.

One way is to take advantage of cold conditions in the transportation media to maintain the freshness of the seeds. Cold conditions can be achieved by reducing the temperature in the transportation container using ice cubes **(Seagrand New York, 2022)**. The principle of cold method transportation is to condition the environmental temperature to remain cool and moist for the organisms during the transportation process. According to the Directorate General of Fishery Product Processing and Marketing in 2007, seaweed can last quite a long time if the temperature remains low and the environment is sufficiently humid. Seaweed is very susceptible to breaking due to pressure, friction, or strong movement during transportation to the cultivation location before planting. Therefore, it was necessary to look for alternative transportation methods that were good and correct to ensure that the seeds arrive at the target site in good condition and to ensure their ability to grow after a long transportation journey.

MATERIALS AND METHODS

1. Research location

This research was carried out for ± 3 months, from May – July 2024, addressing transportation, planting, maintenance, drying and analysis of carrageenan. Planting and maintenance of seaweed seeds was carried out in the waters of Oenaek Village, Kupang Regency, while analysis of carrageenan yield was carried out in the dry laboratory of the Faculty of Maritime Affairs and Fisheries, Nusa Cendana University.

2. Research procedure

The transportation process uses a cold method, namely using ice cubes to reduce the temperature to the range of 22 - 28°C. Ice cubes, totaling 3 bottles of 600mL of mineral water coated with plastic, and newspaper are placed on the inside next to the Styrofoam. The ice cube placement space is designed so that the ice replacement process does not disturb the presence of the seeds being transported, this aims to reduce stress on the seeds. To channel the circulation of cold temperatures produced by ice to the seed storage room, in the partition between the ice storage room and the seed storage room, 6 circulation holes with a diameter of 6cm and a distance between holes of 5cm were made.

The temperature in the transportation container during the transportation process was maintained at no more than 28°C, and to maintain air humidity in the storage room, the bottom and top of the container were lined with filter cotton, and the cover was taped so that there are no gaps for air to escape from the container. Ice cubes were replaced every 24 hours to maintain the temperature within the desired range. A thermometer was installed on the cover of the ice storage room to determine the temperature inside the transportation container.

After the packing process, the transportation process was carried out for 2, 4, and 6 days according to the treatment. After transportation, maintenance was carried out for 36 days, after harvest, the seaweed was dried and the carrageenan content was calculated. Post-transportation survival measurements were carried out at the end of transportation, while seedling growth rate measurements were measured every 9th day during the study. Measurements of the physical and chemical conditions of the water were evaluated every week during the research. Meanwhile, carrageenan analysis was carried out at the end of the research. The design of the container for transporting seaweed seeds using the cold method is shown in Fig. (1).

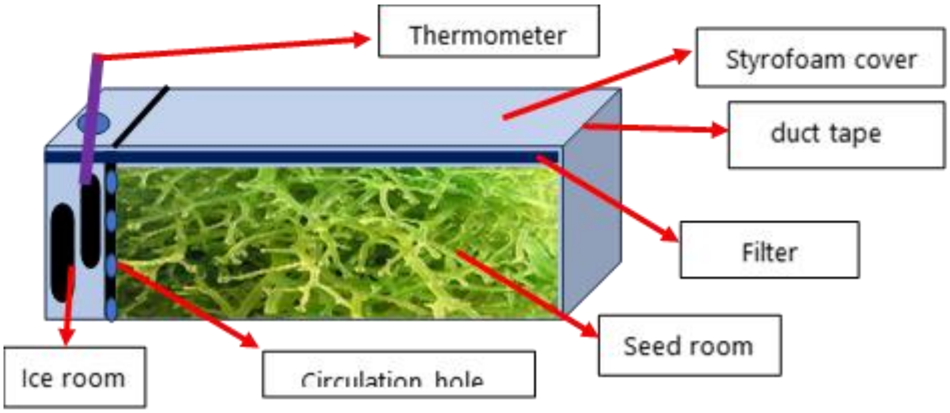

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Fig. 1. Transport container design

This research used a completely randomized design (CRD) with 4 treatments and 3 replications, namely: A) Cold transportation for 2 days; B) Cold transportation for 4 days; C) Cold transportation for 6 days; and K) Without transportation (0 day)

3. Measured variables

3.1 Temperature fluctuations on transportation container

Temperature fluctuations in the transportation container during transportation were measured using a thermometer installed in the transportation container. Based on preliminary research, a change in temperature was detected during 2 hours of storing ice cubes in a styrofoam container, so that observations were made every 2 hours.

3.2 Survival rate of seed seaweed post-transportation

The survival rate (SR) was calculated post-transportation by calculating the weight of the fresh thallus before transportation (fresh green) (N0) divided by the weight of the fresh thallus post-transportation. Thalus that were pale and white after transportation were considered to be parts of dead seaweed seeds. The white thallus was separated from the fresh thallus, and after being separated it was weighed again to obtain the weight of live seaweed seeds (Nt). Survival rates were calculated using the formula of **Yangsong *et al.* (2022)**:

$$\text{Survival Rate} = \frac{\textit{Final number of seaweeds}}{\textit{Initial number of seaweeds}} x100\%$$

3.3 Specific growth rate and absolute growth

Specific growth rate (SGR) seed weight was calculated using the formula of **Luhan and Sollesta (2010)**:

$$SGR = \frac{lnWt - lnW0}{t} x100\%$$

Where:
SGR = specific growth rate (g%/day)
Wt = seed weight at time t (g)
W0 = initial seed weight (g)
t = maintenance time (days)

Absolute growth (W) was calculated using the formula of **Damayanti *et al.* (2019)**:

$$W = Wt – W0$$

3.4 Thallus branching and diameter

The increase in thallus branching was calculated every 9 days, by calculating the additional number of new thallus growing from the primary and secondary thallus.

4. Water quality

As supporting data, water pH measurements were carried out using a pH meter, salinity using a refractometer, temperature using a thermometer, and nitrate using a spectrophotometer. Measurements of pH, salinity, and temperature were carried out every 7 days for 45 days of maintenance, while testing of nitrate content in water was carried out once, namely on the first day of planting.

5. Data analysis

Post-transportation survival and growth data obtained were analyzed using analysis of variance (ANOVA) at a 95% confidence interval, and followed by Duncan's test. Statistical analysis was conducted using IBM SPSS Statistic 24 software.

RESULTS

1. Seaweed transportation

The research results showed that transportation using 1.8L of ice cubes can maintain the temperature in the transportation container in the range of 22-28°C for approximately 24 hours. A graph of temperature fluctuations in the transportation container for 24 hours is shown in Fig. (2).

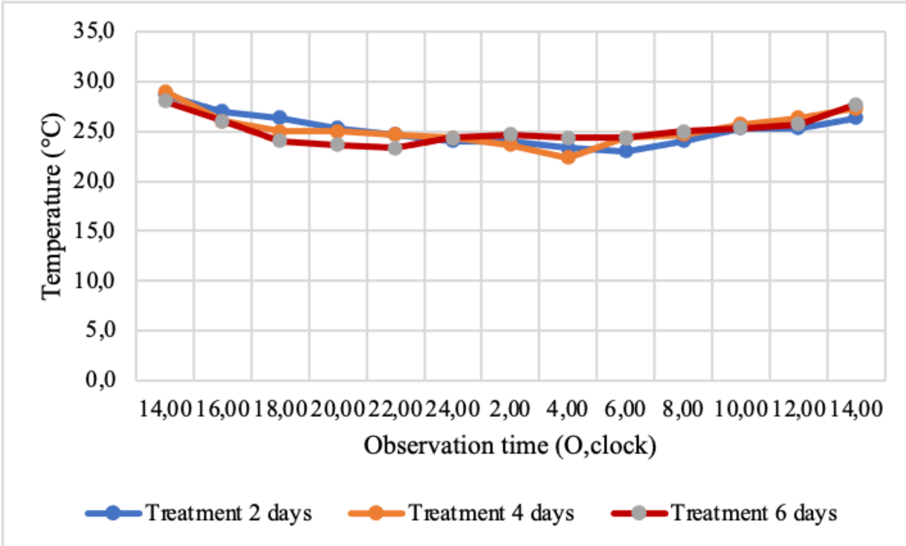

Fig. 2. Temperature fluctuations in the transport container without ice replacement for 24 hours (Note: treatment K without transportation)

Fig. (2) shows that, at hour 0 of transportation, the resulting temperature of 1.8L of ice cubes in the transportation container containing seaweed seeds was in the range of 28-29°C. Post-transportation of 2, 4 and 6 days did not cause bleaching of all the thallus of the transported seedlings. All seedling thallus remained yellowish green, indicating that the thallus were in good condition (Fig.3).

Fig. 3. Condition of seeds after transportation

However, after 6 days of transportation, there was a little mucus on some parts of the seaweed seed thallus, this was thought to be because the seaweed thallus parts with a small surface area experienced a lack of fluid. According to **Aslan (1998)**, *Kappaphycus alvarezii* seaweed is included in the macro algae group, which has a soft thallus substance such as gelatin (gelatinous), so it has a fairly high waters binding ability, but water absorption depends on the surface area of the seaweed, so that different thallus sizes will influence the level of water absorption and storage.

2. Growth and carrageenan content

The growth response of *K. alvarezii* seaweed observed during the 36 days of research was in the form of specific growth rate, absolute growth, and increase in the number of branches on the primary and secondary thallus.

2.1 Specific growth rate (SGR)

The results of the ANOVA analysis showed that there was a real influence of the length of transportation time on the daily growth rate every 9 days for *K. alvarezii* seedlings (Fhit>Ftable). The results of the research showed that there was a reduction in the weight of the seeds after transportation ranging from 70 - 126g due to osmotic events, this is what caused the first week of care for the transported seeds to adjust to the reduced water content **(Aslan, 1998)**. However, after the 18th to the 27th day, the seedlings that were transported for 2, 4, and 6 days showed a faster growth rate compared to the seedlings that were not transported. In accordance with the statement of **Yusnaini *et al*. (2000)** that after going through the adaptation process, seaweed will experience a rapid growth phase and then at a certain time there will be saturation of cell division which will slow down growth, as happened on the 36th day of growth rate in this research.

The results showed that there were fluctuations in the specific growth rate of seaweed during the study, as shown in Fig. (4).

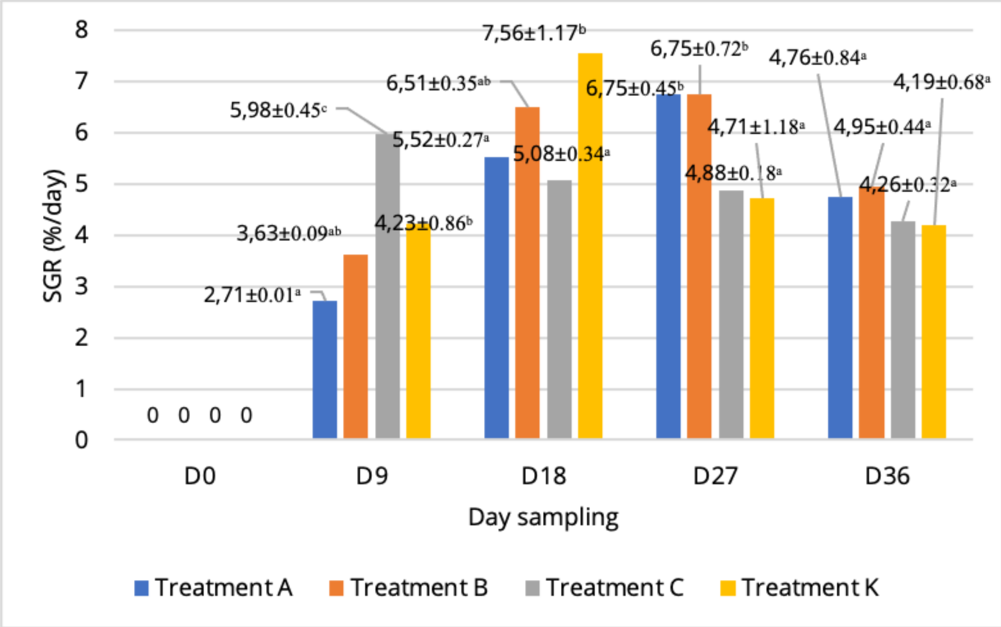

Fig. 4. Graph of growth rate of *K. alvarezii* during 36 days of cultivation

Note: Values in the same column with different superscript differ significantly ($P<0.05$). Data are expressed as Mean ± SE.

2.2 Absolute growth

The results of the research showed that the highest absolute growth occurred in treatment B (transportation time of 4 days), namely 325.83 ± 56.07g; however, the results of the ANOVA analysis showed that there was no real effect of transportation time of 2, 4, 6 days or without transportation on absolute growth. *Kappaphycus alvarezii* seaweed was maintained for 36 days (Fhit<Ftable). This shows that a transportation time of 6 days provides the same growth rate as seaweed seeds that are not transported. The absolute growth of seaweed during 36 days of cultivation is shown in Table (1).

Table 1. Absolute growth of transported and untransported seaweed

Treatment	Absolute growth (g)
A (2 days transportation)	298.33±51.45[a]
B (4 days transportation)	325.83±56.07[a]
C (6 days transportation)	244.17±52.77[a]
K (without transportation)	271.67±50.86[a]

Note: Values in the same column with different superscript differ significantly ($p<0.05$). Data expressed as Mean ± SE.

2.3 Branching and thallus diameter

The results showed an increase in the number of secondary talus and primary talus diameter during 36 days of maintenance. Based on Table (2), increasing the number of secondary thalus affects the diameter of the primary talus. The greater the number of secondary thalus, the smaller the diameter of the primary talus.

Table 2. Average increase in primary talus number and primary talus diameter during 36 days of maintenance

Treatment	Number of Secondary Talus	Primary Talus Diameter on day 36 (cm)	Primary Talus Diameter (cm)
A	15.67±1.15[a]	0.44±0.06[a]	0.23±0.03[a]
B	14.33±2.08[a]	0.53±0.10[a]	0.26±0.08 [a]
C	15.33±1.15[a]	0.53±0.07[a]	0.21±0.05 [a]
K	16.33±1.15[a]	0.47±0.06[a]	0.17±0.06 [a]

Note: Values in the same column with different superscript differ significantly (*P*<0.05). Data are expressed as Mean ± SE.

3. Carrageenan content

Based on the analysis of carrageenan content, it was found that the carrageenan content of *K. alvarezii*, which was reared for 36 days in Oenaek Waters, Kupang Regency, ranged from 24.70 to 26.87% (Fig. 5).

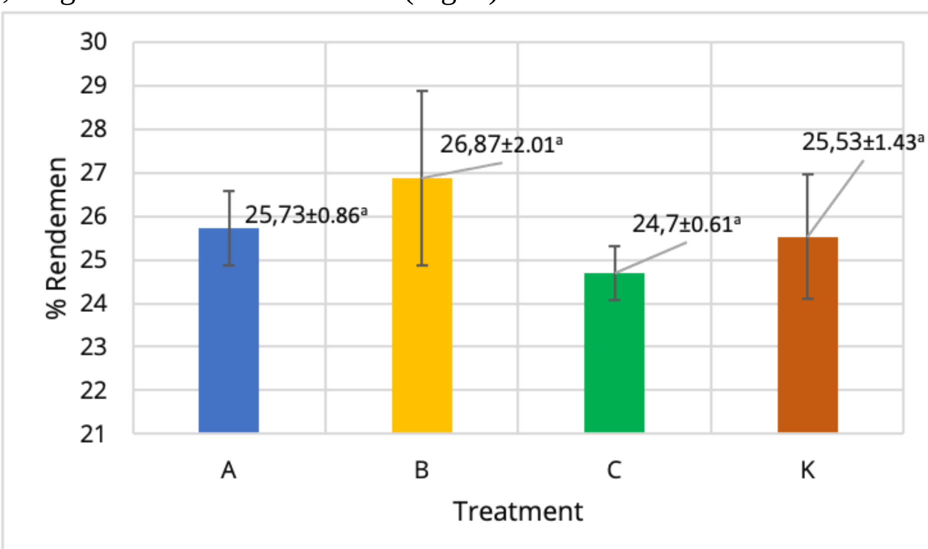

Fig. 5. Percentage yield of *K. alvarezii* during 36 days of cultivation

Note: Values in the same column with different superscript differ significantly (*P*<0.05). Data are expressed as Mean ± SE.

The results obtained showed that seeds transported using the cold method had carrageenan content that was not significantly different from seeds that were not transported. Based on ANOVA analysis, it was found that there was no real effect on the length of transportation time on the carrageenan content of *K. alvarezii* seaweed which was maintained for 36 days (Fhit<Ftable). This shows that *K. alvarezii* seaweed seeds can

be transported using the cold method at temperatures ranging from 22-28°C, over longer distances or transportation time of 6 days (144 hours).

DISCUSSION

According to **Teken *et al.* (2013)**, seaweed seed talus is very susceptible to breaking and bleaching if not handled properly, one of which is during the transportation process. Therefore, proper handling during transportation can ensure that seaweed seeds arrive at the planting site in good and fresh condition.

The temperature in the container continued to decrease until 04.00 am, ranging between 22-24°C. This is because at night until dawn, the temperature is relatively more stable, whereas approaching noon, the temperature again increases to reach the range of 26-28°C. The temperature in the transportation container is kept at <30°C so that the seaweed seeds remain moist and do not experience stress during transportation. Low temperatures ranging from 20-28°C can suppress the rate of decay due to bacterial and fungal contamination **(Geraldine *et al*., 2025)**. In addition, it also maintains the texture of the thallus to remain fresh and dense, and does not undergo oxidation and enzymatic degradation **(Mahfudh *et al*., 2021)**. Stress conditions in other grass seeds are indicated by increasing mucus on the surface of the talus as a result of dry environmental conditions or being attacked by ice-ice disease **(Aslan, 1998)**. According to **Ward *et al*. (2022)**, environmental stress causes the thallus to be susceptible to opportunistic bacteria that cause ice-ice disease, which results in the whitening of the thallus, followed by disintegration of the diseased tissue.

The condition of the seeds, which remained fresh after cold transportation for 2, 4, and 6 days, shows that controlled temperature conditions during transportation can provide comfortable conditions for seaweed seeds so that they do not experience stress. The temperature in the transportation container is maintained at an optimal range for the life of *K. alvarezii* seaweed. According to **Parenrengi *et al*. (2006)**, the optimal temperature for cultivating *K. alvarezii* seaweed is 27–30°C.

Temperature is one of the limiting factors in the life of aquatic organisms **(García-Poza, 2020)**. For seaweed and other marine plants, temperature plays a role in the processes of photosynthesis, respiration, transpiration, and absorption of water and nutrients. Temperature fluctuations can significantly inhibit the growth, both morphology and physiology of seaweed **(Masyahoro *et al*., 2010)**. An increase in temperature beyond the tolerance range of *K. alvarezii* seaweed can cause proteins to denature and damage enzymes and cell membranes which are unstable with high temperatures **(Mamang, 2008)**.

Fig. (4) shows the fluctuations in the growth rate of seaweed seedlings in each treatment. On the 9th day, the highest SGR was experienced by seeds that were not transported since there was no need for time to adapt when planted in natural waters so they experienced a rapid growth phase **(Yusnaini *et al.*, 2000)**. On the other hand, transported seeds require time to adapt to their environment.

A longer transportation time has a positive correlation with the greater distance that can be crossed during the transportation of seaweed seeds among islands. Thus, the problem of a shortage of quality seaweed seeds can be overcome by bringing in superior seeds produced from other regions. Apart from that, the bioecological conditions of seaweed are relatively the same in all waters, making it easier for seaweed seeds to adapt well to the dynamics of water conditions **(Masyahoro *et al*., 2010)**. This allows seaweed seeds to come from anywhere, but the freshness and quality of the seeds need to be maintained during the transportation process. The results showed that the seaweed seedlings remained fresh after 2, 4 and 6 days of transportation and did not suffer from broken talus (Fig. 3). Good condition of the seeds causes osmotic pressure activity to run well, thereby facilitating the exchange of water and nutrients in the waters **(Silviana, 2009; Harahap *et al*., 2022)**.

During 36 days of rearing *K. alvarezii* seedlings, there was an increase in the number of secondary thalus. The increase in the number of thalus was not the same between treatments because the number of growing points or blades on the initial seedling thallus was also different. The blade is the part that provides a photosynthetic surface for seaweed **(Campbell *et al*., 2012; Watung *et al*., 2016; Melinda, 2017)**.

During the research, the temperature was in the optimal range, namely between 27-29°C, this allows the seaweed to receive good light intensity to carry out the photosynthesis process. Apart from that, the pH and salinity of the waters during the research were also in optimal conditions, namely in the range of pH 7.2 - 8.0 and salinity 29 - 32ppt. The nitrate content in the waters during the research was in good condition, ranging between 0.34 - 0.38ppm. According to **Kanglan (2006)**, the nitrate content that is good for seaweed growth ranges from 0.2 - 0.7ppm.

Based on the data depicted in the studies of **Burfeind *et al.* (2009)** and **Susilowati (2012)**, nutrient availability and light intensity can influence the fulfillment of carbon (C), nitrogen (N) and phosphorus (P) needs which are beneficial for seaweed growth. The increase in the number of thalus is strongly influenced by the nitrogen element contained in the water **(Kushartono *et al.*, 2009)**. It was further explained that the effectiveness of nutrient absorption and the photosynthesis process is influenced by the intensity of sunlight reaching the talus.

The diameter of the primary thallus of *K. alvarezii* on day 36 ranged from 0.44 to 0.53cm, with an increase in thallus diameter of 0.17 to 0.26cm. The diameter of the primary thallus was significantly correlated with the absolute growth of *K. alvarezii*. The results showed that transportation of seeds for 4 days gave the highest absolute growth with the largest primary thallus diameter. This took place since the condition of seaweed seeds after 4 days of transportation showed better seed quality performance, compared to the 6-day transportation treatment which resulted in shrunken and slightly slimy seaweed seed thallus. However, the results of the ANOVA test showed that the length of transportation time had no significant effect on absolute growth or primary thallus diameter (Fhit<Ftable).

The length of time that can be used for transportation is correlated with the distance that can be traveled. Therefore, the application of the cold transportation method can be an alternative for providing quality seeds between islands with a travel time of 144 hours or 6 days.

CONCLUSION

Transportation of *K. alvarezii* seaweed seeds using the cold method with temperatures ranging from 22-28°C can maintain the quality of the seeds for 6 days or 144 hours. The growth and carrageenan content of seeds that underwent transportation were not significantly different from seeds that were not transported.

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