

Effects of inorganic nutrient enrichment on the carrageenan yield, growth, and ice-ice disease occurrence of red alga *Kappaphycus striatus*

Jurmin H. SARRI^{1,3}, Yusop A. ABDULMUTALIB¹, Melapearl E. MOHAMMAD TILKA¹, Ertuğrul TERZİ²,
Albaris B. TAHILUDDIN^{1,3}

Cite this article as:

Sarri, J.H., Abdulmutalib, Y.A., Mohammad Tilka, M.E., Terzi, E., Tahiluddin, A.B. (2022). Effects of inorganic nutrient enrichment on the carrageenan yield, growth, and ice-ice disease occurrence of red alga *Kappaphycus striatus*. *Aquatic Research*, 5(2), 99-109.

<https://doi.org/10.3153/AR22009>

¹ Mindanao State University Tawi-Tawi
College of Technology and
Oceanography, College of Fisheries,
Sanga-Sanga, Bongao, 7500
Tawi-Tawi/Philippines

² Kastamonu University, Faculty of
Fisheries, 37200 Kastamonu/Türkiye

³ Kastamonu University, Institute of
Science, Department of Aquaculture,
37200 Kastamonu/Türkiye

ORCID IDs of the author(s):

J.H.S. 0000-0002-4798-0566

Y.A.A. 0000-0001-9238-9372

M.E.M.T. 0000-0002-3503-6674

E.T. 0000-0003-2811-6497

A.B.T. 0000-0002-3237-3552

Submitted: 24.09.2021

Revision requested: 23.11.2021

Last revision received: 06.12.2021

Accepted: 06.12.2021

Published online: 28.01.2022

Correspondence:

Albaris B. TAHILUDDIN

E-mail: albarist20@gmail.com



© 2022 The Author(s)

Available online at

<http://aquatres.scientificwebjournals.com>

ABSTRACT

One of the problems in *Kappaphycus* farming is the slow growth in some farms due to nutrient insufficiency caused by overstocking. In the southern Philippines, most seaweed farmers practice inorganic nutrient enrichment of *Kappaphycus* to boost growth and decrease ice-ice disease occurrence using ammonium phosphate at an average concentration of 8.82 g L⁻¹. In this study, experiments with *Kappaphycus striatus* enriched with inorganic nutrients were carried out at Pasiagan, Bongao, Tawi-Tawi, southern Philippines, using different inorganic nutrients (T₁=8.82 g L⁻¹ of urea, T₂=8.82 g L⁻¹ of phosphorus, and T₃=control) within 45 days. Seaweeds were enriched in these three inorganic solutions for 30 seconds, placed into a large mat, covered with canvas, and left overnight. After 15 days, findings showed that the specific growth rates of T₁ (6.99% day⁻¹) and T₃ (6.72% day⁻¹) groups were significantly higher than the T₂ (5.84% day⁻¹) group ($p < 0.05$). Inorganic nutrient enrichment did not significantly influence the occurrence of ice-ice disease. Moreover, inorganic nutrient enrichment did not affect the carrageenan yield after 45 days. *K. striatus* nutrient-enriched with urea could increase growth at day 15, but no effect on the occurrence of ice-ice disease and carrageenan yield. Hence, inorganic nutrient enrichment using urea provides a positive effect to farmed *K. striatus* by enhancing its growth without affecting its health and carrageenan yield.

Keywords: Carrageenan yield, Ice-ice disease, *Kappaphycus striatus*, Nutrient enrichment, Specific growth rate

Introduction

Kappaphycus striatus is one of the many fishery resources that abound in Tawi-Tawi waters, southern Philippines, mostly of high commercial value in the national and international markets (Arupin, 1997). *Kappaphycus*, a red seaweed locally known as Guso (Cebuano) or Agar-agar (Tausug), is an important export product in Asia. It is one of the country's top three exports of marine-based products. France, China, and the USA are the main markets for seaweed products in the Philippines (BFAR, 2016). Red seaweeds are harvested globally (either from the farm-raised or wild) and have numerous applications as food for human consumption and as a source of two hydrocolloids: carrageenan and agar, which are widely utilized as an emulsifier, binder, gelling and thickening agents as well as food and non-food products (McHugh, 2003).

In the late 1960s, the line and stake method were utilized as the first commercial cultivation of *Kappaphycus* from the southern Philippines, and for over decades, the Philippines was the top producer of *Kappaphycus* until it was surpassed by Indonesia in 2008, although production from the Philippines has been on a downward trend since 2011 (Hurtado et al., 2015). However, in 2019, China was the top producer of aquatic plants, including seaweeds, where the Philippines ranked 4th (FAO, 2020). On the same year, the top fisheries performance in the Philippines was tuna having the export value at US\$ 478 million, followed by seaweed, which went up 13% US\$ 207 million in 2018 to US\$ 250 million in 2019 or 22% total earnings for that year (BFAR, 2019).

The decreased material quality or overstocking is one of the main hurdles in seaweed production, which causes a decrease in nutrients and stunted seaweed growth (Luhan et al., 2015). Temperature, salinity, water movements, turbidity, and light intensity are abiotic factors that can cause ice-ice disease, epiphytes infestation, and poor seedling quality of grown seaweeds (Largo, 2002; Tahiluddin & Terzi, 2021a; Tahiluddin & Terzi, 2021b). One of the important factors in determining seaweed production sustainability and its yield is the fertility of water. The cultivation of *Kappaphycus* is primary dependent on the natural fertility of the water (Hurtado et al., 2001; Munoz et al., 2004; Hayashi et al., 2007a). One of the control measures to reduce the occurrence of ice-ice disease in *Eucheuma* and *Kappaphycus* species is by nutrient enrichment before out-planting (Tahiluddin & Terzi, 2021a). Two nutrients, nitrogen, and phosphorus, are vital supplements for the growth and production of seaweeds (Harrison & Hurd, 2001). Nitrogen combines biologically with carbon, hydrogen, oxygen, and sulfur to form amino acids, which are the protein building blocks and are utilized for the development of the

plant and its growth (Uchida, 2000). Increased source nitrate or ammonium concentrations supplies can result in high nitrogen accumulation, increased growth as well as increased nitrogen sufficiency of the seaweed *Fucus spiralis* (Topinka & Robbins, 1976).

In addition, phosphorus plays a major role in energy storage. It helps to improve plant growth, reduces the incidence of diseases, and improves the quality of some plants (Uchida, 2000). Phosphorus application in agriculture substantially improved the relative water content of plant's leaf, including the rate of photosynthesis of *Alnus cremastogyne* seedlings even under drought period (Tariq et al., 2018). Enrichment of phosphorus significantly increased the photosynthetic rates and growth of *Sargassum fluitans* and *S. natans* (Lapointe, 1986). Sekar et al. (1995) showed that the seaweed liquid fertilizer at 0.25% concentration increased seaweed growth and increased total nitrogen and phosphorus accumulation. In Tawi-Tawi, southern Philippines, farmers are using inorganic nutrients such as ammonium phosphate with an estimated average concentration of 8.82 g L⁻¹ to reduce ice-ice disease occurrence and to enhance the growth of *Kappaphycus*, which likewise proven effective in the field experiment (Tahiluddin, 2018; Tahiluddin et al., 2021a). However, it is still unclear which of the two important nutrients is more essential for *K. striatus*. Thus, this study aimed to determine the effects of urea and phosphorus on carrageenan yield, growth rate, and occurrence of ice-ice disease on the red alga *K. striatus*.

Material and Methods

Study Site and Duration

The study was carried out at the seaweed farm of Pasiagan, Bongao, Tawi-Tawi, southern Philippines (05° 00.424' N, 199° 45.39' E) from February to March 2019 for 45 days.

Preparation of Seedlings

Untreated and healthy *K. striatus* seedlings were purchased from the farmer in the field. Seedlings were placed in styrofoam with *Sargassum* sp. on the top and bottom of seaweeds to maintain the moisture and temperature and transported to the study site via a small boat. After the seedlings were transferred from the source to the study area, the seedlings were conditioned. The styrofoam with seaweeds was gently dipped into the farm area until the seaweeds were completely submerged. The seedlings were planted for three (3) days for acclimatization using the fixed-off bottom method. Seedlings were prepared by cutting with the help of a knife to 50 g per bunch. These were tied into a rope line (5 m) using

a soft straw with a distance of 25 cm (Hurtado et al., 2008). Each line consisted of 20 bunches, and 9 lines were prepared.

Inorganic Nutrient Enrichment

Inorganic nutrient enrichment was carried out late in the afternoon using the method previously reported (Tahiluddin, 2018). Two nutrient solutions were prepared: T₁=8.82 g L⁻¹ of urea, T₂=8.82 g L⁻¹ of phosphorus, and the control group (T₃= control). Simultaneously, all 3 lines were immersed in solutions for 30 seconds, placed into a large mat, and covered with canvas overnight. Seedlings were immersed in seawater for less than 30 minutes. Re-application of nutrient enrichment was done every 15 days (day 0, day 15, and day 30).

Planting

Seedlings were transported to the farm area using a small boat. Wooden poles were placed under the substrate as stakes. Seedlings were planted in Randomized Complete Block Design (RCBD) using the fixed-off bottom method (Trono, 1992). The distance from the seedlings to the bottom was 30 cm.

Farm Maintenance

The farm site was visited every seven days to maintain the cleanliness of the farm by removing epiphytes and debris attached to the seaweeds. The monitoring water parameters such as salinity, temperature, pH, as well as water depth were recorded every seven days using the refractometer (Atago Master), thermometer, pH meter (Smart Sensor), and meter stick, respectively. Water current was determined every seven days using improvised drogoue.

Ice-Ice Disease Monitoring

Monitoring of occurrence of ice-ice disease was done every 15 days (day 0, day 15, and day 30). One or more soft white branches were labeled as an ice-ice disease (Luhan et al., 2015; Tahiluddin & Terzi, 2021a). Seaweeds with soft white branches were summed up and divided by the number of planted seaweeds per line. The occurrence of ice-ice disease was computed using the following formula (Largo et al., 1995a).

$$\text{Percent of ice - ice disease} = \frac{\text{number of infected bunches}}{\text{total number of bunches}} \times 100$$

Growth Sampling

Sampling was done every 15 days of the culture period. Five random subsamples or 25% of seedlings samples per line were taken. To remove excess water, seaweeds were patted with a smooth cloth and weighed using a weighing scale. The

specific growth rate (μ) was computed using the formula below (Luhan et al., 2015).

$$\mu = \frac{\ln(W_f) - \ln(W_i)}{\text{DOC}} \times 100$$

Where:

DOC = days of culture

W_f = final weight

W_i = initial weight

Analysis of Carrageenan Yield

Carrageenan yield was determined every 15 days. Seaweeds were cleaned by removing silt, sand, and other foreign matter. Seaweeds were dried in a solar drier for 3-5 days. The dried seaweeds were brought to the Seaweed Post-harvest Laboratory of the Mindanao State University-Tawi-Tawi College of Technology and Oceanography. Carrageenan yield was determined following the method of Luhan et al. (2015) and calculated by dividing the weight of carrageenan seaweeds treated with an alkali solution to dry weight and times by 100.

Data Analysis

IBM SPSS software version 20 was used to analyze the data of carrageenan yield, growth rate, and occurrence of ice-ice disease of seaweed *K. striatus*. Determination of significant difference was computed through the One-way Analysis of Variance (ANOVA), and Post hoc (Duncan) was used to rank the mean.

Results and Discussion

Physicochemical Parameters

Table 1 shows the environmental status of the farmed area. The temperature ranged from 27.68 ±0.43 to 32.87 ±0.19 °C; pH was measured between 6.93 ±0.03 to 8.43 ±0.03; salinity of the farmed area was 30.17 ±0.44 to 35.00 ±0.29 ‰; water current ranged between 0.05 ±0.00 to 0.16 ±0.03 m s⁻¹; depth of farm area varied between 27.68 ±0.29 to 129.17 ±0.88 cm.

Growth

The specific growth rates (SGR) of T₁, T₂, and T₃ groups were 6.99 ±0.16 % day⁻¹, 5.84 ±0.30 % day⁻¹, and 6.72 ±0.17 % day⁻¹, respectively, at day 15 of the culture period (Figure 1). Statistical analysis revealed that SGR of T₃ and T₁ groups were significantly higher ($p < 0.05$) than the T₂ group. At day 30, SGR of T₁ (5.58 ±0.53 % day⁻¹), T₂ (4.14 ±0.10 % day⁻¹), and T₃ (5.02 ±0.40 % day⁻¹) groups were not differ significantly ($p > 0.05$). At 45 days of the culture period, T₁, T₂, and T₃ groups achieved SGR of 3.90 ±0.46 % day⁻¹, 2.41 ±1.41 %

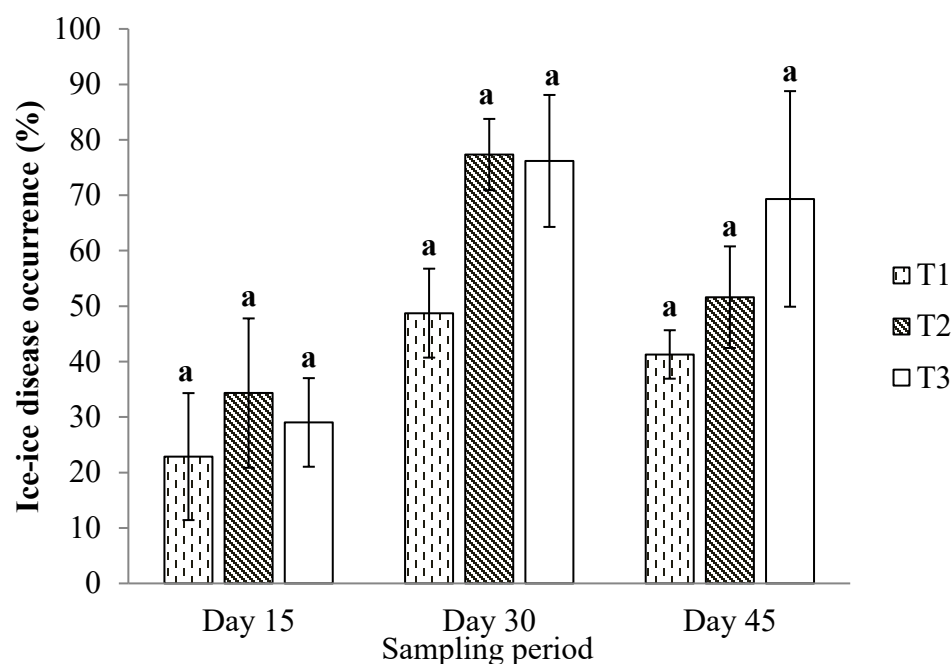


Figure 2. Ice-ice disease occurrence of *K. striatus* in every sampling. T₁=8.82 g L⁻¹ of urea, T₂=8.82 g L⁻¹ of phosphorus, and T₃=control. Bars with the same letters are not significantly different ($p > 0.05$). Error bars in SEM (standard error mean), $n=5-20$.

Carrageenan Yield

Carrageenan yields of alkali-treated seaweeds *K. striatus* in T₁, T₂, and T₃ groups were $28.33 \pm 0.29\%$, $31.53 \pm 1.07\%$, and $29.27 \pm 0.54\%$, respectively on day 15. One-way ANOVA revealed that the T₂ group was significantly higher ($p < 0.05$) than the T₁ group but not significantly different ($p > 0.05$) from the T₃ group. On day 30, carrageenan yields of T₁, T₂, and T₃ groups were $25.65 \pm 0.63\%$, $25.78 \pm 0.19\%$, and $27.11 \pm 0.8\%$, respectively. On day 45, carrageenan yields of T₁, T₂, and T₃ groups were $33.71 \pm 0.83\%$, $36.20 \pm 0.10\%$, and $31.04 \pm 2.49\%$, respectively. There was no significant difference ($p > 0.05$) between treatments as revealed by One-way ANOVA on days 30 and 45 (Figure 3). In terms of change in culture period, carrageenan yield of T₁ significantly dropped ($p < 0.05$) from day 15 to day 30 and significantly increased ($p < 0.05$) from day 30 to day 45. Carrageenan yield of the T₂ group significantly decreased ($p < 0.05$) from day 15 to day 30 and significantly increased ($p < 0.05$) from day 30 to day 45. In the T₃ group, carrageenan yield significantly dropped ($p < 0.05$) from day 15 to day 30. However, there was no significant change ($p > 0.05$) from day 30 to day 45 (Figure 4).

Growth

Phosphorus and nitrogen, which are mostly found in a natural environment, are important nutrients for the growth of seaweeds (Harrison & Hurd, 2001). Many researchers have

stated that the cultivation of *Kappaphycus* spp. is mainly dependent on the natural enrichment of the sea (Hurtado et al., 2001; Munoz et al., 2004; Hayashi et al., 2007a). Fertilization of the water is very important in order to determine the sustainability, yield, and productivity of seaweeds (Luhan et al., 2015). Thus, the addition of nutrients can be beneficial to seaweeds depending on the fertilizer used as well as its concentration. In this study, *K. striatus* nutrient enriched with urea increased the growth ($6.99\% \text{ day}^{-1}$) on day 15 and obtained higher growth ($3.90\% \text{ day}^{-1}$) after 45 days, although not significantly different from the control. According to Luhan et al. (2015), seaweed *K. alvarezii* enriched with sodium nitrate (0.01 g L^{-1}) showed an increase in growth ($2.34\% \text{ day}^{-1}$) after day 45 of culture period in a grow-out cage. They also stated that a lower nitrogen concentration resulted in slower growth, and a higher nitrogen concentration exhibited faster growth. A similar study used nitrate ($1 \text{ mM NO}_3\text{-N}$) to enhance the growth ($0.97\% \text{ day}^{-1}$) of *K. alvarezii* cultured at the laboratory (Sahoo & Ohno, 2003). The used nitrate ($35 \mu\text{g NO}_3\text{ L}^{-1}$) to *Fucus spiralis* enhanced the growth ($0.83\% \text{ day}^{-1}$) after 12 days of culture period in plastic regime rack (Topinka & Robbins, 1976). Uchida (2000) stated that nitrogen is vital because it is the main component of chlorophyll and necessary for photosynthesis. Hence, the enrichment of urea provided an additional nitrogen source to *K. striatus*, thereby enhancing its growth.

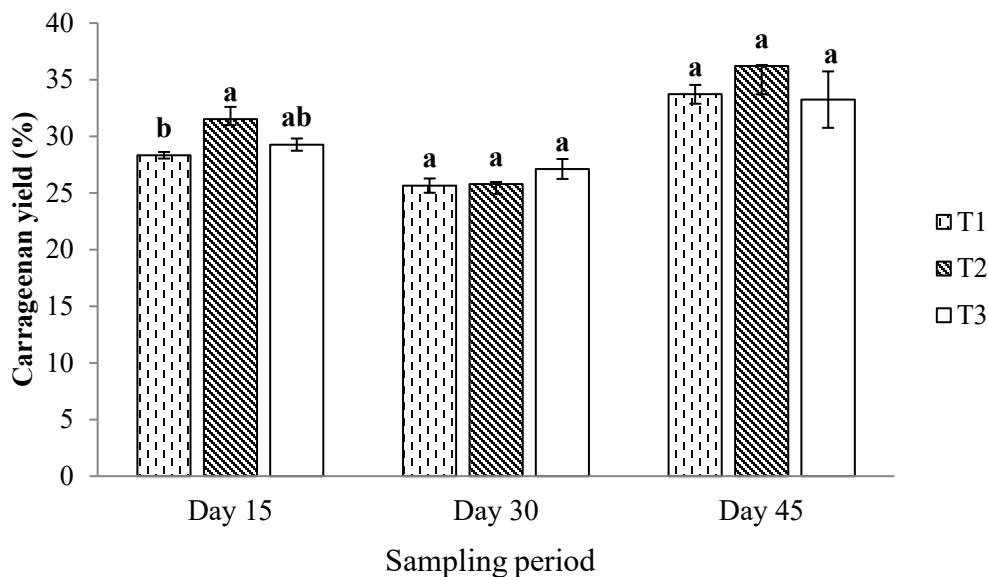


Figure 3. Carrageenan yield of *K. striatus* in every sampling. T₁=8.82 g L⁻¹ of urea, T₂=8.82 g L⁻¹ of phosphorus, and T₃=control. Bars with the same letters are not significantly different ($p>0.05$). Error bars in SEM (standard error mean), n=9.

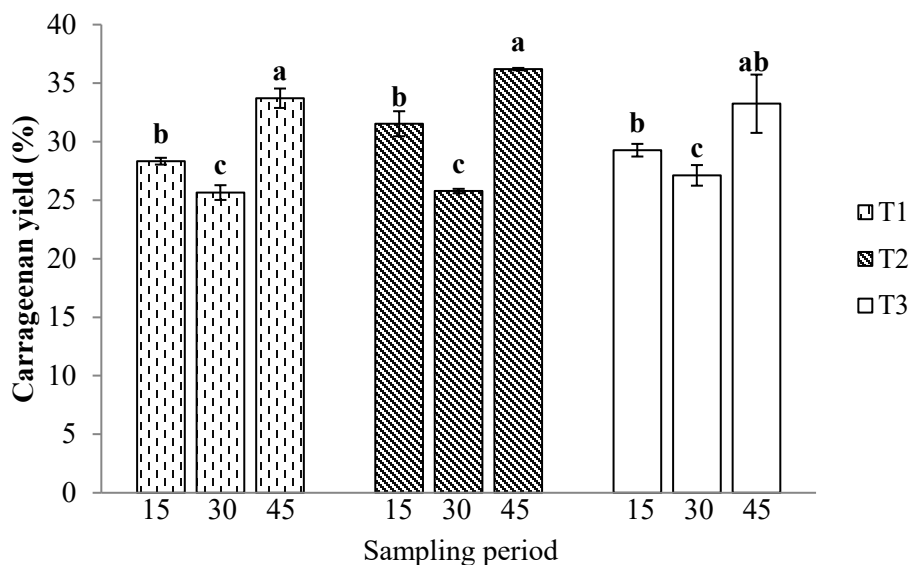


Figure 4. Change in alkali-treated carrageenan yield of *K. striatus* throughout the culture period. T₁=8.82 g L⁻¹ of urea, T₂=8.82 g L⁻¹ of phosphorus, and T₃=control. Bars with the same letters are not significantly different ($p>0.05$). Error bars in SEM (standard error mean), n=9.

On the other hand, stimulation of photosynthetic rates and growth of some algae can be improved by phosphorus enrichment (Lapointe, 1986; Villares et al., 1999; Martin et al., 2011). According to Xu et al. (2010), a high amount of carbon dioxide ($720\mu\text{l L}^{-1}$) and phosphorus ($30\mu\text{M}$) increased the growth of red alga *Gracilaria lemaneiformis* ranged from approximately 1.6 to 2.8% day^{-1} after 16 days cultured in the laboratory. Red alga *Agardhiella subulate* enriched with phosphorus ($6\mu\text{M}$) obtained an SGR of 0.025% day^{-1} (Chopin, 1990). According to Uchida (2000), phosphorus plays a vital role in the transfer of energy and other components of genetic information found in plant photosynthesis and respiration. In our study, phosphorus-enriched *K. striatus* achieved 2.40% day^{-1} growth after 45 days and was lower than the control, indicating that higher phosphorus concentrations may lead to slow growth of seaweed *K. striatus*. Excess phosphorus reduces the plant's ability to take up essential micronutrients, particularly zinc and iron (Provin & Pitt, 2008). They also noted that phosphorus' overuse could become water-soluble and mobile, entering surface water and causing the growth of algae and other undesirable plants. The suggested concentration of the phosphorus fertilizer based on its prescription on the label is 4.5 g L^{-1} . However, this study used 100% phosphate fertilizer (Seachem) with a high concentration of 8.82 g L^{-1} , an average concentration of ammonium phosphate used by the seaweed farmers in Sibutu, Tawi-Tawi, Philippines (Tahiluddin, 2018) in *K. striatus*, which may be the reason of obtaining slow growth of the seaweed.

Ice-Ice Disease Occurrence

Urea (46-0-0) and phosphorus (pure) inorganic nutrient enrichment had no effect in cultured *K. striatus* in terms of ice-ice disease occurrence. However, in other studies, nutrient enrichment reduced ice-ice disease occurrence. Luhan et al. (2015) used sodium nitrate (0.01 g L^{-1}) to reduce the occurrence of *K. alvarezii* ice-ice disease to 8.75% compared to untreated (97%). Ammonium phosphate (8.82 g L^{-1}) used in *K. striatus* significantly lowered the incidence of ice-ice disease by up to 42% compared to untreated (78%) planted during the ice-ice season (Tahiluddin, 2018). Therefore, when there is a combination of these nutrients, seaweed *K. striatus* may lessen ice-ice disease occurrence. Loureiro et al. (2009) showed that Acadian Marine Plant Extract Powder (AMPEP) fertilizers effectively reduced the occurrence of ice-ice disease and epiphytes infestation of *K. alvarezii* cultured in raft method.

The primary cause of the occurrence of ice-ice disease is due to adverse environmental factors such as nutrient insufficiency and high or low salinity, light intensity, and temperature (Largo, 2002; Tahiluddin & Terzi, 2021a; Tahiluddin &

Terzi, 2021b). The increased temperature of $33\text{-}35\text{ }^{\circ}\text{C}$ resulted in the paling and whitening of seaweeds (Largo et al., 1995a). Similar to the current study, where on day 7, the temperature of the farmed area was about $33\text{ }^{\circ}\text{C}$ which could cause the occurrence of ice-ice disease. Less than $50\mu\text{mol photon m}^{-2}\text{ s}^{-1}$ light intensity and less than 20% salinity could lead to the occurrence of ice-ice disease (Largo et al., 1995a). Pathogenic bacteria and fungi are other factors that cause ice-ice disease occurrence (Largo et al., 1995b; Solis et al., 2010; Tahiluddin et al., 2021a; Tahiluddin et al., 2021b). Slow water movement triggered the pathogenic bacteria to colonize the seaweed thalli can also cause ice-ice disease incidence (Largo, 2002).

The occurrence of ice-ice disease is high from May to August (Uyenco et al., 2019). In addition, seaweeds are also susceptible to ice-ice disease during the months of April, October, and December (Tisera & Naguit, 2009). This study was carried out between February and March, where the ice-ice disease appeared throughout the culture period. Intense heating and other environmental factors coupled with the presence of pathogenic microorganisms can cause the occurrence of ice-ice disease of cultivated *K. striatus*.

Production of seaweed, which has been affected by the ice-ice disease, has influenced seaweed farmers and the nation as a whole, particularly affected by the severe decline in production of aquaculture (Tisera & Naguit, 2009). The occurrence of ice-ice disease in seaweed farms could lead to a significant decline in seaweed production. (Doty & Alvares, 1975; Trono, 1993).

Carrageenan Yield

Carrageenan, extracted from red seaweeds and usually obtained by the extraction with water or alkaline water, is widely utilized in the food industry as thickening, gelling, and stabilizing agents, and as ingredients for pharmaceutical, cosmetic, personal care, and among others (Thirumaran et al., 2009; Hayashi et al., 2011; Ahmad, 2014; Husin, 2014). The main source of *kappa*-carrageenan is red alga *K. striatus* (Trono, 1997). Most *kappa*-carrageenan are produced by the presence of potassium ions under a process called potassium precipitation (McHugh, 1987). Inorganic nutrient enrichment used in the present study did not influence the carrageenan yield of *K. striatus* after 45 days of culture. In terms of the culture period, 45 days achieved the highest carrageenan yield compared to 30 and 15 days, but no significant differences were observed between treatments ($p>0.05$). On the contrary, Hurtado et al. (2008) obtained the highest carrageenan yield of *K. striatum* var. *sacol* on day 30 compared to 45 and 60 days. In addition, Hayashi et al. (2007b) revealed that the highest carrageenan yield was higher at day 28 compared

waters of Sao Paulo State, Brazil *Journal Applied Phycology*, 19, 393-399.

<https://doi.org/10.1007/s10811-913-6>

Hayashi, L., Oliveira, E., Bleicher-Lhonneu, G., Boulenguer, P., Pereira, R.T.L., Seckendorff, R. Shimoda, V., Leflamand, A., Vallée, P., Critchley, A. (2007b). The effects of selected cultivation conditions on the carrageenan characteristics of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) in Ubatuba Bay, São Paulo State, Brazil. *Journal of Applied Phycology*, 19, 505-511.

<https://doi.org/10.1007/s10811-007-9163-x>

Hayashi, L., Santos, A.A., Faria, G.S., Nunes, B.G., Souza, M.S., Fonseca, A.L., Barreto, P.L., Oliveira, E.C., Bouzon, Z.L. (2011). *Kappaphycus alvarezii* (Rhodophyta, Areschougiaceae) Cultivated in subtropical waters in Southern Brazil. *Journal of Applied Phycology*, 23(3), 337-343.

<https://doi.org/10.1007/s10811-010-9543-5>

Hurtado, A.Q., Agbayani, R.F., Sanares, R., Castro-Mallare, M.T. (2001). The seasonality and economic feasibility of cultivating *Kappaphycus alvarezii* in Panagatan Cays, Caluya, Antique Philippines. *Aquaculture*, 199(3-4), 295-310.

[https://doi.org/10.1016/S0044-8486\(00\)00553-6](https://doi.org/10.1016/S0044-8486(00)00553-6)

Hurtado, A., Critchley, A., Trespoey, A. (2008). Growth and carrageenan quality of *Kappaphycus striatum* var. sacol grown at different stocking densities, duration of culture and depth. *Journal of Applied Phycology*, 20, 551-555.

<https://doi.org/10.1007/s10811-008-9339-z>

Hurtado, A.Q., Iain, C.N., Alan, T.C. (2015). Developments in production technology of *Kappaphycus* in the Philippines: more than four decades of farming. *Journal of Applied Phycology*, 27(5), 1945-1961.

Husin, A.B. (2014). Extraction of kappa-carrageenan from local seaweeds. University Malaysia Pahang, 1-18.

<https://umpir.ump.edu.my/id/eprint/10682>

Lapointe, B.E. (1986). Phosphorus-limited photosynthesis and growth of *Sargassum natans* and *Sargassum fluitans* (Phaeophyceae) in the Western North Atlantic. *Deep-Sea Research*, 33(3), 391-399.

[https://doi.org/10.1016/0198-0149\(86\)90099-3](https://doi.org/10.1016/0198-0149(86)90099-3)

Largo, D.B. (2002). Recent developments in seaweed diseases. In; Hurtado, A.Q., Guanzon N.G., Castro-Mallare, de

Jr., T.R. & Luhan M.R.J. (Eds) *Proceedings of the National Seaweed Planning Workshop*. Philippines. pp. 35-42.

Largo, D.B., Fukami, K., Nishijima, T., Ohno, M. (1995a). Laboratory-induced development of ice-ice disease of the farmed red algae *Kappaphycus alvarezii* and *Eucheuma denticulatum* (Solieriaceae, Gigartinales, Rhodophyta). *Journal of Applied Phycology* 7(6), 539-543.

<https://doi.org/10.1007/Bf00003940>

Largo, D.B., Fukami, K., Nishijima, T. (1995b). Occasional pathogenic bacteria promoting ice-ice disease in the carrageenan producing red algae *Kappaphycus alvarezii* and *Eucheuma denticulatum* (Solieriaceae, Gigartinales, Rhodophyta). *Journal of Applied Phycology* 7(6), 545-554.

<https://doi.org/10.1007/Bf00003941>

Laurienzo, P. (2010). Marine polysaccharides in pharmaceutical applications: An overview. *Marine Drugs*, 8(9), 2435-2465.

<https://doi.org/10.3390/md8092435>

Loureiro, R.R., Reis, R.P., Critchley, A.T. (2009). In vitro cultivation of three *Kappaphycus alvarezii* (Rhodophyta, Areschougiaceae) variants (green, red and brown) exposed to a commercial extract of the brown alga *Ascophyllum nodosum* (Fucaceae, Ochrophyta) *Journal of Applied Phycology*, 22(1), 101-104.

<https://doi.org/10.1007/s10811-009-9412-2>

Luhan, M.R.J., Avañcena, S.S., Mateo, J.P. (2015). Effects of short-term immersion of *Kappaphycus alvarezii* (Doty) Doty in high nitrogen on the growth, nitrogen assimilation, carrageenan quality, and occurrence of ice-ice disease. *Journal of Applied Phycology*, 27(2), 917-922.

<https://doi.org/10.1007/s10811-014-0365-8>

Martins, A.P., Junior, O.N., Colepicolo, P., Yokota, N.S. (2011). Effects of nitrate and phosphate availabilities on growth, photosynthesis and pigment and protein contents in colour strain of *Hypineea musciformis* (Wulfen in Jacqu.) J.V. Lamour. (Gigartinales, Rhodophyta). *Revista Brasileira de Farmacognosia Brazilian Journal of Pharmacognosy*, 21(2), 340-348.

<https://doi.org/10.1590/S0102-695X2011005000078>

McHugh, D.J. (1987). Production, properties and uses of alginates. FAO Fisheries Technical paper 288, 58-115.

- Neish, A.C., Shacklock, P.F., Fox, C.H., Simpson, F.J. (1977). The cultivation of *Chondrus crispus*. Factors affecting growth under greenhouse conditions. *Canadian Journal Botany*, 55, 2263-2271.
<https://doi.org/10.1139/b77-256>
- Provin, T.L., Pitt, J.L. (2008). Phosphorus--Too much and plants may suffer. Produce by Agricultural Communications, The Texas A&M University System. 2pp.
<https://hdl.handle.net/1969.1/86793>
- Robles, R.J.F. (2020). Effects of different concentrations of ammonium phosphate on the yield and the quality of carrageenan, *Kappaphycus striatus* (Schmitz) Doty ex Silva. *Journal of Fisheries, Livestock and Veterinary Science*. 1(1), 1-9.
<https://doi.org/10.18801/jflvs.010120.01>
- Romero, J.B., Montaña, N.E., Merca, F.E., Rumbaoa, R.G.O., Villanueva, R.D. (2000). Effect of sucrose on some physical properties of different Phillipine agar. *Philippine Journal of Sciences*, 129(1), 7-13.
- Rui, L., Jiajun, L., Chaoyuan, W. (1990). Effect of ammonium on growth and carrageenan content in *Kappaphycus alvarezii* (Gigartinales, Rhodophyta). Thirteenth International Seaweed Symposium, 499-503.
https://doi.org/10.1007/978-94-009-2049-1_71
- Sahoo, D., Ohno, M. (2003). Culture of *Kappaphycus alvarezii* in deep seawater and nitrogen enrichment medium. *Bulletin of Marine Sciences and Fisheries*, 22, 89-96.
- Sekar, R., Thangaraju, N., Rengasamy, R. (1995). Effects of seaweed liquid fertilizers from *Ulva lactuca* on *Vigna unguiculata* L. (walp.), *Phykos*, 34, 49-53.
- Solis, M.J., Draeger, S., Dela Cruz, T.E. (2010). Marine-derived fungi from *Kappaphycus alvarezii* and *K. striatus* as potential causative agents of ice-ice disease in farmed seaweeds. *Botanica Marina*, 53(6), 587-594.
<https://doi.org/10.1515/bot.2010.071>
- Tahiluddin, A.B. (2018). Influence of fertilization on the occurrence of *Vibrio*, “ice-ice” disease and growth of seaweed *Kappaphycus striatus* (F. Schmitz) Doty ex P.C. Silva. [M.Sc. Thesis. University of the Philippines Visayas].
- Tahiluddin, A.B., Terzi, E. (2021a). Ice-ice disease in commercially cultivated seaweeds *Kappaphycus* spp. and *Eucheuma* spp.: A review on the causes, occurrence, and control measures. *Marine Science and Technology Bulletin*, 10(3), 234-243.
<https://doi.org/10.33714/masteb.917788>
- Tahiluddin, A., Terzi, E. (2021b). An overview of fisheries and aquaculture in the Philippines. *Journal of Anatolian Environmental and Animal Sciences*.
<https://doi.org/10.35229/jaes.944292>
- Tahiluddin, A.B., Nuñal, S.N., Luhan, M.R.J., Santander-de Leon, S.M.S. (2021a). *Vibrio* and heterotrophic marine bacteria composition and abundance in nutrient-enriched *Kappaphycus striatus*. *Philippine Journal of Science*, 150(6B), 1549-1761.
- Tahiluddin, A.B., Alawi, T.I., Hassan, N.S.A., Jaji, S.N.A., Terzi, E. (2021b). Abundance of culturable heterotrophic marine bacteria in *Ulva lactuca* associated with farmed seaweeds *Kappaphycus* spp. and *Eucheuma denticulatum*. *Journal of Agricultural Production*, 2(2), 44-47.
<https://doi.org/10.29329/agripro.2021.360.1>
- Tariq, A., Pan, K., Olatunji, O.A., Graciano, C., Li, Z., Sun, F., Zhang, L., Wu, X., Chen, W., Song, D., Huang, D., Xue, T., Zhang, A. (2018). Phosphorus fertilization alleviates drought effects on *Alnus cremastogyne* by regulating its antioxidant and osmotic potential. *Scientific Report*, 8, 5644.
<https://doi.org/10.1038/s41598-018-24038-2>
- Thirumaran, G., Arumugan, M., Arumugan, R., Anantharaman, P. (2009). Effect of seaweed liquid fertilizer on growth and pigment concentration of *Cyamopsis tetragonoloba* (L) Taub. *American-Eurasian Journal of Agronomy*, 2(2), 57-66.
- Tisera, W.L. Naguit, M.R.A. (2009). Ice-ice disease occurrence in seaweed farms in Bais Bay, Negros Oriental and Zamboanga del Norte. *The Threshold*, 4, 1-16.
- Topinka, J.A., Robbins, J.V. (1976). Effect of nitrate and ammonium enrichment on growth and nitrogen physiology in *Fucus spiralis*. *Limnology and Oceanography*, 21(5), 659-664.
<https://doi.org/10.4319/lo.1976.21.5.0659>
- Trono G.C. Jr. (1992). *Eucheuma* and *Kappaphycus*: taxonomy and cultivation. *Bulletin Marine Science Institute College of Science*, 12, 51-65.

Trono G.C. Jr. (1993). Effects of biological, physical and socio-economic factors on the productivity of *Eucheuma/Kappaphycus* farming industry. In: Calumpong H.P. and Menez E.G. (eds), Proc. Second RP-USA Phycology Symp./Workshop., Cebu City and Dumaguete City, Philippines 239-245.

Trono G.C. Jr. (1997). Field Guide and Atlas of the Seaweeds Resources of the Philippines. Bookmark, Inc, Makati City, Philippines 302 pp.

Uchida, R. (2000). Essential Nutrients for Plant Growth: Nutrient Functions and Deficiency Symptoms R. Uchida. From Plant Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture and Human Resources, University of Hawaii at Manoa.

Uyenco, F.R., Saniel, L.S., Jacinto, G.S. (2019). The ice-ice problem in seaweed farming. In Levring T (eds.), Proc. Tenth Int. Seaw. Symp. Walter de Gruyter & Co., Berlin, 625-630. <https://doi.org/10.1515/9783110865271-084>

Villares, R., Puente, X., Carballeira, A. (1999). Nitrogen and phosphorus in *Ulva* sp. in the Galician Rias Bajas (north-west Spain): Seasonal fluctuations influence on growth. *Boletín-Instituto Español de Oceanografía*, 15 (1-4), 337-341.

Zhiguang, X., Zou, D., Gao, K. (2010). Effect of elevated CO₂ and phosphorus supply on growth, photosynthesis and nutrient uptake in the marine macroalgae *Gracilaria lemaneiformis* (Rhodophyta). *Botanica Marina*, 53, 123-129. <https://doi.org/10.1515/BOT.2010.012>