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Original Research Article

Cultivation of Native Tissue-Cultured *Kappaphycus alvarezii* Plantlets at Multiple Sites in The Myeik Archipelago, Myanmar

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ABSTRACT

Commercial cultivation of *Kappaphycus alvarezii* (Cottonii) offers opportunities for Myanmar's coastal economy through sustainable aquaculture and economic development. This study assessed the feasibility of large-scale *K. alvarezii* cultivation in the Myeik Archipelago using tissue-cultured plantlets. Growth performance was evaluated across three sites - Pyin Htet Aw, Thae Chaung, and Don Pale Aw - and the impact of environmental conditions on growth and productivity was examined. Tissue-cultured *K. alvarezii* plantlets were cultivated at three sites over five weeks. Growth performance and daily growth rate (DGR), along with environmental parameters including temperature and salinity, were monitored. Carrageenan yield and viscosity were measured to evaluate seaweed quality. Growth performance varied across sites, with DGRs ranging from 9.10 to 9.32%. Don Pale Aw yielded the highest biomass (963.00 ± 336.19 g), while Thae Chaung had the lowest (848.00 ± 152.65 g). Salinity significantly influenced growth and carrageenan yield, with higher salinities (31.25–33.15 PSU) supporting greater productivity. Growth remained stable across the temperature range (27.05–31.35°C), indicating a slight thermal tolerance over the previously established ideal range (27–29°C). The fresh-to-dry weight ratio was consistent across sites, confirming stable biomass conversion efficiency. Tissue-cultured plantlets exhibited strong adaptability to local environmental conditions, supporting the viability of large-scale *K. alvarezii* farming in Myanmar. Selecting sites with optimal environmental conditions (such as moderate hydrodynamics, stable salinity, high light availability, and favorable temperatures) and combining this with sustainable seaweed farming techniques has the potential to enhance local livelihoods, diversify the economy and advance Myanmar's aquaculture sector.

Keywords*:* Cottonii, Carrageenan, *Kappaphycus alvarezii,* Marine aquaculture, Myanmar Coastal Farming, Seaweed Cultivation, Tissue-Cultured Plantlets.

Introduction

The commercial cultivation of *Kappaphycus alvarezii* (commonly known as Cottonii) is increasing due to its major constituent, carrageenan, which has applications as a gelling, thickening, and stabilizing agent in food products, pharmaceutical formulations, and cosmetics.[1] The commercial success of *K. alvarezii* production in the Philippines and Indonesia has led to its cultivation in over 50 countries across Southeast Asia and China, the Pacific islands, the Western Indian Ocean, Central America, and South America.[2] Seaweed cultivation in Myanmar began in the early 2000s, led by a private enterprise producing carrageenan. From 2004 to 2012, cultivation of *K. alvarezii* was concentrated in the Myeik Archipelago (also known as the Mergui Archipelago), where the region's calm, tropical waters provided suitable growing conditions.[3] Initial seedlings were sourced from Cambodia, likely originating in Vietnam, with subsequent supplies from Indonesia and the Philippines.[2,4] By the late 2000s, commercial production had expanded, particularly during the prime growing season from November to April. However, despite this early growth, production remained modest.[5] Cultivation ceased in 2012 due to recurring outbreaks of ice-ice disease and infestations of endo-epiphytes.[2-4] Since then, efforts to revive the industry have been hindered by limited access to new planting material. In 2021, to meet renewed demand, tissue-cultured plantlets were produced locally from seed.[3] These seed-derived plantlets, which show greater resistance than those propagated through vegetative fragmentation, have since been trialed in Myanmar's open waters.

The present study investigated the cultivation of *K. alvarezii* derived from locally tissue-cultured plantlets at three open sea sites near Pyin Htet Aw, Thae Chaung, and Don Pale Aw villages. The primary objective is to establish a reference for the commercial cultivation of *K. alvarezii* in Myanmar, promoting the country's potential as a viable seaweed producer, while offering sustainable economic opportunities for coastal communities.

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Materials and Methods

Study area

The seaweed cultivation trials were conducted in coastal waters near Pyin Htet Aw village (12°33'06.9"N and 98°28'27.9"E), Thae Chaung village (11°31'14.0"N and 98°16'42.4"E), and Don Pale Aw village (12°20'34.0"N and 98°03'45.8"E) located in Myeik, Tanintharyi, Myanmar (Figures 1A-1B). The trials were conducted from September 2023 to March 2024.

Seaweed seedling preparation

The trial used *K. alvarezii* plantlets regenerated by tissue culture at the Seaweed Research Department in Myeik, Tanintharyi, Myanmar.

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Young *K. alvarezii* plantlets (Figure 2A), weighing between 0.5 and 1.0 g, were initially placed in floating frames as described by Hlaing and Jarukamjorn (2024).[3] Once the plantlets reached approximately 40 g (Figure 2B), they were used as seedlings for seaweed cultivation in multiple sites in Myanmar's open sea.

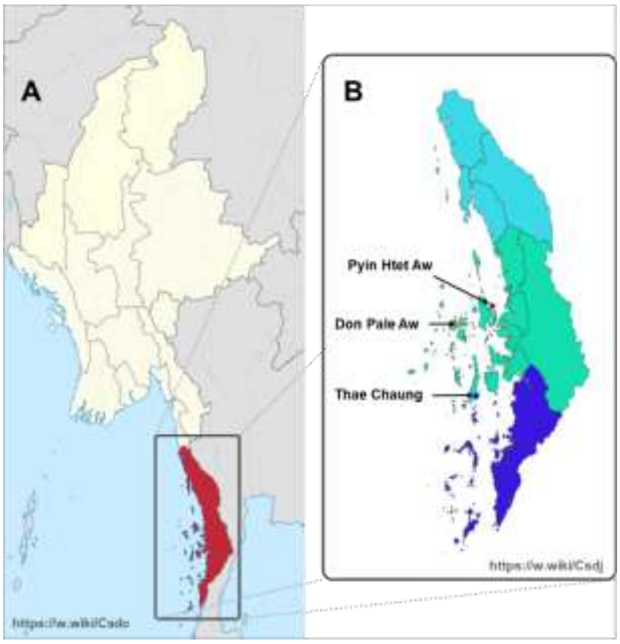

Figure 1: The map (A-B) displays three sites for *K. alvarezii* cultivation in Myeik, Tanintharyi, Myanmar.

Plantlet planting in the floating frame

A floating frame was used to plant young *K. alvarezii* plantlets. The plantlets, with an average weight of 0.67 ± 0.15 g, were placed in perforated plastic bottles suspended across the floating frame (Figures 3A-3C). Once a week, the bottles were cleaned to remove dirt and algae. The plantlets were weighed weekly until they reached around 5 - 10 g, after which they were tied to ropes and hung on the floating frame to continue growing (Figures 3D-3E). This process aimed to support young plantlet development before planting in the long-line frame, to help prevent detachment during the early growth stage.

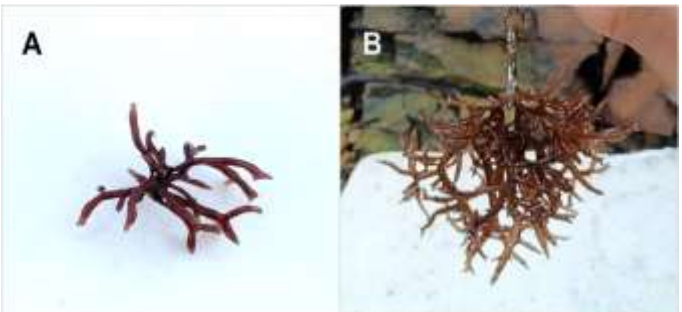

Figure 2: Young plantlets (A) and growing plantlets (B) of *K. alvarezii* regenerated by tissue culture at the Seaweed Research Department in Myeik Archipelago, Tanintharyi, Myanmar.

Seaweed cultivation in the long-line frame

The long-line cultivation method recommended by Simatupang et al. (2021) and Gurunathan et al. (2021) was used.[1-2] The frame was 50 m^2 and consisted of 50 single lines of 4-10 mm-polypropylene rope, each 50 m long and spaced 1 m apart (Figure 4). Each line held 150 seedlings (average weight 40 g) tied at 30 cm-intervals. The lines were floated with plastic bottles and the frame was anchored in the sea with wooden posts.

Seaweed seedlings weighing 40.90 ± 11.04 g, 40.19 ± 6.91 g, and 40.86 ± 6.19 g were tied to ropes using the tie-tie technique.[6] These ropes were transported to Pyin Htet Aw, Thae Chaung, and Don Pale Aw, where the seedlings were individually hung on long-line frames (Figures 5A-5C). The cultivation period lasted five weeks. Cleaning and maintenance were performed two to three times weekly. Seawater parameters such as temperature and salinity were monitored weekly.

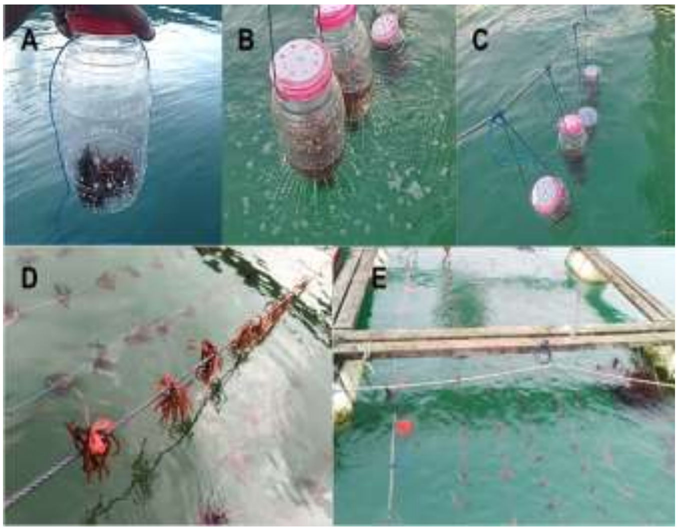

Figure 3: Plantlets in holed plastic bottles (A-C) and tied to ropes (D-E) across the floating frame.

Data observation

The initial weights of the seaweeds were recorded, and growth was monitored weekly for five weeks. The daily growth rate (DGR) was calculated using the formula: $DGR\ (\%day^{-1}) = \left[(W_t/W_0)^{1/t} - 1\right] \times 100\%$, where W_0 and W_t are the initial and the final weights after t days.[7] The fresh-to-dry weight ratio (Fw/Dw) was also determined at each site. Data were collected from ten seedlings per site.[8-9]

Semi-refined carrageenan extraction and physical characteristics

Semi-refined carrageenan content was determined using a potassium hydroxide extraction method.[10] Seaweeds were rinsed with fresh water, dried, and ground into powder. The powder was treated with a 6% potassium hydroxide solution at 1:10 ratio and stirred with a magnetic stirrer at 70°C for 30 minutes to dissolve alkali-soluble sugars. The mixture was centrifuged at 1,800 ×*g* for 5 minutes and the solid residue was rinsed with deionized water to eliminate potassium hydroxide before being dried and powdered to yield semi-refined carrageenan. Carrageenan content was calculated as the percentage of carrageenan per gram of dried seaweed.[11]

The powder was dissolved in deionized water (70 to 80°C) and stirred to obtain 1.5% semi-refined carrageenan solution. Viscosity was measured using a rheometer (HAAKE RotoVisco 1, Thermo Scientific®, Rheology Solutions Pty Ltd., Australia) at 75 ± 5°C with a shear rate of 100 to 500 s^{-1}. The viscosity (η), shear stress (τ), and shear rate (γ) were measured in triplicate to generate viscosity and flow curves.

Statistical analysis

All the results are presented as average ± standard deviation. Statistical analysis was performed using Statistical Package for the Social Sciences version 26.0 (Armonk, New York, USA) with one-way Analysis of Variance and Tukey's *post hoc* test at a significance level of $p<0.05$.

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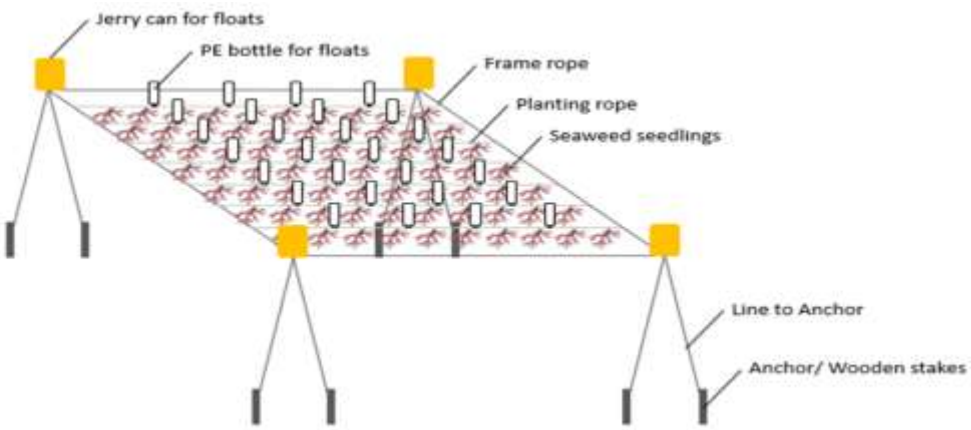

Figure 4: Diagram of the long-line raft method for *K. alvarezii* cultivation.

Figure 5: Cultivation of *K. alvarezii* seaweeds in Myanmar's open seas using a long-line frame (A-C).

Results and Discussion

Environmental variation at the cultivation sites

Kappaphycus alvarezii was successfully cultivated in the coastal waters of the Myeik Archipelago, Myanmar, over a five-week period. During the cultivation period, seawater parameters (temperature and salinity) were monitored at the three cultivation sites: Pyin Htet Aw, Thae Chaung, and Don Pale Aw. From September 2023 to March 2024, seawater temperatures at Pyin Htet Aw ranged from 27.75 to 31.35°C, with the lowest temperature observed in November 2023 and the highest in March 2024. At Thae Chaung, temperatures ranged from 27.85 to 30.85°C, with the minimum in September 2023 and the maximum in March 2024. At Don Pale Aw, temperatures ranged from 27.05 to 29.50°C, with the lowest recorded in December 2023 and the highest in March 2024 (Figure 6A). Table 1 presents the average DGRs in Pyin Htet Aw (9.28 ± 0.40%), Thae Chaung (9.10 ± 0.14%), and Don Pale Aw (9.32 ± 0.60%). The fresh-to-dry weight ratios (Fw/Dw) were 9.68 ± 0.22, 9.60 ± 0.30, and 9.77 ± 0.40, respectively.

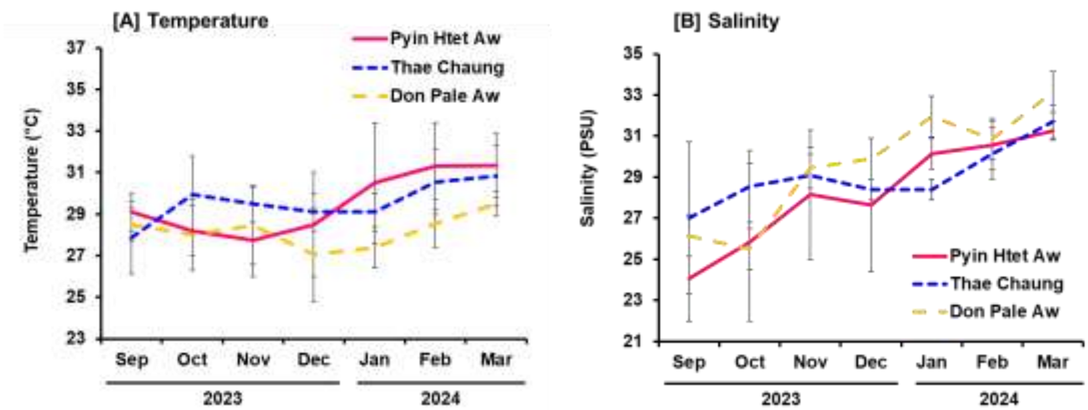

Figure 6: Environmental variations in sea temperature (A) and salinity (B), at the three cultivation sites in Myeik Archipelago, Myanmar, from September 2023 to March 2024.

Temperature across the sites varied within a narrow range (27.05 – 31.35°C), and growth rates (Table 1) remained consistent despite these fluctuations. Don Pale Aw showed the highest growth rate (9.32 ± 0.60%), followed by Pyin Htet Aw (9.28 ± 0.40%), and Thae Chaung (9.10 ± 0.14%). This suggests that *K. alvarezii* can tolerate slight temperature variations within its optimal temperature range without significant impact on growth. Previous studies suggest an ideal temperature of 27 – 29°C, though *K. alvarezii* can tolerate 25 – 30°C.[12-13]

Don Pale Aw (27.05 – 29.50°C) showed the best growth (9.32 ± 0.60%) and Fw/Dw ratio (9.77 ± 0.40), consistent with these findings.

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Salinity was lowest in September 2023, ranging from 24.05 PSU at Pyin Htet Aw, 27.00 PSU at Thae Chaung, and 25.50 PSU at Don Pale Aw (Figure 6B). The highest salinity values were observed in March 2024, ranging from 31.25 PSU at Pyin Htet Aw to 31.70 PSU at Thae Chaung and 33.15 PSU at Don Pale Aw. All three cultivation sites exhibited seawater conditions suitable for *K. alvarezii* cultivation.[3] Despite variations in temperature and salinity across the cultivation sites, *K. alvarezii* exhibited comparable growth rates. This consistency suggests that other environmental factors may have played an important role in supporting growth. Our observations indicated that light availability, water movement, and concentrations of nitrate and phosphate were consistently favorable across all sites. In particular, moderate current flow likely enhanced nutrient uptake and gas exchange, supporting steady growth. These conditions may explain the comparable productivity observed across the different locations.

The ideal salinity range for *K. alvarezii* growth is 30 to 35 PSU.[14-15] This study found that growth occurred at lower salinities (24 – 27 PSU), indicating some tolerance but reduced productivity. Gurunathan et al. (2021) reported that salinities below 25 PSU can reduce carrageenan yield due to osmotic stress.[2] In our study, Pyin Htet Aw (24.05 PSU) had a slightly lower Fw/Dw ratio (9.68 ± 0.22) than Don Pale Aw (25.50 PSU, 9.77 ± 0.40), reflecting this trend. Seasonal variation in salinity was evident, with the lowest values in September 2023 and the highest in March 2024. Higher salinity levels significantly influenced both carrageenan yield and the Fw/Dw ratio, consistent with previous studies.[16-17] Carrageenan production peaked during the period of high salinity in March 2024, when salinity levels reached 31.25 – 33.15 PSU across the three sites. This increase in yield at elevated salinity could be attributed to various physiological and biochemical responses in *K. alvarezii*. Moderate to high salinity is known to stimulate the production of polysaccharides like carrageenan as a protective response against osmotic stress. This process helps maintain intracellular osmotic balance and reinforces cell wall integrity under saline conditions. Elevated salinity has also been associated with increased activity of enzymes such galactosyltransferases, which are involved in carrageenan biosynthesis. These combined mechanisms contribute to the higher yield and improved gelling properties of carrageenan under saline environments.[18] Hayashi et al. (2011) and Rupert et al. (2022) also reported that salinity levels between 30 – 35 PSU enhanced carrageenan synthesis, likely due to enhanced metabolic activity triggered by osmotic stress.[14,18] According to van Oort et al. (2025), salinity has a considerable impact on the metabolic activity of *K. alvarezii*, affecting both growth and carrageenan yield.[19]

Conversely, the lower salinities observed in September 2023 (24.05 – 27.00 PSU) corresponded with reduced carrageenan yields. This suggests that while *K. alvarezii* can tolerate a broad salinity range, suboptimal levels below 30 PSU may impair polysaccharide production, resulting in lower yield and diminished quality. To mitigate this, future cultivation strategies could explore the development of salt-tolerant strains, the use of adaptive farming techniques, or the integration of salinity control in land-based systems to maintain carrageenan productivity. Nevertheless, carrageenan yield is also affected by other environmental and cultivation factors such as nutrient availability.[20] For example, although Pyin Htet Aw and Thae Chaung had similar salinities, their carrageenan yields differed slightly, presumably due to local nutrient dynamics and variations in other site-specific conditions.[16,21]

Biomass production and daily growth rate (DGR)

The biomass of *K. alvarezii* at the three cultivation sites ranged from 848.00 ± 152.65 g at Thae Chaung to 963.00 ± 336.19 g at Don Pale Aw (Figure 7A). After the first week, Pyin Htet Aw showed the highest growth rate, while Thae Chaung had the lowest. By the fifth week, growth rates at all three sites had become nearly comparable (Figure 7B).

The lowest biomass was recorded at Thae Chaung, while Pyin Htet Aw and Don Pale Aw exhibited higher biomass. The DGR values in the present study were slightly lower than the 10.2% previously reported for tissue-cultured seeds in Myanmar(Hlaing & Jarukamjorn, 2024).[3] However, the growth rates observed here were comparable to earlier findings in South East Sulawesi: 4.6% and 7.01%,[22-23] Malaysia: 6.3%,[24] and the Philippines: 5.8 – 7.2%.[25] The growth rates reported here are also higher than those of commercial operations in several countries, including 3.76% in India,[26] 4.07% in Brazil,[14] and 5.46% in Madagascar.[27] The Fw/Dw ratios did not differ significantly across the three cultivation sites, although they were slightly higher than those of 0.17 and 9.42 reported in Indonesia.[22-23]

Table 1: The growth rate and fresh-to-dried weight ratio of *Kappaphycus alvarezii* seaweeds in three cultivation sites in Myeik Archipelago, Myanmar during 5-week cultivation period**.**

Cultivation sites	Initial weight (g)	Final weight (g)	DGR (%w/w)	Fw/Dw
Pyin Htet Aw	40.90 ± 11.04	930.50 ± 306.02	9.28 ± 0.40	9.68 ± 0.22
Thae Chaung	40.19 ± 6.91	848.00 ± 152.65	9.10 ± 0.14	9.60 ± 0.30
Don Pale Aw	40.86 ± 6.19	963.00 ± 336.19	9.32 ± 0.60	9.77 ± 0.40

DGR: the daily growth rate; Fw/Dw: the fresh-to-dry weight ratio.

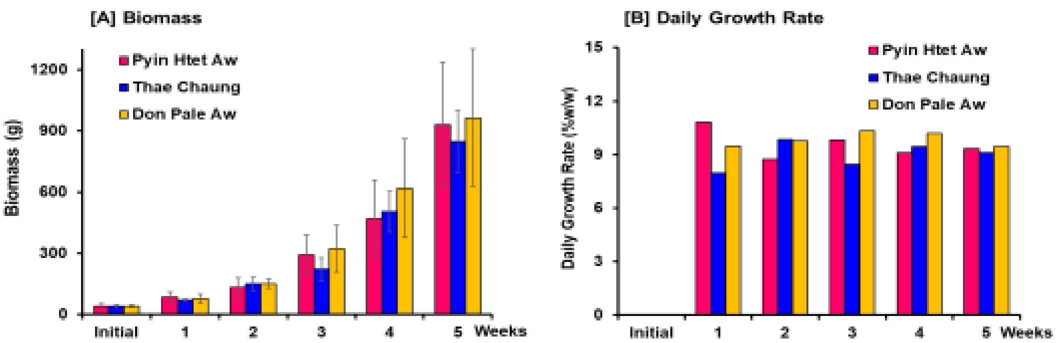

Figure 7: Biomass production (A) and daily growth rate (B) of *K. alvarezii* seedlings over the cultivation period at the three sites in the Myeik Archipelago, Myanmar.

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Carrageenan yields

The carrageenan content of *K. alvarezii* from the three cultivation sites was examined in triplicate. Figure 8 shows the carrageenan yield from each site. The yields were 81.59 ± 5.57% at Pyin Htet Aw, 81.90 ± 6.71% at Thae Chaung, and 67.17 ± 13.98% at Don Pale Aw. Previous research has reported semi-refined carrageenan yields from *K. alvarezii*

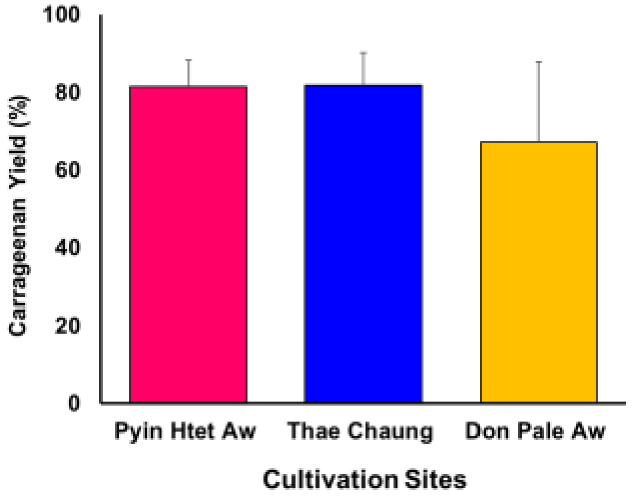

Figure 8: Carrageenan production from *K. alvarezii* at three cultivation sites in the Myeik Archipelago, Myanmar.

of 8.6 to 50%, depending on extraction methods and seaweed quality. Some studies report yields of up to 60% of total biomass: 18.8 – 26.4% in Vietnam,[9] 8.6 – 43.9% in Indonesia,[1] 20 – 32% in Brazil,[16] 30.3 – 40.7% in Mexico,[28] 27.6 – 43.5% in Japan,[29] 44.77 – 63.96% in Indonesia,[30] and 56.9 – 58.0% in China.[31] In comparison, carrageenan production from *K. alvarezii* in this study ranged from 67.17% – 81.90% of the total seaweed biomass, substantially higher than most reported values.

Carrageenan viscosity (η) and shear stress (τ)

The 1.5% semi-refined carrageenan solutions from each site were measured at 75°C under varying shear rates (100 to 500 s⁻¹). The viscosity and the flow curves are presented in Figures 9A and 9B, respectively. The viscosity values obtained in this study align with the range reported for *K. alvarezii* carrageenan from Indonesia (6.1 – 183 cP)[1] but are lower than those reported for Vietnam (83 – 128 cP)[9] and Mexico (110 – 186.8 cP).[28] Notably, the values observed in this study are substantially lower than the maximum viscosity of 890 cP that was reported for Japan.[29] Such variation may result from differences in environmental conditions (water temperature, salinity, and nutrients) and processing factors such as alkali treatment, extraction temperature, and purification methods.[1,28] Pyin Htet Aw exhibited the highest viscosity, indicating that it might provide favorable conditions for the production of carrageenan with superior rheological properties.

Viscosity of the 1.5% semi-refined carrageenan solution decreased with increasing shear rate, indicating a shear-thinning behavior. This non-Newtonian flow characteristic is typical of polysaccharides like carrageenan.[32-33] As shear rate increases, polymer chains align in the flow direction, reducing internal resistance.[34] At low shear rates (100 - 200 s⁻¹), the viscosity was relatively high, indicating that the carrageenan solution maintained a more stable, entangled structure. As the shear rate increased (300 - 500 s⁻¹), the viscosity decreased, indicating that the shear forces were strong enough to overcome the molecular interactions and facilitate more fluid flow.[32-35] This behavior is consistent with previous studies on carrageenan solutions, which typically show shear-thinning properties at similar concentrations and temperatures.[36-37] The non-linear shear-thinning behavior observed in this study suggests complex rheological characteristics such as yield stress or thixotropic effects worthy of further investigation.

The consistency and reproducibility of these findings indicate that the experimental conditions were robust and reliable for evaluating carrageenan viscosity. Overall, *K. alvarezii* could be effectively cultivated within the specified temperature and salinity ranges, with only subtle variations in growth and biomass between sites. While Don Pale Aw showed marginally better performance, all three sites demonstrated favorable conditions for commercial cultivation. These findings confirm that *K. alvarezii* can be commercially cultivated in Myanmar seawater using local tissue-cultured plantlets. These plantlets can be distributed widely from a continual supply of tissue-cultured seeds to develop Myanmar's coastal regions through sustainable aquaculture and raise the standard of living for local communities.

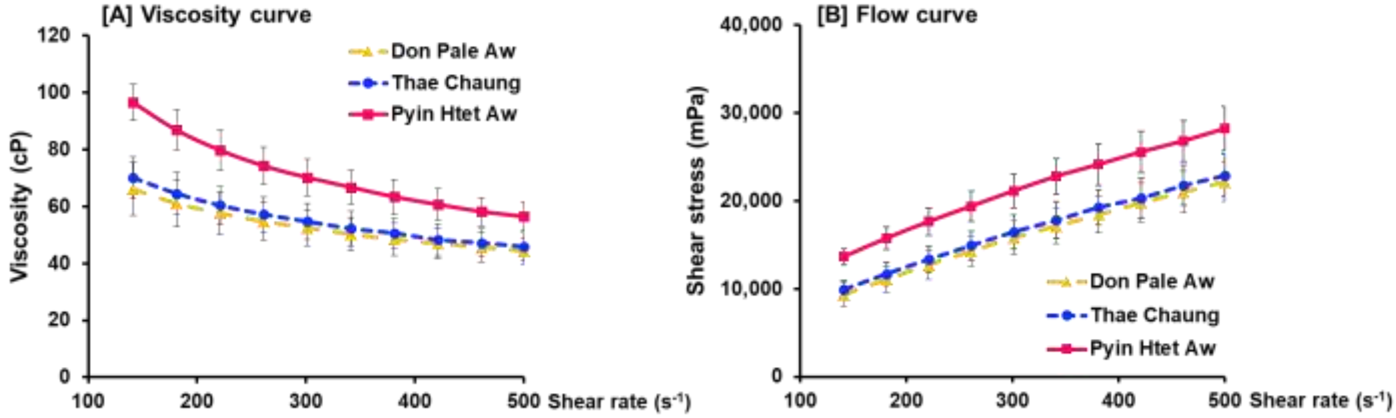

Figure 9: Viscosity (A) and the flow (B) curves of 1.5% semi-refined carrageenan solutions from three cultivation sites in the Myeik Archipelago, Myanmar.

Conclusion

The cultivation of *K. alvarezii* utilizing local tissue-cultured plantlets at various locations across Myanmar's Myeik Archipelago demonstrated successful growth and biomass production under a range of environmental conditions. Temperature and salinity significantly impacted growth and yield. Don Pale Aw, with temperatures of 27.05 to 29.50°C and 25.5 PSU salinity, showed the highest growth rate (9.32 ± 0.60%) and Fw/Dw ratio (9.77 ± 0.40). These results confirm the effectiveness of native tissue-cultured plantlets and support their suitability for large-scale farming in Myanmar's coastal waters. The Myeik Archipelago offers favorable conditions for commercial *K. alvarezii* cultivation, with further improvements possible through research on nutrient dynamics, seasonal variability, and farming methods. Challenges such as epiphytic algal infestations and fish grazing remain obstacles to open-sea farming, highlighting the need for better farm management practices, biosecurity measures, and selective breeding. Long-term monitoring of seasonal and environmental factors is essential to optimize productivity and ensure sustainability. This study provides valuable insights for both scientific research and commercial production. Sustainable seaweed farming in Myanmar has the potential to enhance local livelihoods, support coastal economies, and contribute to global carrageenan markets. To maximize community benefits, future efforts should focus on improved cultivation techniques, efficient post-harvest processing, and developing value-added seaweed-based products. Key improvements include efficient and hygienic drying systems (e.g., solar dryers or raised drying racks), better handling practices to reduce physical damage, and proper storage to maintain carrageenan quality. These measures will help preserve the seaweed's bioactive properties, enable farmers to meet export quality standards, improve profitability, and reduce losses.

Conflict of Interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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