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A preliminary study on the effects of seagrass wrack extract as biofertilizer on the growth, ice-ice disease, nitrogen, and phosphorus assimilation and carrageenan quality of eucheumatoid seaweed *Kappaphycus striatus*

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ABSTRACT

Eucheumatoid seaweed farming is a significant aquaculture activity, not only supplying carrageenan to the global market but also serving as a vital livelihood source for many marginalised coastal communities, particularly in Tawi-Tawi, Philippines. However, the practice of applying chemical fertilisers in eucheumatoid seaweed farming to boost production by enhancing growth performance and mitigating ice-ice disease has become a contentious issue among local stakeholders in this region. This preliminary, proof-of-concept study investigated the potential of utilising seagrass wrack (*Thalassia hemprichii*) as a biofertilizer alternative, evaluating its effects on growth, ice-ice disease prevalence and intensity, nitrogen and phosphorus assimilation, and carrageenan quality (yield and gel strength) in the eucheumatoid seaweed *Kappaphycus striatus*. The experiment employed various concentrations of seagrass wrack extract (SWE): 0 mL L^{-1} (control), 9 mL L^{-1}, 18 mL L^{-1}, and 27 mL L^{-1}. The results revealed no significant effects of SWE on growth or ice-ice disease prevalence and intensity (number and length of ice-ice spots per bundle) after 15, 30, and 45 days of cultivation. Nitrogen and phosphorus assimilation did not significantly impact SWE. Additionally, no impact on gel strength was observed after 45 days. Interestingly, a significant difference was detected in carrageenan yield, with the 27 mL L^{-1} SWE treatment exhibiting a notably higher yield compared to all other treatments at the 45-day mark. While this study demonstrates the potential of SWE to enhance carrageenan yield, its lack of significant effects on *K. striatus* growth and health raises concerns about its overall suitability as a biofertilizer. Therefore, further research is warranted to explore the potential optimisation of the seagrass wrack extract, investigate the use of alternative seagrass wrack species or combinations, and identify strategies to improve the overall effectiveness of SWE as a biofertilizer.

Keywords: Biofertilizer, Carrageenan, Eucheumatoid seaweed farming, *Kappaphycus*, Seagrass wrack

Introduction

Kappaphycus is among the most widely cultivated eucheumatoid seaweeds globally, driven by the growing demand for carrageenan (Bindu & Lavine, 2011). Carrageenan, a polysaccharide derived from red algae like *Kappaphycus*, holds substantial commercial value across diverse sectors, including pharmaceuticals, food, cosmetics, printing, and textiles (Rupert et al., 2022). The cultivation of eucheumatoid seaweeds, particularly *Kappaphycus* and *Eucheuma*, is prevalent in tropical regions like Indonesia, the Philippines, and Malaysia, and in other parts of the world (Hayashi et al., 2017).

In the Philippines, seaweed farming, especially of *Kappaphycus*, provides vital livelihoods for many coastal and marginalised communities (Tahiluddin & Terzi, 2021a; Tahiluddin et al., 2023). Locals also consume these seaweeds as salads due to their nutritional benefits (Dumilag, 2019; Ajik & Tahiluddin, 2024; Tahiluddin et al., 2025). Consequently, seaweeds are the leading aquaculture species in the Philippines by volume, contributing significantly to the national economy (Tahiluddin & Terzi, 2021a). Despite this, farmers face challenges such as slow growth of farmed eucheumatoid seaweeds, particularly *Kappaphycus*, often attributed to poor seedling quality (Luhan et al., 2015) and increased susceptibility to diseases (Faisan et al., 2021; Tahiluddin & Eldani-Tahiluddin, 2024; Faisan et al., 2024; Tahiluddin & Terzi, 2024). Presumptive nutrient deficiencies in farms have led some farmers to use chemical fertilisers to enhance nutrient levels covertly (Tahiluddin et al., 2022a; Tahiluddin & Roleda, 2025).

Historically, nutrient enrichment in *Kappaphycus* farming was not practised, with farms relying solely on the natural nutrient availability. However, after decades of cultivation, nutrient depletion has become evident, leading to slow growth and frequent disease outbreaks among *Kappaphycus* cultivars (Tahiluddin et al., 2022a). Since 2012, inorganic nutrient enrichment has emerged as a practice in the southern Philippines, with farmers increasingly using commercial chemical fertilisers such as ammonium phosphate (16-20-0, N-P-K) and complete fertiliser (14-14-14, N-P-K) to enhance growth and mitigate ice-ice disease, thus enhancing production and profitability (Tahiluddin et al., 2022a). Nonetheless, the Philippine National Standard on “Seaweeds – Code of Good Aquaculture Practices (GAqP)” discourages the use of chemical fertilisers in seaweed farms (BAFS, 2021), highlighting the need for alternative solutions like biofertilizers.

Seagrass wrack presents a promising biofertilizer option. Research has demonstrated that biofertilizers derived from seagrasses can effectively improve agricultural plants (Parente et al., 2013; Grassi et al., 2015; Mininni et al., 2015; Kavitha, 2017; Muniswami et al., 2021). Seagrass wrack, which poses environmental and economic challenges, can be composted and converted into biofertilizer for terrestrial plants, thus reducing reliance on inorganic fertilisers (Emadodin et al., 2020; Mainardis et al., 2021). Given that seagrass wrack is accumulating on the beaches of Sibutu, Tawi-Tawi, Philippines, and has not yet been explored as a biofertilizer for seaweed farming, investigating its potential use for *Kappaphycus* is valuable. This study serves as an exploratory study, offering the first preliminary evidence for the use of *Thalassia hemprichii* extract as a biofertilizer for *K. striatus*. Specifically, it aims to explore the use of seagrass wrack extract from *Thalassia hemprichii* as a potential biofertilizer for the commercial eucheumatoid seaweed *Kappaphycus striatus*, assessing its influence on growth, ice-ice disease prevalence and intensity (number and length of ice-ice spots per bundle), and carrageenan quality (yield and gel strength).

Materials and Methods

Study Site

The study was carried out in the seaweed farm of Sibutu, Tawi-Tawi, Philippines (Figure 1). This region is particularly well-suited for cultivating eucheumatoid seaweeds, such as *Kappaphycus striatus*, a practice that has been established since the 1970s.

Source and Preparation of Seedlings

Healthy seedlings of *K. striatus*, free from diseases and untreated with fertilisers, were sourced from a farmer in the study site. The seedlings were trimmed to weights of 50–55 g each and then attached to 5-m-long rope lines, spaced 25 cm apart. Each rope line had 20 attachment points. In total, 12 rope lines were prepared, representing three different treatments and a control, with three replicates for each.

Formulation of Seagrass Wrack Extract as Biofertilizer

Seagrass wrack (*T. hemprichii*) was manually collected from the coastal beaches of Sibutu, Tawi-Tawi, Philippines. The preparation of seagrass wrack extract as a biofertilizer followed the method outlined by Tahiluddin et al. (2022b). First, the collected seagrass was thoroughly washed to remove any epiphytes, sand, and debris. It was then sun-dried for 30 min to eliminate excess moisture before being cut into small pieces. The seagrass was mixed with distilled water in a ratio of 1:4 (0.5 kg of seagrass wrack to 2 L of distilled water) and boiled for 2 hr. After boiling, the crude aqueous extract was cooled and filtered through muslin cloth. The newly formulated biofertilizer was then transferred to a clean bottle and stored in a cool, dry place.

Immersion of Seedlings in Biofertilizer Solution and Planting

The immersion of seaweeds in the biofertilizer solution was conducted in the late afternoon, between 4 and 6 pm, following the method outlined by Tahiluddin et al. (2022a) as practised by farmers in the study site. Four biofertilizer solutions were prepared for the treatments, with concentrations of 0, 9, 18, and 27 mL L^{-1}, by mixing the biofertilizer with seawater in a 20-L container. Three culture lines (n=3) were simultaneously immersed in each fertiliser solution for 30 sec, then left to sit overnight with a canvas covering. Prior to out-planting, the seaweeds were briefly rested for 10 min beneath the farmer's house to minimise stress before being transported to the farming site by small boat. The lines were set up randomly using the modified fixed-off bottom method (stakes at both ends with floaters), positioned 30 cm above the seabed.

Total Thallus Nitrogen and Phosphorus Determination

After the overnight enrichment of seaweeds, samples from each treatment were sun-dried for 3 days and then stored in ziplock bags. Total thallus nitrogen was measured using the Kjeldahl method (Kjeldahl, 1883), while the phosphorus content was determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Hou & Jones, 2000). Chemical characterisation of the SWE (e.g., nitrogen, phosphorus, and bioactive compounds) was beyond the scope of this preliminary phase and remains a limitation of the study.

Monitoring of Growth Rate, Ice-Ice Disease, and Physicochemical Parameters

Growth sampling was conducted every 15, 30, and 45 days. To assess growth, five randomly tagged branches per line were removed, patted dry with a clean cloth, and weighed. The sampled seaweeds were then re-tied to their original positions. Specific growth rates (SGR) were calculated every 15 days utilising the formula provided by Luhan et al. (2015).

Ice-ice disease prevalence was checked every 15, 30, and 45 days through visual inspections. Each bundle in every line was examined for signs of the disease, which include soft thalli and whitish discolouration. The number of infected bundles per line was measured. Ice-ice disease prevalence (%) was calculated by dividing the number of infected bundles by the total number of bundles per line, then multiplying by 100 (Tahiluddin et al., 2022a). Ice-ice disease intensity was also measured by counting the number of ice-ice spots per bundle and measuring the length of each spot.

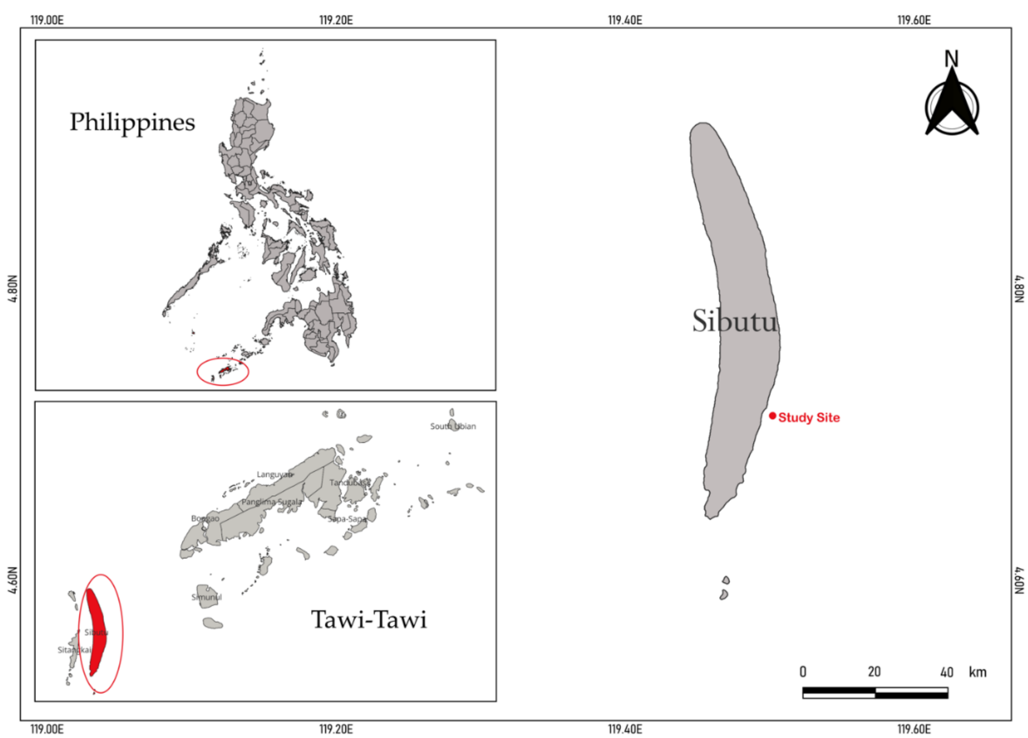

Figure 1. Study site (red dot) showing the location of the farm in Sibutu, Tawi-Tawi, Philippines

Physicochemical parameters, including temperature, salinity, pH, depth, water current, and wind speed, were recorded on days 0, 15, 30, and 45. Various instruments were used: a glass thermometer, a refractometer (Atago Master, Tokyo, Japan), a pH meter (Polsinelli, Kansas, MO, USA), a calibrated rope, a fabricated drogue, and a digital anemometer (BENETECH, Shenzhen, China). Additionally, the farm was maintained weekly by removing silt, debris, and predators from the seaweeds.

Carrageenan Yield and Gel Strength Determination

The seaweeds were carefully cleaned to remove any adhering foreign particles, sun-dried, and then chopped into small pieces. Gel strength and carrageenan yield were determined following the procedure outlined by Muyong & Tahiluddin (2024).

Statistical Analysis

Data are presented as the mean ± standard error (SE). The normality of the data was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated with Levene's test. Significant differences between treatments were evaluated through one-way analysis of variance (ANOVA). Given the exploratory nature of this preliminary, proof-of-concept study, Duncan's Multiple Range Test (DMRT) was selected as the post-hoc test for mean ranking. DMRT was chosen to maximise the sensitivity for detecting potential differences among treatment groups—such as the observed impact on carrageenan yield—where more conservative tests might fail to identify significant effects in a dataset with low replication (n=3) and a single cultivation cycle. All statistical analyses were performed using IBM SPSS software (version 20; SPSS Inc., Chicago, IL, USA). While the replication (n=3) is standard for preliminary seaweed field trials, we acknowledge that this level of replication may limit the statistical power to detect subtle biological variations in nitrogen and phosphorus assimilation.

Results and Discussion

The present study represents the first attempt to explore the potential use of seagrass wrack (*Thalassia hemprichii*), which is abundant on beaches, as a biofertilizer for cultivating Kappaphycus striatus. While our results did not show significant effects on growth, ice-ice disease prevalence, or gel strength, they did reveal a notable increase in carrageenan yield.

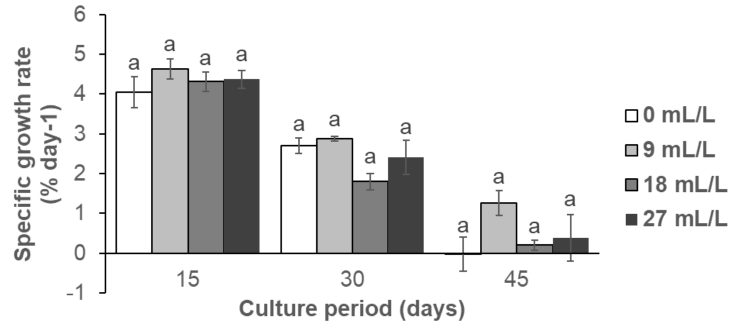

Figure 2. Specific growth rate (SGR, % day^{-1}) of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N= 15

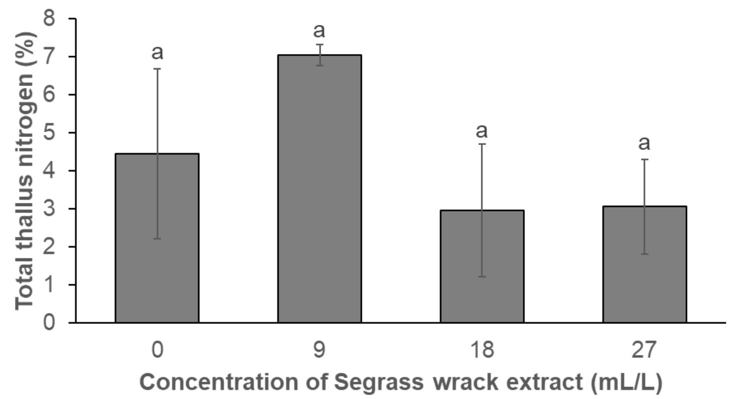

Figure 3. Total thallus nitrogen (%) of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N=3

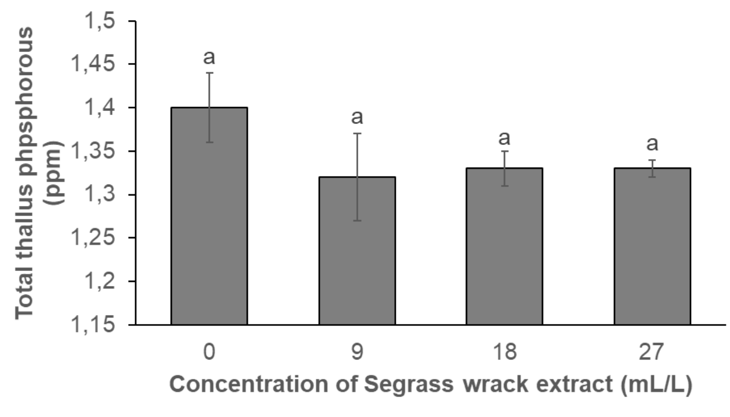

Figure 4. Phosphorus content (ppm) of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N= 3

Previous research has demonstrated that chemical fertilisers such as ammonium phosphate (3.5 and 8.82 g L^{-1}) effectively enhance the growth of eucheumatoid seaweed (Tahiluddin et al., 2022a; Muyong & Tahiluddin, 2024) due to the high nitrogen uptake by the seaweed (Tahiluddin et al., 2021a). In contrast, our study found that using seagrass wrack (*T. hemprichii*) as a biofertilizer had no significant impact on *K. striatus* growth, even though the highest growth rates were consistently observed with a 9 mL L^{-1} biofertilizer concentration on days 15, 30, and 45 (Figure 2). The lack of significant changes in total thallus nitrogen and phosphorus levels (Figures 3 and 4) likely explains the minimal growth impact. Without a chemical profile of the applied SWE, it is difficult to determine if the lack of growth response is due to low nutrient content in the extract or poor uptake by the seaweed. This suggests that seagrass wrack (*T. hemprichii*) is relatively ineffective in promoting the growth of *K. striatus,* although further studies are required. However, seagrass wrack has been recognised as a biofertilizer/biostimulant for crops (Muniswami et al., 2023) and coastal agriculture (Franzén et al., 2019). Additionally, previous studies have shown that brown seaweeds like *Sargassum cristaefolium* and *Turbinaria conoides*, or their combinations, can serve as alternative fertilisers for *K. striatus*, enhancing growth without affecting ice-ice disease occurrence (Tahiluddin et al., 2022b). Nonetheless, in this study, seagrass wrack did not yield significant results for *Kappaphycus* farming, suggesting that it may not be a suitable biofertilizer for *K. striatus*. Different extraction methods, such as fermenting the seagrass wrack and using various concentrations, were tested, but still resulted in non-significant findings (data not shown). Further studies are warranted to further explore the potential of seagrass wrack as a biofertilizer for *Kappaphycus.*

Ice-ice disease is a pathological condition in eucheumatoids, characterised by symptoms like softening and bleaching of the affected thalli (Faisan et al., 2021; Tahiluddin & Terzi, 2021b; Ward et al., 2022; Tahiluddin & Damsik, 2023; Tahiluddin & Terzi, 2024; Tahiluddin & Eldani-Tahiluddin, 2024). This disease is generally caused by a range of factors, including environmental and meteorological changes like temperature, salinity, irradiance, and rainfall (Tahiluddin & Terzi, 2021b; Ward et al., 2022; Tahiluddin & Terzi, 2024; Tahiluddin & Eldani-Tahiluddin, 2024), nutrient deficiencies such as sodium nitrate, ammonium, and ammonium phosphate (Luhan et al., 2015; Tahiluddin et al., 2022a; Tahiluddin & Terzi, 2024), or biological factors like pathogenic marine bacteria and marine-derived fungi (Tahiluddin & Terzi, 2021b; Tahiluddin et al., 2021b; Bermil et al., 2022). Nutrient enrichment has been shown to play a key role in reducing the occurrence of ice-ice disease. For instance, Tahiluddin et al. (2022a) reported that ammonium phosphate fertiliser (8.82 g L-1) significantly minimised the prevalence of ice-ice disease in *K. striatus*. However, it did not affect the severity of the disease in terms of the number and length of ice-ice spots/bundles. In the present study, the seagrass wrack extract biofertilizer (*T. hemprichii*) did not affect the occurrence or severity of ice-ice disease in farmed *K. striatus* throughout the sampling periods (Figures 5, 6, and 7). Similar findings were reported by Tahiluddin et al. (2022b), who found no significant impact on ice-ice disease prevalence when using brown seaweeds (*T. conoides* and *S. cristaefolium*) as biofertilizers. Additionally, Sarri et al. (2022) noted that inorganic nutrient enrichment (urea or phosphorus) had no impact on the prevalence of ice-ice disease. Environmental monitoring showed that pH levels rose from 8.63 to 9.20 over the 45-day study period. This high alkalinity is a significant physiological stressor that can trigger ice-ice disease by depleting the total inorganic carbon required for photosynthesis. Research demonstrates that when pH reaches such levels, seaweed yields can drop fivefold because the plants become carbon-limited, even if other nutrients are available (DeBusk & Ryther, 1984). For *Kappaphycus*, maintaining a stable environment near pH 8.0 is essential to prevent the stress and reduced growth associated with carbon depletion. Since all experimental groups were exposed to these identical high-pH conditions, the SWE did not appear to provide direct protection against these dominant environmental dynamics.

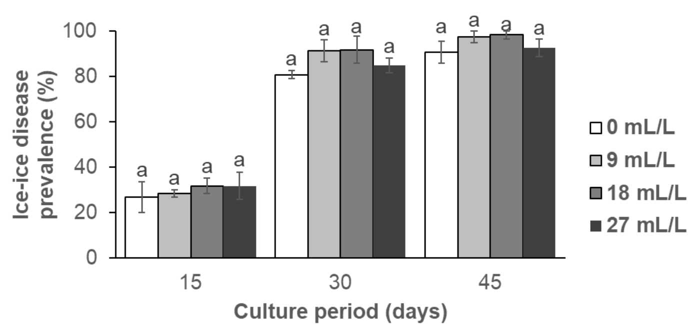

Figure 5. Ice-ice disease prevalence (%) of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N= 3

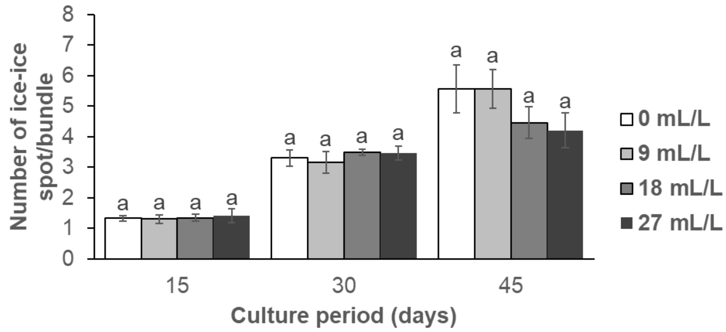

Figure 6. Number of ice-ice spots/bundle of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N= 3

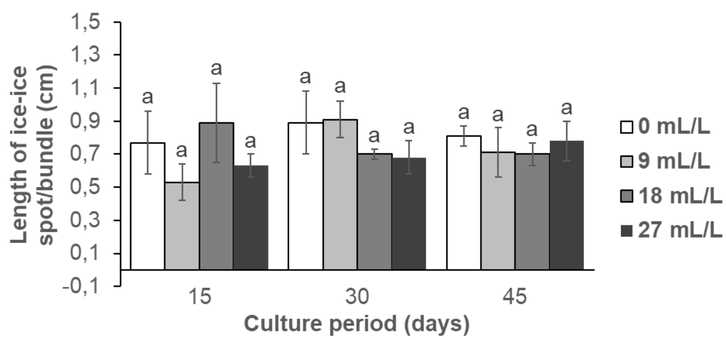

Figure 7. Length of ice-ice spots/bundle (cm) of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N= 3

Table 1. Physicochemical parameters of the farm during the sampling period

Water parameters	Sampling Period (Days)			
	0	15	30	45
Temperature (°C)	30.00 ± 0.00	30.75 ± 0.14	32.00 ± 0.00	29.67 ± 0.08
Salinity (ppt)	34.00 ± 0.00	34.00 ± 0.00	34.00 ± 0.00	34.00 ± 0.00
pH	8.63 ± 0.09	8.80 ± 0.06	9.03 ± 0.03	9.20 ± 0.00
Water current (m s^{-1})	0.25 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
Wind speed (m s^{-1})	4.97 ± 0.15	1.07 ± 0.15	1.87 ± 0.03	0.03 ± 0.03
Farm depth (cm)	51 – 122			

The primary objective of *Kappaphycus* cultivation and processing is to extract carrageenan, a versatile polysaccharide widely used in the food industry (Rupert et al., 2022; Shafie et al., 2022). Carrageenan yield can vary based on species, location, and harvest time (Hurtado et al., 2009; Mendoza et al., 2006; de Góes & Reis, 2012; Sarri et al., 2022; Tahiluddin et al., 2022b), as well as various abiotic (temperature, salinity, irradiance, season, and nutrients) and biotic factors in the farm environment (Rupert et al., 2022). In the present study, carrageenan yield was significantly enhanced by the use of seagrass wrack biofertilizer, with the highest concentration of 27 mL L^{-1} resulting in an elevated yield (Figure 8). This apparent decoupling between nutrient assimilation (which did not change) and carrageenan yield suggests that SWE may contain biostimulants or trigger metabolic shifts that prioritise polysaccharide synthesis over biomass production. This is supported by previous studies showing that biostimulants such as Acadian Marine Plant Extract Powder (AMPEP) significantly increase carrageenan yield while not affecting growth on day 20 and ultimately increasing both yield and growth on day 40 (Loureiro et al., 2014). Here, the biostimulant AMPEP acts as an elicitor, triggering a preventive stress response that prompts the seaweed to increase carrageenan production as a structural defence. Simultaneously, the extract contains antioxidant enzymes that neutralise oxidative stress, thereby allowing higher yields (Loureiro et al., 2012; Loureiro et al., 2014). According to Luhan et al. (2015), nutrient enrichment of *K. alvarezii* could increase carrageenan yield. This suggests that the seagrass wrack used in this study may have the potential to boost carrageenan yield. However, other studies have shown that biofertilizers made from seaweed liquid extracts of *S. cristaefolium* and *T. conoides*, or their combinations, did not impact carrageenan yield in *K. striatus* (Tahiluddin et al., 2022b). Similarly, the use of urea and phosphorus had no significant effect on the carrageenan yield of *K. striatus* (Sarri et al., 2022). Meanwhile, gel strength is a measure of how well a substance can hold its shape and resist falling apart when pushed or stirred (Islam & Hossain, 2021). In this study, the use of seagrass wrack biofertilizer did not affect the gel strength of *K. striatus* (Figure 9), consistent with Luhan et al. (2015), who found that sodium nitrate, as a chemical fertiliser, also did not influence the gel strength of *K. alvarezii*. It should be noted, however, that since the present study was conducted at a single site during one cultivation cycle, these results are specific to the seasonal and environmental conditions of the study period and should not be generalised to all *Kappaphycus* farming systems without further multi-site verification.

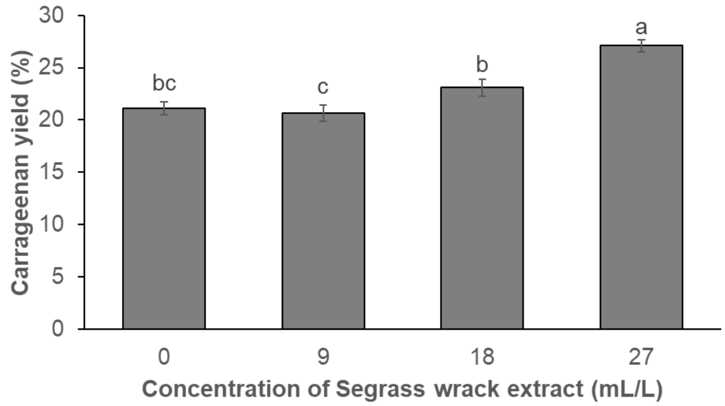

Figure 8. Carrageenan yield (%) of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N= 3

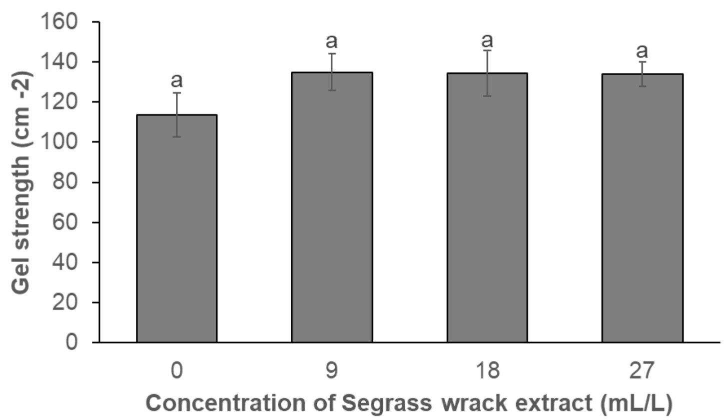

Figure 9. Gel strength (cm^{-2}) of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N= 3

Conclusion

In conclusion, this study provides preliminary observations on the potential of seagrass wrack extract (SWE) in eucheumatoid seaweed farming. While SWE significantly boosted carrageenan yield, the findings for growth and disease resistance were largely non-significant. These results emphasise hypothesis generation regarding biostimulant effects rather than immediate application readiness, and further research with higher replication and chemical characterisation is required to validate these exploratory findings fully.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: Ethical committee approval is not required for this type of study.

Data availability: The data will be made available upon request.

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