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Research Article

Use of Brown Seaweed Extracts as Bio-fertilizers and their Effects on the Ice-ice Disease Occurrence, Carrageenan Yield, and Growth Rate of the Red Seaweed *Kappaphycus striatus*

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Abstract: *Kappaphycus striatus* is one of the most important eucheumatoid species that is widely farmed worldwide. In the southern Philippines, where the initial farm was established, sluggish growth of farmed *Kappaphycus* species brought about by the poor quality of planting materials and extensive farming resulting in unproductive farms and frequent ice-ice outbreaks have been a hindrance in increasing the seaweed production. As a result, farmers have led to the application of inorganic fertilizers as nutrient enrichment for *Kappaphycus*. However, inorganic or chemical fertilizers always pose negative impacts on the environment. Hence, in this study, a preliminary investigation on the potential use of extracts of brown seaweeds *Sargassum cristaefolium* and *Turbinaria conoides* as bio-fertilizers was tested on *K. striatus* for their growth rate, carrageenan yield, and ice-ice disease occurrence. Seaweed liquid extracts (SLE): *S. cristaefolium* (SC), *T. conoides* (TC), combination of SC and TC (MX), and control (C) were utilized as bio-fertilizers for *K. striatus*. SLE-enriched *K. striatus* seedlings were cultivated in a seaweed farm using the fixed-off bottom method for 45 days. Results revealed that the specific growth rates of all SLE treatments were significantly higher than no SLE treatment at day 45. The percentage of ice-ice disease and the yield of carrageenan did not differ among treatments. Enrichment of *K. striatus* using SLE of two selected brown seaweeds before out-planting could improve growth rates while not affecting the ice-ice disease occurrence and carrageenan yield. Hence, formulated SLE from brown seaweeds *S. cristaefolium* and *T. conoides* can be used as potential bio-fertilizers for *Kappaphycus* cultivation.

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1. Introduction

The genus of *Kappaphycus* is an economically important red seaweed cultured in both tropical and subtropical waters. This red seaweed is highly marketable worldwide owing to its carrageenan content, which is extensively utilized in food and non-food products as a binder, emulsifier, gelling, and

thickening agents (Hurtado et al., 2000; Hurtado et al., 2015; Tahiluddin and Terzi, 2021a). The Philippines is a major seaweed producer, ranking fourth globally (FAO, 2020). However, one significant hurdle in increasing the seaweed production in the Philippines is the unproductive seaweed farms which now yield only minimal production compared to before. Reasons could be attributed to lowering seedling stock quality or seaweed overstocking, leading to nutrient depletion and slow seaweed growth. Hence, water fertility is tremendously crucial in regulating the yield and sustainability of seaweed production (Luhan et al., 2015).

The cultivation of *Kappaphycus* species depends primarily on the sea's natural fertility (Hurtado et al., 2001; Muñoz et al., 2004; Hayashi et al., 2007; Luhan et al., 2015). One way of improving seaweed production to meet the phycocolloids demand and improve seaweed farmers' earnings is through the nutrient enrichment of seaweeds (Luhan et al., 2015). Previous studies that utilized inorganic fertilizers and organic biostimulants as nutrient enrichment showed promising results in increasing the growth and ameliorating the health and condition of seaweeds (Neish et al., 1977; Rui et al., 1990; Lavilla-Pitogo, 1992; Dawes et al., 1994; Menéndez et al., 2002; Nagler et al., 2003; Loureiro et al., 2010; Martins et al., 2011; Borlongan et al., 2011; Loureiro et al., 2012; Luhan et al., 2015; Umanzor, 2019; Ali et al., 2020).

Seaweed liquid extracts (SLE) are formulated as a source of bio-fertilizer with huge nutrient contents that affect the diverse physiological processes like seed germination, vegetative growth, and productivity, resistance against pathogens of various crops (Sathya et al., 2010). As originally initiated by Milton in 1952, SLE is now well utilized to enrich crops in agriculture and horticulture (Hurtado et al., 2009). *Sargassum* and *Turbinaria* species have been utilized as SLE for crops, which according to studies, showed remarkable growth, yield, quality, and other properties of crops (Zodape et al., 2008; Erulan et al., 2009; Kumari et al., 2011; Kavipriya et al., 2011; Vijayanand et al., 2014; Selvam and Sivakumar, 2014; Nabti et al., 2017; Layek et al., 2018; Manea and Abbas, 2018; Silva et al., 2019; Sunarpi et al., 2020; Karthik et al., 2020; Ali et al., 2021).

Another problem that hinders eucheumatoid production is the occurrence of ice-ice disease (Ward et al., 2022), which is typically characterized by the appearance of soft and white portions on the infected branches (Tahiluddin and Terzi, 2021a; Tahiluddin et al., 2021b). This disease is brought about by fluctuations of environmental parameters like light intensity, salinity, and temperature resulting in stress on the seaweeds, thus weakening its immune defense system against harmful bacteria *Cytophaga-Flavobacterium* and *Vibrio-Aeromonas* (Largo et al. 1995a and 1995b; Tahiluddin and Terzi, 2021a). In the Philippines, the occurrence of the ice-ice disease is widespread and caused a severe decline in the production of *Kappaphycus* species (Mendoza et al., 2002; Faisan et al., 2021); thus, it is considered a significant threat to the seaweed industry (Tahiluddin et al., 2021c). Nutrient deficiency has been suspected as another factor that may trigger ice-ice disease in the farm (Maryunus, 2018). Moreover, Luhan et al. (2015) reported that nutrient-enriched *K. alvarezii* had significantly lower ice-ice disease occurrence than untreated seaweed.

In Tawi-Tawi, southern Philippines, the ice-ice disease occurrence and slow growth have led to the rampant use of inorganic fertilizers as nutrient enrichment for *Kappaphycus* (Tahiluddin, 2018; Tahiluddin et al., 2021a and 2021b). The diversity of seaweed in Tawi-Tawi is high, with a reported 79 species, including cultured *Kappaphycus* species and wild brown seaweeds (Puig-Shariff, 2015; Yangson et al., 2022). *S. cristaefolium* and *T. conoides* are among the most abundant brown seaweed species. Therefore, exploring the potential use of these seaweeds as alternative environmental-friendly fertilizers is worth investigating. This is also to diminish the use of inorganic fertilizers, which may not only harm the marine environment but also pose negative health risks to consumers since cultivated *Kappaphycus* species are also consumed as salads by local communities. Besides, no studies have been conducted on the potential use of extracts of these brown seaweeds as bio-fertilizer for *K. striatus*. Hence, this study investigated the use of brown seaweed extracts (*S. cristaefolium* and *T. conoides*) as potential bio-fertilizers by evaluating their effects on the ice-ice disease occurrence, growth rate, and carrageenan yield of *K. striatus*.

2. Material and Methods

2.1. Study area

This study was carried out at the seaweed farm of Panglima Sugala municipality, province of Tawi-Tawi, southern Philippines (Fig 1), for 45 days from December 30, 2018, to February 13, 2019.

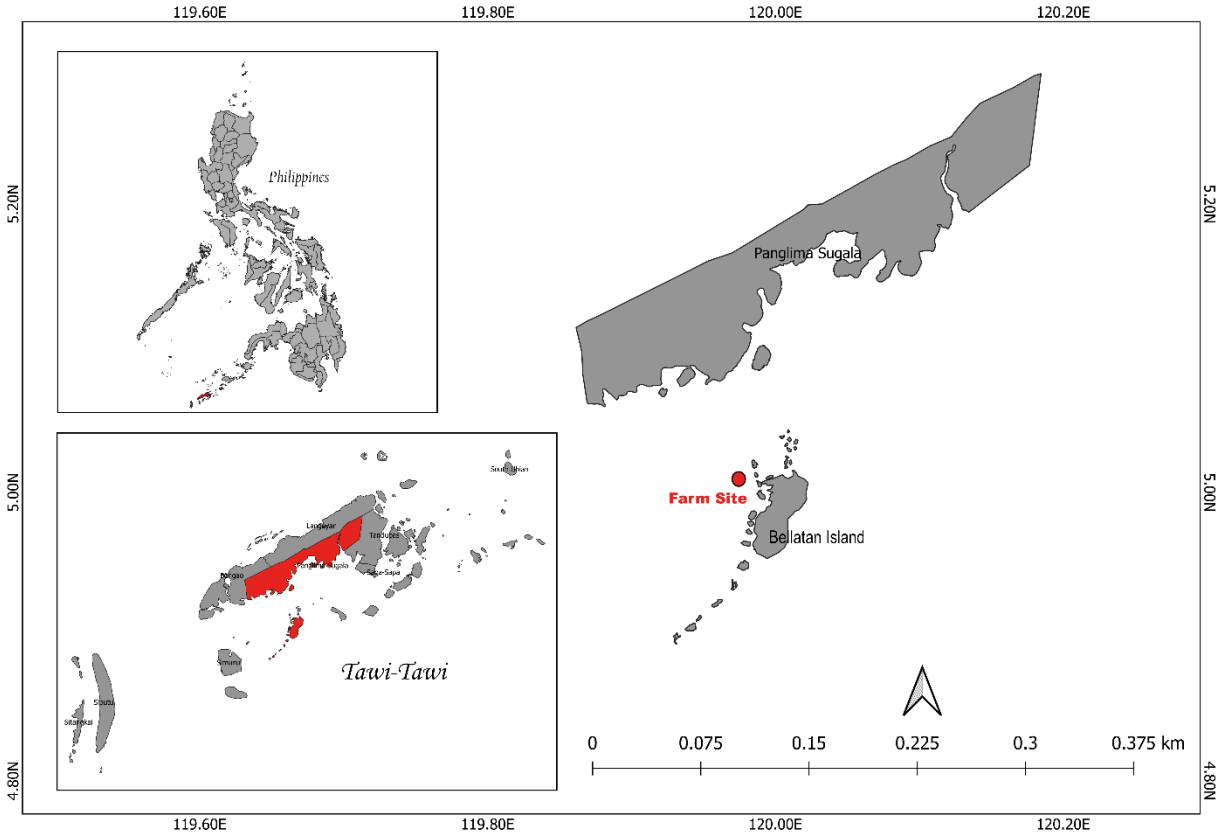

Figure 1. Study area.

2.2. Source and preparation of seedlings

The seedlings of *K. striatu*s were bought from the farmer at the study site. Healthy, disease-free, and untreated seedlings were used in this study. The seedlings were prepared by cutting into 50 g each. These were tied into a rope line measuring 5 m with an interval of 25 cm. There were 20 points per line. A total of 12 lines were prepared to represent the 3 SLE treatments and control, with 3 replicates each.

2.3. Formulation of seaweed liquid extract fertilizers

Formulation of SLE as bio-fertilizers followed the method of Vijayanand et al. (2014). The brown seaweeds *S. cristaefolium* and *T. conoides* were gathered from the rocky area of Sunkist, Pahut, Bongao, Tawi-Tawi, and southern Philippines. The collected seaweeds were cleaned by washing with fresh water 3 times in order to remove any foreign materials like sand, epiphytes, and other associated flora and fauna. SLE was formulated as follows: SC = *S. cristaefolium*, TC = *T. conoides*, MX = combination of SC and TC at a 50:50 ratio. The brown seaweeds were chopped into small pieces. Approximately a kg of each brown alga was sun-dried for 5 min to remove excess moisture. The weight of the chopped seaweeds was determined. Three liters of distilled water were added to chopped seaweeds and boiled for approximately 2 hr. The crude aqueous extracts were cooled and filtered with a muslin cloth to get the SLE. SLE fertilizers were placed in clean bottles and stored in a cool and dry place.

2.4. Immersion of seedlings to SLE solution and planting

The immersion method of seedlings to SLE solution was done in the afternoon from 4-6 pm following Tahiluddin (2018) as practiced by the seaweed farmers in the province. Three bio-fertilizer solutions using SLE (SC = *S. cristaefolium*, TC = *T. conoides*, MX= combination of SC and TC) were prepared at 8.82 mL L^{-1} concentration [average concentration practiced by the farmers (Tahiluddin, 2018)] for each treatment, while only seawater was used for C as control. Three (3) cultivation lines (serving as replicates) were simultaneously dipped into each fertilizer solution for 30 s, then covered with canvas, and left overnight. Before out-planting, the seedlings were immersed in seawater for 10 min to avoid stress, and then these were transported via a small boat to the farming site. Cultivation lines were randomly set up by the fixed-off bottom method, 30 cm above the seabed.

2.5. Monitoring of ice-ice disease occurrence, growth rate, and environmental parameters

Ice-ice disease occurrence and growth rate were determined on days 14, 30, and 45. Growth rate sampling was done by removing tagged branches, patting with a clean cloth, and weighing. Sampled seaweeds were re-tied to their original positions. Ice-ice disease occurrence was monitored through visual inspection. The whitening and softening of the seaweed branches were considered ice-ice disease symptoms (Luhan et al., 2015; Tahiluddin et al., 2021b). The specific growth rate (SGR) was calculated using the formula adopted by Luhan et al. (2015), and the specific growth rate (SGR) was calculated. Ice-ice disease occurrence (%) was calculated by dividing the total number of infected branches by the total of branches per line and multiplying by 100 (Largo et al., 1995).

Seawater conditions in the farm site like salinity, temperature, and pH were monitored using a refractometer, thermometer, and pH meter, respectively, every 7 days. The water current was determined using an improvised drogue, and depth was measured using a calibrated rope every 7 days. In addition, the cultivation setup was maintained every 7 days by removing silt, debris, and predator attached to the farmed seaweeds.

2.6. Carrageenan yield determination

Determination of carrageenan quality was done after 45 days of culture. First, samples were dried under the sun for 3 days. Next, dried seaweeds were cleaned by washing with water to remove silts and other debris. Fifteen (15) g of dried seaweeds were boiled in approximately 250 mL of purified water until all the seaweeds had been dissolved. The dissolved seaweeds were immediately filtered while the slurry was still hot. The filtrate was allowed to cool and freeze overnight. The frozen filtrates of seaweeds were thawed, then dried at 60 ºC in the oven. The resulting product was the native carrageenan. Carrageenan yield was calculated using the formula (Luhan et al., 2015).

$$CY = \frac{Wc}{Wds} x100 \quad (1)$$

Where: CY = Carrageenan yield
Wc = Weight of carrageenan
Wds = Weight of dried seaweeds

2.7. Data analysis

Using IBM SPSS software version 20, data on ice-ice disease, growth rate, and carrageenan yield were subjected to One-way Analysis of Variance (ANOVA). Post hoc (Duncan) was used if significant differences exist among treatments. The data were given as mean ± SE. The statistical significance level was set to 0.05.

3. Results

The specific growth rates (SGRs) of *K. striatus* enriched with different bio-fertilizers are shown in Figure 2. On day 14, the SGRs of SC, TC, MX, and C were 5.1±0.27, 5.23±0.16, 5.01±0.31, and 3.65±0.55% day^{-1}, respectively. On day 30, the SGRs of SC, TC, MX, and C were 4.33±0.14, 4.14±0.17, 4.09±0.3, and 2.87±0.38% day^{-1}, respectively. On day 45, the SGRs of SC, TC, MX, and C were

4.33±0.14, 4.14±0.17, 4.09±0.3, and 2.87±0.38% day^{-1}, respectively. Analysis revealed that the SGRs of *K. striatus* enriched with different bio-fertilizers (SC, TC, and MX) were significantly higher than the control (C) on days 14, 30, and 45 (p<0.05). The mean weights (MWs) of cultured SLE-enriched *K. striatus* are shown in Figure 3. On day 14, MWs of SC, TC, MX, and C were 103.13±4.04, 104.33±2.24, 101.87±4.42, and 86.67±6.04 g, respectively. One-way ANOVA revealed that all SLE-treated *K. striatus* was significantly higher (p<0.05) than control. Higher MWs were also observed on day 30, where SC (185.4±7.49 g), TC (176.2±8.61 g), and MX (180.27±15.13 g) were significantly higher (p<0.05) than C (125.2±15.17 g). On day 45, MWs of SC (238.67±13.12 g), TC (192.07±26.85 g), and MX (180.27±27.47 g) were significantly higher (p<0.05) than C (116.67±22.06 g).

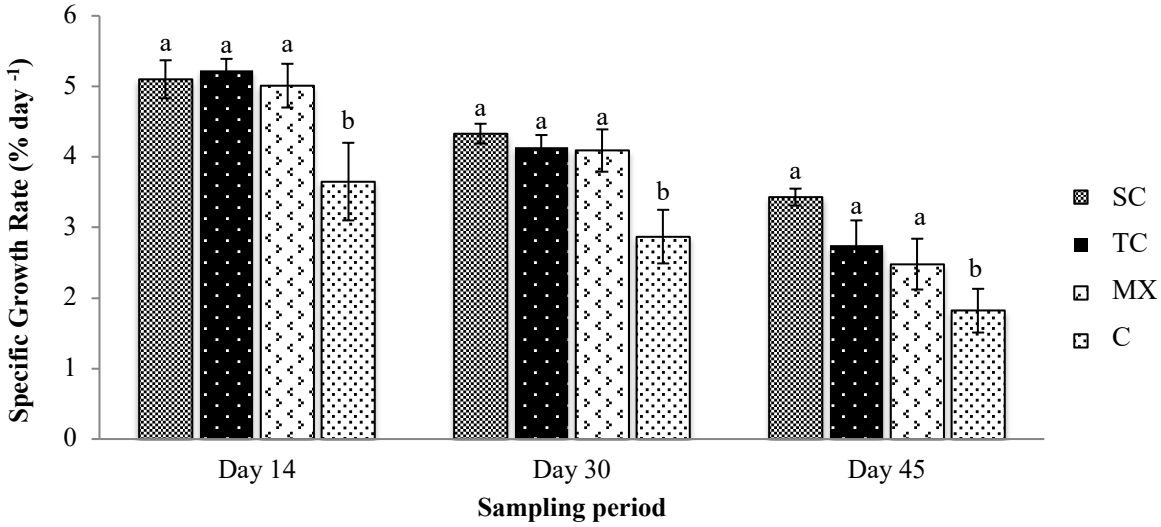

Figure 2. SGR (% day^{-1}) of *K. striatus* in every sampling period. SC= *S. cristaefolium*, TC = *T. conoides*, MX = combination of SC and TC, and C = control. Bars with different letters are significantly different (p<0.05). Error Bars in SEM (standard error of the mean), n=13-15.

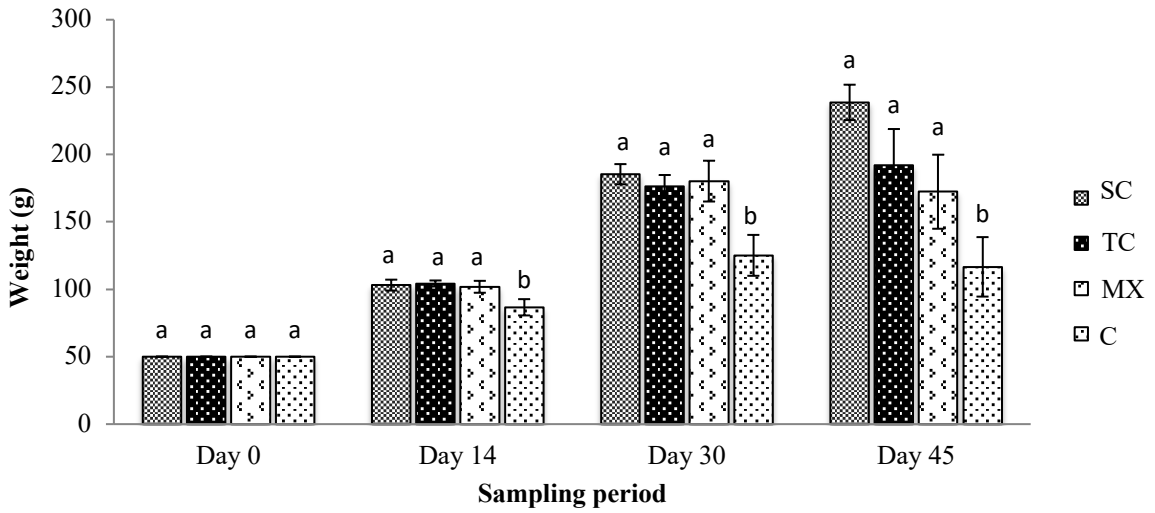

Figure 3. Mean weight of *K. striatus* in every sampling period. SC= SLE (*S. cristaefolium*), TC= SLE (*T. conoides*), MX= SLE (combination of SC and TC), and T_4=Control. Bars with different letters are significantly different (p<0.05). Errors bars in SEM (standard error of the mean), n=13-15.

Ice-ice disease occurrence of all SLE-enriched *K. striatus* treatments did not differ from the control throughout the sampling period, which ranged from about 5-12%, 65-62%, and 8-25% on days 14, 30, and 45, respectively (Fig 4). The percentage occurrence of SC (6.67±6.41%), TC (5±7.38%),

MX (10±2.85%), and C (11.67±1.45%) showed no significant difference (p>0.05) on day 14. On day 30, ice-ice disease occurrences in SC, TC, MX, and C were 45.92±9.96%, 48.09±16.56%, 60.94±10.79%, and 61.81±5.93%, respectively. On day 45, ice-ice disease occurrences in SC, TC, MX, and C were 7.62±6.36%, 20.24±6.07%, 12.31±4.69%, and T4 27.31±3.46%, respectively. No significant differences (p>0.05) in ice-ice disease occurrence were observed on days 30 and 45 among treatments. Change in the occurrence of ice-ice disease of cultured SLE-enriched *K. striatus* is shown in Figure 5. From day 0 to 30, the ice-ice disease occurrence for all treatments significantly increased (p<0.05) but significantly dropped (p<0.05) after 45 days of culture (Fig 5).

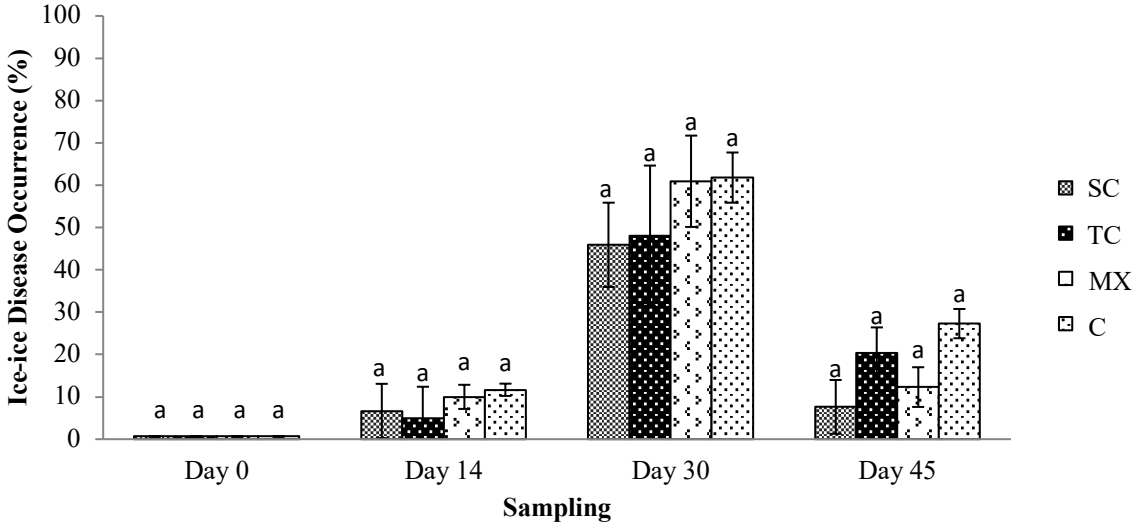

Figure 4. Occurrence of ice-ice disease of *K. striatus* in every sampling period. SC = *S. cristaefolium*, TC = *T. conoides*, C = combination of SC and TC, and C = control. Bars with the same letters are not significantly different (p>0.05). Errors bars in SEM (standard error of the mean), n=14-20.

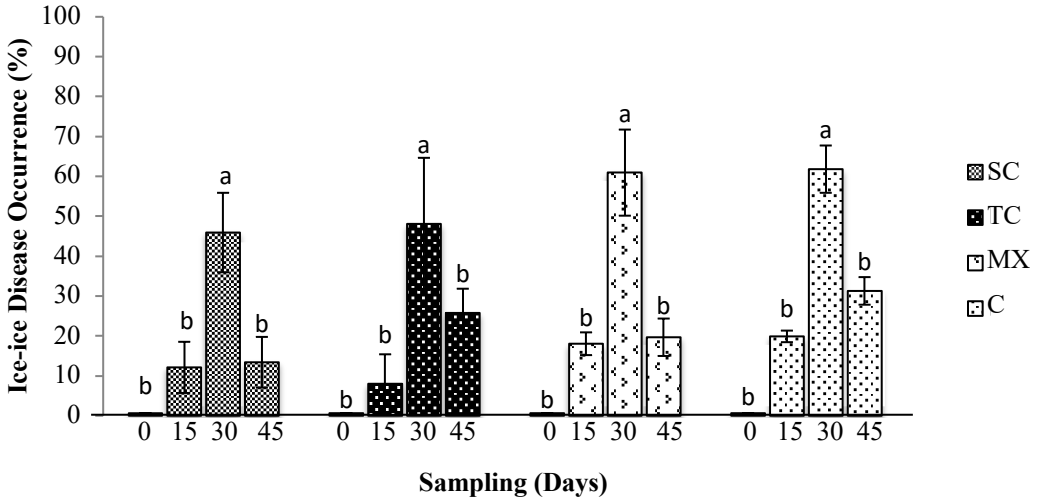

Figure 5. Change in ice-ice disease occurrence of *K. striatus.* SC= SLE (*S. cristaefolium*), TC= SLE (*T. conoides*), MX= SLE (combination of SC and TC), and C=Control. Bars with different letters are significantly different (p<0.05). Errors bars in SEM (standard error of the mean), n=14-20.

Figure 6 shows the carrageenan yield of SLE-enriched *K. striatus* after 45 days (SC, TC, MX, and C), which were 35.07±1.58, 33.44±5.52, 32.98±0.79, and 32.24±1.36 %, respectively, indicating no significant difference (p>0.05) among treatments.

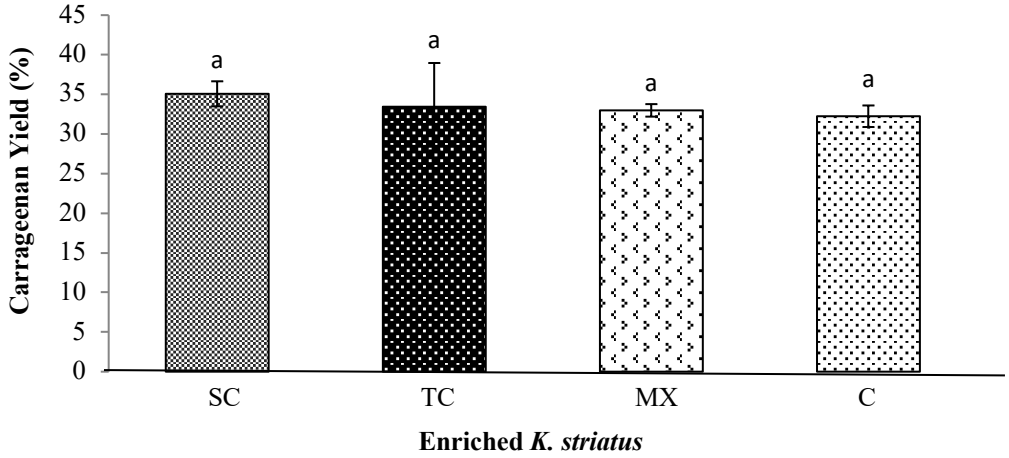

Figure 6. Carrageenan yield of 45-day old *K. striatus*. SC = *S. cristaefolium*, TC = *T. conoides,* MX = combination of SC and TC, and C = control. Bars with the same letters are not significant different (p>0.05). Errors bars in SEM (standard error of the mean), n=3.

The temperature of the farm ranged from 27.23±0.03 to 32.87±0.18 °C, salinity was from 33.33±0.33 to 35±1.0 ‰, pH was from 7.02±0.04 to 8.69±0.01, water current velocity was from 0.08±0.03 to 0.2±0.08 m s^{-1}, and water depth was from 30.67±0.33 to 188.12±0.96 cm throughout the culture period.

4. Discussion

The potential use of brown seaweeds *S. cristaefolium* and *T. conoides* or its combination as liquid extract biostimulants enriched in *K. striatus* before out-planting has demonstrated promising results. Typically, the availability of nutrients in the seawater in the environment allows the seaweed's cell walls to absorb and assimilate additional nutrients (nitrogen) needed to obtain a better growth rate farmed in nutrient-deficient conditions (Luhan et al., 2015). Although we did not determine the nutrient contents of these SLE, studies have found that brown seaweeds such as *T. conoides* and *Sargassum* spp. contain a vast array of nutrients and minerals such as phosphorus, nitrogen, potassium, zinc, magnesium, manganese, iron, calcium, and copper (Santoso et al., 2006; Sutharsan et al., 2017). SLE used in this study may have utilized the nutrients from these extracts or contain biostimulatory properties favoring higher growth than untreated. Besides, the methods and concentration used in this study have proven efficient in providing additional nutrients to *K. striatus*, thereby increasing its growth (Tahiluddin, 2018; Tahiluddin et al., 2021a; Sarri et al., 2022). Ali et al. (2021) reported that seaweed-based bioproducts have phytostimulatory properties that result in improved plant yield and growth parameters in various crops. For instance, SLE of *S. wightii* enriched in cluster bean plant at low concentration (1.5%) promoted growth (Vijayanand et al., 2014). The extract of *S. johnstonii* applied to *Lycopersicon esculentum* showed a positive influence on its yield, growth, and quality (Kumari et al., 2011). The growth and biochemical constituent of pigeon pea *Cajanus cajan* exhibited better results using extract of *S. polycystum* (Erulan et al., 2009). Also, extracts from *S. muticum* and *Ascophyllum nodosum* at 25% concentration showed positive effects in lettuce and rice plants (Silva et al., 2019). On the other hand, a combination of *T. ornata* and *Ulva reticulata* as seaweed liquid fertilizer showed better plant growth and seed germination of *Phaseolus vulgaris* (Green Pea), *Raphanus sativus* (Radish), and *Vigna radiata* (Mung) (Karthik et al., 2020). Similarly, the liquid extract of *T. murayana* has been shown as an effective bio-fertilizer on tomato plants (*Lycopersicum esculentum*) by significantly improving the fruit and flower numbers (Sunarpi et al., 2020).

The application of brown seaweed *Sargassum* and *Turbinaria* as nutrient enrichment for *Kappaphycus* farming has not been explored. Previous investigations focused on using Acadian Marine Plant Extract Powder (AMPEP) to improve the growth of *Kappaphycus*. AMPEP is extracted from

brown alga *A. nodosum*. For instance, *K. alvarezii* enriched with AMPEP obtained an SGR of 1.3 – 4.1 % day^{-1} (Borlongan et al., 2011), lower than the SGR obtained in this study. Loureiro et al. (2010; 2012) reported that brown seaweed *A. nodosum* (liquid form) as enrichment increased the daily growth rate (5.5 – 5.6%) of cultured *K. alvarezii,* which is similar to our result with SGR of 5.23% in enriched *K. striatus*. Also, AMPEP significantly increased the growth rate (7.3%) in *K. alvarezii* (Loureiro et al., 2014), relatively higher than our study.

The ice-ice disease of *Kappaphycus* is usually associated with changes in environmental factors like light intensity, salinity, and temperature manifested with extensive whitening of the branches, which are further degraded by the presence of opportunistic bacteria (Largo et al., 1995a and 1995b; Tahiluddin and Terzi, 2021a and 2021b; Tahiluddin et al., 2021c). Likewise, marine-derived fungi were reported to be a potential causative agent of this disease (Solis et al., 2010). Another factor that seemed to trigger the occurrence of the ice-ice disease is the lack of nutrients in the nutrient-deficient environment, as Luhan et al. (2015) demonstrated. The authors planted the nutrient-enriched *K. alvarezii* in a bamboo raft net cage without maintenance for 45 days and revealed that enriched seaweed had significantly lower ice-ice disease occurrence (8.75%) than control (97%). However, in this study, since enriched *K. striatus* seaweeds and control were cleaned regularly, the effect of used bio-fertilizers in ice-ice disease occurrence was not detected, although a high incidence of ice-ice disease occurred on day 30 but eventually declined on day 45. On the other hand, AMPEP lessened ice-ice disease development in *Kappaphycus* farming (Hurtado and Critchley, 2013). Besides, AMPEP-enriched *K. striatus* with a concentration of 0.01 g L^{-1} and 8.82 g L^{-1} using the same method used in this study significantly decreased ice-ice disease occurrence (Illud, 2020). Moreover, the utilization of AMPEP in *K. alvarezii* as mitigation against epiphytes has been successfully tested (Ali et al., 2020; Borlongan et al., 2011; Hurtado and Critchley, 2013; Loureiro et al., 2017).

The reported carrageenan yield of *Kappaphycus* in the Philippines ranged from 15 to 64% (Hurtado-Ponce, 1995; Mendoza et al., 2002; Luhan et al., 2015; Robles, 2020; Sarri et al., 2022). In this study, the carrageenan yield of nutrient-enriched *K. striatus* (33-35%) did not differ from the untreated seaweed (32%). These findings are parallel to the studies of Loureiro et al. (2012 and 2014), in enriched *K. alvarezii* with AMPEP was about 33 and 38%, respectively. Conversely, in sodium nitrate-enriched *K. alvarezii*, the carrageenan yield was 42% (Luhan et al., 2015), relatively higher than our study. The utilization of SLE of brown seaweeds in *K. striatus* did not influence the carrageenan yield suggesting that these seaweeds can be used as potential bio-fertilizers for *Kappaphycus* farming.

Conclusion

In conclusion, this study revealed that the experimental liquid extracts from the brown seaweeds *S. cristaefolium* and *T. conoides* could be utilized as potential bio-fertilizers that may work as biostimulants since the growth of *K. striatus* improved while not affecting the ice-ice disease and carrageenan yield. Although this is a preliminary study, this practical approach may benefit seaweed farmers who attempt to increase production for better profit and livelihood. However, application refinement, like using different concentrations, mode of application, and soaking period may be explored for enrichment efficiency. In addition, the nutrient contents of these SLE may also be further investigated based on their seasonal availability.

Acknowledgments

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