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Effects of Acadian Marine Plant Extract Powder (AMPEP) and ammonium phosphate as nutrient enrichment on the ice-ice disease occurrence and growth performance of seaweed *Kappaphycus striatus*

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ABSTRACT

Due to the frequent ice-ice disease outbreak and stagnant growth of *Kappaphycus* species, farmers and researchers have been considering nutrient enrichment as one way of easing these issues to increase production to meet the growing demand for carrageenan in the world market. In this study, we determined the effects of Acadian Marine Plant Extract Powder (AMPEP) and ammonium phosphate as nutrient enrichment on the ice-ice disease occurrence and growth performance of seaweed *Kappaphycus striatus*. The study used three treatments: T_1 group = AMPEP at 0.01 g L^{-1}, T_2 group = ammonium phosphate [$(NH_4)_3PO_4$], 16-20-0 at 8.82 g L^{-1}, and T_3 group = control. Nutrient-enriched seaweed *K. striatus* was planted using the fixed-off bottom method for 45 days. Findings revealed that the growth performance of the T_2 group (6.1±0.14 % day^{-1}) was significantly higher ($p<0.05$) after 30 days compared to the T_1 group (5.56±0.16 % day^{-1}) and control group (5.45±0.17 % day^{-1}). In terms of ice-ice occurrence, the T_1 group (0.57±0 %) was significantly lower after 45 days of culture compared to the control group (59.67±27.32 %) but not significant with the T_2 group (23.22±12.83 %). Our study suggests that AMPEP could effectively reduce ice-ice disease occurrence, while ammonium phosphate could enhance the growth performance of *K. striatus*.

KEYWORDS: AMPEP, *Kappaphycus*, ice-ice disease, inorganic fertilizer, biofertilizer, biostimulant

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1. Introduction

Eucheumatoid species, such as *Eucheuma* spp. and *Kappaphycus* spp., are among the important commercial seaweeds farmed globally, especially in tropical regions. Their importance is due to the high content of carrageenan – a phycocolloid that has a vast array of applications in food and non-food industries (Tahiluddin and Terzi, 2021). Roughly 11 million tons, or 34% of the total aquatic plants' world production, came from eucheumatoid production in 2018 (FAO, 2020). About 5% of these aquatic plants' production is mainly contributed by *Kappaphycus* species (FAO, 2020). *Kappaphycus striatus* is one of the popularly cultivated eucheumatoid species globally (Tahiluddin et al., 2022a). However, numerous factors have been determined to affect the sustainability of eucheumatoid aquaculture. Among these are stagnant growth due to less productive farms attributed to nutrient depletion as well as the frequent outbreak of ice-ice disease occurrence (Luhan et al., 2015; Tahiluddin and Terzi, 2021; Tahiluddin et al., 2022b). Hence, farmers in the southern Philippines have been incorporating inorganic nutrient enrichment to help ease these issues (Tahiluddin et al., 2022b).

Nutrient enrichment is usually applied to *Kappaphycus* micropropagation under laboratory conditions, either using biofertilizer/biostimulant or inorganic fertilizer (Hurtado et al., 2009; Luhan and Mateo, 2017). In the natural environment, *Kappaphycus* cultivation typically relies on the natural fertility of the seawater (Luhan et al., 2015). With the aim of skyrocketing the production of *Kappaphycus* to meet the growing demand, the application of biofertilizer/biostimulant, like AMPEP (Borlongan et al., 2011; Hurtado et al., 2012; Loureiro et al., 2012; Hurtado and Critchley, 2013; Loureiro et al., 2017; Tibubos et al., 2017; Hurtado and Critchley, 2018; Ali et al., 2018a; Ali et al., 2018b) and seaweed liquid extract (Tahiluddin et al., 2022a), as well as inorganic fertilizers, such as urea and sodium nitrate (Luhan et al., 2015; Sarri et al., 2022) and ammonium phosphate (Tahiluddin et al., 2021a; Tahiluddin et al., 2021b; Tahiluddin et al., 2022b), have been the focus of the previous and recent studies, which provided promising results in increasing the growth performance and reducing the occurrence of ice-ice disease and epiphytes. Although inorganic fertilizers facilitate easy access due to their availability as commonly used fertilizers for agricultural plants, their uses and practices may threaten the natural environment and compromise the carrageenan quality of seaweeds (Robles, 2020; Tahiluddin et al., 2022b). In this study, we determined the effects of biofertilizer/biostimulant (AMPEP) and inorganic fertilizer (ammonium phosphate, 16-20-0) on the ice-ice disease occurrence and growth performance of *Kappaphycus striatus*.

2. Material and Methods

Study Site, Duration and Seedlings

This study was conducted on the coastal water of Pasiagan, Bongao, Tawi-Tawi, Philippines (Figure 1), with a duration of 45 days from January 14 to February 27, 2019. The healthy and untreated seedlings of *K. striatus* were obtained from the farmer at the study site.

Transporting and Conditioning of Seedlings

The seedlings were placed inside the styrofoam by adding the *Sargassum* sp. at the bottom and top to avoid the stress of seaweeds during transportation. Upon arrival at the farm site, seedlings inside the styrofoam were submerged gradually and planted for three days for acclimatization.

Preparation of Seedlings

Seedlings of *K. striatus* were prepared by cutting them into 50 g each. Using soft straw, the seedlings were tied into a 5 m rope line at an interval of 25 cm. There were 20 bunches (seedlings) in each line, and a total of nine lines were prepared to represent three replicates in each treatment.

Nutrient Enrichment of Seedlings

Nutrient enrichment of seedlings was done late in the afternoon following the procedure of Tahiluddin et al. (2022b). Three fertilizer solutions were prepared; T_1 group represented by biofertilizer/biostimulant (AMPEP) with a concentration of 0.1 g L^{-1} (Hurtado et al., 2012), T_2 group for inorganic fertilizer (16-20-0) with a concentration of 8.82 g L^{-1} (Tahiluddin et al., 2022b), and T_3 group for control. Every three lines were dipped in each solution simultaneously for 30 seconds and covered with canvas overnight (12-15 hours). Used fertilizers were discharged to an excavated land. Every 15 days of culture, seaweeds were re-enriched with nutrients following the same procedure.

Planting of Seedlings

Seedlings were transported from the house to the farm site within 10-15 minutes via a small boat. Long exposure to the sun, rain, and winds was avoided. Seedlings were planted randomly using a fixed off-bottom method in a randomized complete block design.

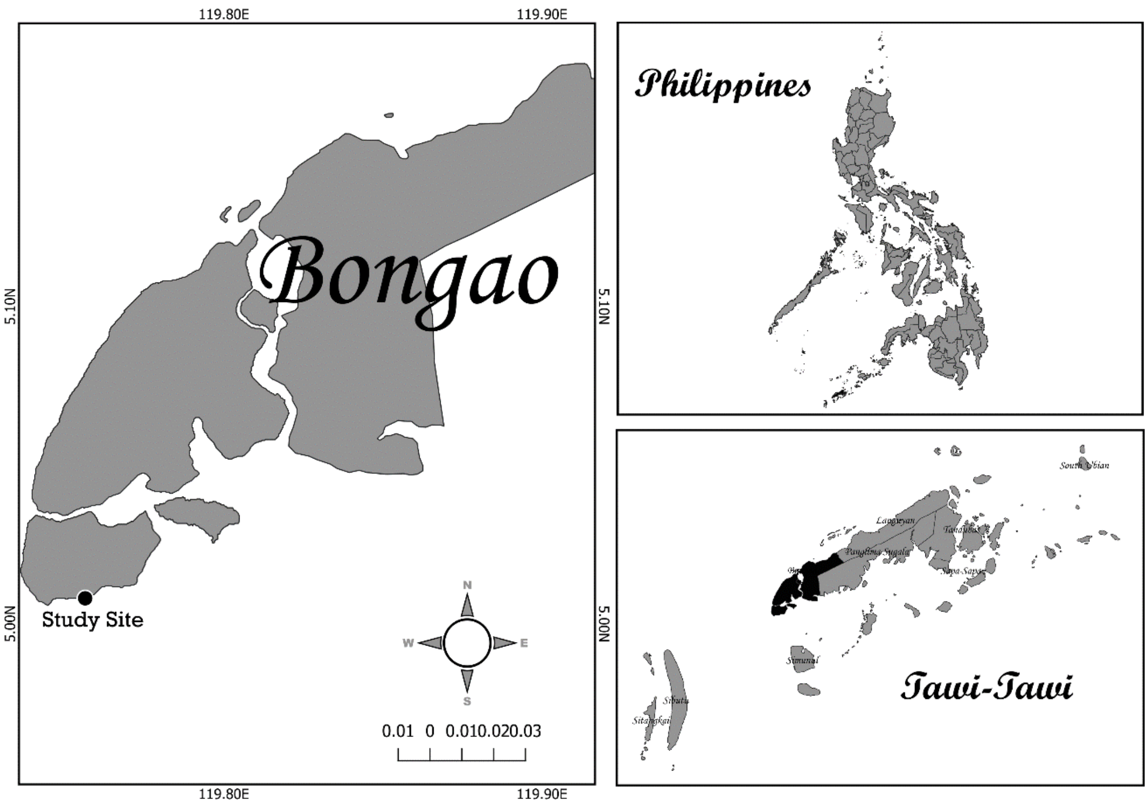

Figure 1. Study site.

Growth Sampling

The growth rate of seedlings was monitored every 15 days. This was done by weighing 25% of the lines (5 bunches per line) for all treatments. Seaweeds were patted with a smooth cloth to remove water from the thalli's surface before weighing. Growth (μ), expressed as the specific growth rate (SGR), was calculated using the following formula (Luhan et al., 2015).

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

Where: W_f = final weigh
W_i = initial weight
μ = specific growth rate

Ice-ice Disease Occurrence Sampling

A sampling of ice-ice disease occurrence was done every 15 days of culture. This was done through visual inspection. Infected bundles were counted, and the percent ice-ice disease occurrence was calculated using the formula below (Largo et al., 1995).

$$\text{Ice-ice \% occurrence} = \frac{\text{Number of infected bunches}}{\text{total number of bunches}} \times 100$$

Monitoring of Water Parameters and Maintenance

On a weekly basis, the farm area was visited to maintain the farm's cleanliness. This was done by removing all attached silt, epiphytes, and predators. Water parameters such as temperature and salinity were determined every seven days using a thermometer and refractometer, respectively. Water current, pH, and depth were measured using an improvised drogue, pH meter (Smart Sensor), and calibrated rope, respectively.

Data Analysis

One-way analysis of variance (ANOVA) was used to test the significance among treatments in terms of growth performance and ice-ice disease occurrence using IBM SPSS version 20. Post Hoc (Duncan) was used to rank the means of the treatments.

3. Results

On day 15, T_1 group (AMPEP), T_2 group (16-20-0), and T_3 group (Control) obtained specific growth rates (SGR) of 5.31±0.28 % days $^{-1}$, 5.82±0.29 % day $^{-1}$, and 5.62±0.22 % day $^{-1}$, respectively (Figure 2). One-way ANOVA revealed that there was no significant difference ($p>0.05$) among the treatments. On day 30, T_1, T_2, and T_3 groups gained SGRs of 5.56±0.16 % day $^{-1}$, 6.1±0.14 % day $^{-1}$, and 5.45±0.17 % day $^{-1}$, respectively. One-way ANOVA showed that the T_2 group was significantly higher ($p<0.05$) than T_1 and T_3 groups. However, on day 45, only T_1 group subsamples remained with an SGR of 4.27±0.01 % day^{-1}. Subsamples of T_2 and T_3 groups were washed out due to an extensive ice-ice disease outbreak.

The mean weight of *K. striatus* is shown in Figure 3. At 15 days of culture, there was no significant difference ($p>0.05$) among the treatments with mean weights of 112±4.23 g (T_1 group), 121±4.95 g (T_2 group), and 118±3.64 g (T_3 group). On day 30, T_1, T_2, and T_3 groups obtained mean weights of 269±12.95 g, 320±13.31 g, and 261±12.04 g, respectively. One-way ANOVA revealed that the T_2 group was significantly higher ($p<0.05$) than T_1 and T_3 groups. At 45 days of culture, however, only T_1 group subsamples were left with a mean weight of 342±18.46 g.

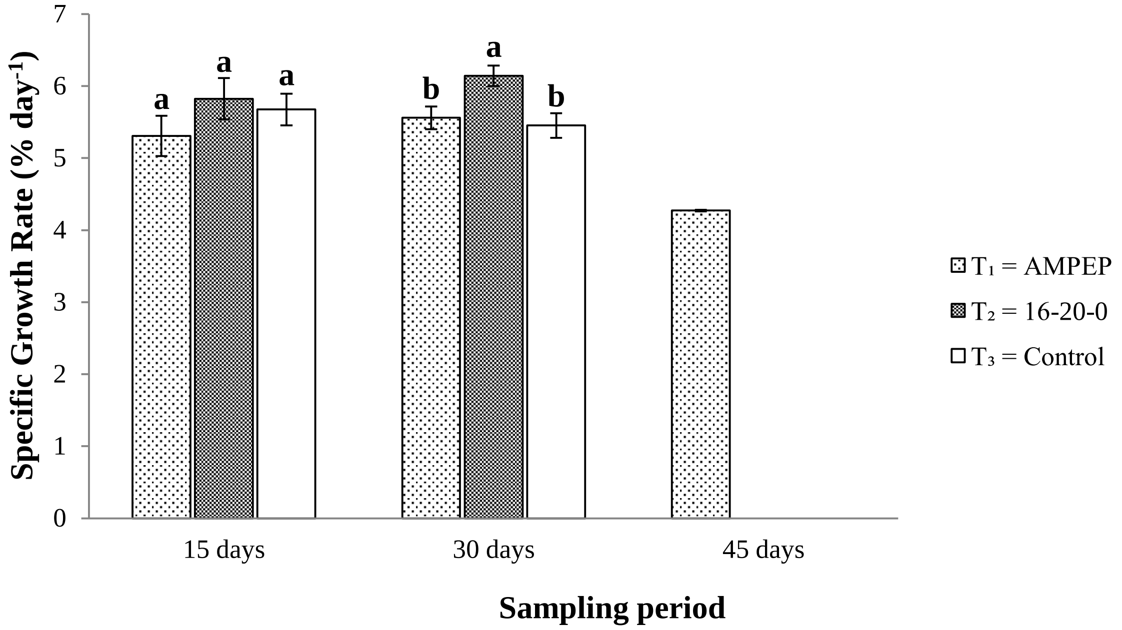

Figure 2. Specific growth rate (% day^{-1}) of *Kappaphycus striatus* in every sampling. Bars with different letters are significantly different at p<0.05. Errors bars in SEM (standard error of the mean), n=14-15.

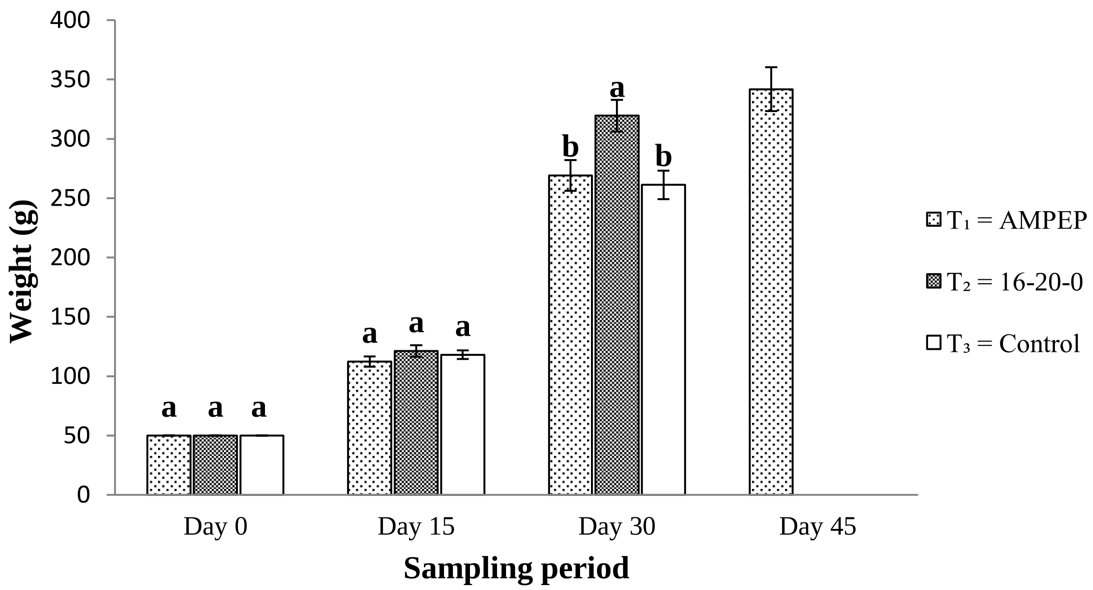

Figure 3. Mean weight of *Kappaphycus striatus* in every sampling. Bars with different letters are significantly different at p<0.05. Errors bars in SEM (standard error of the mean), n= 14-15.

The ice-ice disease occurrence of cultivated nutrient-enriched *K. striatus* was not observed after 15 days of culture. However, on day 30, the ice-ice disease appeared, and the T_2 group (5±0.36 %) recorded the lowest ice-ice disease than the T_1 group (12±0.77 %) and the T_3 group (22±0.74 %), but no significant difference (p>0.05) among the treatments were detected. On day 45, the T_1 group (0.57±0 %) was significantly (p<0.05) lower than the T_3 group (59.67±27.32 %) but not significant as the T_2 group (23.22±12.83 %) (Figure 4).

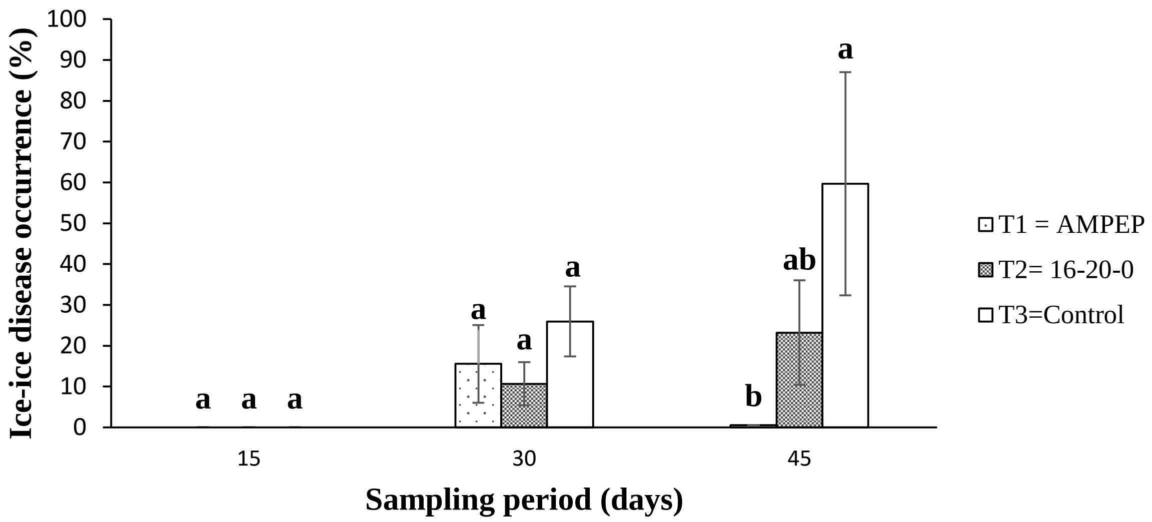

Figure 4. Ice-ice disease occurrence (%) of *Kappaphycus striatus* in every sampling. Bars with different letters are significantly different at $p<0.05$. Errors bars in SEM (standard error of the mean), n= 2-20.

Physico-chemical Parameters of the *K. striatus* Farm

The temperature of the farm water varied during the culture period ranging from 26±0 °C to 31±0.03 °C. The salinity fluctuated throughout the sampling period ranging from 32±0 to 35±0.03‰. pH ranged from 8.18±0.09 to 8.26±0.01. Water current velocity varied depending on depth and tide, ranging from 0.15±0.23 m s^{-1} to 3.92±1.45 m s^{-1}. The water depth of the farm ranged from 7.67±0.06 cm during low tide to 141±1.49 cm during high tide. These indicate that all water parameters were within the optimum condition for cultured seaweed.

4. Discussion

Growth of *K. striatus* enriched with inorganic fertilizer (16-20-0, N P K) was highest (5.82±0.29 % day $^{-1}$) at 30 days of the culture. The concentration of inorganic fertilizer (8.82 g L^{-1}) was higher than the biofertilizer/biostimulant (0.1 g L^{-1}). A similar study revealed that inorganic fertilizer (16-20-0) with a concentration of 8.82 g L^{-1} was effective in increasing the growth rate (4.5 % day^{-1}) after 28 days and consistently obtained a higher growth rate up to 49 days (Tahiluddin et al., 2022b). Ammonium phosphate (16-20-0) at a concentration of 9 g L^{-1}, when enriched to *K. striatus*, significantly improved the total nitrogen content (Tahiluddin et al., 2021); thereby, when planted, it provides additional nutrients essential for increasing the growth of seaweed. In another study, the 12 hours of immersion of *K. alvarezii* in 0.01 g L^{-1} sodium nitrate resulted in a significant specific growth rate (2.34 % day^{-1}) cultured in a grow-out cage for 45 days (Luhan et al., 2015).

Biofertilizer/biostimulant (AMPEP) with a concentration of 0.1 g L^{-1} after 15-30 days of farming did not affect the growth of *K. stiatus* but remained higher in the number of bunches after 45 days, with an SGR of 4.27±0.01 % day^{-1}. In the study of Hurtado et al. (2012), they found out that the AMPEP with a concentration of 1.0 g L^{-1} dipped for 60 minutes obtained a higher growth rate (7.7±0 % day^{-1}) in *K. alvarezii* after 45 days. Similarly, Loureiro et al.

(2013) obtained a positive result on the growth rate (7.3±1.7 % day^{-1}) of *K. alvarezii* after 45 days of culture using AMPEP. Furthermore, *K. alvarezii* and *K. striatus* in a tank culture system enriched with AMPEP resulted in a significant growth rate (2.0±0.03 % day^{-1}) after 40 days of culture (Zuldin and Shapawi, 2015). Borlongan et al. (2011) used AMPEP with a concentration of 0.1 g L^{-1} dipped for 30 minutes and gained a significant growth rate of 1.3-4.1 % day^{-1} in *K. alvarezii* after 45 days.

Ice-ice disease in the *Kappaphycus* farms has been attributed to changes in water parameters, such as temperature, light intensity, salinity, and/or the presence of opportunistic marine-derived fungi and marine bacteria (Tahiluddin and Terzi, 2021a; Tahiluddin and Terzi, 2021b; Bermil et al., 2022). In the Philippines, the ice-ice disease is widespread in eucheumatoid farms (Faisan et al., 2021). In Tawi-Tawi, the ice-ice disease is a lingering issue in *Kappaphycus* farms, and nutrient enrichment using inorganic fertilizers is an emerging treatment for this malaise (Tahiluddin et al., 2022b). In this study, biofertilizer /biostimulant (AMPEP) as nutrient enrichment for *K. striatus* was determined as effective in reducing the ice-ice disease occurrence after 45 days. Loureiro et al. (2012) reported that AMPEP could serve as protection to *K. alvarezii* from the ice-ice and epiphyte *Neosiphonia* sp. (now *Melanothamnus* sp.) in *Kappaphycus* varieties, which has a "vaccine-like effect." Similarly, the percentage of ice-ice disease was significantly lower (28%) in AMPEP-treated *K. striatus* compared with the control (45%) after 45 days (Illud, 2020). It is thought that the mode of action of AMPEP's efficacy in ameliorating damaging disease outbreak outcomes is via elicitating alga's natural defense mechanism against pathogenic microorganisms (Hurtado and Critchley, 2013; Loureiro et al., 2017).

5. Conclusion

The incorporation of nutrient enrichment in the farming of red seaweed *Kappaphycus striatus* renders an improved growth for seaweed enriched with inorganic fertilizer (ammonium phosphate, 16-20-0) and reduced ice-ice disease occurrence for seaweed enriched with biofertilizer /biostimulant (AMPEP). Further studies need to be evaluated using different concentrations, soaking time, species, farm areas, and duration. Carrageenan quality also needs to be determined using these fertilizers.

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