EFFECTS OF ORGANIC FERTILIZERS ON THE GROWTH PERFORMANCE, “ICE- ICE” DISEASE OCCURRENCE AND CARRAGEENAN QUALITY OF FARMED SEAWEED *KAPPAPHYCUS STRIATUS* (F. SCHMITZ) DOTY EX. P.C. SILVA

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ABSTRACT

A study was conducted to determine the effects of organic fertilizers (Acadian Marine Plant Extract Powder [AMPEP] and Padina liquid extract) at different levels of concentrations on the growth performance, “ice-ice” disease occurrence and carrageenan quality of farmed seaweed *Kappaphycus striatus* in seaweed farm of Barangay Taungoh, Sibutu, Tawi-Tawi for a duration of 45 days. The study had five (5) treatments with three (3) replicates: T_1 = control, T_2 = 0.01 g L⁻¹ AMPEP, T_3 = 8.82 g L⁻¹ AMPEP, T_4 = 0.1 ml L⁻¹ Padina liquid extract, and T_5 = 8.82 ml L⁻¹ Padina liquid extract. Results revealed that T_3 was significantly higher ($p < 0.05$) in terms of specific growth rate (3.12% day⁻¹) and carrageenan yield (26.47±1.89%) and significantly lower ($p < 0.05$) in the occurrence of “ice-ice” disease (18.96±2.29%) after 45 days of culture. The Padina liquid extract was also significantly higher ($p < 0.05$) than the control in terms of specific growth rate (2.27% day⁻¹) after 45 days of culture and has no significant difference ($p > 0.05$) with the control in terms of “ice-ice” disease occurrence. The AMPEP and Padina liquid extract could be an efficient organic fertilizer used in seaweed farming.

KEYWORDS: *Kappaphycus striatus*, AMPEP, Padina, growth performance, “ice-ice” disease occurrence, carrageenan quality

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1.INTRODUCTION

The red seaweeds *Eucheuma* and *Kappaphycus* had been harvested from the natural stock in commercial scale for export purposes in the late 1950's and early 1960's. Due to high demand of *Eucheuma* and *Kappaphycus* for carrageenan production from North American and European processing plants, there was an increase in the exploitation of their wild stock. This situation resulted to the depletion of the wild populations of these red seaweeds and subsequently this led to the initiative of Dr. Maxwell S. Doty in establishing commercial seaweed farming in Tapaan Island, Siasi, Sulu in the mid – 1960's and in the early 1970's. The seaweed cultivation has expanded to other parts of the country like the Visayas and Luzon, and to other countries like Micronesia, Fiji, East Africa and China (Hurtado and Agbayani 2000). The eucheumatoid seaweed cultivation was further introduced to over 20 countries in Southeast Asia, in the Pacific and the Western Indian Ocean (Hurtado et al. 2013; Msuya et al. 2013).

Kappaphycus, locally known as 'guso' or 'tambalang' constitutes 80% of the Philippines seaweed export. The Philippines ranked third as major seaweed producer in the world in 2001 after China and Korea (Hurtado 2003). In 2006, the Philippines produced about 1.5 million metric tons equivalent to 70% of the total aquaculture production. In the Philippines, *Eucheuma* and *Kappaphycus* farming increased from around 100,000 metric ton in

1995 to over 1.1 million metric ton in 2012 (White and Wilson 2015).

However, the outbreaks of "ice-ice" disease occurrence and epiphyte infestation in seaweed farms had caused to poor quality crops (Tisera and Naguit 2009; Pang et al. 2015) and the decrease of Philippine *Kappaphycus* production (Hurtado et al. 2006). Seaweed farmers have observed that this "ice-ice" disease occurrence and epiphytic filamentous algae infestation mostly caused by low nutrient levels, light intensity, temperature and salinity (Trono 1986). In recent years, seaweed farmers, particularly in Tawi-Tawi province, have developed culture techniques by using inorganic fertilizers to remedy the poor farming output. With the use of inorganic fertilizers, the farmed seaweeds grow faster and could be harvested in shorter culture period (Tahiluddin 2019).

Considering that chemicals from the synthetic fertilizers might have adverse effects on human health and the environment (Nabti et al. 2016), so the use of organic fertilizers is more preferable because these are biodegradable, non-toxic, non-polluting and non-hazardous to humans and other animals (Dhargalkar and Pereira 2005). The AMPEP and brown seaweeds such as *Sargassum* and *Padina* were proven to be environmental-friendly fertilizers. Thus, this study was conceived using organic fertilizers for seaweed cultivation. The result of this study could serve as guide to sustainable seaweed farming industry in Tawi – Tawi as well as other seaweed producing regions in the country.

This study aimed to investigate the effects of organic fertilizers on growth performance, "ice-ice" disease occurrence and carrageenan quality of farmed seaweed *Kappaphycus*. This study investigated the effects of organic fertilizers on growth performance, "ice-ice" disease occurrence and carrageenan quality of farmed seaweed *Kappaphycus striatus*.

2. LITERATURE CITED

Status of World Seaweed Farming

The top seven most cultivated seaweed genera, three are used mainly for hydrocolloid extraction. The red algae *Kappaphycus* and *Eucheuma*, are major sources of carrageenan which account for over 80% of world's carrageenan production (Pereira and Yarish 2008). China produces seaweeds mostly for food i.e. *Saccharina japonica* (formerly *Laminaria japonica*), *Undaria pinnatifida*, *Pyropia* spp. (formerly *Porphyra*) and *Sargassum fusiforme*. On the other hand, Indonesia and the Philippines produce mainly the carrageenophytes *Kappaphycus* and *Eucheuma* and *Gracilaria* spp. for carrageenan and agar respectively (FAO 2016).

In 2014, China and Indonesia were the leading seaweed farming countries, each produced more than 10 million tonnes, the Philippines and the Korean Republic over 1 million tonnes, while the People's Democratic Republic of Korea, Japan, Malaysia and Zanzibar produced over 100 000 tonnes (FAO 2016). *Kappaphycus* and *Eucheuma* have been cultivated in Indonesia that contributed over 9.0 million tons, over 83% of global production, mostly *Eucheuma*; followed by the Philippines nearly 1.4 million tons, 13% of global production, mostly *Kappaphycus*. Approximately 340,000 tons of these carrageenophytes were produced from Malaysia, Cambodia, Vietnam, China, Tanzania, Madagascar, Belize and Brazil (Valderrama et al. 2015, Bjerregaard et al. 2016, FAO 2017).

Effects of Fertilization on Seaweeds

The success of seaweed (*Eucheuma/Kappaphycus*) farming depends primarily on the natural fertility of the farm site. Seaweed farming fertilization is not yet a common practice throughout the world. In China, the nitrogen enrichment has

been practiced in *Laminaria japonica* cultivation since 1956 using the unglazed porous clay bottles, the porous plastic bags, splashing and spraying liquid fertilizers, soaking young sporelings in a fertilizer solution, and natural fertilization through polyculture (FAO 1989).

The nutrient enhancement could promote growth, improve carrageenan quality and reduce the occurrence of "ice-ice" diseases in farmed *Kappaphycus alvarezii*. The *K. alvarezii* enriched with sodium nitrate fertilizer could decrease "ice-ice" to 8.75% and become more resilient to diseases. The *K. alvarezii* fertilized with 10ppm sodium nitrate could increase the specific growth rate (SGR) of 1.4% day⁻¹ (Luhan et al. 2014) while *K. striatus* also fertilized using ammonium phosphate (16-20-0) every 10 days at high concentration (8.82g L⁻¹) doubled the SGR of 3.75% day⁻¹ even during "ice-ice" season (Tahiluddin 2019). Fertilization is not a common practice worldwide. In the Philippines, particularly in Tawi-Tawi province, the seaweed farmers have started *Kappaphycus* fertilization using agricultural fertilizers to increase production and minimize "ice-ice" occurrence since 2013 (Tahiluddin 2019). Sodium nitrate enrichment in farmed *Kappaphycus alvarezii* could provide better growth, good quality carrageenan and protection against "ice-ice" disease (Luhan et al. 2014). The seaweed based organic fertilizers generally contain 60 trace minerals which are essential for plant's health and growth. The use of organic fertilizers is a sustainable alternative to chemical fertilizers which might have detrimental effects in the environment. The synthetic fertilizers such as Nitrogen – Phosphorous – Potassium (NPK) may have different nutrient concentrations (Vitosh 1996). Every nutrient has specific role and function for plant growth. The nitrogen (N), phosphorous (P) and potassium (K) are necessary for photosynthesis, metabolism and physiological activities (Uchida 2000). The seaweed based organic fertilizers also contain nutrients, minerals and several important growth stimulators such as auxins, hormones, gibberellins, indoles, and cytokinins (Nabti et al. 2016).

The Acadian marine plant extract powder (AMPEP) is extracted from brown seaweed *Ascophyllum nodosum* and it is now used worldwide for large scale seaweed cultivation (Rodrigues 2016). Brown seaweed *Ascophyllum nodosum* extract as contained in AMPEP has been discovered to potentially activate the natural defense system of *K. alvarezii* against (Pramanick et al. 2017). The AMPEP used to fertilized *Kappaphycus species* can increase daily growth rate of 1.3 – 4.1 % day⁻¹ and decrease occurrence of epiphyte infestation (Borlongan et al. 2010) and the *K. alvarezii* fertilized with 10ppm ammonium could attain the daily growth rate of 4.6 % day⁻¹ (Rui et al. 1990). The thalli of *K. alvarezii* infested with "ice-ice" disease reduced in the concentration of photosynthetic so this would cause the loss of its photosynthetic efficiency and resulted to poor growth performance (Ganzon-Fortes et al. 1993). Several studies on the nutrient enrichment reported to have improved growth and health on cultivated seaweeds (Topinka 1976; Neish et al. 1977; Harlin and Thorne-Miller 1981; Lapoite 1986; Rui et al. 1990; Dawes et al. 1994; Troell et al. 1999; Villares et al. 1999; Menéndez et al. 2002; Borlongan et al 2010; Loureiro et al 2010; Martins et al. 2011; Reef et al. 2012; Zuldin and Shapawi 2015). The fertilization using *Ascophyllum nodosum* extract for farmed *Kappaphycus alvarezii* could improve growth and reduce epiphyte infestation (Loureiro et al. 2010). The use of Acadian Marine Plant Extract Powder (AMPEP) as fertilizer could also reduce the occurrence of "ice-ice" disease and epiphyte infestation in farmed.

Kappaphycus striatus (Zuldin and Shapawi 2015). The seaweed fertilizers also play a protective role in salinity tolerance by retaining moisture and nutrients in the soil (Sofy et al. 2017). The organic fertilizer derived from brown seaweed *Padina pavonica* also contains macronutrients such as nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg) and other trace elements which are necessary for development and growth of plants (Chbani et al. 2013).

Occurrence of Disease on Farmed Seaweeds

The "ice-ice" disease and infestation of epiphytic filamentous algae (EFA) are two major problems in *Eucheuma* and *Kappaphycus* farming. The "ice-ice" disease develops as a result of stresses on the seaweed brought about by unfavorable environmental factors (De San 2012; Largo et al. 1995a). The signs of the disease are that the thalli degenerate, turn into a pale white color and eventually rot (De San 2012). In a farm with slow water movement, the "ice-ice" disease can be also caused by pathogenic bacteria through invading the seaweed thalli (Largo et al. 1995b).

The high incidence of epiphytic filamentous algae (EFA) can also contribute to the occurrence of "ice-ice" disease (Uyenco et al. 1981; Trono and Ohno 1989). The epiphyte (*Neosiphonia savatieri*) infestation happens at high temperature, low salinity and slow water movement and during the period when the farmed seaweeds are vulnerable to "ice-ice" disease (Tisera and Naguit 2009; Hung et al. 2009). The "ice-ice" disease in eucheumatoid seaweed farming usually occurred simultaneously with *Neosiphonia savatieri* infestation (Pang et al. 2015). Pests and diseases are considered as the major problems in seaweed farming (Azanza and Ask 2017). Hurtado et al. (2006) reported the *Polysiphonia* outbreaks in the Philippines that caused a decrease in *Kappaphycus alvarezii* production. The occurrence of epiphytic filamentous algae had also caused a large scale die-off of *K. alvarezii* in China (Pang et al. 2015). The "ice-ice" disease has become a major problem in seaweed industry. The "ice-ice" affected seaweeds become whitish, soft and eventually disintegrate and hence resulted to poor quality crops (Tisera and Naguit 2009). If "ice-ice" is observed at seaweed farm, it is recommended to harvest the entire crop and replace them with the new seedlings or transfer the plants to the unaffected farming area (Arasamuthu and Edward 2016).

Carrageenan Quality of Seaweeds

The Eucheumatoid species including *Eucheuma denticulatum*, *Kappaphycus alvarezii* and *K. striatus* (formerly *K. striatum*) are primary source of the valuable hydrocolloid carrageenan (Bixler and Porse 2011). Carrageenan is a good source of soluble fiber (Burtin 2003). These are species of seaweeds as source of different types of carrageenan: (a) *Eucheuma denticulatum* (*spinosum*) – iota carrageenan, (b) *Kappaphycus alvarezii* (*cottonii*) and *K. striatus* – kappa carrageenan, (c) *Gigartina radula*, *Gigartina skottsbergii*, *Sarcothalia crispata* and *Chondrus crispus* – kappa/lambda carrageenan. These types of carrageenan slightly vary in chemical structures and properties (McHugh 2003). The iota, kappa, and lambda carrageenan have the highest commercial interest. Carrageenan is a versatile product widely used for food and nonfood industries (Van de Velde et al. 2002; Heertje 1993; Hood & Allen 1977; Swaisgood 1982).

3. MATERIALS AND METHODS

Research Locale

The study was conducted in the seaweed farm of Barangay Taungoh, Sibutu, Tawi-Tawi (4° 42' 21" N; 119° 29' 34" E) for 45 days from February 9 to March 23, 2019 (Fig. 3.1).

Figure 1: Map of the study site (Extracted from Google Earth)

Design

This study made use of experimental research design. There were five (5) Treatments with three (3) replicates: Treatment T_1 – control, T_2 – Acadian Marine Plant Extract Powder (AMPEP) (0.01g L^{-1} low concentration), T_3 – Acadian Marine Plant Extract Powder (AMPEP) (8.82 g L^{-1} high concentration), T_4 – *Padina* liquid extract (0.1 ml L^{-1} low concentration) and T_5 – *Padina* liquid extract (8.82 ml L^{-1} high concentration).

Procedures

Fertilizers

The Acadian Marine Plant Extract Powder (AMPEP) is extracted from brown seaweed *Ascophyllum nodosum* and it is now commercially available organic fertilizer in global market (Rodrigues 2016). The formulated fertilizer was prepared using a brown algae *Padina* sp. as sole ingredient since it has been proven as good organic fertilizer (Chbani et al. 2013). *Padina* sp. was collected from Kalang, Sanga-Sanga, Bongao, Taw-Tawi. The *Padina* was thoroughly washed using tap water to remove sea salts on the surface of the seaweed. Fresh *Padina* was cut into small pieces and boiled with 1 liter distilled water for an hour and the extract was filtered through a fine cloth (Kumar and Sahoo 2011). The extract was kept in an empty bottle and stored at room temperature. The formulated fertilizer was in the form of *Padina* liquid extract.

Farming of Seaweeds

Healthy, epiphyte – free, unfertilized propagules were purchased from the seaweed farmer in the study area. A bunch of 50g propagules was tied into a 5m cultivation line (PE rope #6) with 25cm interval (Trono 1992). Three replicate lines were prepared with 20 bunches each line. A total of 15 lines were prepared.

Fertilization

Fertilization was done late in the afternoon (2 – 6 pm). Four (4) different fertilizer solutions were prepared using AMPEP and *Padina* liquid extract with 0.01 g L^{-1} (Hurtado and Critchley 2013) and 8.82 g L^{-1} (Tahiluddin 2019) concentrations for both fertilizers and the control without fertilizer. The tied propagules were dipped into the ten (10) liter fertilizer solutions for 30 seconds, and then covered with canvass overnight (12-15 hours) (Tahiluddin 2019 unpublished data). Refertilization was done every 15 days using newly mixed solutions.

Planting of Seaweeds

The propagules were planted randomly in the farm using a modified fixed – off bottom method at 0.3m above the seabed (Fig. 2). Planting of seaweeds was done in the morning during low tide. Planted seaweeds were monitored every 7 days for cleaning and removal of the attached epiphyte algae and other debris. The environmental parameters were recorded every 7 days. Temperature and salinity were measured using thermometer and refractometer, respectively. The water depth using calibrated rope line and water current velocity using an improvised drogue.

Figure 2: Experimental layout of the farm

Growth Sampling

Sampling was done every 15 days to determine specific growth rate (SGR). Five (5) bunches of propagules were randomly taken from three replicates for all treatments. The propagules were blot-dried on smooth cloth and then weighed using weighing scale. Growth (μ) expressed as specific growth rate was computed using formula below (Luhan and Sollesta 2010):

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

where W_f is the final weight, W_i is the initial weight and $\ln$ is the natural logarithm.

The occurrence of "ice-ice" disease was monitored every 15 days through visual inspection. Any sign of white and soft branches was considered "ice-ice" (Luhan et al. 2014). The "ice-ice" percentage occurrence of infected seaweeds was calculated using the formula below (Largo et al. 1995a).

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

Extraction of Carrageenan from *K. striatus*

Alkali-treated Carrageenan

The seaweeds *K. striatus* was sun-dried for three days. A 20g dried seaweed samples from each treatment was prepared for moisture content analysis. The samples were mixed thoroughly. Three (3) replicates of 5g samples were taken and analyzed with moisture analyzer. A weight of 200g of sun-dried seaweeds *K. striatus* was taken from each treatment for carrageenan extraction. The dried seaweeds were cut into small pieces. Fifteen (15) g samples of dried seaweed from each treatment were soaked in 5% KOH solution for 2 hours. The alkali solution was decanted. The treated thalli were heated for one hour. The thalli were washed and 250ml of purified water was added. The thalli were boiled for one hour and then osterized. The decalite was added to the solution before it was filtered. The filtrate was allowed to cool and gel. The gel was frozen overnight and thawed the following day. The product was alkali-treated carrageenan and it was dried in an oven (Romero 2009; Romero et al. 2000).

Carrageenan Yield

The dried carrageenan was weighed and recorded. Carrageenan yield was determined using equation below (Mohammad-Akhmal et al. 2017; Luhan et al. 2014; Webber et al. 2012; Muñoz et al. 2004.):

$$Y = \frac{W_c}{W_{ds}} \times 100$$

where Y is the yield of crude carrageenan extract (%), W_c is the amount of the extracted alkali-treated carrageenan (g) and W_{ds} is the weight of dried seaweed (g) used for extraction.

Viscosity

A 2.25g dried carrageenan was placed in a tare-weight of 250ml beaker. A 0.30g KCl and distilled water were added to the carrageenan until a 150g tare weight was reached. The beaker was placed in the water bath to dissolve the carrageenan. The solution was heated using microwave oven. After re-weighed, the solution was heated again to more than 75°C. The viscosity jar was filled with solution to the marked level. The jar was inserted in Brookfield Synchro-lectric nViscometer Model LVF. The viscosity of the solution was read at 75°C (Romero 2009; Romero et al. 2000).

Gel Strength

Thirty (30) ml of carrageenan solution was placed in a 50ml beaker and allowed to cool overnight. The gel strength was measured using Gel texture analyzer and it was computed using equation below (Romero et al. 2000).

$$GS = \frac{F}{A}$$

where GS is gel strength (g cm^{-2}), F is the force (g) and A is an area which was obtained from the gel tester analyzer with a constant value of 1.26677cm^2 .

Gelling and Melting Temperatures

The 10ml/tt remaining hot solution was put in a test tube. A thermometer with its bulb situated just below the solution surface was inserted through a stopper. The solution was allowed to cool and glass beads (dia: 2.85mm; wt: 30g) was dropped at intervals of 0.5°C. The temperature was recorded at which a bead failed to drop through the agar solution. This

is the gelling temperature of the solution. The gel set-up used in gelling temperature determination was allowed to cool overnight. The set-up was placed in an electric heated water bath and heated slowly. A lead shot (dia: 4.30mm; wt: 50mg) was placed on the gel surface. The temperature at which the lead shot dropped to the bottom of the test tube was recorded as the melting temperature (Romero 2009; Romero et al. 2000).

Syneresis Index

Ten (10) g gel solution was placed in a cylindrical tube (5cm length) and allowed to cool overnight and then, it was weighed. The gel was individually placed on filter papers and allowed to stand for 2 hours. The test tube was weighed to get the weight of the gel and the gel was weighed after 2 hours. The difference in the weight represents the amount of water exuded by the gel upon standing. Syneresis index was determined using equation below (Romero et al. 2000).

$$I = \frac{W_i - W_f}{W_i} \times 100$$

where I is the syneresis index (%), W_i is the initial weight of the gel (g) and W_f is the final weight of the gel after standing for 2 hours.

Statistical Analysis

The significant difference on the growth rate, "ice-ice" disease occurrence and carrageenan quality of *Kappaphycus striatus* was subjected to Two-way Analysis of Variance (ANOVA) using SPSS software version 20 (SPSS Inc.). A Post hoc test (LSD) was used to rank the means.

4. RESULTS

Growth

After 45 days of culture, the average final weight, weight gained, daily growth increment (DGI) and specific growth rate (SGR) of *K. striatus* enriched with organic fertilizers with two levels of concentrations are presented in Table 1. Results revealed that T_3 was highly significant ($p < 0.05$) than those of the four treatments. Results also showed that T_2 , T_4 and T_5 were significantly higher ($p < 0.05$) than T_1 .

Table 1: Average final weight, weight gained, daily growth increment (DGI) and specific growth rate (SGR) of *K. striatus* enriched with organic fertilizers after 45 days culture periods. Values with different letters are significantly different at $p < 0.05$

Treatments	Final Wt. (g)	Wt. Gained (g)	DGI (g)	SGR (% day ⁻¹)
T_1 – Control	134.87±1.27	84.07	1.87	2.17±0.020 ^a
T_2 – 0.1 g L ⁻¹ AMPEP	141.60±0.20	90.70	2.02	2.27±0.003 ^b
T_3 – 8.82 g L ⁻¹ AMPEP	148.40±0.31	97.50	2.17	2.38±0.003 ^c
T_4 – 0.1 g L ⁻¹ <i>Padina</i>	139.13±0.29	88.53	1.97	2.27±0.012 ^b
T_5 – 8.82 g L ⁻¹ <i>Padina</i>	140.33±0.18	89.73	1.86	2.27±0.003 ^b

The average specific growth rates (SGR) of farmed *K. striatus* treated with organic fertilizers with two levels of concentrations in three sampling periods are presented in Table 2. Results revealed that T_3 was significantly higher ($p < 0.05$)

than those of the four treatments throughout the sampling periods.

Table 2: Average specific growth rate (% day⁻¹) of *K. striatus* enriched with organic fertilizers in three sampling periods. Values with different letters are significantly different at p<0.05

Treatments	Day 15	Day 30	Day 45
T ₁ – Control	2.45±0.133 ^a	2.51±0.035 ^a	2.17±0.020 ^a
T ₂ – 0.1 g L ⁻¹ AMPEP	2.80±0.067 ^{ac}	2.59±0.011 ^a	2.27±0.0003 ^b
T ₃ – 8.82 g L ⁻¹ AMPEP	3.97±0.103 ^b	3.00±0.003 ^b	2.38±0.003 ^c
T ₄ – 0.1 g L ⁻¹ <i>Padina</i>	2.87±0.087 ^{ad}	2.55±0.003 ^a	2.27±0.012 ^b
T ₅ – 8.82 g L ⁻¹ <i>Padina</i>	2.96±0.272 ^{cd}	2.51±0.085 ^a	2.27±0.003 ^b

Occurrence of "Ice-ice" Disease

The percentage of "ice-ice" disease occurrence of farmed *K. striatus* enriched with AMPEP and *Padina* liquid extract with two levels of concentrations in three sampling periods is shown in Table 4.3. The "ice-ice" disease occurrence was observed after 30 days of culture. Results revealed that T₃ (18.96±2.3%) was significantly lower compared to T₂ (19.98±1.7%), T₁ (20.1±1.6%), T₅ (27.84±0.89%) and T₄ (28.38±1.03%) but T₃ was not significantly different with T₂ on the 30th day of culture. On the 45th day of culture, results revealed that T₂ and T₃ were significantly lower with 28.17±2.4% and 27.63±2.8%, respectively (p<0.05).

Table 3: Percentage (%) of "ice-ice" disease occurrence in farmed *K. striatus* in three samplings during 45 days of cultivation. Values with different letters are significantly different at p<0.05

Treatments	Day 15	Day 30	Day 45
T1 – Control	0	24.10±1.583 ^{ad}	45.27±3.948 ^a
T2 – 0.1 g L ⁻¹ AMPEP	0	19.98±1.689 ^{ac}	28.17±2.405 ^b
T3 – 8.82 g L ⁻¹ AMPEP	0	18.96±2.287 ^c	27.63±2.755 ^b
T4 – 0.1 g L ⁻¹ <i>Padina</i>	0	28.38±1.030 ^d	43.99±5.097 ^a
T5 – 8.82 g L ⁻¹ <i>Padina</i>	0	27.84±0.892 ^d	39.22±1.963 ^a

Carrageenan Quality

The moisture content, carrageenan yield, viscosity, syneresis, gel strength, gelling temperature and melting temperature *K. striatus* are presented in Table 4.5. Results showed that T₃ was significantly higher on carrageenan yield and syneresis index with 26.47±1.89% and 13.7±0.34%, respectively, and significantly lower in gel strength with 249.03±3.98 g cm⁻² (p<0.05), but it was not significantly different (p>0.05) on the moisture content, viscosity, gelling and melting temperatures with 32.0±0.55%, 15.0±2.84%, 40°C and 50.0±3.46°C, respectively.. Results also revealed that T₂ was significantly higher in gel strength with 333.34±4.43 g cm⁻² than T₁, T₃ and T₄ but it was not significantly different with T₅ (p<0.05).

Table 4: Carrageenan quality of dried *K. striatus* after 45 days of culture.Values with different letters are significantly different at $p < 0.05$

Treatment s	Moisture content of dried seaweed (%)	Yield (%)	Viscosity (cPs)	Syneresis index (%)	Gel strength (g cm^{-2})	Gelling temperature ($^{\circ}\text{C}$)	Melting temperature ($^{\circ}\text{C}$)
T ₁ – Control	33.75±0.20 ^a	21.27±0.83 _b	15.50±2.26 _a	10.74±0.39 _c	302.61±11.92 ^b _c	39.00±0.58 _a	51.33±0.67 ^a
T ₂ – 0.1 g L ⁻¹ AMPEP	31.85±0.58 ^b	22.11±0.16 _b	12.67±1.67 _a	12.25±0.45 _{ab}	333.34±4.432 ^a	40.67±0.33 _a	51.67±4.10 ^a
T ₃ – 8.82 g L ⁻¹ AMPEP	32.00±0.55 ^{ab}	26.47±1.89 _a	15.00±2.84 _a	13.70±0.34 _a	249.03±3.987 ^d	40.00±0.00 _a	50.00±3.46 ^a
T ₄ – 0.1 g L ⁻¹ Padina	32.57±0.66 ^{ab}	24.09±0.36 _b	12.00±1.26 _a	10.95±.60 ^{bc}	300.16±2.637 ^c	40.00±0.00 _a	48.67±3.84 ^a
T ₅ – 8.82 g L ⁻¹ Padina	32.15±0.77 ^{ab}	21.91±1.05 _b	13.00±2.02 _a	10.42±0.54 _c	320.76±4.604 ^a _b	40.00±0.00 _a	45.33±2.96 ^a

5.DISCUSSIONS

Growth

In this study, the farmed seaweed *Kappahycus striatus* enriched with AMPEP (8.82 g L⁻¹) obtained highest specific growth rate (2.38±0.003% day⁻¹) after 45 days of culture. Enriched *K. striatus* grew faster due to an efficient absorption of nutrients during fertilization with AMPEP (Hurtado et al. 2011). Nutrient enrichment could significantly increase growth for *K. alvarezii* (Luhan et al. 2014). The result of this study was similar to 2.4–3.0% day⁻¹ *K. alvarezii* (Borlongan et al. 2010), 3.1–4.1% day⁻¹ *K. alvarezii* (Hurtado et al. 2011), 2.00% day⁻¹ in the *K. alvarezii* dipped with AMPEP (Zulidin and Sapawi 2015), and 1.3–4.1% day⁻¹ for *K. alvarezii* enriched with AMPEP under laboratory conditions (Loureiro et al. 2009). There have been few studies on the use of inorganic fertilizers and seaweed extract as a growth promoter in farmed seaweed *Kappaphycus*. A recent study conducted by Tahluddin (2019) on farmed *K. striatus* enriched with ammonium phosphate (16-20-0) at high concentration (8.82 g L⁻¹) attained the SGR of 3.75% day⁻¹ during poor season. Luhan et al. (2014) obtained SGR of 0.53–0.40% day⁻¹ enriched with nitrogen (N) for *K. alvarezii* cultured in tank. Rui et al. (1990) obtained SGR of 4.6 % day⁻¹ fertilized with 10ppm ammonium *K. alvarezii*.

The *Padina* liquid extract applied to farmed seaweed *K. striatus* could also increase growth rate (2.28% day⁻¹). All these of available information on seaweed *Padina* extracts were applied in agricultural crops. *Padina* liquid extract can provide macronutrients and trace elements necessary for the growth of plants (Chbani et al. 2013). Seaweed *Padina* liquid fertilizers also contain higher amount of water-soluble potash, other minerals and trace elements which are readily absorbed by plants (Kumareswari and Rani 2015).

Occurrence of "Ice-ice" Disease

It was observed that "ice-ice" disease occurred after 30 days of culture. The seaweed *K. striatus* treated with AMPEP obtained the lowest percentage (18.96±2.29%) and (27.63±2.76%) of "ice-ice" disease occurrence during 30 day and 45 days of culture, respectively. The AMPEP is more efficient organic fertilizer in reducing the occurrence of "ice-ice" disease in farmed *K. striatus* (Zulidin and Shapawi 2015). Despite the optimum physio-chemical parameters of the seaweed

farm, the "ice-ice" disease occurred during March. The optimum physio-chemical parameters for *Kappaphycus* should range between 33–35 ppt (Largo 2002), 20 – 32 °C (Largo et al. 1995a; De San 2012). High stocking density and seaweed overcrowding (Largo et al. 1995a) and high incidence of epiphytes could contribute to the occurrence of "ice-ice" disease (Uyenco et al. 1981; Trono and Ohno 1989; Largo et al. 1995a).

The application of AMPEP to seaweed farming could inhibit occurrence of "ice-ice" disease and pathogenic and epiphyte infestations (Borlongan et al. 2010). Luhan et al. (2014) reported that occurrence of "ice-ice" disease reached as 97% for control while *K. alvarezii* enriched with sodium nitrate could significantly lower to 8.75% in cage culture. The "ice-ice" affected seaweeds would disintegrate and resulted to poor quality crops and production (Tisera and Naguit 2009). The "ice-ice" disease outbreak has become a major problem for seaweed farmers who do have other alternative means of livelihood.

Analysis of Carrageenan Quality

Carrageenan quality and properties can vary among seaweed species depending on the culture technique (Azanza-Corrales 1990; Trono and Lluisma 1992), farm site and its environmental parameters (Hayash et al. 2007), the extraction process (Villanueva et al. 2003) and the nutrient enrichment (Luhan et al. 2014).

The moisture content obtained in this study (Table 4) met the standard moisture content requirement (ranging 30 – 39%) for top quality dried seaweed. The average 35% moisture content referred to as the most stable dried seaweed. Moisture content of the seaweed is very critical factor which can affect its carrageenan quality and eventually its market price (Hurtado and Agbayani 2000). Highest carrageenan yield ($26.47 \pm 1.89\%$) was obtained from *K. striatus* enriched with AMPEP (8.82 g L^{-1}) followed by $24.09 \pm 0.36\%$ from *Padina* liquid extract (8.82 g L^{-1}). The carrageenan yields may vary depending on the extraction method and the strain or variant species of seaweeds (Mohammad-Akhmal et al. 2017). In this study, carrageenan yield was quite similar to that reported by Mohammad-Akhmal et al. (2017) which ranged from 20.4–28.4% and Hayashi et al. (2007) obtained 15–20% for *Kappaphycus sp.*. Munoz et al. (2004) reported higher carrageenan yield (30.3 to 40.7%) of brown, green and red strains of *K. alvarezii*. Hurtado-Ponce (1995) reported that yield could be as low as 15.00% and Mendoza et al. (2002) as high as 64.00% in *Kappaphycus*. Luhan et al. (2014) obtained 42.55% for *K. alvarezii* enriched with sodium nitrate and 33.05% for control. Pathik et al (2006) reported that *K. alvarezii* extract yielded 62.40% treated with KOH and 2.8 – 72.9% with NaOH. The variation in carrageenan yield could possibly differ due to the extraction method and process (Mohammad-Akhmal et al. 2017).

The organic fertilizers did not affect the viscosity of the alkali-treated carrageenan. The viscosity obtained from this study was similar to that reported by Pathik et al. (2006) which ranged from 12.94 – 25.25 cPs in *K. alvarezii*. Other studies obtained viscosity of 16.17 ± 17 cPs in *K. alvarezii* (Dewi et al. 2014), 18.00 cPs in *K. striatum* (Mendoza et al. 2006), and 20.0 cPs in *K. striatum* (Mendoza et al. 2002). Hurtado-Ponce (1995) obtained viscosity ranged from 23.0 ± 2.0 – 34.5 ± 1.5 cPs in *K. alvarezii*. Krechhoff et al. (2015) obtained viscosity ranged from 30.0–60.0 cPs in *K. alvarezii*. The organic fertilizers could affect gelling temperature of the alkali-treated carrageenan. *K. striatus* treated with organic fertilizers obtained high gelling temperature. Gelling temperature is the temperature for initial development of gel networks through formation of helices and junction zones upon cooling (Lai and Lii 1997). The gelling temperature obtained in this study was similar with other studies such as 39.3 ± 0.3 °C in *K. striatum* (Mendoza et al. 2006) and 32.7 ± 0.3 – 34.5 ± 0.9 °C in *K. striatum* (Mendoza et al. 2002). Hurtado-Ponce (1995) obtained gelling temperature in *K.*

alvarezii ranged from 8.6 ± 1.6 – 17.3 ± 1.3 °C. The organic fertilizers did not affect melting temperature of the alkali-treated carrageenan. The melting temperature corresponds of the dissociation of the most cross-linked junction zones of gel upon heating (Lai and Lii 1997). The melting temperature obtained in this study was similar with other studies such as 53.0 ± 2.6 °C in *K. striatum* (Mendoza et al. 2006) and 52.0 ± 1.8 – 56.2 ± 2.5 °C in *K. striatum* (Mendoza et al. 2002). Hurtado-Ponce (1995) obtained melting temperature in *K. alvarezii* ranged from 46.5 ± 0.5 – 67.5 ± 0.5 °C.

The AMPEP enhanced the syneresis index of the alkali-treated carrageenan (Table 4). The syneresis index (%) expresses the amount of water exuded from the gel matrix after standing for certain period of time (Rees 1969; Armisen and Galatas 1987). The syneresis index (Fiszman and Duran 1992) as modified by Romero et al. (2000) indicates the extent of interaction between and among polymers. The index represents the water holding capacity of the gel. Less water exuded from the gel means high water holding capacity. The amount of synerized water may vary among species of seaweed and between the extraction methods (Romero 2009). These results were lower than to the syneresis index reported by Mendoza et al. (2002) which ranged (15.4 ± 3.3 – $25.2 \pm 2.7\%$) extracted from *K. striatum*.

The organic fertilizers could affect the gel strength of the alkali-treated carrageenan. *K. striatus* enriched with 0.01 g L^{-1} AMPEP obtained the highest gel strength of $333.34 \pm 4.43 \text{ g cm}^{-2}$ followed by $320.76 \pm 4.60 \text{ g cm}^{-2}$ from 8.82 ml L^{-1} *Padina* liquid extract. The gel strength of agar or carrageenan is an important parameter in distinguishing the gel quality for the use in bacteriological and biomedical applications and food industry (Yaphe and Duckworth 1972). The gel strengths obtained from this study were higher than other studies such as $131.0 \pm 8.0 \text{ g cm}^{-2}$ in *K. alvarezii* (Hurtado-Ponce 1995) and 37.0 ± 5.6 – $146.5 \pm 25.6 \text{ g cm}^{-2}$ in *K. striatum* (Mendoza et al. 2002). These results were lower than those reported by previous studies such as $504.3 \pm 11.8 \text{ g cm}^{-2}$ in *K. striatum* (Mendoza et al. 2006) and $776.53 \pm 37.15 \text{ g cm}^{-2}$ in *K. alvarezii* (Dewi et al. 2014). Luhan et al. (2014) obtained gel strength of 672 g cm^{-2} for *K. alvarezii* enriched with 10 ppm sodium nitrate and 613 g cm^{-2} for control. Mohammad-Akhmal et al. (2017) reported gel strength ranged from 484 – 712 g cm^{-2} for *Kappaphycus spp.* treated with KOH. Krechhoff et al. (2015) obtained gel strength which ranged from 557–1,391 g cm^{-2} in *K. alvarezii*.

6. CONCLUSIONS

The use of Acadian Marine Plant Extract Powder (AMPEP) with high concentration has very efficient effect on growth performance on farmed *Kappaphycus striatus*. The AMPEP could also be an effective agent in minimizing the occurrence of "ice-ice" disease on farmed *K. striatus*. High concentration of AMPEP could produce higher carrageenan yield of *K. striatus*. *Padina sp.* could also be good alternative as organic fertilizer used in seaweed farming because this could increase growth on *K. striatus* and produce high carrageenan yield. Thus, organic fertilizers (AMPEP and *Padina* liquid extract) could enhance faster growth and reduce occurrence of "ice-ice" disease on farmed *K. striatus*.

7. RECOMMENDATIONS

It is recommended that other studies on the use of organic fertilizers in seaweed farming should be conducted at different level of concentrations and with different farming methods and techniques. Other studies should also investigate on the carrageenan quality and properties in terms of culture periods using the same fertilizers in seaweed cultivation.

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