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Performance of Red Seaweed (*Kappaphycus* sp.) Cultivated Using Tank Culture System

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ABSTRACT

Little is known on the performance of seaweed cultivated in land-based facilities. The present study was conducted to determine the performance of *Kappaphycus* sp. cultivated in tank culture system with the effect of seaweed density, usage of Acadian Marine Plant Extract Powder (AMPEP), AMPEP fertilizer and the observation on the disease occurrence. Two red seaweed species (*K. alvarezii* variety Brown Tambalang, BT and *K. striatum* variety Green Flower, GF) were selected and mutually cultivated using hanging culture method. Three 40 days trials were conducted with different seaweed density (Trial 1:2.40, Trial 2:1.92 and Trial 3:0.96 g L^{-1}). Besides, four treatments were performed in each trial: T1 (filtered seawater only), T2 (filtered seawater enriched with 13 mg L^{-1} AMPEP fertilizer), T3 (filtered seawater enriched with sands and corals and T4 (filtered seawater enriched with 13 mg L^{-1} AMPEP fertilizer, sands and corals). Based on the result, the maximum daily growth rate (2.00±0.03% day^{-1} for *K. striatum* and 1.46±0.06% day^{-1} for *K. alvarezii*) was recorded during Trial 3 with the lowest seaweed density using T4 treatment under natural culture parameters of light intensity, DO level, pH, temperature and salinity. In the present study, the performance of seaweed culture in tank was challenged by diseases such as "ice-ice" and seawater epiphyte infection that might cause bleached stems and weight loss to the seaweed. These findings are significant to provide a baseline data and facilitate the land-based seaweed farming in the future.

Key words: *Kappaphycus* sp., *K. alvarezii* variety Brown Tambalang *K. striatum* variety Green Flower, land based cultivation, seaweed density

INTRODUCTION

Seaweed farming has been identified as one of the high impact aquaculture activities in Malaysia due to the increasing world demand for raw and processed seaweed with reported global world demand in 2012 of about 350,000-400,000 mt (Yassir, 2012). Most of the seaweed farming in Malaysia and the neighbouring countries such as Philippines, Indonesia, Vietnam and Myanmar involve open sea cultivation (Bindu and Levine, 2011). The most common cultivation method practiced by local seaweed farmers in Semporna, Sabah involve fixed long-line "tie-tie" culture system or also known as hanging culture method for a period of 30-45 days before harvest (Ali *et al*., 2014). In Sabah, Malaysia, seaweed farming in the open sea is challenged by the

ambiguous dependency on the seasonal growth variation, epiphytic infection, grazer's threats of turtles and fishes (Neish, 2003) and also safety issues of the labour. The land-based cultivation method of seaweed might provide an alternative to the open sea cultivation. In addition, the land-based culture facility is important for seaweed research work such as acclimatization of seedling produced from the tissue culture and genetics study.

Little is known about the performance of seaweed cultivated in land-based culture methods. Recently, few researches studied the nutritional content, micropropagation and favourable cultivation method with promising findings (Chan *et al*., 2006). In 2009, researchers from China had invented a small raceway tank for the cultivation of brown seaweed (*Sargassum horneri*) in an indoor space (Pang *et al*., 2009). The brown seaweed was reported to have the ability to survive in tank with accelerated reproduction rate under an optimum condition. Therefore, comparable experimental design could also be applied for *Kappaphycus* sp. in order to investigate their ability to grow in tank culture system.

In the present study, *Kappaphycus* sp. was selected as the experimental subject based on their commercial value as carrageenophytes producing marine biopolymer known as carrageenan that acts as binding, gelling and thickening agent. *Kappaphycus* sp. is also abundantly cultivated in the East Coast waters of Sabah, Malaysia. In terms of reproduction, *Kappaphycus* sp. has the ability to reproduce through both asexual and sexual ways (Bulboa *et al*., 2008) and undergo spores germination and vegetative propagation (Ask and Azanza, 2002). In fact, vegetative propagation is highly applicable for seaweed cultivation in tank culture method (Halling *et al*., 2005). Therefore, *Kappaphycus* sp. might be able to survive and grow normally in tank through vegetative reproduction or growing thalli with the presence of dividing cells at the tip of each thallus growing on the seaweed branchlets. The present study aims to determine the performance of *Kappaphycus* sp. cultivated in tank culture system with the effect of seaweed density, usage of acadian marine plant extract powder, AMPEP fertilizer and the observation on the disease occurrence.

MATERIALS AND METHODS

Seedling selection, collection and translocation: The red seaweed species (*Kappaphycus* sp.) was selected based on their abundance, availability and quality based on previous physiological experimental data covering growth rate, survival rate, nutritional value and carrageenan content (Neish, 2003). Sampling was done using manual seaweed selection at the local seaweed farm located at Selakan Island, Semporna (N04°34.066', E118°40.673'). *Kappaphycus alvarezii* variety Brown Tambalang (BT) and *K. striatum* variety Green Flower (GF) were then translocated from the farm to University Malaysia Sabah (UMS) Shrimp Hatchery (Fig. 1). The young and healthy seaweeds with good quality and high number of potential growing thalli were selected and weighed about 50-60 g as initial seedlings. The seedlings were washed thoroughly in fresh sea water before being transferred to the tank for visible epiphytes removal purpose.

Experimental design: Three 40 days trials were conducted with different seaweed density (Trial 1:2.40, Trial 2:1.92 and Trial 3:0.96 g L^{-1}) and four different treatments were tested in each trial. The trials were performed from July to August 2013 (Trial 1), September to October 2013 (Trial 2) and February to middle of March 2014 (Trial 3). The seaweed density was

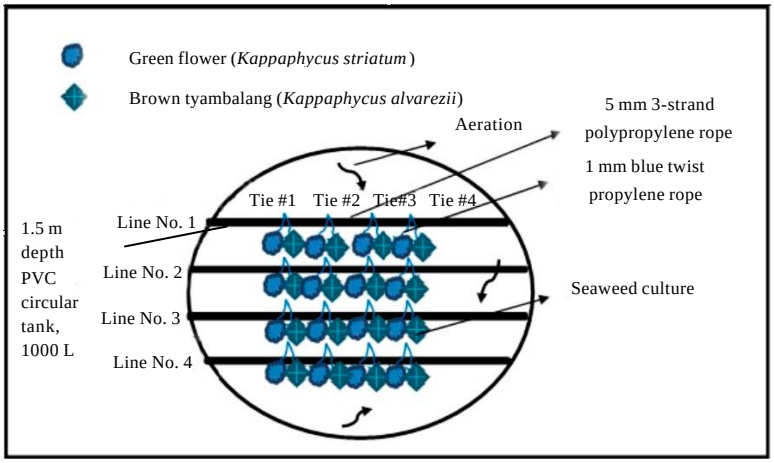

Fig. 1: Basic design of individual tank culture system used during every trial for each treatment tank

calculated based on the wet weights of seaweed plants tied onto the hanging culture ropes divided by the volume of the seawater inside the tank. The seaweeds were then placed in tanks with different treatment of fertilizer and bottom substrate.

Two-factor experimental design was applied in the study with two seaweed species (*K. striatum* and *K. alvarezii*) mutually cultivated in one tank culture. The seaweeds were tied in pair (mixed species) across the 1 m hanging polyethylene ropes in quadruplicates (four lines) to investigate the possibility of growing the two different species in the same line. Tank culture system was designed (Fig. 1) using four 1000L PVC (Polyvinyl chloride) tanks with 1.11 m depth placed at the outdoor area of the hatchery. The tank culture applied running seawater using flow through system with filtered seawater source supply (flow rate = 50 mL sec^{-1} and also equipped with continuous aeration. Flow through system occurred when the seawater was added into the tank from the inlet pipe of the filtered seawater source supply and discharged back to the drain through the outlet pipe.

The tanks were labelled according to the treatment type as T1 (filtered seawater only), T2 (filtered seawater enriched with 13 mg L^{-1} Acadian Marine Plant Extract Powder, AMPEP fertilizer), T3 (filtered seawater with added bottom substrate of sands and corals) and T4 (filtered seawater enriched with 13 mg L^{-1} AMPEP fertilizer, added bottom substrate of sands and corals). For T2 and T4 treatments, seaweed were immersed at 13 mg L^{-1} for 2 min at the beginning of the culture period and sprayed onto the plant every 20 days during the culture period. Acadian Marine Plant Extract Powder (AMPEP) was added in each tank treated with fertilizer enrichment. AMPEP is an extract of *Ascophyllum nodosum* that can promote plant growth and improve crop health and nutritional quality (Hurtado *et al.*, 2009). For T2 and T4 treatments, seaweed were immersed at 13 mg L^{-1} of liquefied AMPEP (500 g AMPEP diluted in 1 L distilled water) for 2 min at the beginning of the culture period and sprayed onto the plant every 20 days during the culture period. The bottom substrates added in tank were composed of coral rubbles and coarse sands collected from the shoreline nearby the UMS Shrimp Hatchery.

Observation of disease occurrence: The disease infections on both *K. alvarezii* var. Brown Tambalang and *K. striatum* var. Green Flower cultured in each treatment tank during Trial 1, 2 and 3 were observed physically by examining the conditions of each seaweed plant. Two types of diseases were observed: "ice-ice" and epiphytes infections. The number of infected plants were randomly calculated and recorded towards the final day of the culture trial (Day 40).

Tank seawater quality analysis: The water quality parameters in each culture tank were monitored *in-situ* (Dissolved Oxygen level (DO), pH, temperature, salinity and light intensity) twice daily (8 am in the morning and 3 pm in the afternoon) using HANNA Multi-parameter Equipment, Lux meter and *ex-situ* nutrient concentration (concentration of nitrite, nitrate and ammonia) was measured using the colorimetric analysis within the 40 days trial (Yong *et al*., 2013).

Daily growth rate analysis: In terms of the growth performance, the wet weight measurement for each seaweed plant was recorded every 10 days. The growth performance was measured by calculating the Daily Growth Rate (DGR, %) for each tank. The equation used to calculate DGR was stated as (Yong *et al*., 2013):

$$\text{DGR (\%)} = \left(\frac{W_t}{W_o^{1/t}} - 1\right) \times 100$$

Where:
W_t = Final fresh weight at t day (g)
W_o = Initial fresh weight (g)
t = Number of culture days

Statistical analysis: Whenever appropriate, data was subjected to further analysis of significant differences by two way ANOVA and a *Post-hoc* test using SPSS software version 21 (SPSS Inc.).

RESULTS

After 40 days of culture, *K. striatum* var. GF and *K. alvarezii* var. BT of four different treatments (T1, T2, T3 and T4) showed significant differences in their daily growth rate, DGR ($p<0.05$). The maximum Daily Growth Rate (2.00±0.03 % day^{-1}) was achieved by *K. striatum* var. GF cultured during Trial 3 with lowest seaweed density of 0.96 g L^{-1} and treated with the enrichment of AMPEP and placement of sands and corals in the tank, T4 followed by T2, T3 and T1 (1.60±0.06, 1.49±0.03 and 1.44±0.02% day^{-1}, respectively) as shown in Fig. 2. Similarly, the DGR of *K. alvarezii* var. BT was also the highest in T4 (1.46±0.06% day^{-1}) followed by T2, T3 and T1 (1.11±0.02, 0.89±0.13 and 0.41±0.33% day^{-1}, respectively). From Trial 1 at 2.40 g L^{-1} seaweed density, negative growth for *K. striatum* var. GF and *K. alvarezii* var. BT were observed in all treatments except for the *K. striatum* var. GF (0.21±0.23% day^{-1}) cultured in T4 (Fig. 3). Meanwhile, Trial 2 at 1.92 g L^{-1} seaweed density exhibited negative growth for *K.alvarezii* var. BT and minimal growth improvement for *K. striatum* var. GF in each treatment where the highest growth rate was achieved in T4 (0.46±0.40% day^{-1} followed by T2, T3 and T1 (0.30±0.39, 0.28±0.38 and 0.08±0.28% day^{-1}, respectively) (Fig. 4). Based on the results from Table 1, the number of *Kappaphycus* sp. affected by "ice-ice" and epiphyte infections was higher in Trial 1 (48 plants) and Trial 2 (30 plants) compared to the seaweed cultured during Trial 3

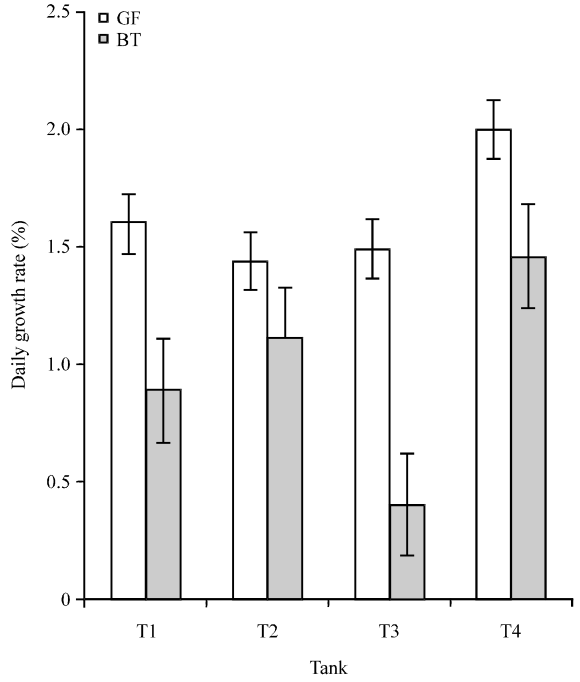

Fig. 2: Daily growth rate of *K. striatum* var. GF and *K. alvarezii* var. BT cultures exposed to different tank treatments at the seaweed density of 0.96 g L^{-1} during Trial 3 (February 2014 to middle of March 2014). Error bars correspond to the standard error

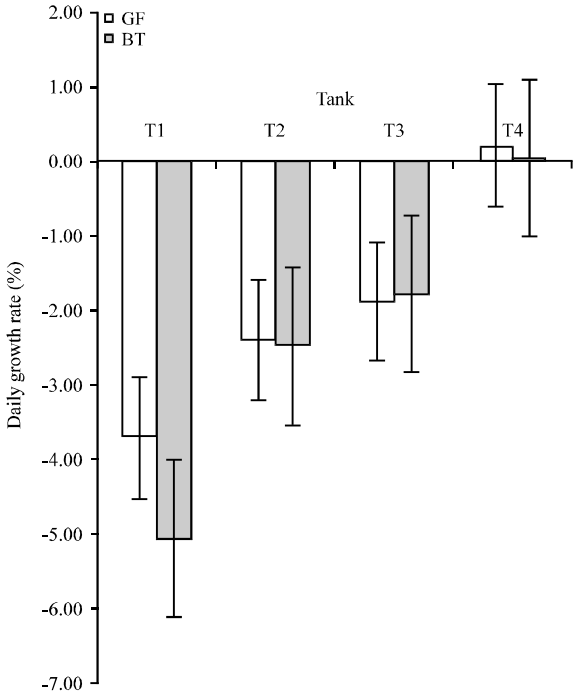

Fig. 3: Daily growth rate of *K. striatum* var. GF and *K. alvarezii* var. BT cultures exposed to different tank treatments at the seaweed density of 2.40 g L^{-1} during Trial 1 (July 2013 to August 2013). Error bars correspond to the standard error

Table 1: No. of Seaweed Plants Infected by "Ice-ice" and epiphyte infections (disease occurrence) in each treatment tank during trial 1, 2 and 3

		No. of infected seaweed plant	
Treatment tank	Total No. of seaweed plant in tank	*K. alvarezii* var. BT	*K. striatum* var. GF
Trial 1			
T1	40	11	7
T2	40	9	4
T3	40	6	5
T4	40	4	2
Total	160	30	18
Trial 2			
T1	32	9	4
T2	32	5	1
T3	32	6	-
T4	32	3	-
Total	128	23	5
Trial 3			
T1	16	2	1
T2	16	-	-
T3	16	1	-
T4	16	-	-
Total	64	3	1

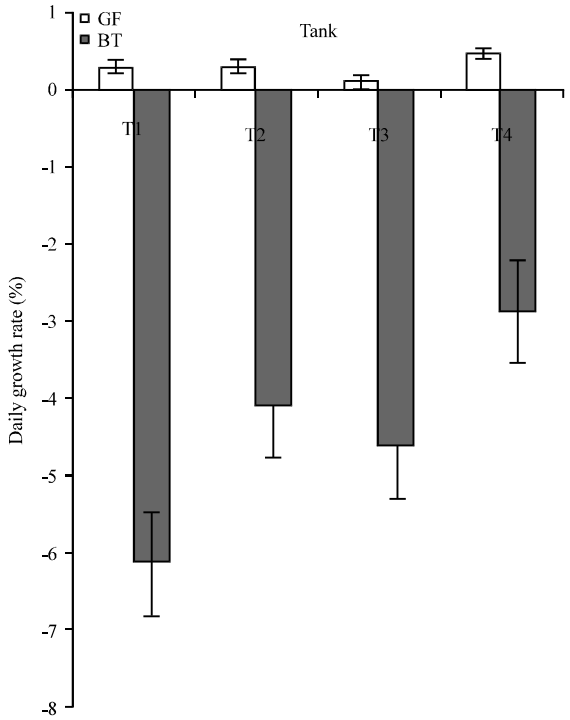

Fig. 4: Daily growth rate of *K. striatum* var. GF and *K. alvarezii* var. BT cultures exposed to different tank treatments at the seaweed density of 1.92 g L^{-1} during Trial 2 (September 2013 to October 2013). Error bars correspond to the standard error

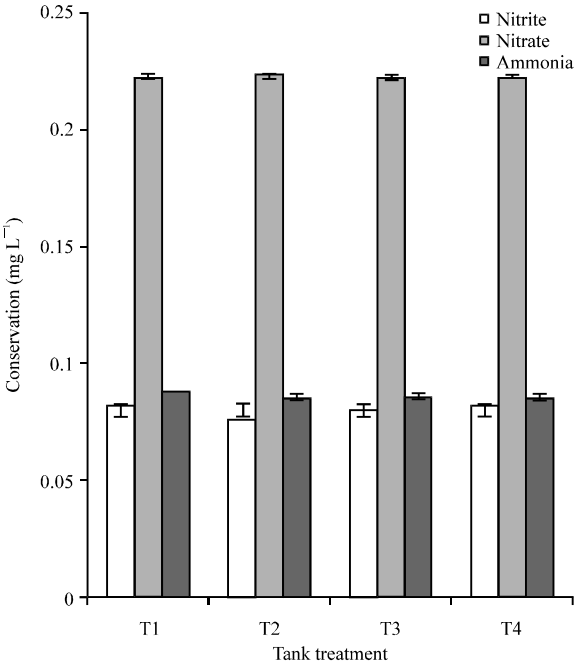

Fig. 5: Average nitrogen-nitrite, nitrogen-nitrate and nitrogen-ammonia content measured in all treatment tanks during Trial 1, 2 and 3

Table 2: *In-situ* water quality analysis with average value of pH, DO level, temperature, salinity and light intensity taken during Trial 1, 2 and 3 within July 2013 until April 2014

Water parameters trial	pH value	DO	Level temperature	Salinity (ppt)	Light intensity (lux)
1	7.66±0.48	5.00±0.35	27.68±1.92	29.20±0.49	5842.11±1081.23
2	8.19±0.36	5.51±0.17	29.71±1.31	30.18±1.29	5945.62±1033.95
3	8.82±0.44	6.95±0.65	32.24±0.47	34.97±0.40	6117.02±547.880

(4 plants) and the most infected seaweed was *K. alvarezii* var. BT. In other words, the number of disease occurrence in Trial 1 and 2 was higher compared to Trial 3. In terms of the seawater quality used as a culture media in tank, the readings of salinity, temperature, dissolved oxygen level, pH and light intensity for all trials ranged in average from 29.20±0.49 to 34.97±0.40 ppt, 27.68±1.92 to 32.24±0.40°C, 5.00±0.35 to 6.95±0.65, 7.66±0.48 to 8.82±0.44 and 5842±1081.23 to 6117±547.88 lux, respectively as presented in Table 2. Besides, the average concentration of nitrite, nitrate and ammonia in all treatment tanks were 0.08±0.01, 0.22±0.01 and 0.09±0.02 mg L^{-1}, respectively (Fig. 5).

DISCUSSION

Based on the results, the study suggested that the good performance of *K. alvarezii* var. BT and *K. striatum* var. GF was found in tanks with lowest seaweed density and treated with the enrichment of AMPEP fertilizers and placement of sands and corals in the tank. In general, *K. striatum* var. GF showed slightly better performance in tank compared to *K. alvarezii* var. BT. However, further scientific research is needed to explore the true potential of *Kappaphycus* sp. in tank culture system. There is very limited report on the performance of seaweed in land-based tank culture system. Indeed, little is known on the growth of *Kappaphycus* sp. in land-based hatchery

using tank culture system. Land-based seaweed culture is significant for research purposes which will support the sustainability of the industry. To our knowledge, this is the first report on the performance of grown seaweed using tank culture system in Malaysia. However, a few studies reported the growth performance of seaweed farmed at open sea.

The Daily Growth Rate (DGR) of *K. alvarezii* and *K. striatum* was reported to be 3-4% (Hayashi *et al*., 2011) and 1.75-3.5% (Ali *et al*., 2014), respectively when cultured at open sea. Based on the results from this study, both *K. striatum* var. GF and *K. alvarezii* var. BT exhibited high growth rate at lower seaweed density (0.96 g L^{-1}) in culture tank with AMPEP fertilizers and placement of sands and corals. The addition of AMPEP fertilizer with the presence of sands and corals at the bottom of the culture tank might facilitate the growth of *K. alvarezii* and *K. striatum*. AMPEP has been reported to be widely used as a fertilizer in *K. striatum* cultivation (Hurtado *et al*., 2009; Hayashi *et al*., 2010). According to Hurtado *et al*. (2009), AMPEP was extracted from *Ascophyllum nodosum*, a brown seaweed extract that contain more essential macronutrients and micronutrients which are also required for the growth of *K. alvarezii*. Borlongan *et al*. (2011) stated that the addition of AMPEP to the seaweed culture not only can promote growth but also can control the epiphytic infections.

In the present study, the lowest seaweed density at 0.96 g L^{-1} showed higher growth rate of *Kappaphycus* sp. compared to higher seaweed density at 2.40 and 1.92 g L^{-1}. According to Yong *et al*. (2014), higher seaweed density in culture tank may cause in depleting nutrients in the culture facility. Besides, the light penetration to the tank might decrease with higher seaweed density causing lower photosynthesis rate for the seaweed in which reducing the growth rate (Bidwell *et al*., 1985). Therefore, the study suggested that *Kappaphycus* sp. should be cultivated at lower stocking density in land-based tank culture system in order to promote a higher daily growth rate. Besides, the findings from this study also suggested that both *K. striatum* var. GF and *K. alvarezii* var. BT could be mix-cultured in a similar culture line or tie, preferably at lower seaweed density, with enrichment of additional organic fertilizers such as AMPEP and also presence of sands and corals. According to Preisig and Hans (2005), areas with coarse sandy to corally bottom substrate are good sites for seaweed cultivation. Therefore, the placement of sand and corals might aid in promoting the growth of *Kappaphycus* sp. in tank.

The number of disease occurrence in each culture trial affects the performance of *Kappaphycus* sp. culture in tank. In fact, the tank with lowest seaweed density showed lower number of disease occurrence and good performance of *Kappaphycus* sp. cultured in tank indicated by the maximum daily growth rate obtained from Trial 3. According to Vairappan *et al*. (2008), "ice-ice" is a type of bacterial infection that could affect the growth of high density seaweed culture. Besides, Hayashi *et al*. (2010) reported that "ice-ice" is a symptom of *K. alvarezii* undergoing stress such as abrupt changes in temperature or salinity. Both *K. alvarezii* and *K. striatum* cultured during Trial 1 and 2 showed an extremely slow and negative growth where most of the seaweeds experienced damaged parts of the branched stems and fell off from the culture tie after 30 days causing the weight loss (Fig. 6). Previous study conducted by Vairappan (2006) in Balambangan Island, Kudat, Sabah from January to December 2003 addressed the phenomenon of epiphyte infection that is prevalence in commercial red seaweed farming and strongly influenced by seasonal factor whereby cultured seaweed became susceptible to epiphytes in the period between March until June and September until November. In the present study, the seaweed culture in Trial 1 was carried out from July until August 2013 and Trial 2 was carried out from September until October 2013. Thus, the negative growth rate of *K. alvarezii* var. BT and *K. striatum* var. GF cultured

Fig. 6(a-c): Photos of (a) The epiphyte infection found on *K. alvarezii* var. BT cultured in T2 during Trial 2, (b) The "ice-ice" infection found on *K. alvarezii* var. BT cultured in T3 during Trial 1, and (c) Arrow pointing at bleached stem of *K. alvarezii* var. BT that fall off from hanging tie after being infected by epiphytes

during Trial 1 and 2 might be affected by the seasonal epiphytic infections. Meanwhile, the *Kappaphycus* sp. cultured during Trial 3 was least affected by the seasonal epiphytic infection since the culture period was from February 2014 until middle of March 2014.

Several important seawater quality parameters and factors need to be considered throughout the seaweed cultivation in tank including the light intensity, solar irradiance, temperature, water motion, salinity, DO level, pH and availability of nutrients. In this experiment, selected parameters were monitored comprised of salinity, temperature, dissolved oxygen (DO) level, pH, light intensity and water nutrient content (concentration of nitrite, nitrate and ammonia) as presented in Table 2 and Fig. 5. Salinity, temperature, dissolved oxygen level, pH and light intensity for all trials ranged in average from 29.20±0.49 to 34.97±0.40 ppt, 27.68±1.92 to 32.24±0.40°C, 5.00±0.35 to 6.95±0.65, 7.66±0.48 to 8.82±0.44 and 5842±1081.23 to 6117±547.88 lux, respectively. Based on the observations, the recorded water quality parameters in the culture tank fall within the range of optimum requirements for *Kappaphycus* sp. cultured at open sea except for the light intensity. According to Preisig and Hans (2005), the optimum mariculture condition of *Kappaphycus* sp. at open sea includes the range of temperature of about 27-30°C, salinity of 30-33 ppt, DO of 5-6, pH value from 7-9, light intensity of 6000-8000 lux and also water level of 0.5-1.0 m during low tide and 2.0-3.0 m during high tide.

The light intensity during Trial 1 and Trial 2 were slightly lower than in Trial 3 (Table 2). The difference in light intensity might also affect the seaweed growth performance in each trial. The higher daily growth rate of *Kappaphycus* sp. in Trial 3 showed that the light intensity rate during the culture period was suitable for seaweed growth since the optimum light intensity for seaweed culture ranged from 6000-8000 lux (Preisig and Hans, 2005). During the culture period in Trial 1 and Trial 2, the light intensities were lower than Trial 3 due to the frequent rainfall from June until November 2013. Seaweed is a type of marine plant that undergoes photosynthesis just like other photosynthetic plants. Seaweeds are also categorized as marine macroalgae distributed only at the coastal region of the sea due to the benthic characteristic (attached to the bottom) and undergoing photosynthesis (Mine, 2008). Photosynthesis in seaweed occurs similar as other higher plants with the presence of pigment mainly chlorophyll and others such as carotenoids and phycobilins that absorb sunlight and convert them into food and energy source (Rabinowith and Govindjee, 1965). Therefore, the amount of available light for absorption is very significant in ensuring the efficiency of photosynthesis in seaweed plant that leads to the growth improvement through sufficient energy and nutrients required for seaweed cell division. In terms of the seawater nutrient content in the tank, the average concentrations of nitrite, nitrate and ammonia in all treatment tanks were below 1.0 mg L^{-1}, indicating good water quality with low concentration of seaweed waste. However, the concentration of nitrate is slightly higher compared to nitrite and ammonia due to the nature of seaweed in assimilating nitrate as nutrient source in the form of fixed dissolved inorganic nitrogen. In natural aquatic ecosystems, 95% of the nitrogen which occurs as dissolved dinitrogen gas (N_2), is not directly accessible to most photosynthetic-oxygen organisms. Nitrate is the principal form of fixed dissolved inorganic nitrogen assimilated by organisms. Therefore, nitrate constitutes the prevailing available nitrogen source for macroalgae in the marine environment (Chow, 2012).

In summary, both *Kappaphycus* sp. (*K. striatum* var. GF and *K. alvarezii* var. BT) have high potentials to be mutually grown in land based tank culture system at lower seaweed density and optimum water quality. The seaweed research area might be expanded with the data provided from the present study on land-based (hatchery) tank culture system. Indeed, the land-based seaweed cultivation might also create new opportunities for the industry to produce high quality seedlings in the hatchery using land-based tank culture system. Therefore, further research on different tank designs should be considered to explore more potential in land-based seaweed farming.

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