# SEASONAL CHANGE IN GROWTH RATE, CARRAGEENAN YIELD AND QUALITY FROM THE RED ALGA (*Kappaphycus striatus*) CULTIVATED IN CAM RANH BAY

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#### SUMMARY

The red alga *Kappaphycus striatus* was cultivated at Camranh Bay, KhanhHoa, Vietnam. For a 30 day cultivation period, the alga showed the higher growth rate  $(4.1 - 5.8 \% \text{ day}^{-1})$  from October 2014 to April 2015 and in September 2015, while the lower growth rate  $(2.5 - 3.1 \% \text{ day}^{-1})$  was found from May to August 2015. The carrageenan quality was done for a 60-day cultivation period, the carrageenan yield and gel strength showed little variation with the higher values  $(28.1 - 28.7 \% \text{ and } 855 - 935 \text{ g cm}^{-2}$ , respectively) obtained from November 2014 to March 2015 and lower ones  $(25.1 - 26.7 \% \text{ and } 555 - 758 \text{ g cm}^{-2}$ , respectively) from April to September 2015. The data on moisture content of alga (33.5 - 36.8 %), clean anhydrous algal/salt ratio (0.92 - 1.07), and carrageenan yield obtained in this study fulfilled those of standards for carrageenophytes. This study shows that the red alga *K. striatus* can be grown in the tropical waters of the Camranh Bay during the northeast monsoon and a part of the southwest monsoon as a source of materials for carrageenan production.

Keywords: Kappaphycus striatus, carrageenan yield, environmental factors, gel strength, growth rate

#### INTRODUCTION

carrageenophytes, The red algae, Kappaphycus alvarezii, Kappaphycus striatus and Eucheuma denticulatum are farmed as raw material for the extraction of carrageenan and are considered important as an commercial commodity in the Philippines, Indonesia, Malaysia, Vietnam and Tanzania (Critchley et al., 2004; Hurtado et al., 2008). The increasing demand for carrageenan by the industry because of its diverse product applications makes Kappaphycus an important marine commodity. More than 90 % of the global carrageenophyte

culture farms are located in the coastal waters of South East Asian countries (Hurtado *et al.*, 2014; Zuldin *et al.*, 2016). Kappaphycus farming is a labor-intensive activity, and the yield is often dependent on culture conditions and disease outbreak. Recently, *K. striatus* has preferably been cultivated than the other carragenophytes, because this species showed a more resistant to epiphytes, Ice–Ice disease and tolerated a wide range of environmental conditions, especially during sudden changes in water conditions such as increases in temperature (Critchley *et al.*, 2004; Hurtado *et al.*, 2006), though its growth rate was lower than that of *K. alvazerii* (Ali *et al.*, 2018).

In Vietnam, K. striatus has been cultivated since 2005, due to transport of algal seeds from Bohol, Philippines. The estimated total area and production in Vietnam of Kappaphycus algae were reached more 700 ha and 2,000 dried tons, respectively (McHugh, 2006). Red alga K. striatus may now contribute as a source of not only carrageenan, but also other bioactive compounds for biochemical and medicinal uses (Yuan, Song, 2005; Yuan et al., 2011; Le Dinh Hung et al., 2015, 2020). However, little information is known about factors which effected to quality of this cultivated alga. Thus, the purpose of this study is to report on effect of environmental factors to the growth rate, carrageenan yield and quality of K. striatus cultivated in Camranh Bay, Vietnam, which will provide more valuable information for cultivation of this alga (Tran Mai Duc et al., 2007; Tran Kha et al., 2007).

### MATERIALS AND METHODS

#### Materials

*Kappaphycus striatus* alga was collected from a seaweed farm at Ninhthuan province of Vietnam, which has been cultivated since 2005 (material originally originated from Bohol, Philippines). Initially, 20 kg algal material was transported from seaweed farm at Ninhthuan and domesticated at Camranh Bay during August and September 2014 to obtain sufficient seed material for further cultivation.

#### **Cultivation site**

Camranh Bay (109°12'16.2" E, 11°50'21.12"

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N), the habitat was characterized by flat sandy bottom. Water depth varied from 1.2 to 2.2 m. The water motion/current during northeast monsoon (mid-November to mid-January) was heavy and the rest of the time was relatively calm. Water was clear most of the times in the experimental site except during northeast monsoon period. The cultivation was carried out from October 2014 to September 2015 to evaluate the daily growth rate and carrageenan quality of *K. striatus* alga.

# The cultivation method and determination of growth rates

The cultivation was carried out using the floating-raft method (Trono, Ohno, 1989; Subba Rao et al., 2008) in an area of 50 m<sup>2</sup> (5 m x10 m). The raft contained 12 monolines of 10 mm polypropylene rope of 11 m length at 0.4 m intervals. Each line consisted of 20 seedlings of 100 g (fresh weight) at 0.5 m intervals and thus each line contained (100 g x 20) = 2.0 kg (fresh weight) seed material. The seedling was tied to a monoline using monofilament ropes (3 mm in diameter). The floats at 2.0 m intervals were tied with polypropylene ropes and adjusted to the depth of the water, so that the plants were under seawater level and were not exposed during low tides. The raft was fixed with help of anchors on both sides. Periodical maintenance of the rafts was done by removing the unwanted weeds and cleaning the rafts. At the 30 day culture period, the daily growth rate (DGR = % day<sup>-1</sup>) were measured and expressed as the percent increase in wet weight per day for each line according to the following formula (Dawes et al., 1994):

DGR (% day <sup>-1</sup>) =  $[(W_t/W_0)^{1/t} - 1] \times 100$  %

Where  $W_0$  = average wet weight at start,  $W_t$  = average wet weight at time t and t = time intervals (days).

#### **Environmental factors**

Environmental factors were measured at the depth where the algae were growing. Water temperature and salinity readings were determined twice per month at cultivation site and vicinity using a mercury thermometer and a refractometer. At each sampling time, pH was recorded "in situ". Two separate seawater samples were collected in the cultivation area every 2 weeks, stored in a cool box at 4°C, and transported rapidly to the laboratory for nutrient analysis. The seawater samples were analyzed in triplicate for nitrate and phosphate according to the method of Parsons *et al.* (1984). The average values for seawater temperature, salinity, pH, nitrate and phosphate for each month were finally obtained.

### Determination of dry algal quality

At the end of the culture period (60 days), the harvested materials were sun-dried, and the moisture content (MC (%) = dry weight/original weight  $\times$  100) was measured by drying the sample at 105°C for 3 h. Clean anhydrous alga was also obtained by washing the known material in tap water to remove sand and salt. Washed material was dried at 105°C for 3 h. Then the clean anhydrous algal/salt ratio was calculated. The experiments for moisture content and clean anhydrous algal/salt ratio were done in triplicate (Periyasamy *et al.*, 2014).

# Extraction and determination of carrageenan yield and quality

Alkali treated carrageenan extraction was carried out according to the method of Ohno *et* 

*al.* (1994). Algae were washed with tap water to eliminate sand, debris and epiphytes, and dried by sunlight. Dry algae (20 g) were incubated in 400 mL 6% KOH at 80°C for 2 h. The algae were then washed and extracted in 400 mL distilled water at 90°C for 2 h. Thereafter, the solution was filtered through nylon tissue and the extract was gellified with 0.2% KCl, frozen and thawed at least twice, and dried at 60°C to constant weight. The weights were recorded and expressed as percentage of dry algal weight. The experiments for carageenan yields were done in triplicate.

## **RESULTS AND DISCUSSION**

#### Growth rate of K. striatus alga

*K. striatus* alga was cultivated in tropical waters of Camranh Bay, Vietnam from October 2014 to September 2015. The daily growth rate varied from  $2.50 \pm 0.19\%$  to  $5.75 \pm 0.28\%$  over the study period. The highest growth rate was obtained in January 2015 and the lowest daily growth rate in May 2015 (Fig. 1). The mean annual daily growth rate was  $3.91 \pm 0.24\%$ .



Figure 1. Daily growth rates (% day<sup>-1</sup>) of Kappaphycus striatus (October 2014 to September 2015).

The daily growth rate recorded from *K. striatus* was comparable to those reported for eucheumatoid farming by other authors. For example, the daily growth rate of *K. striatus* cultivated in Tosa Bay, Southern Japan was from

3.1 - 5.2% (Gerung, Ohno, 1997), at different depths in the Philippines from 0.7 - 4.7% (Hurtado *et al.*, 2008) and at Saurashtra, India from 2.5 - 7.2% (Mairh *et al.*, 1995). For *K. alvarezii* cultivation at tropical and subtropical

waters, different growth rates were recorded from 0.13 - 8.12% in the subtropical waters of Shikoku, Japan (Ohno et al., 1994), 3.16 -10.80% and 1.6 - 4.6% in tropical waters at Nhatrang and Camranh, Vietnam (Ohno et al., 1996; Le Dinh Hung et al., 2009), 4.50 - 8.20% and 1.4 - 5.3% in the subtropical waters of Brazil (De Paula et al., 2002; Goés, Reis, 2012), 2.00 -7.10% in the tropical waters of Mexico (Munoz et al., 2004), 0,1 - 8.4% and 2.3 - 4.2% in the Philippines (Dawes et al., 1994; Hurtado et al., 2001), 1.5 - 6.2% in Hawaii (Glenn, Doty, 1990, 1992), 3.64 - 13.98% in the subtropical waters of Northwest coast of India (Subba Rao et al., 2008) and 3.76 - 4.92% at Ramanathapuram, India (Periyasamy et al., 2014). The high growth rate was found during October 2014 to April 2015 in the present study while the same results were reported during September to February in Philippines (Hurtado et al., 2001), October to March at Northwest coast of India (Subba Rao *et al.*, 2008), from December to April at Ramanathapuram, India (Periyasamy *et al.*, 2014) and from June to August at Southeast Sulawesi, Indonesia (3.52 - 3.69 %) (Kasim, Mustafa, 2017; Budiyanto *et al.*, 2019).

#### **Environmental factors**

Seawater temperature varied from  $24.9 \pm 0.6^{\circ}$ C to  $31.2 \pm 0.5^{\circ}$ C at cultivation site (Fig. 2). The maximum seawater temperatures were recorded in June 2015 and the minimum temperatures in December 2014. The salinity varied from  $29.3 \pm 0.8\%$  at the cultivation site. The maximum seawater salinities were recorded in May 2015 and the minimum salinities in December 2014 (Fig. 2). The mean annual salinity was 33.0%. The pH ranged from 7.9 to 8.2 and showed no significant variation in this study.



**Figure 2.** Monthly variation in temperature (---) and salinity (--) at cultivation site (October 2014 to September 2015).

The high growth rates recorded in this study were found in the range of seawater temperature from 24.9 - 28.5°C and this was in agreement with the results reported (Trono, Ohno, 1989; De Paula, Pereira, 2003; Subba Rao *et al.*, 2008; Periyasamy *et al.*, 2014). The reduction in growth rate during May to July 2015 in the present study could be attributed to a high seawater temperature (30.5 - 31.2°C). Temperature appeared to be the main factor affecting the growth rates of the seaweed as was noticed in the present study and this was also reported at Yucatan, Mexico (Munoz *et al.*, 2004), Shikoku, Japan (Ohno *et al.*, 1994), the Northwest coast of India (Subba Rao *et al.*, 2008) and at Ramanathapuram, India (Periyasamy *et al.*, 2014). The seasonal variation in seawater temperature from 24.9 - 31.2°C at this cultivation

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site, 21 - 28°C at Kaneohe Bay, Hawaii (Glenn, Doty, 1990), 17 - 33°C at Ubatuba, Brazil (De Paula, Pereira, 2003), 28 - 31°C at Yucatan, Mexico (Munoz *et al.*, 2004), 19.3 - 26.9°C at the Northwest coast of India (Subba Rao *et al.*, 2008), and 27 - 36°C at Ramanathapuram, India (Periyasamy *et al.*, 2014) may explain the difference in growth rates in subtropical and tropical waters.

The nitrate content varied from  $2.1 \pm 0.4$  to  $6.7 \pm 0.6 \ \mu M \ L^{-1}$  at the cultivation site (Fig. 3). The maximum nitrate content was recorded in January 2015 and the minimum in May 2015. The mean annual nitrate content was  $4.8 \pm 0.5 \ \mu M \ L^{-1}$ . The phosphate content ranged from  $0.9 \pm 0.3$  to  $4.7 \pm 0.5 \ \mu M \ L^{-1}$  (Fig. 3). The maximum phosphate content was recorded in December 2014 and the minimum in July 2015. The mean annual phosphate content was  $2.4 \pm 0.3 \ \mu M \ L^{-1}$ . Generally, seawater at this experimental site showed high contents of nitrate and phosphate. In the present study, the salinity and pH were in the range of 29.3 - 34.3‰ and from 7.9 - 8.2,

respectively, and was within the required levels for eucheumatoid farming reported (Glenn, Doty, 1992; Hurtado-Ponce, 1992; Ohno *et al.*, 1994; Zudin *et al.*, 2016). The high growth rates were found during October 2014 to April 2015, coinciding with periods of the high nutrient concentrations at cultivation site. The same results have also been reported for *K. alvarezii* cultivation during September to February in Philippines (Hurtado *et al.*, 2001), October to March at Northwest coast of India (Subba Rao *et al.*, 2008) and December to April at Ramanathapuram, India (Periyasamy *et al.*, 2014).

It is believed that temperature and nutrients are the most important parameters governing the growth of *Kappaphycus* and a low correlation was recorded between growth rates and lectin contents with environmental factors in this seaweed (Glenn, Doty, 1990, 1992; Subba Rao *et al.*, 2008; Le Dinh Hung *et al.*, 2009) and this seemed to be similar to correlation observed in this study.



Figure 3. Monthly variation in nitrate (→) and phosphate (→) concentration at cultivation site (October 2014 to September 2015).

#### Dry algal quality

The moisture content of alga ranged from 33.5 to 36.8%. The highest moisture content was recorded during December-January 2015 and the lowest moisture content during April-May 2015 (Table 1). The mean annual moisture content was 35.2%. The clean anhydrous algal/salt ratio ranged from 0.92 to 1.07 in this study (Table 1) and showed no significant variation. The mean annual clean anhydrous algal/salt ratio was 0.97. The moisture content and clean anhydrous algal/salt ratio of this alga in the present study from 34.4 - 36.8% and 0.92 - 1.07, respectively, are in the range as reported for moisture content from 31.3 - 37.7% and clean anhydrous weed/salt ratio from 0.92 - 1.40 of K. alvarezii at Ramanathapuram, India, (Periyasamy et al., 2014), and in accordance with the accepted values as the standard specification for *K. alvarezii* alga of CP Kelco, Philippines (Moisture content less than 42%, clean anhydrous weed/salt ratio from 0.92 - 1.40).

# Carrageenan extraction and determination of viscosity and gel strength

The yield and gel strength of alkali treated carrageenan varied from 25.1 to 28.7% and from 555 to 935 g cm<sup>-2</sup>, respectively (Table 1). The highest carrageenan yield and gel strength was recorded during February-March 2015 and the lowest carrageenan yield and gel strength during June-July 2015. The mean annual carrageenan yield and gel strength was 27.2% and 788 g cm<sup>-2</sup>, respectively. Viscosity of alkali treated carrageenan ranged from 23.8 to 37.6 cp and showed no significant variation (Table 1).

**Table 1.** Moisture content, clean anhydrous algal/salt ratio, yield, viscosity and gel strength of carrageenans from *K. striatus* alga cultivated from October 2014 to September 2015

Months	Moisture content (%)	Clean anhydrous algal/salt ratio	Yield of carrageenan (%)	Viscosity of carrageenan (cp)	Gel strength of carrageenan (g. cm <sup>-2</sup> )
Oct –Nov	35.6	0.95	28.1	31.5	855
Dec -Jan	36.8	0.99	28.4	34.6	925
Feb-Mar	36.1	1.07	28.7	37.6	935
Apr-May	33.5	0.92	26.7	27.3	700
Jun-Jul	34.4	0.93	25.1	23.8	555
Aug-Sep	34.9	0.93	26.3	30.7	758

The alkali treated carrageenan yields obtained in the present study ranged from 25.1 - 28.7% and these are comparable with those reported for other carrageenophytes, although higher carrageenan yields from have been reported from 31 - 43% and 41.16% in Brazil (Hayashi *et al.*, 2007; Goés, Reis, 2012), 34.5 - 45.30% in Vietnam (Ohno *et al.*, 1996), 45% in Indonesia (Trono, Ohno, 1989), 54.5% in the Philippines (Ohno *et al.*, 1996), 30.3 - 40.7% in Mexico (Munoz *et al.*, 2004), 42.4 - 58.3% in India (Subba Rao *et al.*, 2008), 31.2 - 38.1% in the Philippines (Hurtado *et al.*, 2008), and 40.8 -

54.3% in Malaysia (Zuldin *et al.*, 2016), whereas yields from 17.1 - 56.3% were recorded in Indonesia (Iskandar *et al.*, 2013) and 24.5 - 31.1% in India (Periyasamy *et al.*, 2014). The gel strength of *K. striatus* alga ranged from 555 - 935 g cm<sup>-2</sup>, within the levels required for gel strength as reported by other authors, 480 - 1,960 g cm<sup>-2</sup> (Ohno *et al.*, 1994, 1996), 1,158 g cm<sup>-2</sup> (Santos, 1989), 688 - 926 g cm<sup>-2</sup> (Hayashi *et al.*, 2007) and 503 - 1004 g cm<sup>-2</sup> (Le Dinh Hung *et al.*, 2009). Despite the many reports on carrageenan yields, quantitative and qualitative comparisons are difficult, since yields vary depending on the

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extraction method, age of alga and/or the extracted raw material (Ohno *et al.*, 1994, 1996; Hayashi *et al.*, 2007; Hurtado *et al.*, 2008; Subba Rao *et al.*, 2008; Le Dinh Hung *et al.*, 2009; Periyasamy *et al.*, 2014; Zuldin *et al.*, 2016). Such differences can be attributed to the extraction methodology used in each study, and the time of algal harvest.

#### CONCLUSION

The present study showed a variation in the growth rate, moisture content and clean anhydrous algal/salt ratio, carrageenan yield and gel strength from cultivated *K. striatus* alga throughout year. The results suggest that commercial cultivation of this alga can be grown during the northeast and a part of the southwest monsoon in Camranh Bay as a source of carrageenan, for application.

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# SỰ THAY ĐỔI THEO MÙA CỦA TỐC ĐỘ TĂNG TRƯỞNG, HÀM LƯỢNG VÀ CHẤT LƯỢNG CARRAGEENAN TỪ RONG ĐỔ (*Kappaphycus striatus*) NUÔI TRỒNG Ở VỊNH CAM RANH

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### TÓM TẮT

Rong đỏ *Kappaphycus striatus* được nuôi trồng ở vịnh Cam ranh, Khánh Hòa, Việt Nam. Qua một giai đoạn nuôi trồng 30 ngày, rong đã cho thấy tốc độ tăng trưởng cao (4,1 - 5,8%/ngày) từ tháng mười năm 2014 đến tháng tư năm 2015 và trong tháng chín năm 2015, trong khi đó các tốc độ tăng trưởng thấp (2,5 - 3,1%/ngày) được ghi nhận từ tháng năm đến tháng tám năm 2015. Chất lượng và hàm lượng carrageenan được xác định qua giai đoạn nuôi trồng 60 ngày, hàm lượng và độ bền gel của carrageenan cho thấy có sự biến động nhẹ với các giá trị cao (tuần tự là 28,1 - 28,7% và 855 - 935 g. cm<sup>-2</sup>) được ghi nhận từ tháng 10 năm 2014 đến tháng 3 năm 2015 và các giá trị thấp hơn (tuần tự là 25,1 - 26,7% và 555 - 758 g. cm<sup>-2</sup>) từ tháng tư đến tháng 9 năm 2015. Các số liệu về độ ẩm của rong (33,5 - 36,8%), tỉ lệ rong khô sạch/muối (0,92 - 1,07), và hàm lượng carrageenan thu nhận trong nghiên cứu này đã đáp ứng về các tiêu chuẩn của rong carrageenophyte. Nghiên cứu này cho thấy rằng rong đỏ *K. striatus* có thể phát triển trong vùng nước nhiệt đới ở Vinh Cam Ranh trong mùa đông bắc và một phần của mùa Tây nam được xem là một nguồn nguyên liệu để sản xuất carrageenan.

**Từ khóa:** Kappaphycus striatus, độ bền gel, hàm lượng carrageenan, tốc độ tăng trưởng, yếu tố môi trường