Establish Cultivation by Mixing Crops of Different Strains of *Eucheuma* and *Kappaphycus* Species

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Abstract Species Kappaphycus alvarezii (Doty) Doty, Kappaphycus striatum (Schmitz.) Doty and Eucheuma denticulatum (N. L. Burman) Collins et Harvey, which was brought to Vietnam from Japan in 1993 and Coco island, Martan Sea, Cebu, Philippines in 2005 have been cultivated in the different coasts of South Central Vietnam. Their growth rates and physical properties of carrageenan, then, were analyzed. The obtained results showed that the growth rate of E. denticulatum and K. striatum strains is higher than those of K. alvarezii. Species of K. striatum could grow over wide range of temperature and tolerate more strongly to high temperature compared with K. alvarezii, but their content and gel strength of kappa-carrageenan were almost the same and high. For purpose of the Kappahycus cultivation farms with stable and high production all year round (especially in the seawaters of shallow, semi-closed Lagoons where the water movement is not good and with high temperature in the hot season), mixed cropping of K. alvarezii and K. striatum as seeds stock during different cropping seasons was established. Our results suggested that K. alvarezii and K. striatum could be grown in the cool season (from Oct. to next March) with the same and high content and gel strength of kappa - carrageenan, but in the hot season need to chose K. striatum for cultivation only (from Apr. to Sept.).

Key words : Cultivation, Eucheuma denticulatum, Kappaphycus alvarezii, Kappaphycus striatum, mixing crops

Introduction

The demand for carrageenan is now going increasingly world-wide. *Eucheuma* and *Kappaphycus* genera are economically important seaweed resources for carrageenan extraction in tropical areas. The seaweed industry is successful due to the large - scale cultivation of *K. alvarezii*, *K. striatum*, a kappa-carrageenan-producing species, and *Eucheuma denticulatum*, an iota-carrageenan-producing species which have been promoted in the Philippines for 1969-1970, in Indonesia since 1985 and in Tanzania since 1989 (Ohno *et al.*, 1996). The relative successes of these seaweed cultivations are apparently due to similar environmental conditions in the respective transplanted sites where the *Euchema* and *Kappaphycus* were originally collected

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Species of *K. alvarezii*, *K. striatum* and *E. denticulatum* have not been found on the Vietnamese coasts, though the environmental conditions are considered suitable for their cultivation. The seed stock of *K. alvarezii* (Doty) Doty, brown strain and original from Philippines, was transferred to Vietnam by Prof. Masao Ohno (Usa Marine Biological Institute, Kochi Universtity, Japan) in 1993. It has being cultivated in large scale mainly in the coast line of South Central Vietnam such as Ninh Thuan, Khanh Hoa, Phu Yen... province for the last few years. The estimated total area and production of *Kappaphycus* cultivation in large scale reached 700 hectares and 1.300 dried tons, respectively, from 2003 to 2005 (Huynh and Tsutsui, 2006). About 200 dried tons of Kappaphycus are supplied to the domestic market annually for local food such as jelly, salad with other vegetables and more than 1200 dried tons were exported in 2005. However, the growth of K. alvarezii is significantly effected by high temperature in the hot season (from Apr. to Sept.). In these months, water temperature (WT) ranges from 30°C to 34°C, daily growth rate (DGR) of K. alvarezii average ranges from 1.5 % to 2 % per day, the seaweed is very prone to ice-ice disease, especially in the shallow - closed lagoons where seawater temperature is high and water movement is not good. This causes a serious problem of K. alvarezii cultivation in Vietnam. Various methods have been used in planting Kappaphycus and Eucheuma along the coast of central to Southern Vietnam which include the floating raft, fixed off-bottom monolines, broadcasting seedlings directly onto the bottom or fixed off-bottom nets methods. The average size of one raft is approximately 1000 to 2500 square meters, and the productivity is 20 to 30 dried tons per hectare in a year (Huynh and Tsutsui, 2006). The fixed off-bottom raft method is used for shores that continuous water movement, strong wave action and in cool areas (achieving 8-10 % dried tons per hectare within 6 months). In semi-closed or closed lagoons, inlet with deep waters and coast areas with sand or mud bottom, Kappaphycus is cultivated using fixed off-bottom monolines method. In this method, the productivity is 8-9 % per day (in the cool season, from Oct. to next Mar.), 4-6% per day (in the hot season, from Apr. to Sept.), and 10-12% per day in the sea abundant in nutrient. In addition, Kappaphycus can also be cultivated in shrimp cultivation ponds with the methods above mentioned (Huynh and Tsutsui, 2006).

During more than 10 years for *Kapaphycus* cultivation in the different coast of South Central Vietnam under different cultural conditions, different strains of *K. alvarezii* have been generated from brown strain of *K. alvarezii* which were original from Philippines and transferred to Vietnam. They have differences in morphology such as size, number of branches and color. Within the coastal areas of Central to Southern part of Vietnam, maybe, there are eight strains of *K. alvarezii, K. striatum* and *E. denticulatum* comprising three

strains of *K. alvarezii* (green, dark green and brown strains); two strains of *E. denticulatum* (green and brown strains); three strains of *K. striatum* (payaka brown strain with short and long branches, Sacol green and Sacol brown strains). Morphological differences between the strains studies were found to be based on cultivation conditions like water and temperatures. Color was however shown to be genetics based. Genetically, the Sacol green was closer to the brown and green strains of *K. alvarezii* than to the Sacol brown strain of *K. striatum* (Dang Diem Hong *et. al.*, 2008).

In this report, cultivation properties of *E. dentic-ulatum*, *K. alvarezii* and *K. striatum* under different conditions of seasonal temperature and depths in the sea water using the floating cage in Nha Trang Bay and fixed bottom racket in Cam Ranh Bay (Khanh Hoa province) in South Central Vietnam was investigated which based on their biological characteristics such as the DGR and physical properties of carrageenan.

Material and Methods

Material

Brown strain of *K. alavarezii* (Doty) Doty which is being cultivated in large scale along the coast of South Central Vietnam (Nha Trang and Cam Ranh Bay, Khanh Hoa province) were original from Philippines and transferred to Vietnam in 1993 by Prof. Masao Ohno (Usa Marine Biological Institute, Kochi University, Japan).

All strains belonging to *K. striatum* and *E. denticulatum* which were collected in cultivated areas of Coco Island, Martan Sea, Cebu, Philippines and transferred to Vietnam in 2005 by Huynh Quang Nang (Institute of Research and Applied Technology, Nha Trang, Vietnam) were used in this report.

The strain of *K. striatum* (Schmitz) Doty such as brown strain (local name payaka brown with short branches in Bohol, Philippines) cultivated by floating cage at Nha Trang and Cam Ranh Bay, Khanh Hoa province on 2005-2006. On the other hand, in order to have enough materials used for an iota – carrageenan extraction and food, there are other two strains of *E. denticulatum* (N. L. Burman) Collins et Harvey such as green and brown strain cultivated in Cam Ranh Bay, Khanh Hoa province, Vietnam on 2005 -2006 were used as materials in this report.

Cultivation methods and places

In Nha Trang Bay (Khanh Hoa province) with the depth of 5-8 m: species of *K. alvarezii* and *K. striatum* were cultivated in floating cage, surrounded by nilon net (for protecting fishes eating) from Sept, 2005 to Jun, 2006. The seeds were tied to the ropes, hanging at the depth of 0.3-0.5 m near the seawater surface.

In Cam Ranh Lagoon (Khanh Hoa province): the semi-closed Lagoon and shallow (1-2 m depth): species of *E. denticulatum, K. alvarezii and K. striatum* were cultivated by fixed off bottom racket with floats in the hot season (from Mar. to Jun., 2006).

Determination of environmental parameters and growth rate

- *Environmental parameters:* temperature of seawater (WT) was measured by pH meter (Horiba O.12, Japan) and Conductivity meter (Yokogawa SC 82, Japan); the salinity (S) was measured by Refractor meter (Atago K. K., Tokyo); the light intensity (LI) was measured by lux meter; the concentration of nutrients in water such as NH₄, NO₃, PO₄ were measured by electro-photometer (HACH DR/2010, USA) at 400-950 nm using reagents of Nessler, molipdate amon and phenoldisunfonic acid, respectively (Standard methods for examination of water and waste water, 1997).

- *Growth rate*: the growth rate (GR) were measured by increases in fresh weight and presented as percentage growth per day using the formula of Penniman *et al* (1986), for the every 15 days.

$$GR = \left[\left(\frac{Wt}{Wo} \right)^{\frac{1}{t}} - 1 \right] \times 100$$

Where GR = % increase in fresh weight per day; Wo = initial weight (gr) and Wt = weight after t days (gr)

Analyzing the content and quality of carrageenan were followed the methods described in report of Ohno *et al.*, (1996).

Results

The effects of temperature and salinity in seasons on the growth rates and a kappa-carrageenan of brown strain of *K. alavarezii* and brown strain (local name payaka brown with short branches in Bohol, Philippines) of *K. striatum* cultivated by floating cage at Nha Trang Bay

The seawater temperature in the cold season (from Nov. 2005 to Jan., 2006) and in the hot season (from Apr. to Jun., 2006) is different. Temperature ranged from $25.76 \pm 1.02^{\circ}$ C to $26.52 \pm 0.95^{\circ}$ C in the cold season and $30.16 \pm 1.12^{\circ}$ C to $30.68 \pm 1.20^{\circ}$ C in the hot season. The results of the effect of temperature and salinity in the cool and the hot seasons on the growth rate and physical properties of kappa-carrageenan of brown strain of K. alvarezii and payaka brown with short branches strain of K. striatum cultivated in the floating cage at Nha Trang Bay from Sept., 2005 to Jun., 2006 are presented in Table 1 and 2. Table 1 indicated that the DGR of both brown strain of K. alvar*ezii* (ranged from 5.73 ± 0.52 % day⁻¹ to 6.25 ± 0.50 % day⁻¹) and payaka brown with short branches strain of K. striatum (ranged from 4.43 \pm 0.65 % dav⁻¹ to 5.04±0.43% day⁻¹) cultivated by floating cage were high in the cool season (during Nov. to next Jan.). In these months, the DGR of brown strain of K. alvarezii is higher than that of payaka brown with short branches strain of K. striatum (about 1 % day⁻¹ more). On the contrary, the DGR of K. alvarezii strain is 2-3% day⁻¹ lower than that of K. striatum in the hot season. These values ranged from $0.87 \pm 0.52\%$ day⁻¹ to 1.14 \pm 0.45 % day⁻¹ in K. alvarezii strain and from 2.96 \pm 0.67 % day⁻¹ to 3.28 \pm 0.66 % day⁻¹ in strain of K. striatum. It showed that strain of K. striatum can grow with wider range of temperature and tolerance to the temperature higher than that of brown strain of K. alvarezii. The physical properties of kappa - carrageenan from K. alvarezii and K. striatum cultivated in the difference temperature seasons by floating cage in Nha Trang Bay (from Nov. 2005 to Jan. 2006 in the cool season and from Apr. to Jun., 2006 in the hot season) were showed in Table 2. Although the DGR were difference between brown strain of K. alvarezii and

Table 1. The growth rate of brown strain of *Kappaphycus alvarezii* (Doty) Doty and payaka brown with short branches of *Kappaphycus striatum* (Schmitz.) Doty cultivated in the seasonal temperature by floating cage method in Nha Trang Bay (2005-2006)

	Seasons	Seawa	ater tempe	erature ¹ (°C)		Salinity $(^{0}/_{00})^{1}$ Growth rate (%		$e (\% day^{-1})^1$	
		Max.	Min.	Aver.	Max.	Min.	Aver.	K. alvarezii	K. striatum
	Nov., 2005	27.8	25.1	26.52±0.95	33	30	31.21±0.68	6.18±0.35	5.04±0.43
Cool	Dec., 2005	27.9	24.5	26.14±110	34	30	31.65±1.20	6.25±0.50	4.68 ± 0.60
	Jan., 2006	27.4	24.6	25.76±1.02	34	29	31.31±1.56	5.73±0.52	4.43±0.65
	Apr., 2006	32.6	28.9	30.21±0.96	35	32	33.42±0.62	1.14±0.45	3.24±0.58
Hot	May, 2006	32.9	28.7	30.68±1.20	35	32	33.62±0.71	0.87 ± 0.52	2.96 ± 0.67
	Jun., 2006	32.7	28.6	30.16±1.12	35	31	33.20±1.28	1.12±0.50	3.28 ± 0.66

¹Average value (mean±SD)

Table 2. The physical properties of kappa-carrageenan from brown strain of *Kappaphycus alvarezii* (Doty) Doty and payaka brown with short branches of *Kappaphycus striatum* (Schmitz.) Doty Doty cultivated in the seasonal temperature by floating cage method in Nha Trang Bay (2005-2006).

Seasons	Physical properties of Kappa-Carrageenan ¹								
		Brown strain of		Payaka brown with short branches of					
	Ka	ppaphycus alvare	zii	Kappaphycus striatum					
	Content	Gel strength	Viscosity	Content	Gel strength	Viscosity			
	(%dr.wt.)	$(g/cm^{2})^{*}$	(cps)	(%dr.wt.)	$(g/cm^{2})^{*}$	(cps)			
Cool (from Nov. 2005 to Jan. 2006)	27.06±1.59	1436.34±291.46	26.52±0.95	26.38±2.56	1166.40±188.49	26.52±0.95			
Hot (from Apr. to Jun., 2006	25.15±2.59	1051.06±279.35	26.52±0.95	25.79±3.39	1085.86±212.86	26.52±0.95			

¹Average value (mean \pm SD); * after 2 months cultivation, 1.5% kappa-carrageenan solution in water, measured at 20^oC.

payaka brown with short branches of K. striatum in the hot season, but the content, gel strength and viscosity of kappa-carrageenan in K. alvarezii (27.06 ± 1.59% dr. weight, 1436.34 ± 291.46 g/cm² and 26.52 ± 0.95 cps in cool season, respectively; $25.15 \pm 2.59\%$ dr. weight, 1051.06 ± 279.35 g/cm² and 26.52 ± 0.95 cps in the hot season, respectively) and in K. striatum (26.38 \pm 2.56% dr. weight, 1166.40 ± 188.49 g/cm² and 26.52 \pm 0.95 cps in the cool season, respectively; 25.79 \pm 3.39% dr. weight, 1085.86 ± 212.86 g/cm² and 26.52 \pm 0.95 cps in the hot season, respectively) were almost the same and high. These values were also high and no difference between species of K. alvarezii (27.06%, 1436.34 g/cm² and 26.52 cps, respectively) and K. striatum (26.38 %, 1166.40 g/cm² and 26.52 cps, respectively) cultivated in the cool season.

The effects of temperature and salinity in seasons on the growth rate and physical properties of carrageenan of brown strain of *K. alvarezii*, payaka brown with short branches strain of *K. striatum* and green and brown

strain of *E. denticulatum* (brown and green color) cultivated in Cam Ranh Bay

The results of the effect of temperature and salinity in the cool and the hot seasons on the DGR and physical properties of kappa and iota-carrageenan of brown strain of K. alvarezii, payaka brown with short branches strain of K. striatum, green and brown strains of E. denticulatum cultivated in the fixed bottom racket in shallow-semi closed lagoon of Cam Ranh Bay on 2006 are showed in Table 3, 4, 5 and 6. The DGR of brown strain of K. alvarezii ranged from 0.89 ± 0.58 to 1.06 \pm 0.47 % per day in the hot season, while the DGR of payaka brown with short branches strain of K. striatum ranged from 3.21 ± 0.81 to 3.64 ± 0.84 % per day and green and brown strain of E. denticulatum ranged from 3.78±0.51 to 5.34±0.49% per day and 3.80 \pm 0.53 to 4.84 \pm 0.39 % per day, respectively, which cultivated in the same place and time with species of K. alvarezii were higher about 3 % day⁻¹ (Table 3). No difference in the DGR was seen between brown

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Table 3. The growth rate of brown strain of *Kappaphycus alvarezii* (Doty) Doty, payaka brown with short branches of *Kappaphycus striatum* (Schmitz.) Doty and green and brown strain of *Eucheuma denticulatum* (N.L.Burman) Collins et Harvey cultivated in hot season by the fixed bottom racket method in shallow- semi closed lagoon in Cam Ranh (2006).

Seasons	Seawater temperature ¹ (⁰ C)					inity ¹ / ₀₀)	Growth rate ¹ (%day ⁻¹)			
	Max.	Min.	Aver.	Max.	Min.	Aver.	K. alvarezii	K. striatum	<i>E. denticulatum</i> (green strain)	<i>E. denticulatum</i> (brown strain)
Apr., 2006	33,7	29.4	31.19±1.30) 35	30	32.25±1.27	0.89±0.58	3.21±0.81	3.78±0.51	3.80±0.53
Hot May, 2006	33.4	29.5	30.87±1.01	35	31	32.62±0.85	1.06 ± 0.47	3.64 ± 0.84	4.76±0.48	4.85±0.39
Jun., 2006	33.5	29.2	31.01±1.23	34	30	31.78±0.92	0.95±0.55	3.35±0.82	5.34±0.49	4.65±0.60

¹Average value (mean±SD)

and green strain of *E. denticulatum* in the cultivated months on Cam Ranh Bay (from Mar. to Dec., 2006) (Table 4).

The content, gel strength and viscosity of kappa-carrageenan in brown strain of *K. alvarezii* were not significantly different to those of payaka brown with short branches strain of *K. striatum* cultivated in the hot season from Apr. to Jun., 2006 (Table 5). Similarly, no significantly difference in the content and moisture of an iota-carrageenan was identified between green and brown strain of *E. denticulatum* cultivated in Cam Ranh Bay in hot and cool season of 2006 (Table 6). However, in hot season, the viscosity was significantly higher in brown strain of *E. denticulatum* (274 ± 21.41 cps) than that in green strain of this species (167 ± 19.42 cps) and also was indicated in Table 6.

Discussion

Among *Kappaphycus* species, two species of *K. alvarezii* and *K. striatum* which are cultivated the most widespread in the world possess the same useful and

Table 4. The growth rate of brown and green strain of *Eucheuma denticulatum* (N.L.Burman) Collins et Harvey cultivated in Cam Ranh Bay in 2006 (% day⁻¹)

Strain	Months										A
(color)	March	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	- Aver.
Green strain	2.56	3.78	4.76	5.34	5.89	5.03	3.38	3.71	5.15	5.62	4.52±0.21
Brown strain	1.85	3.80	4.85	4.65	6.85	6.71	2.88	3.33	4.56	6.12	4.56±0.30

Table 5. The physical properties of a kappa-carrageenan from brown strain of *Kappaphycus alvarezii* (Doty) Doty and payaka brown with short branches of *Kappaphycus striatum* (Schmitz.) Doty cultivated in the hot season at the shallow semi-closed lagoon in Cam Ranh (in 2006)

Hot season	Physical	- Note		
(From Apr. to Jun., 2006)	Content (% dr. wt.)	Gel strength (g/cm ²)	Viscosity (cps)	- Note
K. alvarezii	24.15±2.55	1025.86±266.19*	111.42±27.15	After 3 months cultivation
K. striatum	25.84±2.58	1117±260.56	103.32 ± 18.30	After 3 months cultivation

* 1.5% Kappa-Carrageenan solution in water, measured at 200C.

 Table 6. The content and viscosity of an iota-carrageenan from brown and green strain of *Eucheuma denticulatum* (N.L.Burman)

 Collins et Harvey cultivated in different seasons in Cam Ranh Bay (in 2006).

Season	Strain	Moisture (%)	Content (%)	Viscosity (cps)
Hot	Brown	22.0±2.10	17.5±1.98	274±21.41
(Apr Sept.)	Green	21.0±1.96	15.2±2.13	167±19.42
Cool	Brown	30.0±3.42	15.5±1.21	217±15.93
(Oct March)	Green	33.0±2.12	14.5±1.82	195±24.56

economic values. Until now, total of twelve strains of K. alvarezii and K. striatum and three strains of E. denticulatum have been determined in cultural regions in the Philippines. Among them, Tambalang strain of K. alvarezii and Sacol Tawi - tawi, Sacol Bohol of K. striatum possess economically and ecologically important variable (colloid quality and content, growth rate, warm susceptibility, disease resistance, invasive potential in these commercially important seaweeds) (Aguilan et. al., 2003; Hurtato et. al., 2005; Trono et. al., 2000; Zuccarello et. al., 2006). In order to establish the Kappaphycus cultivation farms with stable and high production all year round using mixed cropping of different strains/species as seed stock during different cropping seasons (with different environmental conditions, especially temperature), some strains of K. striatum from cultivation areas of Coco Island, Martan Sea, Cebu, Philippines were collected for this goal and transferred to Vietnam in 2005 by Huynh Quang Nang. Three strains of K. striatum (Schmitz.) Doty are payaka brown with short and long branches, Sacol brown and green were transferred to Vietnam in 2005. On the other hand, there are two strains (brown and green strains) of E. denticulatum were also transferred to Vietnam in 2005 for getting enough materials for an iota-carrageenan extraction and food in Vietnam.

When temperature and salinity of seawater were low (about WT: 24°C-28°C and S: 29-34‰), the DGR of brown strain of *K. alvarezii* was higher than that of payaka brown with short branches of *K. striatum* (about 1 % per day more). In contrast to the observed result above, the DRG of brown strain of *K. alvarezii* was 2-3 % per day lower than that of payaka brown with short branches of *K. striatum* in hot season (about WT: 29°C-33°C and S: 31-35‰). It showed that the species of *K. striatum* can grow with wider range of temperature and has tolerance to high temperature higher than that of *K. alvarezii*.

The content and gel strength of kappa-carrageenan in brown strain of *K. alvarezii* cultivated in the difference seasonal temperature by floating cage in Nha Trang Bay in 2005, 2006 (27.06 \pm 1.59 % dry weight and 1436.34 \pm 291.46 g/cm², respectively, in the cool season; 25.15 \pm 2.59 % dry weight and 1051.06

 ± 279.35 g/cm², respectively, in the hot season) and in pavaka brown with short branches of K. striatum (in the cool season: 26.38 ± 2.56 % dry weight, and 1166.40 ± 188.49 g/cm², respectively; in the hot season: 25.79±3.39 % and 1085.86±212.86 g/cm², respectively) were almost the same and high. The DGR of the species of K. alvarezii (ranged from 0.89±0.58 % day⁻¹ to 1.06 ± 0.47 % day⁻¹) cultivated by fixed bottom racket in shallow, semi-closed Lagoon in the hot season (during Apr. to Jun., with the WT ranged from 30.87±1.01°C to 31.19±1.30°C) is low and the seaweed is very prone to ice-ice disease (average 90 % of total plants were infected), while the DGR of species of K. striatum and E. denticulatum (ranged from 3.21±0.81% dav^{-1} to 3.64±0.84 % dav^{-1} and from 3.78±0.51 to 5.34 ± 0.49 % day⁻¹, respectively), which were cultivated in the same place and time with K. alvarezii, is higher (about 3% day⁻¹ more). Furthermore, these seaweed species are few prone to Ice-ice disease (average only 5-10% of total plants were infected). The content and gel strength of a kappa- carrageenan in species of K. alvarezii were not significantly different to those of K. striatum and high. Our obtained results are similar to reports of other authors (Ohno et al., 1994; Lluisma et al., 1995; Ohno et al., 1996, Gerung and Ohno, 1997; Zuccarello et al., 2006). From Mar. to Dec. the DGR average of green and brown strain of E. denticulatum cultivated on Cam Ranh (Khanh Hoa province) was 4.52±0.21 and 4.56±0.30 % day-1, respectively, and were almost the same and very high compared with that of K. alvarezii and K. striatum.

The obtained results showed that *K. striatum* could grow over wide range of temperature and could tolerance more strongly to high temperature, compared with *K. alvarezii*. But their content and gel strength of kappa-carrageenan were almost the same and high. It created the *Kappaphycus* cultivation farms, (especially, in the seawater of shallow, semi-closed Lagoons, where the water movement is not good and with high temperature in the hot season), with stable and high production in all year round by mixed cropping of *K. alvarezii* and *K. striatum* species as seed stock during the different cropping seasons: *K. alvarezii* and *K. striatum* could grow well in the cool season (from Oct. to next Mar.), and need to chose only *K. striatum* species growing in the hot season (from Apr. to Sept.).

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