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Short Paper

Greenhouse Cultivation of *Gracilaria verrucosa* (Hudson) Papenfuss and Determination of Chemical Composition

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Abstract

The agarophyte *Gracilaria verrucosa* (Hudson) Papenfuss was cultivated under greenhouse conditions in Modified Johnson Medium over a 5-month period. Biomass productivity ranged between 5.00 g L^{-1} to 16.37 g L^{-1} and the highest biomass was obtained in December. Relative growth rate (RGR) varied from 4.03±1.63 to 1.21±0.34% day^{-1}. While the highest percentages of protein were found in December (20.28±0.94% of dw), the lowest percentages were in March (14.99±0.14% of dw). Phosphorus content ranged from 101.66±3.11 ppm (march) to 114.03±5.44 ppm (december). The lipid concentrations of *G. verrucosa* were measured within 2.39±0.77% dw and 2.66±0.94% dw. The agar yiel were determined between 9.65±1.12 and 18.64±2.38% of dw in december and march. The melting and gelling temperatures were stable through the experiment. The highest and the lowest values for both melting and gelling temperatures were 39.00±0.35°C (december) – 34.00±0.32°C (march) and 86.50±0.30°C (march) – 85.50±040°C (february), respectively. As a result of this study *G. verrucosa* could be cultivated in greenhouse conditions succesfully. Depending on the high growth rates, high concentrations of crude protein, phosphorus and agar of *Gracilaria* we conclude that it can be cultivated in greenhouse conditions.

Keywords: *Gracilaria verrucosa*, macro algae, tank cultivation, growth parameters, agar - agar.

Gracilaria verrucosa (Hudson) Papenfuss'nın Sera Koşullarında Yetiştiriciliği ve Kimyasal İçeriğinin Belirlenmesi

Özet

Agarofit *Gracilaria verrucosa* (Hudson) Papenfuss değiştirilmiş Johnson ortamı kullanılarak sera koşullarında 5 ay boyunca yetiştirilmiştir. Biyomas üretimi 5,00 g L^{-1} ile 16,37 g L^{-1} arasında değişmiştir ve en yüksek biyomas aralık ayında elde edilmiştir. Büyüme hızı (RGR) %4,03±1,63 ile 1,21±0,34 $gün^{-1}$ olarak değişmiştir. En yüksek protein yüzdesi aralık ayında (%20,28±0,94 kuru ağırlık) saptanırken en düşük yüzde mart ayında (%14.99±0.14 kuru ağırlık) belirlenmiştir. Fosfor içeriği 101,66±3,11 ppm (mart) ile 114,03±5,44 ppm (aralık) arasında değişmiştir. *G. verrucosa* 'nın yağ içeriği kuru ağırlığın %2,39±0,77 ile %2,66±0,94'ı arasında ölçülmüştür. Agar verimliliği aralık ve mart aylarında kuru ağırlığın %9.65±1.12 ile %18,64±2,38 arasında belirlenmiştir. Erime ve jelleşme sıcaklıkları çalışma süresince sabit kalmıştır. Erime ve jelleşme sıcaklıklarının en yüksek ve en düşük değerleri sırasıyla 39,00±0,35°C (aralık)- 34,00±0,32°C (mart) ve 86,50±0,30°C (mart)- 85,50±0,40°C (şubat)'dır. Çalışma sonuncunda *G. verrucosa* sera koşullarında başarıyla yetiştirilmiştir.

Anahtar Kelimeler: *Gracilaria verrucosa*, makro alg, tank yetiştiriciliği, büyüme parametreleri, agar – agar.

Introduction

Seaweeds play an important role in primary production and they are widely used in various industries such as food, agriculture, cosmetic and pharmacy (Ak and Cirik, 2004; Cirik and Cirik, 1999). *Gracilaria* (Gracilariales, Rhodophyta) is one of the most important seaweeds in terms of commercial value because of the agar extracted (Castro and Guanzon, 1993). *Gracilaria* is being increasingly used in the production of food grade agar (Armisen, 1995). Its availability has greatly increased mainly through the development of cultivation techniques in several countries (Critchley, 1993). Commercial cultivation is done on a large scale in Chile, China and Taiwan. Pilot scale cultivation is carried out in medium sized farms mainly in Namibia, Venezuela and Malaysia. At present, the culture

methods for *Gracilaria* rely on vegetative fragments (Uriostequi and Robledo, 1999). Previous studies also mentioned that *Gracilaria* can be cultivated in outdoor systems (Hanisak, 1982, 1987, 1990; Hanisak and Ryther, 1984; Cirik and Cirik, 1999) and the biomass is used in food or bio fuel industries (Hanisak, 1982, 1987; Hanisak and Ryhter, 1984). Biomass yield changes depending on the cultivation methods. Water temperature, salinity, light intensity, nutrients, water exchange and mixing are also the most important factors affecting seaweed biomass (Agadi, 1983; Castro and Guanzon, 1993; Neori *et al*., 2000; Cirik *et al*., 2006). The growth of this species was found to be both eurythermal, with growth over the temperature range of 12-36°C and euryhaline with growth over the salinity range of 6-42‰ (Cirik and Cirik, 1999; Turan *et al*., 2006; Cirik *et al*., 2006). Optimum production was determined at pH 8 (DeBusk and Ryther, 1984). In cultures, if the nutrient level is limited at the beginning, then algal growth starts to diminish. Hanisak (1990) showed that addition of nutrients twice a week into the culture vessels can be effective as much as daily addition of nutrients. Periodic nutrient introduction also prevents the cultures from epiphyte growth and algal blooms. In addition, *Gracilaria* species needs large amounts of aeration of sufficient intensity to keep the seaweed in suspension and to rotate in the water. Knowledge of proximate composition of seaweed is both important for the assessment of nutritional value to marine herbivores (Hawkins and Hartnoll, 1983), and for the evaluation of potential sources of protein, carbohydrate and lipid for commercial use (Chapman and Chapman, 1980) or for possible human consumption (Abott, 1988). Proximate composition and agar content of *Gracilaria* vary according to culture techniques, and it was shown that there is a positive correlation between protein content and nitrogen availability (Marinho-Soriano and Bourret, 2005).

G. verrucosa is naturally found in Turkish coastal waters, but cultivation studies on this alga are limited (Cirik *et al*., 2006). Ak and Cirik (2004) determined the abundance of *G. gracilis* between 0.7 kg ww.m^{-2} and 7.7 kg ww.m^{-2} in İzmir Bay from April 2001 to July 2002. The potential for over harvesting of wild stocks in a looming and farming of *Gracilaria* is therefore desirable supplement production from natural stocks.

Aim of this study is to determine growth rate, proximate composition and agar content of *Gracilaria verrucosa* under greenhouse conditions.

Materials and Methods

Seaweed Material

Gracilaria verrucosa (Hudson) Papenfuss fragments were collected in İzmir Bay (38°45´N, 27°05´E), Turkey. Collected seaweeds were transported to the greenhouse, washed with seawater and the epiphytes were cleaned. The fragments were maintained for four weeks in the greenhouse conditions in aerated 100 L seawater.

Experimental Design

The study was conducted in ellipsoidal polyester tanks having 50 cm width, 100 cm length and 40 cm depth. The tanks containing 50 L enriched seawater were placed in the greenhouse in Dardanos Campus of Çanakkale Onsekiz Mart University, Turkey, from November 2007 to March 2008. In order to avoid growth limitation, biomass density was kept approximately at 5 g wet weight (ww) per liter throughout the culture period (Vergara *et al*., 1993; Hanisak, 1987). During the experiments, the water exchange level was kept very low (1 volume per week). Modified Johnson Medium (MJM) was applied in the tanks weekly (Johnson *et al*., 1968).

Environmental Parameters

During the study period, the environmental parameters such as water temperature, salinity and light intensity were recorded weekly. Water temperature was measured by a hand help thermometer and salinity was determined by a hand reflactometer (Nippon, Japan). Light intensity was measured with a LI-250 a light meter (Li-Cor, USA).

Growth Estimation

During the experiments, wet weight of *G. verrucosa* was measured weekly with a digital balance (Kern 440-49N) weekly. Before the biomass was weighed, the fresh *G. verrucosa* was covered by a paper towel to remove the excess water from the surface. The mean relative growth rate (RGR), expressed as % day^{-1}, was calculated according to the exponential model

$$RGR=[ln\ (N_2/N_1)/(t_2-t_1)*100]$$

where N_2 and N_1 are wet weights at times t_2 and t_1. According to growth rates, fragments were harvested at 50th (December), 90th (February) and 140th (March) days of experiment.

Proximate Analysis

G. verrucosa fragments were analyzed for proximate composition according to AOAC (2002). Samplings were done in December (Day 50th), February (Day 90th) and March (Day 140th) when RGRs were starting to decrease. Moisture was determined in an oven at 105°C while the ignition of samples for ash determination was carried out in a muffle furnace at 550°C overnight for 12 h. Crude protein and total lipid values were determined found

using the Kjeldahl and Folch methods respectively (AOAC, 2002; Falch, 1957).

Agar Extraction and Gel Properties

The agar extraction was performed according to the method described by İlyas (1989). Each dried sample was grounded and homogenized well before use. 10 g of dw (dried weight) algae was added to 200 mL of distilled water in an Erlenmeyer flask and heated for 6 h at 90°C in a shaker. The extract was pre-filtered through plankton net (125 µ). The residue was centrifuged at 3,000 rpm for 3 min. The extract was allowed to gel at room temperature and then placed in freezer (-20°C) overnight. The frozen gel was thawed, washed with distilled water and dried for 24 h at 60°C. The agar yield was calculated as the percentage of dry matter. Melting and gelling temperatures were determined according to Young and Percival (1974).

Statistical Analysis

Growth, proximate and agar contents were statistically analyzed using ANOVA. The data was then analyzed using Tukey's multiple comparison of means. Data was tested for homogenous variance using Cochran's test (Zar, 1999).

Results

Water temperature in tanks ranged from 11.80±2.11°C (December) to 17.90±3.10°C (March). The minimum and the maximum salinity values were 38.13±3.44‰ (October) and 40.25±0.68‰ (January). Levels of pH were stable throughout the experiment and ranged 8.03±0.24–8.44±0.12. Average daily light intensities in greenhouse were found to be 469±92 µmol photon m^{-2} s^{-1} in January and 946±143 µmol photon m^{-2} s^{-1} in March (Table 1).

Biomass Yield and Relative Growth Rates

Biomass gain and relative growth rates (RGRs) of *G. verrucosa* cultivated under greenhouse conditions were shown in Figure 1. Biomass production varied from 5.00 g L^{-1} to 16.37 g L^{-1} over the cultivation period. The highest RGR was calculated in December (4.03±1.63 % day^{-1}) and the lowest RGR was obtained in March (1.21±0.34 % day^{-1}). During the experiment, average RGR was recorded as 2.46±1.70 % day^{-1}.

Proximate Analysis

Mean changes in proximate values were shown in Table 2. Mean protein content ranged from

Table 1. Water quality parameters measured during the cultivation of *G. verrucosa* in the greenhouse conditions (mean ± SD and minimum - maximum, n=25)

Months	Temperature (°C)	Salinity (‰)	pH	Light Intensity (µmol photon/m^2/s)
October	15.10±3.11 (12.40–18.50)	38.13±3.44 (32.50–41.50)	8.44±0.12 (8.31–8.63)	595±112 (467–635)
December	11.80±2.11 (8.50–14.50)	39.50±1.09 (38.00–41.5)	8.37±0.23 (8.07–8.56)	469±92 (432–567)
January	12.50±2.00 (9.00–14.50)	40.25±0.68 (39.50–41.50)	8.03±0.24 (7.98–8.32)	515±132 (467–732)
February	16.50±3.21 (13.00–21.00)	39.69±0.92 (38.00–41.00)	8.38±0.26 (8.11–8.61)	719±129 (634–893)
March	17.90±3.10 (12.00–22.00)	39.19±4.19 (30.50–39.50)	8.42±0.12 (8.29–8.56)	946±143 (789–1032)

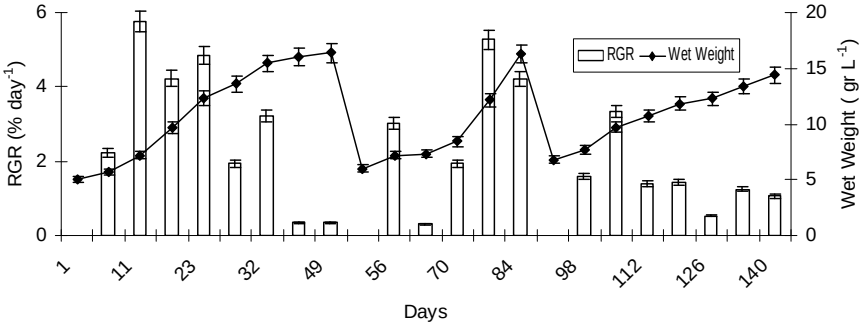

Figure 1. Mean Relative growth rates and wet weight (±SD) of *Gracilaria verrucosa* grown in greenhouse conditions (n=3).

Table 2. Proximate contents and agar properties of *G. verrucosa* (mean ±SD, minimum - maximum, n=5)

	Months			ANOVA	
	December	February	March	F	Prob.
Protein	20.28[a]±0.94 (19.34–21.22)	17.22[b]±0.32 (16.9–17.54)	14.99[c]±0.14 (14.85–15.13)	63.13	<0.001
Phosphorus	114.03[a]±5.44 (108.59–119.47)	111.32[ab]±4.56 (106.76–115.88)	101.66[b]±3.11 (98.55–104.77)	6.34	<0.05
Lipid	2.66[a]±0.94 (1.72–3.60)	2.39[a] ±0.77 (1.62–3.16)	2.63[a]±0.34 (2.29–2.97)	0.12	NS
Moisture	11.71[a] ±0.23 (11.48–11.94)	11.59[a]±0.43 (11.16–12.02)	10.90[a]±0.55 (10.35–11.45)	3.18	NS
Ash	12.08[a]±0.11 (11.97–12.19)	11.25[a]±0.58 (10.67–11.83)	12.14[a]±0.78 (11.36–12.92)	2.33	NS
Agar	9.65[b]±1.12 (8.65–10.65)	16.79[a]±2.01 (14.78–18.78)	18.64[a]±2.38 (16.30–20.99)	19.32	<0.05
Gelling	39.00[a]±0.35 (38.65–39.35)	36.00[b]±0.20 (35.80–36.20)	34.00[c]±0.32 (33.68–34.32)	215.18	<0.001
Melting	86.00[ab]±0.32 (85.68–86.32)	85.50[b]±0.40 (85.10–85.90)	86.50[a]±0.30 (86.20–86.80)	6.38	<0.05

NS indicates, non significant at 5% level.

14.99±0.14% in March to 20.28±0.94% in December. There was a significant difference (P<0.05) within the months. Also phosphorus content (ranging from 101.66±3.11 to 114.03±5.44 ppm) was significantly different within the months (P<0.05). The highest and the lowest lipid contents were observed in December (2.66±0.94%) and in February (2.39±0.77%), respectively.

No significant difference was noted on the lipid contents (P>0.05). The moisture content of *Gracilaria* varied from 10.9±0.55 to 11.71±0.23%. While the highest ash content was 12.08±0.11% in December, the lowest ash content was 11.25±0.58% in February. There was no significant difference on the moisture and ash contents between the months (P>0.05).

Agar

The agar yield from three harvest periods ranged between 9.65±1.12% (December) and 18.64±2.38% (March) of dry weight. Agar yield of *G. verrucosa* varied significantly between the months (P<0.05). It was noticed that agar content decreased when protein content increased. The agar yield was also correlated negatively with gelling temperature. The highest and the lowest values of melting temperatures were 39.00±0.35°C (December) – 34.00±0.32°C (March). Maximum gelling temperature was obtained as 86.50±0.30°C (March) and minimum gelling temperature was measured as 85.50±0.40°C (February). There was a significant difference on the melting and gelling temperatures within months (P<0.05) (Table 2).

Discussion

Algal Growth

The highest RGRs observed in this study were also similar to RGRs reported by other investigators for other species of *Gracilaria* (Critchley, 1993; Halling *et al*., 2005). Hanisak (1987) reported that optimum light intensity for *G. tikvahiae* was about 100 $\mu Em^{-2}\ s^{-1}$ and RGR was limited by light intensity. In seaweeds, nitrogen concentration in tissue was influenced by light intensity (Lapointe and Duke, 1984; Shivji, 1985). Increasing light intensity causes crude protein contents to decrease (Lapointe and Duke, 1984). *Gracilaria* grows well in October – July when the water temperature range between 10 to 20°C (Cirik *et al*., 2006; Turan *et al*., 2006). According to Cirik *et al*. (2006) and Turan *et al*. (2006), *G. verrucosa* grows better below 20°C, and our study agrees with the findings of these studies. It was found that that *G. verrucosa* can tolerate changes in salinity and pH levels. Optimum production was determined at 42‰ salinity (Cirik and Cirik, 1999; Turan *et al*., 2006) and at pH 8 (DeBusk and Ryther, 1984). In our experiment, there was significant effect of salinity on RGR, but there was no significant affect of pH on RGR.

Proximate Contents

In the study of Marinho-Soriano and Bourret (2005), a positive correlation was found between protein and nitrogen contents, and negative correlation was found between temperature and salinity. Briggs and Smith, (1993) point out that the protein content for most *Gracilaria* species is between 7–13% where as Marrion *et al*. (2005) and Ova-Kaykac, (2007) found 19.13% and 27.38%. Our study shows likeness with the results of Marrion *et al*. (2005) and Ova-Kaykac (2007). Furthermore, the mean of crude protein content (c.a. 20.28±0.94%) recorded was higher than the concentrations found in higher plants (Norziah and Ching, 2000).

Most studies on *Gracilaria* nutrient content

concentrate on nitrogen, making it difficult to compare our phosphorus values with others. Phosphorus contents varied between 101.66±3.11 ppm and 114.03±5.44 ppm and significant differences were found among the harvest periods (P<0.005). Ilyas (1989) determined that *G. verrucosa*'s phosphorus contents vary between 138.45 ppm (June) and 65.56 ppm (September); and this shows similarities to our values.

In general, seaweeds exhibit low lipid contents (Dawes, 1998). In fact, in comparison to other proximate constituents, lipid contents were the smallest component observed for the species studied. Mabeau and Fleurence (1993) and Norziah and Ching (2000) reported that total lipid content of seaweed changes between 1% and 3%. In our study, lipid contents ranged from 2.39±0.77% to 2.66±0.94 and there were no significant differences among harvest periods (P>0.05). Also, these findings agree with previous studies.

Moisture content of *G. verrucosa* ranged from 10.90±0.55% to 11.71±0.23% and there were no significant differences within harvest periods. Marinho-Soriano *et al*. (2006) determined the moisture content of *G. cervicornis* as 14.66±1.78%. Also, Ova-Kaykaç (2007) stated the content of *G. verrucosa* varying between 11.73±0.62% (summer) and 10.67±1.65% (autumn). Our results are in agreement with these previous studies.

Ash content of algae differs according to the species and seasons. According to the Marinho-Soriano *et al*. (2006), the maximum percentage of ash contents was 7.72% for *G. cervicornis*. Ova-Kaykac (2007) reported the minimum value as 19.13±0.23% (summer) and the maximum value as 28.71±0.71% (winter). In this experiment, ash content varied between 11.25±0.58% and 12.14±0.78% and there were no significant differences between harvest periods (P>0.05). Our results reveal the ash contents of *G. verrucosa* collected from the nature differ from *G. verrucosa* cultivated.

Agar Contents

Agar yields fluctuate between 6% and 71%, while 20% being common for agarophytes (Whyte and Englar, 1980; Ilyas, 1989). In other studies, agar yields of *Gracilaria* were reported to be 30.0–34.8% dw (Marinho-Soriano, 2001), and 19.0–30.0% dw (Marinho-Soriano and Bourret, 2003). Higher yields of agar from *G. chilensis* were found by Matsuhiro and Urzúa (1990) who reported up to 43% of dw. The highest yield and the strongest gels of agar contents were found to coincide with a period of low nutrients and slow down in growth (Dawes, 1987). Maximum agar yield was obtained as 18.64±2.38% dw in March, when RGR was the lowest (1.21±0.34 day^{-1} %). This figure is comparable to the results of Dawes (1987). *Gracilaria* is a source of phycoccolloid agar and a number of studies have determined yields, gelling and melting temperatures. Because of great variation in culture methods, it is difficult to make specific comparisons among the studies. Gelling temperature was found 37-48°C for *G. gracilis* and 34-46°C for *G. bursa-pastoris* (Marino-Soriano and Bourret, 2003). According to the Marinho-Soriano and Bourret (2005) the minimum and the maximum gelling temperature of *G. dura* was 38°C and 43°C. In our study, gelling temperature ranged from 34.00±0.02°C to 39.00±0.35°C and there were significant differences among harvest periods (P>0.05). Meena *et al*. (2006) reported that melting temperature is 82°C for *G. crassa* and 76°C for *G. edulis*. Our results for melting temperature were between 85.50±0.40°C and 86.50°C and there were significant differences among the harvest periods (P<0.005). Our results are higher than previous studies and these differences come from different *Gracilaria* species.

In this study, *G. verrucosa* was cultivated in greenhouse conditions and analyzed for its proximate composition and agar yield. Depend on the high growth rates, percentage of crude protein, phosphorus and agar, *Gracilaria* can be cultivated in greenhouse conditions.

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