

# Agar Production by Macroalga *Gracilariaopsis persica* in the Coastal Waters of the Persian Gulf

Fatemeh Eidighaleghazi

Payam Noor University, Bandar Abbas Center, I.R. Iran, P. O. Box: 7913613915

Received: May 13, 2014

Accepted: July 22, 2014

## ABSTRACT

Production of agar from marine macroalga *Gracilaria*, makes it important for local enterprise of coastal communities. The aim of this study is to gather basic technical data for the development of *G. oopsis persica* mariculture in Persian Gulf of Iran. Monoline and net methods were applied to determine biomass and the agar productivity. Biological parameters of chlorophyll, thallus length, position along the thallus, and color; and environmental parameters of temperature, pH, salinity, TDS and TSS were measured during cultivation. The changes of  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{2-}$  ions were monitored daily. STATISTICA 10 Software was used for statistical analyze of data. The experiments were performed from May through June 2010 in Qeshm Island. Biomass values increased during experiment period and reached to maximum  $5890\text{-g Fw m}^{-1}$  (30<sup>th</sup> day) and  $5300\text{-g Fw m}^{-1}$  (36<sup>th</sup> day) in monoline and net methods, respectively. Mean growth rate (GR) and relative growth rate (RGR) for the mono and net methods were  $4.4\text{-}\% \text{d}^{-1}$ , ( $4.0 \pm 0.30$ ) and  $4.3\text{-}\% \text{d}^{-1}$ , ( $3.0 \pm 0.25$ ), respectively. Positive correlations were found between biomass ( $r = 0.59$ ;  $p < 0.001$ ) and GR ( $r = 0.61$ ;  $p < 0.001$ ). During the study period, temperature was changed in the range of  $25.35 - 30.75^\circ\text{C}$  (Mean:  $28.03 \pm 1.23$ ), pH in the range of  $7.9 - 8.4$  (Mean:  $8.2 \pm 1.14$ ), and salinity from  $41.2 - 49.75$  (Mean:  $46.13 \pm 2.11$ ) PSU. Extracted agar yield in the cultivation period ranged from  $40.4$  to  $15\text{-}\%$ . These results demonstrated the great potential of *G. oopsis persica* for mariculture system and its economic potential use for agar production by Persian Gulf coastal communities.

**KEYWORDS:** *G. oopsis persica*, Macroalgae Mariculture, Agar Production, Monoline Technique, Net Technique.

## 1. INTRODUCTION

Macroalgae, as a group of seawaters lives, were used both as a human food and animal feed in many countries such as Japan, Philippine, Chili etc. [1-4]; and as industry feedstock such as agar, alginate and the chitosan for a long time [5]. In agriculture, the biomasses of macroalgae were used as fertilizer and, as a source for energy generations. In the environment sector, various algae are used for sensing, monitoring, and bioremediation activities to attain sustainability in marine and maritime programs [6, 5]. A popular macroalga with high commercial impacts in mariculture is genus *Gracilaria*. Some species of *Gracilaria* are the most useful algae in the world, combining the production of valuable polysaccharide agar with a fast growth rate, ease of vegetative reproduction and other attributes favoring their cultivation [3]. Genus *Gracilaria* has been farmed successfully in many countries because of its importance for producing agar. In addition, *Gracilaria* cultivation is also gaining popularity in developing countries as a source of foreign exchange and a means of broadening the livelihoods of coastal communities [7, 8]. By the way, in the best of our knowledge, there are no reports or cultivation history of *Gracilaria* mariculture in Iran in the pilot scale and there is no information of its mariculture for local entrepreneurs.

Agar is the main component of the cell matrix and walls of some red algal species, particularly from the families *Gracilaria* [9, 10]. The genus *Gracilaria* was reported as the best sources for agar production. *Gracilaria* species are a major source of agar, particularly the agar used by the food industry [11] and approximately 60% of all agars are produced from this alga [12]. *Gracilaria* has been farmed on a large scale in several countries, including Chile, China, Taiwan and Vietnam by enterprise.

The commercial cultivation of *Gracilaria* has been shown to be technically feasible using suspended rafts, ropes and net [13-17]. Suspended cultivation has some advantages over bottom stocking, such as surface harvesting and easy control. Monoline and net methods are main mariculture systems of this alga [17]. The monoline method is extensively used in the culture of sea weed and it has some important advantages such as simplicity, use of inexpensive and readily available materials, and good plant growth [10]. To supply great demand of the global market, the cultivation technologies of *Gracilaria* were developed in the last years. However, still many technical challenges remain to be solved which makes the production process expensive for commercial applications and beneficiary of businesses in local communities. Process optimization results higher agar productivity and reduce the commercial production expenses. Environmental parameters such as light irradiation, depth of cultivation, nutrients, configurations, water temperature, etc. have large impacts on the alga growth rate and agar accumulation and constrain simple systems to manage these parameters makes it feasible

\*Corresponding Author: Fatemeh Eidighaleghazi (MSc). Payam Noor University, Bandar Abbas Center, I.R. Iran, P.O. Box: 7913613915; Email: fatemeheidi57@yahoo.com

business by reducing the capital of the business. These parameters also affect the current expenditures and the maintenance of the farming system for the local people [15]. This study, as the first trial of mariculture of *G. ophis persica* in Persian Gulf area, is going to resolve the practical challenges happen in set up of pilot scale cultivation. In addition, it will investigate the effects of depth of cultivation, mass of initial thallus, and the method of cultivation of *G. ophis persica* for cell growth and agar production. This work also tries to guide the farmers, to find the best coastline start point for mariculture of *G. ophis persica* based on the tidal cycle. Finally we discussed the technical and commercial feasibility of *G. ophis persica* mariculture for the local communities

## 2. MATERIAL AND METHODS

### Experimental design

The cultivation experiments were conducted in Qeshm Island, Hormozghan province, Iran with the latitude and altitude of 26 56 12 23N, 56 16 45 12E, from May, 1<sup>st</sup> to June, 30<sup>th</sup> 2010, at the concrete rectangular channel with dimensions of 200-cm x 200-cm x 10000-cm and the water flow of 3000-m<sup>3</sup>day<sup>-1</sup>. Specimens of *G. ophis persica* vegetative thalli were harvested from the sandy intertidal zone of the Qeshm Island, on the coast of the Persian Gulf. The apical parts of thalli were rinsed thoroughly several times with filtered seawater to remove rock debris and attached epiphytes; layered between seawater-saturated blotting paper, and transported to the laboratory of Payam Noor University in isothermal boxes (the journey took 2-h), where they were kept in a 100-L aquaria for 24-h at 40-‰ salinity seawater and 25 - 27°C in darkness and constant aeration [13, 18]. The healthy apical thalli were excised into 10-cm lengths, from the vegetative thalli and inserted between the braids of polypropylene cultivation rope at 20-cm intervals. For the net culture, the thalli were used without any changes. *G. ophis persica* were transplanted in nine monoline modules (27 rope with 200-cm long, N = 270 thalli). In monoline modules, polyethylene ropes of 1.2-cm wide, with 10 fragments of thallic with the weight of 10, 20 and 25 ± 1.0-g (Mean ± SD, N=3) individuals and totally 100, 200 and 250 ± 3.0-g (Mean ± SD, N=3) per rope, were inserted between the twists of the rope. In the net module, 3 fronds with total weight of 1000-g were placed in the net. Modules made of 27 lines in 3 water surface levels and the 100-cm horizontal intervals. The modules were suspended at 60, 80 and 100-cm below the water surface with buoys attached to the ends and fixed to the bottom by concrete blocks. The layout of experiments is shown in Figure 1.

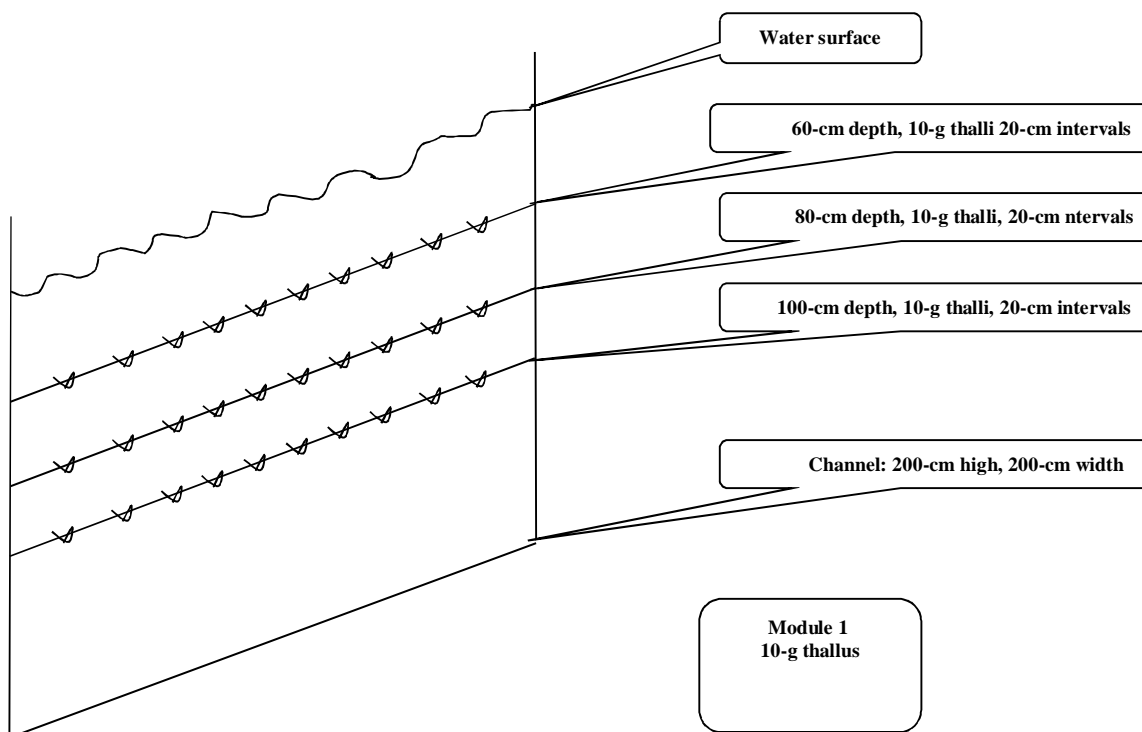


Figure 1. Diagrammatic representation of experimental set-up

### Measurement of growth rate

A correlation line was obtained from set biomasses before and after rinsing water by paper towel. Every week rope samples were weighted and dry weight was calculated from the correlation line. To obtain correlation line, various amounts of alga biomass selected and minuses the vessel and the excess water after 5 minutes. Then its dry weight was obtained. Based on the results, correlation line and equation was obtained, then alga were blotted on paper towel to remove excess water and the biomass was determined (fresh weight; Fwt.m<sup>-1</sup>). Each line

was weighed individually. The fresh weight was obtained from correlation line. The growth was expressed as biomass fresh weight obtained during cultivation time. Relative growth rate (RGR) expressed as % day<sup>-1</sup>, and was calculated according to the exponential model:

$$\text{RGR} = [\ln (w_2/w_1) / (t_2 - t_1)] * 100$$

where  $w_1$  is the initial weight,  $w_2$  is the final weight,  $t_2$  and  $t_1$  is the interval between the final and initial date and  $\ln$  is the Napierian logarithm. The specific growth rate (SGR), expressed as the percentage of increase of fresh weight (Fw d<sup>-1</sup>), and was calculated as:

$$\text{SGR} = n (m_2/m_1) * 100 / (t_2 - t_1),$$

Where  $m_1$  and  $m_2$  refer to the initial or previous average wet weight of fragments and after time  $t$ , respectively, and  $t$  is time in days. The biological parameters of the fronds including color, length, appearance of sporeling and healthy conditions were observed daily. The channel water movement was relatively low and the transparency of water was approximately 0.8–2.0-m.

### Measurement of environmental and nutrient parameters

Environmental conditions of water surface including temperature, water surface salinity and the pH were also measured simultaneously to study of biomass, twice a day at 0900 and 1700 hours from the depth of 80-cm. Temperature, salinity and pH were measured with (SENSDIRECT, SN 051796 GERMANY) and pH meter (ph 3150 JENWAY UK), respectively. The NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup> ions were measured with Spectrophotometer (model 1367, Germany). Triplicate samples were collected every 2 h over a complete tidal cycle (12 h) from each site. All collections were made with buckets rather than Niskin bottles. The buckets were used because they allowed one investigator to obtain all of the water samples at the three rafts during the tidal sampling period. The SW was filtered with 0.22 µm acetate filters (Osmonics Inc., Minnetonka, MN) into 30 mL scintillation vials. The vials were frozen within 1 h of collection, which stops the conversion of NH<sub>4</sub><sup>+</sup> to other forms of nitrogen. Nutrient analysis was performed using a Bran and Lubbe AA3 Auto Analyzer (SPX Process Equipment, Inc., Delavan, WI).

### Agar extraction and analyzing

The agar extraction was performed according to the method described by Iyas [18]. To determine agar yield and quality, the algal biomass was taken to the laboratory in isothermal boxes, washed thoroughly several times with tap water to remove impurities and encrusted organisms, and kept 12-h in distilled water to remove salinity. Then, the alga poured into a plastic tray and were air-dried to constant weight at room temperature for 24-h for subsequent extraction and analysis of the agar. For native agar extraction, 100-g whole dry alga was treated with 100-mL of 0.5-M NaOH at 90°C for 90-min with constant stirring. After washing, 1.0-N HCl was used to neutralize excess NaOH. The residue was rinsed thoroughly with running distilled water, and kept in 0.5-% (v/v) acetic acid for 1-h. The pH of the mixture was adjusted to 6.3 ± 0.2 with HCl or NaOH as appropriate, and the resulting extracted pellet was vacuum-filtered (Whatman paper and diatomaceous earth), poured to the tray and frozen for 12-h, then thawed at room temperature, and the resulting liquid was discarded. The gel was washed twice with 70-% ethanol for 15-min and twice with concentrated ethanol for 30-min; finally, it was dried at 60°C for 72-h, weighed, and the agar yield was calculated relative to the initial 100-g sample as the percentage of dry weight. In all cases, agar extraction was carried out with three replicates. Gel strength was determined for 1.5-% (w/w) agar cylinders (2.4-cm height, 4-cm diameter) using a plunger with a contact area of 2.4-cm<sup>2</sup> [19]. The gelling and melting temperatures were measured as described [20- 25].

### Statistical analysis of data

The statistical analyses were carried out using STATISTICA 10 Software (StatSoft, Tulsa, OK). Analysis of variance (ANOVA) was used to test the effects of cultivation depth and initial thallus weight on agar accumulation and the growth rate of *G. oopsis persica*. A one-way ANOVA was used to determine the differences in weight, and branch number for the cultivated plants. The data were tested for normality (Lilliefors tests) and homogeneity of variances using the Leven test, and non-normal data were transformed accordingly. Turkey's honest significance difference (HSD) test was used for comparison of means [16, 17].

## 3. RESULTS

### Biomass and growth rate

The geographical location of *G. oopsis persica* harvesting site is shown in Figure 2. 270-thallus for monoline and 10-thallus for net modules were selected after cutting and weighting based on the morphological observations for cultivation. The mean value of wet weight was measured as 10, 20 and 25 ± 0.01-g at the beginning of the experiments each 30. The longest thallus reach to 700-cm in monoline cultures. During the study period the highest biomass value was recorded in the 30<sup>th</sup> days of cultivation in monoline and at 36<sup>th</sup> days of cultivation in net culture, respectively. Afterwards, these values declined and remained constant until the end of the experiment. The cell mass has been reached to 5387-g Fw m<sup>-1</sup> in monoline and 5368-g Fw m<sup>-1</sup> in net culture system. The profile of *G. oopsis persica* growth in monoline and net culture are shown in Figure 3.

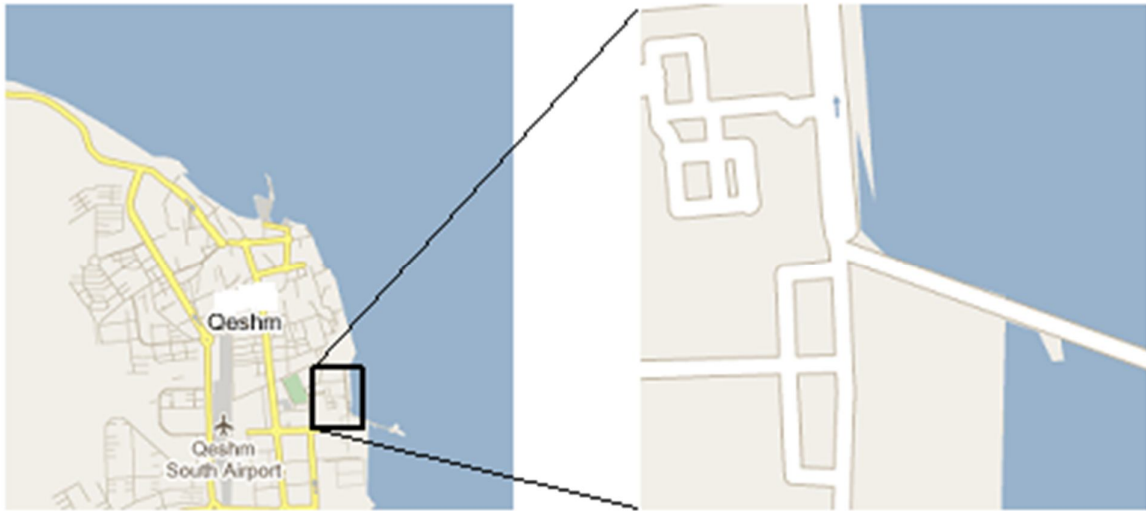
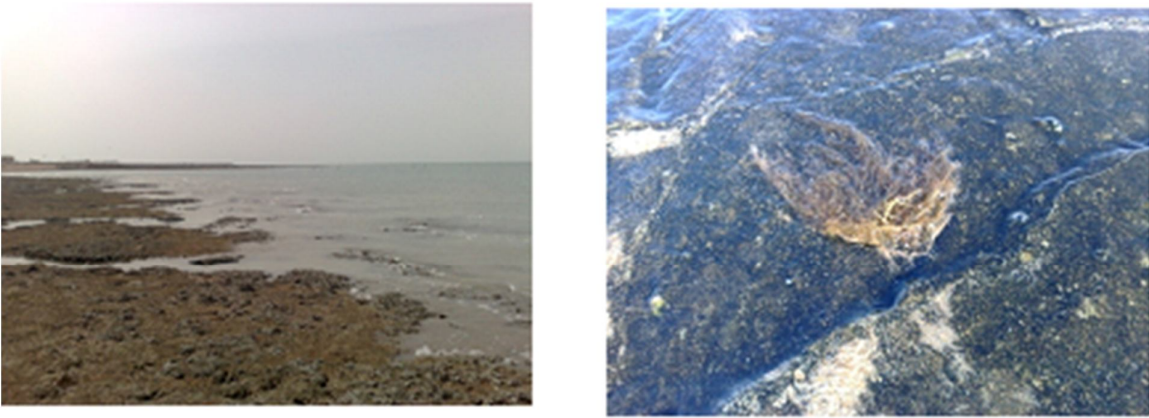


Figure 2. The geographical location of *G.opsis persica* harvesting site

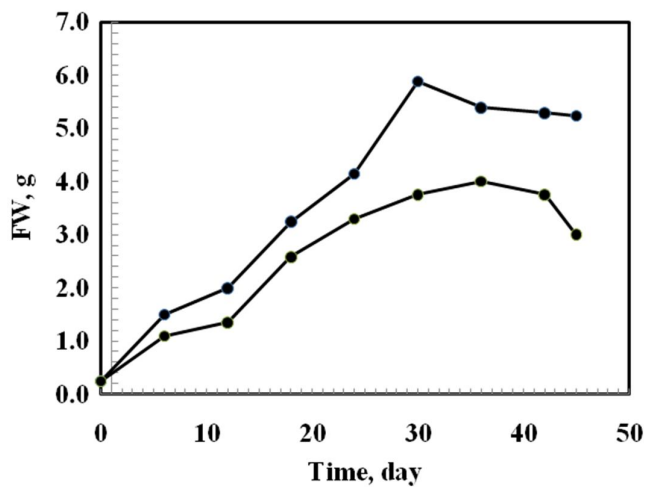
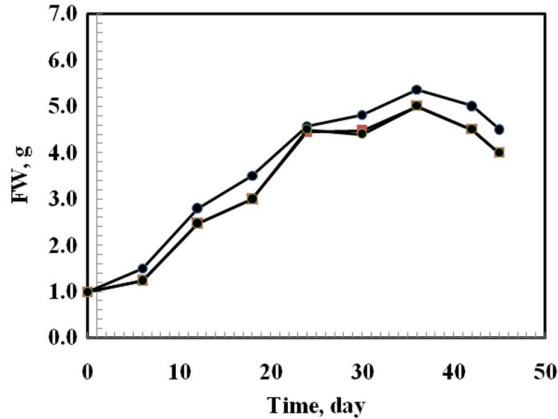


Figure 3 The profile of *G.opsis persica* growth in monoline and net culture

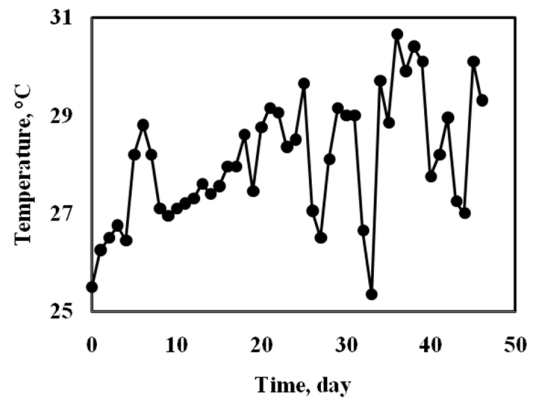
Relative growth rate (RGR) during the whole period was 4.4-%  $d^{-1}$ , ( $4.0 \pm 0.30$ ) in the monoline and 4.3-%  $d^{-1}$ , ( $3.0 \pm 0.25$ ) in the net culture. Positive correlations were found between biomass ( $r = 0.59$ ;  $p < 0.001$ ) and

GR ( $r = 0.61$ ;  $p < 0.001$ ). The growth of alga with various initial weights of 10, 20 and 25-g shows different final biomass as shown in Figure 4.

The correlations were found,  $r = 0.64$ ;  $p < 0.001$ ,  $r = 0.71$ ;  $p < 0.001$ , and  $r = 0.61$ ;  $p < 0.001$  for the 10, 20 and 25-g initial weights, respectively. No differences were recognized among replicates ( $N = 3$ ). The highest cell mass obtained in the depth of 60-cm from the water surface and lowest in the depth of 100-cm from the water surface. No interaction effects were found between the initial thallus weight and depth of cultivation. The bars show the variance of experiments.



**Figure 4.** The growth of alga with various initial weights of 10, 20 and 25-g

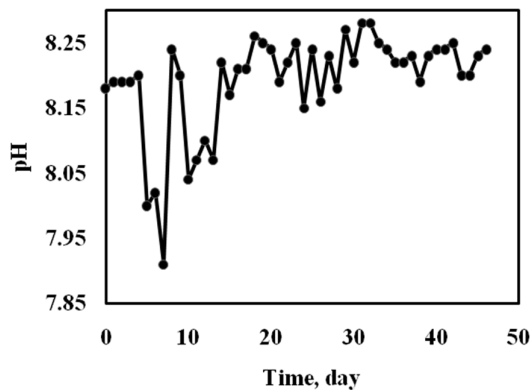


**Figure 5.** The average daily temperature of 0900 and the 1700 hours during study period

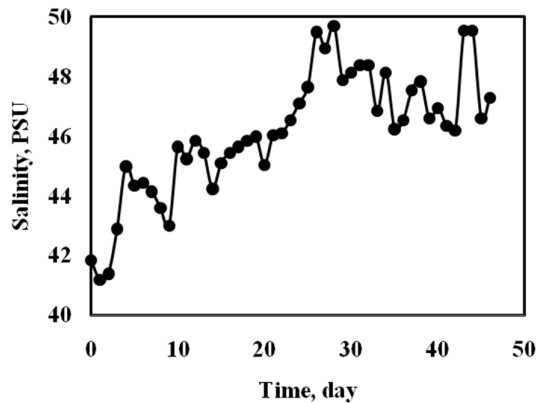
**Effects of environmental parameters**

In the study period, water temperature had 5 degree fluctuations from 25.35-30.75°C (Mean 28.03 ± 1.12). The lowest temperature was in the beginning, while slightly increased during experiments from May through June. Some of the fluctuation in water temperatures happened because of weather change and rain fall (around 7<sup>th</sup> day). No major difference was observed between surface and bottom water temperature. The average daily temperature of 0900 and the 1700 hours during study period are shown in Figure 5.

Water temperature and biomass wet weight had a significant correlations ( $r = 0.76$ ). The pH value was varies in the experiment period with relatively small fluctuations from 7.9 - 8.4 (Mean 8.2 ± 1.12). The average pH variations of 0900 and the 1700 hours during experiments are shown in Figure 6.



**Figure 6.** The average pH variations of 0900 and the 1700 hours during experiments



**Figure 7.** The average variations of 0900 and the 1700 hours in the salinity of culture medium

pH values and biomass fresh wet weight had no significant correlations ( $r = 0.06$ ). The salinity was varied between 41.2 - 49.75 (Mean 46.13 ± 1.12) PSU. Similar to temperature changes, it slightly increased during the experiment period as the weather temperature had almost a same pattern. The average variations of 0900 and the 1700 hours in the salinity of culture medium are shown in Figure 7. Water salinity and biomass fresh wet weight had a significant correlations ( $r = 0.49$ ).

**Agar accumulation**

*G.opsis persica* yielded 40.4-% of native agar with gel strength 850 g cm<sup>-2</sup>. Agar production during study period is shown in Figure 8.

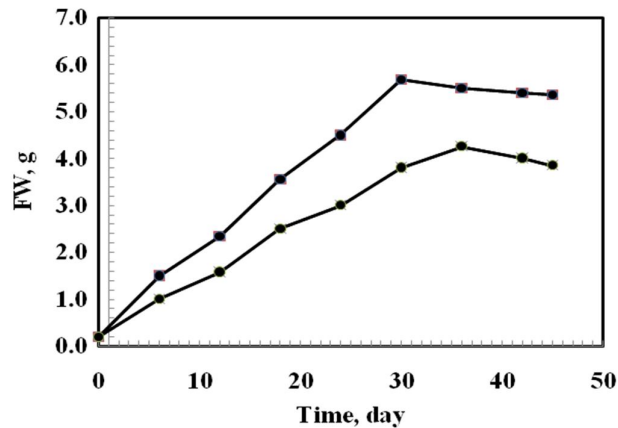


Figure 8. Agar productions during study period

There are significant relations between cell biomass and the quantity and quality of produced agar. Tables 1 and 2 Summarize the descriptive variance of the agar yield and physical properties of agar from *G.opsis persica* extracted in two different conditions: alkali treated and not treated (native).

Table 1. Environmental parameters (range and mean  $\pm$  standard deviation, n = 12) recorded during the study period

Environmental parameters	Range	Mean $\pm$ SD
Water temperature ( $^{\circ}$ C)	25.35 - 30.75	28 $\pm$ 1.15
Salinity (PSU)	41.2 - 49.75	34.36 $\pm$ 1.87
pH	7.9 - 8.4	7.9 $\pm$ 0.4
Nitrate ( $\mu$ mol L $^{-1}$ )	1.212 - 1.503	1.404 $\pm$ 0.120
Nitrite ( $\mu$ mol L $^{-1}$ )	0.000 - 0.658	0.125 $\pm$ 0.242
Ammonium ( $\mu$ mol L $^{-1}$ )	1.862 - 2.556	2.273 $\pm$ 0.243
Phosphate		

Table 2. Agar yield and gel strength (range, mean standard error and ANOVA from *G.opsis persica* during the study period

Items	Range	Mean $\pm$ SD	ANOVA	
			F - ratio	p - value
Agar yield (AY) (%)	17 - 22	19.83 $\pm$ 1.60	7.70	0.002
Gel strengths (GS) (g m-2)	850 - 700	750 $\pm$ 54.47	9.000	0.1001

#### 4. DISCUSSION AND CONCLUSION

The results of the present study revealed that *G.opsis persica* can be cultivated along the southeast coast of Persian Gulf, Iran. January to June is the ideal period for the large scale cultivation of the *G.opsis persica*. The observed maximum growth period for *G.opsis persica* in cultivation coincides with the ideal cultivation period reported elsewhere. Cultivation in surface water is the other criteria for successful cultivation of this alga. As the entire conditions of Qeshm Island are almost similar from climate viewpoint, the all-around of the island would be ideal for cultivation of *G.opsis persica*.

As far as we know, there is no research to analysis the effects of depth on growth and biomass production of *G.opsis persica* in Persian Gulf, but as depth has interrelations with two competitive important parameters of cultivation, temperature and sunlight uptake, that are critical to growth and biomass accumulation, we concluded that there is no optimized fix depth with growth. In the other word, the trends show that in the summer season, deeper cultivation is better compare to winter season and vice versa, lower cultivation depth is more preferable in the colder seasons.

The cultivation performed in the channel after the primary experiments of cultivation system of *G.opsis persica* with monoline and the net systems in open-water failed as the thallus were consumed by turtle and fishes and some other grazers such as epiphytism, lobsters, Phytes, other algae, small lobsters, amphipuda, isopuda as crustacea, nereis and nemertin as worm were found among thallus. The results of net system shows safety of cultures from grazers compare to monoline system. The net system protects the cultures, but the biomass growth is less as of high algal density. The present results of the experiments in open water suggested that herbivory in the cultivation area might be high and the cultivation would be feasible only if protected by the nets. The present experimental design was not planned to study the herbivory, but to approach a feasible way to cultivate *G.opsis persica*.

The increase in depth in cultivation will need higher maintenance and care in which the total production price goes higher. Our results show that is no meaningful deviance between these two parameters and cultivation can be done in less depth of water results less expensive production. Other reports almost used less depth for

cultivation and did not mention about the effect of depth on the growth. Low control of environmental conditions is a problem for cultivation of alga in open waters [26]. The effects of temperature on growth were extensively studied in various research work to determine the optimized seawater temperature and variations for cultivation [18, 27]. The average temperatures of 25-30°C would be the best for cultivation in most cases.

This study examined the feasibility of introducing *G.opsis persica*, as a potentially superior candidate for cultivation in the Persian Gulf coastal area to produce high-quality raw material for the agar industry. Even we used a channel for cultivation, our experiments are belongs to natural cultivation categories as we did not add or remove to sea water to algae. Higher nutrient levels seem to have some effect on growth of seaweed leading to slightly higher growth rate. Stocking density is one of the major factors that control the growth of the two seaweeds; lower density results in higher growth rates but high density gives higher biomass yield. For better growth of the seaweeds, four weeks seem to be the best time to harvest the seaweeds. Results of the carrageenan properties will give the best time to harvest the seaweed for more quantity and quality of the carrageenan. The agar yield and gel strength of *Gracilaria* species were determined in relation to environmental factors by several research groups [28]. The agar accumulation follows the same trends with growth. The agar yield and gel strength extracted from *G.opsis persica* in the present study is almost similar to the earlier works in *Gracilaria* species determined Pickering [15].

Due to unpredictable weather conditions during the past year, a continuation of this study for another year or a similar study are being recommended so as to have more data to give more concrete conclusions. From the operation view, it is easier and less expensive to operate at the upper level of sea water, for compete the price of produced agar. In addition, as macroalgae, absorb sun light for photosynthesis and growth and formation of the materials, the depth of cultivation is a parameter of interest for optimization of cultivation. Further studies are necessary to investigate the cell growth, agar productivity and gel strength during various seasons in the Qeshm Island. There is a great market for agar obtained from *G.opsis persica* in the country and the region. Cultivation of the *G.opsis persica* will help fetch additional income besides supplying continuous source of raw material to the seaweed based industries but future studies should evaluate the cost-effectiveness of a commercial *G.opsis persica* production in various cultivation modes. Cultivation technique still need to be developed and productivity needs to be improve, in order to outweigh costs for nursery areas, labor, etc., before the suspended seaweed culture will become a practiced cultivation technique in integrated open seawater systems [15].

#### Acknowledgments

The authors acknowledge the support of the National Institute of Genetic Engineering and Biotechnology (NIGEB) and express their sincere thanks to three reviewers for their very valuable comments on the manuscript.

#### REFERENCES

1. Mazaheri, M. 2000. The role of adult attachment in marriage functioning. Journal of Educational Psychology, Shahid Chamran University, Year IV, Number III.
2. Masakazu Murata, J.-i. N. 2001. Production and use of marine algae in Japan. JARQ 35(4): 281-290.
3. Liebezeit, G. (2005). "Aquaculture of "non - food organisms" for natural substances production." Adv Biochem Engin/Biotechnol 97: 1-28.
4. Yu-Feng Yang, X.-G. F., Jin-Ming Song, Hai-Yan Hu, Guang-Ce Wang, Ik Kyo Chung 2006. Growth of *Gracilaria lemaneiformis* under different cultivation conditions and its effects on nutrient removal in Chinese coastal waters. Aquaculture 254: 248-255.
5. Sasidharan Sreenivasan, d. I., Mohd Jain Noordin Mohd Kassim 2007. Free radical scavenging activity and total phenolic compounds of *Gracilaria changii*." International Journal of Natural and Engineering Sciences 1(3): 115-117.
6. Jose Pedrassoli Salles, F. S., Cristalina Yoshie Yoshimura, Mauricio Fanganiello, Zenilda Laurita Bounzon, Paulo Antunes Horta 2010. Cultivation of native seaweed *Gracilaria domingensis* (Rhodophyta) in Southern Brazil." Brazilian Archives of Biology and Technology 53(3): 633-640.
7. Ask, E. I. and R. V. Azanza 2002. Advances in cultivation technology of commercial eucheumatoid species: a review with suggestions for future research." Aquaculture 206(3-4): 257-277.
8. Harris J. Bixler, H. P. 2010. A decade of change in the seaweed hydrocolloids industry." Journal of applied Phycology.
9. Ask, E. I., Ledua E., Batibasaga A., Mario S. 2003. Developing the Cottonii (*Kappaphycus alvarezii*) cultivation industry in the Fuji Islands." *Kappaphycus Cultivation in Fuji Islands*: 81-85.
10. Vivek Kumar, R.F. 2009. Agar extraction process for *Gracilaria cliftonii*." Carbohydrate Polymers 78: 813-819.

11. Craigie, J. S. and A. Jurgens 1989. Structure of agars from *Gracilaria tikvahiae* rhodophyta: Location of 4-O-methyl-l-galactose and sulphate. *Carbohydrate Polymers* 11(4): 265-278.
12. Santelices, B. and M.S. Doty 1989. A review of *Gracilaria* farming." *Aquaculture* 78(2): 95-133.
13. Santos, G.A. and M.S. Doty 1983. Agar from some Hawaiian red algae." *Aquatic Botany* 16(4): 385-389
14. Tseng, C. 2001. Algal biotechnology industries and research activities in China." *Journal of applied Phycology* 13: 375-380.
15. C. Halling, G.A., M. Cifuentes, A.H. Buschmann, M. Troell 2005. Comparison of spore inoculated and vegetative propagated cultivation methods of *Gracilaria chilensis* in an integrated seaweed and fish cage culture." *Aquaculture International* 13: 409-422.
16. Eun Kyoung Hwang, C.S.P., Jae Min Baek 2006. Artificial seed production and cultivation of the edible brown alga, *Sargassum fulvellum* (Turner) C. Agardh: Development a new species for seaweed cultivation in Korea." *Journal of Applied Phycology* 18: 251-257.
17. Pickering, T. 2006. Advances in seaweed aquaculture among Pacific Island countries." *Journal of Applied Phycology* 18: 227-234.
18. Thomas J. Lyimo, E. F. M., Charles Lugomela, Mats 2006. seagrass biomass and productivity in seaweed and non-seaweed farming areas in the east coast of Zanzibar, tanzania. *western Indian Ocean Journal Marine science* 5(2): 141-152.
19. M. Ganesan, N. s., K. Eswaran 2011. Raft culture of *Gracilaria edulis* in open sed along the south-eastern coast of India. *Aquaculture*.
20. Satoshi Suzuki, K. F., Tadashi Kawai, Ichiro Takeuchi 2007. Growth and annual production of the brown alga *Laminaria religiosa* introduced into the Uwa sea in southern Japan." *Phycological Research* 55: 272-277.
21. Anna V. Skriptsova, Y.V.N. 2009. Comparison of three gracilarioids: growth rate, agar content and quality. *Journal of Applied Phycology* 21: 443-450.
22. Lahaye, M. and C. Rochas 1991. Chemical structure and physico-chemical properties of agar. *Hydrobiologia* 221(1): 137-148.
23. Oyieke, H.A. 1994. The effect of phenotypic plasticity on agar from *Gracilaria salicornia* (J.A.G.) Dawson (*Gracilariales*, rhodophyta) in Kenya. *Bioresource Technology* 49(3): 267-271.
24. Marinho-Soriano, E. and Bourret E. 2003. Effects of season on the yield and quality of agar from *Gracilaria* species (*Gracilariaceae*, Rhodophyta). *Bioresource Technology* 90(3): 329-333.
25. Hilliou, L., F.D.S. Larotonda, et al. 2006. Effect of extraction parameters on the chemical structure and gel properties of [kappa]/[iota]-hybrid carrageenans obtained from *Mastocarpus stellatus*. *Biomolecular Engineering* 23(4): 201-208.
26. Pereira-Pacheco, F., D. Robledo, et al. 2007. Optimization of native agar extraction from *Hydropuntia cornea* from Yucatán, México. *Bioresource Technology* 98(6): 1278-1284.
27. Romero, J. B., R. D. Villanueva, et al. 2008. Stability of agar in the seaweed *Gracilaria eucheumatoides* (*Gracilariales*, Rhodophyta) during postharvest storage. *Bioresource Technology* 99(17): 8151-8155.
28. Eggert A., West J.A., Michalik D., Karaten U. 2007. Acclimation of the intertidal red alga *Bangiopsis subsimplex* (*Stylonematophyceae*) to salinity changes. *Journal of Experimental Marine Biology and Ecology* 343: 176-186.
29. Timothy D. Pickering, M.E., Lennard G., Tong J. 1995. A preliminary trial of a spray culture technique for growing the agarophyte *Gracilaria chilensis* (*Gracilariales*, Rhodophyta). *Aquaculture* 130: 43-49.
30. Manivannan K., G> Karthikai Devi G. T., hemalatha A., Anantharaman P. 2008. Biochemical composition of seaweeds from mandapam coastal region along southeast coast of India. *American-Eurasian Journal of Botany* 1(2): 32-37.