Journal of Plant Process and Function, Vol. 10, No. 46, Year 2022 **Page: 61-70**

Research Article

Growth of red alga *Gracilariopsis persica* (Rhodophyceae) in desalination pools of Qeshm Island

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(Received: 26/07/2021-Accepted: 30/11/2021)

Abstract

Gracilariopsis persica **is one of the most abundant red algae distributed the in Persian Gulf, and despite increasing use in medicine as a major source for natural biomaterials and bioactive compounds in recent years, the researches on growth parameters of this alga remain limited. The main aim of this research was to study the favorable conditions for *G. persica* growth. Also, it was to find the suitable primary storage density for the establishment of algal thallus in the appropriate depth which causes increase in biomass in the concrete pool of Qeshm Island. Tall of *G. persica* were grown for 45 days on polyethylene ropes with primary storage weight of 100 and 200 gm^{-1} in two depths of 1 and 2 m. The effect of environmental factors such as temperature, salinity, pH, and nutrient absorption were investigated for different treatments. Growth rate during the cultivation period increased for both densities of 100 g and 200 g of *G. persica* at first but decreased along with the time till sixth week. The highest growth rate (percentage of increase in fresh weight d^{-1}) was obtained for the density of 100 g in 1 m depth in 45 days and the lowest growth rate was observed for the density of 200 g in 2 m depth. Relative growth rate (RGR) was measured weekly based on the changes in fresh weight and showed the highest efficiency in 1 m depth and 100 g density (15.47% d^{-1}). The results showed that nutrients, especially those containing nitrogen and affecting algae growth at low depth, were inversely related (Kendall and Spearman negative correlation coefficients) to growth rate at 1 m depth. At depth 2 m, alga growth showed negative correlation with NO_3^- and NO_2^-. Findings provided novel evidence concerning growth and culturing conditions of *G. persica*, which can aid in the development of cultivating protocols for commercial agar production by this alga.**

Keywords: Biomass, Algal density, Depth of water, Nutrient, Salinity, Temperature of water

Introduction

Seaweed (or macroalgae) refers to several species of macroscopic, multicellular, and marine algae (Hurd *et al*., 2014). The ecosystem of seaweed is a dynamic and active setting, such that its physical and chemical variables are dynamic, so that the responses of the algae to these changes are not always obvious. Sustainable management of seaweed aquaculture requires understanding of the underlying biological mechanisms controlling macroalgal life cycles using diverse approaches requiring a broad range of technological tools (Charrier *et al*., 2017). In addition to light and water, generally seaweeds need a combination of nitrogen (N), inorganic carbon (C), phosphate (P), iron, cobalt, manganese, and other marine elements to grow. N, C and P are among the major known substances for the growth of the algae (Lobban and Harrison, 2000). Nutrients limiting growth of algae in the sea are nitrogen and phosphorus, both of which are present in low concentrations in natural ocean waters (Cordover, 2007).

Seaweed is usually harvested from natural beds but excess harvesting can disturb the balance of natural populations. A sustainable alternative capable of avoiding exhaustion of natural populations is to cultivate species capable of producing seaweed in a large scale (Buschmann *et al*., 2008). However, the rapid development of aquaculture resulted in excessive increase of nutrients, especially nitrogen and phosphorus in marine ecosystems (Beveridge, 1996), generally results from fertilizing pools and ponds and various metabolic residuals. In this sense, minimizing the negative effects of these activities is a main challenge for developing non-polluting strategies. Seaweeds are biologically extractable beings which use the excessive nutrients produced by fish and shrimps in their growth. Integrated cultivation of fed aquaculture (fish and shrimp) with extractable aquaculture (seaweeds and shell) is called integrated multi-trophy aquaculture (IMTA). The general concept of IMTA is a model based on an environment in which pairs of biologically extractable inorganic beings (seaweeds) or biologically extractable organic beings (shell) are used to balance the densely cultivated fed beings (fish and

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shrimp) in order to obtain a sustainable, clean and diverse aquaculture system (Neori *et al*., 2007).

Fishes produce N, P, and carbon dioxide (CO_2) through metabolic processes (Neori *et al*., 2007). In coastal waters, high levels of these nutrients may increase harmful algae growth which in turn may have significant negative consequences on coastal ecosystem as well as economy. In contrast, these nutrients could be used for supporting economic growth of seaweeds (Yarish and Pereira, 2008). The success of seaweed cultivation in shrimping pools significantly depends on nutrient concentration, cultivation type, and water flow (Buschmann *et al*., 2001). Development based on new environment guarantees sustainable cultivation technology, employment of coastal communities in the future, balance of coastal ecosystems and protection of important wild populations (Yarish and Pereira 2008).

Gracilariopsis persica has universal distribution as it can be found in polar, moderate, and tropical regions, but most of its species have been reported in tropical waters (Sahoo and Yarish, 2005; Belloni *et al*., 2008). In studies conducted in southern coastal regions of Iran, *G. persica* was also identified in the Persian Gulf (Sohrabipour and Rabiei, 1999). Thallus matures of *Gracilariopsis persica* are 30–60 cm and with cartilaginous, leathery or stringy elastic in texture, purple, red-greenish pale to yellowish; erect axes are terete throughout and 1–2 mm in diam, arising from small disk-like holdfast. The thalli are freely and loosely branched to 1-4 orders; branchlets not abruptly constricted at bases, tapering gradually toward apices. A range of protocols are available to cultivate seaweeds, thanks to previous physiological studies carried out in an applied phycological context. However, more focused and on-demand approaches are always required (Charrier *et al*., 2017). Although *G. persica* is one of the most abundant red algae distributed in the Persian Gulf, there has been only limited literature around its growth under various conditions. Due to the importance of genus *Gracilaria* in agar production, the aim of this study was to test growth and biomass production of this alga as well as the effects of depth and algal density on the growth of this alga. The capacity of *G. persica* to remove nutrients from seawater and the effect of nutrients on the growth of this species were also investigated.

Materials and methods

Sampling of alga *G. persica*: To cultivate the alga, *G. persica* transplants were obtained on April 17th, 2013 in full tide from their natural habitat in Sorou, Bandar Abbas (56˚ 17’ 16” E and 27˚16’ 48” N). Three transects were chosen in a parallel manner to the coast for ring growth of *G. persica*. Sampling was conducted by randomly throwing a 50 × 50 cm quadrant at five replications. Alga thalluses present in each quadrant were separated from the sand bed connection region using a spatula. The samples were placed between successive layers of wet hempen (hempen dampened with seawater) and were transferred to the laboratory in Styrofoam boxes (Liu, 1987). Then, Alga thalluses habitats were placed in an aquarium for 24 h. The aquarium was filled with seawater with 40 ppt salinity at temperature 25-27 ºC with aeration. Alga thalluses were washed with the same aquarium water to eliminate the mud and undesirable epiphytes.

Cultivation was performed in concrete pool of desalination canal of Qeshm Island (56˚ 16’ 45.12” E and 26˚ 56’ 12.23” N). The concrete canals had 45 m^2 surface area with 2.5 m depth in which water flow was active in 6 hours each day. Cultivation and growth period was 45 days from April to June in 2013. In this cultivation, the moonlight method using a series of ropes with 4 inches thickness as alga cultivation bed, was used. Alga thalluses were spun off on 2 m long ropes 20 cm from each other in two 10 and 20 g groups with 15 cm length and were cultivated (Friedlander and Levy, 1995). Totally, two densities of 100 and 200 g alga transplants were stored on the ropes. Ropes were located horizontally in two depths of 1 and 2 m from free water level and tied to the concrete supports on the two sides of the canal. Three replications were employed for the two variables of water depth and algal density (Fig. 1).

Growth Estimation: The relative growth rate (RGR) was calculated by the following equation (Dawes, 1998):

$RGR = [(W_f - W_i)/t] \times 100$

W_i = Initial fresh weight (g); W_f = final fresh weight (g); t = cultivation time (days)

Evaluation of environmental factors changes during the growth: Physical and chemical factors such as pH, temperature and salinity were measured twice daily, 8 AM and 4 PM, in both depths. Temperature and pH were measured with a digital portable pH meter model JENWAY 3150 (England) and saltiness was measured using a digital portable salinity meter for sea water (model Send Direct, Germany) (Laing *et al*., 1989).

Measurement of nutrients present during the growth: Measuring nutrients dissolved in seawater was performed every seven days in both depths using a spectrophotometer (model CECIL-CE3091, UK). Ruthner sampling bottles were used to collect seawater samples. The bottles were 1.8 L in volume and were equipped with a mercury thermometer. Then, The samples were then transferred to the laboratory in polyethylene vessels in 4 ºC in the absence of light. The samples were rapidly filtered and frozen to prevent the biological and chemical reactions. Therefore, the samples were filtered using a vacuum pump and a filter with 4.7 cm in diameter and 0.45 µm pore diameter and then were frozen in a freezer at -20 ºC. Nitrogen, nitrate, nitrite as well as phosphate were measured spectrophotometrically using water and wastewater standard methods (APHA, 1995).

Data analysis: Statistical analysis was conducted using the Statistical Package for the Social Sciences

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Fig. 1 Cultivation of *G. persica* with 100 g density in the concrete canals (1 m water depth). A) In first day; B) after the first week; C) after week 4.

(SPSS). A two-way analysis of variance (ANOVA) was conducted. Kendal and Spearman correlation tests were used to examine the relationship between relative growth rate (RGR) and environmental variables. To investigate the effect of environmental variables on algal growth in different weeks, one-way ANOVA was used and Student-Newman-Keuls test was used for classification. Also, to investigate the differences of RGR in different weeks, the Kruskal-Wallis test was used. In all graphs, the results were expressed in average values of three replications ± standard deviation (SD). The significance level for all test was $P< 0.05$.

Results

Once *G. persica* seaweed was stored in desalination canals, the first event observed was the discoloration of the tip point in some thallus in 2-3 days; after a week from the beginning of the alga growth, their color returned to normal.

Investigation of the effects of algal density and water depth on the growth of alga *G. persica*: The growth rate during the cultivation period was higher at a water depth 1 m than at a water depth 2 m for both algal densities 100 g and 200 g of *G. persica* (Fig. 2). The highest growth rate (% of increase in fresh weight d^{-1}) was observed for the algal density of 100 g in 1 m water depth in 45 days and the lowest growth rate was reported for the algal density of 200 g in 2 m water depth (Fig. 2). On the other hand, Algal density did not have significant effect on the growth ($P< 0.05$). The mean relative growth rates were similar for both algal densities; therefore primary algal density did not affect growth ($P< 0.05$). Reduced growth rates were observed by increasing the cultivation depths for *G. persica* in 45 days of cultivation period. The highest growth rate was recorded in 1 m water depth for 100 g algal density in 45 days and the lowest growth rate was recorded in 2 m water depth in 45 days. According to Fig. 2 the mean relative growth rates in 1 m and 2 m water depths were not similar therefore it could be concluded that the depth factor affected the growth ($P< 0.05$). Water depth was an effective factor on biomass and relative growth rate during the study period and there was a meaningful relation between depth and weight.

Evaluation of mean relative growth rate in different weeks in alga *G. persica*: Investigation of mean relative growth rate in algal density 100 g and water depths 1 and 2 m showed that the amount of RGR during the cultivation period was increased (Fig. 3). RGR in different weeks in both 1 and 2 m water depths showed significant differences ($P< 0.05$). In both investigated water depths the highest growth was obtained in week 3 whereas the lowest growth was reported in week 6, and according to Fig. 3 water depth 1 m with algal density 100 g had higher growth compared to that obtained in water depth 2 m and algal density 100 g, especially in weeks 1, 3, and 4.

Evaluation of mean relative growth rate in density 200 g in depths 1 and 2 m: The relative growth in water depths 1 and 2 m with algal density 200 g in

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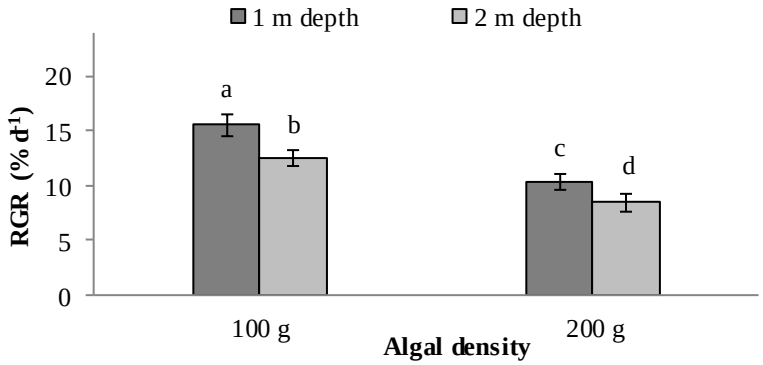


Fig. 2. Relative growth rate (% d^{-1}) of *G. persica* grown with two algal densities in two water depths during 45 days. Different letters within each density show significant differences between treatments according to the Duncan's test at P<0.05.

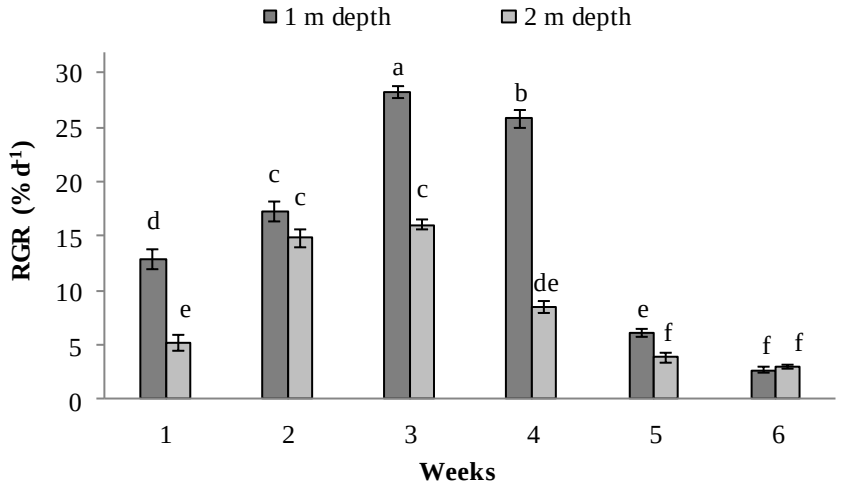


Fig. 3. RGR difference for *G. persica* grown during 6 weeks in two water depths with algal density 100 g. Different letters show significant differences between treatments according to the Duncan's test at P<0.05.

different weeks is shown in Fig. 4. RGR had significant differences in different weeks (P< 0.05) at both water depths 1 and 2 m. In both water depths the highest and lowest growths were reported in weeks 3 and 6 respectively and according to the obtained results depth 1 m with density 100 g had higher growth compared to the water depth 2 m and algal density 200 g, especially in weeks 3 and 4.

Measurement of temperature during the growth period: Water temperature variations were similar in depths 1 and 2 m, regardless time and day (Table 1). Temperature increased with growth time in the morning, while this was not consistent in the afternoon (e.g. week 3). Water temperature ranged from 25.27 to 28.25 ^{o}C both in the morning and in the afternoon (4 PM) (Table 1). Temperature variations in depths 1 and 2 m in the morning and in the afternoon showed significant differences in the most of the weeks (P< 0.05).

Evaluation of water salinity during the growth period: Variations of water salinity in water depths 1 and 2 m were similar and the water salinity variation ranges were between 50.94-51.67 ppt and 51.20-51.62 ppt in the mornings and in the afternoons respectively. According to the results shown in Table 2 water salinity variations in the mornings and in the afternoons in water depths 1 and 2 m did not show any significant differences in most of the weeks (P< 0.05).

Evaluation of water pH during the experiment period: Variations of water pH were similar in both water depths and water pH variation ranges were between 7.98-8.25 and 7.92-8.32 in the mornings and in the afternoons respectively. According to the results shown in Table 3 water pH variations in the mornings and in the afternoons in water depths 1 and 2 m did not showed significant differences in most of the weeks (P< 0.05).

Measurement of nitrate concentration during the experiment period: The nitrate concentration in water depth 1 m was decreased by increasing the biomass during the cultivation period (28 days) and in the final 2 weeks of the cultivation it was increased due to the decreased biomass. Therefore, according to Fig. 5 nitrate concentration in water depth 1 m was significantly different in the final 2 weeks compared to the first 28 days of the cultivation (P< 0.05). Also, nitrate concentration in water depth 2 m in the same period (28 days) was decreased by increasing the biomass but did not show significant difference (P<

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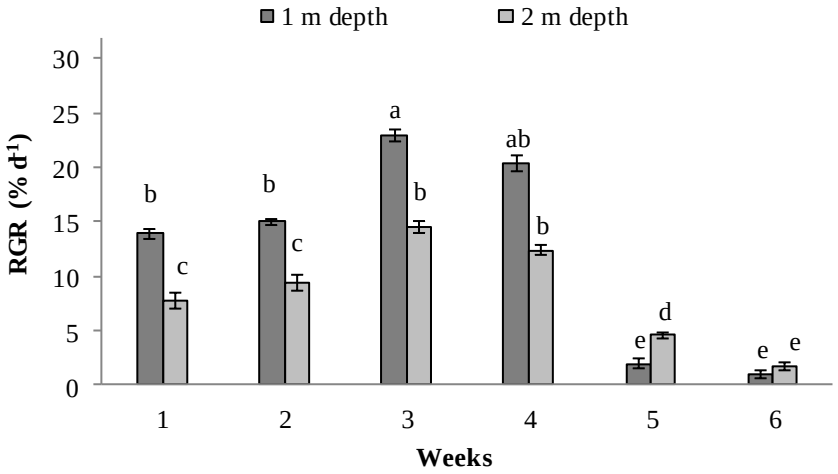


Fig. 4. RGR difference for *G. persica* in water depths 1 and 2 m with algal density 200 g. Different letters show significant differences between treatments according to the Duncan's test at P<0.05.

Table 1. Water temperature during the experiment period (mean ± standard deviation, n = 42)

Week	Morning (8 AM)		Afternoon (4 PM)	
	1 m water depth	2 m water depth	1 m water depth	2 m water depth
1	25.27 ± 0.399 ^{d}	26.07 ± 0.399 ^{d}	26.55 ± 0.62 ^{c}	26.95 ± 0.62 ^{d}
2	25.54 ± 0.289 ^{d}	26.14 ± 0.289 ^{d}	26.20 ± 0.29 ^{c}	27.00 ± 0.29 ^{c}
3	26.10 ± 0.71 ^{c}	26.80 ± 0.71 ^{c}	27.05 ± 0.15 ^{b}	27.95 ± 0.15 ^{b}
4	26.40 ± 0.18 ^{c}	26.98 ± 0.18 ^{c}	26.40 ± 0.25 ^{c}	27.08 ± 0.25 ^{c}
5	27.42 ± 0.52 ^{b}	28.02 ± 0.52 ^{b}	27.40 ± 0.46 ^{b}	28.03 ± 0.46 ^{b}
6	28.25 ± 0.69 ^{a}	28.95 ± 0.69 ^{a}	28.34 ± 0.07 ^{a}	29.08 ± 0.07 ^{a}

Different letters show significant differences between the weeks according to the Duncan's test at P<0.05.

Table 2. Water salinity during the experiment period (mean ± standard deviation, n = 42)

Week	Morning (8 AM)		Afternoon (4 PM)	
	1 m water depth	2 m water depth	1 m water depth	2 m water depth
1	50.94 ± 0.43 ^{a}	51.14 ± 0.43 ^{a}	51.21 ± 0.14 ^{a}	51.31 ± 0.14 ^{a}
2	51.21 ± 0.13 ^{a}	51.51 ± 0.13 ^{a}	51.35 ± 0.20 ^{a}	51.45 ± 0.20 ^{a}
3	51.32 ± 0.26 ^{a}	51.82 ± 0.26 ^{a}	51.15 ± 0.20 ^{a}	51.25 ± 0.20 ^{a}
4	51.22 ± 0.19 ^{a}	51.72 ± 0.19 ^{a}	51.21 ± 0.13 ^{a}	51.31 ± 0.13 ^{a}
5	51.32 ± 0.16 ^{a}	51.82 ± 0.16 ^{a}	51.20 ± 0.19 ^{a}	51.40 ± 0.19 ^{a}
6	51.37 ± 0. 22 ^{a}	51.77 ± 0. 22 ^{a}	51.61 ± 0.28 ^{a}	51.71 ± 0.28 ^{a}

Different letters show significant differences between the weeks according to the Duncan's test at P<0.05

Table 3. Values of water pH in experiment period (mean ± standard deviation, n=42)

Week	Morning (8 PM)		Afternoon (4 PM)	
	1 m water depth	2 m water depth	1 m water depth	2 m water depth
1	8.07 ± 0.08 ^{b}	8.17 ± 0.06 ^{b}	8.07 ± 0.07 ^{b}	8.18 ± 0.09 ^{b}
2	8.15 ± 0.09 ^{ab}	8.13 ± 0.04 ^{ab}	8.16 ± 0.03 ^{ab}	8.06 ± 0.05 ^{ab}
3	8.11 ± 0.04 ^{ab}	8.12 ± 0.02 ^{ab}	8.16 ± 0.04 ^{ab}	8.17 ± 0.04 ^{ab}
4	8.18 ± 0.04 ^{ab}	8.19 ± 0.03 ^{ab}	8.18 ± 0.05 ^{ab}	8.19 ± 0.06 ^{ab}
5	8.11 ± 0.03 ^{ab}	8.11 ± 0.02 ^{ab}	8.16 ± 0.02 ^{ab}	8.17 ± 0.02 ^{ab}
6	8.22 ± 0.03 ^{a}	8.32 ± 0.02 ^{a}	8.28 ± 0.03 ^{a}	8.32 ± 0.04 ^{a}

Different letters show significant differences between the weeks according to the Duncan's test at P<0.05

0.05) and in the final 2 weeks nitrate concentration was increased which was significant compared to the previous weeks (P< 0.05). Also, in the investigation of nitrate concentration in both water depths there were no significant differences except for fifth week 5.

Evaluation of nitrite concentration during the experiment period: The nitrite concentration in water depth 1 m was decreased by increasing the biomass during the cultivation period (28 days) and the highest decrease was observed in week 3; in the final 2 weeks of the cultivation nitrite concentration was increased due to decreased biomass. Therefore, according to Fig. 6 nitrite concentration in water depth 1 m was significantly different in most of the weeks during the cultivation period (P< 0.05). Also, nitrite concentration in water depth 2 m in the same period (28 days) was decreased by increasing the biomass whereas this decrease was not statistically significant (P< 0.05) and

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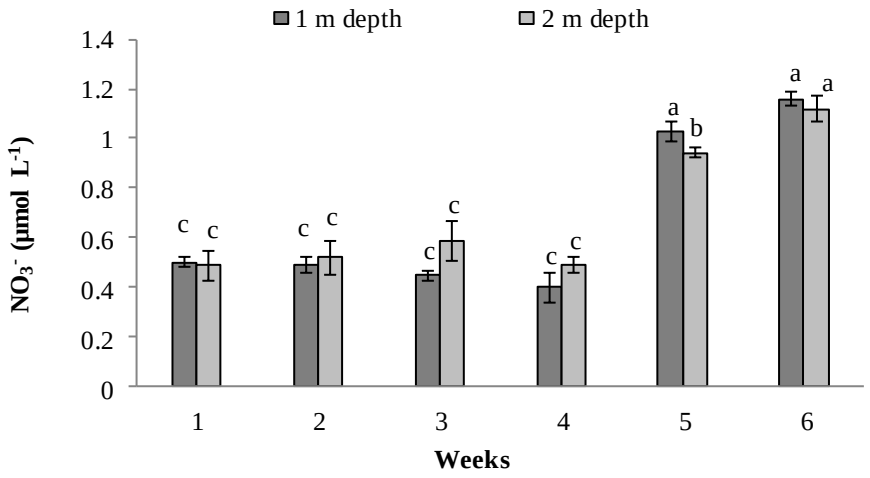


Fig. 5. Nitrate concentration variation in water depths 1 and 2 m during the experiment period (6 weeks). Different letters show significant differences between treatments according to the Duncan's test at P<0.05.

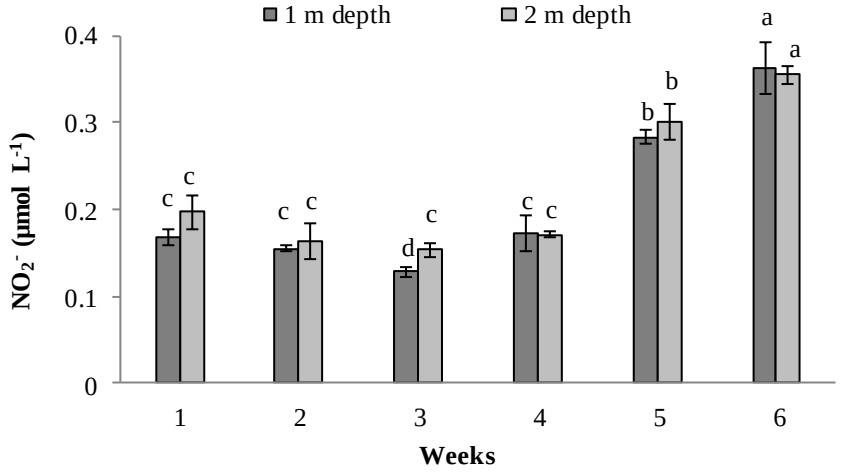


Fig. 6. Nitrite concentration variation in depths 1 and 2 m during the experiment period (6 weeks). Different letters show significant differences between treatments according to the Duncan's test at P<0.05.

in the final 2 weeks nitrite concentration was increased which was significant compared to the previous weeks (P<0.05). Also, in the measurement of nitrite concentration in both water depths there were no significant differences in most of the weeks.

Evaluation of phosphate concentration during the experiment period: The phosphate concentration in water depth 1 m was increased and then decreased by increasing the biomass during the cultivation period (42 days). Therefore, according to Fig. 7 phosphate concentration in water depth 1 m was significantly different in most of the weeks during the cultivation period (P< 0.05). Also, phosphate concentration in water depth 2 m in the same period (42 days) did not show any variations in weeks 2, 3 and 4, therefore there were no significant differences in these weeks (P< 0.05). Also, in the evaluation of phosphate concentration in both water depths only in week 5 there was significant difference and in other weeks there were no significant differences observed.

Evaluation of ammonia concentration during the experiment period: The ammonia concentration in depth 1 m was decreased by biomass during the cultivation period (28 days) and in the final 2 weeks of the cultivation ammonia concentration was increased by decreased biomass. Therefore, according to Fig. 8 ammonia concentration in water depth 1 m was significantly different in most of the weeks during the cultivation period (P<0.05). Also, ammonia concentration in water depth 2 m in the cultivation period (42 days) showed significant differences in most weeks (P< 0.05). Meanwhile, in the evaluation of ammonia concentration in both depths only in weeks 3, 4, 5 and 6 there was significant difference and in other weeks there were no significant differences.

Relationship between algal growth rate and environmental factors in 2 different water depths: Kendal and Spearman invariant test showed that nutrients had meaningful relationship with growth rate in depth 1 m. According to Table 4 there was an inverse

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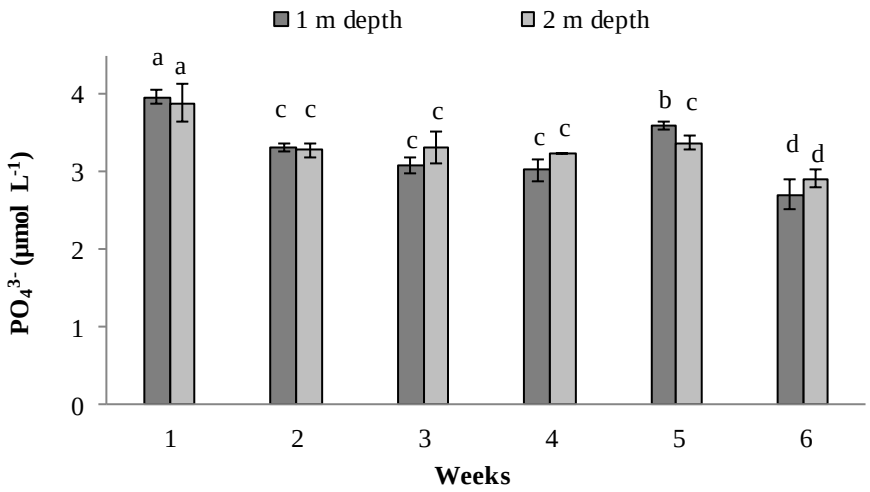


Fig. 7. Phosphate concentration variation in water depths 1 and 2 m during the experiment period (6 weeks). Different letters show significant differences between treatments according to the Duncan's test at P<0.05.

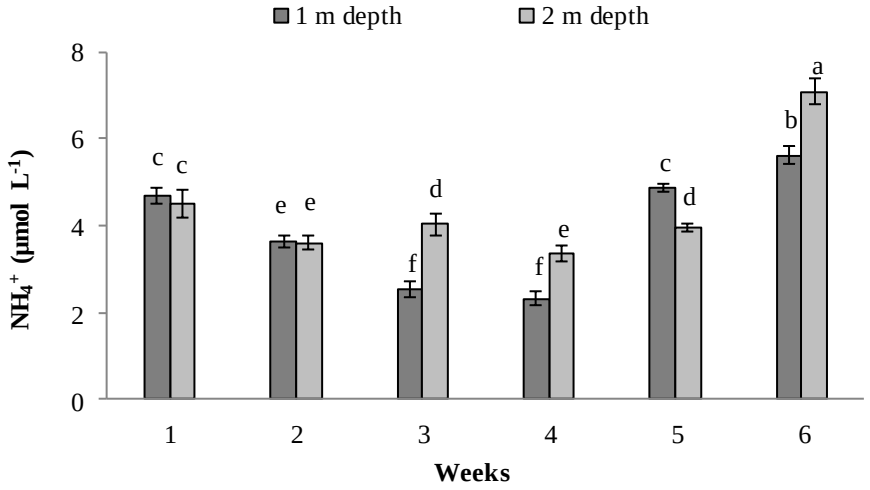


Fig. 8. Ammonia concentration variation in water depths 1 and 2 m during the experiment period (6 weeks). Each column represents the mean value for 3 replications and vertical bars show standard deviation. Different letters show significant differences between treatments according to the Duncan's test at P<0.05.

Table 4. Relationship among RGRs of *G. persica* during the growth period at the conditions including different variables.

Correlation	Variable						
	pH	Salinity	Temp	NO_3^-	NO_2^-	PO_4^-	NH_4^+
1 m depth							
Kendall's tau b	0.467	0.078	-0.276	-0.867*	-0.733*	-0.733*	-0.867*
Spearman's rho	0.203	-0.600	-0.551	0.943**	-0.829*	-0.829*	-0.943**
2 m depth							
Kendall's tau b	0.467	0.078	-0.276	-0.569**	-0.612**	-0.033	-0.315
Spearman's rho	0.203	-0.600	-0.551	0.701**	-0.790**	-0.127	-0.434

**** Significant at P<0.01; * significant at P<0.05**

relationship between these variables and the growth rate in depth 1 m. In other words, alga growth was increased by the decreased in the amounts of nutrients (P<0.05). Therefore, it can be concluded that this species had the capacity to eliminate nutrients in depth 1 m. But, according to Kendal and Spearman invariant test (Table 4), there was no meaningful relationship (P<0.05) among the environmental parameters (pH, saltiness and temperature) in depth 1 m.

According to Table 4 Kendal and Spearman invariant test in 2 m showed that nutrients nitrate and nitrite had meaningful relationship with growth (P< 0.05) whereas phosphate and ammonia did not show meaningful relationship with growth (P<0.05). Also,

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according to the invariant test there was no meaningful relationship ($P<0.05$) among the environmental parameters (pH, salinity and temperature) and growth rate in depth 2 m.

Discussion

This study investigated RGR, growth, and the effects of water depth and algal density (base on the algal fresh weight) on the growth of *G. persica* alga. We also investigated the capacity of *G. persica* in removing nutrients such as phosphate, nitrogen, nitrite and ammonium from seawater and the effect of nutrients on its growth. In other places, displacement and transfer of natural species for growth purposes has always been accompanied by the environmental stresses or other pathogens (Anderson, 2005). These stresses which have been reported as whitening or necrosis of thallus populations in natural and grown *Gracilaria*, are mostly observed during the early cultivation and are then disappeared in a few days when the algae were adapted to their new environment. It is possible that these variations were due to the physiological changes in the oxidative activity resulted from H_2O_2 production which was performed by the thallus themselves or the presence of pathogenic bacteria in the environment (Tenorio-Rodriguez *et al*., 2018). Also, during the cultivation, the stored algae use the nutrients present in seawater and the content of nitrogen and phosphorus can affect the phycocolloids as well as the amount of phycoerythrin and chlorophyll pigments (Oliveira *et al*., 2000). In the present study, discoloration observed in the tip point of some thallus returned to normal color after a week.

RGR in alga *G. Persica*: The highest RGRs observed in this study were similar to those reported by other researchers for other species of *Gracilaria* such as *G. chilensis* (Halling *et al*., 2005). Daily growth rates of alga *G. persica* which were reported indifferent literature are very diverse. Oliveira *et al*. (2011) showed that *G. birdiae* had higher productions in depth 10 cm and this showed that this alga preferred an environment with moderate light. Yang *et al*. (2006) recorded the variations in seaweed growth cultivated in different depths (0-1, 0.5-1.5, 1-2, 1.5-2.5 m) when *G. lemaniformis* was grown in eutrophic waters and the highest production was achieved in depths 0-1 and 0.5-1.5 m. Different depths in eutrophic environment belonged to *Gracilaria* growth factor. Marinho-Soriano (2012) showed that depth had significant effect on biomass and SGR from *G. bursa-pastoris* and based on their results the highest growth was achieved in depth 1 m and the lowest growth rate was obtained for depth 4 m, these differences could be described by the availability of light in the water levels; decrease in the light intensity with depth results in decrease in photosynthesis of algae and therefore decrease in their growth. Hurtado *et al*. (2008) showed that, *Kappaphycus* in depth 100-500 cm during 30 days, showed the best performance compared to higher depths and longer periods considering growth rate, molecular weight and carrageenan content.

Kotta *et al*. (2008) showed that the growth rates of two red algae *Furcellaria lumbricalis* and *Cocotylus truncates*, were related to the density and their growth were considerably high in low densities. In this study during 45 days, growth rates were decreased in weeks 5 and 6 due to higher densities and even in the last week some algae were separated from the ropes and then were fragmented maybe due to the high weights of the separated biomasses of the algae and maybe this is one reason why farmers prefer shorter time periods for seaweed growth. Also, in this research, considerable differences in biomass variations from *G. persica* in different conditions of tested depths and densities were observed which were considered as a factor dependent on algal growth. The results showed, biomass and growth rate in different conditions of cultivation showed that growth during 45 days was increased. The growth rate obtained from this study was totally satisfactory and was comparable with the amount obtained in commercial scale (yields about 35 g d. wt m^{-2} d^{-1} and with RGR 18% d^{-1}) (Capo *et al*., 1999). Also, it was observed that variable depth was an effective factor on growth rate, biomass of *G. persica* and in depth 1 m better growth was obtained compared to that of depth 2 m, and the primary density did not affect growth rate. These differences could be described by the availability of light in the water column; decrease in light intensity with depth results in decrease in photosynthesis of algae and therefore decrease in growth. On the other hand, it possible the lower RGR at 200 g is due to the greater competition for growth and nutrient uptake than at 100 g density.

In the present study, although a decrease in NO_3, NO_2, and NH_4^+ concentrations was observed in the first 4 weeks, their concentrations increased in the sixth week. According to Jones *et al*. (2001), this increase may be related to the mineralization of organic matter.

Environmental factor during the growth period: The growth rate in genus *Gracilaria* may be different in the various locations depending on the environmental parameters. Marinho-Soriano (2012) showed that *G. bursa-pastoris* had different responses under different environmental factors regarding biomass, growth, pigment and nutrient content, the growth of this species in temperatures below 5ºC and above 25ºC was decreased significantly and the optimum growth was obtained in 15ºC. In this study, it was also shown that temperature showed meaningful differences during the cultivation period in different weeks. According to results of Kendal and Spearman invariant test in this study, the growth rate in each depth significantly depended on environmental variables (nitrate, nitrite, ammonium and orthophosphate). This fact was verified by investigating the effects of depth variations on the growth rate of alga *G. persica* and also interactions and features of environmental variables in the development of seaweeds. In this study the environmental parameters such as pH, temperature and salinity were shown to

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have no significant effect ($P< 0.05$) on the growth of *G. persica* and possibly *G. persica* was adapted to these factors in the canals.

Most macroalgae tend to store nutrients in their tissues for the times when there were not enough nutrients available for them to grow. Generally, increase in nitrogen and phosphorus occur when light and temperature decreases. Some researchers have shown that nutrient storage period is correlated with maximum growth and development of algae (Gagne *et al*., 1982). Previous studies have shown that *Gracilaria* can rapidly absorb the dissolved nutrients from water and store them in the form of pigments and other organic compounds and thereforecan use them during the growth (Deboer, 1981). Bezerra and Marinho-Soriano (2010) showed that *G. parvispora* in contact with high ammonium concentrations showed increased biomass and growth rate. The effects of nitrogenated nutrients on the growth of macroalgae have been reported by different authors. Also, it has been shown that macroalgae can use soluble nitrogen which result in increased photosynthesis capacity and efficiency (Yang *et al*., 2006). When seaweeds are located in nitrogen and phosphorus rich environments they generally show high growth rates. However they cannot tolerate nutrients in rich environments for long time which results in an reduced efficiencies (Yu and Yang, 2008).

The maximum growth rate in this study was recorded for the increased concentration of nutrients in water which shows the effect of this parameter on the efficiency; this observation verified the positive relation between the nutrient concentrations, biomass as well as RGR. Also, there was significant elimination of nutrients by seaweed *G. persica* in depth 1 m with high biofiltration yields for NH_4^+ and NO_3^- compared to PO_4^{3-}. The maximum absorption yields obtained in this study [NH_4^+ and NO_3^- (94.3%); NO_2^- and PO_4^{3-} (82.9%)] were almost similar to those obtained in other studies (Marinho- Soriano *et al*., 2009). Previous studies have shown that *Graciliaria* species were capable of removing NO_3^- (Yang *et al*., 2006; Jones *et al*., 2001). In another study, biofiltration capacity of NO_3^- was shown to be 100% at the end of the experiment; this is to say that all the NO_3^- present in water column was eliminated (Marinho-Soriano *et al*., 2009). In this case, factors associated with environmental factors such as age of the plant and the storage of the nutrients in the alga, tissue may have helped these differences. In present study, alga biomass as well as RGR was increased by time. This increase showed that *G. persica* had high growth rates in nutrient rich waters. Alga cultivation in integrated systems was proved to be a suitable alternative for improving water quality in a variety of aquaculture activities. In this regard *G. persica* species used in this study had a great potential efficiently in the removing of nitrogenated nutrients in a moderate level and therefore improving water quality. Biofiltration capacity of *G. persica* was significant and this showed that this species had a great dependency on nutrients. During the study period, a 94.3% decrease in nitrogen removal indicated the high efficiency of this algae in removing this nutrient.

There is an increase in RGR in algae after weeks 6 or 7 of cultivation, but it is less than in weeks 3 and 4, so it is suggested that if the cultivation of this alga is done for biofiltration, the harvest is after the fourth week. In this study, after 45 days, growth rates were decreased in weeks 5 and 6 due to higher densities and even in the last week some algae were separated from the ropes and then were fragmented maybe due to the high weights of the separated biomasses of the algae and maybe this is one reason why farmers prefer shorter time periods for seaweed growth and this short period is also very suitable for biofiltration.

Conclusion

Findings provide novel evidence concerning growth and culturing conditions of *G. persica* growth rate of *G. persica* which was totally satisfactory in this study and could be used in the commercial scale. The results showed the significant effect of depth as an integral of different parameters capable of affecting the growth rate of *G. persica*. Moreover, elimination of nutrients by seaweed *G. persica* with high biofiltration yield in lower depth, therefore, it can be concluded that this species was significantly capable of eliminating the nutrients and can be used as a suitable biofilter in aquaculture complexes and integrated cultivation.

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