SEASONAL PRODUCTIVITY AND COSTS PRODUCTION OF *DUNALIELLA BARDAWIL* UNDER EGYPTIAN CONDITIONS

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Abstract

Microalgae are the natural feeds of many aquaculture species and are the basis of the natural food. This work aimed at the investigation of the possibility cultivation green algae *Dunaliella bardawil* under outdoor conditions (Egyptian conditions) was studied, variation in chemical composition of algal cells due to season of cultivation was also investigated and costs production. Average cell mass productivity reached the maximum in summer followed by autumn and spring. The algae were grown in batches using successively larger containers, the yield of the biomass, initial ($1.5x10^6$ cells/ml) and final density ($12 x10^6$ cells/ml) of *Dunalilla bardawil* were obtained at four days culture 96 hours. Methods of drying significantly affected amino acid composition. Growth and chemical composition of *Dunalilla bardawil* cells are maximum crude protein content was around 52% for all seasons. Amino acid composition showed no significant difference between the batch cultures. Methionine was the limiting amino acid. The calculated average and maximum productivity of algal cells and crude protein showed the highest value in summer culture followed by autumn and spring seasons. The chemical composition of the algal powder was similar for all batches in percent of dry weight, 51.8 % crude protein(C.P), 8.9 % ether extract (E.E), 6.5 % crude fiber (C.F), 9.2% (ash), 23.6% N. free extract (NFE),and algal extracts containing antioxidant vitamins. The high cost of labor in the outdoor cultivation which represents approximately 49.26 % from the total operating costs, followed by the nutrients, which recorded about 26.10 % from the total costs, while the electricity had minimum cost recorded about 24.63 % from the total costs. The harvesting of ton lives algae and oven dried gave 950 g dry biomass. The 1.052 ton live algae produced 1kg dry biomass. The costs of Ingredient outdoor culture for producing live *Dunalilla bardawil* and operating dry biomass were about 15.30 Pound \ ton of live algae and the costs of dry weight were LE 20.30 Pound, while operating costs were LE 21.37 Pound/kg dry biomass.

Key Word: *Dunaliella bardawil*, outdoor conditions, growth, chemical composition, Egypt.

Introduction

Microalgae have been investigated as a human and animal food for over 40 years, the use of microalgae in aquaculture has several potential advantages over the production of microalgae for human foods or terrestrial animal feeds such as high conversion efficiencies and no need for harvesting, drying and storage, as the animals or food chains could use the algae as produced. However, the production of microalgae for aquaculture feeds has been relatively neglected, mainly, because the aquaculture system themselves were generally poorly developed (**Dam *et al*., 2002**). A few years ago, microalgae have been increasingly produced for commercial purposes which include human and animal consumption, bioactive compounds for medicine, fuel production, biofertilizers, and as live

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feeds for the cultivation of filter feeding organisms. Currently, microalgal biomass production is economically feasible only when product values are relatively high, such as special chemicals and pigments, or when the microalgae play a critical role in aquaculture production (**Spectorova *et al*., 1997**). Different techniques of axenic algae production were developed, growing blue-green algae usually occur in abundance during the warm months of the year (**Fogg, 1984**). Out of the intensive research in many countries, one can conclude that, microalgae provide a valuable source of protein and other chemical compounds (**Becker, 1994**). Microalgae may provide such countries with a potential direct and indirect protein source which can be locally produced. In the commercial and semi-commercial production of algae for feed and food, important advances have been made in South East Asia (**De-Pauw *et al*., 1998; De-Pauw and Persoone, 2006**).

Egypt has good potential for mass production of algae. Algae might be a good source for poultry and fish feed which is now being partially imported. Open-door mass production of algae is carried out in Egypt on experimental scale. This work aimed at the investigation of the possibility of the mass production of *Dunalilla bardawil* in outdoor cultivation under Egyptian conditions and to study, how successfully an algae production system for cultivation period and the growth rate during seasonal variations. Also variation in chemical composition of algal cells due to season of cultivation was also investigated.

Materials and Methods

Basic medium and Algae culture:

Bold basal medium according to **Bischoff and Bold** (**1963**) was used as nutrient solution for multiplication green microalgae Table (1). Algae cultures were incubated in the growth room at 25 ºC, adjusted by air condition, continuous illumination provided by 6 fluorescent lamps (osram, cool white, 40 w120 cm) which gave a light intensity of 5000 lux, light was supported by one side of light bank under photoperiods 14 light/10 hours, cycles. The pH was adjusted daily to 7.0 with additional 5 pellets of KOH (hydroxide potassium), while light intensity was measured daily and adjusted with Lux meter, as well as photoperiods were adjusted by electricity timer. The inoculum was prepared in laboratory, 250 ml Erlenmeyer flasks each containing 100 ml. of sterilized media was inoculated with ***Dunaliella bardawil*** in a concentration of 1.5 x 10^6 cells/ml.

Table (1): Chemical composition of B.B.M. medium.

Chemicals	Final concentration
A. Macronutrients: Consists of six stock solutions:	
1-Sodium nitrate ($NaNO_3$)	**25 g/l**
2- Potassium dihydrogen phosphate ($KH_2 PO_4$)	**17.5 g/l**
3-Dipotassium hydrogen phosphate ($K_2H PO_4$)	**7.5 g/l**
4-Magnesium sulfate ($MgSO_4$)	**7.5 g/l**
5-Calcium chloride ($CaCl_2$)	**2.5 g/l**
6-Sodium chloride ($NaCl_2$)	**2.5 g/l**

B. Micronutrients: Consists of four stock solution:	
1-Na_2-EDTA + KOH	**50 g plus 31 g/l**
2-$FeSO_4.7H_2O$	**4.98 g/l**
3-H_3BO_3 Boric acid	**11.42 g/l**
4-The following four salts: All dissolved in one liter volume distilled water	
- $ZnSO_4.7H_2O$	**8.82 g/l**
- $MnCl_2$	**1.44 g/l**
- $CuSO_4$	**1.57 g/l**
- $Co(NO_3)_2.6H_2O$	**0.49 g/l**

Glass aquaria culture stages (outdoor):

At the beginning of the experiment carboy bottle was used until cultures reached the harvestable density (log phase), then they were used to inoculate 100-liter glass aquaria (75 x 40 x 60 cm). Carboys and mass culture aquaria using air compressor of a 5-hp air blower. This aeration supplies suitable oxygen density and carbon dioxide concentration needed for algae propagation, keeps algal cells in suspension form and helps to stabilize pH. The glass aquaria are cleaned and prepared for the experiment by filled it with tap water and added sodium hypochlorite to disinfect the system. After 24 hours the residual hypochlorite is neutralized with sodium thiosulfate. Atmospheric temperature was recorded daily by thermometer at three times intervals (9 a.m., 12 noons and 3 p.m.). Tap water was used to prepare the medium for all large scale culture experiments. The chemical analysis of tap water was presented in (**Table 2**).

Table (2): Physical and chemical analysis of tap water during three months.

Item	**Months**		
	September	**October**	**November**
Temperature ºC	**25.4 ± 2.7**	**24.2±1.9**	**22.7±1.2**
Dissolved Oxygen	**6.3± 0.3**	**5.16±0.8**	**6.73±0.4**
pH	**8.67 ±0.04**	**8.36±0.02**	**7.93±0.07**
Salinity (mg/l)	**0.20±0.00**	**0.22±0.00**	**0.19±0.00**
NH_4 (mg / l)	**0.58±0.09**	**0.47±0.06**	**0.54±0.07**
NH_3 (mg / l)	**0.06±0.01**	**0.08±0.03**	**0.05±0.02**
NO_3 (mg / l)	**0.15±0.03**	**0.11±0.07**	**0.13±0.04**
Total solids(mg/l)	**272.3±12**	**266±13**	**265.3±11**
Total suspended solids	**145±2.4**	**138±2.5**	**151±3.0**
Total Alkalinity	**340±5.6**	**348.6±6.1**	**358±6.3**
Total Hardness	**299.3±5.2**	**241.3±6.4**	**249.6±5.9**
Total phosphorus	**0.28±0.01**	**0.22±0.01**	**0.29±0.02**

Data are represented as mean of three samples replicates± standard deviation.

Also, the large scale cultures were disinfected according to **Hemerick *et al*. (1973)** by the addition of the commercial bleach, Clorox ® at the rate of 5.25% solution as

sodium hydrochlorite (NaOCL) and kept for one day under continuous aeration, amount of Clorox and sodium thiosulfate used to disinfect water presented in (Table 3)

Table (3): Amount of Clorox and sodium thiosulfate used to disinfect water.

Chemicals	Type		
	Carboy (20 l)	Glass aquaria (100 l)	Tank (1000 l)
Clorox (ml)	5	25	100
Sodium thiosulphate (g)	0.2	1.0	5

Sodium thiosulfate is not added until 4 hours after the addition of clorox.®

Nutrients for outdoor algae cultures:

The outdoor cultures were grown on a liquid foliar fertilizer which is marketed under "Complesal formula" amount of the following salts per 1000 liters tap water, each chemical dissolved separately in 100 ml warm water before added to water tank, algae culture medium presented in **Table (4)**. The Proximate chemical analysis was determined according to the methods described by **A.O.A.C.** (**1995**).

Table (4): Chemical composition of culture medium for outdoor cultivation.

Chemicals	Elements	Composition		Final concentration (g/1000 l)
		Weight (%)	Volume (%)	
Sodium nitrate	N	12.0	14.0	127
Ammonium sulfate/ or substituted with potassium nitrate	P- S	4.00-0.20	4.70-0.24	100 187
Mono potassium phosphate	K-B	6.00-0.02	7.10-0.024	30.0
Fe-EDTA(Ethylene diamine tetra acetic acid disodium salt)	Mg Fe	0.16- 0.010	0.20 0.012	15.0
Minerals mixed mg\ 1000 l) zinc sulfate Cobalt chloride	Zn	0.001	0.000	2.2
Manganese Chloride	Mn	0.010	0.012	35.0
Sodium mohybdate	Mo	0.005	0.006	0.36
Cupric sulfate	Cu	0.010	0.012	9.8

Algal biomass harvesting and Drying:

The quality of the algal biomass dried by the two methods was compared by determining their moisture and crude protein contents. Since cells of microalgae at the stationary phase of growth tended to settle to the bottom of the cultivation tank, primary

separation of the algal cells from the liquid phase was achieved by gravity separation. After the algal culture reached maximum growth, the circulation provided by the pumping system was stopped and the algal cells settled and formed thick sediment at the bottom of the cultivation tank within 24hours (**Allen and Nelson, 1974**).

Concentrated algal suspension:

Centrifugation was used to concentrated algal suspension by automatic superspeed centrifuge at 5000 r.p.m for 10 min. In order to find a suitable drying method, the algal pellet obtained by centrifugation was dried either: in an oven at 110 °C for 15–20 minutes, or oven-dried at 85 °C for 4 h, then at room temperature. Crude protein was determined by using microkjeldahl methods according to **APHA (1985).**

Statistical analysis

Correlation coefficients, regression and Least Significant Difference (LSD) were carried out using the Analysis of variance (ANOVA) and Duncan's multiple Range Test (1996) to determine differences between treatments means at significance rate of $P < 0.01$. The standard errors of treatment means were also estimated. All statistics were carried out using Statistical Analysis system **(SAS, 2000)**.

The present study was carried out in the Central Laboratory for Aquaculture Research (CLAR) and International Central Laboratory for Aquaculture Research Management. The World Fish Center, Regional Center for Africa and West Asia, Abbassa, Abu-Hammad, Sharkia, Egypt.

Results and Discussion

1-Chemical composition and essential amino acid:

The chemical composition of alga (table 5) showed that, the crude protein percentage was (51.8 %), 8.9 % ether extract, 6.5 % crude fiber, 9.2% ash, 23.6% N. free extract and algal extracts containing antioxidant vitamins, B1, B2, B6, B12, E, C, Biotin, B-carotene and Folic acid (µg\g dry weight) were found to be 28.4, 18.6, 23.0, 0.8, 12.0, 166, 0.9, 85 and 4.6 respectively these finding was more correlated to the content of *Scenedesmus spp.* and slight different to *Chlorella spp*. published by **Tartiel** (**2005**). As shown in Table (5), calcium content in the alga was 18 mg/g dry matter. This result was nearly similar to that of (**Behr and Soeder, 2002**). **Broun** (**2000**) reported nearly equal to figures to phosphorus and magnesium contents (12.5 and 10.2 mg/g dry wt. respectively) found in the present investigation. Also, potassium, sodium, iron, zinc, manganese, copper and lead contents are similar to those reported by (**US EPA, 2007**).

Table (5): Chemical composition, minerals content and vitamins content of *Dunalilla bardawil.*

Chemical composition	% on dry matter bases	Minerals	mg/g DW	Vitamin	µg/g DW
Crude protein	**51.8**	**Calcium**	**18.0**	**B1**	**28.4**
Ether extract	**8.9**	**Phosphorus**	**10.2**	**B2**	**18.6**
		Magnesium	**12.5**	**B6**	**23.0**

Crude fiber	**6.5**	**Potassium**	**7.80**	**B12**	**0.8**
		Sodium	**3.00**	**E**	**120**
Ash	**9.2**	**Iron**	**2.10**	**C**	**166**
		Zinc	**1.85**	**Biotin**	**0.9**
Nitrogen free extract *	**23.6**	**Manganese**	**0.22**	**β-carotene**	**85**
		Copper	**0.20**	**Nicotinate**	**77**
Gross energy (Kcal/100g)**	**473.2**	**Lead**	**0.08**	**Folic acid**	**4.6**
				Panthothe	**12.9**

*Nitrogen free extract = 100 – (protein + lipid + ash + crude fiber).
**Gross energy: Calculated according to NRC (1993) as 5.64, 9.44 and 4.11 kcal / g for protein, lipid and Nitrogen free extract, respectively.

From Table (6), Protein chemical score of samples taken from culture of spring and summer seasons was nearly the same and was relatively higher in the autumn season. Maximum crude protein content was around 52% for all seasons. This was attained at the age of 8 days for spring and summer and at 12 days in autumn seasons. Nitrogen is the major component of proteins and amino acids, and is, after C, H and O, the most abundant element in living cells (**Persoone *et al.*, 2009**). Total nucleic acid content was about 4%. Summer temperature naturally ranged higher that other two seasons and consequently, resulted in higher growth rate and protein productivity. However, analysis of variance comparing algal growth rate and yields of spring summer and autumn cultures were not significant. Maximum protein productivity value of *Dunalilla* culture was obtained in summer season followed by autumn and spring respectively (Table 6). Such data agree with higher growth rate in summer of green algae, investigated by **Broun** (**2000**). The start of the protein and the extent to which it declined varied with the season of cultivation. Lipids extracted by ether and total carbohydrates in the cells showed a spontaneous increase during the same period. The amino acid composition of the 3 species compared well with the FAO amino acid pattern except for methionine and isoleucine, in the study carried by **Dam *et al*.** (**2002**). They reported that bioassay evaluation of the algal proteins gave the following values: protein efficiency ratio 1.9 to 2.1, net protein ratio 2.4 to 2.8, biological value 75 to 78, digestibility coefficient 88 to 89 and calculated net protein utilization 67 to 69, (**Abd El-hakim and Badawy, 2008**).

Table (6): Effect of seasonal variation on the combined amino acid of *Dunalilla bardawil*

Amino acid	Season						FAO Pattern*
	Spring	S.%	Summer	S.%	Autumn	S. %	
Threonine	**6.4**	**7.4**	**6.2**	**6.6**	**6.6**	**7.4**	**2.8**
Isolecucine	**4.2**	**5.5**	**5.8**	**6.1**	**4.6**	**5.1**	**4.2**
Leucine	**9.6**	**11.2**	**8.2**	**8.7**	**7.8**	**8.6**	**4.8**
Lysine	**6.5**	**4.9**	**5.4**	**5.7**	**4.0**	**4.5**	**4.2**
Methionine**	**0.1**	**0.12**	**0.1**	**0.1**	**1.0**	**0.01**	**2.2**
Phenylalanine**	**2.8**	**3.2**	**4.2**	**4.4**	**3.5**	**3.9**	**2.8**
Valine	**4.9**	**5.7**	**6.7**	**7.0**	**6.2**	**6.9**	**4.2**
Tryptophane	**1.4**	**1.6**	**1.5**	**1.0**	**1.5**	**1.7**	**1.2**
Histidine	**1.2**	**1.4**	**1.3**	**1.4**	**1.4**	**1.6**	

Arginine	**6.8**	**7.4**	**6.5**	**6.9**	**6.3**	**7.0**	
Aspartic acid	**8.5**	**9.8**	**10.0**	**10.5**	**10.0**	**11.2**	
Serine	**3.2**	**3.7**	**5.0**	**5.3**	**4.1**	**4.6**	
Glutamic acid	**12.0**	**13.9**	**12.4**	**13.1**	**10.4**	**11.6**	
Proline	**4.0**	**4.6**	**4.2**	**4.5**	**4.4**	**4.9**	
Glycine	**5.1**	**5.9**	**4.8**	**5.0**	**5.1**	**5.7**	
Alanine	**8.2**	**9.5**	**8.1**	**8.6**	**8.3**	**9.3**	
Tyrosine	**3.5**	**4.0**	**3.8**	**4.02**	**4.1**	**4.6**	
Ornithine	**0.1**	**0.1**	**0.2**	**0.2**	**0.2**	**0.22**	
Total amino acids	**88.5**		**94.4**		**89.5**		

S. %= Score% of total amino acids;*Recommended essential amino-acid content for an ideal protein for animal consumption (WHO/FAO) (**Bhumiratana, 1976**). **Essential amino acids in ***Dunalilla bardawil*** g/100g protein. Algal samples containing crude protein level (dry weight basis).

2-Seasonal variations:

The growth of algae is essentially dependent on nutrient composition, H-ion concentration of the medium, temperature, light and adequate agitation of the culture (**Munoz *et al.*, 2007; Edmondson, 2006**). Table (7) indicates that the calculated average and maximum productivity of algal cells and crude protein showed the highest value in summer culture followed by autumn and spring seasons (**Felts and Heath., 1984; Dam *et al*., 2002; Yang and Huang, 2007**). Growth curves showed a lag phase from zero to three days for spring and autumn seasons. Logarithmic growth phase continued until 15 days for spring, 30 days for summer 18 days for autumn seasons. Decline of growth rate started after 24 and 21 days for summer and autumn seasons. **Becker** (**1994**) suggested that, the nutritional status as well as surrounded environmental growth conditions limited both growth and cellular component of green algae. In this respect, the environmental factors may be both physical, such as temperature and light, and chemical, which provide all raw materials used for the structural and protoplasmic synthesis of the algal cells. The amino acid composition of the 3 species compared well with the FAO amino acid pattern except for methionine and isoleucine, in the study carried by **Dam *et al*.** (**2002**).

The growth of algae can be limited by insufficient illumination, low temperature, and low concentration of a biologically important element on the medium or its low rate of diffusion from the medium into the cells. It is suggested that the variation in temperature and light intensities resulted in lower productivity in spring season **Dam *et al*.** (**2000**) reported that green algae cultured in summer have been characterized by higher growth rate, followed by autumn and winter. This phenomenon has been attributed to the decrease of temperature in these seasons. Maximum protein productivity value of culture was obtained in summer season followed by autumn and spring respectively, (Tables 6 and 7). Such data agree with higher growth rate in summer of green algae, investigated by **Soeder *et al*.** (**2003**). The start of the protein and the extent to which it declined varied with the season of cultivation. Lipids extracted by ether and total carbohydrates in the cells showed a spontaneous increase during the same period.

Table (7): Variation in the amino acids composition with level of crude protein in *Dunalilla bardawil* grown at outdoor conditions. (Data expressed as g/100g protein).

Amino acid	FAO pattern	Maximum protein		Minimum protein	
		Amino acid	S.%	Amino acid	S..%
Threonine	**2.8**	**6.4**	**228**	**5.1**	**182**
Isolecucine	**4.2**	**4.8**	**30.7**	**3.7**	**88**
Leucine	**4.8**	**9.7**	**202**	**7.5**	**156**
Lysine	**4.2**	**4.2**	**100**	**3.0**	**71**
Methionine	**2.2**	**0.1**	**4.5**	**0.7**	**31**
Phenylalanine	**2.8**	**2.8**	**100**	**2.9**	**103**
Valine	**4.2**	**4.9**	**116**	**4.6**	**109**
Tryptophane	**1.2**	**1.4**	**117**	**1.2**	**100**
Histidine	**--**	**1.2**	**--**	**--**	**--**
Arginine	**--**	**6.4**	**--**	**--**	**--**
Aspartic acid	**--**	**8.5**	**--**	**--**	**--**
Serine	**--**	**3.2**	**--**	**--**	**--**
Glutamic acid	**--**	**12.0**	**--**	**--**	**--**
Proline	**--**	**4.0**	**--**	**--**	**--**
Glycine	**--**	**5.1**	**--**	**--**	**--**

*Recommended essential amino-acid content for an ideal protein for animal consumption (WHO/FAO) (Bhumiratana, 1976). * S. % Score% of FAO pattern.**Essential amino acids in ***Dunalilla bardawil*** g/100g protein.

3-Mass production of *Dunalilla bardawil*

The duration of each batch population depended on growth rate (g/m^2) which varied from one batch to another according to climatic condition (especially temperature), the obtained results showed that highest productivity was recorded during May- September and lowest during December-March. The algal yield (ton/feddan/year) calculated according to the average of daily productivity during the cultivation period (12-17g/m^2) was 14-21 ton/feddan/year (300 days duration) are shown in Table (8). The time required for air-drying the algae was dependent on some environmental factors such as relative humidity, temperature, evaporation rate and light intensity. Qualities of the algal biomass dried by the two methods were compared by determining the moisture and crude protein contents of the dried algal biomass. Oven drying at 110ºC was for drying the harvested algal cells because a much shorter drying time was required. **El-Fouly *et al***. (**1998**) reported that total ash of *Chlorella* decreases proportionally with the decrease in crude protein. However, we might explain these results on the basis that *Anabaena* culture was uncovered. Consequently contamination with atmospheric dust was possible. In this respect **De Pauw *et al.*** (**1998**) mentioned that site selection is one of the most important factors which can increase net productivity of algae, added that micro algae plant should be located at site where climatic conditions provide optimal growth conditions for the longest possible period. The yield from 3 large-scale cultures of *Scenedesmus acutus, Chlorella vulgaris and Coelastrum*

proboscideum was drum dried. **Delorenzo *et al.*** (**2009**) reviewed that; commercially the large-scale production of *H. pluvialis* must also carefully controlled. Where, growth rate was reduced at higher temperature and the best growth was obtained between 15 and 25^{o}C. However, at 28^{o}C growth was inhibited and at 35^{o}C the culture died. Also, relatively high culture temperature may lead to enhanced carotenoids formation and or higher reactivates of active oxygen in the algal cell (**Yan *et al*., 2010**). In natural habitat, the aplanospore formation in *H. lacustris* in small ponds is directed more or less at with standing during out, high irradiation and light, results indicated that the sulphur amino acids are the most limiting amino acids in algae, while histidine and iso-leucine were the second and third limiting amino acids. From the aforementioned results, it is evident that the green alga *Dunalilla bardawil* can be cultivated under the Egyptian outdoor conditions. The cultivation can be carried out all year around. The modified production system proved to be efficient and the technology used in adequate and appropriate. All conditions for algal growth turbulence and agitation, Co_2, nutrient solution, recycling, pH adjustment were regularly checked. The produced alga dry material can be used as a supplement to poultry and fish feed (**Badawy *et al*., 2008**).

4- Construction and running the production unit

El-Fouly *et al*. (**1998**) described The unit with 245m^2 of basins surface area, consists of four small ground basins 5m^2 / each and three big basins of 75 m^2 / each. These varying increases are proportional with decreases of crude protein content of algal cells cultivated in different seasons. Meanwhile, the increase of indigestible polysaccharides content is related to the increase of carbohydrates in the same samples. The high percentage of silica content (about 50% of total ash as shown in results were also in accordance with those obtained by **Wang *et al*.** (**2007**), they reported that the context of threats to fragile environments, there is a need in animal production to identify alternative feed resources, which are environmentally friendly, but at the same time utilize natural resources efficiently. When a culture grows under optimum conditions its growth curve follows a sigmoid pattern in which at least five phase in the life of the culture may be recognized. They are namely: log phase, logarithmic phase, linear phase, stationary phase and death phase (**Becker, 1994**). This phenomenon could be attributed to high temperature and high sunlight intensity during these seasons, which led to photo-inhibition. Other studies on growth of green and blue-green algae under outdoor cultivation conditions gave similar results as reported by **El-Fouly *et al.*** (**1998**). The green algae require, for optimum growth, temperature in the culture liquid between 26°C and 32°C, the irradiation should exceed 600 Cal/m^2 per day. Conditions like these exist in Egypt all the round, which allow high and stable yield with optimum efficiency of equipment utilization. On the other hand, it was observed that the amount of protein was within the same range for all cultures (1.3 – 1.6 g/L) in spite of the differences in algal yields. The latter values were 3.18, and 1.8 g/L. for spring and autumn seasons, respectively. Lower dry matter yield in summer and autumn seasons resulted probably form the death of algal cells at the age of 36 and 30 days respectively. Death of cells could be attributed to photosynthesis inhibition which has been reported in other studies. **Fan *et al*.** (**1994**)**; Yan *et al*.** (**2010**) and **Delorenzo *et al.*** (**2009**) reported that production of *Spirulina* in Southern France was 12 g/m^2/day, and its protein

content was 64 – 70 % of the dried matter and variation in the raw protein content might be observed, especially when nitrogen was lacking in the medium.

Table (8): Average values of atmospheric and growth rate during cultivation months.

Season	Month	Average daily temperature °C	Radiation Cal.cm^2/day	Cell yield g/m^2	Growth rate g/m^2/day
Winter	December	12-19	250-300	120-135	12-14
	January	10-17	270-300	120-140	12-15
	February	14-20	330-360	110-140	10-14
Spring	March	18-22	450-500	110-140	10-13
	April	19-24	450-570	120-145	12-14
	May	24-28	600-630	120-155	13-15
Summer	June	28-30	650-680	140-160	14-17
	July	28-32	650-680	130-160	13-17
	August	28-34	600-640	125-155	14-16
Autumn	September	25-28	520-560	125-145	14-16
	October	23-26	420-460	120-140	13-15
	November	17-24	310-340	110-130	12-14

5-Economic evaluation:

The operating costs of producing one ton of microalgae using the multi-step method were LE 12.25/ton live algae (Table 9). The harvesting of ton live algae and oven dried gave 700 g dry biomass. The 1.43 ton live algae produced 1kg biomass. Various artificial feeds such as freeze-dried or processed natural products have been developed to substitute, if not eliminate, the use of microalgae in the hatcheries, but the technique was not adopted by hatchery operators due to the additional skill and equipment needed.

Table (9): Final density and biomass yield of *Dunalilla bardawil* after 76 hour.

Item	*Dunalilla bardawil*
Initial density (cells/ml)	1.5 X 10^6
Final density (cells/ml)	12 X 10^6
Dry weight (g/l)	0.950
Dry weight of biomass yield (g/ton) 1 m^3 water	950
Biomass yield / kg dry weight	1052.63 L. culture

Shelef *et al.* (2005) reported that depending on the production and use of microalgae as live food for commercially important fish, mollusks and crustaceans during at last part of their life cycle.

Table (10) and Figure (1) shows the costs of 1kg dry biomass of *Dunalilla bardawil*. The multi-step method is an efficient; it is a simple technique and can easily be adopted by fish hatchery operators **(Fulks and Main, 1996).** The minimal costs involved using this method make it an applicable technique for the mass culture of *Dunalilla bardawil* showed that examined algae could be utilized in Nile tilapia (*Oreochromis niloticus*) diets up to 50% instead of the dietary ingredient without any adverse effects on fish growth performances, feed utilization parameters and body composition **(Spectorova *et al*., 1997 and Badawy *et al.*, 2008)**. The use of microalgae as fish feed inputs has been studied with encouraging results. (**Broun, 2000)**, reported positive growth performance in fish feed diets containing algae cells. Algae have attention as a possible alternative protein source for cultured fish, particularly in tropical and subtropical regions where algae production high and their good protein, vitamins and essential fatty acids contents.

Table (10): Total Costs of producing live microalgae and operating dry biomass of *Dunalilla bardawil* grown outdoor culture after 3 days for cultivation.

Ingredient	Total cost (Pound)	Percentage (%)of total
Inoclum of outdoor	**1.70 LE**	**26.10%**
Clorox	**0.75 LE**	
Nutrient	**2.85 LE**	
Labor	**10.0 LE**	**49.26%**
Electricity	**5.0 LE**	**24.63%**
Cost of live algae/ton	**15.30 LE**	
Cost of dry weight	**20.30 LE**	
Total operating cost/1kg dry weight	**21.37 LE**	

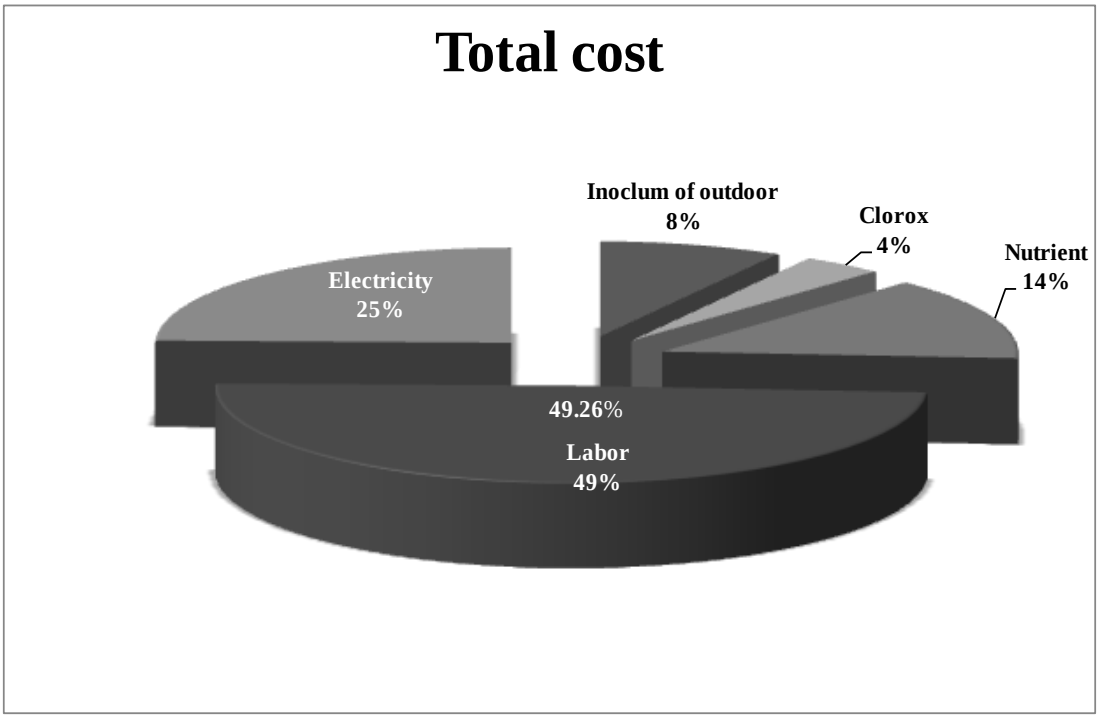

Figure (1): Percentage of total costs producing examined grown in outdoor at four days for cultivation

In conclusion, Egypt has an arid climate with maximum irradiation, offering the best conditions for photosynthetic productivity. Variation of day-length is not considered to be of importance with respect to algal yields. The fluctuations of temperature during the seasons might be compensated by choice of appropriate algal species. Algae have been utilized traditionally as food supplements and as a source of fine and valuable chemicals for various medicinal purposes. Uses include traditional cosmetics as antiseptic compounds. Microalgae are now considered one of the non-conventional source of vitamins, they contain several water and lipid-soluble vitamins, vitamins are presented in higher concentrations in *Dunalilla bardawil* than in conventional foods, some of the vitamins, are of particular commercial interest such as vitamin E(tocopherol), vitamin C(ascorbic acid), β-carotene (pro-vitamin A) and vitamin B12. Ascorbic acid has been shown to reduce some potentially harmful genetic alterations. It was clear that Egypt has good potential for mass production of algae.

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الانتـــاجيـــة المـوسـمية لطحـلب الديـونيلا وتكـالـيف الانتـــاج تحت الظــروف الــبيئية المصريــة

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قسم بحوث البيئة و البيولوجى- المعمل المركزى لبحوث الثروة السمكية بالعباسة – مركز البحوث الزراعية- وزارة الزراعة- الدقى- مصر.

لقد اتجهت أنظار العلماء فى الاونة الاخيرة الى تنمية وانتاج بعض الكائنات الحية وخاصة الطحالب بصورة مكثفة واستخدامها كأعلاف لثروات حيوانية ويلى ذلك استخدامها كغذاء للانسان وبالنظر الى طحلب الديونيلا الذى خضع للبحث العلمى الحالى هو طحلب أخضر وحيد الخلية ويتميز بقيمة غذائية لما يحتويه من نسبة عالية من المركبات العضوية والبروتينات والكاروتينيدات والفيتامينات المضادة للاكسدة ويعتبر أحد أهم فوائده استخدامه كغذاء متكامل للمراحل الاولى للاسماك ويتم اسخدام العلائق المكونة من الطحالب الدقيقة فى الاستزراع السمكى فى الوقت الحالى بصورة أساسية فى انتاج اليرقات والاصبعيات . تهدف هذه الدراسة الى امكانية تحقيق أعلى معدل للانتاج المكثف لطحلب الديونيلا بأقل التكاليف تحت ظروف الجو الخارجى للبيئة المصرية. تم عزل هذا الطحلب من بحيرة البردويل بشمال سيناءوتقدر مساحتها بحوالى 650 كم^2 وتتميز بنظام بيئى فريد حيث انها بحيرة ضحلة فقيرة فى العناصر الغذائية خاصة نسب الفسفور والنتروجين منخفضة ولاتدعم نمو الهائمات مما اثر ذلك على الانتاج السمكى حيث تستغل البحيرة كمصدر رئيسى للاسماك ولتحسينه نوصى باستزراع الهائمات النباتية(الطحالب الهائمة) مع المحافظة على أتزان العوامل البيئية والبيولوجية بها مع التنظيف المستمر للفتحات الطبيعية للبحيرة لتقليل الملوحة، وتقع البحيرة تحت تهديد تأثير التلوث عند اتصالها بترعة السلام وهذا مايجب اخذه فى الاعتبار لحماية النظام البيئى للبحيرة حيث انها احدى الثروات الطبيعية فى مصر. أوضحت النتائج ان هناك اختلافات فى التركيب الكيماوى لخلايا الطحلب النامية فى المواسم المختلفة وقد كان هناك ارتفاع فى متوسط الانتاجية فى شهور الصيف يليها الخريف ثم الربيع . اثبتت النتائج ان البيئة المستخدمة فى الاستزراع الخارجى المضاف اليها 35 جم منجنيز/1000 لتر تعتبر افضل وسط غذائى للانتاج المكثف لطحلب الديونيلا تحت الظروف البيئية المصرية حيث حصلنا على اعلى كتلة طحلبية 12 X 10^6 خلية /مللتر فى فترة زمنية قصيرة عند بداية اليوم الرابع بعد مرور 76 ساعة من بداية التحضين بكثافة 1.5 X 10^6 خلية /مللتر، وكان الاستزراع فى تنكات فيبر جلاس حجم 1000لترفقط (1متر مكعب من المياه). اثبتت النتائج ان التركيب الكيماوى لمسحوق الطحالب كان متشابها فى المواسم المختلفة حيث سجلت احتواء الخلايا على 51.8 % بروتين خام (C.P)، 8.9% مستخلص ايثرى (E.E)، 6.5% الياف خام (C.F)، 9.2% رماد (Ash)، 23.6% المستخلص الخالى من النتروجين (NFE). وكذلك على نسبة عالية من الفيتامينات المضادة للاكسدة وبيتا كاروتين (بادىء فيتامين أ) ولذلك فانه يمكن القول ان استخدام هذه السلالة من الطحالب الخضراء فى تغذية الكائنات الحية وخاصة الاسماك سوف تتراكم فى انسجتها مجموعة الفيتامينات المثبطة للاكسدة وبالتالى فان تغذية الانسان على هذه الاسماك يؤدى الى رفع قدرة الكائن على مقاومة الامراض السرطانية ومن ثم فانه يمكن الاستفادة من مستخلصات فيتامينات هذه السلالة من الطحالب بطريقة غير مباشرة. لم يظهر تحليل الاحماض الامينية اية اختلافات خلال النمو فى المواسم المختلفة. اشارت النتائج الى ان تكلفة انتاج 1 طن طحالب حية كانت 15.30 جنيه مصرى بينما كانت تكلفة الحصول عليها جافة كانت 20.30 جنيه مصرى (950 جم طحالب جافة)، كان تقدير تكلفة انتاج 1 كجم طحالب جافة كانت 21.37 جنيه مصرى ، ولذلك فقد اثبتت نتائج هذه الدراسة امكانية تنمية طحلب الديونيلا تحت الظروف البيئية المصرية وتحقيق أعلى معدل انتاج لخلايا الطحلب بأقل التكاليف.