The Influence of Different Light Wavelengths on Growth, Enzymes Activity and Photosynthesis of the Marine Microalga *Dunaliella parva* W.Lerche 1937

Mostafa El-Sheekh*   *Samha Dewidar*   *Azza Hamad*

Botany Department, Faculty of Science, Tanta University, Tanta 31527, Egypt
*Corresponding author: mostafaelsheikh@science.tanta.edu.eg, samhadowidar@hotmail.com, drazzaaa@yahoo.com
*ORCID ID: https://orcid.org/0000-0002-2298-6312,

Abstract:

Light is an important factor that influences the growth and photosynthetic efficiency of microalgae; however, little is known about how light intensity together with the wavelength affect the photosynthetic capacity and growth of marine microalgae. In the present study, the growth of the marine green microalga *Dunaliella parva* was studied and optimized under different light intensities (25 ~ 70 μmol m^{-2} s^{-1}) and qualities (blue, green, and red) in comparison with white light at 40 μmol m^{-2} s^{-1} as a control. The growth was monitored by counting the cell number, pigment content, Chl *a*, Chl *b*, and carotenoids concentrations. The optimal growth and highest photosynthetic efficiency (Fv/Fm) were recorded at a light intensity of 40 μmol m^{-2} s^{-1}, white light, and 1.25 M NaCl (1.47 and 0.678×10^6 cell mL^{-1}, respectively). The activity of antioxidant enzymes, including catalase and peroxidase, as well as ascorbate content, showed the highest values of 0.190 μM/min.mg Chl, 0.434 and 13.3 mg/g f.wt. respectively, under the green light, which confirmed the presence of environmental stresses.

Key words: Antioxidant enzymes, *Dunaliella*, Light intensity, Light quality, Photosynthesis.

Introduction:

The halotolerant, unicellular biflagellate green alga *Dunaliella* is distinguished morphologically by the absence of a rigid cell wall, contractile vacuoles, and its large pyrenoid. It has two equal flagella, one cup-shaped chloroplast, an anterior nucleus, an eye-spot [1]. *Dunaliella* is the only eukaryotic photosynthetic marine microalga that exhibits special characteristics by synthesis and varying the intracellular concentration of glycerol to enable it to grow over an extremely wide range of salt concentrations [2]. Light is the most important factor influencing the growth of all photosynthetic organisms, including *Dunaliella* sp. [3]. It is well known that *Dunaliella salina* is significantly affected by the light intensity, which is an important factor regulating pigment production in the cells. When light intensity increased the chlorophyll-a production increased as compared with the low light intensity [4]. Coesel *et al.*, [5] observed a linear increase in the total carotenoids content of *Dunaliella salina* when light intensity increased.

The low light intensity is known to increase the large photosynthetic unit, chlorophyll antenna size of both PSI and PSII, and cells is found to be normally converted to deep green as pigments increased in *Dunaliella salina* [6]. On the other hand, the high light intensity promotes the photosynthetic smaller chlorophyll antenna size, and cells are yellow-green in color [7]. Exposure of *D. bardwill* to photon flux density ranges over 100 to 4000μ mol m^{-2}s^{-1} increasing total carotenoids reaching 4.5 fold. The over-accumulation of β-carotene in *Dunaliella* cells protect the algal cells against damage mainly to protect the principal pigment Chl. *a* by high irradiation [8].

The effect of different light qualities (White, Blue, Green, Red, and yellow light) on the red alga *Porphyra leucosticta* was investigated by Korbee *et al*. [9] by measuring the photosynthetic efficiency of PSII (F_v/F_m), pigments, protein, and the UV-absorbing mycosporine-like amino acids (MAAs). They found that the lowest photosynthetic efficiency and growth rate were detected under blue light, but the higher value was detected under red and white lights. They also concluded that blue light promoted the highest accumulation of nitrogen metabolism derived compounds i.e., MAAs, phycoerythrine, and proteins in *Porphyra*

leucosticta previously grown in ammonium enriched medium. Recently, Palacios *et al.* (10) studied the effect of light intensity on the photosynthetic and growth of the marine alga *Nannochloropsis oculata* (Phylum: Ochrophyta), and they found that cultures were grown under a range of light intensities from 20 to 200 μmol photons m^{-2} s^{-1} showed a doubling in the content of Chl. *a*, and consequently increased the oxygen evolution capacity.

The aim of this work is to assess the optimal growth conditions of *Dunaliella parva* under different light intensities and light qualities as monitored by optical density, cell number, and determination of pigments content. The photosynthetic efficiency (Fv/Fm), the activity of enzymatic antioxidant (Catalase, Peroxidase, and ascorbic acid oxidase), and the content of non-enzymatic antioxidant (Ascorbate content) were also studied under different light quantities and qualities.

Material and Methods:
Algal culturing
The pure and identified culture of *Dunaliella parva* was obtained from NIES Collection, Tsukuba, JAPAN. The alga was maintained on MH medium (11) and, after being checked with the sterilized medium, it was used as an inoculum for algal growth in a liquid nutrient medium (MH) under sterilized conditions.
For the proper maintenance of the algal cultures, the agar slants were inoculated and left under the light of 40 μ mol m^{-2} s^{-1} at room temperature (25 ±3 °C).

One liter of the liquid nutrient medium was poured into two liter Erlenmeyer Pyrex- glass flasks. The culture flasks were plugged with cotton and sterilized in an autoclave at 121 °C and 1.5 atoms for 20 min. After cooling, the flasks were inoculated with 200 ml of bacteria-free stock culture and incubated under continuous light provided by fluorescent lamps at a light intensity of 40 μ mol m^{-2} s^{-1}. The growth and pigment production were determined until the end of the experimental period (16 days) for the growth curve. However, determination of growth and pigment content for all subsequent experiments was carried out on the 10th day.
Effect of light intensity
The culture flasks (batch culture) of *D. parva* were placed on shelves illuminated by fluorescent lamps. The light intensity was adjusted to (25, 40, and 70 μ mol m^{-2} s^{-1}) by changing the distance from fluorescent lamps. The light intensity was measured using LI-185 B Quantum/Radiometer photometer.
Effect of light quality

Erlenmeyer flasks (500 ml) containing 300 ml MH medium were inoculated with an initial volume (10 ml) of a pre-culture of *Dunaliella parva*. The flasks were covered with blue (BL), red (RL), and green (GL) cellophane paper according to Vijaya and Anand (12) and incubated under controlled laboratory conditions (25 ±3 °C and light intensity of 400 μ mol m^{-2} s^{-1}). White light (WL) was used as the control.
Determination of growth
The growth of *D. parva* was determined by monitoring cell count using the haemocytometer slide. Counts were estimated as a number of cells/ml culture, and counts were made with at least 5 replicates (13).
Relative growth rate
The relative growth rate (k') was calculated according to Robert (13) from the following formula:
$$K' = [Log\ N - Log\ N0]/t$$
N = Number of cells/ml after time (t) (days).
N_0 = Number of cells/ml at the initial time.

Generation time: The generation time (G) is the time needed for doubling the number of cells. It was calculated according to the following formula proposed by Fogg (14):
$$G.T = 0.301/K'$$
Where:
K'= the relative growth rate.

Number of recycling: The number of recycling for doubling the cells within a definite time could be calculated as follows:
$$\text{No. of recycling} = T/G$$
Where:
T= time from the beginning to the end of the experiment and G= mean doubling time during that time.

1.1.1. *Growth rate*: The growth rate (R) (number of divisions/ day) was calculated by using the equation proposed by Robert (13):
$$R = (3.322/\ (t_2 - t_1)) \times log\ (N_2/N_1)$$
Where:
3.322 = growth constant, t_1= time at the beginning of the experiments, t_2= time at the end of the experiment, N_1= number of cells/ml culture at t_1 and N_2= number of cells/ml culture at t_2.
Mean growth rate: the mean growth rate (R') (number of divisions / definite time "days") was calculated from the formula proposed by Robert (13):
$$R = (3.322/\ (t - t_0)) \times log\ (N/N_0)$$
Where:
3.322 = growth constant, t_0= time at the beginning of the experiments (days), t = time at the end of the

experiment (days), N_0= number of cells/ml culture at t_0 and N= number of cells/ml culture at t.

Estimation of photosynthetic pigments
Chlorophyll *a, b* and carotenoids were determined spectrophotometrically using the method recommended by Mckinney (15). The extract was measured against blank of free methanol (100%) at 650, 665 and 452 nm. It was possible to determine the concentration of each pigment fraction Chlorophyll *a, b* and carotenoids as µg/ml algal suspension using the following equations:

Chlorophyll-$a = 10.3E_{665} - 0.918E_{650}$

Chlorophyll-$b = 19.7E_{650} - 4.87 E_{665}$

Carotenoids $= 4.2E_{452} - (0.0246$ chl-a $+0$ .426 chl-b)

Where E = The reading of sample on Unico UV-2000 spectrophotometer.

Measurement of Chlorophyll a fluorescence
The chlorophyll a fluorescence was measured at room temperature. A known volume of algal culture having a known chlorophyll concentration was pipetted out and centrifuged at 3000 rpm for 20 min. The supernatant was decanted, and the pellet was placed on a piece of dressing imposed on filter paper with a known and constant diameter at all measurements. Using a modulated light MINI-PAM portable fluorometer (Walz, Germany), the initial fluorescence (F_0), maximum fluorescence (F_m) were analyzed, and quantum efficiency of open photosystem II centres-quantum yield (F_v/ F_m) was calculated. The algal discs were previously adapted to the dark for 1 min. The fluorescence variable (Fv) was calculated from the difference between F_m and F_0. The F_v and F_m values were used to obtain the maximum photochemical efficiency of PSII (Fv/Fm) ratio.

Enzyme assay

Assaying catalase*:* Catalase (EC-1.11.1.6) was assayed according to Kato and Shimizu (16) by spectrophotometric measuring the initial rate of disappearance of H_2O_2 (U mL^{-1}).

Assaying peroxidase*:* Peroxidase (EC 1.11.1.7) activity was measured according to Kato and Shimizu (16). Enzyme activity was expressed in units of µM of the substrate converted per min per mg chlorophyll.

Ascorbic acid Oxidase (AO)
The activity of ascorbic oxidase (EC1.10.3.3) was assayed, according to Oberbacher and Vines (17). Enzyme activity was expressed in units of µM of the substrate converted per min. per gram f.wt.

Determination of ascorbic acid (AA)
Non-enzymatic antioxidant, ascorbic acid, was estimated, according to Oser (18). The ascorbic acid content was calculated as mg/g f.wt using a calibration curve of ascorbic acid.

Results:
Effect of light intensity on growth parameters
Table 1 shows the effect of light intensity (25 and 70 µmol m^{-2} s^{-1}) on the growth parameters of *Dunaliella parva*. It is clear from the results that there was a decrease in cell number at a light intensity of 25 µmol m^{-2} s$^{-1,}$ which amounted to 14.39 %, and a slight decrease (3.03%) at 70 µmol m^{-2} s^{-1} as compared with control, meanwhile the decrease in cell number at (25 µmol m^{-2} s^{-1}) was accompanied by a decrease in the relative growth (K'), the number of recycling, and the mean growth rate (R').

Table 1. Effect of light intensity on growth parameters of *Dunaliella parva* after 10 days of growth

Light intensity (µmol m^{-2} s^{-1})	Cell no.* 10^6/ml	K'	G	No. of recycling	R	R'
25	1.13±0.01	0.067	4.473	2.235	0.180	0.223
40 (control)	1.32±0.04	0.074	4.065	2.459	0.489	0.245
70	1.28±0.05	0.072	4.140	2.415	0.312	0.241

Each value is the mean of five readings ± standard deviation. K'= the relative growth, G= generation time, R= rate of growth and R'= mean growth rate.

Effect of light intensity on photosynthetic pigments and photosynthetic activity (F_V/F_m)
The data in Fig. 1 (a-c) revealed a highly significant decrease in the content of Chl.a in response to the light intensity of (25 and 70) µmol m^{-2} s$^{-1,}$ amounting to 44.07 and 51.95 %, respectively as compared with control. The Chl. *b* content was highly significantly decreased by 7.89 and 26.13 % as compared with control on exposure of *D. parva* to the intensity of 25 and 70 µmol m^{-2} s^{-}

^{1}, orderly compared with the control. Exposure of *Dunaliella* cells to the light intensity of 25 and 70 µmol m^{-2} s^{-1} induced a highly significant decrease in carotenoids content, reaching 19.99 and 33.71%, respectively, relative to the control. Furthermore, total chlorophyll was significantly decreased by 31.78 % and 42.84 %, respectively, at 25 and 70 µmol m^{-2} s^{-1} compared with control. Concerning Chl. *a*/Chl. *b* ratio, exposure of the alga either to light intensity of 25 or 70 µmol m^{-2} s^{-1} had resulted in a highly significant decrease of 39.28 and 34.32

%, respectively, with respect to the control. Meanwhile, the total chlorophyll/carotenoids ratio was significantly decreased by 13.75 %, at the light intensity of 70 µmol m^{-2} s^{-1} as compared to the control. On the other hand, low light intensity (25 µmol m^{-2} s^{-1}) did not induce any significant change in the ratio of total chlorophyll/carotenoids.

Regarding the photosynthetic efficiency of the photosystem II as indicated by the (F_V/F_m), it was high significantly decreased by 30.38 %, at 70 µmol m^{-2} s^{-1}, compared with the control. However, there was no significant change in F_V/F_m at the low light intensity (25 µmol m^{-2} s^{-1}).
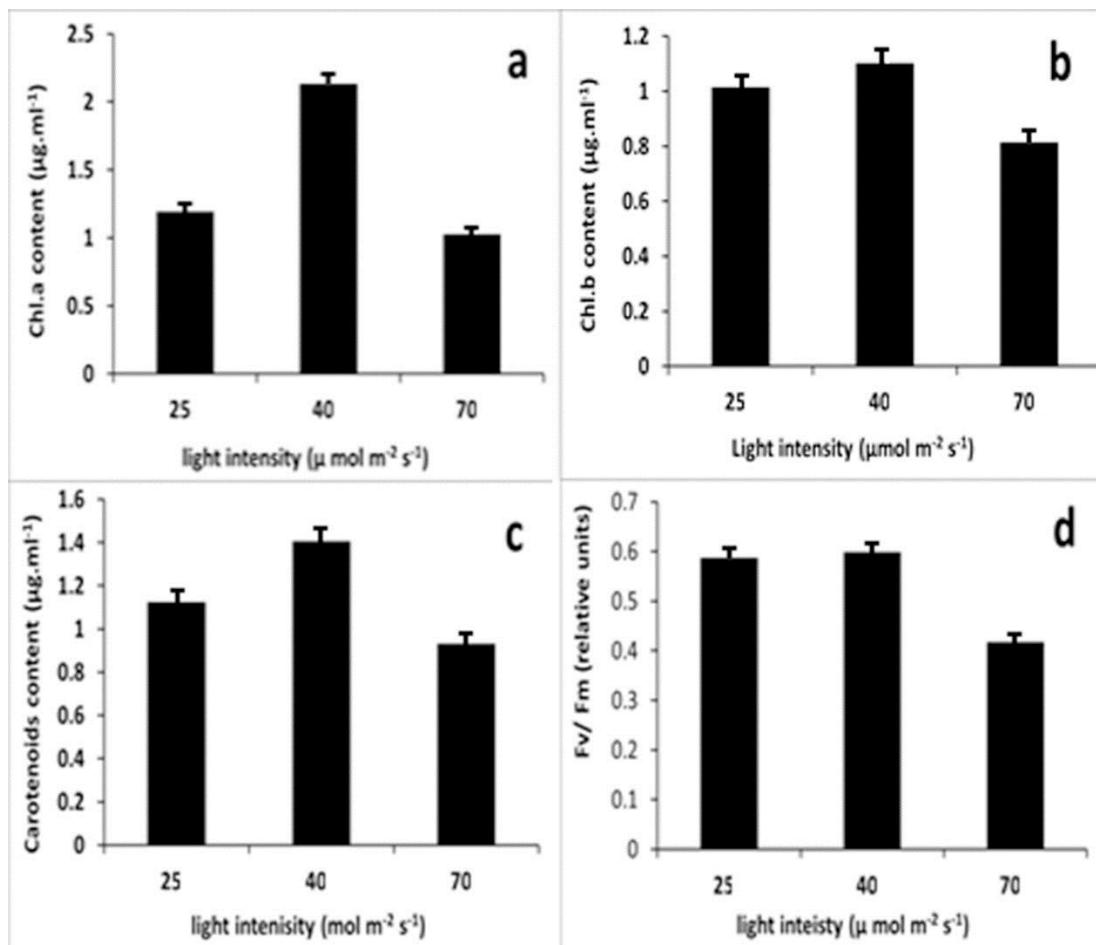

Figure 1. Effect of light intensity on pigments content of *Dunaliella parva* after 10 days of incubation. (a) Chlorophyll *a*, (b) Chlorophyll *b*, (c) Carotenoid and (d) Photosynthetic efficiency F_v/F_m

Effect of light intensity on the activity of enzymatic and nonenzymatic antioxidants.

The effect of light intensity on the activity of antioxidant enzymes and non-enzymatic antioxidants (Ascorbate) is presented in Fig. 2. The current results indicated that the low light intensity (25 µmol m^{-2} s^{-1}) did not induce any significant change in the activity of all antioxidant enzymes (Catalase, Peroxidase, and ascorbic oxidase) and the content of non-enzymatic antioxidant (Ascorbate).

The high light intensity 70 µmol m^{-2} s^{-1}, has significantly increased the activity of both catalase and peroxidase and the content of ascorbate. The percentage of stimulation was 38.25 and 21.93 %, for catalase and peroxidase, respectively, compared with control, and the content of ascorbate was increased by 46 %. However, the activity of ascorbic acid oxidase was reduced by 18.44 % with respect to the control.
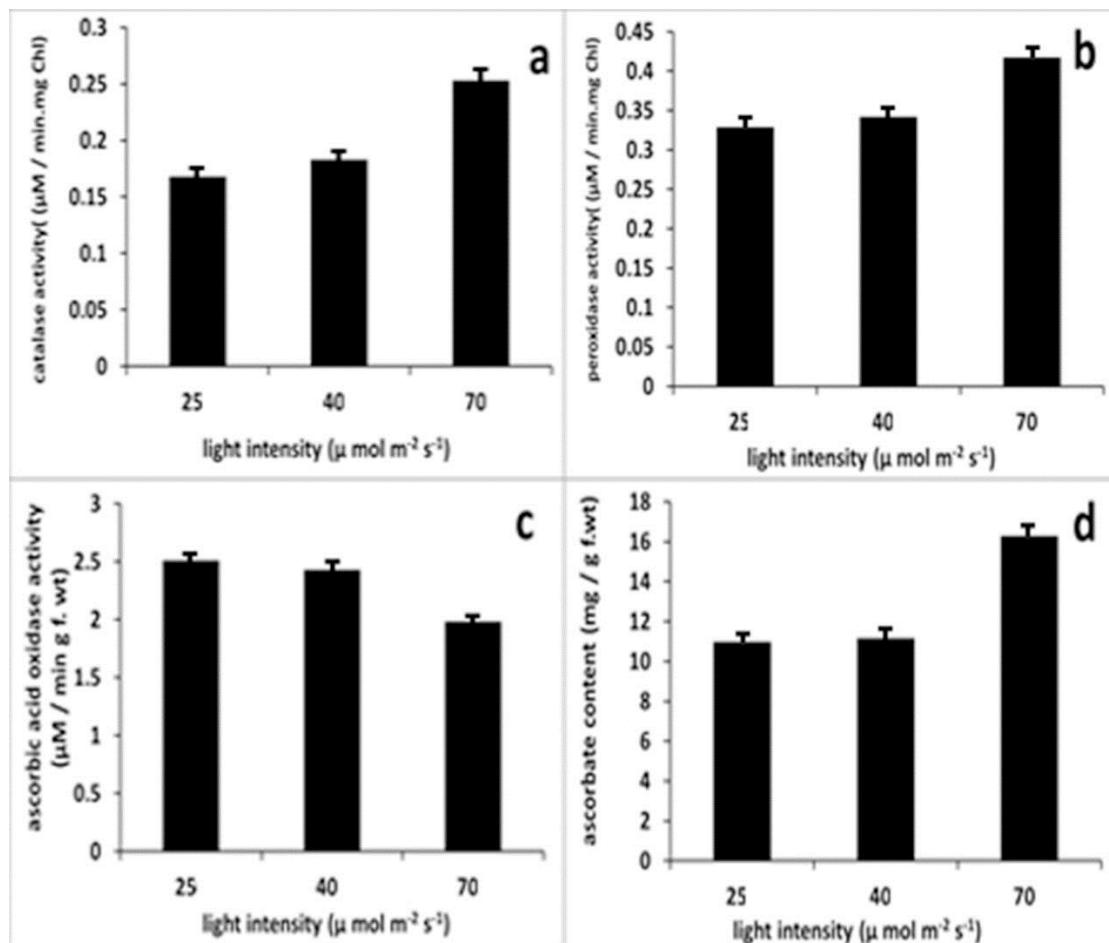
Figure 2. Effect of light intensity on the activity of enzymes and nonenzymatic n antioxidant of *Dunaliella parva* **(a) Catalase, (b) peroxidase, (c) Ascorbic acid oxidase and (d) Ascorbate content.**

Effect of light quality on cell number of Dunaliella parva

Table 2 shows the effect of different light qualities (White light (WL) control, Blue light (BL), Green light (GL), and red light (RL)) on the cell number. The data revealed that there was a decrease in cell number under (BL), (RL), and (GL) reaching 40.82, 48.30, and 64.63 %, respectively, as compared with its corresponding value of the control (WL). Meanwhile, the decrease in cell number under (BL), (GL), and (RL) was accompanied by a decrease in the relative growth (K'), number of recycling, and the mean growth rate (R').

Table 2. Effect of light quality on growth parameters of *Dunaliella parva* **after 10 days of incubation**

Light quality	Cell no. * 10^6/ml	K'	G	no of recycling	R	R'
White (control)	1.47±0.01	0.078	3.824	2.614	0.395	0.261
Blue	0.87±0.08	0.055	5.381	1.858	0.330	0.185
Red	0.76±0.05	0.050	6.012	1.663	0.233	0.166
Green	0.52±0.09	0.033	8.963	1.115	0.137	0.111

Each value is the mean of five readings ± standard deviation. K'= The relative growth, G= Generation time, R= Rate of growth and R'= Mean growth rate.

Effect of light quality on pigments content and photosynthetic activity (F_V/F_m) of Dunaliella parva

The changes in Chl.*a*, Chl.*b*, carotenoids, total Chl., Chl. *a*/Chl. *b* and total Chl./carotenoids of *D. parva* under light spectra (BL), (RL), and (GL) are presented in Table 3. The present results indicated that the content of Chl.a was significantly decreased by 10.30, 25.56, and 31.95 %, under BL, RL and GL, orderly relative to the control. In the meantime, BL, RL, and GL induced a highly significant decrease in chl. *b* content, which reached 7.09, 26.15, and 38.31 %, respectively, compared with the control. The carotenoids content was also significantly decreased under BL, RL, and GL. The percentage of decrease was 16.56, 27.70, and 34.65 %, respectively with respect to the control. The data

also revealed that the total chl. was significantly decreased below the control value under all light spectra. The results indicated that no change in the ratio of chl. *a*/chl. *b*, under BL, RL, and GL. The ratio of total chlorophyll/carotenoids was significantly increased by 8.81, 13.65 % under BL, and RL, respectively compared with the control. On the other hand, this ratio was significantly decreased

by 8.86 % under (GL) with respect to control. Concerning the photosynthetic efficiency of PSII (F_V/F_m), the data indicated a highly significant decrease in F_V/F_m under BL, GL, and RL. The percentage of decrease was 8.11, 17.40, and 52.80 %, orderly at BL, RL, and GL, relative to the control.

Table 3. Effect of different light quality on pigments content and photosynthetic activity (F_V/F_m) of *Dunaliella parva* after 10 days of growth

Light quality	Chl.*a* ($\mu g.ml^{-1}$)	Chl. *b* ($\mu g.ml^{-1}$)	Carot. ($\mu g.ml^{-1}$)	total chlorophyll ($\mu g.ml^{-1}$)	Chl. *a/b*	Total chlorophyll/ carotenoids	F_V/F_m
White (control)	2.019± 0.092	1.044± 0.03	1.498± 0.01	3.062± 0.157	1.936	2.044	0.678 ±0.001
Blue	1.811***± 0.011	0.970± 0.011(ns)	1.250***± 0.005	2.781***± 0.008	1.866(ns)	2.224*	0.623*** ±0.002
Red	1.503± 0.009***	0.771± 0.011***	1.083± 0.005***	2.274***± 0.021	1.948(ns)	2.323*	0.560*** ±0.001
Green	1.374***± 0.009	0.644***± 0.003	0.979***± 0.007	2.018***± 0.020	2.134(ns)	1.863*	0.320*** ±0.0005
F value	115.51	83.14	2396.28	106.79	33.54	56.16	1783.98
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
LSD	0.129	0.095	0.021	0.217	0.094	0.128	0.017

Each value is the mean of three readings ± standard deviation.
One way analysis of variance (ANOVA) (*** highly significant at $P \le 0.05$ and (ns) non-significant at P> 0.05).

Effect of light spectra on the antioxidant and non-antioxidant enzymes of Dunaliella parva:

Table 4 shows the influence of light spectra on the activity of antioxidant enzymes (Catalase, Peroxidase, and ascorbic acid oxidase) and the content of non- enzymatic antioxidant (ascorbate). It is evident from the results that the activity of catalase was stimulated under GL and RL. The magnitude of increase was 18.01 and 12.42 %, orderly compared with the control. However, BL did not induce any significant change in catalase activity. Peroxidase activity was significantly

stimulated under GL and RL, but there was no significant change under BL. The percentage of stimulation under GL and RL reached 18.58 and 4.10 %, with respect to the control. Regarding the activity of ascorbic acid oxidase, the results revealed no significant change under BL. On the contrary, under GL and RL, there was a highly significant decrease in the activity of ascorbic acid oxidase, the highest magnitude of inhibition was recorded under GL, and it amounted to 50.21 %, relative to the control.

Table 4. Effect of light spectra on the antioxidant enzymes (catalase, peroxidase, ascorbic acid oxidase) and non-antioxidant (ascorbate) of *Dunaliella parva*

Light quality	Catalase ($\mu M/min.mg$ Chl)	Peroxidase ($\mu M/min.mg$ Chl)	Ascorbic acid oxidase ($\mu M/min$ g f. wt)	Ascorbate (mg/g f.wt)
White (control)	0.161 ± 0.022	0.366 ± 0.006	2.396 ± 0.8	11.250 ± 0.05
Blue	0.167 ± 0.12(ns)	0.370 ± 0.003 (ns)	2.383 ± 0.1(ns)	11.361 ± 0.09(ns)
Red	0.181 ± 0.011***	0.381 ± 0.006***	1.984 ± 0.8***	11.514± 0.04*
Green	0.190 ± 0.012***	0.434± 0.03***	1.193 ± 0.08***	13.284 ± 0.1***
P	0.002	0.0001	0.0001	0.0001
LSD	0.02	0.013	0.223	0.204

Each value is the mean of three readings ± standard deviation. One way analysis of variance (ANOVA) (*** highly significant at $P \le 0.05$ and (ns) non-significant at P> 0.05).

Concerning the impact of light spectra on ascorbate, the results indicated that GL increased its content by 18.08 %. Also, RL exhibited a slightly significant increase in ascorbate content, relative to control, while BL had no significant effect.

Discussion:

Dunaliella is a halotolerant green alga that now belongs to the phylum Chlorophyta and family Dunaliellaceae (19). Cell number is a helpful indicator to clarify the role of biochemical mechanisms underlying the responses observed (20). The present results indicated that *Dunaliella parva* cells attained their maximum growth after 10 days of incubation. This result agreed with those of Taha et al., (21), who reported that at the 8-10th day, the cells of *Dunaliella salina* were at their start of a stationary phase of growth and so harvested at this time. The results also indicated that there was a gradual and highly significant increase in all pigments (Chl. *a*, Chl. *b* and carotenoids) with increasing time logical and expected due to an increase in living biomass. This may be related to the ability of new cells to synthesize pigments using nutrients derived from dead cells and/or the low degradation rate of pigments at a late stage of growth under normal conditions (22).
Light is the ultimate energy source for photosynthesis, and light intensity is one of the most important environmental factors for all photosynthetic organisms (3). In the present work, the cell number of *Dunaliella parva* was decreased by 14.39 and 3.03 % at 25 and 70 µmol m^{-2} s^{-1} on the 10th day of growth. This result was not in harmony with Zarandi Miandoab *et al*. (20), who found that the growth and division of *Dunaliella salina* were increased significantly under 1000 µmol m^{-2} s^{-1}. This may be related to adequate and effective cell adaptation (5). The growth rate of *Dunaliella virids* was extremely affected by light intensity; it increased from darkness to 70 µmol m^{-2} s^{-1} but decreased at 150 µmol m^{-2} s^{-1} (23).

A deep insight into the results of photosynthetic pigments showed that the content of all pigment fractions (Chl. *a*, Chl. *b*, Carotenoids, and total chlorophyll) were high significantly decreased under both light intensities (250 and 700 µmol m^{-2} s^{-1}). This finding is in accordance with the results of Ilkhnur *et al*. (24), who found a decrease of both chlorophylls and carotenoids contents with increasing light intensity (50-75) µmol m^{-2} s^{-1}. They also reported that chlorophyll a and carotenoids concentrations were the highest at the light intensity of (50 µmol m^{-2} s^{-1}). They speculated that growth at higher light (75 µmol m^{-2} s^{-1}) intensity was faster, so pigment accumulation could not be promoted by

the adaptation of microalgae to high light, the dimensions of light-harvesting antenna lessen, and thylakoid membranes become more efficient, which is a natural process. During the so-called photoacclimation phenomenon, cellular chlorophyll components come to a minimum, and thylakoid membranes start to work more efficiently (25). In this respect, Aro *et al*. (26) reported that non-optimal light intensity could adversely affect metabolism and excitation of the photosynthetic apparatus, which can induce photoinhibition, photo-damage, and degradation of the photosynthetic proteins in the cell.

In addition, the results revealed that Chl *a/b* ratio was high and significantly decreased under both light intensities. This result was in agreement with the findings of Levy *et al*. (27), who reported that when *Dunaliella bardawili* grew under sunlight spectra with high light intensities Chl *a/b* ratio was decreased and accompanied by 60 % increase in β carotene. They further concluded that light is a limiting factor influencing pigment synthesis. However, the present data are not in agreement with the increase in carotenoids, as reported in the above- mentioned algae. This may be related to the ability of *Dunaliella* cells to adapt to different stresses as they have the mechanism to change their intracellular chemicals between glycerol and carotenoids (28).

The results indicated that photosynthetic efficiency F_v/F_m was high and significantly reduced at both light intensities, but the magnitude of reduction was great at a high light intensity (70 µmol m^{-2} s^{-1}). Changes in light intensities during plant and algal growth results in a reversible structural and functional adjustment in the photosynthetic apparatus (29). Photosynthetic organisms acclimate to the level of irradiance by adjusting the size of the chlorophyll antenna associated with photosystems (30). When plants are grown under high light intensity, photosystems contain small antennas and relatively low amounts of Chl *b* in comparison with low light intensity (31). Thus, the mechanism of chloroplast photoacclimation involving the regulation of Chl. *b* biosynthesis and changing in chlorophyll antenna size is a dynamic irradiance dependent recovery response (6).

Endogenous antioxidant enzymes, such as catalase and peroxidase, play an important role in the repair of oxidative damage (32). When photosynthetic pigments absorb more energy than is used in photosynthesis, the excess electron reacts with the abundantly present oxygen under those conditions; the chlorophyll molecules coupled with

the electron transport system are the primary source for the creation of singlet oxygen (33).

High irradiance also may lead to over-reduction of the photosynthetic electron transport chain and hence increases the production of several free radicals referred to as reactive oxygen species (ROS) (20).

The increase in the activity of catalase, peroxidase, and ascorbate content in the present work may be due to the oxidative stress imposed on *Dunaliella parva* at the high light intensity. The abovementioned reasons for the production of ROS under high light intensity may account for the recorded stimulation of catalase and peroxidase activities and the increase in the content of ascorbate as a defense mechanism. Biological systems respond to elevated ROS levels by activating several antioxidative defense mechanisms. Because of the central role of photosynthesis, photosynthetic organisms had developed strategies to acclimate to a broad range of environmental conditions such as high light by modulating their metabolism (34).

Algal photosynthetic apparatus quickly adapt to radiation quality as well as to irradiance (35), light quality, and carbon source (36). The results of this study indicated that cell number was decreased under different light qualities in the following order BL > RL > GL compared with control. This may be related to the difference in energy provided by different light spectra. Overall, the white light, which has the energy for the activation of photosynthetic pigments, provides enough energy for optimal maintenance and growth (37). This suggests that the red part of the spectrum is not by itself able to support the growth, but is not inhibitory either to growth or to the maintenance of cell integrity. However, the energy is not enough for other essential metabolic processes (38), which explains the low cell number recorded under RL in the present work. On the other hand, blue light, which is absorbed by photosynthetic pigments, can run both the photosynthetic and respiratory cycles (39). As a result, the cell integrity process performed well in the BL. The lowest cell number recorded under green light may be related to very low energy reaching PSII via the reflection of GL by photosynthetic pigments.

This study indicated that the content of all pigment fractions (Chl. *a*, Chl. *b* and carotenoids) was highly significantly decreased under all studied light spectra, with respect to white light, with the exception of Chl. *b* under BL. This finding may be attributed to structural changes in chloroplastic thylakoid membranes and their integrity. In this respect, Dowidar (40) found that grana of *Chlamydomonas reinhardii* were well organized under BL, but disorganized under RL. Also, Muthuvelan *et al.,* (37) reported that RL induced the disorganization of thylakoid via the low energy provided to maintain cell integrity. Disorganization of thylakoid membranes, which is the protective system of photosynthetic pigments, may explain the recorded low content of all pigment fractions in this study.

Algal adaptation to different light spectra is known to induce variation in the thylakoid components, particularly the antennae size and a number of photosystem complex (25). The reduction in photosynthetic efficiency (F_v/F_m), in the present work, under different light spectra was associated with a decrease in Chl. *b* content. This result was in harmony with Dowidar (40) and Hamada *et al*. (36), who found high Chl. *b* content in cells grown under BL and high Chl. *a* concentration in those cells grown under RL. In addition, they found that Emerson enhancement and the fraction of photons delivered to PSII (β) measured from cells grown under BL were significantly higher than under RL and were lower than those grown under WL.

Kowallik and Schürmann (41) reported that high Chl.b concentration is necessary for an efficient function of PSII. On the other hand, Dowidar (40) reported that the fraction of photons delivered to PSI (α) and absorbed by RL grown cells might result in an increased cyclic flow of PSI. Consequently, electron flow from PSII may slow down the oxygen evolution of these cells when compared with those under WL, even though RL contains both wavelengths required for stimulation of PSII and PSI (40).

Blue light is known to stimulate protein synthesis (38), and the metabolic processes and morphological development rely on the role of protein and lipid (37). In addition, the non-significant change in the antioxidant system in the present work may be explined by the enough energy provided to the cell by BL for metabolic activities. On the other hand, the energy for the photosynthesis process may not be available in the red light; therefore, it might alter the products of photosynthesis in such a way to halt growth and continue the cell maintenance process with the limited source of other substances (37). It has been shown that such loss cannot be compensated by supplementation of growth medium with sugar acetate or amino acids (42). This low energy provided by RL and GL might impose a stress on the studied organism, which responds by stimulating the activity of catalase and peroxidase and increased the content of ascorbate.

Conclusion:

In conclusion, the optimal growth and highest photosynthetic efficiency (Fv/Fm) of the marine alga *Dunaliella parva* were recorded at a light intensity of 40 μ mol m^{-2} s^{-1}, white light, and 1.25 M NaCl. The activity of antioxidant enzymes, including catalase and peroxidase and ascorbate content, showed the highest values of 0.190 μM/min.mg Chl, 0.434 and 13.284 mg/g fresh weight, respectively under the green light. The induction in catalase and peroxidase activity indicated that *D. parva* responded to low energy provided by red light and green light to overcome this environmental stress.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: NA.

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تأثير أطوال موجات ضوئية مختلفة على النمو ونشاط الإنزيمات والبناء الضوئي للطحالب البحرية الدقيقة ديوناليلا بارفا *Dunaliella parva*

مصطفى محمد الشيخ   سمحة دويدار   عزة حماد

قسم النبات، كلية العلوم، جامعة طنطا، مصر.

الخلاصة:

يعتبر الضوء عاملاً هامًا يؤثر على نمو الطحالب الدقيقة وكفاءة التمثيل الضوئي لها ؛ ومع ذلك ، لا يُعرف الكثير عن كيفية تأثير شدة الضوء مع الطول الموجي على قدرة التمثيل الضوئي ونمو الطحالب البحرية الدقيقة. في هذه الدراسة ، تمت دراسة نمو الطحالب البحرية الخضراء الدقيقة ديوناليلا بارفا واقلمته تحت شدة الضوء المختلفة ($70 \sim 25$ µmol m^{-2} s^{-1}) ونوعية الضوء (الأزرق والأخضر والأحمر) مقارنة بالضوء الأبيض عند 40 µmol m^{-2} s^{-1} كتجربة ضابطة (كنترول). تمت مراقبة النمو عن طريق حساب عدد الخلايا ومحتوى الصبغة وتراكيز Chl a و Chl b والكاروتينات. تم تسجيل النمو الأمثل وأعلى كفاءة التمثيل الضوئي (Fv / Fm) بكثافة ضوء 40 µmol m^{-2} s^{-1}، ضوء أبيض ، و 1.25 مولار كلوريد الصوديوم (1.47 and 0.678×10^6 cell mL^{-1} ، على التوالي). أظهر نشاط إنزيمات مضادات الأكسدة ، بما في ذلك الكاتلاز والبيروكسيديز وكذلك محتوى الأسكوربات ، أعلى قيم بلغت 0.190 µM/min.mg Chl, 0.434 and 13.3 mg/g f.wt. على التوالي ، تحت تأثير الضوء الأخضر ، الذي أكد وجود ضغوط بيئية.

الكلمات المفتاحية: انزيمات الأكسدة- ديوناليلا بارفا- شدة الضوء- نوعيو الضوء- البناء الضوئى