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Effect of Different Temperature and Light Spectrum on the Growth and Chlorophyll Pigment Content of Green algae

Heba Mahmoud Shaker [1]
Sadiq Kadhum Lafta Alzurfi [2]

Department of Ecology and pollution - Faculty of science - University of Kufa-Iraq[1,2]

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*Corresponding Author:
Email:
sadiqk.alzurfi@uokufa.edu.iq

Abstract: *Chlorophyll produced by photosynthetic organisms such as plants, algae, and microbes can serve as a chemoprotective agent with potential health benefits when consumed as a dietary supplement. The current study aimed to investigate the effects of temperature and light spectrum on chlorophyll production in the microalga Chlorella vulgaris. An incubator with three compartments providing distinct red, blue, and white light spectra was designed to grow algal cultures. Four replicates per treatment were incubated for 21 days, with optical density, growth rate, biomass, and chlorophyll pigments quantified at regular intervals. Over time, optical density, growth rate, and biomass increased significantly at temperatures of 20°C, 25°C, and 30°C, while doubling time decreased. Maximum chlorophyll concentrations were attained on day 18 under red light at 25°C (1.22 µg/L), while minimum concentrations occurred on day 1 under white light at 25°C (0.06 µg/L). For chlorophyll b, highest levels were reached on day 18 with red light at 30°C (1.23 µg/L) and lowest on day 1 with white light at 30°C (0.04 µg/L). In summary, this study demonstrates that light spectrum and temperature significantly influence C. vulgaris growth and chlorophyll production, with red light and moderate temperatures promoting optimal chlorophyll biosynthesis. Careful control of cultivation conditions could maximize yields of this health-promoting pigment.*

.**Keywords*: Algae, Chlorophyll, Light, Temperature, Spectrum.***

1.Introduction

Chlorophylls are distinct green pigments that are present in cyanobacteria, algae, and a variety of plants[1]. The Greek words chloros, which means "green," and phyllon, which means "leaf," are the sources of the word chlorophyll. French pharmacist Joseph Bienaimé Caventou and chemist Pierre-Joseph Pelletier isolated and named chlorophyll for the first time in 1817 [2]. The green spectrum is reflected by chlorophyll pigment, which absorbs blue and red light from solar radiation at wavelengths of 430 and 660 nm, respectively [1]. Chlorophyll was formerly divided into four types: chlorophyll a, b, c, and d [3]. However, a new type of chlorophyll, is f, was identified within a stromatolite (a hard rock structure formed by cyanobacteria) in western Australia. Consequently, ultimately.

Research has revealed that chlorophylls are tetrapyrroles, a cyclic form of porphyrin and chlorin (the parent molecule of all chlorophylls). This cyclic form creates an isocyclic ring with the help of CH bridges. Chemically, chlorophylls possess a magnesium ion in the central position, which is found connected with the tetra- pyrrole ring [4]. Moreover, chlorophylls are hydrophobic molecules because they contain phytol, an esterified isoprenoid C20 alcohol. The phytol (C20H30OH) possesses a double bond in the trans configuration [5].

Four pyrrole rings are joined together by methane (CH=) bridges, and four nitrogen atoms are coordinated with a central metal atom. this structure contains a chromophore of several conjugated double bonds responsible for absorbing light in the visible region, i.e. red (peak at 670–680nm) and blue (peak at 435–455 nm). The reflection and/or transmission of the non-absorbed green light (intermediate wave- length) give the characteristic green color to plants and chlorophyll solutions. Higher plants, ferns, mosses, green algae, and the prokaryotic organism prochloron only have two chlorophylls (a and b); the remaining chlorophylls are present in algae and bacteria. Light properties (spectral quality, quantity, and duration) have a profound impact on the metabolism and development of plants and seaweeds)[6]. Effect of different light qualities on growth, pigment content, chlorophyll fluorescence, and antioxidant enzyme activity in the red alga *Pyropia haitanensis* (Bangiales, Rhodophyta) [7].

Previous research results indicate that red, green and blue light have a special effect on regulating algal growth and the synthesis of photosynthetic pigments. Although there is also some research on the effects of different light wavelengths on photosynthesis efficiency, chlorophyll content, and growth in photosynthetic prokaryotes and eukaryotes [8,9]. Photosynthesis, like all metabolic processes, is affected by temperature [10]. Microalgae manifest a range of physio- logical responses to temperature changes [11].

Chlorophyll-a serves as a sensitive indicator of physiological status in microalgae. The dependence of chlorophyll-a can be described based on photon flux density (PFD or I) and temperature in nutrient-sufficient microalgae, which grow significantly through an empirical equation with four coefficients. Chlorophyll-a increases linearly with rising light levels at a constant temperature and decreases significantly with increasing temperature at a constant light level [12]. The objective of current work is to study effect of temperature and light spectrum on chlorophyll pigments content of *Chlorella vulgaris.*

Materials and Methods:

All equipment and media were sterilized by autoclaving at 121°C and 1.5 bar pressure for 15 minutes prior to use. Algal cultures were grown in modified BG-11 medium prepared by[13] combining 2.5 ml of each stock solution per liter of distilled water, followed by sterilization. The pH of the resulting BG-11 medium was adjusted to 6.4 with hydrochloric acid or sodium hydroxide (0.01N).

Cultures were incubated in a custom-built chamber with three compartments providing distinct red (152 µmol/m2/s), blue (1194 µmol/m2/s), and white (303 µmol/m2/s) light spectra. Four replicate samples were placed in each compartment and incubated for 21 days under the specified light conditions. *Chlorella vulgaris* was cultured in the cultivation medium (BG11) after being prepared and sterilized using an autoclave. 10 ml of culture was taken from a pure algal strain and added to liquid culture mediums of size 400 ml and incubated in an algal growth chamber at three temperatures (20, 25, and 30 °C) and three spectra (red, blue, and white) under different light intensities at the light system (16–8)l/d. Subsequently, four replicates were prepared for each of the three treatments. the culture shaking twice daily to prevent algae aggregate

on walls. Daily samples were drawn for density assessments, growth rate and duplicate time with samples taken on the first day, the ninth day, and the 18th day for chlorophyll examinations. Two samples were drawn for measuring lutein and β-carotene pigments on the ninth and 18th days. The samples were incubated continuously until the death stage was reached [14].

Estimated growth rate of C. vulgaris is appraise daily with a spectrophotometer by optical density (OD) measurements at 680 nm, the Optical Density of cells was measured from the (time = 0) and every 24 hours for a different period [15] In order to determine the growth constant K, the following formula [16] was used:

$$K=(\log Odt - \log OD\,o) \times 3.332 / t$$

K: growth rate

T: time

Od0: optical density at zero time (the start of the experiment)

Odt: optical density following a day (t).

The following equation [17] is used to calculate the generation time of the multiplication of G.

$$G = 0.301 / K$$

G: doubling time

Twenty-five ml of each chlorophyll foliar extract were taken and filtered directly through a 0.45-micron GFC type filter paper. The filtered sample was then placed in 10 ml test tubes with tight covers. 10 ml of acetone (90%) was sealed tightly. The tubes were kept in the refrigerator at 4°C for 24 hours. Afterward, the samples were centrifuged using a Chania-made centrifuge at a speed of 3000 revolutions per minute. The filtrate was collected, and its absorbance was measured using an American-made AQU A Mate spectrophotometer (thermo_Electron corporation) at wavelengths of (750, 647, 664, and 630) nm. The following formulas were used to calculate the chlorophyll a, b, and c concentration [18].

Calculation:

The following formula is used to determine the water sample's total chlorophyll, chlorophyll a and b levels:

$$Chl\ a\ mg/gm = \frac{11.85(D664 - D750) - 1.54(D647 - D750) - 0.08(D630 - D750) \times VE}{Vs \times \delta.}$$

$$Chl.\ b = \frac{\{-5.43(D664 - D750) + 21.03(D647 - D750) - 2.66(D630 - D750) \times VE}{Vs \times \delta.}$$

where Chl-a and Chl-b denote the relative µg/L amounts of chlorophyll a and b in the water sample. The absorbance at wavelengths of 630, 647, 664, and 750 nm is represented by the numbers D630, D647, D664, and D750, respectively. % is the cuvette's optical path length, and VE, mL, Vs, and L are the extract and filtered water sample volumes, respectively.

Statistical analyses:

Using IBM SPSS Statistics v26.0, univariate analysis of variance (ANOVA) was used to statistically examine all the data. The significant difference between the means at $P < 0.05$ was found using Tukey test. Pearson's correlation analysis was used to quantitatively assess the relationship between pigment contents and cell growth. Each and every value is given as the mean standard deviation (SD).

Results and Discussion:

Growth rate: Figure (1-a) shows the growth rate during cultivation of Chlorella vulgaris algae at 20 ºC, the Exponential phase began to increase from the fourth day, there was a steady increase in growth, continuous increase in cell number to reach to stationary phase after ten day. It was decrease in growth decrease phase

began after seventeen day and decrease continues in growth to reach died of cells.

According to the results, the algae exposed to the blue spectrum had the highest growth rate, measuring 0.294 cells/hour; this was followed by the red spectrum wave, which had a value of 228 cells/hour; the white spectrum wave showed a decrease that day, measuring 0.207 cells/hour; the blue spectrum wave continued to rise higher than the white and red, and on the eighteenth day, all three spectra reached the stage of decline and stability. The statistical study reveals noteworthy variations between spectra and days. While demonstrating how quickly Chlorella vulgaris grows when cultivated at 25 oC, the red, blue, and white spectrums appear first, then the white one (Figure 1 b).

As for the value of the growth rate, we find its behavior follows the optical density values, as the growth rate is a coefficient used to describe the speed of growth of a specific strain or population over time. The growth rate of microalgae can vary based on algae types and the impact of environmental conditions. It depends on factors such as temperature, light, nutrient concentrations, and others [19].

Optical density readings are used to estimate the growth of algae particles or living cells in the cultivation medium. Changes in OD values over time can be used to calculate the growth rate using specific equations. An increase in the growth rate usually correlates with an increase in OD values over time, and vice versa[20](Table 1,2 and 3).

In current study, the maximum value of growth rate was at 30 ºC with the red light spectrum, while the minimum value was at 20 ºC with the white light. This indicates that as the temperature decreases, the growth rate also decreases.

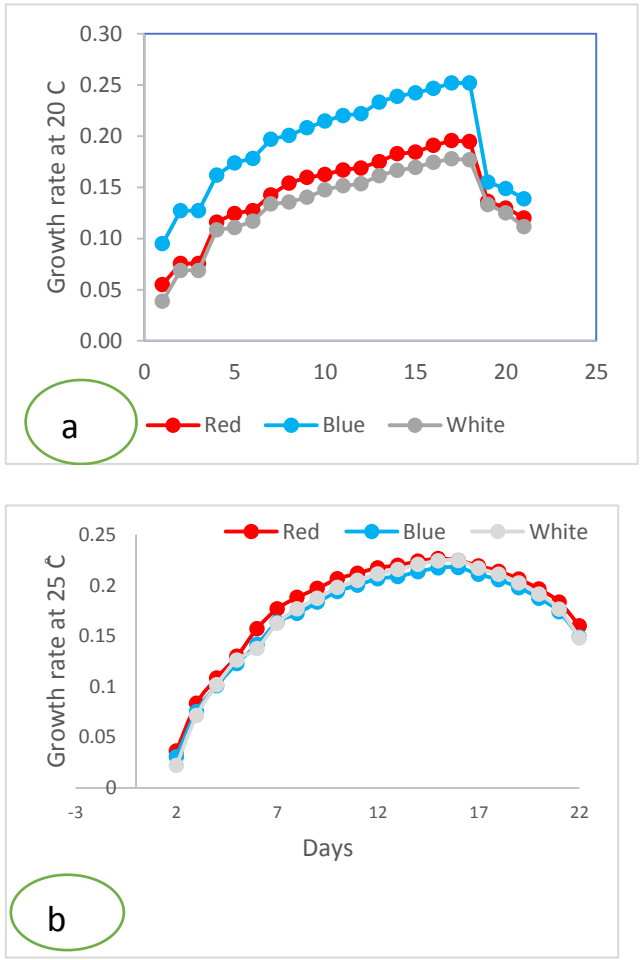

Fig1: Growth rate of *Chlorella vulgaris* a) Incubated at 20 °C b) Incubated at 25 °C during the experiment period.

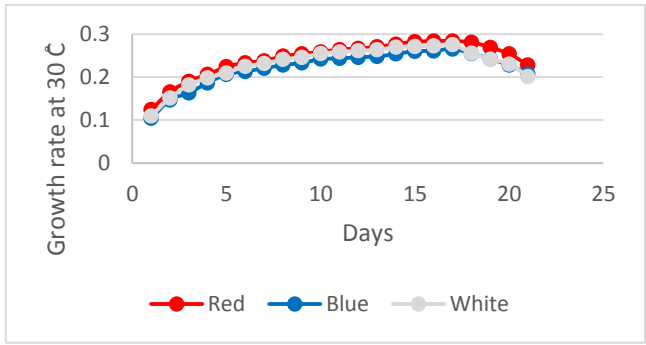

Fig2: Growth rate of *Chlorella vulgaris* that was incubated at 30 °C during the experiment period.

Duplication time:

It is noted from figure 3 that the doubling time at 20 ºC gradually decreases throughout the experimentation period. where it recorded a higher level in the white spectrum on the first day, where it reached 9.563 hr. The red spectrum was 4.752 hr, and the blue spectrum was 2.82 hr. The lowest rate was recorded on the eighteenth day for all three spectra. As noted from figure 4, the doubling time at a temperature of 25 ºC decreases throughout the study period. The highest doubling time was on the first day, when the white spectrum had a value of 12.812 hr, followed by the blue spectrum with a value of 8.498 hr, followed by the red spectrum with a value of 6.339 hr. Then the rate began to decrease until it reached the lowest amount on the 15th day for the three spectra, then increased again on the sixteenth day for all three spectra. While figure 5 shows that the doubling time at 30 ºC decreased throughout the study period, The highest doubling time was on the first day, when the blue spectrum had a value of 2.488 hr., followed by the white spectrum with a value of 2.362 hr., followed by the red spectrum with a value of 2.088 hr. Then the rate began to decrease until it reached the lowest amount on the 17th day for the three spectra, then increased again on the eighteenth day for all three spectra. The statistical analysis shows significant differences between days and spectra.

Doubling time is the duration it takes for a population to double in size or value. When the relative growth rate (not the absolute growth rate) is constant, the quantity undergoes exponential growth with a fixed doubling time, which can be directly calculated from the growth rate[21]. As evident in the current study's results, the doubling time was consistently reduced across all days of C. vulgaris algae. The highest doubling time value was observed on the first day at a temperature of 30 ºC with blue light. This finding aligns with [22], who mentioned the highest doubling time rate on the first day, especially with blue light.

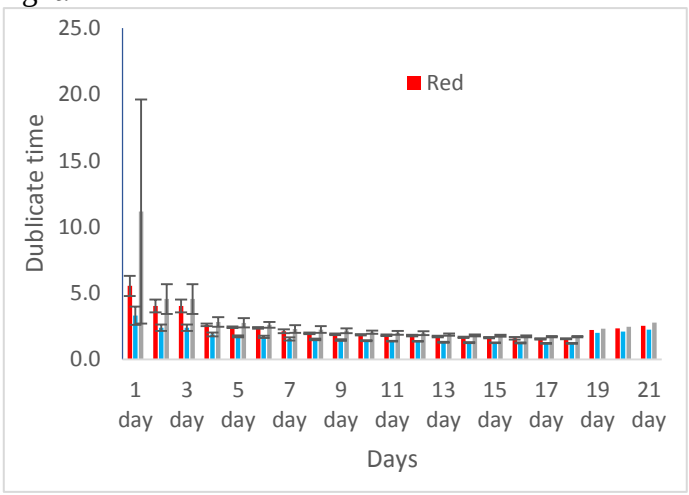

Fig3: Duplication time of *Chlorella vulgaris* that was incubated at 20 °C during the experiment period.

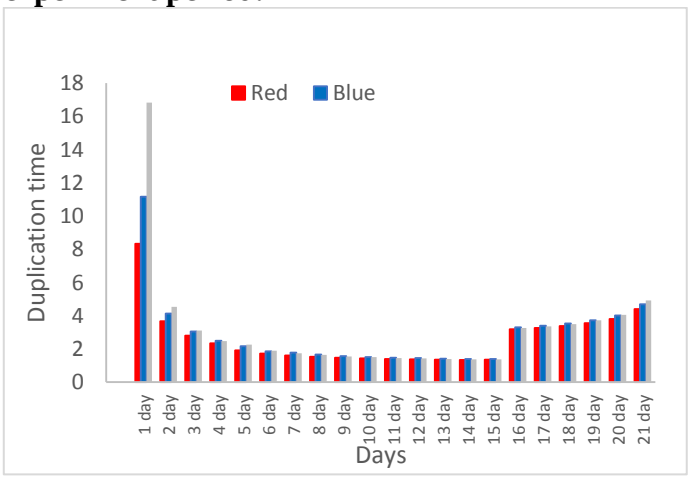

Fig4: Duplication time of *Chlorella vulgaris* that was incubated at 25 °C during the experiment period.

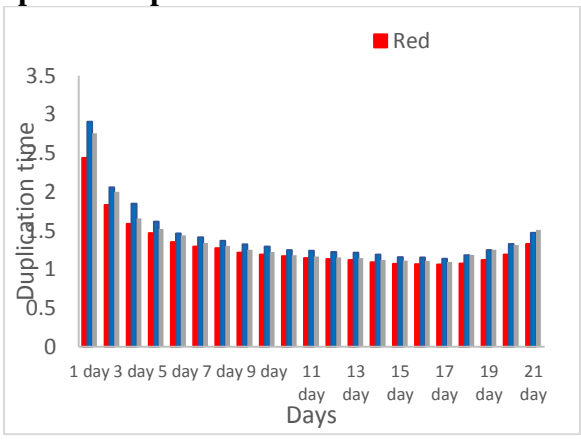

Fig 5: Duplication time of *Chlorella vulgaris* that was incubated at 30 °C during the experiment period.

Chlorophyll content:

Figure (6) shows the concentration of chlorophyll a pigment when *Chlorella vulgaris* algae was incubated at a temperature of 20 degrees, the highest concentration of chlorophyll a was recorded on the 18th day in the blue spectrum and was 0.66 µg/l, while the lowest value of chlorophyll a was recorded during the first day in the white spectrum, 0.31 µg /l. The statistical analysis shows that there are significant differences at the level of $P < 0.05$. As for the concentration of chlorophyll b pigment when *Chlorella vulgaris* was incubated at a temperature of 20 degrees, the highest value of chlorophyll b was recorded on the 18th day in the blue spectrum and was 0.78 µg /l, while the lowest value of chlorophyll a was recorded during the first day in the white spectrum, 0.18 µg/l. The statistical analysis shows that there are significant differences at the level of $P < 0.05$ (Fig.7).

The current study results reveal that the highest chlorophyll a content was observed at 25°C with the red light wave, reaching 1.22 µg /l on the 18th day. In contrast, the lowest content was found under the white light wave at the same temperature 0.16 µg /l on the 1st day. While that the highest chlorophyll b content was observed at 25°C with the white light wave, reaching 0.52 µg /l on the 18th day. In contrast, the lowest content was found under the white light wave at the same temperature 0.06 µg /l on the 1st day. As for at 30 C that the highest chlorophyll a content was observed with the red light wave, reaching 1.21 µg /l on the 18th day. In contrast, the lowest content was found under the white light wave at the same temperature 0.29 µg /l on the 1st day. While that the highest chlorophyll b content was observed at 30°C with the red light wave, reaching 1.23 µg /l on the 18th day. In contrast, the lowest content was found under the white light wave at the same temperature 0.04 µg /l on the 1st day. Moreover, the study indicates a decrease in chlorophyll a content as the temperature decreased, with the highest rate at 30 °C seen with the red light wave and the lowest at the same temperature and day with the white light wave 0.04 µg /l. Additionally, the content decreases with a lower temperature.

In the current study, maximum values of chlorophyll a and chlorophyll b were measured when C. vulgaris algae were treated with LED waves in the colors white, blue, and red at varying temperatures (20, 25, and 30 °C).The optimal conditions for the highest productivity of C. vulgaris algae were observed at 30 °C with the red light wave, aligning with Sharma et al. [23], mentioning the optimal temperature range of 30–35 °C. So, our study suggested that the ideal lighting for chlorophyll pigment production could be within the red light wave, and these results differed from those of Baidya et al. [24], who suggested the blue light wave is optimal. This variation might be attributed to conducting their study under a constant temperature range between 20 and 26 °C, where our results showed that the highest values were under the influence of the blue light wave.

At 20°C, the current study agreed with [24] that the blue light has a short wavelength compared to other LED lights, and this wavelength might activate specific photoreceptor proteins, namely phototropin (phot1 and phot2) [25]. Blue light is also known to activate phytochrome A (the phyA gene), which positively modulates phototropism [26]. This phot1, phot2, and phyA gene might specifically regulate the growth of *Chlorella sp*. Blue LED light might be greatly consumed by algal photosynthetic pigments and act as a vital catalyst to stimulate the pigment contents in microalgae[27]. The activity of the enzyme-assisted principal gene in the pigment contents was stimulated by exposure to blue light and tremendously

enhanced the pigment accumulation as well as cryptochrome action [28]. The blue LED light deeply penetrated the batch culture of microalgae, which enhanced growth and cell density[27]. As a result, the wavelength (475 nm) of blue LED can significantly enhance the cell density of *Chlorella sp*. Plant cell photoreceptors are activated by blue light, and these photoreceptors regulate targeted gene expression[29]. Seyfabadi et al. [30] mention that the blue color (low radiation) has a higher concentration of β-carotene, while the high radiation (red color) has a lower concentration at harvesting time (the 18th day of exposure), and that also agrees with the current study. Moreover, the white color was in between because this color contains all visible waves [31]. Through our study, we found that there is a positive, significant relationship between the content of chlorophyll pigments and growth rate and biomass and an inverse relationship with doubling time (Table 1).

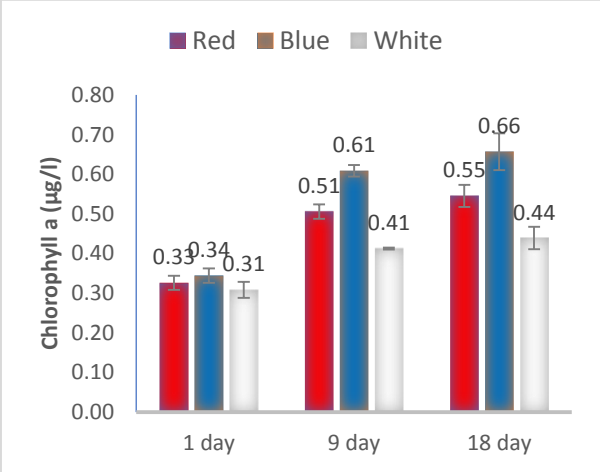

Fig 6 : Chlorophyll a (µg/l) that was incubated at 20 C° during experiment period.

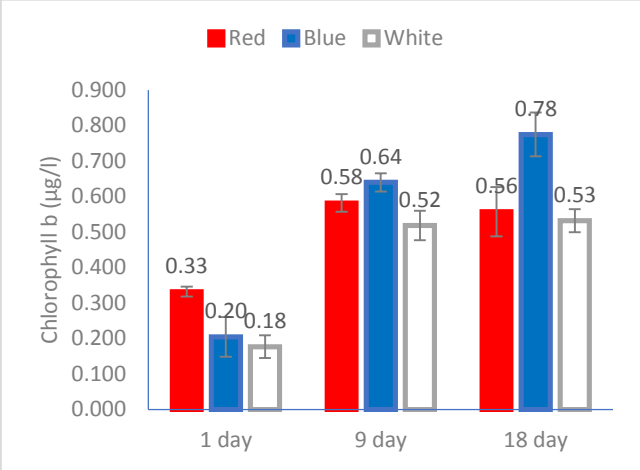

Fig7: chlorophyll b pigment concentrations(µg/l) that was incubated at 20 C° during experiment period.

The differences in chlorophyll a and chlorophyll b concentrations were mainly comparable. From the first day of inoculation until the eighteenth day, both photosynthetic pigments grew steadily and achieved their maximum values under various light wavelengths. Cultures exposed to blue light consistently had larger concentrations of both chlorophyll A and B than cultures exposed to white or red light. One significant source of pigments is microalgae. A number of variables influence how pigments build up within the cell. Numerous research have looked into these issues [32]. One of the most significant elements thought to be involved in the build-up of chlorophyll is light[33].

The quality, wavelength, and emission spectra of light affect chlorophyll production as well as algal cultivation. For this reason, the amount of chlorophyll was found to be different in Chlorella vulgaris cultures illuminated with different wavelengths (Fig. 6). The amount of total chlorophyll a and b in Chlorella vulgaris cultures illuminated with different wavelengths of light increased until the 18th day of experimentation. However, the amount of chlorophyll was higher in cultures illuminated with blue light than in those illuminated with white and red light (Figs. 6and 7). Previous

works have shown that blue light stimulates chlorophyll accumulation [33,34].

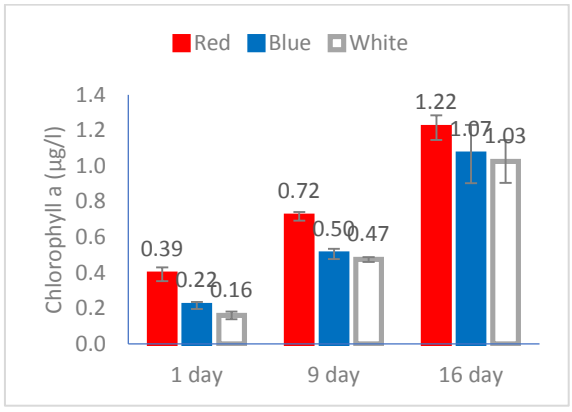

Fig8: Chlorophyll a pigment (µg/l) that was incubated at 25 C° during experiment period.

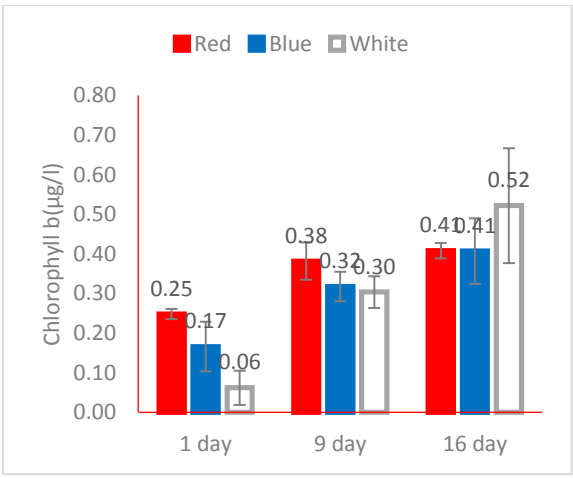

Fig9: Chlorophyll b pigment concentrations(µg/l) that was incubated at 25 C°during experiment period.

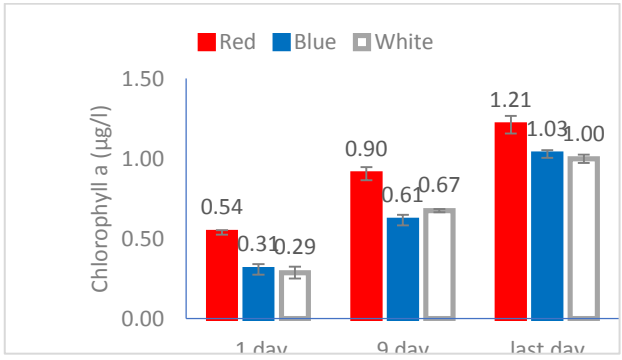

Fig10: Chlorophyll a pigment (µg/l) that was incubated at 30 C° during experiment period.

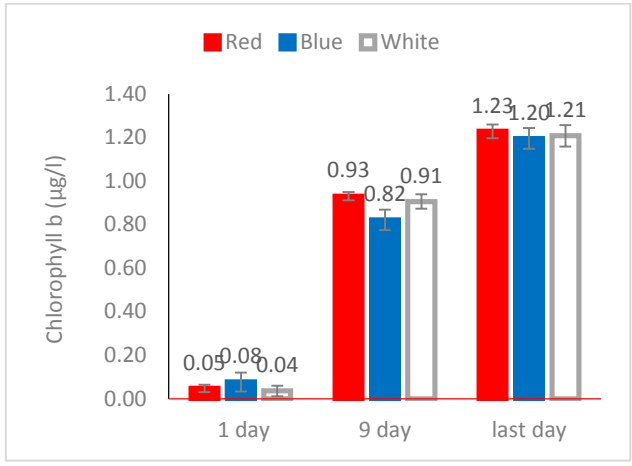

Fig 11: Chlorophyll b pigment concentrations(µg/l) that was incubated at 30 C° during experiment period.

Table(1):Correlation test between chlorophyll content and growth parameters at incubated temperature 20 C°

		Chlorophyll a	Growth rate	Duplicate time	Chlorophyll b
Chlorophyll a	Pearson Correlation	1	.842**	-.689-*	.927**
	Sig. (2-tailed)		.004	.040	.000
Growth rate	Pearson Correlation	.842**	1	-.847-**	.871**
	Sig. (2-tailed)	.004		.004	.002
Optical density	Pearson Correlation	.756*	.898**	-.686-*	.821**
	Sig. (2-tailed)	.018	.001	.041	.007
Duplicate time	Pearson Correlation	-.689-*	-.847-**	1	-.765-*
	Sig. (2-tailed)	.040	.004		.016
Chlorophyll b	Pearson Correlation	.927**	.871**	-.765-*	1
	Sig. (2-tailed)	.000	.002	.016	
	Sig. (2-tailed)	.000	.002	.020	.000
Biomass	Pearson Correlation	.646	.845**	-.679-*	.727*
	Sig. (2-tailed)	.060	.004	.044	.026

Table (2):Correlation test between chlorophyll content and growth parameters at incubated temperature 25 C°

At 25 C		Chlorophyll a	Growth rate	Duplicate time	Chlorophyll b
Chlorophyll a	Pearson Correlation	1	.808**	-.653-	.887**
	Sig. (2-tailed)		.008	.056	.001
Growth rate	Pearson Correlation	.808**	1	-.906-**	.873**
	Sig. (2-tailed)	.008		.001	.002
Optical density	Pearson Correlation	.879**	.986**	-.854-**	.883**
	Sig. (2-tailed)	.002	.000	.003	.002
Duplicate time	Pearson Correlation	-.653-	-.906-**	1	-.838-**
	Sig. (2-tailed)	.056	.001		.005
Chlorophyll b	Pearson Correlation	.887**	.873**	-.838-**	1
	Sig. (2-tailed)	.001	.002	.005	
Biomass	Pearson Correlation	.570	.691*	-.466-	.699*
	Sig. (2-tailed)	.109	.039	.207	.036

Table (3):Correlation test between chlorophyll content and growth parameters at incubated temperature 30 C°

		Chlorophyll a	Growth rate	Duplicate time	Chlorophyll b
Chlorophyll a	Pearson Correlation	1	.897**	-.865-**	.915**
	Sig. (2-tailed)		.001	.003	.001
Growth rate	Pearson Correlation	.897**	1	-.992-**	.980**
	Sig. (2-tailed)	.001		.000	.000
Optical density	Pearson Correlation	.913**	.977**	-.956-**	.990**
	Sig. (2-tailed)	.001	.000	.000	.000
Duplicate time	Pearson Correlation	-.865-**	-.992-**	1	-.961-**
	Sig. (2-tailed)	.003	.000		.000
Chlorophyll b	Pearson Correlation	.915**	.980**	-.961-**	1
	Sig. (2-tailed)	.001	.000	.000	
Biomass 30 C	Pearson Correlation	.624	.582	-.573-	.663
	Sig. (2-tailed)	.073	.100	.107	.051

Conclusions

The current study demonstrates that blue light has an effective role in the production of chlorophyll a and b at a temperature of 20 degrees Celsius, while red light is effective in producing the green pigment content at temperatures below 30 degrees Celsius. The optimal conditions for the highest productivity of *C. vulgaris* algae were observed at 30 °C with the red light wave.

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