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**The role of light intensity in stimulating lutein
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Haifaa M. Jawad, Haider A. Alghanmi



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The role of light intensity in stimulating lutein production in *Coelastrella saipanensis*

Haifaa M. Jawad, Haider A. Alghanmi

Biology Department, College of Education, University of Al-Qadisiyah, Iraq

Algae are of particular importance because they synthesize a variety of biomaterials of high commercial value, such as lipids, carbohydrates, amino acids, pigments, and carotenoids. One of the several carotenoids produced by microalgae, lutein is a very important pigment for the retina of the eye, used as a dietary supplement and pigment for diet, cosmetic, and medicinal purposes. Most ecological parameters, including light, which is an important growth regulator affecting the productivity of algal cells and synthesis and distribution of their by-products under different qualities and intensities, have great influence on lutein production and accumulation in algae. In the present study, we investigated the effect of different light irradiance (120, 100, 80, 60, and 40 $\mu\text{mol}/\text{m}^2/\text{s}$ using white LEDs) on the increment of growth and lutein content in the algae *Coelastrella saipanensis* grown in Chu 10 media at a steady condition of 28° C and pH 7. The results of the present study showed that light irradiance influences both growth efficiency and lutein content in the alga. The highest value of lutein was 0.562 $\mu\text{g}/\text{mg}$ at 80 $\mu\text{mol}/\text{m}^2/\text{s}$, while the lowest was 0.055 $\mu\text{g}/\text{mg}$ at 120 $\mu\text{mol}/\text{m}^2/\text{s}$. It can be concluded from this that *Coelastrella saipanensis* has a good production of lutein when exposed to appropriate light intensity, as these results are supported by statistical analyses at $P \leq 0.05$.

Keywords: Chlorophyta, *Coelastrella saipanensis*, light Intensities, lutein production

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Haider A. Alghanmi,
Biology Department, College of Education,
University of Al-Qadisiyah, Iraq
Email: haider.ghanmi@qu.edu.iq
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INTRODUCTION

Algae are autotrophic organisms, grow in different environmental conditions, and vary in shape and size (Badar et al., 2021). They are either unicellular with a length of about 1-0.5 microns (Skriptsova and Kalita, 2020), or multicellular macroalgae, such as kelp, which can reach a length of about 60 meters (Jayasankar, 2022). It comprises of approximately 25,000 species with the majority being eukaryotic, except the cyanophytes which are prokaryotic. Cyanophyta and Chlorophyta are two of the most important type of algae, and it is found to be very common in several environments, which include aquatic, terrestrial, and atmospheric environments, (Sahoo and Seckbach, 2015). Algae are active CO₂-reliant microorganisms with high efficiency of photoautotrophic relative to land-based vegetation, producing commercially valuable biomaterials such as pigments, amino acids, lipids, and carbohydrates (Cruz-Balladares et al., 2021). In addition, algae have higher levels of carotenoids compared to those in terrestrial plants (Gong and Bassi, 2016; Singh et al., 2019). Among the various carotenoids produced by microalgae that are in high market demand are beta-carotene, astaxanthin, and lutein, due to the fact that beta-carotene is related to vitamin A and is an industrially important coloring agent, while astaxanthin has antioxidant and anti-inflammatory characteristics useful for health care (Razz, 2024). Lutein is an important pigment for the retina (Li et al., 2020). Lutein is important for enhancing immunological defenses, improving eyesight and eye-care, and preventing cancer, diseases of the retinal

nerve, degeneration and disorders of the macula, cardiovascular diseases, arterial sclerosis, coronary heart disease (acute and chronic), apoplexy, retinopathy of diabetes, diseases of Alzheimer's, and many more (Chen et al., 2019a). Based on its recognized bioavailability as an effective bio-antioxidant and anti-inflammatory agent, lutein is widely used as a source of natural food color and as a dietary pigment in the food, cosmetic, and pharma industries (Muhammad et al., 2024). Lutein is a xanthophyll of the oxygenated carotenoids, found in high amounts in vegetables and algae, it is a yellowish-orange crystalline and lipophilic pigment (Mora-Gutierrez et al., 2018). Microalgae, because of their superior lutein content, short-term lifespan, and resistance to seasonal fluctuations, have been recognized as an alternative source of lutein production (Zheng et al., 2022).

The genus *Coelastrella* sp. was discovered almost a century ago and contains only a few species. It is a unicellular green alga, or rarely in small groups, its cells are oval to lemon shaped. The cell wall of *Coelastrella* species bears 16-40 longitudinal ribs, sometimes with polar thickenings. In culture media, this species accumulates several carotenoids, especially in the stationary phase, such as neoxanthin, pheophytin A, astaxanthin, canthaxanthin, lutein and violaxanthin (Goecke et al., 2020). This pigment profile is a very important taxonomic feature of microalgae (Serive et al., 2017), and in this case, these pigments support the classification of the genus *Coelastrella* sp. (Ali et al., 2022). Also, Chang et al. (2021) proposed that

Coelastrella sp. has been a candidate for the commercial production of carotenoids, which are important in many medical, pharmaceutical and therapeutic aspects. Lutein is the major xanthophyll in the green algae and is tightly bound to the light harvester apparatus of the photosynthesizing machinery. In *Chlamydomonas reinhardtii*, it was noted that the pigment is responsible for maintaining the light-harvesting apparatus against oxidative damage brought about by high intensity light (Erickson et al., 2015). Therefore, lutein is crucial for microalgae to adapt to varying regimes of light (Camarena-Bernard and Pozzobon, 2024). The production and enrichment of algal carotenoids is influenced by light intensity; however, the effects of light intensity vary between different microorganisms, regardless of whether an increase or decrease in light duration or intensity results in improved carotenoid productivity (Maltsev et al., 2021). Light quality and intensity is considered an important growth factor and has fundamental effects on algal cells in terms of productivity, synthesis and diffusion of their by-products (Wu et al., 2024). Several investigations have proven that light sources have strong impacts on cell growth rate, productivity and pigment formation in algae, as different algal species respond differently to light spectra (Saefurahman, 2015; Singh and Singh, 2015).

Light intensity is considered one of the most important factors that influence carotenoid production in microalgae, according to (Xie et al., 2013b). Different algal species respond differently to light regimes, and changes in different photoperiods affect productivity and efficiency (Grobbelaar, 2009). Many scientists have observed that the length of light and darkness affects microalgae development and its biochemical components, where the optimal length of light increased the efficiency of photosynthesis, promoting lutein formation. (Krzemińska et al., 2014; Chen et al., 2019b). In addition, the use of optimized photoperiods instead of continuous lighting is able to save energy, since the majority of the researchers employ 16:8-hour light-dark periods in their studies (McClure et al., 2019; Rajendran et al., 2020). Light intensity for ideal formation of lutein within the algae is strain-dependent for microalgae, hence microalgae *Tetraselmis* sp. reached an increased lutein content under optimal light conditions (Schüler et al., 2020). Due to the lack of studies on the lutein pigment produced by *Coelastrella saipanensis* and the influence of environmental factors on it, therefore, the current study aimed to investigate the effect of

light intensity on lutein production by *Coelastrella saipanensis*.

Algal sampling and purification

Water samples were collected from the Al Diwaniyah River in central Iraq (31° 59' 34.40" N, 44° 55' 31.87" E), and for the purpose of obtaining a unialgal culture, the method of streaking agar plates described by (Stein-Taylor, 1973) was used, placing 1 ml of the sample containing algae in one edge of a Petri dish containing the previously prepared Chu 10 nutrient medium solidified with agar. Using a sterile loop of inoculum sterilized by fire with a burner lamp, cool it at the edge of the dish, then use it to spread the algae sample in the form of lines on the agar in all directions. When growth appears, a sample is taken from the edge of the dish containing the target algae and the streaking method is repeated several times until a single agar culture is obtained. In order to obtain an axenic culture of the algae under study, the method of density gradient centrifugation was used, it was followed for 10ml of liquid from the unialgal culture to be purified were placed in a 15 ml thick-walled centrifuge tube and placed in centrifuge for 3000 rpm for about 5 minutes, the supernatant was discarded and the precipitate was washed with sterilized distilled water and the procedure was repeated as 12 times minimally (Wiedeman et al., 1964). The final test was the inoculation of a sample of the algal culture on the solid culture medium Nutrient Agar for bacterial testing, incubated at 37°C for (72 hours) to ensure that the culture is free of bacteria and fungi (Andersen, 2005). The identification of *Coelastrella saipanensis* was based on compound light microscopy and taxonomic keys (Wang et al., 2019 a,b).

Algal cultivation and biomass production

Pure culture from *Coelastrella saipanensis* under study as shown in (Figure 1) was obtained from the Advanced Environment Laboratory at the University of Al-Qadisiyah. For biomass production, the algal isolates which was isolated from water and identified according to Hanagata (2001) were cultivated into a 500 ml sterile flask containing 400 ml of Chu10 medium (Chu, 1942) adapted from (Kassim et al., 1999). To grow and propagate green algae cultures, and incubated at 25°C and 40 µmol/m²/s light intensity, and the algal samples and were incubated until use about 10 days after cultivation (Tredici, 2004).

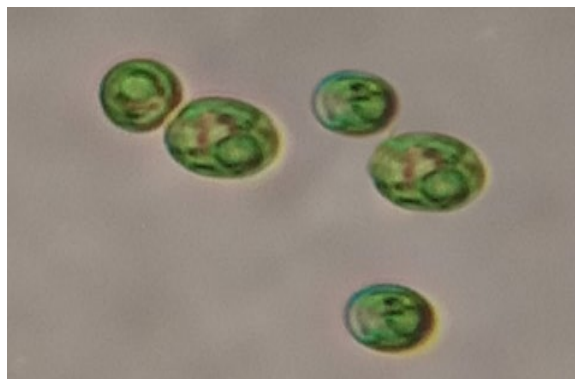


Figure 1. Photograph of *Coelastrella saipanensis* at 40x magnification Using light microscope.

Light intensity treatment

For light treatments, algal isolates were grown under various light intensities (40, 60, 80, 100, 120) $\mu\text{mol}/\text{m}^2/\text{s}$ using white LEDs to study its effect on lutein synthesis of *Coelastrella saipanensis* grown in Chu10 medium at 28°C, pH 7.2 and 16:8 light: dark period.

Estimation of chlorophyll a

Chlorophyll a concentration were determined according to Jeffrey and Humphrey (1975), where 10 ml from the algal sample were centrifuged at 5000 rpm for 5 min., the supernatant was discarded, the debris of algal cell were collected and washed with distilled sterile water for several consecutive times, this process of precipitating and washing is repeated numerous consecutive times in order to dispose of the supernatant and take the precipitate representing the algae, adding 5 ml of acetone (90%) V / V vortex for (90) seconds, then put in a shaker water bath at 25°C for one hour or more until it turns white. If the algae precipitate doesn't turn white, repeat the process, then centrifuge at 6000 rpm for 10 min and collect the filtrate to measure the optical density at wavelengths (664, 647, and 630 nm) using a spectrophotometer according to the chlorophyll 'a' were calculated using the following equation.

Chlorophyll a $\mu\text{g}/\text{L} = 11.85 \text{ E}_{664} - 1.54 \text{ E}_{647} - 0.08 \text{ E}_{630}$

Lutein quantification by HPLC

Lutein values was estimated by an HPLC device adapted by (Xu et al., 2023); A C18 column, 5 μm 250 x 4.6 mm was eluted using acetone and water as a mobile phase. The chromatogram was recorded on 450 nm at flow rate 1.0 mL / min. Identification of lutein was confirmed by comparability of its retention

time and absorption spectra with a lutein standard (Sigma Aldrich, 07168-5MG). Quantification of lutein amount was performed from calibration curve constructed from the peak area of the standard according to the following formula (Figure 2).

Statistical analysis

For statistical analysis, the LSD ANOVA test was applied to compare the treatments regarding different light intensities and white light and their impacts on lutein production by the studied algae.

RESULTS AND DISCUSSION

Growth determination

The growth curve of *Coelastrella saipanensis* is given by the chlorophyll 'a' content in terms of absorbency values and represented in Figure 3. It was observed that the exponential growth phase of the algae began after the fourth day and the growth increased and continued until it reached the stationary phase after the tenth day, but the growth decreased after the 13th day and continued until the death of the algae. Since the findings showed that the highest growth rate was detected when the algae were exposed to a light intensity of 80 $\mu\text{mol}/\text{m}^2/\text{s}$, while the lowest growth rate was registered when the algae was exposed to 40 and 120 $\mu\text{mol}/\text{m}^2/\text{s}$, respectively.

Lutein production

The results of lutein production from *Coelastrella saipanensis* under different light intensity conditions are tabulated in a table1 from which it was concluded that the maximum value of lutein was 0.562 $\mu\text{g}/\text{mg}$ at 80 $\mu\text{mol}/\text{m}^2/\text{s}$ and the lowest value was 0.055 $\mu\text{g}/\text{mg}$ at 120 $\mu\text{mol}/\text{m}^2/\text{s}$, as shown in Table 1. there was a significant difference between the various light intensities used in the experiment and their impact on lutein production at $P \leq 0.05$. Light is closely related to microalgal photosynthesis under different growth conditions and therefore considered a critical variable that will affect cell development and lutein accumulation (Dinh et al., 2022). Lutein production in algae differs not only between species, but even among various environmental parameters, such as light intensity and culture media composition (Yeh et al., 2017). Among the physicochemical factors affecting lutein biosynthesis in microalgae, light intensity, carbon dioxide and several other components such as nitrate, manganese, and copper are the main parameters that significantly affect the growth rate and lutein accumulation (Dineshkumar et al., 2015).

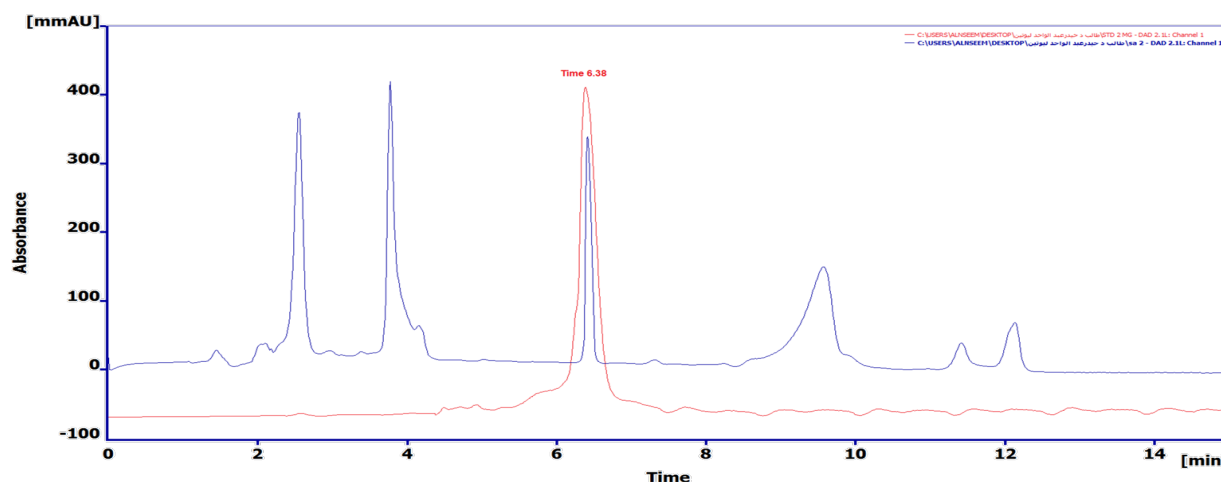


Figure 2. *Coelastrella saipanensis* produced lutein (blue color), as confirmed by its identical retention time (6.38 minutes) and absorption spectrum to the lutein standard (red color).

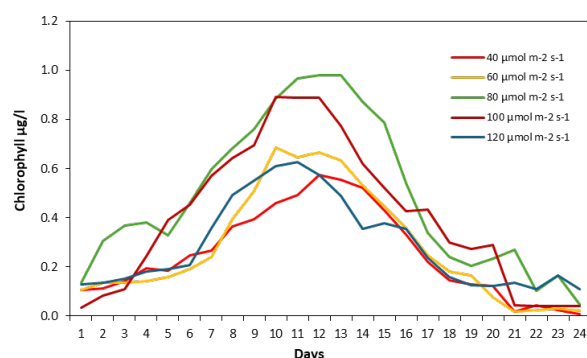


Figure 3. Growth curve related to chlorophyll a of *Coelastrella saipanensis*.

Table 1. Lutein production by *Coelastrella saipanensis* at different light intensities

Light Intensities $\mu\text{mol m}^{-2} \text{s}^{-1}$	Lutein $\mu\text{g/mg}$
40	0.085 ± 0.006 ^{CD}
60	0.184 ± 0.040 ^C
80	0.562 ± 0.062 ^A
100	0.443 ± 0.051 ^B
120	0.055 ± 0.006 ^D
LSD	0.103

The results of the present study showed that the optimal lutein Production of *Coelastrella saipanensis* occurred at $80 \mu\text{mol/m}^2/\text{s}$, which may be due to the optimum light intensity for the growth of algae. Lutein biosynthesis can be optimized by providing appropriate and optimal light intensity since the photosynthetic antenna complexes contain the lutein component (Leong and Chang, 2023). In most studies related to light intensity, the effect on lutein production in various algal species showed that increased lutein content can be observed at higher

light intensities compared to low irradiance (Xie, 2015). The results obtained by Sibi et al. (2020) in his study of different low, medium and high light intensities $50, 125, 160, 268, 300 \mu\text{E/m}^2/\text{sec}$, showed that optimal growth conditions for carotenoid produced by *Chlorella vulgaris* were at $160 \mu\text{E/m}^2/\text{sec}$.

Also, light ratios necessary for the greatest algal productivity and high carotenoid content photovoltaic systems since in *C. vulgaris* light intensity at $160 \mu\text{E/m}^2/\text{sec}$ was suitable for a good proportion of lutein which was 8.8%, with the production of astaxanthin which was 4.6% and Beta-carotene 3.9% (Nwoba et al., 2020). Xie et al. (2013a) investigated the effect of light intensity on lutein accumulation in *Desmodemus* sp. F51 cultured under different light intensities. Highest amount of lutein value was 5.05 mg/g at light intensity of $600 \mu\text{mol/m}^2/\text{s}$, and lutein yield decreased at higher and lower light intensities than $600 \mu\text{mol/m}^2/\text{s}$. This means that $600 \mu\text{mol/m}^2/\text{s}$ is an appropriate light intensity for lutein accumulation. In a study by Gayathri et al. (2021), the effect of different light intensities, 100, 200, and $400 \mu\text{mol/m}^2/\text{s}$, on *Chlorella salina*, was studied and observed that $200 \mu\text{mol/m}^2/\text{s}$ was very suitable for lutein generation, reaching a maximum level of 30% of dry weight, which was twice as high as that found at low light intensities.

Lutein content started to decrease when the light intensity was exceeded above $200 \mu\text{mol/m}^2/\text{s}$. Furthermore, illustrated that an abrupt variation of light intensity from low, high to medium or moderate increased the lutein content in the microalgae

Coccomyxa onubensis (Vaquero et al., 2014). The present study also showed that lutein also decreased under low and high exposure levels, which can be associated with the fact that less oxygen radicals are produced under low irradiance and when under high exposure, cells cannot manage to utilize all the energy produced, so a drop of lutein may be a result from the functional group in lutein molecule, as found by (Guedes et al., 2011). Moreover, low light limits photosynthesis, while high light hampers cell division with lysis of cells since the photosynthetic apparatus is impaired by excess light (Sibi et al., 2020).

Low and high light intensities affect microalgae cell growth and production of lutein, low light due to photoperiodic limitation, and high light due to photosynthetic inhibition (Dineshkumar et al., 2016).

On the other hand, in the present study, a decrease in lutein content in algae exposed to 120 $\mu\text{mol}/\text{m}^2/\text{s}$ may be due to increased oxidative stress. Indeed, it has been reported that under conditions of high light intensity, microalgae suffer increased oxidative stress. This decreases key reaction center proteins, photosynthetic rates, respiration rates, photochemical performance associated with a deterioration of photosystem II (Li et al., 2011). Photooxidative stress caused by light intensity fluctuation and environmental stressful agents may damage the photosynthesis mechanism of chloroplasts (Ledford and Niyogi, 2005).

Mild light stress can irreparably injure the physiology of algal cells, causing reduced biomass production (Carvalho et al., 2011). In some cases, algae do not need high light intensities throughout the growth phase because of light inhibition, as the dose of light exceeds the absorptive capacities of the chloroplasts and reach the light saturation point (Han et al., 2000). Also, the excessive light can result in photo-saturation, photoinhibition or photo-oxidation leading to cell damage, in same time led to carotenoids accumulate to avoid photooxidative damage (Toyoshima et al., 2020). In green algae, lutein is the major carotenoid with additional light-harvesting and light-protecting functions. Many researchers have suggested that the decline in microalgal content of lutein at high light intensity of the medium is due to the decrease in the magnitude of the light-harvesting complex, which is the storage and synthesis site of lutein (Mulders et al., 2014). Ma et al. (2020) showed that, even though the higher light intensity resulted in higher biomass concentrations for some species of algae, lutein

formation was inversely related to light intensity, likely due to a light suppression action where the size of the light-harvesting complexes was reduced at higher light intensities.

Heo et al. (2018) reported that a decline in the lutein content in *Tetraselmis* sp. and *Schizochlamydeella* sp. with the increase in light intensities. It is further confirmed by the work of (Del Campo et al., 2004), where lutein content in *Chlorella zofingensis* diminished by 50% due to increased light intensity from 90 $\mu\text{mol}/\text{m}^2/\text{s}$ to 460 and 920 $\mu\text{mol}/\text{m}^2/\text{s}$, respectively. Ho et al. (2014) showed that the *Scenedesmus obliquus* cells developed highly with increased light intensity, while lutein contents declined. Chen and Liu (2018) found that the maximum yield of lutein by *Chlorella sorokiniana* was 4.1 mg/L/d at a light intensity of 150 $\mu\text{mol}/\text{m}^2/\text{s}$, and at higher light intensities it decreased slightly.

CONCLUSION

This study concludes that light intensity has an appreciable effect on lutein production. Thus, 80 $\mu\text{mol}/\text{m}^2/\text{s}$ light intensity gave the best growth and productivity of lutein in *Coelastrella saipanensis* and is considered ideal and suitable intensity while high and low light intensities resulted in low lutein content.

CONFLICT OF INTEREST

There is no conflict of interest

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