Influence of Different Light Intensities on the β-carotene Production by Green Alga Coelastrella oocystiformis

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Abstract

Microalgae are a broad, underutilized source of beneficial compounds to the pharmaceutical industry with both preventive and therapeutic applications in pharmacy and medicine. The concentration of β-carotene in algae is heavily influenced by light intensity. The present work investigated the varying light intensity's effect (26, 43, 60, 86) µmol m-2 s-1 on the β-carotene composition and growth rate of the green algae Coelastrella oocystiformis cultured in Chu-10 medium with a fixed temperature of 28°C. In addition, the green algae Coelastrella oocystiformis, one of the Chlorophyta species, are distinguished by a spindle-shaped with pointed ends. In contrast, the mature cells are usually ellipsoid or oval. The results showed that the light intensity that reaches the algae positively impacts its pigment concentration and growth rate. As a result, the greater concentration of β-carotene reached 0.33 µg/mg was observed at 60 µmol m-2 s-1, while the smaller concentration of β-carotene at 0.04 µg/mg was detected at 26 µmol m-2 s-1. This resulted in the optimum light intensity, leading to a good production of β-carotene by alga understudy. Also, the statistical analyses at $P<0.05$ supported these consequences. Besides, the BLAST findings of microalgae 18S rRNA gene sequences indicated high percentage similarities 99% that showed the species Coelastrella oocystiformis strain SAG 277-1. The isolate Coelastrella oocystiformis with accession number: MW929196.1. is deposited in the NCBI GenBank.

Keywords: *light intensity, Chlorophyta, Coelastrella oocystiformis, β-carotene.*

1. INTRODUCTION

Algae are an organisms group known since ancient civilizations. They are autotrophic organisms due to their photosynthesis as a result of their nature of containing the pigment chlorophyll. They are often aquatic, while few live on land (Sahoo & Seckbach, 2015). They used light, energy, carbon dioxide (CO2), and ions dissolved in water to manufacture complex compounds and produce biomass. They are spread in various environments, whether in the seas, freshwater, transitional water, or the thin layer (Tomaselli, 2004).

Algae are composed of about 25,000 species, most of which are (eukaryotic) except the blue-green Alga (Cyanobacteria) (Sahoo & Seckbach, 2015). For hundreds of millions of years, algae have been acting as a crucial part of the preservation of the world ecosystem. They generate oxygen during photosynthesis, capture large quantities of dehydrating atmospheric carbon dioxide in the ocean and provide food for the rest of its life (Leliaert et al., 2011). In addition, green algae produce bioactive compounds, some of which are rich in fats and proteins. Therefore, edible algae have been considered a complete food as they

promote the proper ratio of carbohydrates, proteins, minerals, and vitamins. It has also been utilized in medicine for generations, not just as food but also in other industrial uses, extracts in food, and cosmetics. Its therapeutic and medicinal properties relate to its antioxidant, anti-cancer, anti-viral properties and its therapeutic properties in promoting health (Pooja, 2014).

Algae contain carotenoids, granules that give red, orange, or yellow color that does not dissolve in water and dissolve in alcohol, acetone, and others. Carotenoids are usually found inside the plastid and can be divided into two groups, orange; either in the form of β-carotene, alpha (α) or gamma (γ) and yellow xanthophylls (Sahoo & Seckbach, 2015). Also, β-carotene is one of the crucial natural carotenoid types made by microorganisms and plants such as yeasts, as it reaches about 75% of the total carotene (Eldahshan & Singab, 2013).

Interest in carotenoids has expanded considerably recently due to the abundance of evidence revealing their significance and advantages for human health. It is a vitamin A source in the body as it is converted in the human intestine, which is essential to protect eyesight. This is because it is an antioxidant substance that guards the body against free radicals, improves the body's immune system, and contributes to stimulating cell growth as well as differentiation. It is also highly important and has many uses as it is used in medicines, food supplements, and cosmetics (Johnson & Schroeder, 1996). The algae biochemical composition and growth are influenced by nutrients, salinity, temperature, light, as well as pH. As a result, carotenoids are also affected by these environmental factors (Seyfabadi et al., 2011).

Photosynthesis, growth, lipid accumulation, fatty acid composition, and carotene content are all optimized differently in distinct microalgae's taxonomic groups, in addition to different strains of the same species. Light is required for microalgae to grow photosynthetically. From a biological standpoint, spectral composition, light intensity, frequency of illumination, and duration can all be utilized to regulate microalgae metabolism, production, and growth of valuable chemicals such as Beta carotene, fatty acid, lipids (Maltsev et al., 2021). In both terrestrial plants and microalgae, the wavelength dependency of carotenoid-related gene translation and the carotenoid biosynthesis's light-mediated regulation (inclusive of the dark/light cycle) were examined. The light intensity regulates the β-carotene accumulation, facilitating Euglena gracilis acclimate to the light environment in night and day settings (Tanno et al., 2020).

Carotenoids are required for photosynthesis, light-harvesting, and light protection in photosynthetic systems. Different wavelengths of light alter the photosystems' structure and composition, enabling narrow-band optical spectra to be used to control carotenoid composition and concentrations in photosystems (Frede et al., 2019).

Light is among the crucial factors influencing the algae biochemical and growth composition, increasing the efficiency of β-carotene. Furthermore, Wu et al. (2016) noted that increasing the intensity of light with a decrease in the concentration of nitrates leads to an increment in the β-carotene aggregation in green Algae Dunaliella Salina. Also, Gayathri et al. (2020) used the response surface method to investigate the interaction and impact of lighting cycle (photoperiod), light irradiance strength (light intensities), as well as aeration

rate on lutein (carotenoids) production activity and biomass concentration of the green alga Chlorella salina in an enclosed, small-scale airlift photobioreactor. They found that light intensity and aeration rate have a substantial impact on concentration of cell, although a simultaneous rise in light intensity resulted in a noticeable fall in lutein content.

In general, the agricultural mass system is affected by light. For this reason, light is easily absorbed, and it is dispersed through the algae cells. According to research, algae's capacity to photosynthesize is influenced by both major variations in dye content and the number of thylakoids (Danesi et al., 2004; Jeon et al., 2006; Khotimchenko & Yakovleva, 2005; Litchman et al., 2003). This present study aimed to evaluate the light's impact on β-carotene synthesis by locally isolating algae Coelastrella oocystiformis under different light intensities.

2. MATERIALS AND METHODS

2.1 Identification and isolation

Samples from river in Al Diwaniyah city south of Iraq are collected to isolate Coelastrella oocystiformis that belongs to the Chlorophyta class. In order to be able to isolate a single alga (unialgal culture), the research followed the dilution method described by Stein (1973). Then, to obtain the axenic culture of the study, a treatment method was performed on the alga with antibiotics as well as to get an isolate free of contamination, according to Andersen (2005). The algal was then relocated to a 500 mL sterile glass flask consisting of 100 mL Chu 10 sterilize medium (Kassim et al., 1999). Subsequently, it was incubated in a growth chamber at 25 µmol m-2 s-1 and 25°C with photoperiodic 16:8. The alga was identified according to morphological features by some taxonomies key (Guiry, 2020) and genetically identified using the 18S rRNA gene. For light treatments, different light intensities (26, 43, 60, 86) µmol m-2 s-1 was utilized to assess their effects on the β-carotene composition and growth rate of the green algae Coelastrella oocystiformis cultured in Chu-10 medium with constant temperature of 28°C, pH of 7.2 and 16:8 preperiodic.

2.2 Genomic DNA extraction, PCR Thermocycler and DNA Sequencing

For 18S rRNA gene analysis and sequencing, the microalgal strain's genomic DNA was extracted utilizing a DNA Mini Bacteria Kit, liquid nitrogen, and glass beads (Geneaid, Korea) as per the guidelines of its manufacturer. Chloro-R (5-GAATCAACCTGACAAGGCAAC-3), PCR master mix (Bioneer, Korea) and 20 µl reaction tube with primers for green alga Chloro-F (5-TGGCCTATCTTGTTGGTCTGT-3) (Valiente Moro et al., 2009) were employed for the PCR amplification, which was conducted utilizing conventional PCR Thermal Cycler Bio-Rad T100, USA. First, there is the initial denaturation step (5 min at 95°C), followed by 35 incubation cycles, each comprising of 1 min at 95°C, 1 min at 72°C, and 1 min at 60°C. The final step includes an extension at 72ºC for 5 min.

The PCR products sequencing, in which the PCR products were purified from agarose gel by utilizing (EZ-10 Spin Column DNA Gel Extraction Kit, Biobasic. Canada). Afterwards, the purified PCR products samples were transported to Macrogen Company in Korea, where DNA sequencing was conducted on AB DNA sequencing equipment. By utilizing the BLASTN search, the isolate's 18S rRNA gene sequence was differentiated to 18S rRNA gene sequences accessible on the NCBI site. In addition, MEGA X. was employed to examine

a phylogenetic tree and multiple sequence alignment (Molecular Evolutionary Genetics Analysis).

2.3 Chlorophyll-a Estimation

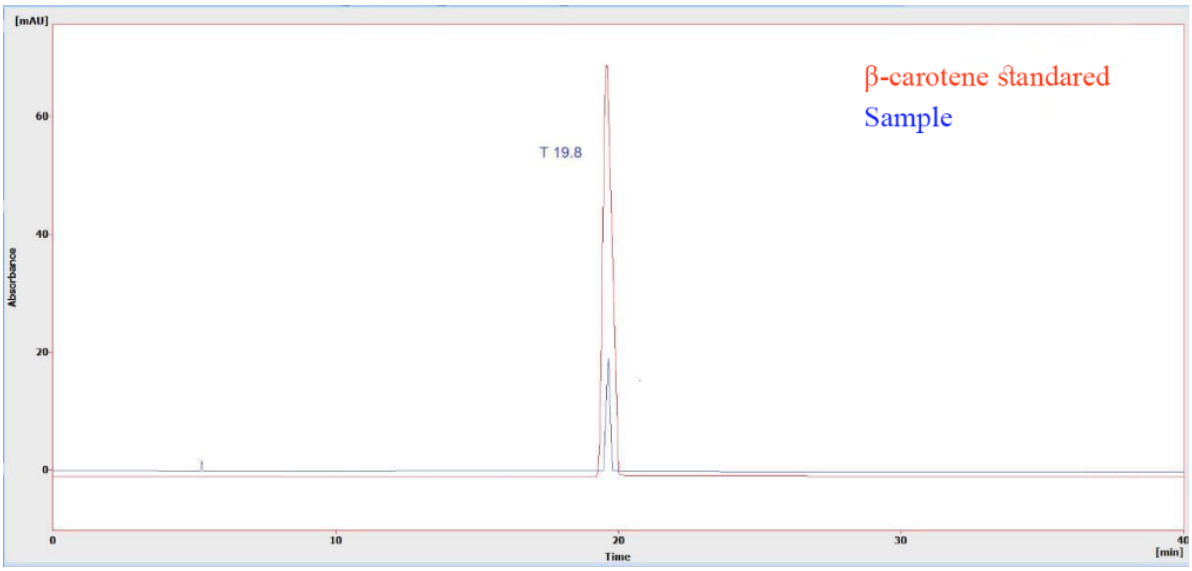
About 10 mL of Coelastrella oocystiformis culture was centrifuged for 5 min at 5000 cycles/min to evaluate the chlorophyll-a content of the alga. By using ddH2O, the process was repeated numerous times. Then, the supernatant was extracted, and 5 mL of 90% acetone was added to the precipitate, whirled for 90 sec, and put in a water bath at 25°C. Following that, the supernatant was centrifuged for 10 min at 6000 cycles/min, and it was calculated by utilizing a spectrophotometer at varying wavelengths (664, 647, 630) nm. The (Jeffrey & Humphrey, 1975) equation was used to calculate chlorophyll a.

Chlorophyll a µg/mL=11.85 E664 - 1.54 E647 - 0.08 E630

2.4 Carotenoids Estimation

The extraction, detection, and quantitation of β-carotene were performed according to (Barba et al., 2006). The separation on a C18 column (Knauer, Germany) (250 * 4.6 nm I.D., 5 µm particle size, 80 Å pore size) mobile phases were methanol/ACN (90/10 v/v) +TEA 9 lM, flow rate 1 mL/min detection on 475 nm. Each compound was identified by matching the absorbance spectrum and retention time of the standards, which were purchased from Sigma Aldrich Company (C9750-10G) (Fig.1). Standard external materials' serial concentrations calculated the concentration to construct a calibration curve between concentration as well as its corresponding peak area.

Fig. 1 matching of β-carotene standard (red color) and β-carotene of algal samples (blue color) with retention time 19.8 by HPLC device



2.5 Statistical analysis

Least significant differences (LSD) and One-way Analysis of Variance (ANOVA) were used in the statistical analysis between four light intensity and their effect on β-carotene production by algae under study.

3. RESULTS AND DISCUSSION

3.1 Morphological and genetical identification

The morphological analysis findings showed that the alga under study belongs to the class of Chlorophyceae, which was Coelastrella oocystiformis (Fig. 2). In addition, the genotypical analysis revealed that sequences of strains under investigation were related closely to the green algae sequences found in the NCBI site. The BLAST findings of microalgae 18S rRNA gene sequences displayed high percentage similarities of 99% that showed the species Coelastrella oocystiformis strain SAG 277-1. The isolated Coelastrella oocystiformis with accession number: MW929196.1. is deposited in the NCBI GenBank. At the same time, the phylogenetic tree analysis by the MEGAX program showed a match ratio of about 57% with the same algae (Fig. 3). Currently, molecular techniques are employed in a variety of fields. They enhanced the level of trust in investigations besides morphological taxonomy in the classification of algae (Kadar et al., 2018). This explained that it could depend on genetic information and phylogenetic investigations. A molecular marker is useful for distinguishing and accurately detecting microalgae at the lowest taxonomic level. Additionally, the conventional techniques that focus on morphological features may not be able to accurately characterize the species type with high sensitivity. This is why molecular approaches should be employed in species identification, as more new insights and accurate findings on the phylogeny of algae are provided by molecular data (Soylu & Gönülol, 2012).

Fig. 2 Light microscopic view of algal strain identified as Coelastrella oocystiformis at magnification 40x

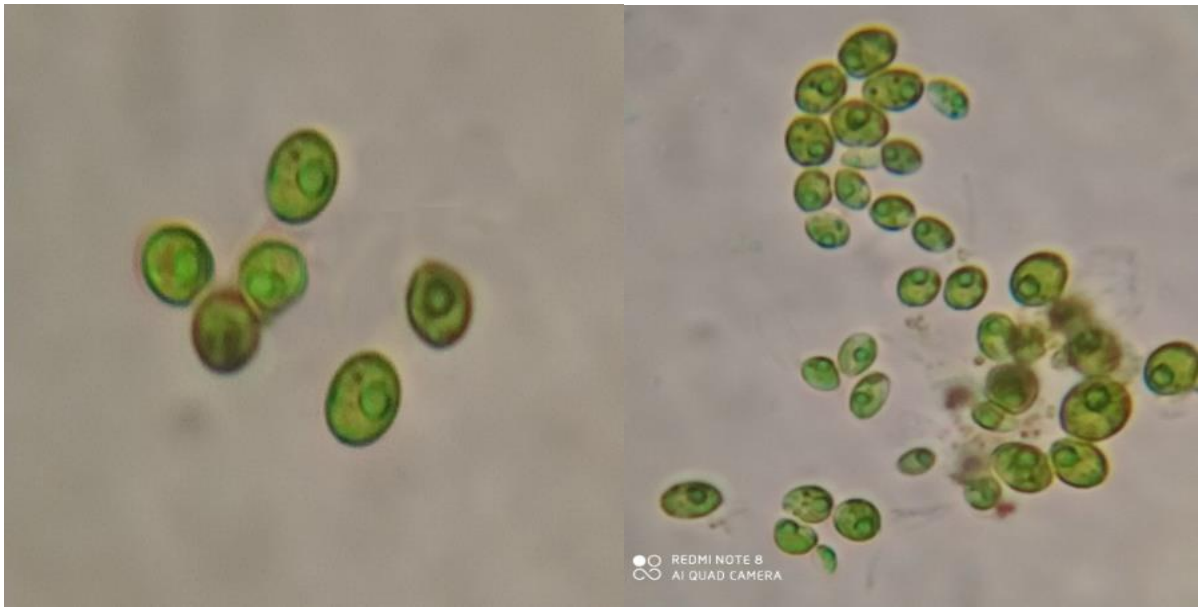
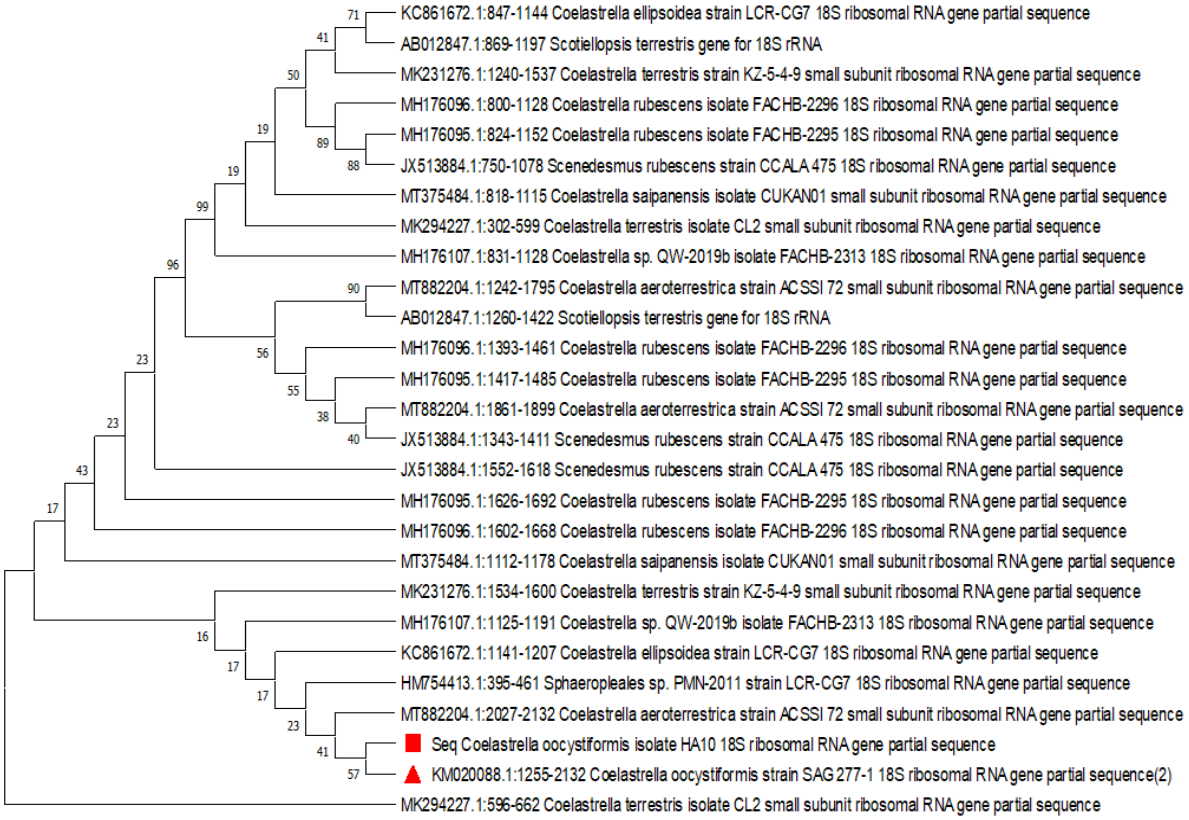


Fig. 3 Dendrogram phylogenetic UPGMA tree of green alga Coelastrella oocystiformis assessed utilizing MEGAX program at Bootstrap 100.



3.2 Chlorophyll and β-carotene production

The growth curve was estimated by chlorophyll a concentration (Figure 4). The algae give a good biomass production under four light intensities. Also, β-carotene production of Coelastrella oocystiformis has been investigated (Fig. 5 and Table 1). The results showed that β-carotene composition registered a high value at light intensity 60 µmol m-2 s-1, meanwhile the lowest value was documented at 26 µmol m-2 s-1. Additionally, it was noted that the light intensity greater than 60 µmol m-2 s-1, specifically 80 µmol m-2 s-1, substantially decreased β-carotene production.

Moreover, Xie et al. (2020) mentioned that high light settings resulted in a large boost in β-carotene concentrations (46 µmol m-2 s-1). All photosynthetic organisms produce carotenoids, which play an essential role in light-harvesting, photoprotection, and photosystem assembly (Li et al., 2009). Also, it was reported that the optimum light intensity to accumulate β-carotene in green algae Coelastrella striolata was 65 µmol m-2 s-1 (Abe et al., 2007). At the same time, another study showed that light, as well as optimal temperature for growing algae, were 22°C and 135.3 µmol m-2 s-1. Meanwhile, the greatest level of β-carotene (117.99 mg L-1) was produced at 22°C and 245.6 µmol m-2 s-1 conditions in three Dunaliella salina strains (Wu et al., 2016). Furthermore, Singh et al. (2019) discovered a 3.5 mM phosphate and 10 mM nitrate, irradiated with blue light having 60 photon mol m-2 s-1 with 0.17 mM salinity, these were the

most favorable conditions for carotenoid synthesis.

Apart from that, Seyfabadi et al. (2011) found that the highest amount of β-carotene (0.07 pg cell−1) was achieved at 100 µmol m-2 s-1. However, from a practical standpoint, the 16:8-h light/dark photoperiod and light regime of 62.5 µmol m-2 s-1 might be more advantageous compared to the other regimes because cell number is maintained longer in the exponential phase. They described their research's outcomes as highlighting the significance of regulating photoperiod and irradiance in phytoplankton cultures. This is because, based on the species, these factors might alter metabolic processes. As a result, microalgae's nutritional and composition values are crucial to be considered in aquaculture techniques.

Carotenoids have long been known to play an important influence in photosynthetic light-harvesting. In addition, carotenoids may also protect cells from damage caused by excessive light intensity. As a result, the light was thought to be a significant element in algal development and beta-carotene accumulation (Stamatakis et al., 2014). Also, Tanno et al. (2020) proposed that the light intensity controls the buildup of β-carotene, which might help Euglena gracilis acclimate to the light environment in day-night situations. According to Ben-Amotz (1987), increasing light intensity in this range had a lower effect on biomass increase but a strong effect on β-carotene accumulation.

Photosynthesis is driven by light, yet it can also be destructive. To adapt to intense light, oxygenic photosynthetic organisms have been cultivated in several photoprotective mechanisms. In most algae and higher plants, the zeaxanthin and β-carotene concentrations substantially rose due to greater light conditions in Pyropia yezoensis (Bangiales, Rhodophyta) sporophytes. In order to assist in light-harvesting, low-light plants formed bigger photosynthetic units, while highlight plants produced lesser photosynthetic units to avoid photodamage (Major & Dunton, 2002).

Fig. 4 The result of various light intensities on Chlorophyll a concentration of Coelastrella oocystiformis

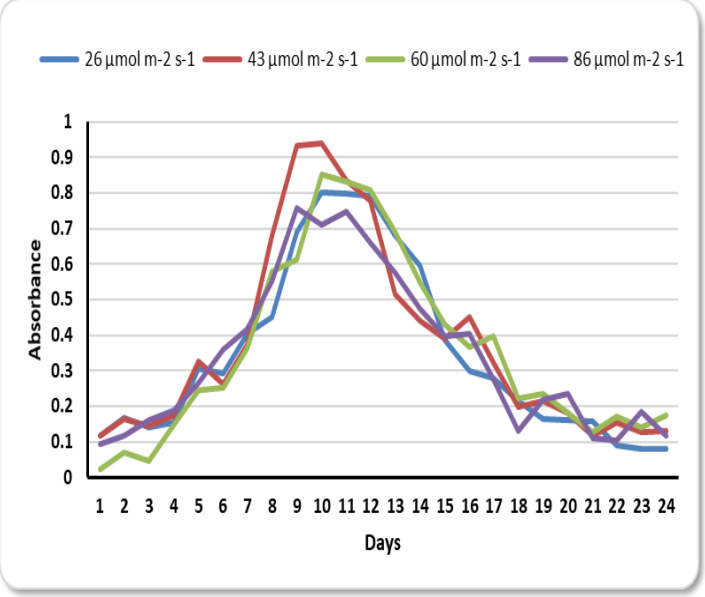
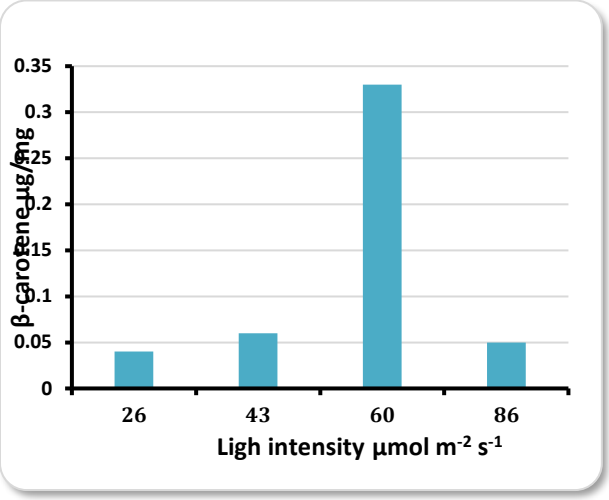


Table 1. The result of various light intensities on β-carotene production by Coelastrella oocystiformis

Light intensity µmol m^{-2} s^{-1}	26	43	60	86	LSD
β-carotene µg/mg	0.04±0.00058 b	0.06±0.003 a	0.33±0.0057 b	0.05±0.00067 b	0.024

lowercase letters indicate the differences between the light intensities

Fig. 5 Result of different light intensities on β-carotene production by Coelastrella oocystiformis



4. CONCLUSION

The findings of this investigation revealed that light is critical for β-carotene accumulation. Low light intensity enhanced production of β-carotene, and an increase in the light intensity promoted its production further. Coelastrella oocystiformis generated the highest amount of β-carotene when the light intensity was at 60 µmol m-2 s-1.

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