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Zeaxanthin Production by the Cyanobacterium *Oscillatoria amoena* Under Different Light Intensities

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ABSTRACT

Microalgae have attracted particular interest since they represent a rich biological resource for many applications and one of the most promising natural sources of zeaxanthin, which is used in a wide range of pharmaceutical and food applications. Environmental factors such as light affect the production and accumulation of zeaxanthin in cyanobacteria. This study investigated the effect of different light intensities (40, 60, 80, 100 and 120 µmol m-2 s-1) on growth rate and zeaxanthin production in the cyanobacterium *O. amoena*.The results of zeaxanthin production by *O. amoena* showed that the highest value of zeaxanthin was 0.9390µg/ mg, which was recorded at a light intensity of 80µmol m-2 s-1, while the lowest value (0.0807µg/ mg) was recorded at 40µmol m-2 s-1. Based on the research results, it can be deduced that *O. amoena* shows a good production of zeaxanthin upon exposure to an appropriate light intensity. While, statistical analysis showed no differences in zeaxanthin production among the light intensities used in the current study at $P > 0.05$.

INTRODUCTION

The prokaryotic cyanobacteria and the eukaryotic photosynthetic microorganisms (microalgae) are responsible for photosynthesis, the process by which light energy is converted into chemical energy. The value-added byproducts derived from microalgae include proteins, lipids, polysaccharides, minerals, vitamins, pigments and polyunsaturated fatty acids, all of which have significant economic and health benefits **(Sun *et al.*, 2023)**. A number of biologically active secondary metabolites from algae have attracted scientific interest. Particular attention has been paid to carotenoids, which are found in large quantities in a wide range of photosynthetic organisms, including numerous species of algae. With countless health benefits, carotenoids are among the most important natural pigments. A sharp increase has been recorded in the amount of carotenoids in algae and the interest in their potential industrial uses **(Generalić Mekinić *et al.*, 2023)**.

Algae are a particularly significant source of the natural pigments, viz. chlorophyll, phycoerythrin, zeaxanthin, carotene and other natural pigments. In addition to providing color, pigments have powerful physiological activities such as antioxidant, anti-

inflammatory, anti-obesity and lipid-lowering properties **(Chen *et al*., 2023)**. Zeaxanthin is a carotenoid pigment; it is a possible by-product of microalgae; it is used in dietary supplements and in the food industry to treat age-related macular degeneration owing to its antioxidant properties; it can dramatically reduce the risk of developing eye diseases such as cataracts **(Pereira *et al*., 2021)**.

Microalgae are photosynthetic microorganisms that are a potentially attractive source for zeaxanthin production. Therefore, carbon dioxide and light are the primary feedstocks for the process. In their study, **Mohammed *et al*. (2023)** suggested that a strategy to shift light and temperature stress in the cultivation of Arthrospira platensis C1 could increase its nutraceutical value. They found that an optimal condition (35°C and 100μmol photons m-2 s-1) resulted in a specific growth rate (μ) of 0.024h- 1, with a carotenoid content of 4.66mg g-1, via studying the effect of light and temperature stress on zeaxanthin production in *A. platensis* C1 (PCC9438). Furthermore, **Tanno *et al*. (2020)** discovered that exposure to blue or red light at different intensities had an impact on carotenoid accumulation in *Euglena gracilis*. They observed that blue light irradiation (300μmol photons m−2 s−1) increased the cellular content of all trans-diatoxanthin and zeaxanthin. Another study has shown that the content of zeaxanthin also increases in response to an increase in light intensity. The authors detected a significant difference in zeaxanthin content between *Dunaliella bardawil* and *Dunaliella salina* CCAP 19/18, with the greatest difference in zeaxanthin content observed at 200μmol photons m-2 s-1 light **(Park *et al*., 2013)**.

The current study aimed to investigate the effect of light intensity on the production of zeaxanthin by the blue-green algae Oscillatoria sp. since zeaxanthin is an important pigment and is widely used in industry in addition to light that plays an important role in increasing its production by algae.

MATERIALS AND METHODS

Algal cultivation and biomass production

An axenic culture of the cyanobacterium *O. amoena* (Fig. 1) was obtained from the Advanced Environmental Laboratory, Department of Biology, University of Al-Qadisiyah. The strain was isolated from a freshwater body. *O. amoena* was cultured on nutrient agar and incubated for 72 hours at 37°C to confirm its purity and absence of bacteria and fungi **(Andersen, 2005)**. For the purpose of biomass production, the cyanobacterium was transferred to a steriled 500ml glass flask containing 400ml of BG-11 culture medium and incubated at a temperature of 28°C and a light intensity of 60μmol. Photon m-2 s-1; the incubation of the algae samples was continued until growth ceased **(Tredici, 2004)**.

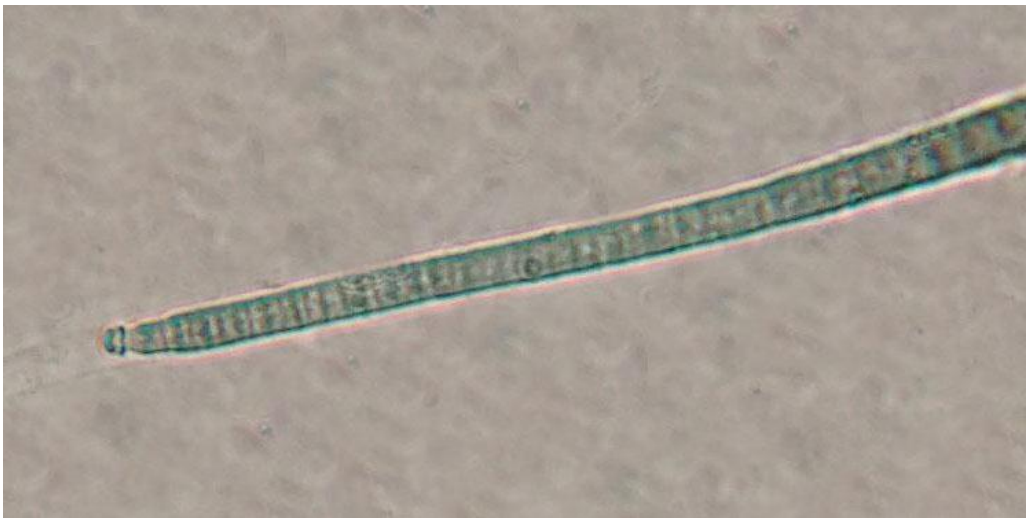

Fig. 1. *Oscillatoria amoena* under the light microscope at magnification power 40X

Light intensity treatment

In order to study the effects of five different light intensities (40, 60, 80, 100 and 120µmol. Photon m-2 s-1) on the growth rate and zeaxanthin production of *Oscillatoria amoena*, a cyanobacterium was cultivated in BG-11 medium using white LED lamps, with a pH of 7.2; a temperature of 28°C±2, and a 16:8 light period for Light: Darkness.

Chlorophyll a estimation

A volume of 5ml was taken from the cyanobacterial culture and centrifuged at 5,000rpm for 5 minutes, and the supernatant was discarded. An amount of 5ml of acetone 90% was added to the algal cell precipitate, then placed in a shaking water bath at 25°C for one hour, and centrifuged at 6000rpm for 10 minutes, and only the supernatant was collected. The optical density of the supernatant was measured using a spectrophotometer at a wavelength of 664nm, and the concentration of chlorophyll A was calculated using the following equation of **Ritchie (2006)**:

$$\text{chl a } [\mu g/mL] = 11.4062 * A664$$

Zeaxanthin estimation by HPLC device

The detection and quantification of algal zeaxanthin was carried out using the **Zhao *et al.* (2013)** method, separated by a column (C18 PAH, 3µm, 250 x 4.6 mm) (Agelent, Germany). The mobile phase consists of dichloromethane: acetonitrile: methanol (20:70:10, v/v/v), with a flow rate of 1.0ml/ min at room temperature, and the chromatograms were recorded at 450nm. The detection of the zeaxanthin was carried out by matching the retention time, and absorption spectrum of the standard substance zeaxanthin that was purchased from SIGMA-ALDRICH (14681-1MG-F). In addition, the quantitative measurement of the sample was calculated by measuring the integrated peak area, while the content was calculated using a calibration curve by plotting the peak area against the respective standard sample concentration (Fig. 2).

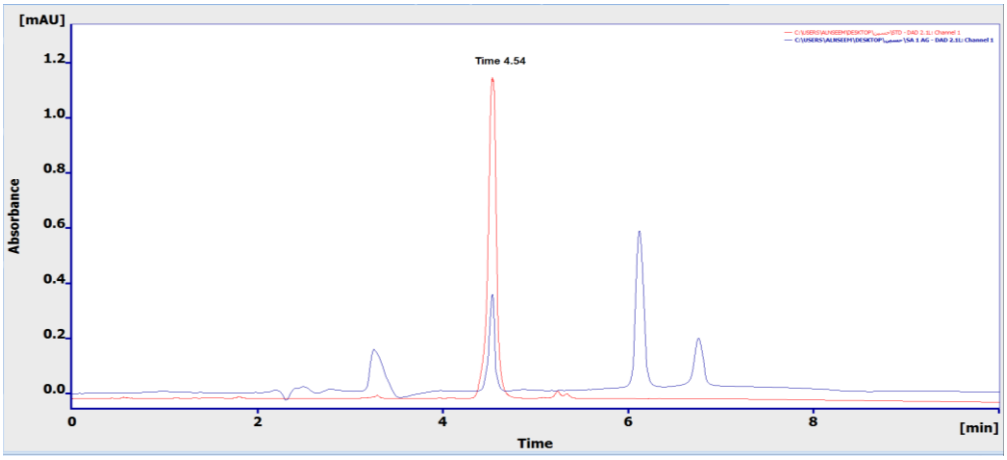

Fig. 2. Zeaxanthin production by *Oscillatoria amoena* with blue color matching to zeaxanthin standard with red color at retention 4.54min by HPLC

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) with least significant differences (LSD) to compare the treatments with different light intensities and their effect on the production of zeaxanthin by the cyanobacterium. All treatments were performed in triplicate.

RESULTS AND DISCUSSION

Growth curve determination

The chlorophyll a values were used to estimate the growth curve of the alga *O. amoena* (Fig. 3). It was found that the highest cyanobacterial growth rate was recorded at a light intensity of 80µmol m-2s-1, while the lowest growth rate was recorded at light intensities of 40 and 120µmol m-2s-1.

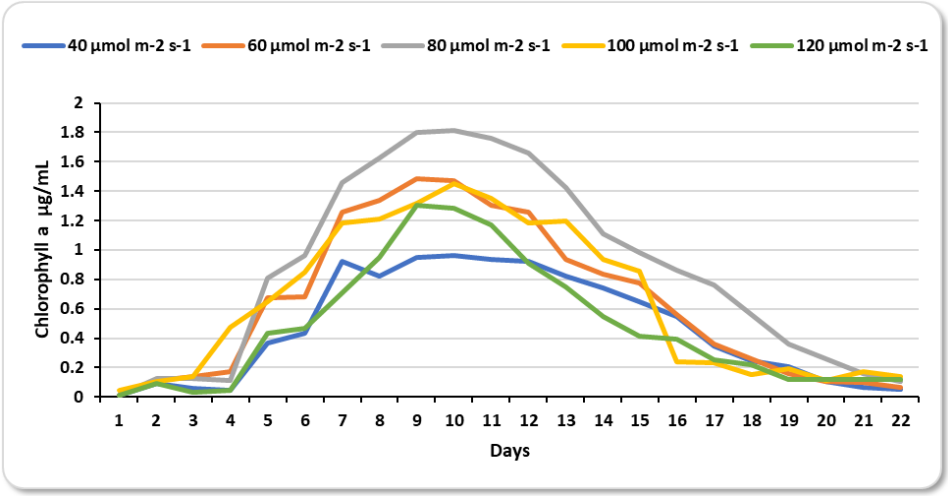

Fig. 3. Growth curve of *Oscillatoria amoena* based on the chlorophyll a value

Zeaxanthin production

The results of the production of zeaxanthin by *O. amoena* showed that the highest value of zeaxanthin was 0.9390µg/ mg, which was recorded at a light intensity of 80µmol m-2 s-1, and the lowest zeaxanthin production was recorded at light intensity of 40µmol m-2 s-1, with a value of 0.0807µg/mg, respectively (Table 1, Fig. 4). Furthermore, statistical analysis showed that the light treatment used in the study was different in zeaxanthin production at *P*> 0.05.

Table 1. Zeaxanthin production µg/mg by *Oscillatoria amoena* at different light intensities

Light intensities	Zeaxanthin µg/mg
40 µmol m-2 s-1	0.0807± 0.00176 d
60 µmol m-2 s-1	0.1323 ±0.00219 c
80 µmol m-2 s-1	0.9390±0.03579 a
100 µmol m-2 s-1	0.1743±0.00273 b
120 µmol m-2 s-1	0.1310±0.00346 c
LSD	0.041

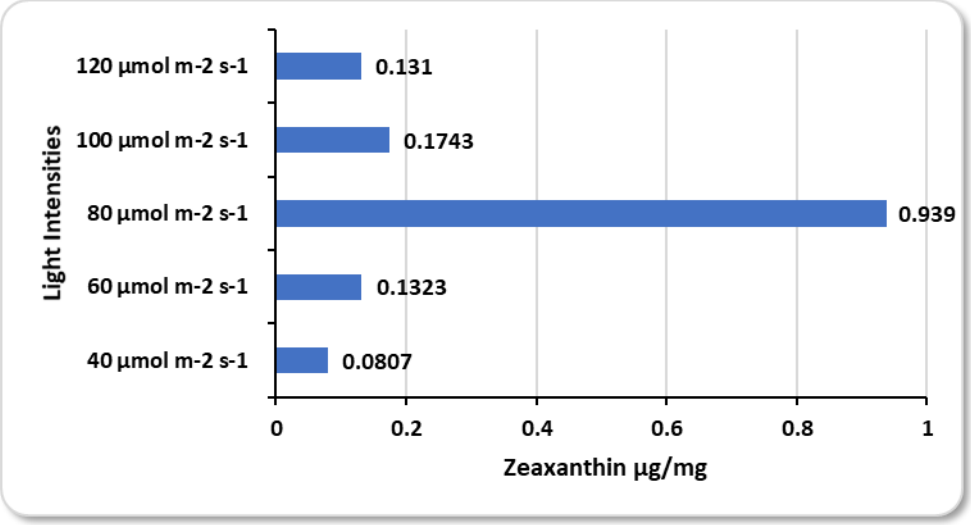

Fig. 4. Zeaxanthin production µg/mg by *Oscillatoria amoena* at different light intensities

Microalgae are increasingly recognized as potent and persistent natural antioxidants containing bioactive compounds, such as phenolic compounds, polysaccharides and

carotenoids. Through exposure to light, carbon dioxide and other environmental stressors, these microalgae can produce a wide range of bioproducts. Light is therefore considered an important factor in increasing the production of active ingredients such as pigments **(Plaza *et al*., 2009; Yang *et al*., 2023)**.

The findings of this research were similar to those of other studies, which found that the best production of the pigment zeaxanthin occurred at an illumination level of 80, including the study by Bourdon et al. (2021), who examined two species of cyanobacteria (*Synechococcus* sp. PCC7002 and *Synechocystis* sp. PCC6803) and a rhodophyte (*Rhodosorus* sp.). At a light intensity of 80 µmol photons m-2 s-1, the specific zeaxanthin content was 2.30, 1.61 and 2.16mg/ g for all the algae mentioned above, respectively. The highest specific growth rate (0.74) was observed for *Synechococcus* PCC7002.

Further results obtained by **Diaz-MacAdoo *et al.* (2022)** showed that growing under blue light at 80µmol photons/m2/s mainly stimulated carotenoid synthesis by microalgae *Muriellopsis* sp. (MCH-35). In addition, **Singh *et al.* (2019)** elucidated that the optimized conditions of culture medium and light: 80 µmol photon m-2 s-1 have a high carotenoid production by the microalgae *Asterarcys quadricellulare*, which reached 118mg/ mg dry weight. Another study showed that the maximum carotenoid astaxanthin content per dry weight produced by *Haematococcus pluvialis* was 10.3mg/ g at 85µmol m-2 s-1, with light being the best inducing carotenogenic factor observed **(Cifuentes *et al*., 2003)**.

The reason why the light intensity of 80 micromoles gave the highest production of zeaxanthin may be because this is the optimal intensity for cyanobacterial growth, as suggested by **Maltsev *et al*. (2021)**, is that adequate light intensity can stimulate the synthesis of pigments such as carotenoids and phycobiliproteins, they also found that different taxonomic groups of microalgae, as well as different strains of the same species, have different optimal light conditions for photosynthesis, growth, lipid accumulation, fatty acid composition and content of carotenoids. Similarly, **Ali and Alghanmi (2023)** postulated that algae pigment content and growth rate were positively influenced by the ideal light intensity reaching the algae. According to **Hong *et al*. (2023)**, there are differences in the optimal conditions for microalgal *Mychonastes* sp. to produce lutein and zeaxanthin, and optimizing the salinity and light intensity of the culture can aid in the development of carotenoids.

It was also found that zeaxanthin production decreased in low and high light conditions. This may be traced back to its impact on the photosynthetic process and thus the growth rate of the algae, leading to a decrease in the production of this pigment. This explanation was mentioned by **Xu *et al*. (2016)** who found that, as the light intensity increased, the cells appeared to become increasingly stressed; this was evidenced by a decrease in both chlorophyll and carotenoid content. On the other hand, **Park *et al*. (2013)** reported that cells produce less zeaxanthin and accumulate violaxanthin under low light conditions. Low light levels cause changes in chloroplast structure and photosynthetic metabolism;

therefore, prolonged exposure to low light levels can stunt plant development by providing insufficient energy, hence it has an impact on the production of pigments **(Lu *et al*., 2021)**.

CONCLUSION

The results of the current study showed that light intensity plays a crucial role in zeaxanthin production; therefore, low and high light intensities decreased zeaxanthin productivity, but the optimal light intensity of 80µmol m-2 s-1 increased the growth rate and showed optimal zeaxanthin production by the cyanobacterium *Oscillatoria amoena*.

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