MALAYSIAN JOURNAL OF SCIENCE
MJS
ISSN 1394-3065

Regular Issue

Malaysian Journal of Science 42 (1): 1-6 (February 2023) https://mjs.um.edu.my

EFFECTS OF VARIOUS LIGHT INTENSITIES ON PHYCOCYANIN COMPOSITION OF CYANOBACTERIUM *LIMNOSPIRA FUSIFORMIS* (VORONICHIN) NOWICKA-KRAWCZYK, MÜHLSTEINOVÁ & HAUER

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Abstract: Phycocyanin denotes a photosynthetic pigment discovered in Rhodophyta and cyanobacteria, which has been used in medical, industrial, and agricultural applications. In general, phycocyanin production by cyanobacteria depends on many environmental conditions, mainly light during the cultivation period. The goal of this research was to see how various light intensities of 47, 52, as well as 60 µmol m-2 s-1, affected the Phycocyanin production of cyanobacterium Limnospira fusiformis cultured in Zarrouk medium with a maximum temperature of 28°C. The outcomes revealed that with mild light intensity (52 µmol m-2 s-1), increased phycocyanin production of 11.94 ng/mg took place. With regard to greater light intensity (60 µmol m-2 s-1), the lesser phycocyanin production of 0.57 ng/mg took place. These results give a good impression that moderate lighting increases phycocyanin production, but high light intensity inhibits it. The statistical analysis results also showed that there are significant differences between the light intensities used in the study at a level of $p<0.05$. Therefore, this study concluded that phycocyanin was affected by light intensity. Light regime optimization gives a good yield of this pigment. In this study, high phycocyanin production by cyanobacterium Limnospira fusiformis occurred in mild light intensity (52 µmol m-2 s-1).

Keywords: *Limnospira fusiformis, cyanobacteria, light intensity, photosynthetic pigment, phycocyanin*

1. Introduction

Algae that grow in high solar radiation habitats have auxiliary pigments that shelter them from oxidation as well as radiation damage, owing to the double conjugate bonds in the chromophore. The combination of the attachment pigments and their arrangement gives the algae various colors, for instance, red, brown, green, and golden algae (Barsanti & Gualtieri, 2014; Lee, 2018). Carotenoids, chlorophyll, and phycobiliproteins are among the colorful components discovered in algae and cyanobacteria. Moreover, phycoerythrin, allophycocyanin, and phycocyanin are the most common phycobiliproteins, and they are built up of distinct α and β polypeptides subunits (Ferreira & Gouveia, 2020).

Some microalgae such as *Limnospira* (Nowicka-Krawczyk et al., 2019) showed an excellent application in commercial, medical, and pharmacological because it has excellent nutritional properties as well as a wide variety of active compounds. They include several natural pigments and compounds with functional qualities and high protein content. Allophycocyanin (AP) and phycoerythrin (PE) are minor amounts of phycobiliproteins derived from *Spirulina*, while phycocyanin is plentiful. Phycocyanin is a bright blue pigment that, depending on its purity, has a variety of vital uses (Barsanti & Gualtieri, 2014; Becker & Venkataraman, 1984; Berg, 2002; Silva, 2008).

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Phycocyanin is an algae-derived light-harvesting pigment-binding protein. It is a common coloring additive in nutritional and dairy items, for instance, cosmetics, beverages, candies, gums, and jellies in China and Japan. Phycocyanin is heat and light-sensitive. In comparison to gardenia or indigo, it has a pure blue colorant. Phycocyanin can be utilized to treat a number of malignancies, such as squamous cell carcinoma. Also, phycocyanin isolated from *Spirulina platensis* showed anticancer efficacy (Tripathi et al., 2021). The antioxidant compounds present in Spirulina microalga, for instance, phycocyanin, phenolic compounds, as well as polyunsaturated fatty acids, may explain its ability to reduce blood lipid levels and boost HDL cholesterol (Colla et al., 2007; Colla et al., 2008).

Nutrients like nitrogen, phosphorus, calcium, magnesium, iron, manganese and light affect the pigment biosynthesis (Norena-Caro et al., 2021). Light is one of the most critical components that affect microalgae and cyanobacteria pigments production because it allows them to gather energy and carry out photosynthesis (Atta et al., 2013). However, the majority of cultivations rely on sunlight as a source of energy. Artificial light is more often utilized in cultivations for high added value bioproducts like phycocyanin, with effective and standardized photosynthetic regulation photon flux density, leading to high productivities of these pigments (Blanken et al., 2013). The light intensity impact as well as the quality of the phycocyanin amount in *Synechococcus* sp. NKBG042902 cells were studied by Takano

Received: January 23, 2022
Accepted: July 14, 2022
Published: February 28, 2022

DOI:https//doi.org/10.22452/mjs.vol42no1.1
Malaysian Journal of Science 42(1): 1-6 (February 2023)

et al. (1995), which indicated that the phycocyanin concentration was maximum as the cyanobacterium was cultivated under 25 µmol m^{-2} s^{-1} illumination utilizing a cool-white fluorescent light, as well as the phycocyanin and biomass productivities were 21-100 $mg1^{-1}$ day^{-1}, accordingly. The light spectrum and nutritional content affect *Spirulina platensis* production. According to Wicaksono et al. (2019), when the red and nutritional spectrums were mixed, the optimal production of phycocyanin, as well as dry weight of Spirulina, were achieved, producing 0.6677/0.5 g L^{-1} and 5.1078 mg g^{-1}, accordingly.

The purpose of this research was to see how varying light intensities affected the growth and production of phycocyanin in the cyanobacterium *Limnospira fusiformis*.

2. Methods and Material

2.1 Cyanobacterial strain

The axenic culture of a *Limnospira fusiformis* isolated from wastewater (Photo 1) was obtained from the Biology department laboratory, College of Education, the University of Al-Qadisiyah/Iraq. It was identified morphologically and genetically, according to Alghanmi (2020). The strain was maintained in Zarrouk medium described in Vonshak (1997) and cultivated in a 100 ml flask containing Zarrouk medium with pH 9.6 in a growth chamber having a controlled temperature of 28°C, 25 µmol m^{-2} s^{-1} of light intensity, as well as photoperiod 16 hours, light/8 dark.

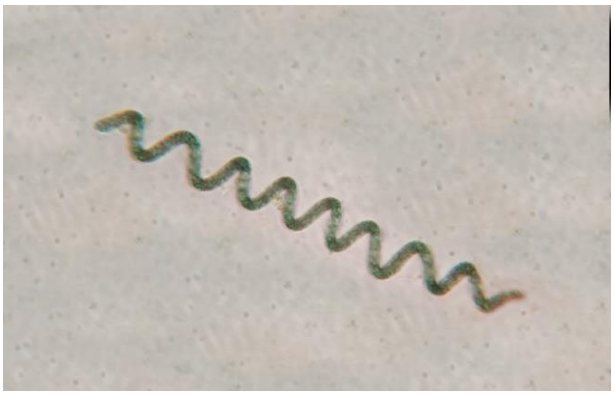

Photo 1. Cyanobacterial strain identified as *Limnospira fusiformis* under light microscope (magnification at 40x).

2.2 Effect of light intensity on phycocyanin production

The influence of various light intensities, 47, 52, and 60 µmol m^{-2} s^{-1}, on the growth rate, including phycocyanin production of cyanobacterium *L. fusiformis* cultured in Zarrouk medium (pH 9.6) with an ideal temperature of 28°C and a photoperiod of 16 light:8.

2.3 Growth curve estimation

The growth curve of *L. fusiformis* was estimated depending on the chlorophyll-a pigment determination in accordance with the Zavrel et al. (2015) method. The samples were gathered every day for 30 days from the culture of *L. fusiformis*, then treated with 90% acetone for extracted chlorophyll-a later determined using a spectrophotometer at wavelength 665 and A720 nm. The chlorophyll was estimated by the equation below:

Chl a [µg/L] = 12.9447 (A665 − A720).

2.4 Pigment extraction and analysis

L. fusiformis dried powder was suspended in a 1:15 w/v ratio in (0.1 M) sodium phosphate buffer with pH 7. First, the suspension was homogenized for 10 min utilizing sonication. Next, three RFT freezing and thawing cycles were performed, then the suspension was centrifuged at 4000 *g* for 30 mins, the crude extract with a blue color comprising C-phycocyanin was recovered. Following that, the samples were then filtered utilizing Millipore 0.45 µm filter paper, which was dried in an oven at 40°C for 8 hrs. Note that the dried paper was weighed and placed in a tube, to which 5 ml of 0.1 M phosphate buffer (pH 7) was administered. Next, the mixture was homogenized for 10 minutes before being frozen at -20°C for an hour and subsequently thawed at room temperature for another hour. This procedure was carried out three times. Lastly, the sample was centrifuged at 8,000 *g* for 10 minutes and filtrated using 0.45 µm Millipore filter paper and then was used for the measurement by the HPLC device (Moraes et al., 2011).

2.5 High-performance liquid chromatography (HPLC)

A Shimadzu HPLC system (Kyoto, Japan) was employed for executing chromatographic analysis. The HPLC system included a SIL-9A autosampler injector (Shimadzu), as well as an LC-9ADvp pump (Shimadzu). The chromatographic separations were accomplished employing an Orbit 100 C4, 5 µm, 250 × 4.0 mm. Note that the mobile phase was made up of water having 0.1% v/v TFA (solvent A) as well as CH_3CN with 0.1% v/v TFA (solvent B). Moreover, at a constant flow rate of 0.8 mL min^{-1}, the subsequent gradient program was utilized from 0 to 10 min, and the components were raised from 45% - 100% in B. The injection had a volume of 100 µL. The photodiode array detector set at 620 nm was utilized to observe the column effluent. The detection of c-phycocyanin was analyzed by matching the retention time and absorbance spectrum of the standard (Figure 1). The standard of phycocyanin was purchased from Sigma Aldrich with CAS-Number P2172-10MG. The concentration of phycocyanin extracted from *L. fusiformis* was calculated by serial concentrations of standard external materials to build a calibration curve between concentration and its equivalent peak area (Moraes et al., 2011).

DOI:https//doi.org/10.22452/mjs.vol42no1.1
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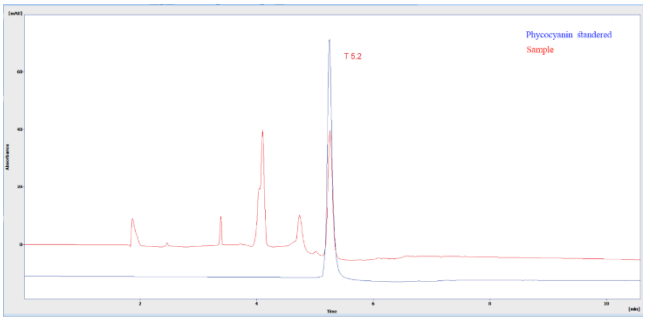

Figure 1. Phycocyanin of *Limnospira fusiformis* with red color matching the standard phycocyanin in blue color at retention time 5.2 min.

2.6 Statistical Analysis

Every experiment was analyzed in triplicate to ensure that the findings were reproducible. The data was evaluated utilizing a one-way analysis of variance (ANOVA), which was then subsequently followed by the least significant differences (LSD). All analyses were carried out with SPSS 26 at $p < 0.05$.

3. Findings and Discussion

Algal pigments like C-phycocyanin (C-PC), among the key primary pigments of the cyanobacterium *Spirulina*, a microalga utilized as a dietary supplement in various countries, include a wide spectrum of pigments, which include phycobiliproteins, aside from their health advantages, algal pigments possess tremendous commercial potential in the pharmaceutical industry as natural colorants, cosmetics, as well as nutraceutical industries (Kuddus et al., 2013). Moreover, light is one of the optimum conditions affecting pigment production in cyanobacteria. Here, microalgae provide an effective technique for turning solar energy into biomass since phototrophic cell factories are driven by sunlight to produce bioproducts, antioxidants, bioactive compounds, vitamins, proteins, dyes, fats, and sugars (Brennan & Owende, 2010).

The growth curve estimated by chlorophyll concentration (Figure 2) showed good growth of *L. fusiformis* under three light intensities 47, 52, and 60 µmol m^{-2} s^{-1}, with the highest growth registered at 52 µmol m^{-2} s^{-1}. The results of phycocyanin production by *L. fusiformis* in table 1 and Figure 3 show higher production of phycocyanin of 11.94 ng/mg occurred in the mild light intensity 52 µmol m^{-2} s^{-1}. At a greater light intensity of 60 µmol m^{-2} s^{-1}, however, a lesser phycocyanin content (0.57 ng/mg) was found. These findings are consistent with those of Ma et al. (2015). When incubating cyanobacterium *Nostoc sphaeroides* at different light intensities 10, 30, 60, 90, as well as 120 µmol m^{-2} s^{-1}, they discovered that the cyanobacterium colonies were soft and pale when exposed to low (10,30 µmol m^{-2} s^{-1}) as well as high (120 µmol m^{-2} s^{-1}) light intensity. Furthermore, towards the conclusion, the spherical aggregation was disrupted. Under the strong light intensity, this was much more severe. White light at 90 µmol m^{-2} s^{-1}, on the other hand, provided the best light conditions for phycobiliprotein accumulation and growth in *N. sphaeroides*. Moreover, Schipper et al. (2020) investigated the phycocyanin production by the desert cyanobacteria *Leptolyngbya* sp. QUCCCM56 under various light intensities. Their findings revealed that the maximum light intensity for phycocyanin production was observed at minimal light intensity (80 µmol m^{-2} s^{-1}) and rising light intensities of 300 and 1800 µmol m^{-2} s^{-1} resulted in substantial decreases in phycocyanin content of 53.0% and 78.7%, accordingly.

The elevation in phycocyanin content at 52 µmol $m^{-2}s^{-1}$ might be linked to an adjustment necessary to protect the chlorophyll molecules from light, as clarified by Grossman (2003) that cyanobacteria modify their phycobiliprotein ratios to maximize photosynthetic efficiency in response to changes in light quality.

Cyanobacteria may evolve to change their light absorption qualities in response to light availability in various environments like freshwater, wastewater and soil, allowing them to regulate photosynthesis. As a result, the pigment concentration involved in light absorption, such as phycocyanin, may fluctuate based on the particular climatic conditions of light intensity (Khandual et al., 2021). Moreover, Szwarc and Zieliński (2018) reported that the light spectrum influenced both the phycocyanin content as well as the biomass of *Limnospira platensis* biomass. High light intensity alters the features of every photosynthetic system. However, as per Eriksen (2008), extremely intense light can cause photoinhibition. Subsequently, the pigment that absorbs particular light wavelengths is prioritized (Boisen & Eggum, 1991).

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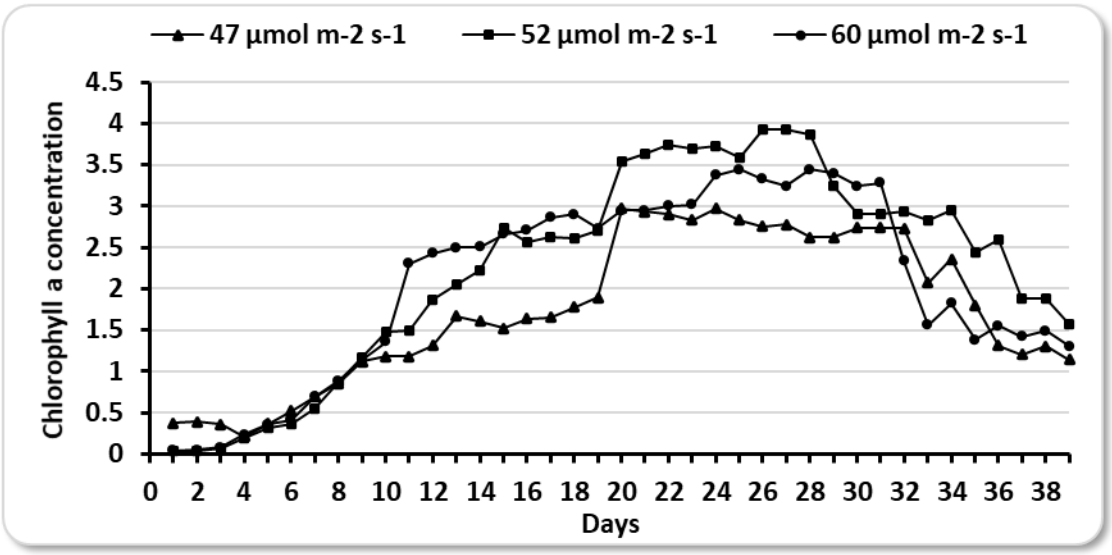

Figure 2. Growth curve estimated by chlorophyll production of *Limnospira fusiformis*

Table 1. Different light intensity effects on phycocyanin production by *Limnospira fusiformis*

Ligh intensity µmol m-2 s-1 / *Limnospira fusiformis*	47 Mean±SE	52 Mean±SE	60 Mean±SE	LSD
Phycocyanin ng/mg	1.76±0.058 b	11.94.0205 a	0.57±0.0001 c	0.05

**lowercase letters indicate the differences between the light intensities*

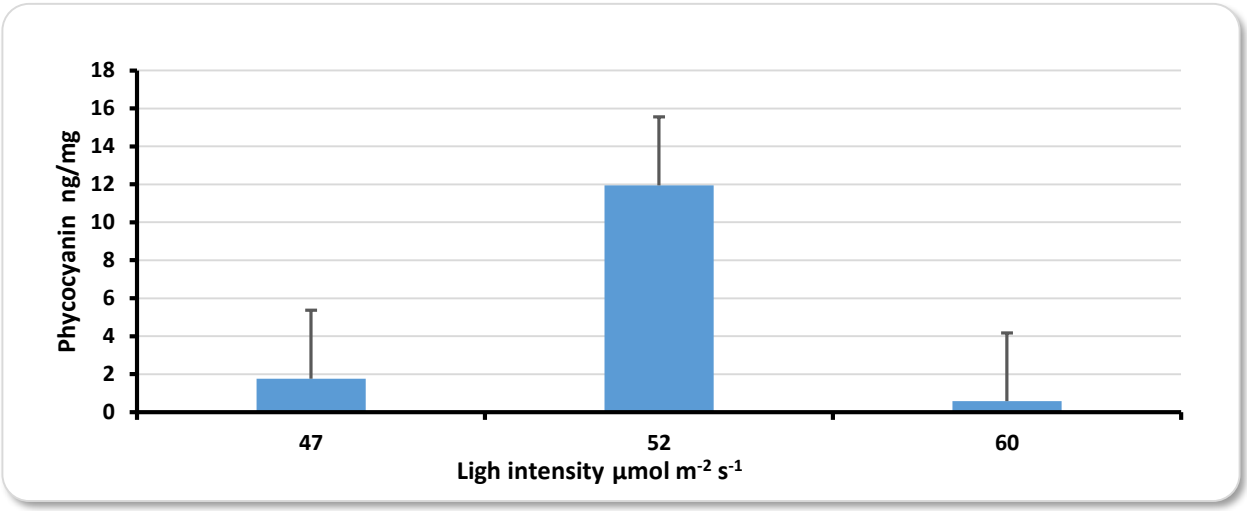

Figure 3. Different light intensities effects on phycocyanin production by Limnospira fusiformis

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4. Conclusion

Phycocyanin is an important pigment that poses pharmacological, medical. Moreover, economic values, the production of this pigment is affected by light intensity. Hence, the current study suggested that light stress may influence the production of phycocyanin where they noticed that high phycocyanin production by cyanobacterium Limnospira fusiformis occurred at light intensity (52 µmol m-2 s-1). In contrast, the low production of phycocyanin occurred at low and high light intensities.

5. Acknowledgement

We appreciate the assistance of Assist Prof. Ahmed J. Hassan (University of Al-Qadisiyah) with the statistical analysis.

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