MODELING THE INFLUENCE OF LIGHT QUALITY ON GROWTH AND SYNTHESIS OF NATURAL PRODUCTS BY THE DIAZOTROPIC BLUE-GREEN ALGA *CYLINDROSPERMUM MUSCICOLA* MTC-30602

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Abstract:

The present communication deals with light quality of different colours (Blue, Green, Red and White) on the growth and synthesis of photosynthetic pigments along with other value added products by the diazotropic blue-green alga *Cylindrospermum muscicola* MTC-30602. *C. muscicola* batch cultured for 10 days under controlled culture conditions. All four colours of light were observed to have an impact on the yields of dry biomass, chlorophyll, total carotenoids, phycobilin (c-phycocyanin, c-allophycocyanin, and c-phycoerythrin), total carbohydrate, total protein, and total fat. The results obtained indicated that increased production of biomass (dry weight), chlorophyll-a, total carotenoids, total protein was obtained when illuminated with white light. However, maximum synthesis of total carbohydrate, c-phycocyanin and c-phycoerythrin were observed under the influence of green light while c-allophycocyanin and lipid under the influence of red light. The findings suggested that exposure to various light colours may be in charge of the synthesis of additional naturally occurring compounds with added value, such as photosynthetic pigments.

Keywords: Cyanobacteria- light quality- photosynthetic pigments- natural products.

Introduction

Cyanobacteria (Blue-green algae/ Cyanoprokaryotes) are well known, phototrophic prokaryotic microorganisms. They are the largest group of Gram negative photosynthetic prokaryotes and well known primary colonizer **(Tiwari, 1972; Kant *et al*., 2004)**. They are the most diversified and ancient photosynthetic

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micro-organisms that contribute significantly to the ecosystem and capable to grow in wide range of habitats **(Kant *et al*., 2005, 2020 a & b; Sarma *et al*., 2020)**.Cyanobacteria are also well known for their value added products **(Kant *et al*., 2006; Kant, 2011)**. Cyanobacterial value added products are widely now used as cosmetics, fluorescent markers in biomedical research, natural dyes, nutritional ingredients and pharmaceuticals **(Venugopal *et al*., 2005)**.

Cyanobacteria are one of the functional microorganisms widely used in biotechnological applications and food industries **(Venkataraman and Becker, 1985)**. Cyanobacterial reserved food materials can be used as natural source of pigments, certain secondary metabolites and proteins **(Cardozo *et al*., 2007; Tan, 2007; Pagels *et al*., 2019)**. They are also wealthy source of vitamins and amino acids **(Ciferri and Tiboni, 1985)** and contain considerable quantity of lipids and fatty acids **(Sarma *et al*., 2020)**. Screening results of cyanobacteria demonstrated it as the potential source of new antibiotic **(Sarma *et al*., 2023)** and pharmacologically active compounds **(Fish and Codd, 1994; Browitzka, 1995; Jaiswal *et al*., 2011)**.

The genus *Cylindrospermum* Kutzing ex Bornet et Flahault is a filamentous, unbranched and heterocystous cyanoprokaryote well known for its biological applications. The genus *Cylindrospermum* has a vast amount of potential for managing the environment. It can be utilised as powerful protein supplements, animal feed, soil conditioner, bio-fertilizer, and biomonitors of soil fertility **(Whitton and Pots, 2000)**. They also contain nitrogenase enzyme capable of fixing atmospheric nitrogen and make it available to the plants. The members of the genus *Cylindrospermum* is well known for its application as bio-fertilizer to maintain and improve soil status **(Muthukumaran *et al*., 2022)**. Under light inadequacy level of photosynthetic pigments typically boosts up to facilitate energy acquisition **(Macintyre *et al*., 2002)**. However, the photosynthetic apparatus's macromolecular makeup and ultrastructure acclimatise

to avoid photoinhibition when exposed to enough light **(Falkowski and Raven, 2013)**.

The present study is focused on evaluation of the effect of monochromatic light such as Blue Light (BL), Green Light (GL), Red Light (RL) and White Light (WL) on the growth and synthesis of photosynthetic pigments along with total carbohydrates, total lipids and total proteins in *C. muscicola*.

Materials and Methods

Experimental Organism:

The experimental organism used in the present study was isolated from moist soil collected from bank of Ganga river located at Hastinapur, Meerut, Uttar Pradesh, Bharat and deposited as *Cylindrospermum muscicola* MTC-30602 at Algal Germplasm Collection Center, Department of Botany, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, Bharat.

Experimental culture media formulation and conditioning:

In the present investigation, all the experiments were performed in 150 ml conical flasks (Borosil), each containing 100 ml of sterilized BG-11 medium **(Stainer *et al.*, 1971)**. *C. muscicola* was cultured for exponential growth for one week on magnetic stirrer (LABQUEST-BOROSIL MHPS15P) at 28±2ºC, light 100µmol photons/m^2/s, 14:10 hours light:dark regime for 10 days for uniform growth of *C. muscicola.*

Experimental design:

The experiment was done in complete randomized design in triplicates. The experimental organism *C. muscicola* was batch cultured under the influence of four different colours of light *i.e.* BL, GL, RL and WL. For providing different coloured light effect, cellophane sheets of four colours *i.e.* Blue, Green, Red and White were used for covering the culture flasks to give different monochromatic light effect under controlled conditions (Temp. 28±2ºC, light 100 µmol photons/s/m^2, 14:10 hrs. light:dark hours photoperiod).

Batch Culturing of the experimental organism:

C. muscicola was batch cultured for 10 days harvesting a single batch every 5^{th} day till second harvesting. All the batch experiment was terminated after a period of 10 days.

Estimation of dry biomass:

Dry biomass of *C. muscicola* was determined with the help of method described by **Stumpf *et al.* (2016)**. To obtain dry biomass, homogenized cyanobacterial culture was filtered by prewashed, dried and pre weighed filter paper (Whatman No.1) and then the fresh weight was obtained by filtering the biomass. The dry weight of biomass was obtained by drying fresh biomass of *C. muscicola* in oven (REMI-RDHO-80) at 60 ºC for 24 hours to obtain a constant weight and the dry weight was measured from the obtained final value of the biomass.

Estimation of chlorophyll-*a*:

The estimation of Chlorophyll-*a* was done by the method of **Tuba *et al*. (1994)** using spectrophotometer (SYSTRONICS 118) under dark condition to avoid photoreaction and loss of pigments.

Estimation of total carotenoids:

Total carotenoids were determined with the help of method described by **Jensen (1978)** under dark condition to avoid loss of pigments.

Estimation of phycobiliproteins:

Phycobiliproteins were extracted by the method described by **Bennett and Bogorad (1973)**. Estimation of phycobiliprotein was done using phosphate buffer by freezing and thawing the contents repeatedly. The OD was measured at 562 nm, 615 nm and 652 nm in spectrophotometer (SYSTRONICS 118).

Estimation of total lipid:

Total lipid content was determined using a mixture of Chloroform: Methanol (2:1 v/v) as described by **Bligh and Dyer (1959)**.

Statistical analysis:

The data obtained were subjected to statistical analysis of variance (ANOVA) in Microsoft Office Excel 2007 by using completely randomized design **(Armstrong and Hilton, 2010)**. Each mean was calculated from three different values. Standard deviation and standard error were calculated against the values obtained.

Results

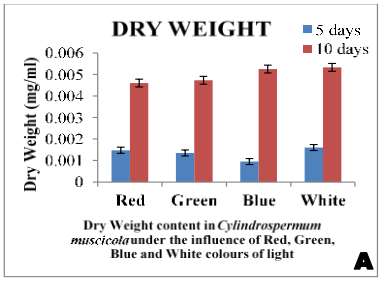
An increasing trend in dry weight was observed in the culture flasks illuminated with Red, Blue, Green and White light. The amount of accumulated biomass (dry weight) indicated the growth of *C. muscicola* under the influence of different colours of light. Maximum dry weight was observed 0.0016 mg/ml and 0.005 mg/ml respectively after 1st and 2nd harvesting under the influence of WL. Minimum amount of dry weight after 1st harvesting was observed 0.0095 mg/ml under the influence of BL while after 2nd harvesting the amount of dry biomass under the influence of BL increased significantly almost near to that of culture flask illuminated with WL. Minimum dry weight was observed 0.0046mg/ml was observed under the influence of RL. A detailed result in the growth of biomass under four different colours of light is given in Graph 1(A)

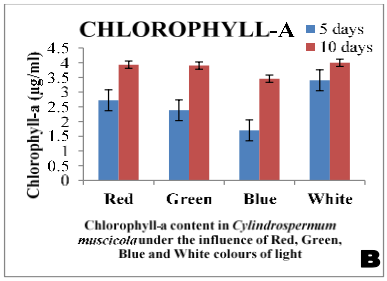
Synthesis of chlorophyll-*a* under the effect of monochromatic light, the cells of *C. muscicola* show an increasing trend up to 2nd harvesting in all the culture flasks illuminated with different colours of light. Culture flask illuminated with WL show maximum synthesis of chlorophyll-*a* after 5 days. Maximum chlorophyll-*a* content was observed 3.4064µg/ml and 3.9981µg/ml after 5 days and 10 days respectively under the influence of WL. Minimum chlorophyll-*a* content was observed 1.7018 µg/ml and 3.456µg/ml after 5 days and 10 days respectively under the influence of BL. Significant differences were observed in production of chlorophyll-*a* content in *C. muscicola* under monochromatic effect of light. The results obtained shows that different colours of light are responsible for the variation in the synthesis of chlorophyll-*a* pigment. Detailed result on the synthesis of chlorophyll-*a* under the influence of four different colours of light is given in Graph 1(B).

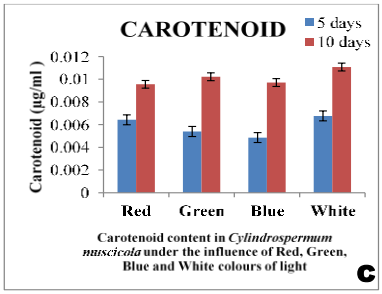
In the synthesis of total carotenoids pigment under four different colours of light a continuous increasing trend was observed till 10 days. On the first harvesting synthesis of carotenoid pigment was observed 0.006µg/ml, 0.005µg/ml, 0.004µg/ml and 0.007µg/ml under the influence of RL, GL, BL and WL respectively. Maximum carotenoid pigment was observed 0.011µg/ml after 10 days of culturing under the influence of WL. Minimum synthesis of carotenoid pigment was observed 0.0095µg/ml under the influence of RL. Detailed result on the synthesis of carotenoid pigment under the influence of four different colours of light is given in Graph 1(C).

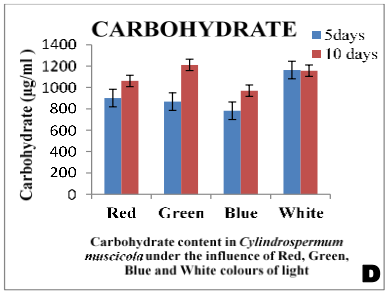
In the synthesis of total carbohydrate in cells of *C. muscicola* show an increasing trend under the influence of RL, BL, GL and WL. Maximum amount formation of the carbohydrate after 5 days is found in WL (1162.94 µg/ml) while less amount is found in BL (701.176 µg/ml) condition. But after 10 days the high synthesis of carbohydrate is found in GL (1156.47 µg/ml) and less is found in BL (969.411 µg/ml). A detailed result on the synthesis of carbohydrate content in the cells of *C. muscicola* under monochromatic light is given in Graph 1(D).

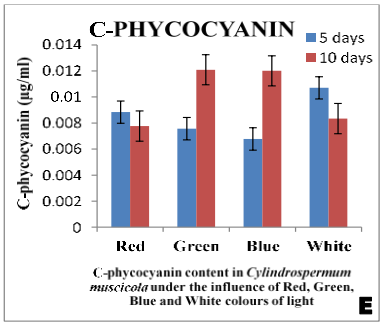
C-phycocyanin synthesis by *C. muscicola* show increasing trend till 5 days in all the culture flasks while after that a decreasing trend in the synthesis of c-phycocyanin was observed after 10 days illuminated by RL and WL. But the culture flask illuminated with GL and BL show an increasing trend till 10 days. The formation of high amount of c-phycocyanin after 5 days treatment is found 0.010704µg/ml when *C. muscicola* culture illuminated under WL and minimum synthesis was found 0.0006775µg/ml when illuminated with BL. But after 10 days, maximum c-phycocyanin content was observed 0.120918µg/ml when illuminated with GL and minimum amount was observed 0.07766µg/ml when illuminated with RL. Detailed result on the synthesis of c-phycocyanin in the cells of *C. muscicola* under monochromatic light is given in Graph 1(E)











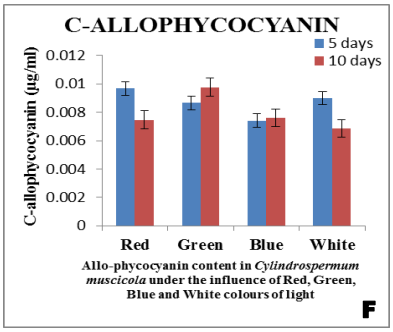


Figure-1(A-E): Showing the effect of different colours of light in synthesis of Biomass (dry weight); chlorophyll-a; carotenoid, carbohydrate, c-phycocyanin, callophycocyanin in *C. muscicola*.

Synthesis of accessory light harvesting pigment c-allophycocyanin was observed under the influence of RL, GL, BL and WL and it was observed that an initial increasing till 5 days and then decreasing in the synthesis of c-allophycocyanin after 10 days illuminated by RL and WL. But, a gradual increasing trend in c-allophycocyanin content was observed when illuminated with GL and BL. Maximum formation of c-allophycocyanin after 5 days treatment is found 0.0096µg/ml under the influence of RL while minimum concentration was observed 0.0074µg/ml when illuminated with BL. But after 10 days, high c-allophycocyanin content was found 0.0098µg/ml under GL and minimum c-allophycocyanin content was observed 0.0069µg/ml when illuminated with WL. Detailed result on the synthesis of c-allophycocyanin content in the cells of *C. muscicola* under the effect of monochromatic light is given in Graph 1(F).

A gradual increasing trend in the synthesis of c-phycoerythrin pigment by *C. muscicola* was observed in the culture flask till 10 days when illuminated with GL while all other flasks illuminated with RL, BL and WL showed gradual increase till 5 days after that, a decline in the synthesis of c-phycoerythrin was observed. Maximum c-phycoerythrin content after 5 days was observed 0.0068µg/ml when illuminated with WL and minimum content was observed 0.0051µg/ml when illuminated with GL. But after 10 days, maximum c-phycoerythrin content was observed 0.0051µg/ml illuminated with GL and minimum amount 0.0048µg/ml was observed when illuminated with BL. A detailed result on the synthesis of c-phycoerythrin content in the cells of *C. muscicola* under monochromatic light is given in Graph 2(A).

Lipid content in *C. muscicola* under the influence of monochromatic light was observed over a period of 10 days and it was found that lipid content in *C. muscicola* under the influence of RL, GL, BL and WL increased till 10 days. Maximum lipid content after 10 days was observed 0.003775µg/ml when

illuminated with RL and minimum lipid content was observed 0.00315µg/ml under the influence of BL. A detailed result on the synthesis of total lipid content by *C. muscicola* under the effect of monochromatic light is given in Graph 2(C).

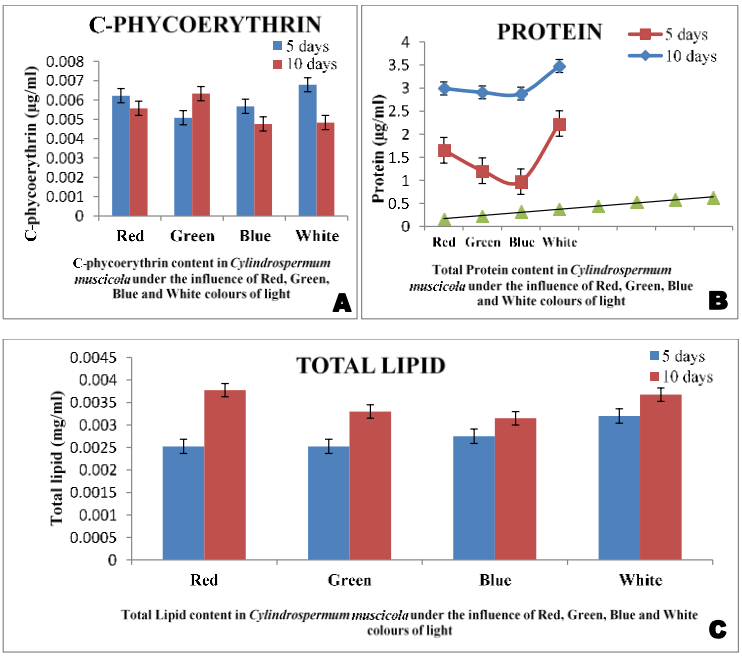


Figure-2 (A-C): Showing the effect of different colours of light in synthesis of c-phycoerythrin, total protein and total lipid in *C. muscicola*

A gradual increase in the amount of total protein was observed till 10 days in all the flasks illuminated with RL, BL, GL and WL. Maximum total protein content in *C. muscicola* after 10 days was observed 2.2313µg/ml illuminated with WL and minimum content was observed 0.9738µg/ml when illuminated with BL. A detailed result on the synthesis of total protein content in the cells of *C. muscicola* under the effect of monochromatic light is given in Graph 2(B).

Discussion

Cyanobacteria are greatly influenced by the light colour in terms of the output of biomass and synthesis of pigments like chlorophyll-*a*, carotenoids and phycobiliproteins. Light conditions processes cellular proliferation including growth, phototaxis, cell aggregation and photosynthesis by these photoreceptors **(Wiltbank and Kehoe, 2019)**. Several studies on effect of different colours of light on cyanobacterial species such as *Phormidium*, *Cyanothece*, *Pseudanabaena*, *Cylindrospermum*, *Spirulina*, *Arthrospira*, etc indicate that light colour have potential impact on the synthesis of pigments **(Van Liere and Walsby, 1982)**. Monochromatic exposure of different colours of light also has influence on the synthesis of carbohydrates, proteins and lipid molecules in cyanobacteria **(Park and Dinh, 2019)**.

Response to different colors of light during growth is a crucial factor for the growth in biomass in cyanobacteria **(Hauschild *et al.*, 1991)**. The effect of different colours of light in biomass assimilation in *Arthrospira* sp. studied by **Park and Dinh (2019)**. Their study on biomass assimilation indicated that, growth in biomass is maximum under RL and WL while minimum under BL and GL. Our present study on *C. muscicola* under different colours of light also revealed the same result that the maximum biomass content could be obtained

under the influence of WL and minimum biomass content was observed under RL.

When growth is constrained by the availability of an essential resource, cells upregulate synthesis of the molecular machinery that acquires this limiting resource **(Tempest *et al*., 1983; Ludwig and Bryant, 2012)**. Chlorophyll-*a* is considered an important parameter for evaluation of photosynthesis in cyanobacteria. Different colours of light could influence the concentration of Chlorophyll-*a* in cyanobacteria **(Hotos and Antoniadis, 2022)**. The highest chlorophyll-*a* concentration was obtained under RL and WL, while the lowest growth and chlorophyll-*a* concentration were observed under BL **(Park and Dinh, 2019)**. From our study on monochromatic effect of light same results were observed. *C. muscicola* grown under the influence of WL could be best for the maximum synthesis of chlorophyll-*a* pigment. Carotenoids are photoprotective pigments responsible for protection against oxidative damage. The results obtained on the study on photoprotective or UV ray protective pigment carotenoid under the influence of WL is similar to the results obtained by **Khatoon *et al*. (2018)** indicating that carotenoid production could be maximized under the influence of WL.

The effect of different colours of light and nutrient media on the growth of *Pseudanabaena mucicola* and its phycobilin production was studied by **Khatoon *et al*. (2018)**. The results obtained by them indicated that *P. mucicola* grown in WL using wastewater as medium attributed higher biomass and when extracted with water, also showed significantly higher production and purity of phycobilin. **Parmar *et al*. (2013)** worked on RL and GL effect on phycocyanin and phycoerythrin production by *Lyngbya* sp. A09DM, *Phormidium* sp. A27DM and *Halomicronema* sp. A32DM and concluded that both GL and RL could be suitable for the maximum synthesis of phycobilin. Similar results were obtained from our experiment showing maximum

synthesis phycobilin comprising of c-phycocyanin, c-allophycocyanin and c-phycoerythrin from *C. muscicola* under GL indicating that GL could be most suitable for phycobiliprotein production and can also maximize the synthesis of phycobilin.

Influence of light on the synthesis of total carbohydrate was studied by **Singh and Das (2011)** and observed that under the influence of WL *Nostoc calcicola* RDU-3 can produce high amount of extracellular polysaccharide. Similar results were observed in our experiment indicating that WL is responsible for enhanced production of carbohydrate. The effect light on synthesis of photosynthetic pigments and total protein in *Anabaena ambigua* was studied by **Vijya and Anand (2009)** and revealed that BL could enhance the yield of total proteins. But from our experiment it could be revealed that WL could enhance the production of total protein.

Effect of monochromatic light on the growth and synthesis of photosynthetic pigments and other value-added natural products by *Cylindrospermum* sp. (MTC-30601) was done by **Neha *et al.* (2021)** and revealed that WL could enhance the production of total lipid content. Similar results were obtained in our present experiment indicating that WL could be responsible for enhanced production of lipid in *C. muscicola*.

Conclusion

On the basis of the results obtained from the experiment it is concluded that different light colours severely influence the amount of biomass, chlorophyll-a, carotenoids, phycobiliproteins, proteins, carbohydrates and lipids in *C. muscicola* and the yield of desired natural products can be enhanced by different the light colours.

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