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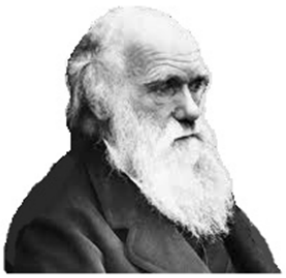

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Preliminary evaluation of impact of monochromatic light on the biosynthesis of astaxanthin in green alga *Haematococcus pluvialis*

Ashaq Hussain Rather* and **Surendra Singh**

Algal Biotechnology Laboratory, Department of Post Graduate Studies and Research in Biological Sciences, Rani Durgavati University, Jabalpur - 482001, (M.P.), India

*E-mail address: ashaqabl2015@gmail.com

*Phone No. 09340892141

ABSTRACT

Astaxanthin biosynthesis in *Haematococcus pluvialis* was enhanced by different monochromatic light patterns. Among the monochromatic light, red light was found most effective for facilitation of astaxanthin - up to 0.055 μg mL^{-1}, followed by blue light, while the biosynthesis of astaxanthin in unfiltered white fluorescent light was found to be 0.04 μg mL^{-1}. Astaxanthin use as drawn from *Haematococcus*, will expand not only in the consumer market, but also in the worldwide medical market, therefore, an optimal biosynthesis method for astaxanthin production is important.

***Keywords*:** Monochromatic, *Haematococcus pluvialis*, Astaxanthin, Photo damage

1. INTRODUCTION

Photosynthetic cells are important for the production of organic matters. Microalgae have umpteen source of important pharmaceuticals pigments and biochemicals (Metting *et al*., 1986). *Haematococcus pluvialis* is considered as best natural source of astaxanthin Astaxanthin (3,3'-dihydroxy-β,β'-carotene-4,4'-dione) is a red ketocarotenoid, occurs in cytoplasmic lipid bodies. Cultivation of *H. pluvialis* is very difficult on a large scale due to its slow growth and risk of contamination in open cultures.

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The life cycle of unicellular green microalga *Haematococcus pluvialis* has two stages depending on its environmental conditions, green motile and red non-motile stage (Kobayashi *et al*., 1991; Margalith 1999). Nutrient limitation or supplement (Boussiba *et al*., 1991), high light intensity (Fabregas *et al*., 2000; Park *et al*., 2001), cell concentration, light path, mixing rate, and the geometry of the cultivation vessel (Richmond *et al*., 2003), are the factors which influence on the accumulation of astaxanthin in *Haematococcus pluvialis*. The effect of light is undoubtedly the most important factor in the astaxanthin accumulation (Park *et al*., 2001; Hall *et al*., 1999).

In our previous study (Singh *et al*., 2011), screening, production, optimization and characterization of cyanobacterial polysaccharide, was investigated. In the present investigation, the efforts were made to study the impact of monochromatic light on the biosynthesis of astaxanthin in green alga *Haematococcus pluvialis.*

2. MATERIALS AND METHODS

2. 1. The procurement and maintenance of *Haematococcus pluvialis* culture

The *Haematococcus pluvialis* used in the present investigation, was procured from the Culture Collection of Algae at the University of Texas, Austin, USA. Culture of *Haematococcus pluvialis* was maintained in both liquid and solid modified form of Bold's basal medium (BMM) (Kanz *et al*., 1969). The axenic stock cultures were incubated in controlled air-conditioned culture room maintained at 25 ±2 °C under 16:8 h (L/D) of light intensity of 35 µmol m^{-2} s^{-1}.

2. 2. Chemicals

All the media chemicals used for the experiment were analytical grade, obtained from companies-Sisco Research Laboratories Pvt. Ltd., Mumbai; HiMedia Laboratories Pvt. Ltd., Mumbai; Loba Chemie Pvt. Ltd., Mumbai. Qualigens Fine Chemicals, Mumbai.

2. 3. Design of experiment for the culture of *H. pluvialis*

Monochromatic light was adjusted by filtering the white fluorescent light by sevan colored cellophane filters such as blue, red, green, yellow violet, green, black and unfiltered white fluorescent light was taken as control. For the preparation of the inoculum, the cells from the stock culture were centrifuged at 5000 rpm for 5 minutes the supernatant was discarded and the pellet was washed with the sterilized double distilled water thrice. The pellet was homogenized in 1 ml BBM and transferred aseptically in a 250 ml conical flask containing 100 ml of fresh BBM (KH_2PO_4, 17.5; $CaCl_2{\cdot}2H_2O$, 2.5; $MgSO_4{\cdot}7H_20$, 7.5; $NaNO_3$, 25; K_2HPO_4, 7.5; NaCl, 2.5; Na_2ETDA, 10; $FeSO_4{\cdot}7H_2O$, 4.98; H_3BO_3, 11.5 g/L, pH 6.8) and incubated under continuous illumination of 35 µmol m^{-2} s^{-1} at 25 ±2 °C for 4 days. A 4-day old culture was used as an inoculum for the experiment.

The experiment was performed in 250 ml conical flasks. 4-day old culture approximately 1×10^6 cells mL^{-1} was inoculated into 100 ml sterilized fresh medium in 250 ml flasks and incubated separately in blue, red, green, yellow violet, green, black in controlled air conditioned culture room at 25 ±2 °C. All the cultures were shaken thrice a day with rotary flask shaker.

2. 4. Extraction of astaxanthin

The harvested biomass of *H. pluvialis* was first treated with a solution of 5% KOH in 30% methanol to destroy the chlorophyll. The supernatant was discarded and remaining pellet was treated with DMSO for the extraction of astaxanthin (Boussiba *et al*., 1991). The absorbance of the combined extracts was determined at 492 nm, and the amount of the pigment was calculated according to Davies, (1976).

3. RESULTS AND DISCUSSION

The effect of light is undoubtedly the most important factor of the astaxanthin accumulation in *Haematococcus pluvialis*. A suitable light source with adequate light intensity is required to accumulate a high-level of astaxanthin The quality of light, such as wavelength and/or emission spectra of light also affects the performance of algae cultivations (Taiz *et al*., 2002) as well as astaxanthin production. In present investigation, fluorescent light was filtered into individual light. The selective irradiation of red light was most effective for the facilitation of biosynthesis of astaxanthin upto 0.055 µg mL^{-1} in *H. pluvialis* followed by blue light i.e., 0.04 µg mL^{-1} as shown in (Fig. 1).

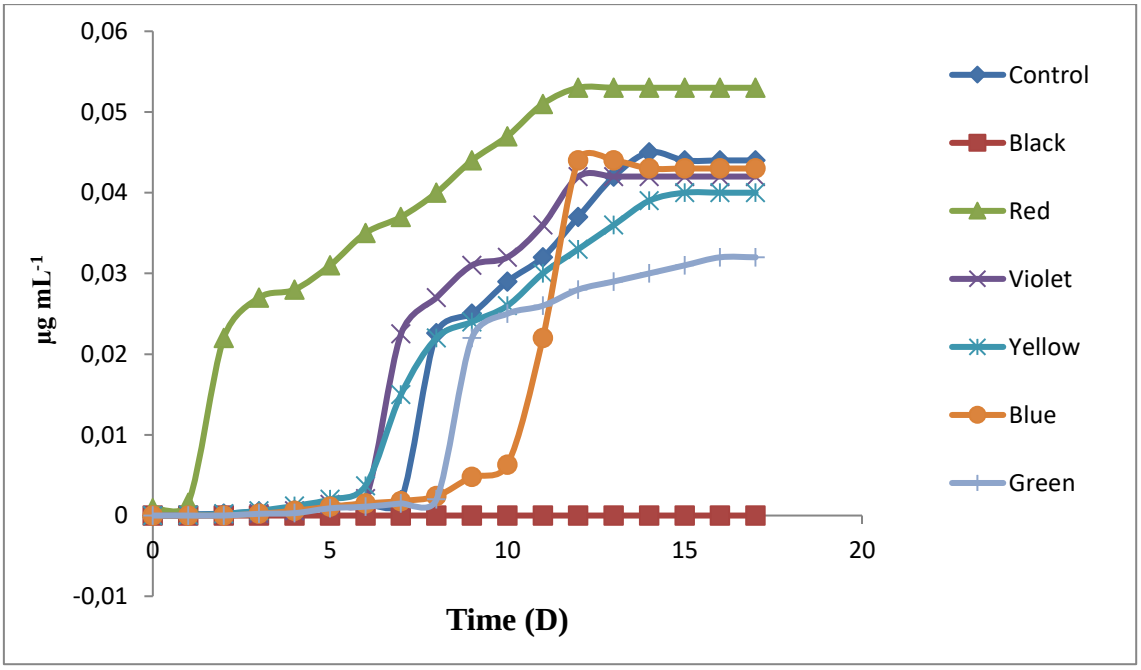

Fig. 1. Impact of monochromatic light on the biosynthesis of astaxanthin

In *Dunaliella bardwawii*, it was observed that massive accumulation of β-Carotene and it was found that this massive accumulation of β-Carotene in *Dunaliella bardwawii* prevent its photosynthetic apparatus against photo damage caused by red light (Ben-Amotz *et al*., 1989). Similar results were reported by Kobayashi (1992), using selective irradiation of blue, red and red plus blue. According to the study of Kobayashi (1992), the induction of

astaxanthin occurred in the red plus blue light conditions. However it was imprecise that which light condition was responsible for the increment of astaxanthin in *H. pluvialis*, also it was reported that the red light was found efficient for photosynthesis and more stressfull than the blue light in *H. pluvialis* ((Katsuda *et al*., 2004). This could be a reason for enhanced astaxnthin biosynthesis in cultures incubated in red light. The formation of astaxanthin in the *H. pluvialis* cells incubated under different monochromatic light were observed under microscope dailywise as seen in (Fig. 2).

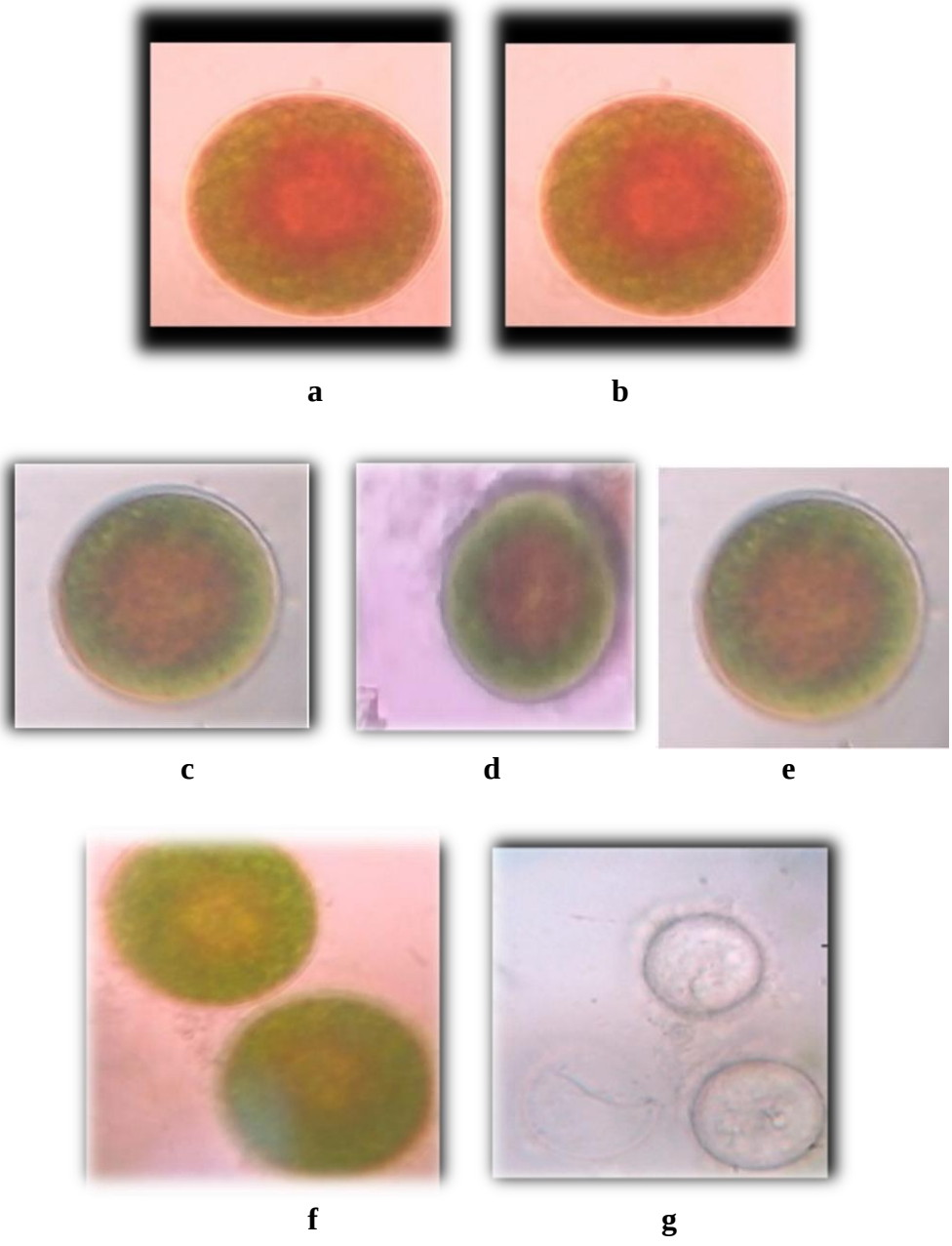

Fig. 2. Microscopic view of *H. pluvialis* under the : Red (**a**) Blue (**b**) Normal fluorescent light (**c**) Violet (**d**) Yellow (**e**) Green (**f**) and Black light(**g**)

4. CONCLUSION

From our present investigation, it is apparent that the red light is more effective in promoting the biosynthesis of astaxnthin content in green alga *Haematococcus pluvialis.* Astaxanthin from *Haematococcus* will expand not only the consumer space but also medical institution worldwide, therefore optimal biosynthesis method of astaxanthin is important for the human welfare

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