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ASSESSMENT OF UV-B INDUCED CHANGES IN GROWTH, PHOTOSYNTHETIC PIGMENTS AND BIOCHEMICAL RESPONSE OF TWO CYANOBACTERIA *NOSTOC MUSCORUM* AND *SYNECHOCYSTIS* PCC 6803

Md. Akhlaqur Rahman and Shanthy Sundaram
Centre of Biotechnology, Nehru Science Centre, University of Allahabad,
Allahabad – 211002, Uttar Pradesh, India

Abstract— **In the present work, impact of UV-B radiation (280-315nm: 0.4 W m-2) on growth, photosynthetic pigments, protein, ascorbate, proline and lipid peroxidation have been studied in two cyanobacteria *Nostoc muscorum* and *Synechocystis* PCC 6803. UV-B radiation (2 to 6 hrs) leads to 55% inhibition of growth in *Synechocystis* PCC 6803 in comparison to control where as in *Nostoc muscorum* growth reduces up to 45%. This UV-B treatment also significantly decreased the contents of chlorophyll, carotenoids and phycocyanin. Photosynthetic pigments decreased with increasing doses of UV-B (2 to 6 hrs) radiation. However, the inhibitory effect in *Synechocystis* PCC 6803 was more pronounced than in *Nostoc muscorum*. With increasing UV-B exposure period, production of ascorbate (19-45%), proline (12-29%) and lipid peroxidation was significantly higher in *Synechocystis* PCC 6803 as compared to control sample. It was observed that lipid peroxidation enhanced 33 % than control sample of *Synechocystis* PCC 6803. Our result shows that photosynthetic apparatus is the main target of UV-B radiation causing degradation of photosynthetic pigments. This study concluded that *Synechocystis* PCC 6803 was the susceptible organism for survival in stress condition than *Nostoc muscorum*.**

Key words— **carotenoids, chlorophyll, cyanobacteria, lipid peroxidation, proline, UV-B radiation.**

I. INTRODUCTION

As we know the global reduction of stratospheric ozone layer due to release of atmospheric pollutants such as chlorofluorocarbons (CFCs), chlorocarbons (CCs), and organobromides (OBs) causing an increase level of UV-B (280-315nm) radiation on earth surface [1,2]. This increased UV-B radiation induces hazardous effect on physiological and morphological traits of plants [3, 4].

In the natural environment, the microorganisms including cyanobacteria may experience many kinds of stresses such as UV-B radiation. UV-B is a potential abiotic stress negatively affecting crop productivity and living organisms. UV-B radiation not only affect photosynthesis and related metabolic processes including biological nitrogen fixation but also affect growth, survival, pigmentation, protein profile and membrane damage of cyanobacteria. Photosynthetic process is a universal feature of algae and higher plants, and PS II has been found to be sensitive to environmental changes [5].

Enhanced levels of UV-B radiation have also been demonstrated to cause damaging effects in cyanobacteria [6]. Aquatic photosynthetic organisms such as cyanobacteria are affected from ultraviolet radiation, because they lack protective UV absorbing epidermal layer like higher plants [7, 8]. In some photosynthetic organisms, it has been demonstrated that high levels of white and blue light mediate photo repair and ameliorate UV-B-induced damage.

UV-B radiation arrests growth, suppresses chlorophyll biosynthesis and inhibits electron transport, but little attention has been paid to lipid peroxidation and antioxidant enzyme in the photosynthetic organism cyanobacteria. Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequently tissue damage. UV-B induced oxidative stress and oxidative damage increases with irradiation time and can be reversed after long-term irradiation. In order to prevent harmful effects caused by stress, organism develops radical quenchers and antioxidants that provide protection by scavenging harmful radicals [9].

With this aim, we have compared studied the effect of UV- B radiation on growth, photosynthetic pigment, protein, proline and lipid peroxidation in two different cyanobacteria *Nostoc muscorum* and *Synechocystis* PCC 6803.

II. MATERIALS AND METHODS

A. Organisms and Culture conditions

Nostoc muscorum, a filamentous, heterocystous cyanobacteria and *Synechocystis* PCC 6803, unicellular, non-nitrogen fixing cyanobacteria were obtained from the stock culture kept in the Algal Laboratory, Botany Department, University of Allahabad, India. *Nostoc muscorum* was grown axenically in nitrogen free BG-11 medium, [10] at27±2 °C and *Synechocystis* PCC6803 was grown in nitrate containing BG-11 medium [11] at 30 ±2°C.The cultures were illuminated under fluorescent light of 72 µmol m^{-2} s^{-1} Photo synthetically Active Radiation (PAR) with a photoperiod of 14:10 (light: dark).

B. UV-B radiation Treatment

For UV-B exposure the cyanobacterial strain *Nostoc muscorum* and *Synechocystis* PCC 6803 were taken in open

petridishes (75mm) occupying a depth of 0.5cm. The source of UV-B radiation at influence rate of 0.4 W $^{m-2}$ was obtained from single Philips (TL- 40W/12, RS UV-B Medical, made in Holland) tubes with main output at 315nm. Petridishes containing algal suspension were kept at a distance of 20 cm (+ UV-B) for 2, 4 and 6 hours in triplicate covered with 0.14 mm cellulose acetate filter (Johnston Industrial Plastics, Toronto, Canada) to remove all incidents UV-C (< 280 nm). During UV-B exposure culture were gently agitated by magnetic stirrer to ensure uniform exposure. Culture maintained at27±2 °C and 30 ±2°C under fluorescent light were considered as control.

C. Growth measurement

The cyanobacteria were inoculated at the initial absorbance of 0.7 at 750 nm. To determine the growth, homogenized cyanobacterial cell were exposed to UV-B for 2, 4 and 6 hours. After predetermined time interval liquid culture was withdrawn from each test sample and growth of cyanobacteria was recorded by measuring the absorbance of cell suspension at 750 nm using UV-Visible spectrophotometer (Ultrospec 4000 UV/Visible, Pharmacia Biotech).

D. Photosynthetic pigments measurement

For determination of photosynthetic pigment, 10 ml of cyanobacterial culture was centrifuged and the pellet was suspended in acetone (80%). The cells were incubated overnight at 4ºC.The suspension was centrifuged at 10,000 rpm for 5 minutes and the supernatant was used for the measurement of chlorophyll a and carotenoid contents by determining O.D. at 665nm and 480 nm, respectively, with Ultrospec 4000 UV-Visible spectrophotometer (Pharmacia Biotech). Cyanobacterial Pellet was used for the extraction of phycocyanin by repeated freezing and thawing, and the blue supernatant formed in Phosphate buffer (pH 7.5) was recorded at 620 nm. Chlorophyll a content was calculated according to Mackinney [12]. The total amount of carotenoid was calculated using the specific absorption coefficient, described by Myers and Kratz [13] and phycocyanin according to Brody and Brody [14].

E. Protein estimation

The Protein was estimated at 700 nm spectrophotometricaly following the method of Lowry et al [15].

F. Determination of osmoprotectants

Ascorbate was extracted from pellet of cell (treated and untreated) of 10 mL test samples with 5% w/v sulfosalicylic acid and the amount of ascorbate was determined in the supernatant after centrifugation at 10,000 rpm for 15 min, methodology was followed of Oser [16] and expressed as μmol g-1 protein.

G. Proline estimation

For Proline estimation cyanobacterial cells (treated and untreated) were suspended in 10 ml of 3% Sulphosalicylic acid and centrifuged at 10,000 rpm for 10 min to remove cell debris. For reaction mixture 2 ml of supernatant, 2 ml of ninhydrin was added, followed by addition of 2 ml glacial acetic acid and incubated at boiling temperature for one hour. The mixture was extracted with toluene. Proline was measured spectrophotometrically at 520 nm from organic phase (Bates et al) [17].

H. Lipid peroxidation

Lipid peroxidation was measured according to method of Heath and Packer [18]. Harvested cyanobacterial cells were homogenized in 1% Trichloroacetic acid (TCA) and then centrifuged at 10,000 rpm for 15 min at room temperature. Same volume of supernatant and 0.5% Thiobarbituric acid (TBA) in 20% TCA solutions (freshly prepared) were added into a new test tube and incubated at 95 °C for 30 minutes in water bath. The supernatant were transferred into ice bath, after cooling centrifuged at 10,000 rpm for 10 minutes. The absorbance of the supernatant was recorded at 532nm and 600nm. 0.5% TBA in 20% TCA was used as the blank. MDA contents were determined using the coefficient 155 $mm^{-1}cm^{-1}$.

I. Statistical analysis

All the procedures were performed under aseptic conditions. Percentage reductions in activities were calculated by comparing with control samples % reduction= [(control-treatment)/control] x 100. Data were statistically analyzed and the results were expressed as averages (±SD) of three independent replicates. Comparison of UV treatment effects were conducted by standard ANOVA analysis and multiple comparison tests were performed using Excel software (Microsoft, USA).

J. Results and Discussion

The growth response of two cyanobacterial species *Nostoc muscorum* and *Synechocystis* PCC 6803 after UV-B exposure showed inhibitory effect (Fig.-1).

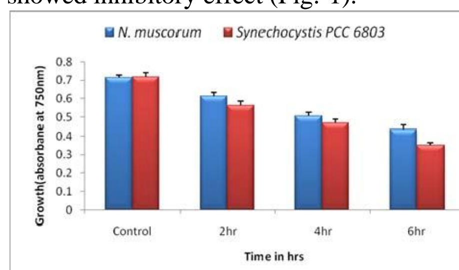

Fig. 1: Effect of UV-B radiation on growth of two cyanobacteria *Nostoc muscorum* and *Synechocystis* PCC 6803

It was observed that growth pattern of test organism *Synechocystis* PCC 6803 decreased with gradual increase in UV-B radiation exposure duration as compared to *Nostoc muscorum*. In *Nostoc muscorum* growth decreased by 10-45% of control and in *Synechocystis* PCC 6803 by 20-55% on 2 to 6 hrs of UV-B exposure. The growth response of cyanobacteria to UV-B radiation resulted in differences probably due to different degree of damage caused by UV-B directly or indirectly on DNA, protein and photosynthetic apparatus as reported by [19, 20, 21]. The decreasing levels of photosynthetic pigments, Chl a, carotenoids and phycocyanin increased with UV-B exposure time. *Synechocystis* PCC 6803 showed decrease in chlorophyll at 6 hrs UV exposure (45%). On the other hand, *Nostoc muscorum* showed 16-38% inhibition (Fig.-2).

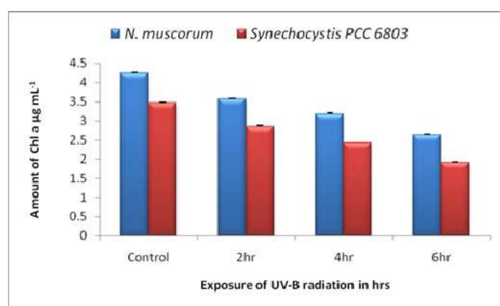

Fig. 2: Effect of UV-B radiation on Chlorophyll a of two cyanobacteria *Nostoc muscorum* and *Synechocystis* PCC 6803

Carotenoids showed varied response to UV-B exposure. Exposure for 2 to 6 hrs radiation caused decrease by18-42% in *Synechocystis* and 16-32% in *Nostoc muscorum* (Fig.-3).

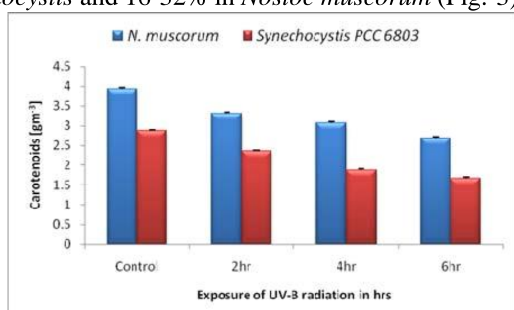

Fig. 3: Effect of UV-B radiation on Carotenoids of two cyanobacteria *Nostoc muscorum* and *Synechocystis* PCC 6803

Phycocyanin was severely affected in comparison to chlorophyll a and carotenoid. *Synechocystis* PCC6803 showed decrease of phycocyanin from 18-32% and 12-28% in *Nostoc muscorum* (Fig.-4).

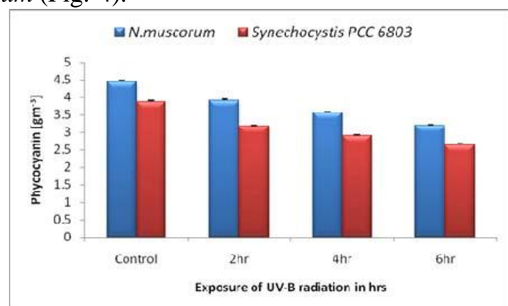

Fig. 4: Effect of UV-B radiation on Phycocyanin of two cyanobacteria *Nostoc muscorum* and *Synechocystis* PCC 6803

It was suggested that the damaging effect on photosynthetic pigments was due to bleaching caused by UV-B radiation or through active oxygen mediated peroxidation [22]. The loss of phycocyanin was caused due to direct interaction of UV-B with phycocyanin. Phycocyanin directly absorbs radiation in UV-B region (280 nm and above) causing damage, being it is protenacious in nature and localized on the outer surface of thylakoid membrane. The effect of UV-B radiation on protein content in both cyanobacteria *Nostoc muscorum* and *Synechocystis* PCC 6803 was found to have significant alteration as compared to control sample. In *Nostoc muscorum* the protein content decreased to 9% at 6 hrs of exposure to UV-B radiation. While, on the other hand in *Synechocystis* PCC 6803 the protein content decreased up to 29% at 6 hr of UV-B radiation (Fig.-5).

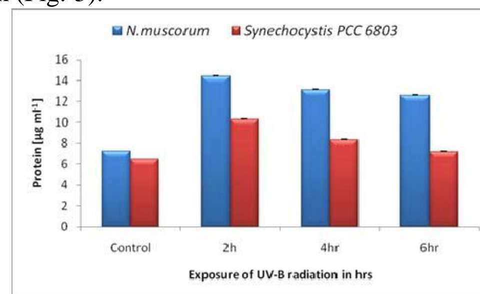

Fig. 5: Effect of UV-B radiation on Protein content of two cyanobacteria *Nostoc muscorum* and *Synechocystis* PCC 6803

The increase in ascorbate, proline and MDA contents after stress are indicative of a correlation between free radical generation and proline accumulation. Proline is an iminoacid essential for primary metabolism whose accumulation occurs in unfavorable and stress condition in cyanobacteria and plants. This is also in agreement with earlier report on Spirulina platensis and *Westillopsis prolifera* [23, 4]. In the experimental organisms, *Synechocystis* PCC 6803 and *Nostoc muscorum*, ascorbate and proline content drastically increased with increasing dose of UV-B radiation. In the case of *Nostoc muscorum*, ascorbate increased by 14-39% whereas, in *Synechocystis* PCC 6803 by 19-45% in comparison to control (Fig.-6).

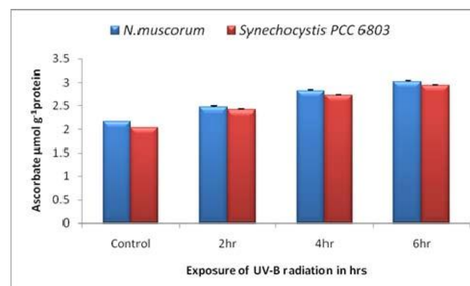

Fig. 6: Effect of UV-B radiation on Ascorbate activity of two cyanobacteria *Nostoc muscorum* and *Synechocystis* PCC 6803

The proline content was higher than control in *Nostoc muscorum* by 25% and in *Synechocystis* PCC 6803 by 29% of control (Fig.-7).

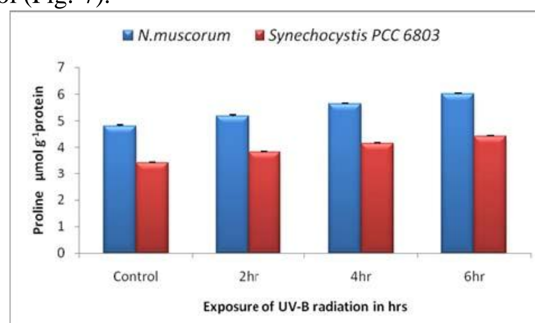

Fig. 7: Effect of UV-B radiation on Proline of two cyanobacteria *Nostoc muscorum* and *Synechocystis* PCC 6803

High accumulation of cellular proline has been reported in many higher plants and cyanobacteria due to increased synthesis and decreased degradation under various stress conditions [24, 25]. The accumulation of proline after stress is probably due to decrease in the activity of electron transport system [26]. Lipid peroxidation (estimated as malondialdehyde) that is used as reliable marker of oxidative stress [27] was measured after UV-B stress to cyanobacteria. The free hydroxyl radical reacts with deoxyribose to produce deoxyribose propanols which react with thiobarbituric acid and forms red color complex. The result indicates high degree of lipid peroxidation in *Synechocystis* PCC 6803. Content of MDA increased by 13-30% in *Nostoc muscorum* and 15-33% in *Synechocystis* PCC 6803 (Fig.-8).

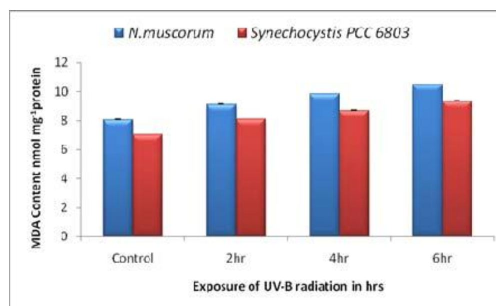

Fig. 8: Effect of UV-B radiation on Lipid peroxidation of two cyanobacteria *Nostoc muscorum* and *Synechocystis* PCC 6803

Increased MDA content in cyanobacteria is caused due to oxidative degradation of polyunsaturated fatty acids in cyanobacterial membrane as reported by [28, 29] who also, observed an increase in MDA content in Cylendospemum sp. upon UV-B exposure.

III. Supplementary Material

Value represent mean of three independent determinants ± S.D

Experimental Organisms	UV-B exposure (hr)	Growth OD at 750 nm	Chl a [gm^{-3}]	Carotenoids [gm^{-3}]	Phycocyanin [gm^{-3}]	Protein [µg ml^{-1}]	Ascorbate µmol g^{-1}protein	Proline µmol g^{-1}protein	MDA content nmol mg^{1} protein
N.muscorum	Control	0.715±0.014	4.25±0.015	3.94±0.033	4.45±0.034	6.70±0.032	2.17±0.002	4.81±0.023	8.06±0.034
	2 hr	0.637±0.023	3.57±0.014	3.31±0.032	3.91±0.032	6.55±0.022	2.47±0.032	5.19±0.031	9.11±0.023
	4 hr	0.542±0.022	3.18±0.024	3.07±0.022	3.56±0.022	6.40±0.041	2.82±0.021	5.63±0.023	9.83±0.022
	6 hr	0.434±0.024	2.64±0.013	2.69±0.031	3.20±0.023	6.15±0.030	3.02±0.024	6.01±0.022	10.48±0.014
Synechocystis PCC 6803	Control	0.719±0.02 3	3.47±0.015	2.87±0.031	3.87±0.032	7.25±0.033	2.03±0.013	3.42±0.013	7.02±0.013
	2 hr	0.615±0.023	2.85±0.024	2.35±0.022	3.17±0.031	6.42±0.032	2.41±0.023	3.83±0.022	8.07±0.031
	4 hr	0.507±0.021	2.42±0.014	1.87±0.032	2.90±0.032	6.40±0.023	2.72±0.022	4.14±0.023	8.71±0.032
	6 hr	0.410±0.013	1.91±0.023	1.67±0.031	2.64±0.032	6.15±0.022	2.94±0.013	4.42±0.014	9.33±0.041

Table-1: Effect of UV-B radiation on growth, photosynthetic pigments, protein content, osmoprotectants And lipid peroxidation of *Nostoc muscorum* and *Synechocystis* PCC 6803

IV. Conclusion

On the basis of the present study, it is concluded that UV-B radiation caused significant alteration in biochemical process of cyanobacterial species, *Synechocystis* PCC 6803 and *Nostoc muscorum*. Among both of the species cyanobacteria, *Nostoc muscorum* was found to withstand at long exposure period of UV-B radiation as compared to *Synechocystis* PCC 6803.

V. Acknowledgment

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