Indian Journal of Experimental Biology
Vol. 44, April 2006, pp. 330-335

Effect of UV-B on photosynthesis, membrane lipids and MAAs in marine cyanobacterium, *Phormidium corium* (Agardh) Gomont

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Received 26 July 2005; revised 31 January 2006

UV-B radiation (0.8±0.1 mW cm^{-2}) and UV-B radiation supplemented with low intensity PAR (~80 μ mol m^{-2} s^{-1}) affected photosynthesis at the level of antenna system as well as PS II reaction centre (F_o and F_m declined) in *Phormidium corium* (Agardh) Gomont. UV-B radiation resulted in decline in sugar content, peroxidation of membrane lipids as well as quantitative and qualitative changes in phosphoglycolipids and neutral lipids. Fatty acid profile did not show any qualitative changes due to the treatment, however, UV-B supplemented with low PAR resulted in slightly higher level of unsaturation. *P. corium* synthesized MAAs in response to UV-B. Quantity of MAAs increased when UV-B treatment was supplemented with low level PAR.

Keywords: Chlorophyll fluorescence, Cyanobacteria, Lipid peroxidation, Mycosporine like amino acids, Phosphoglycolipids, Sugars, UV-B radiation.

Depletion of the stratospheric ozone layer has resulted in an increasing incidence of solar UV-B radiations (280-320 nm) at the Earth's surface[1]. Since cyanobacteria use sunlight for photosynthesis, they are also exposed to UV-B radiation. UV-B is a small component of the solar radiation reaching the earth's atmosphere and is known to penetrate to different levels in fresh water as well as marine environments[2]. The energy level of UV-B photon is high which makes it photochemically active form of radiation and it is largely absorbed by DNA, protein, chromophores, pigments, plastoquinone and plastoquinol. This lead to wide ranging effects, including alteration in the structure of proteins, DNA and depression of key physiological processes[3]. A number of physiological and biochemical processes in cyanobacteria such as growth, pigmentation, photosynthesis, enzymes of nitrogen and carbon metabolism have been reported to be susceptible to UV-B[4-5]. In this study we investigated the effect of UV-B and UV-B radiation supplemented with low intensity PAR on photosynthesis, lipids, sugars content and mycosporine like amino acids (MAAs) in a cyanobacterium, *Phormidium corium* (Agardh) Gomont, isolated from coral reefs of Lakshadweep Island, India.

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Materials and Methods

Phormidium corium was obtained from Dr C Raghukumar, National Institute of Oceanography, Goa. It has been isolated from corals of Lakshadweep Island, India. The cultures were grown in ASN III medium (Rippka *et al.*[6]) and maintained at 30°±2°C under fluorescent light at 80 μmol m^{-2} s^{-1} PAR with 12h light/dark cycle. All experiments were carried out during active growth phase inoculating 30 days old culture of the organism.

Exposure to UV-B radiation—UV-B exposure of 0.8±0.1 mW cm^{-2} was given to the cells while stirring in a BOD chamber for 0-6 h. UV-B radiation was measured using a UV-B radiometer (Radiometer model VLX-312 and light source model T-6M with a λ-max at 312 nm were from Vilbour-Lourmat, France). For certain experiments, white light of 80 μmol m^{-2} s^{-1} PAR was supplemented using light source with fibre optic at 60° angle to the culture during UV-B treatment.

Photosynthesis measurement—Photosynthesis was measured using chlorophyll fluorometer (PAM, Walz, Germany) according to Sharma *et al*[7]. Culture was dark adapted for 10 min prior to measurement. The dark-adapted culture was exposed to a modulated light at 4 μmol m^{-2} s^{-1} to measure initial fluorescence (F_o) followed by exposure to a saturating pulse of white light of 4000 μmol m^{-2} s^{-1} to provide the maximum fluorescence (F_m). Variable fluorescence

(F_v) was determined by deducting the F_o from F_m ($F_v = F_m - F_o$) and the F_v/F_m ratio was calculated.

Cultures were harvested by centrifuging at 8000 × *g* for 15 min. The pellet was homogenized in a tissue homogenizer and resuspended in fresh culture medium and used for analysis. Total sugar content was determined using anthrone reagent and the amount of reducing sugars was determined using 2-4, dinitrosalicylic acid following Plummer[8]. Glycogen content was determined according to Liotenberg *et al*[9]. Lipid peroxidation was quantified by TBA-MDA (thiobarbutaric acid-malonaldehyde) adduct formation which signifies the formation of polyunsaturated fatty acid peroxides according to method of Sankhalkar and Sharma[10].

Extraction and determination of lipids—Total lipids were extracted according to Turnham and Northcote[11]. Lipid extract was centrifuged for 5 min at 2000×*g* to get rid of cell debris. To the supernatant, 0.8 ml of distilled water was added followed by 5 ml of chloroform and 5 ml of 0.88% KCl in a separating funnel to make the ratio of chloroform: methanol:water (1:1:0.9). The mixture was shaken and allowed to separate for 30 min. The solvent phase was collected in screw-capped vials and concentrated under nitrogen gas. The dried lipid extract was redissolved in chloroform and was used for qualitative and quantitative determination of different class of lipids. Separation of glycolipids and phospholipids into individual lipid classes was carried out in a thin layer chromatography (TLC) on silica gel H according to Liljenberg and Von Arnold[12]. The eluting solvents were the following: chloroform: methanol:glacial acetic acid:water (85:15:10:3.5) for phospho glycolipids and diethyl ether: water (90:1) for neutral lipids. The spots were visualized in an iodine chamber and identified against standards and R_f values[13]. Total sugars in glycolipids were determined in total lipid extract by the phenol-sulfuric acid method according to Kushawa and Kates[14]. Phospholipid was estimated by determining the amount of phosphorus in total lipid extract according to Bartlett as described by Christe[15].

Esterification of fatty acids—The fatty acid methyl esters were prepared for analysis in a Gas chromatograph (Nucon, 5765) with FID detector (column 6 mm×2 mm ss, with DEGS 10% on 80-100 mesh chromosorb W-HP; column temperature 180°C; injector temperature 220°C; carrier gas nitrogen, 30 ml/min). The internal standard (1 m*M* heptadeconoic acid) was added to sample and were subjected to methanolysis in the presence of methanoic-HCl at 68°-70°C for 2 h. The methyl esters were extracted with hexane, treated with sodium bicarbonate and washed with distilled water. Upper (hexane) solution was evaporated to dryness in a water bath at 35°-40°C with nitrogen gas. The methyl esters were extracted in hexane and injected to the injector port of GC. The fatty acid methyl esters peaks were identified by comparing their retention time of fatty acid standards and were quantified.

Extraction and purification of mycosporine like amino acids (MAAs)—Extraction and purification of MAAs was carried out according to the method of Sinha and Häder[4]. Lyophilized cells (0.1 g) were homogenized using 20% (v/v) aqueous methanol (HPLC grade) and incubated at 45°C for 2.5 h. The pellet was removed and supernatant was evaporated to dryness. The dried supernatant was dissolved in 0.2% acetic acid and analyzed using HPLC (Waters Spherisorb ODS 25 μm×4.6 mm×250 mm; detection programme Waters 2996 PDA detector) at 330 nm with a flow rate of 1.0 ml/min using isocratic mobile phase of 0.2% acetic acid. Spectra of MAAs was measured using PDA detector (Waters, 2996) at the wavelength range of 200-700 nm.

Results and Discussion

Effect of UV-B and UV-B supplemented with light on chlorophyll fluorescence (F_v/F_m ratio) given in Fig. 1 showed a linear decrease in the F_v/F_m ratio in response to increasing duration of the UV-B treatment. A 6 h UV-B treatment resulted in complete inhibition of F_v/F_m ratio, however, supplementing UV-B radiation with low PAR showed 95% decrease in F_v/F_m ratio. Decrease in F_v/F_m ratio was due to decrease in F_o as well as F_m level. UV-B treatment for

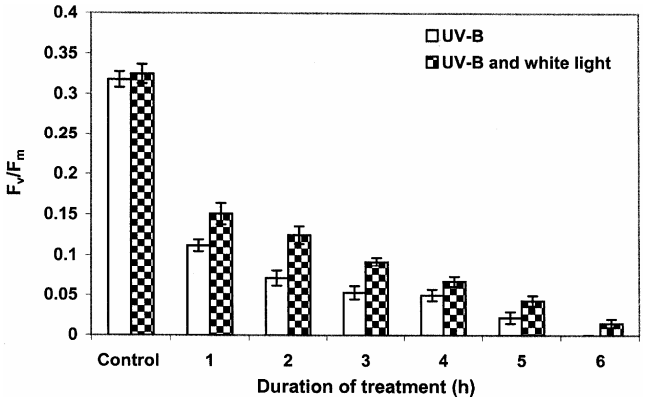

Fig. 1—Effect of UV-B radiation and UV-B supplemented with low visual light up to 6 h on F_v/F_m ratio in *P. corium*. [Bar represents the mean ±SD n=6].

5 h showed 26% decrease in F_o level and 49% decrease in F_m level. UV-B supplemented with low visual light treatment for 5 h resulted in 41% decrease in F_o level and 58% decline in F_m level (data not shown).

Decrease in F_o is an indicator of decrease in the excitation energy reaching the photosynthetic reaction centre II probably due to loss of pigments in the light harvesting complex II[16]. Decrease in F_m is an indicator of damage to the PS II reaction centre itself and may represent direct damage to key components within the photosystem such as D2 protein of PS II[17]. Fluorescence excitation and emission spectral studies by Nedunchezhian *et al.*[18] have also shown UV-B induced decrease in the photosynthetic activity in *Anacystis* sp.

Exposure to UV-B radiation and UV-B supplemented with low light decreased total sugar, reducing sugar and glycogen content to varying degree (Fig. 2). UV-B exposure for 6 h resulted in 75% decrease in reducing sugar (Fig. 2a), 50 % decrease in total sugar content (Fig. 2b) and 44% decrease in glycogen content (Fig. 2c).

Decline in sugar content due to UV-B and UV-B supplemented with low light treatment could be due to the damage caused to photosynthesis. Stored sugar in the form of glycogen declined upon longer duration of treatment suggesting conversion of stored sugar to meet the energy and metabolic requirement. Similar to this study exposure of cyanobacteria, *Nostoc, Anabaena* and *Scytonema,* to UV-B resulted in decrease in total starch content in accordance with the inhibition of photosynthesis[19].

UV-B treatment for 6 h resulted in an increase of peroxidation of cell membrane lipids by 73% as compared to control, while supplementing the UV-B radiation with low level visual radiation decreased the level of lipid peroxidation to 35% (Fig. 3). Increased level of lipid peroxidation of cell membrane is an indicative of oxidative damage as well as level of unsaturation of fatty acids, which may affect the fluidity of membrane. Salmon *et al.*[20] have reported that UV-B radiation promotes the formation of lipid oxidation products, which destroys the lipid soluble antioxidants and causes oxidation of membrane lipids. He *et al.*[21] have also reported that UV-B treatment increased oxidative damage, decreased photosynthetic efficiency and inhibited growth in cyanobacteria.

UV-B induced changes were observed in profile of phospho-glycolipids and neutral lipids of *P. corium.* (Fig. 4a, b). UV-B treatment for 6 h resulted in 56% decline in the glycolipid content, whereas in UV-B supplemented with low visual radiation, the decrease was only 6 % as compared to control (Fig. 5). Effect of UV-B treatment and UV-B radiation supplemented with low visual radiation resulted in almost similar decrease in the phospholipid content (Fig. 5). Quantitative changes in phospho and glycolipids could be because of oxidative damage affecting the integrity of the membrane[22]. Funteu *et al.*[23] have also reported significant decrease in the level of

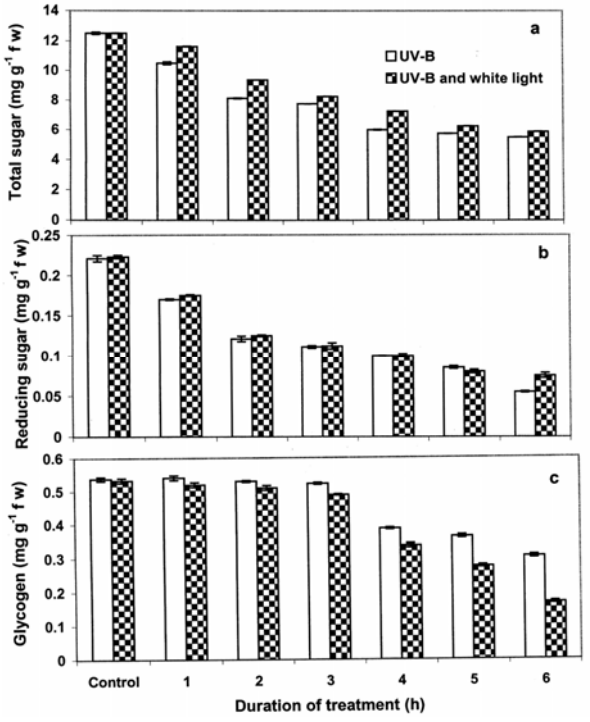

Fig. 2—Effect of UV-B radiation and UV-B supplemented with low visual light up to 6 h on (a) total sugar, (b) reducing sugar and (c) glycogen content in *P. corium.* [Bar represents the mean ±SD n=4].

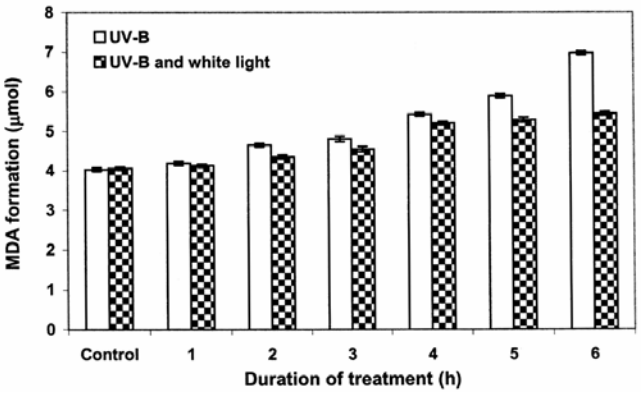

Fig. 3—Effect of UV-B radiation and UV-B supplemented with low visual light up to 6 h on lipid peroxidation of cell membrane in *P. corium*. [Bar represents the mean ±SD n=5].

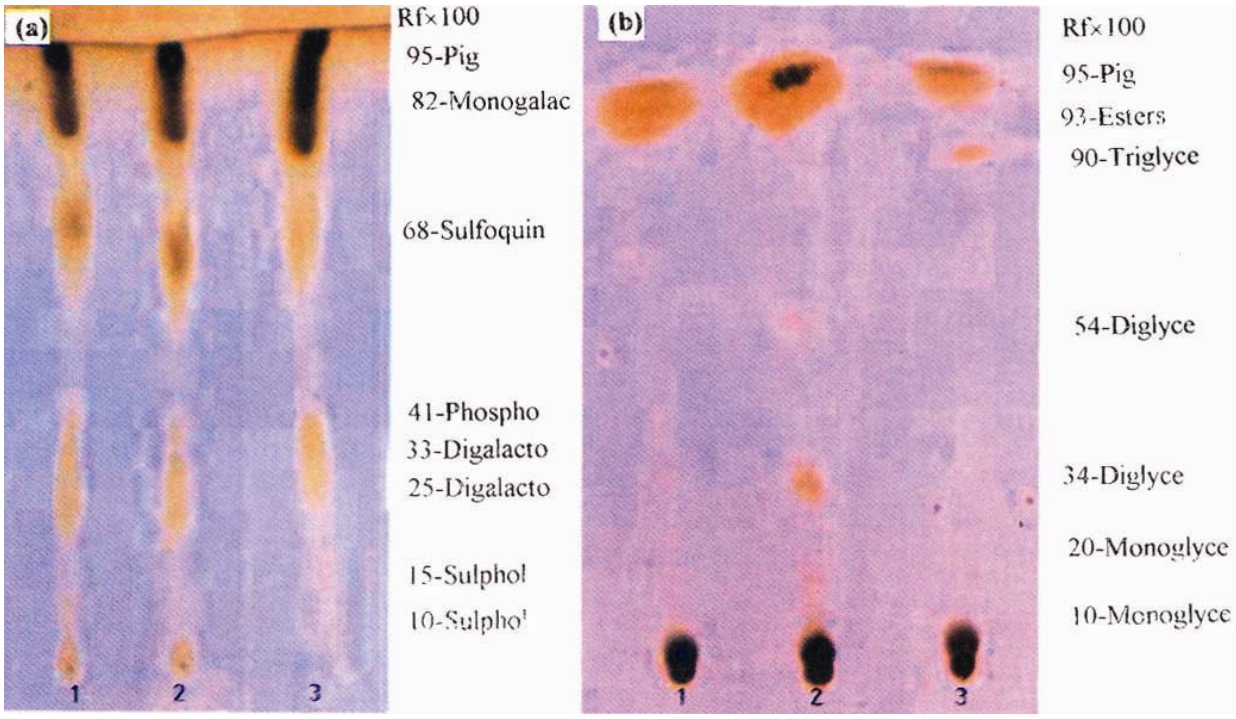

Fig. 4—TLC profile of UV-B radiation and UV-B supplemented with low visual light on (a) phosphoglycolipids and (b) neutral lipids in *P. corium.* (Lane 1- control, Lane 2-UV-B treatment for 6 h, Lane 3-UV-B treatment supplemented with low visual light for 6 h). [95-Pig: 95 pigments; 82-Monogalac: 82 monogalactosyl-diglyceride; 68-Sulfoquin: 68 sulfoquinosyldiglycerol; 41-Phospho: 41 phosphotidylglycerol; 33-Digalacto: 33 digalactosyl-diglyceride; 25-Digalacto to: 25 digalactosyldiglyceride; 15-Sulphol: 15 sulpholipid; 10-Sulphol: 10 sulpholipid; 90-Triglyce: 90 triglyceride; 54-Diglyce: 54 diglyceride; 34-Diglyce: 34 diglyceride; 20-Monoglyce: 20 monoglyceride; and 10-Monoglyce: 10 monoglyceride]

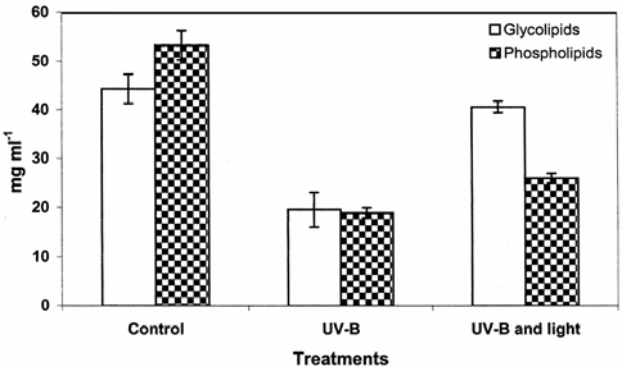

Fig. 5—Effect of UV-B radiation and UV-B supplemented with low visual light for 6 h on glycolipids and phospholipid content in *P. corium.* [Bar represents the mean ±S.D. n=4].

sulfoquinosyl diacylglycerol and phosphatidylglycerol in the cyanobacterium, *Spirulina platensis*.

Fatty acids (lauric acid C_{12}, myristic acid C_{14}, palmitic acid C_{16}, stearic acid C_{18} oleic acid $C_{18:1}$, linoleic acid $C_{18:2}$ and linolenic acid $C_{18:3}$) were detected in the control cultures of organism. There was no qualitative change in the fatty acid composition due to UV-B treatment. Quantity of saturated and unsaturated fatty acids also did not show appreciable change due to UV-B. However, UV-B supplemented with low visual light resulted in an increase in unsaturated fatty acid composition of lipids compared to control (Table 1). The fluidity of membrane lipids is directly correlated with the level of unsaturated fatty acids in biological membranes and increase in unsaturation of fatty acids in membrane lipids might have helped in providing protection to *P. corium* against UV-B radiation.

Absorption spectra of methanolic extracts showed absorbance in the UV region of the spectrum at 322 nm due to MAAs (Fig. 6, inset). HPLC chromatogram of MAAs showed a single peak with retention time of 2.8 min (Fig. 6). Exposure of UV-B radiation for 6 h considerably increased the amount of MAAs (80%), which was further increased to 230% due to UV-B treatment in combination with low visual radiation as compared to control. Spectra of MAAs peak showing

Table 1—Effect of UV-B radiation and UV-B supplemented with low visual light for 6 h on composition of fatty acids of total lipids and glycolipids in *P. corium* (% mole).

Treatment	Fatty acid composition (% mole)						
	Lauric acid 12:0	Myristic acid 14:0	Palmatic acid 16:0	Stearic acid 18:0	Oleic acid 18:1	Linoleic acid 18:2	Linolenic acid 18:3
Total lipids							
Control	11.38	17.63	14.83	9.14	3.28	19.98	3.38
UV-B	11.36	17.61	14.81	9.13	3.28	19.97	3.38
UV-B+L)	11.48	17.72	14.92	9.44	3.65	21.36	4.38
Glycolipids							
Control	11.62	13.40	9.82	16.63	5.76	35.06	5.93
UV-B	11.48	13.24	9.71	16.43	5.76	35.06	5.93
UV-B+L	13.12	15.12	11.24	18.78	7.80	39.75	8.95

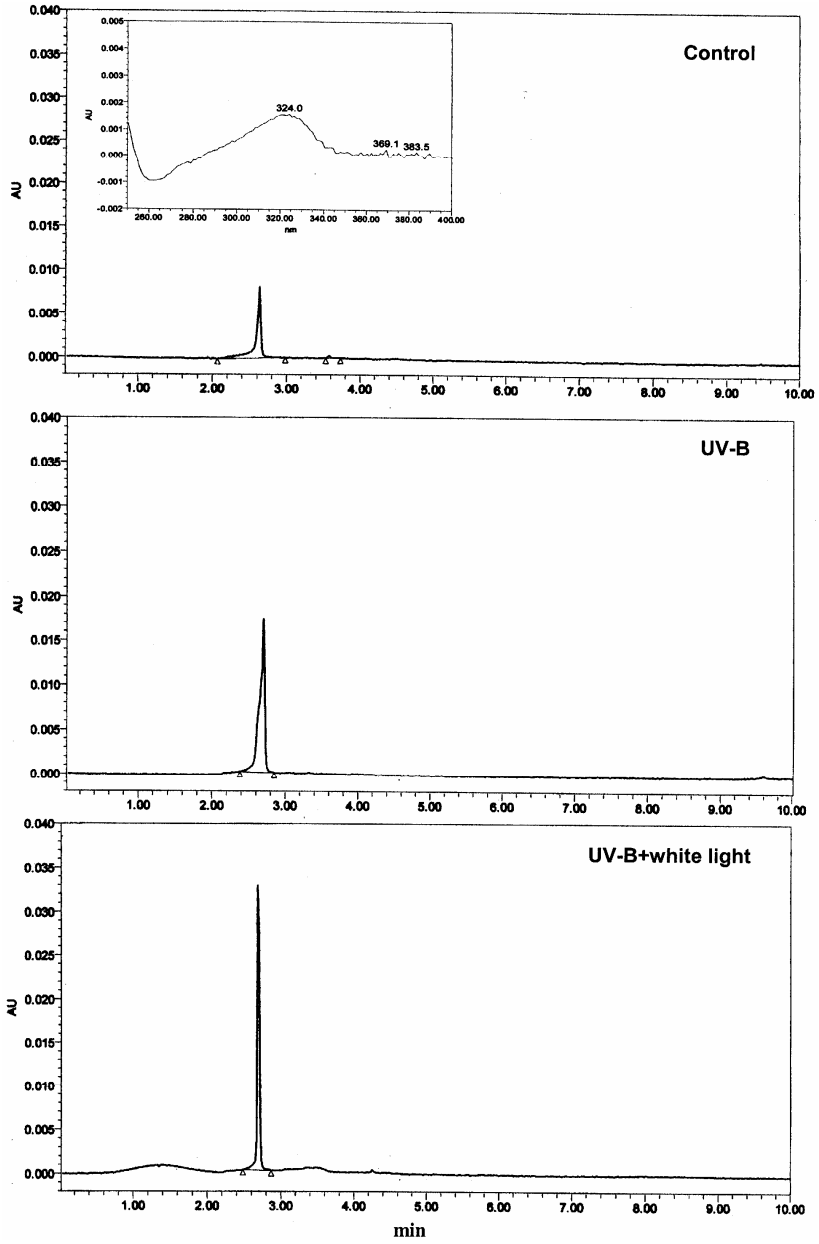

Fig. 6—HPLC profile of mycosporine like amino acids (MAAs) extracted from *P. corium* after exposure to UV-B radiation and UV-B supplemented with low visual light treatment for 6 h. Inset shows absorption spectra of methanolic extracts (20%, v/v). Quantitation of MAAs was calculated on absorbance (AU) basis.

absorbance in the UV region is given (Fig. 6 inset). MAAs have been proved to provide protection against UV-B radiation in certain cyanobacteria[24-25]. Sinha *et al.*[26] have shown that MAAs prevent 3 out of 10 photons from hitting cytoplasmic targets and provide protection as a UV sunscreen pigment.

The present results showed that upon UV-B treatment along with low-level visual radiation the damage to photosynthesis, sugar and lipid peroxidation of *P. corium* was attenuated to some extent. Data also showed that in response to UV-B radiation, there was increase in UV-B absorbing compounds like MAAs to provide protection against the damage. Role of change in fatty acid composition of membrane due to UV-B radiation has also been discussed.

Acknowledgement

We thank to Dr C Raghukumar, NIO, Goa for the gift of *Phormidium corium* culture, Professor Sivkumar Swamy, Karnatak University, Dharwad for help in fatty acid analysis and to Council of Scientific and Industrial Research, New Delhi for financial assistance (grant No. 38(1030)/02/EMR-II).

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