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UV-B radiation and high light induced oxidative damage in *Phormidium corium* may cause bleaching to associated coral reefs.

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Effect of UV-B and photosynthetic active radiation (PAR) flux at 30°C in *Phormidium corium* (Agardh) Gomont, a cyanobacterium isolated from coral, *Porites lutea* (Milne-Edwards and Haime) on the Kavaratti reef of the Lakshawdeep Island, (11° N; 71°E), India had been examined. Exposure of *P. corium* to UV-B and PAR decreased photosynthetic efficiency and increased oxidative damage measured as F_v/F_m ratio and lipid peroxidation of cell membrane. Data indicate little activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) in response to the UV-B and PAR treatment. Activity to quench reactive oxygen species (ROS) was much less in *P. corium* as compared to other cyanobacteria and higher plants. Though mycosporine like amino acids (MAAs) increased significantly as a result of UV-B treatment, PAR caused decrease in the MAAs content, thus neutralizing the beneficial effect of MAAs. Present study suggest that one of the factors of bleaching of coral reefs off the Lakshawdeep Island, may be due to oxidative damage caused to it by production of ROS as *P. corium* lacks efficient antioxidant system.

[Keywords: antioxidant enzymes, coral bleaching, cyanobacteria, lipid peroxidation, mycosporine like amino acids, reactive oxygen species, UV-B]

Introduction

Corals are small animals those live in immense colonies, harvesting nourishment and energy from micro and macro algae, which inhabit their cells[1]. The pigments of associated micro and macro algae in combination with pigments of various hosts such as zooxanthallae lend their coral hosts a spectacular variable coloured appearance[2]. Potential pharma-ceuticals products are also being studied from coral reef ecosystems[3].

Corals are one of the most sensitive ecosystems to climate change on earth[4]. This ecosystem is suffering from coral bleaching. When stressed, corals may lose much of their symbiotic algae. Preliminary assessments indicate that the Indian Ocean is severely impacted region due to coral bleaching. More than seventy percent mortality observed off the coasts of the Maldives, the Andamans, and the Lakshadweep and Seychelles Marine Park System[5]. The outer-atoll seaward slopes of Kadmat in the Lakshadweep Islands, India, had heavy mortality with only 3% live coral cover and 87% dead branching forms.

Coral reef bleaching is caused by various anthropogenic and natural variations in the reef environment including sea temperature, solar irradiance, sedimentation, pollution, disease, excess shade, salinity changes etc[6]. Solar radiation and temperature stresses are reported to be principal causes of coral bleaching[7]. Solar radiation, in the form of both photosynthetic active radiation (PAR) and ultraviolet (UV), can elicit a stress response in symbiotic associations. UV-B penetrates oceanic waters sufficiently to have both direct and indirect effects[8]. UV-B is probably more harmful than high levels of PAR[9,10] and when high light is combined with high temperature synergistic effects may be observed.

Present study consists data to show that both UV-B and high PAR results in increased level of oxidative damage due to inability of *Phormidium corium* (Agardh) Gomont, one of the cyanobacteria found in association with coral *Porites lutea* on the Kavaratti reef of the Lakshadweep island (11°N; 71°E), India, to metabolize reactive oxygen species (ROS) generated due to the stress. This may well be one of the reasons for the coral's bleaching.

Materials and Methods

Phormidium sp. was isolated in association with normal and bleached coral, *Porites lutea* (Milne-Edwards and Haime) on the Kavaratti reef of the

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Lakshadweep group of island (11°N; 71°E), India by Ravindran & Raghukumar[11]. The culture was routinely grown in autoclaved liquid ASN III culture medium[12] and maintained in 100 ml conical flasks filled to 40% of their volume and kept on a environmentally control shaker set to a temperature of 30°C under cool white fluorescent light tubes providing 120 µmol m^{-2} s^{-1} PAR at the culture level with a 14th of photoperiod. *P. corium* was allowed to grow for 30 days to obtain its logarithmic phase of growth (balance growth). All experiments were set with 30 days old culture. The isolated cyanobacterium was identified as *Phormidium corium*

The algal culture was transferred to a petri-plate. The UV-B treatment of 0.8±0.1 mW cm^2 (Vilbour-Lourmat, France T-6M source with a λ-max at 312 nm) was given in a BOD chamber at 30°C up to 6 h while keeping the algal culture continuously stirred using a magnetic flea (0.1×1 cm). The UV-B radiation was measured using a UV-B radiometer specific to 312 nm, from the same manufacturer.

The algal tissue was exposed to a relatively high irradiance of 500 µmol m^{-2} s^{-1} PAR (Li-cor, Model Li-189) at the culture level at 30°C up to 6 h in a double walled cuvette circulated with temperature controlled water. The light treatment was given using two slide projectors from opposite sides. Culture was kept constantly stirred during the treatment at a slow speed in order to avoid mechanical damage.

Photosynthesis measurement was taken using chlorophyll fluorometer (PAM 101-102, Walz, Germany) according to Sharma *et al*.,[13]. Culture was dark adapted for 10 minutes prior to measurements at room temperature. The dark adapted cultures were exposed to a modulated light with an intensity of 4 µmol m^{-2} s^{-1} to measure initial fluorescence (F_o). This was followed by an exposure to a saturating pulse of white light of 4000 µmol m^{-2} s^{-1} to obtain 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.

Lipid peroxidation was determined by the production of TBA-MDA adduct formation according to method described[14]. Algal culture was harvested by centrifuging at 8000 g for 15 min. The algal pellet was homogenized in a tissue homogenizer and redissolved in fresh culture medium with a ratio of 1:5 (w/v). Resuspended algal culture (5 ml) was again centrifuged and algal pellet was homogenized in 0.5% TCA. The homogenate was made up to 5 ml and centrifuged at 8000 *g* for 15 minutes. The supernatant was collected and used for measuring the peroxidation of membrane lipids. 1 ml of the supernatant was added to the test tube containing 2.5 ml of freshly prepared (0.5%) TBA in (20%) TCA and allowed to incubate for 30 min at 90°C in a water bath. After incubation, it was allowed to cool at room temperature and centrifuged for 2 min at 1000 *g* to settle the debris and non-specific precipitation. The optical density was taken at 532 nm (Schimadzu, UV-250). Peroxidation of lipids was measured using an extinction coefficient of 155 mM^{-1} cm^{-1}. Quenching of ROS was determined using epinephrine according to the method described by Boveris[15]. Ascorbic acid content was determined using DCIP titrated with metaphosphoric acid according to Reiss[16]. Activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) was assayed according to method described by Sankhalkar and Sharma,[17]. Extraction and purification of UV-B absorbing compounds such as mycosporine like amino acids (MAAs) was carried out using HPLC according to the method by Sinha and Häder[18]. The HPLC profile was extracted at 330 nm from spectral range of 200-400 nm.

Results

A linear decrease in the photosynthesis (PS II efficiency) measured as F_v/F_m ratio in response to UV-B as well as high light treatment was observed. Six hours of UV-B treatment to *P. corium* resulted in complete inhibition of F_v/F_m ratio while high light treatment to the same duration resulted in a decrease of 97% in the efficiency of photosynthesis (Fig. 1). Peroxidation of membrane lipids was studied by measurement of malondiadehyde (MDA) formed as a

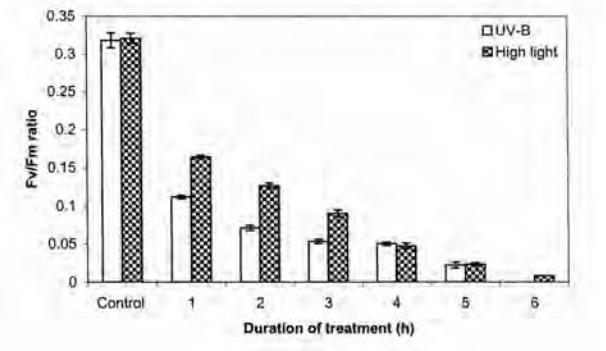

Fig. 1—Effect of UV-B radiation and high light up to 6 h duration of treatment on F_v/F_m ratio in *P. corium.* Each bar represents the mean ±S.D. n=5.

product of membrane peroxidation. A 6 hour of UV-B and high light treatment resulted in an increase of peroxidation of cell membrane lipids by 73% and 44% respectively (Fig. 2).

It was seen that *P. corium* grown under control growth condition did not show significant ability to prevent generation of ROS (Fig. 3a). Only slight increase in ascorbate content (Fig. 3b), a non-enzymaic antioxidant, and activity of SOD (Fig. 3c) as well as APX (Fig. 3d), enzymatic antioxidant, was observed in *P. corium* as a result of the UV-B and high light treatment. Six hours of UV-B or high light treatment resulted in approximately 20% increase in the activity of SOD as compared to their control (Fig. 3c). APX activity was slightly higher as compared to SOD. UV-B or high light treatment up to 3 h resulted in an increase in APX activity by 32% and 46% respectively as compared to control. However, further increase in the duration of the UV-B treatment (up to 6 h) reduced the increase in the APX activity to only 28% (Fig. 3d).

After exposure to UV-B radiation for 6 h, considerable increase (80%) in the amount of UV-B absorbing compound such as MAAs was observed as compared to control. These MAAs could absorb in the range of 200-330 nm (Fig. 4). However, high light treatment (500 PAR) for the same duration resulted in 40% decrease in the amount of MAAs as compared to control (Fig. 4).

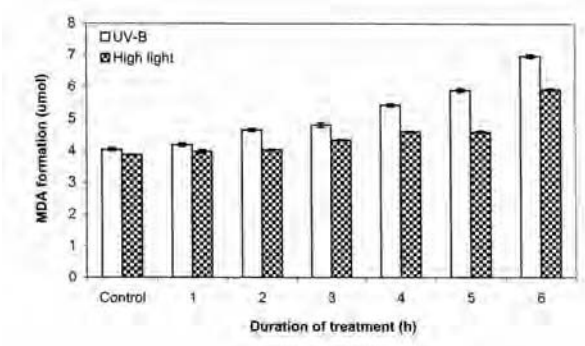

Fig. 2—Effect of UV-B radiation and high light up to 6 h duration of treatment on lipid peroxidation in *P. corium.* Each bar represents the mean ±S.D. n=5.

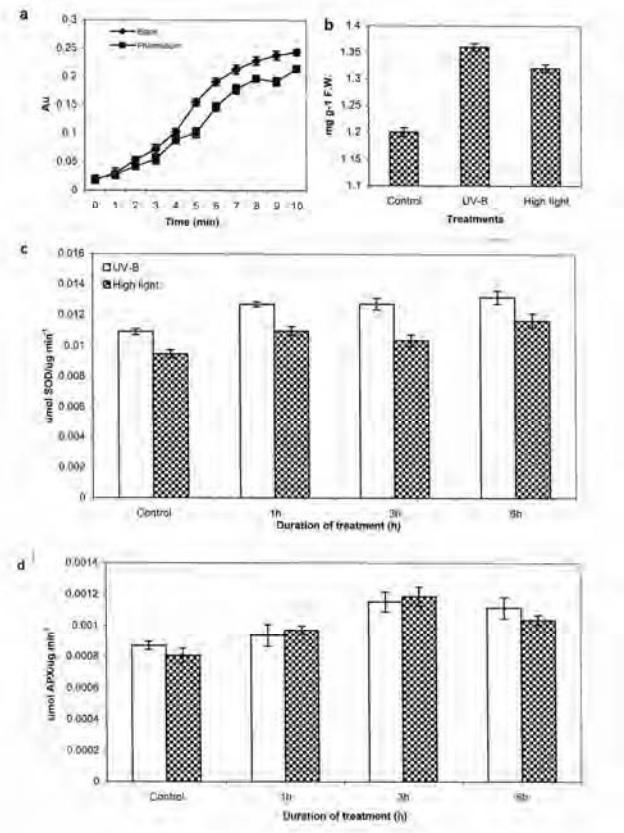

Fig. 3—Effect of UV-B radiation and high light for 6 hours on (b) ascorbic acid content, (c) superoxide dismutase (SOD) and (d) ascorbate peroxidase (APX) in *P. corium.* (a) Quenching of free reactive oxygen species (ROS) in *P. corium.* Each bar represents the mean ±S.D. n=4.

Discussion

Our results indicate that UV-B and high light both affected photosynthesis (Fig. 1) and thereby growth and productivity of the cyanobacterium. Decrease in the F_o is an indicator of decrease in the excitation energy reaching the photosynthetic reaction centre II largely due to loss of pigments in the light harvesting complex II, while decrease in the F_m is an indicator of damage to the PS II reaction centre itself[19]. The results obtained may represent direct damage to key components within the photosystem such as D1 and D2 protein of PS II[20] as well as loss of photosynthetic pigments due to generation of ROS[21]. It is known that both oxidizing as well reducing conditions in the photosynthetic electron transport are source of generation of ROS, which may lead to bleaching of surrounding pigments and peroxidation of lipid membranes[22]. This study indicates significant oxidative damage to the cyanobacteria under our experimental conditions as seen with level of lipid peroxidation, which is an indicative of oxidative damage and was significantly higher in UV-B and PAR treated *P. corium* (Fig. 2). Lipids are some of the oxidative targets attacked by the elevated ROS and lipid peroxidation occurs especially at sites where

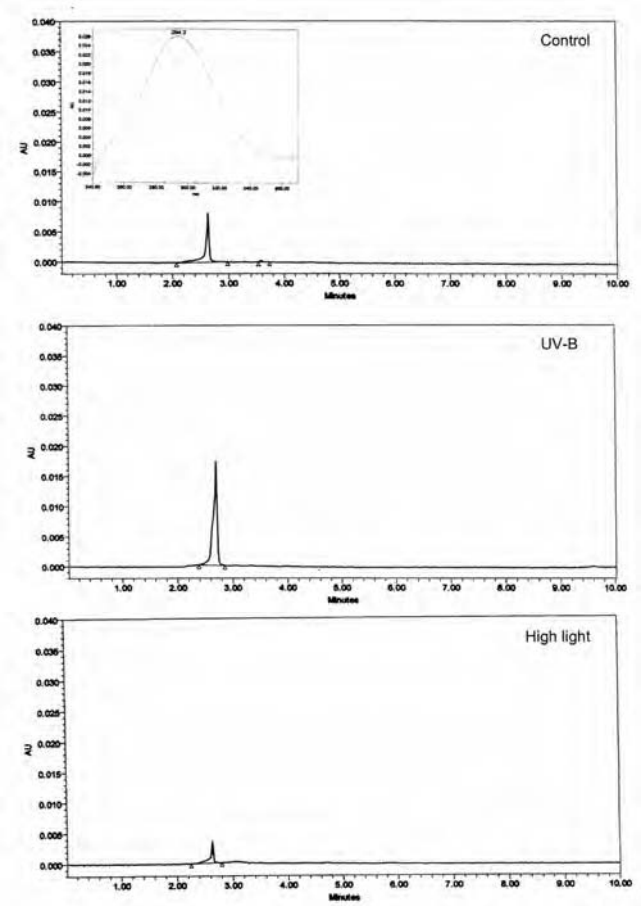

Fig. 4—HΠΛX profile and spectral characteristics of mycosporine like amino acids (MAAs) in *P. corium*. (a) control, (b) 6h exposure to UV-B radiation and (c) 6 h exposure to PAR. Inset shows absorption spectra at 330 nm.

polyunsaturated fatty acids occur in high concentrations. Ultraviolet radiation has been shown to induce free oxygen radicals[23] which are very effective in inducing lipid oxidation of biological membranes[24], polyunsaturated fatty acids[25] and phospholipid liposomes[26] and damage to the photosynthetic antennae and photobleaching of the cells of cyanobacteria[27].

He and Häder,[28] observed induction of ROS under *in vivo* condition in cyanobacterium, *Anabaena* sp. and reported that impaired photochemical reactions of PS II can enhance the production of ROS in cyanobacterium. UV-B was found to reduce the amounts of photosynthetic pigments and cause photoinhibiton (a reduction in the rate of photosynthesis) in the red alga *Porphyra leucosticta*[29]. Shick *et al*.,[30] reported that the octocoral *Clavularia* exhibited a 50% decrease in photosynthesis when exposed to high levels of UV-B. Gleason and Wellington,[31] used an underwater spectroradiometer to determine that increased dosages of UV could induce bleaching in the stony coral *Montastraea annularis*. Additionally, other coral reef inhabitants, including algae (macro and micro), invertebrates, and fish, all can be affected directly by UV exposure and indirectly by changes in coral condition. High solar irradiance is thought to be especially stressful to corals when coupled with elevated sea surface temperatures[32]. The association of *Phormidium* sp. with polyps affected with pink line syndrome (PLS)[11] may also result in hyperactive defense mechanism where organism generate burst of oxygen radicals through NADH oxidase to kill the pathogen.

Present study consists less ability of quenching of ROS existed in *P. corium* (Fig. 3a) which leads to increase in the formation of ROS, a natural product of aerobic system, exacerbated under UV-B and high light conditions due to impaired photochemical reaction[10]. This is substantiated by increased oxidative nature (observed as lipid peroxidation) of damage to *P. corium,* which may be one of the reason why corals, largely associated with *P. corium,* are getting bleached. It will also be interesting to study the other micro and macro algae found in association with corals for their antioxidant activity and extent of photo bleaching.

Level of increase in the SOD and APX was considerably less and was found to be only for short duration (Fig. 3c & d). Increase to the extent of 400% has been observed in APX activity in wheat in response to UV-B treatment in order to protect against UV-B damage[14]. It was seen that tissue homogenate of *Phormidium corium* could quench very little ROS as compared to three times more seen in *Nostoc spongiaeforme* and seven times more observed in *Cassia tora* (data not shown). It seems that inability of *P. corium* to quench ROS on account of low inherent level of antioxidant enzymes and ascorbic acid and their limited induction on imposing of the stress, since the quenching of ROS is mainly due to enzymatic (SOD and APX) and non-enzymatic (ascorbic acid) antioxidant system, resulted in greater formation of ROS that led to oxidative damage and subsequent bleaching and death of the coral reefs. Ascorbate in normal plants was reported to be in the range of 5-9 mg g^{-1} F.W.[33] which can be increased to the level of 20 mg g^{-1} F.W. under stress conditions. However, no such level were observed in our study again indicating limitation of ascorbate as antioxidant in *P. corium*.

Lesser,[7] had reported generation of reduced oxygen intermediates within both the algal symbionts and host, resulting in oxidative stress which causes decrease in photosynthesis and subsequent bleaching. Lesser and Shick,[34] showed generation of ROS under conditions which lead to bleaching of symbiotic *Anthopleura elegantissima.* Brown *et al*.,[35] cited solar irradiation as a possible cause of bleaching in corals either acting alone or in conjunction with other environmental factors. Downs *et al*.,[36] also observed strong positive correlations between accumulation of oxidative damage products and bleaching in corals.

Reef-building corals also contain UV absorbing compounds capable of blocking potentially damaging UV radiation as a first line of defense. Our study showed that *P. corium* was able to synthesize MAAs having absorbance in UV region, in response to UV-B radiation but was inhibited by high light (Fig. 4). Many reef animals can produce natural sunscreens (MAAs) to protect themselves against UV. Synthesis of MAAs in response to UV has been reported by various workers[37] and he also observed that amount of MAAs in a corals tissues helps to determine how much UV it can withstand without bleaching. Lesser and Farrell,[38] observed that both photosynthetic pigments and MAAs are depressed after experimental exposure to high solar radiation and thermal stress to common Caribbean coral, *Montastraea faveolata*.

Present study showed that when cyanobacterium was exposed to UV-B radiation there was considerable increase in the MAAs content, however, high light resulted in considerable decline in the MAAs content (Fig. 4). This antagonistic effect of UV-B and high light on MAAs may probably explain greater damage to *P. corium* under *in vivo* conditions even when having ability to produce higher MAAs under UV-B conditions.

It seems that MAAs in *P. corium* is the primary molecule which prevent biological damage mainly by screening the UV-B radiation and thus prevent damage to sensitive biological molecules within the cell and also act as antioxidant system[39], which may explain less inherent ability of SOD, APX and ascorbate to respond under stress conditions in the organism. However, presence of high light may degrade or inhibit MAAs production (Fig. 4).

Present study suggest that bleaching of coral reefs observed, off the Lakshadweep Island, India, which are largely associated with *P. corium,* may be due to at one hand by oxidative damage caused as a result of generation of ROS under high UV-B and PAR conditions and at another hand due to inefficient enzymatic (SOD and APX) and non-enzymatic (ascorbic acid) antioxidant system present in the associated cyanobacterium. The two processes together lead to formation of excess ROS which results in repelling of algae from symbiont corals as a protection to itself against ROS, since MAAs is a primary protecting molecules against UV-B radiation as well as against ROS loading, was also found to be less under combination of UV-B with high light, resulting in starving and death of the corals, as autotrophic algae are the source of providing food to corals.

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References

1 Goreau T F & Goreau N I, Distribution of labelled carbon in reef building corals with and without zooxanthellae, *Science,* 181 (1960) 668-669.

2 Kleppel G S, Dodge R E & Reese C J, Changes in pigmentation associated with the bleaching of stony corals, *Limnol. Oceanogr.,* 34 (1989) 1331-1335.

3 Hay M E & Fenical W, Chemical ecology and marine biodiversity: Insights and products, *Oceanography,* 9 (1996) 10-20.

4 Reaka-Kudla M L, The global biodiversity of coral reefs: A comparison with rainforests. In: Reaka-Kudla ML, Wilson DE, Wilson EO (eds). *Biodiversity II: Understanding and Protecting Our Natural Resources*, (Joseph Henry/National Academy Press, Washington, D.C), (1996) 83-108.

5 Wilkinson C R, Linden O, Cesar H, Hodgson G, Rubens J & Stong A E, Ecological and socioeconomic impacts of 1998 coral bleaching in the Indian Ocean: An ENSO impact and a warning of future change? *Ambio.,* 28 (1999) 188-196.

6 Hoegh-Guldberg O, Berkelmans R & Oliver J, Coral bleaching: Implications for the Great Barrier Reef Marine Park, CRC January 1997, *Conference in research and reef management proceedings,* (1997) 21-43.

7 Lesser M P, Oxidative stress causes coral bleaching during exposure to elevated temperatures, *Coral Reefs,* 16 (1997) 87-192.

8 Menon H B, Lotliker A & Nayak S R, Pre-monsoon bio-optical properties in estuarine, coastal and Lakshadweep waters, *Estuarine Coastal and Shelf Science,* 63 (2005) 211-223.

9 Gleason D F & Wellington G M, Variation in UV-B sensitivity of Planula larvae of the coral *Agaricia agaricites* along a depth gradient, *Mar. Biol.,* 123 (1995) 693-703.

10 Sharma P K, Response of plants to ultraviolet-B radiation: Impact on photosynthesis and productivity, *J. Plant Biol.,* 30 (2003) 271-283.

11 Ravindran J & Raghukumar C, Pink like syndrome (PLS) in the scleractinian coral *Porites lutea*, *Coral Reefs,* 21 (2002) 252-256.

12 Rippka R, Deruelles J, Waterbery J B, Herdman M & Stanier Y R, Generic assignments, strain histories and properties of pure cultures of cyanobacteria, *J. Gen. Microbiol.,* 111 (1979)1-61.

13 Sharma P K, Anand P, Sankhalkar S & Shetye R, Photochemical and biochemical changes in wheat seedlings exposed to supplementary UV-B radiation, *Plant Sci.,* 132 (1998a) 21-30.

14 Sharma P K, Anand P & Sankhalkar S, Oxidative damage and changes in activities of antioxidant enzymes in wheat seedlings exposed to ultraviolet radiation, *Cur. Sci.*, 75 (1998b) 359-366.

15 Boveris A, Determination of the production of superoxide radicals and hydrogen peroxide in mitochondria, *Method. Enzymol.,* 105 (1984) 429-435.

16 Reiss C, Determination of ascorbic acid content. In: Reiss C (ed) *Experiments in Plant Physiology,* (Prentice-Hall. Inc), (1994) p 1-8.

17 Sankhalker S & Sharma P K, Protection against photooxidative damage provided by enzymatic and non-enzymatic antioxidant system in sorghum seedlings, *Indian J. Exp. Biol.*, 40 (2002) 1260-1268.

18 Sinha R P & Häder D-P, Effects of UV-B radiation on cyanobacteria, *Rec. Res. Devel. Photochem. Photobiol.,* 4 (2000) 239-246.

19 Krause G H, Photoinhibition of photosynthesis, An evaluation of damaging and protective mechanisms, *Physiol. Plant.,* 74 (1988) 566-574.

20 Jansen M A K, Aba V, Greenberg B M, Matto A K & Edelman M, Low threshhold levels of UV-B in a background of photosynthetically active radiation trigger rapid degradation of D2 protein of PS II, *Plant J.,* 9 (1996) 693-699.

21 Bischof K, Krabs G, Wiencke C & Hanelt D, Solar ultraviolet radiation affects the activity of ribulose-1,5-bisphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green algae *Ulva lactuca* L, *Planta*, 215 (2002) 502-509.

22 Sharma P K, Photoinhibition of photosynthesis and mechanism of protection against photodamage in crop plants, *Everyman's Science,* 36 (2002) 237-252.

23 Palmer H, Ohta M, Watanabe M & Suzuki T, Oxidative stress induced cellular damage caused by UV and methyl viologen in *Euglena gracilis* and its suppression with rutin, *Photochem. Photobiol. B:Biol*., 67 (2002) 116-129.

24 Malanga G, Calmanovici G & Puntarulo S, Oxidative damage to chloroplasts from *Chlorella vulgaris* exposed to UV-B radiation, *Physiol. Plant*.,101 (1997) 455-462.

25 Yamashoji S, Yoshida H & Kajimoto G, Photooxidation of linoleic acid by ultraviolet light and effect of superoxide anion quencher, *Agric. Biol. Chem*. Tokyo, 43 (1979) 1249-1254.

26 Pelle E, Maes G A, Padulo E K & Smith W P, An *in vitro* model to test relative antioxidant potential: Ultraviolet induced lipid peroxidation in liposomes, *Arch. Biochem. Biophys.,* 283 (1990) 234-240.

27 Xenopoulos M A, Frost P C & Elser J J, Joint effect of UV radiation and phosphorus supply on algal growth rate and elemental composition, *Ecology,* 83 (2002) 23-435.

28 He Y Y & Häder D-P, Involvement of reactive oxygen species in the UV-B damage to the cyanobacterium *Anabaena* sp, *Photochem. Photobiol. B: Biol*, 66 (2002) 73-80.

29 Figueroa F L, Salles S, Aguilera J, Jiménez C, Mercado J, Vinegla B, Flores-Moya A & Altamirano M, Effects of solar radiation on photoinhibition and pigmentation in the red alga *Porphyra leucosticte*, *Mar. Ecol-Prog. Ser.,* 151 (1997) 81-90.

30 Shick J M, Lesser M P & Stockaj W L, Ultraviolet radiation and photooxidative stress in zooxanthellate anthozoans: the sea anemone semoni and the octocoral *Clavulari*a sp, *Symbiosis,* 10 (1991) 145-173.

31 Gleason D & Wellington G M, Ultraviolet radiation and coral bleaching, *Nature,* 65 (1993) 836-838.

32 Glynn P W, Coral reef bleaching: Facts, hypotheses and implications, *Global Change Biol.,* 2 (1996) 495-509.

33 Okeri H A & Alonge P O, Determination of the ascorbic acid content of two medicinal plants in Nigeria, *Pak J Pharm Sci.,* 19 (2006) 44-48.

34 Lesser M P & Shick J M, Effects of irradiance and ultraviolet radiation on photoadaptation in zooxanthellae of *Aiptasia pallida:* Primary production, photoinhibition, and enzymatic defenses against oxygen toxicity, *Mar. Biol.,* 102 (1989) 243-255.

35 Brown B E, Dunne R P, Scoffin T P & LeTissier M D A, Solar damage in intertidal corals, *Mar. Ecol. Prog. Ser.,* 105 (1994) 219-230.

36 Downs C A, Fauth J E, Halas J C, Dustan P, Bemiss J & Woodley C M, Oxidative stress and seasonal coral bleaching, *Free Radical Biol. Med.,* 33 (2002) 533-543.

37 Gleason G M, Effects of disturbance on coral communities: Bleaching in Moorea, French Polynesia, *Coral Reefs,* 12 (1993) 193-201.

38 Lesser M P & Farell J H, Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress, *Coral Reefs,* 23 (2004) 367-377.

39 Yakovleva I, Bhagooli R, Takemura A & Hidaka M, Differential susceptibility to oxidative stress of two scleractinian corals: Antioxidant functioning of mycosporine-glycine, *Comp. Biochem. Physiol. B,* 139 (2004) 721-730.