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Effect of UV-B radiation on growth, photosynthetic activity and metabolic activities of *Chlorococcum* sp.

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Abstract - The impact of two intensities (2.5 W m^{-2} and 5 W m^{-2}) of ultraviolet-B (UV-B) radiation on growth, photosynthetic pigments, photosynthetic activity and membrane leakage has been studied in *Chlorococcum* sp. isolated from El-Kased fresh water canal, Tanta, Egypt. Exposure of *Chlorococcum* sp. for 60 and 35 min of 2.5 and 5 W m^{-2}, respectively, inhibited growth by 50%. Chlorophyll *a* and chlorophyll *b* were decreased in *Chlorococcum* sp. by exposure to UV-B, but the effect was more pronounced on chlorophyll *b*. On the other hand, carotenoids were stimulated at small doses (time of exposure and intensity) of UV-B whether at 2.5 or 5 W m^{-2}. However, oxygen amount, total soluble carbohydrates, and total soluble proteins were inhibited by UV-B treatment at all exposure times but the effect of 5 W m^{-2} was more than that of 2.5 W m^{-2} and O_2 was completely abolished after 90 and 120 min at 2.5 and 5 W m^{-2}, respectively. With regard to extracellular polysaccharides, there was a significant increase at small doses of UV-B but reduction observed at high doses. Exposing cells to 2.5 and 5 W m^{-2} UV-B caused 22.7 and 39.6% increase, respectively, in electrolyte leakage than in control.

Key words: *Chlorococcum* sp., electrical conductivity, photosynthetic pigments, survival, ultraviolet-B radiation.

INTRODUCTION

Ultraviolet-C (UV-C) is the most dangerous radiation to life on earth of the three bands (Garde, 1998), but since it is entirely absorbed in the atmosphere, this radiation is regarded as unimportant for biological processes on the earth. UV-B is the most harmful radiation reaching the earth surface, due to its absorption by biologically important components. UV-B is partly absorbed by the stratospheric ozone layer and accounts for no more than 1.5% of the total radiation, while UV-A and photosynthetic active radiation (PAR) are poorly absorbed in the atmosphere. The levels of UV-B will therefore increase during periods of stratospheric ozone depletion, while the PAR and UV-A levels will remain unchanged. Therefore, this study will concentrate on the effect of UV-B radiation. Depletion of the ozone layer in the stratosphere owing to increasing emission of chlorinated fluorocarbons (CFCs) and other man-made chlorocarbons is today accepted as a reality (Madronich *et al.,* 1995; Prather *et al.,* 1996). The ozone absorbs UV-B radiation (280-320 nm), and the thinning of the ozone layer has created a concern for the potential impact of increased UV-B radiation on the earth's ecosystems.

High doses of PAR cause photoinhibition which has been extensively studied (Aro *et al.,* 1993; Hideg and Vass, 1996). On the other hand, increasing doses of UV-B radiation reaching the earth's surface may also be deleterious for plants (Teramura and Sullivan, 1994; Bornman and Sundby-Emanuelsson, 1995). One of the most important processes in algal cells is the photosynthesis. Kulandaivelu (1993) suggested that UV-B radiation predominantly attacks PSII. Sinha *et al.* (1997) studied the impact of UV-B irradiation on RuBISCO activity and $NaH^{14}CO_3$ uptake in five N_2-fixing cyanobacterial strains and revealed that UV-B exposure for as little as 30 minutes resulted in a considerable decrease in the RuBISCO activity, which further decreased with increasing exposure time. The pigments are important molecules in plant cells. Some pigments are involved in the capturing of light energy, i.e. chlorophylls, phycobilins, and some carotenoids, while other carotenoids can protect the cell from UV-B radiation or excessive PAR irradiance, e.g. diadinoxanthin and zeaxanthin. UV-B radiation can affect the pigment concentrations in algal cells by inducing a photodegradation of light-absorbing pigments, resulting in a loss of photosynthetic capacity (Post and Larkum, 1993).

The aim of this work was to study the stress response of the green alga *Chlorococcum* sp. cells isolated from fresh water sample to two intensities of UV-B radiation for different periods that were cultured, under laboratory conditions., Effects on survival, pigment content, carbohydrates, proteins, and oxygen amount and membrane leakage were studied also directly after UV-B irradiation, in addition to photoreactivation studies.

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MATERIALS AND METHODS

Experimental organism and growth conditions. *Chlorococcum* sp. was isolated from Egyptian fresh water sample and identified according to Prescott (1975). Purification of the organism was done by subculturing, antibiotic treatment according to Venkataraman (1969) and ultraviolet irradiation according to Gerloff *et al*. (1950). The alga was cultured in a medium described by Kuhl (1962). The culture was grown in an air-conditioned culture room at 25 ± 2 ºC and illuminated with fluorescent white light (3000 lux). UV-B irradiation was done to log phase cultures having optical density from 0.15-0.20 at 560 nm.

Mode and source of UV-B radiation. The tested organism grown in liquid culture was transferred into a sterilised Petri dish (17 x 2.5 cm) and exposed individually to artificial UV-B radiation. The UV-B radiation system comprised an array of three ultraviolet long lamps, UV-A, UV-B and UV-C. The UV-B lamp (T-8 M) manufactured by Vilbar-lourmat, France. The spectral emission of UV-B source ran from 280-320 nm with a peak at 312 nm. The suspension was gently agitated by a magnetic stirrer during irradiation to facilitate uniform exposure.

Measurement of survival and LD_{50}. This experiment was carried out according to Rai *et al.* (1995) to evaluate the lethal dose (LD_{50}) which causes death of 50% of algal populations. The algal cells exposed to 2.5 W m^{-2} and 5 W m^{-2} UV-B radiation were withdrawn at 15 min intervals and then plated onto agar plates for measuring their survival by plate colony count method.

Extraction and estimation of photosynthetic pigments. Chlorophyll *a* and chlorophyll *b* contents were measured spectrophotometrically using the method described by Jeffrey and Humphrey (1975). Also, carotenoids were measured spectrophotometrically according to Jensen and Liaan Jensen (1959).

Carbohydrates.
Total soluble carbohydrates. After pigment extraction, the algal cells were extracted with 1 N NaOH in a boiling water bath for 2 h as described by Payne and Stewart (1988). Total soluble carbohydrates were quantitatively determined by the method of phenol-sulphuric acid described by Kochert (1973) using a calibration curve of glucose as a standard.

Extracellular total carbohydrates. According to Kaplan *et al*. (1987), a known volume of the medium was hydrolyzed in sulphuric acid (1 N) in a boiling water bath for 1 h, and then total soluble carbohydrates were determined according to Kochert (1973).

Total soluble proteins. Total soluble proteins were quantitatively determined in the algal cells according to the method described by Lowry *et al*. (1951) using a calibration curve of bovine serum albumin as a standard protein.

Measurement of oxygen amount. The photosynthetic activity was measured as oxygen amount evolved in the culture using oxygen meter (Jenway, model 9070 DO2 meter). After preparation and calibration of the oxygen meter, the amount of oxygen in the samples was measured as a percentage of oxygen against control, 100% oxygen (Tyagi *et al*., 1993).

Membrane leakage. For measurements of membrane leakage, the electrical conductivity of 5 ml of algal suspension, immediately after UV irradiation, was measured by EC meter as µmohs/cm at 26 °C.

Statistical analysis. Results are presented as mean ± standard deviation (SD) from three different readings. The statistical analyses were carried out using SPSS 10.0 and Minitab 14. Data obtained were analysed statistically to determine the degree of significance between treatments using two way analysis of variance (ANOVA). Additionally, the LSD test was used to determine treatment differences comparing with control at $P \leq 0.001$ level of significance.

RESULTS

Survival and LD_{50}

From the results in Table 1, it was obvious that, approximately 50% (LD_{50}) survival of the tested organism was observed after 60 and 35 min of UV-B exposure at 2.5 and 5 W m^{-2}, respectively.

TABLE 1 - Determination of LD_{50} of *Chlorococcum* sp. at two UV-B intensities (2.5 and 5 W m^{-2})

Exposure time (min)	Percent survival	
	2.5 W m^{-2}	5 W m^{-2}
0	100 ± 3.95	100 ± 5.65
15	94.86 ± 4.74$^{(ns)}$	78.25 ± 5.69***
30	88.42 ± 6.01**	61.19 ± 5.79***
45	71.7 ± 5.81***	31.56 ± 5.41***
60	49.84 ± 5.63***	23.45 ± 5.35 ***
75	38.59 ± 4.56***	7.46 ± 3.9***
90	21.22 ± 5.17***	5.54 ± 3.1***
105	16.08 ± 4.64***	1.07 ± 1.29***
120	7.4 ± 3.91***	0.64 ± 0.96***
135	5.79 ± 2.89***	0
150	2.57 ± 1.96***	0
165	3.54 ± 2.01***	0
180	2.89 ± 2.01***	0
195	1.29 ± 1.11***	0
210	0.64 ± 0.85***	0
225	0.32 ± 0.32***	0
240	0	0

Each value is the mean of three replicates ± standard deviation.
*** Highly significant at $P \leq 0.001$ using one way analysis of variance (ANOVA).
** Significant at $P \leq 0.001$ using one way analysis of variance (ANOVA).
$^{(ns)}$ Non significant at $P \leq 0.001$ using one way analysis of variance (ANOVA).

Photosynthetic pigments

Results in figure 1A revealed that, chlorophyll a content shows subsequent decreases as the time of exposure increased, and the reduction was 47.46% as compared to control after 120 min of exposure to 2.5 W m^{-2} UV-B and 63.03% at 5 W m^{-2} UV-B. Exposure to 5 W m^{-2} induces a subsequent decrease in chlorophyll content more than 2.5 W m^{-2}. In addition, figure 1B, revealed that, UV-B exposure caused reduction in chlorophyll *b* content. The reduction was 82.87 and 90.88% compared to control after 120 min at 2.5 and 5 W m^{-2}, respectively. With regard to carotenoid content after UV-B exposure, it can be observed from figure 1C that, in cells exposed to 2.5 W m^{-2}, UV-B enhanced carotenoid production at all exposure time intervals. The increase in carotenoid content was of 13.3, 30.5, and 26.6% as compared to the control after 30, 60 and 120 min of exposure. Also in cells exposed to 5 W m^{-2}, UV-B initially enhanced carotenoid production. After 30, 60 and 90 min of exposure, the stimulation was of 35.2, 27.7 and 6.5% as compared to control. However, a reduction in carotenoid content was observed after 120 min of exposure (15.5% as compared to control).

In the suggested statistical models (data not shown), Two way analysis of variance showed that, UV-B intensities and exposure times revealed highly significant effect ($P \leq 0.001$) on all pigment fractions while the interaction revealed highly significant effect on carotenoids, significant effect on chlorophyll *b*. One way analysis of variance showed that, exposure to 2.5 and 5 W m^{-2} UV-B revealed highly significant effect on chlorophyll *a*, chlorophyll *b* and carotenoids at all the exposure times except 2.5 W m^{-2}/30 min which revealed significant effect on chlorophyll *a*.

Total soluble carbohydrates

Results in figure 2 revealed that, exposure of *Chlorococcum* sp. to UV-B determined a decrease in the total soluble carbohydrate content. Exposure to 2.5 and 5 W m^{-2} UV-B revealed highly significant effect ($P \leq 0.0001$) on total soluble carbohydrates at all the exposure times except 2.5 W m^{-2}/30 min which revealed non significant effect on total soluble carbohydrates. After 120 min of UV-B irradiation cells exposed to 2.5 and 5 W m^{-2} showed a decrease in carbohydrate content of 29.0 and 64.0% as compared to control, respectively.

Extracellular carbohydrates

Results in figure 3 revealed that, exposure to UV-B stimulated the synthesis of extracellular carbohydrates at the first times of exposure. Exposure to 2.5 W m^{-2} resulted in stimulation of extracellular carbohydrate content after 30 min and 60 min (21.3 and 15.4%, respectively, as compared to control) then exposure of 90 and 120 min resulted in inhibition by 3.6 and 39.4%, respectively, as compared to control. In a similar way, in cells exposed to 5 W m^{-2}, UV-B stimulated extracellular polysaccharides by 32.3 and 17.0% as compared to control after 30 min and 60 min, respectively. The inhibition was 11.8 and 57.6% as compared to control after 90 min and 120 min, respectively. Exposure to 2.5 and 5 W m^{-2} UV-B revealed highly significant effect on the extracellular polysaccharides at all the exposure times except 2.5 W m^{-2}/90 min which revealed non significant effect on the extracellular polysaccharides ($P \leq 0.001$).

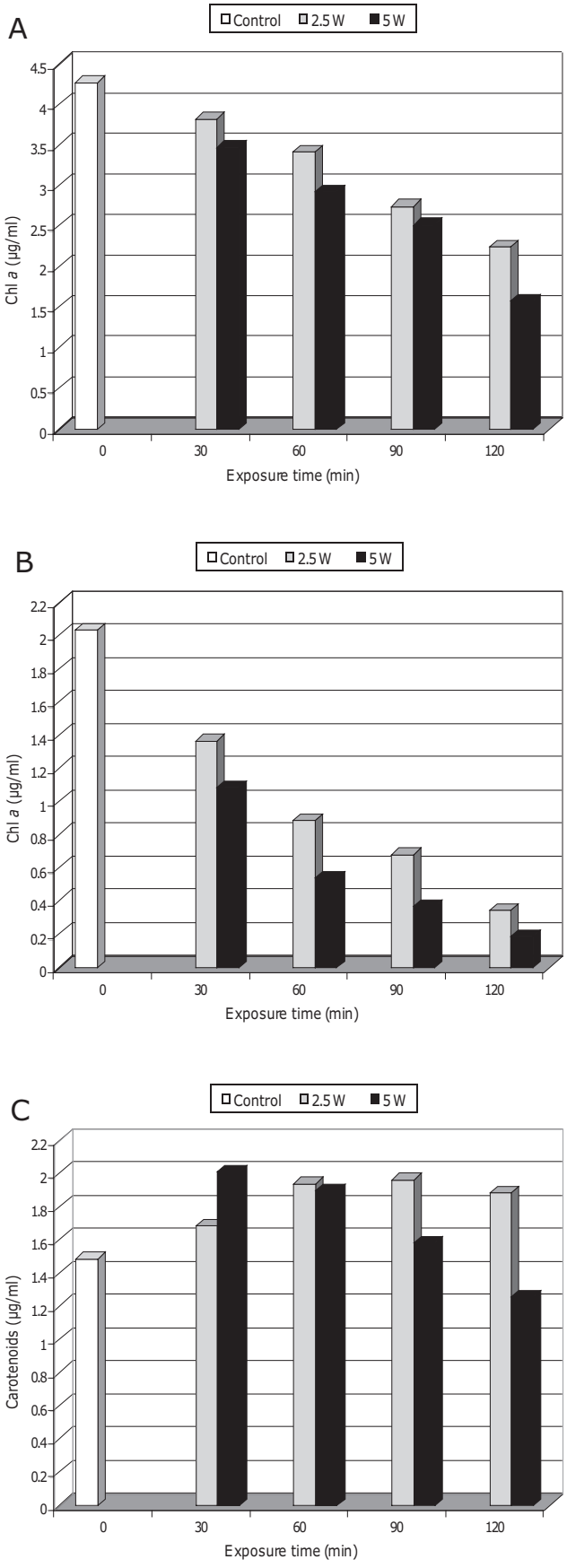

FIG. 1 - Effect of UV-B irradiation on different pigment fractions of *Chlorococcum* sp. at two UV-B intensities, 2.5 and 5 W m^{-2}. A: effect on chlorophyll *a*; B: effect on chlorophyll *b*; C: effect on carotenoids.

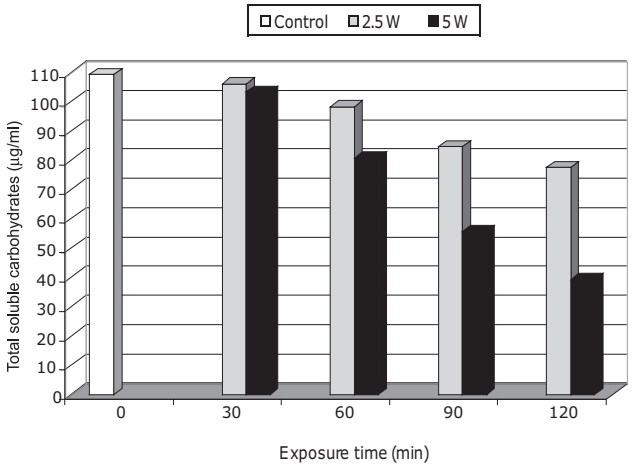

FIG. 2 - Effect of UV-B irradiation on total soluble carbohydrates of *Chlorococcum* sp. at two UV-B intensities, 2.5 and 5 W m^{-2}.

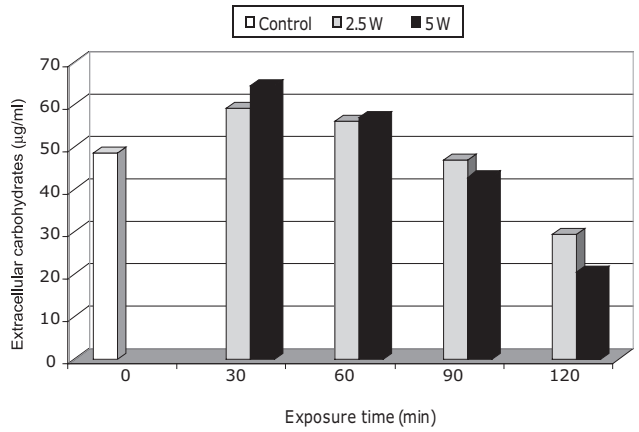

FIG. 3 - Effect of UV-B irradiation on extracellular carbohydrates of *Chlorococcum* sp. at two UV-B intensities, 2.5 and 5 W m^{-2}.

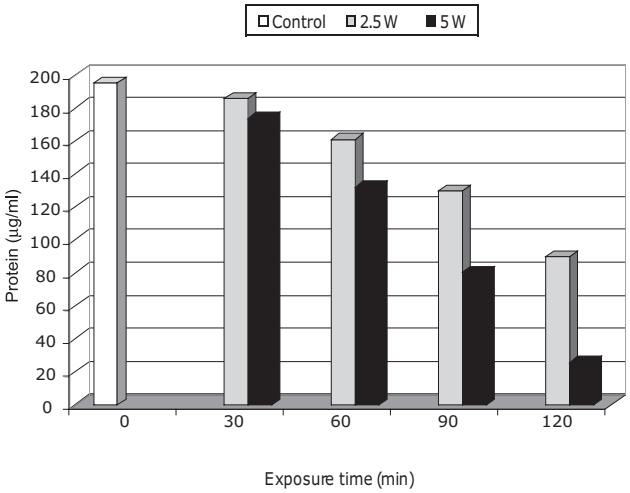

FIG. 4 - Effect of UV-B irradiation on total soluble proteins of *Chlorococcum* sp. at two UV-B intensities, 2.5 and 5 W m^{-2}.

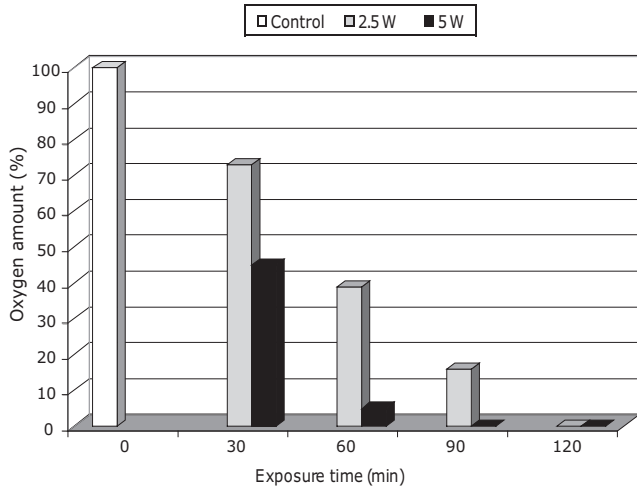

FIG. 5 - Effect of UV-B irradiation on oxygen content in culture of *Chlorococcum* sp. at two UV-B intensities, 2.5 and 5 W m^{-2}.

Total soluble proteins

From figure 4, it can be observed that, UV-B exposure led to reduction in the total soluble proteins. The reduction was 53.9 and 86.7% below the control after 120 min at 2.5 and 5 W m^{-2}, respectively. Exposure to 2.5 and 5 W m^{-2} UV-B revealed highly significant effect ($P \leq 0.001$) on total soluble proteins at all the exposure times except 2.5 W m^{-2}/30 min which revealed non significant effect on total soluble proteins.

Photosynthetic activity

Results in figure 5 revealed that, cells of *Chlorococcum* sp. exposed to 2.5 W m^{-2} UV-B intensity showed highly significant($P \leq 0.001$) reduction in oxygen amount 27, 61, and 84% as compared to control after 30, 60 and 90 min, respectively, and stopped evolving oxygen completely after 120 min. Cells exposed to 5 W m^{-2} UV-B intensity showed highly significant reduction in oxygen amount (55 and 95% as compared to control after 30 and 60 min, respectively), and stopped evolving oxygen completely after 90 min.

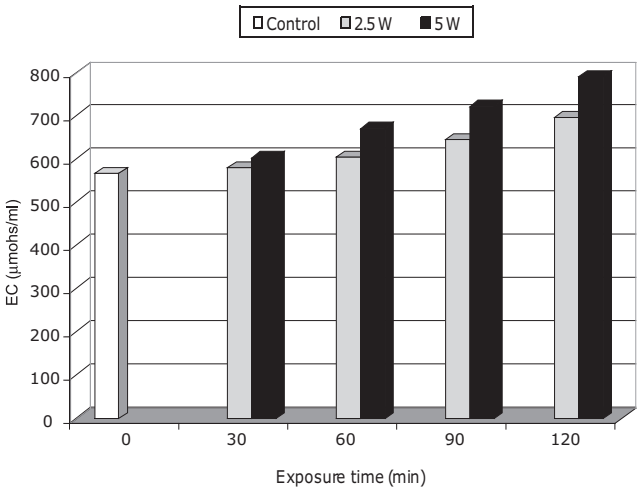

FIG. 6 - Effect of UV-B irradiation on electrical conductivity (µmohs/ml) stimated in *Chlorococcum* sp. exposed to two UV-B intensities, 2.5 and 5 W m^{-2}, for 30, 60, 90 and 120 minutes.

Membrane leakage

Statistically analysed data presented in figure 6 showed a highly significant ($P \leq 0.001$) increase in the electrical conductivity detected in the culture of *Chlorococcum* sp. with all UV-B doses compared to the control. At the UV-B dose of 2.5 W m^{-2}/120 min the increase in the membrane leakage reached 22.7% and at 5 W m^{-2}/120 min, 39.6%.

DISCUSSION

This study is aimed to reveal the influence of UV-B radiation on the growth as well as the different metabolic activities of the green alga *Chlorococcum* sp.

The present results showed pronounced inhibitory effects of UV-B on growth and survival of *Chlorococcum* sp., where the inhibition increased with increasing of UV-B intensity and exposure time. In accordance with our results, lethal effect of artificial UV-B radiation at irradiances ranging from 2 to 5 W m^{-2} has been reported in several algae (Sinha and Häder, 2000; Kumar *et al.*, 2003; Bancroft *et al.,* 2007).

The present investigation clearly demonstrated that, UV-B irradiation at 2.5 and 5 W m^{-2} significantly decreased chlorophyll *a* and *b* contents of *Chlorococcum* sp. However, the effect of 5 W m^{-2} was more significant than that of 2.5 W m^{-2}. These results agree with those obtained by Figueroa *et al.* (2003) on *Ulva*. The damaging effect of UV-B on photosynthetic pigments may be due to the bleaching caused by UV-B irradiation or may be attributed to the damage of the enzymes involved in chlorophyll biosynthesis (Prasad and Zeeshan, 2004).

Results showed also that, UV-B irradiation (2.5 and 5 W m^{-2}) significantly increased carotenoids at low UV-B doses. Similar results were recorded by Bhargava *et al.* (2007) who reported that, the synthesis of pigments absorbing wavelength in the UV range is an important protective strategy against UV-B radiation displayed by algal cells.

In this investigation it was observed that, inhibition of photosynthetic activity, measured as oxygen content, increased with the increase in intensity and duration of exposure to UV-B. The negative effect of UV-B on photosynthetic processes has been demonstrated in several publications (Correia *et al*., 2005). The decrease in chlorophyll contents by UV-B, observed here, may result in the damage of PSII through their effect on the composition and structure of the light harvesting complex causing a disturbance in the chloroplast architecture (El-Shintinawy, 2000; Vani *et al.*, 2001). It was argued that UV-B induced depression of photosynthesis that could be a result of the structural alteration of the D1:D2 polypeptide matrix without any detectable loss of the D1 protein (Babu *et al.*, 1999). Such an alteration may block the coordination of the functional manganese, thus leading to impairment of the functions of the PSII (Vasiliokiotis and Melis, 1994). An alternative possibility of somehow delay of the D1 protein loss than decline of photosynthetic activity in response to UV-B exposure may be due to the fact that the protein is linked by the radiation and rendered non-functional before it is degraded by protease (Aro *et al.*, 1993). Prasod and Zeeshan (2004) indicated that, whole chain and PSII activity was inhibited more than PSI activity. Melis *et al.* (1992) on the basis of their observations have concluded that the quinines of PSII are also likely target to UV-B radiation as they have absorption maxima in that range. The reduction in the PSII activity could be due to the UV-B induced damaging effect on reaction centre complex as Rai *et al.* (1995) suggested that the integrity of membranous structure including thylakoid membrane got affected following UV-B exposure. Estevez *et al.* (2001) and Malanga *et al.* (1997) studied the effects of UV-B on *Chlorella* sp. and reported that, many deleterious effects to photosynthesis by UV-B radiation could be caused by the generation of free radicals that can accumulate in the thylakoids and be responsible for oxidative stress and peroxidative reactions that destroy various components of the photosynthetic apparatus. In addition, Correia *et al.* (2005) demonstrated a reduced photosynthetic rate promoted by enhanced UV-B which may have resulted from changes in photosynthetic apparatus composition.

The present results showed that, all exposure times, except 2.5 W m^{-2}/30 min, of UV-B radiation brought about a significant decrease in total soluble carbohydrates. Again, the decline in total soluble carbohydrate contents increased at 5 W m^{-2} more than at 2.5 W m^{-2} UV-B dose. These results are in agreement with those of Mackerness *et al.* (1997) and Correia *et al*. (2005) who observed decreases in total soluble sugars and starch of maize, especially the former, by UV-B irradiation; they concluded that this reduction in total soluble sugars and starch indicates that the main response is mediated by lower net photosynthetic rate. Babu *et al.* (1998) studied the effect of UV-B radiation on total starch content of cyanobacteria; they stated that, the level of total starch decreased progressively as the intensity of UV-B increased. They explained this by the low photosynthetic pigment content of the cells that could also lead to decrease in the level of other parameters, a result of decreased carbon and N_2 assimilation or due to enhanced catabolism. Furthermore, results revealed that, small doses of UV-B stimulated the synthesis of extracellular carbohydrates in *Chlorococcum* sp. However, higher doses inhibited the extracellular carbohydrate production. The previous results were generally in agreement with the results of Ehling-Schulz *et al.* (1997) who studied the effect of UV-B irradiation on the production of extracellular polysaccharides in *Nostoc commune*. In addition, the production of polysaccharides by *Chlorella stigmatophora*, as a green alga, was monitored by Kaplan *et al.* (1987). Shick and Dunlap (2002) and Shick (1993) denoted that, green algae have higher proportion of genera containing mycosporines which have sunscreening and antioxidant functions for UV protection.

Measurement of electrolyte leakage is a classic method to assess the damage in the plasma membrane induced by stress, e.g. by frost stress (van Hasselt *et al*., 1996). The present data indicated that the damage in cell membranes was also correlated to the UV-B dose. The higher UV-B dose (5 W m^{-2} for 120 min) caused the most leakage compared to lower UV-B treatments. These results are in accordance with those of van Hasselt *et al.* (1996) and Shi *et al.* (2005). This may indicate that UV-B radiation can cause some injuries (including the peroxidation of lipids of membranes) in the cell membrane, which lead to a loss of its selective permeability. Shi *et al.* (2005) and Mishra *et al.*

(2006) concluded that, ion leakage reflects the membrane injury as a result of oxidative damage. When considering the relation between many essential processes and cellular membranes, it could be predicted that the irreversible damage of membranes can lead ultimately to a reduction in growth or even to cell death, as in the present study.

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