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EFFECT OF ULTRAVIOLET-B RADIATION ON GROWTH AND PIGMENTS OF *CHLORELLA VULGARIS*

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Ultraviolet radiation (UV) is known to affect the biological processes and thereby production of metabolites and overall growth of organisms including phytoplankton. Algal responses to UV radiations are broadly species and dose specific. The impact of artificial UV-B light from the source to cultures has been studied in *Chlorella vulgaris*. The cultures of *C. vulgaris* were exposed at two distances (50 and 100 cm) from the radiation source for different periods and cultured under laboratory conditions. The effect on growth and pigment contents were studied after irradiation and compared with untreated control cultures. The exposure of UV-B at short distance (50 cm) with increasing the duration from 1 to 10 min showed decreasing trend of growth (optical density, cell count and dry weight) and photosynthetic pigments (chlorophyll-a, b and carotenoids) as compared to the controls. Chlorophyll-b was found more sensitive to radiation. Growth and pigment contents were found increased up to 5 min exposure at long distance (100 cm) thereafter reduction was observed on subsequent exposures as compared to unexposed cultures. The UV-B exposure for 2 min had maximum growth and pigment content. These experiments revealed that the growth and pigment contents of *C. vulgaris* was inversely proportionate to the exposure time and directly proportionate to distance of source.

KEY WORDS: Carotenoids, *Chlorella vulgaris*, Chlorophylls, Ultraviolet radiation

The green micro-alga, *Chlorella vulgaris* has existed since the very beginning of life on Earth. Fossil evidence shows that the algae have existed since the Precambrian era 2.5 Billion years ago. Since 1919, when Otto Warburg chose *Chlorella* for his experiments on photosynthesis, the green micro-alga became more and more popular. At the beginning, researcher's interest was focused on the micro-algae themselves – their nutritional and cultivation requirements were defined. After that *C. vulgaris* has been commercially exploited for the production of food supplements, animal feed, cosmetic colorants and pharmaceuticals because of its ability to produce large quantities of valuable products (Sharma and Sharma 2013).

The effect of UV irradiation on biological matter became an important issue over the past three decades since the first reports of man-made changes in the stratospheric ozone layer, which covers and protects the earth's surface from harmful UV radiation (Rai and Mallick 1998; Holzinger and Lutz 2006). Stratospheric ozone loss above the Arctic has also raised concern about the impact of UV irradiation on the Northern biosphere (Hessen 2002). Anthropogenically released atmospheric pollutants such as halogenated carbons particularly chlorofluorocarbons are responsible for the continued depletion of the stratospheric ozone layer (Platt and Honninger 2003).

Variety of alterations may occur as a consequence of UV irradiation in the different experimental setups and likely in a naturally enhanced UV scenario. However, UV does not always have a harmful effect on plants and algae. Due to evolutionary adaptation, plants and algae can sense short wave irradiation and have found mechanisms to adjust to a sometimes quickly changing solar input. It is surprising, however, that more and more experiments describe a positive, stimulating effect of UV irradiation (Bjorn 2004).

Pioneer studies on radio sensitivity of algae towards UV radiation have been carried out by Kumar (1963). UV radiation is known to affect phytoplankton's motility, orientation, growth, development, pigments and photosynthesis, respiration etc. in negative, neutral and positive ways depending on the species or levels of irradiation energy (Rai and Mallick 1998). Dose dependent effects of UV on pigments of marine diatoms (Dohler and Stolter 1986) and in *Chlamydomonas microsphaera* (Zhang *et al*. 2013) were studied. Pattern of pigments differ in dependence on the applied wavelengths of the used UV source (Dohler and Lohmann

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1995). Effects on ultra structure and related metabolic function of algae (Holzinger and Lutz 2006) and physiological and structural changes in the green alga, *Micrasterias denticulate* induced by UV-B radiation have already been reported by Lutz *et al.* (1997).

A few contributions on chlorococcalean algae are citied here; Arnold (1933) studied the effects of 2537 Å on the rate of photosynthesis and respiration of *C. pyrenoidosa.* Vaidya and Vijayakumari (1978) studied the effect of UV on metabolism of *Chlorella*. Prasad *et al*. (2006) studied the protective role of sodium chloride and sodium dithionite against UV induced damage in *C. vulgaris* and in *C. pyrenoidosa* and Nitric oxide alleviates oxidative damage caused by UV radiation (Chen *et al*. 2003). The effect of UV light upon various physiological activities of *Chlorella* cells at different stages in their life cycle (Sasa 1961) and ultra structural changes of *Chlorella* sp. (Juan *et al.* 2005). Estevez *et al.* (2001) are also reported the effects of UV on Antartic *Chlorella* sp. on growth and the activity of lipid-soluble antioxidants.

The objective of this work was to study the impact of ultraviolet B (UV-B) radiation on green algae represented by *Chlorella vulgaris* as model organism.

MATERIALS AND METHODS

Test Organism and Growth Conditions

The experimental organism, *Chlorella vulgaris* was isolated by Pringsheim method (1946) from Mawtha, a fresh water pond, pH 7.3, near Amber Fort in Jaipur (26°98'59''N to 75°85'07"E), Rajasthan (India). The isolated *C. vulgaris* was cultured on Modified Chu-10 medium and maintained on the same medium by regular sub-culturing every two weeks as described (Sharma *et al.* 2011). The irradiated cultures were placed under dark for 24 hr to avoid photo-repair and then transferred to culture cabinet receiving light 12hr/day by fluorescent tube lights (40 W) having 2500 Lux intensity at 25±2°C (Sharma *et al.* 2012). The identification of the algal species was provided by Algae Orphanage Research Initiative (AORI) Pune (India).

Source and Mode of UV-B Treatment

The source of artificial ultraviolet-B radiation (UV-B light) was a Philips UV tube (TUV 30W). The spectral emission of UV-B source range was from 280-315 nm with a peak at 312 nm. The 20 ml culture suspension of *C. vulgaris* was transferred in sterile glass Petri-plates (9 cm in diameter) and exposed with UV radiation by adjusting the distance 50 cm and 100 cm between UV source and cultures. UV-B irradiation was applied to log phase of cultures having optical density 0.12 at 670 nm. Observations were carried out for a period of 4 weeks at weekly interval. Cultures were exposed upto 10 minutes with a gap of 1 minute in addition to the untreated cultures used as controls.

Growth Measurement

Growth was followed through optical density (OD), cell count and dry weight. The OD of cultures was recorded with the help of Photo-colorimeter (Century Instruments Private Limited, Chandigarh) at 670 nm. Cell count was determined using a Neubauer improved Haematocytometer according to Guillard and Sieracki (2005). For dry weight measurement, a known volume from the culture sample was filtered on a previously dried pre-weighed Whattman filter paper No. 1 of 10 cm diameter. The filter paper containing the algal biomass was rinsed with distilled water and weighed after drying for 24 h at 60°C in hot air oven and dry weight calculated in terms of mg/ml (Richmond and Gobbelaar 1986).

Estimation of Pigments

Chlorophyll contents were determined by procedure and equation as suggested by Parson and Strickland (1965) and carotenoids were measured according to Jensen (1978).

Statistical Analysis

Results of the analyses were compared by one way analysis of variance (ANOVA). The significance between pairs of variable means

was analyzed using least significant difference (LSD) test at 5% level of significance (Gomez and Gomez, 1984).

OBSERVATIONS AND RESULTS

Growth

The effect of ultraviolet-B radiation of short distance (i.e. 50 cm) on the growth of *C. vulgaris* showed decreasing trend with increasing duration of UV-B exposure from 1 to 10 min (Table-1). It was observed that 10 min exposure of UV-B at 50 cm distance reduced the growth in terms of optical density (OD), cell count (CC) and dry weight (DW) to 2.90, 2.93 and 2.91 times, respectively, as compared to control cultures after 4 weeks of culture (Table-1).

Table 1. Effect of Ultraviolet radiation (at 50 cm) on growth of *Chlorella vulgaris* in 4th week Values are mean ± Standard deviation (n=3). For each individual experiment variable means with the same letter are not significantly different (p>0.05).

Time	OD	CC	DW
Control	0.32 ±0.017 ^{a}	355±9.8 ^{a}	23.0±1.0 ^{a}
1 Min	0.30 ±0.016 ^{b}	328±8.0 ^{b}	20.8±0.72 ^{b}
2 Min	0.29 ±0.017 ^{bc}	324±11.0 ^{b}	20.4±0.40 ^{b}
3 Min	0.29 ±0.015 ^{bc}	319±8.1 ^{b}	20.3±0.43 ^{b}
4 Min	0.28 ±0.014 ^{c}	298±9.6 ^{c}	19.2±0.26 ^{c}
5 Min	0.26 ±0.016 ^{d}	274±10.5 ^{d}	17.8±0.62 ^{d}
6 Min	0.24 ±0.013 ^{e}	257±6.0 ^{e}	16.2±0.43 ^{e}
7 Min	0.22 ±0.017 ^{f}	245±11.5 ^{e}	15.6±0.52 ^{e}
8 Min	0.21±0.017 ^{f}	230±9.5 ^{f}	14.5±0.43 ^{f}
9 Min	0.14±0.016 ^{g}	160±7.0 ^{g}	10.0±0.55 ^{g}
10 Min	0.11±0.015 ^{h}	121±8.1 ^{h}	7.9±0.43 ^{h}

OD- Optical Density at 670 nm; CC - Cell Count in cells/ml; DW - Dry weight in mg/50ml

The effect of UV-B at long distance (i.e. 100 cm) up to 10 min of exposures (with a gap of 1 minute) showed the increased growth of *C. vulgaris* up to 5 min as compared to unexposed cultures. However, the growth was reduced on subsequent exposures after 5 min. Maximum growth was observed after 2 min of exposure. After 2 min, OD and cell count were increased to 1.37 times and dry weight to 1.34 times in irradiated cultures as compared to control cultures (Table-2).

Table-2 Effect of Ultraviolet radiation (at 100 cm) on growth of *Chlorella vulgaris* in 4th week Values are mean ± Standard deviation (n=3). For each individual experiment variable means with the same letter are not significantly different (p>0.05).

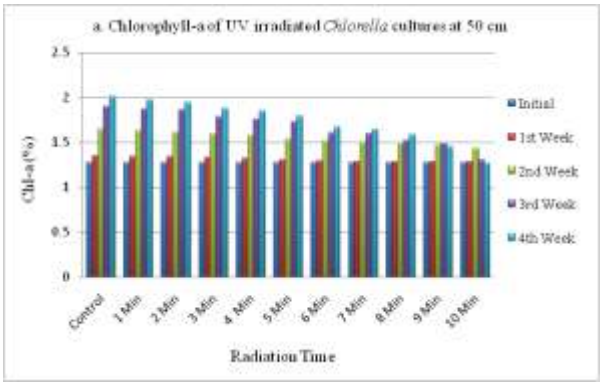
Time	OD	CC	DW
Control	0.32 ± 0.017 ^{e}	360 ± 8.8 ^{e}	23.0± 1.0 ^{f}
1 Min	0.40 ± 0.015 ^{b}	429± 11.0 ^{b}	28.0± 1.0 ^{b}
2 Min	0.44 ± 0.012 ^{a}	494 ± 10.3 ^{a}	30.9± 0.43 ^{a}
3 Min	0.38 ± 0.010 ^{c}	412 ± 7.5 ^{c}	26.9 ± 0.79 ^{c}
4 Min	0.37 ± 0.010 ^{c}	409 ± 7.9 ^{c}	26.0 ± 0.45 ^{d}
5 Min	0.35 ± 0.015 ^{d}	389 ± 7.9 ^{d}	24.6 ± 0.51 ^{e}
6 Min	0.30 ± 0.014 ^{f}	327 ± 6.0 ^{f}	21.1 ± 0.52 ^{g}
7 Min	0.28 ± 0.013 ^{g}	302 ± 6.0 ^{g}	19.3 ± 0.36 ^{h}
8 Min	0.26 ± 0.012 ^{h}	298 ± 8.0 ^{g}	19.0 ± 0.43 ^{h}
9 Min	0.24 ± 0.014 ^{i}	268 ± 7.5 ^{h}	17.7 ± 0.26 ^{i}
10 Min	0.20 ± 0.015 ^{j}	224 ± 7.2 ^{i}	14.3 ± 0.36 ^{j}

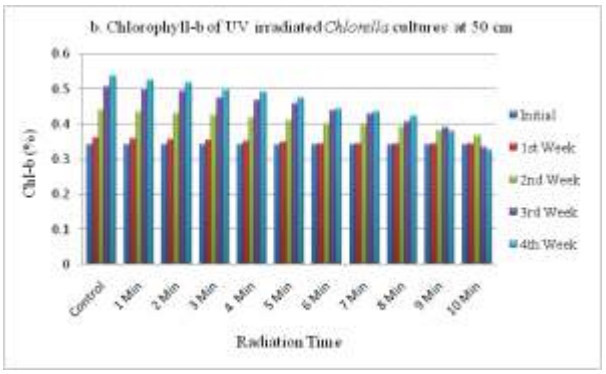
OD- Optical Density at 670 nm; CC - Cell Count in cells/ml; DW- Dry weight in mg/50ml

Photosynthetic pigments

The effect of UV-B (at 50 cm) on photosynthetic pigments were showed declining trend with increasing duration of exposures of 1 to 10 min. Although, the exposures of 1 to 8 min, chlorophyll-a, b and carotenoids were increased linearly up to the fourth week of experiment but lower as compared to controls. In 9 min irradiated cultures, these pigments increased up to third week then steadily decreased. In 10 min treated cultures after second week onwards chlorophyll-a, b and carotenoids decreased and then dropped slowly (Figures 1 a, b, c).

Chlorophyll-a, b and carotenoids contents subsequently decreased as the time of exposure





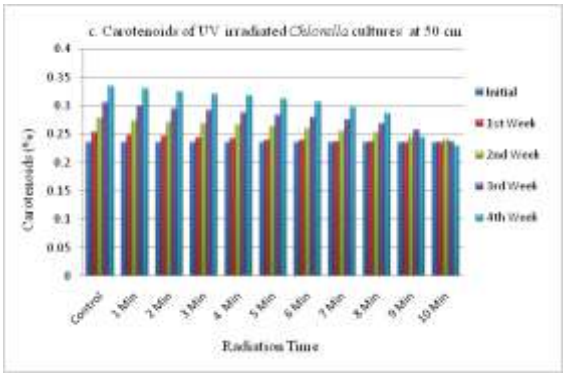
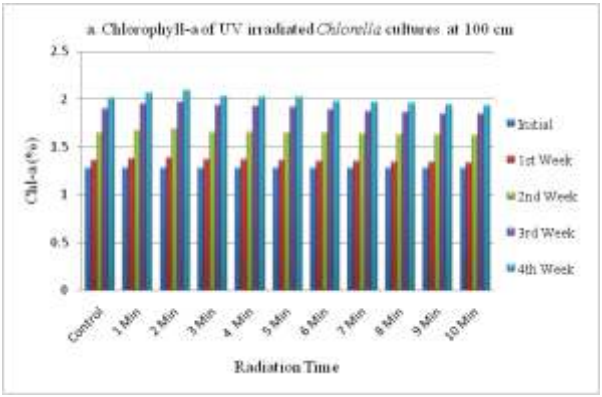
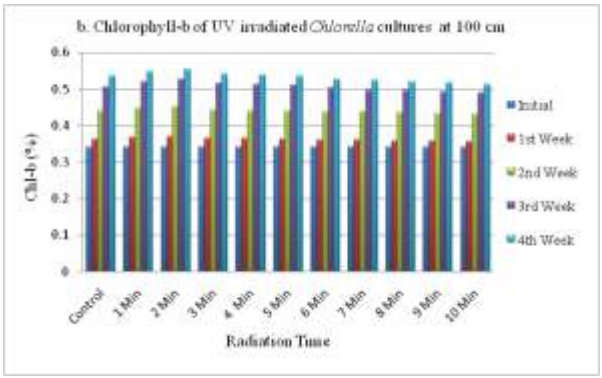


Figure 1a-c. Effect of Ultraviolet Radiation (at 50 cm) on the photosynthetic pigment contents of *Chlorella vulgaris*. Values are mean ± Standard deviation (n=3). For each individual experiment variable means with the same letter are not significantly different (p>0.05).





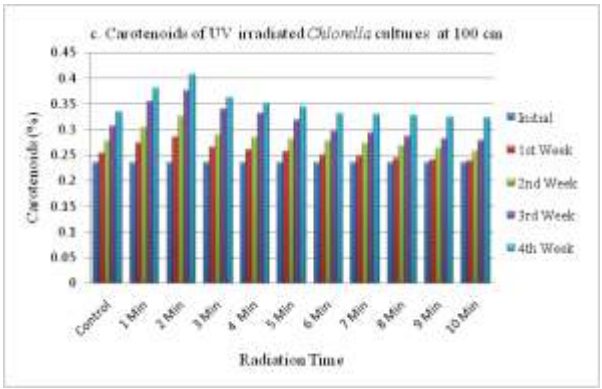


Figure 2 a-c. Effect of Ultraviolet Radiation (at 100 cm) on the photosynthetic pigment contents of *Chlorella vulgaris.* Values are mean ± Standard deviation (n=3). For each individual experiment variable means with the same letter are not significantly different (p>0.05).

increased and the reduction was 1.58 times in chlorophyll-a, 1.64 times in chlorophyll-b and 1.45 times in carotenoids as compared to control after 10 min of exposure at the end of experiment. Chlorophyll-b was more sensitive to UV exposure then chlorophyll-a and carotenoids were less affected by UV exposure (Figures 1 a, b, c).

The effect of UV (at 100 cm) on pigments showed that longer distance exposure revealed some positive results. One to 10 min UV irradiated cultures showed that chlorophyll-a, b and carotenoids were increased up to 5 min exposures as compared to unexposed culture However, on subsequent exposure after 5 min pigments were reduced. Although after 5 min exposure chlorophyll-a, b and carotenoids were increased linearly up to the fourth week of experiment but lower as compared to control

cultures.

Two min UV treated cultures showed more positive results with maximum contents of chlorophyll-a, b and carotenoids. At this treatment, chlorophyll-a, b and carotenoids were increased 1.03 and 1.21times, respectively, as compared to control at the end of experiment (Figures 2 a, b, c).

DISCUSSION

The effect of UV-B on living algae depends upon the irradiance, the wave band and the duration of exposure (Rai and Mallick 1998). In the present study, the effect of UV-rays at short distance (50 cm) from the source which is higher dose exhibited that all treatment 1 min to 10 min had negative or detrimental effect on growth as well as photosynthetic pigment contents of *Chlorella vulgaris* as compare to unexposed cultures.

The reduction in growth (OD, DW and CC) with 50 cm distance showed that this dose was higher for *C. vulgaris.* Growth measurements are helpful in estimating possible changes of productivity, as growth is considered an important parameter that integrates stress effects in several biochemical processes within the cell (Anastazia and Robert 1995).

UV induced growth delay has been reported for the cyanobacterium (Garcia-Pichel *et al*. 1993). Prolonged UV exposure led to decreased cell number in comparison with control cultures (Ehling-Schulz *et al*. 1997).

Chlorophyll and carotenoid contents were also decreased with this higher dose, although chlorophyll was more sensitive than carotenoids. Our findings are in agreement with the earlier findings of Dohler *et al*. (1986) and Tiyagi *et al*. (1992). Agrawal (1992) also reported reduction in chlorophylls and carotenoids in some green algae.

The reductions in pigment contents due to UV radiation have been ascribed either to an inhibition of chlorophyll biosynthesis or to a breakdown of pigments or their precursors in higher plants (EI-Mansy and Salisbury 1971; Teramura 1983). The damaging effect of UV on photosynthetic pigments may be due to the bleaching caused by UV irradiation or may be attributed to the damage of the enzymes involved in pigment synthesis in *Chlorococcum sp*. (ABO-Shady *et al*. 2008).

Less inhibition of carotenoids by UV might be due to its antioxidant property which neutralizes the singlet state of oxygen produced and quenches the chlorophyll and xanthophyll to prevent adverse effect of UV-B (Prasad *et al*. 2006).

When *C. vulgaris* was exposed to UV radiation at 100 cm distance (lower dose) for 1 min to 10 min showed increased growth and biochemical contents up to 5 min exposures as compared to unexposed cultures, whereas 2 min UV exposure has maximum growth and highest biochemical contents. However, on subsequent exposures after 5 min the growth and biochemical contents were reduced.

UV exposure at long distance led to the positive mutational changes in *C. vulgaris* with high pigment contents with 1-5 min that means their synthesis is induced, directly or indirectly by UV radiation that indicate it's not detrimental as short distance exposure because long distance favors low dose of radiation.

Several green algae appear to be well adapted to low levels of UV irradiation. This might be due to highly efficient avoidance mechanisms. Surface mucilage sheaths and internal UV-absorbing compounds or accessory pigments may protect cytoplasm and the chloroplast from harmful irradiation, thus enabling single cells to withstand under low UV irradiation (Holzinger and Lutz 2006).

In low doses, UV radiation with 1-5 min in *C. vulgaris* showed enhancement in growth (OD, DW and CC) which was also reported by Vimalabai and Kulandaivelu (2002) in some marine microalgae.

Chlorophyll and carotenoids were also increased upto 5 min treatment in *C. vulgaris*. An increase in photosynthetic pigments was also observed in *Nostoc* and *Spirulina platensis*

under UV treatment (Bhandari and Sharma 2007; Rawat and Singh 2008). The increased in carotenoids may be to protect the organism against the singlet oxygen species generated from oxidative damage by UV. Increased proportion of pigments especially carotenoids could play an important role in UV ray protection, functioning as antioxidant and quenching photosensitization products (Cockell and Knowland 1999) as in the present study.

CONCLUSION

The results obtained with short distance (at 50 cm) and long distance (at 100 cm) UV-B exposure experiments revealed that the growth and pigment contents of *C. vulgaris* was inversely proportionate to the exposure time and directly proportionate to the distance of source.

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