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Effect of UV-B Radiation on Growth, Photosynthetic Activity and Metabolic Activities of *Chlorella vulgaris*

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ABSTRACT

The impact of different intensities (1 Wm^{-2}, 3 Wm^{-2} and 5 Wm^{-2}) of ultraviolet-B (UV-B) radiation on growth, photosynthetic pigments, and metabolic activity has been studied in *Chlorella vulgaris* isolated from fresh water sample of Kulavoi lake, Chengalpet. The experimental alga *Chlorella vulgaris* was exposed to different intensities of 1, 3 and 5 W m^{-2}, and different durations of 10, 15, 20, 25, 30 mins of UV-B radiation respectively. Among the different intensities and durations tested inhibited growth by 50% Chlorophyll 5 Wm^{-2} of UV-B after 15 min followed by decrement in cell growth. On the other hand, carotenoids were stimulated at small doses (time of exposure and intensity) at 3 or 5 W m^{-2} of UV-B radiation. However, total proteins and total carbohydrates were inhibited by UV-B exposure times but the effect of 5 Wm^{-2} was more than other two intensities. The results suggest that *Chlorella vulgaris* is resistant to UV-B radiation damage at lower exposure time even at higher intensity and the possible negative effect of additional UV-B radiation on the growth of microalgae may have been effectively balanced by the UV-B radiation stress through increase in UV-absorbing compounds.

INTRODUCTION

The reduction of the stratospheric ozone layer allows more UV-B radiation to reach the Earth's surface and to a significant depth in the ocean [1]. India is among those countries that are close to the equator, thus faces high fluxes of UV radiation with sunlight. The average latitude of India is 20°C North of the equator and maximum ultraviolet-B (UV-B; 280–320 nm) irradiance near the equator (solar elevation angle <25°C) under clear, sunny skies is approximately 2.5 Wm^{-2}, which may affect the major occupation of the country, i.e., agriculture. A significant declining trend in total ozone column (TOC) over numerous stations lying in the Northern part of India is highly alarming [2]. Scenario-based chemistry-climate models show in 21st century, UV-B radiation will be enhanced due to high concentration of greenhouse gases [3]. The microalgae are single cell autotroph, require sunlight for their growth and exposed to elevated levels of UV radiation in their natural habitat, and simultaneously mechanisms were developed to lessen the damage effects of UV-B during long term of acclimation. The harmful effects of UV-B radiation on microalgae are always intervened by reactive oxygen species (ROS) which induce oxidative strain [4].

The most important processes in algal cells are the photosynthesis. It is also suggested that UV-B radiation predominantly attacks Photosystem II and the photosynthetic activity [5]. UV-B radiation affects the pigment concentrations in algal cells by inducing a photo degradation of light-absorbing pigments, resulting in a loss of photosynthetic capacity [6]. Because UV radiation is absorbed by biomolecules including nucleic acids, proteins, lipids and carbohydrates which is essential for biological and physiological activity within cells, it can affect many biological processes [7]. Inspite of adverse effects of solar UV radiation, cyanobacteria are not defenseless and have developed various strategies such as formation of antioxidants or efficient DNA repair mechanisms to counteract the damaging effects of UV-B radiation [8].

The UV screening compounds such as Mycosporine-like Amino Acids (MAAs) are usually accumulated intracellularly in cyanobacteria. *Aulosira fertilissima* exhibited the induction of three folds of MAAs when exposed to 20 min/day exposure of UV-light as compared to control [9]. It has been reported that UV-B radiation not only impairs the motility and photo orientation of cyanobacteria but also affects a number of physiological and biochemical processes, such as growth, survival, pigmentation and nitrogen metabolism [10]. Studies have stated that exposure to mild doses of UV-B radiation has induced resistance in some microalgae. UV-B radiation induced resistance in microalgae has the potential to serve as a source of biodiesel, antioxidants and nutraceutical substances. The effect of such induced UV-B resistance has impact on the carbon capture and carbon allocation efficiency for the synthesis of the molecules for biodiesel production [11].

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The aim of this work was to study the stress response of the green alga *Chlorella vulgaris* cells isolated from fresh water sample of Kulavoi Lake, Chengalpet, where it was exposed to three different intensities of UV-B radiation for different periods under laboratory conditions. Effects on pigment content, carbohydrates, proteins and lipid content were studied also directly after UV-B irradiation.

MATERIAL AND METHODS

Growth Conditions

C. vulgaris was isolated from fresh water sample of Kulavoi Lake, Chengalpet, and identified using standard manual. Purification of the organism was done by sub culturing, antibiotic treatment and ultraviolet irradiation [12]. The culture was grown and kept under 30 μE m^{-2} s^{-1} light intensity, 12/12 light dark cycle and at 24 ± 1°C. UV-B irradiation was done to log phase cultures having optical density from 0.15 - 0.20 at 600 nm.

Mode and Source of UV-B Radiation:

The tested organism grown in liquid culture was transmitted into a sterilised Petri dish and exposed individually to artificial UV-B radiation. The UV-B radiation comprised an array of three ultraviolet long lamps, UV-B lamps- 280/320 nm, Philips TL 20 W/12, Philips Gleolampenfabriken, USA. The suspension was gently agitated during irradiation to facilitate uniform exposure.

Measurement of Growth Rate

This experiment was carried out to evaluate the lethal dose (LD50) which causes death of 50% of algal populations. The algal cells exposed to 1 Wm^{-2}, 3 Wm^{-2} and 5 Wm^{-2} UV-B radiations were withdrawn at intervals and then counted using Neubauer chamber for measuring their survival [13].

Determination of Pigment

Five mL of culture sample was taken and centrifuged at 5000 rpm for 10 min and the supernatant was discarded. The algal pellet was then added with 5 mL of 80% acetone and homogenized in a sonicator. Then it was covered with black paper and kept overnight at 4°C. The sample was then centrifuged at 5000 rpm for 15 minutes collect the supernatant and the optical density was measured at 644.8 λ, 661.6 λ and 470 λ in Ultraspec UV-Visible spectrophotometer [14].

Extraction and Estimation of Total Protein

Five mL of algal sample was taken and centrifuged at 5000 rpm for 15 minutes. The pellet was homogenized in 5 mL of 0.1 M sodium phosphate buffer at pH 7.0 in a sonicator and then centrifuged at 5000 rpm for 15 minutes. The supernatant was taken for the estimation of total protein. To 0.2 mL of sample protein 5 mL of CBB reagent (100 mg of CBBG-250 dissolved in 50 mL of 93% ethanol. To this 100 mL of 85% Phosphoric acid was added and diluted to 1000 mL with glass distilled water) mixed well. The absorbance was read at 595 nm against a reagent blank. The amount of protein was calculated by using a standard graph with BSA ranging from 10 to 100 μg mL^{-1} [15].

Extraction and Estimation of Total Carbohydrate

Five mL of algal culture was taken and centrifuged at 5000 rpm for 15 minutes. The pellet was homogenized with 5 mL of 0.1 M sodium phosphate buffer at pH 6.8 in a sonicator and then centrifuged at 5000 rpm for 10 minutes. The supernatant was collected for the estimation of carbohydrate. To the 1 mL of sample 1 mL of 5% phenol and 5 mL of H_2SO_4 was added and mixed thoroughly. The solution was allowed to stand at room temperature for 30 minutes. The Optical Density was read at 490 nm. Standard graph was prepared with different concentrations of D-glucose ranging from 10 to 100 μg.mL^{-1} [16].

Extraction and Estimation of Total Lipid

Five mL of culture was taken and centrifuged at 5000 rpm for 15 min. The pellet was homogenized in a sonicator with 6 mL of chloroform: methanol (2:1). It was then transferred to a separating funnel and added with 2 mL of 0.9% NaCl solution and mixed well. This mixture was left undisturbed for overnight. Then from the lower chloroform phase, 0.5 mL was collected in a clean vial and the pellet was collected. To the pellet 0.5 mL of concentrated sulfuric acid was added and mixed well. The tubes were closed with glass marbles kept in a boiling water bath for 10 min and allowed to cool at room temperature. To 0.2 mL of sample 5 mL of vanillin reagent (0.2 g vanillin in 80 mL of ortho phosphoric acid and 20 mL of distilled water) was added and mixed well. It was allowed to stand for 30 minutes and the colour developed was read at 520 nm. Standard graph was prepared using cholesterol ranging from 5.0 to 50 μg/mL and the values are expressed as μg.mL^{-1} [17].

Extraction of UV-B Absorbing Compound MAA–Mycosporine like Amino Acid

Thirty grams of UV-B adapted A. platensis biomass was taken and ground with 30% methanol and left at 4°C overnight. The extract was filtrated through filters paper (Whatman No. 1) and the filtrate was centrifuged at 5000 rpm for 20 min at 4°C. The supernatant was vacuum-filtered using a Membrane filter apparatus. The filtrate was concentrated to 300 mL. The methanol

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insoluble fraction was removed by centrifugation at 7000 rpm for 10 min at 4 °C and the supernatant was concentrated and suspended with 100% methanol. After centrifugation at 21,000 rpm for 10 min for 4 °C, the supernatant was obtained as crude MAA.

Statistical analysis

Results are presented as mean with standard deviation (SD) from three different readings. The statistical analyses were carried out using SPSS 21.0 obtained were analyzed statistically to determine the degree of significance between treatments using two way analysis of variance (ANOVA).

RESULTS

The experimental organism *Chlorella vulgaris* was isolated from fresh water samples of Kulavoi lake, Chengalpet. The culture was maintained in the Bold Basal medium at less than 25 ± 1 °C at 30 µE $m^{-2}.s^{-1}$ light intensity and 12/12 light/dark photoperiod **(Figure 1)**. In the present investigation, Ultraviolet light – B induced changes in the growth, pigment and protein content of *Chlorella vulgaris* was studied. The test organism was given exposure to Ultraviolet radiation (UV-B) for different time intervals of 10, 15, 30, 45 and 60 minutes. Soon after UV-B treatment the change in growth, pigment and protein content were analyzed. It was observed that the growth characteristic of test species *Chlorella vulgaris* decreased with gradual increase in time of exposure of UV-B radiation as compared to control (untreated cultures). The growth remains static up to 15 minutes in 5 W m^{-2} UV-B exposure time followed by decline in subsequent 60 minutes time of UV-B exposure **(Figure 2)**.

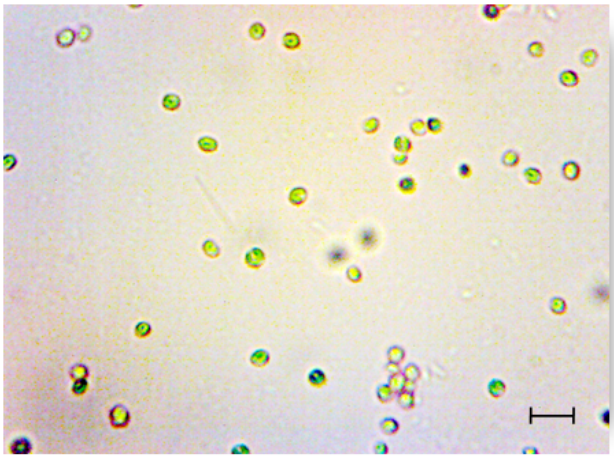

Figure 1. Microscopic Observation of *Chlorella vulgaris*.

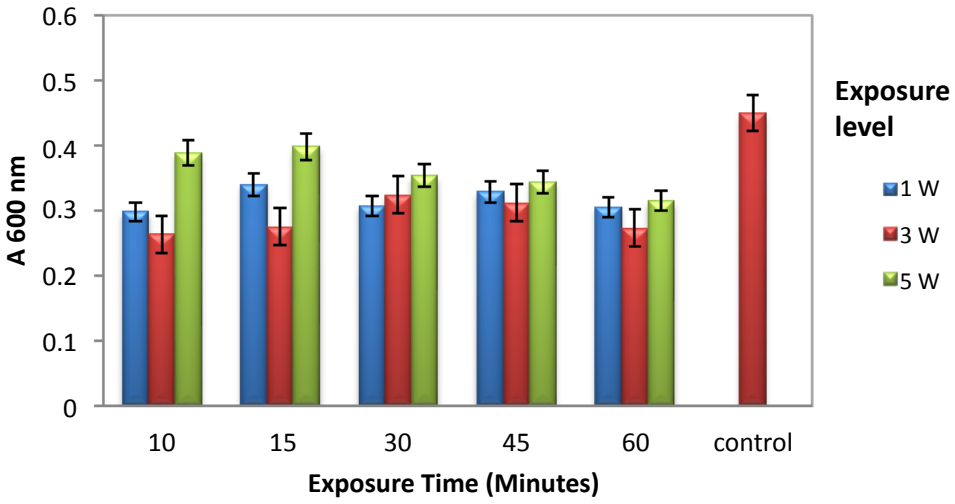

Figure 2. Effect of UV-B radiation on the growth rate of *Chlorella vulgaris*.

Survival

In order to select a lethal dose (LD50), cultures of *C. vulgaris* were exposed to 1, 3 and 5 Wm^{-2} UV-B radiation for different time periods such as 10, 15, 30, 45 and 60 minutes **(Table 1)**. There was 50% survival at 5 Wm^{-2} of UV-B after 15 min treatment and therefore this dose was used in all further experiments.

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Table 1. Percent survival based on colony counts after UV-B treatment for different periods.

Exposure Time (min)	Percent Survival		
	1 Wm^{-2}	3 Wm^{-2}	5 Wm^{-2}
0	100	100	100
10	90	81	78
15	71	62	53
30	38	58	32
45	33	33	21
60	29	27	16

Pigments

The effects of UV-B on photosynthetic pigments chlorophyll and carotenoids content showed decreasing trend with increasing duration of UV-B exposure. However, carotenoids were less affected than total chlorophyll. The chlorophyll content decreased in all the UV exposure time intervals as compared to control but there was a notable increase in chlorophyll content up to 30 minutes exposure to 5 W m^{-2} UV-B radiations followed by decrease after 15 days of growth. It was observed that even at the highest UV - B exposure time (30 minutes) chlorophyll content was much higher than that of control **(Figure 3)**.

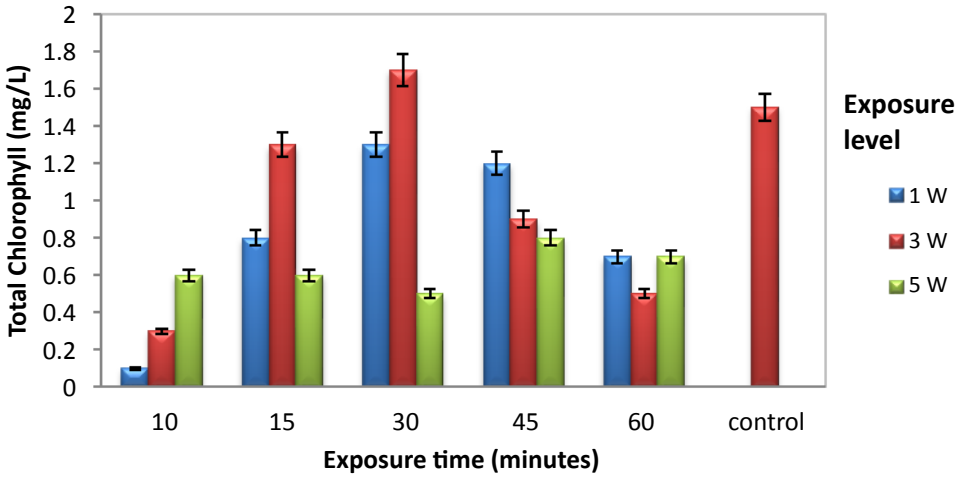

Figure 3. Effect of UV-B radiation on the Total chlorophyll content of *Chlorella vulgaris.*

Carotenoid Content

With regard to carotenoid content after UV-B exposure, it can be observed that, in cells exposed to 3 Wm^{-2}, UV-B enhanced carotenoid production at all exposure time intervals. The increase in carotenoid content was of 6.5%, 13.3%, 30.5%, 26.6% and 21.7%, as compared to the control after 10,15,30,45, and 60 min of exposure. Also in cells exposed to 5 $W.m^{-2}$ of UV-B radiation initially enhanced carotenoid production. However, a reduction in carotenoid content was observed after 60 min of exposure (15.5%) as compared to control **(Figure 4)**.

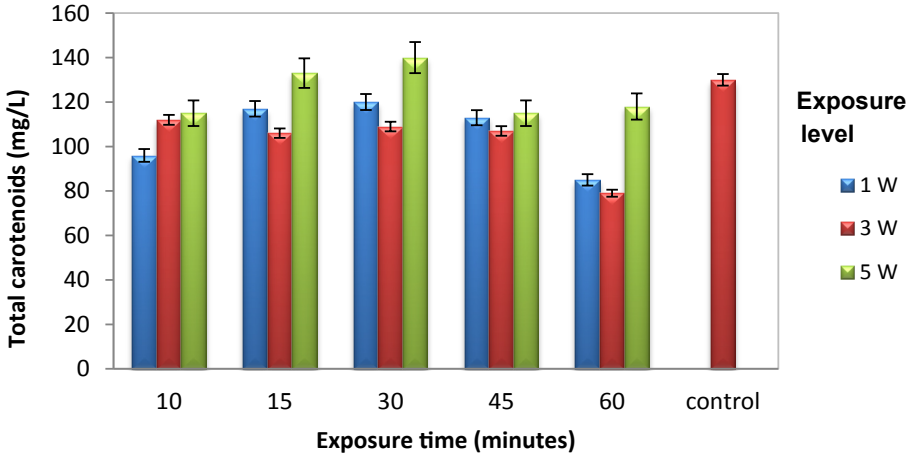

Figure 4. Effect of UV-B radiation on Total carotenoids content of *Chlorella vulgaris*.

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Total Proteins

UV-B exposure led to reduction in the total proteins. The reduction was 86.7and 53.9 % below the control after 60 min at 3 and 5 Wm^{-2}, respectively. Exposure to 3 and 5 Wm^{-2} UV-B revealed highly significant effect on total soluble proteins at all the exposure times except 1 W m^{-2}/30 min which revealed non-significant effect on total proteins **(Figure 5)**.

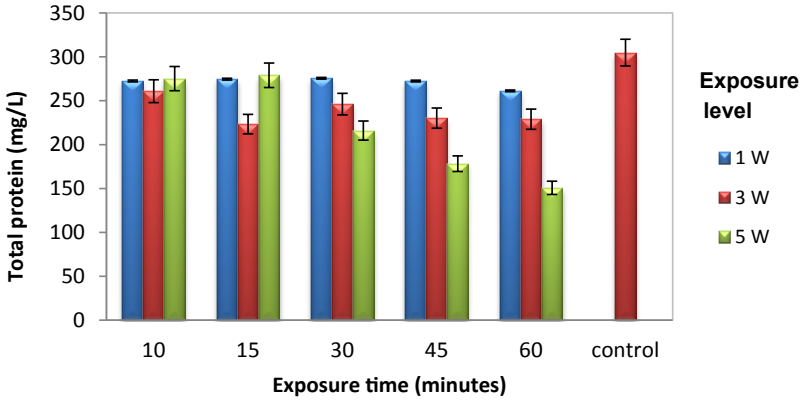

Figure 5. Effect of UV-B radiation on the protein content of *Chlorella vulgaris*.

Total Carbohydrates

Exposure of *C. vulgaris* to UV-B radiation showed a decrease in the total carbohydrate content. After 60 min of UV-B irradiation cells exposed to 3 and 5 Wm^{-2} showed a decrease in carbohydrate content of 29.0 and 64.0% as compared to control, respectively **(Figure 6)**.

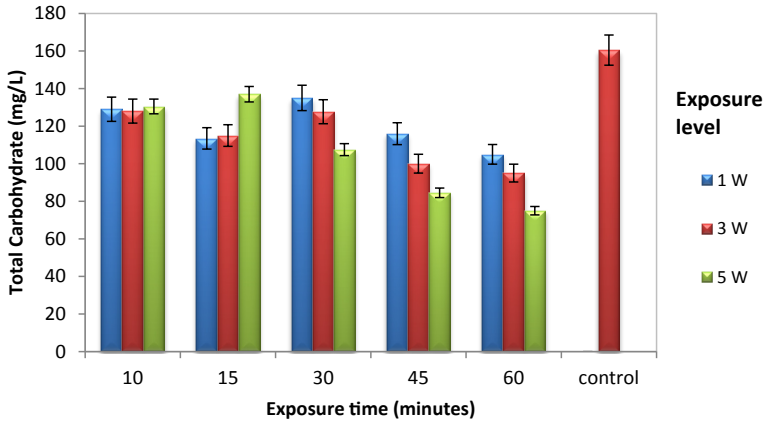

Figure 6. Effect of UV-B radiation on Total carbohydrate content of *Chlorella vulgaris*.

Total Lipid

In *Chlorella vulgaris*, the total Lipid content in control (without UV radiation exposure) becomes 138 mg/L whereas after 15 minute 5 W m^{-2} UV B exposure the lipid content followed by decrease in subsequent treatment of UV-B exposure **(Figure 7)**.

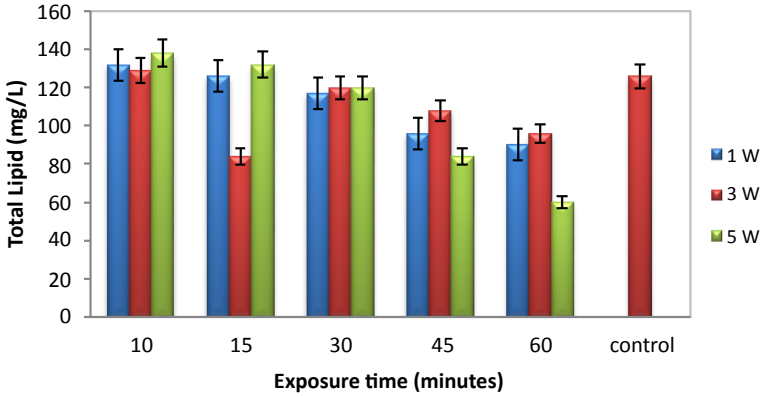

Figure 7. Effect of UV-B radiation on Total lipid content of *Chlorella vulgaris.*

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The experimental microalgae exposed UV-B radiation were screened for the compounds like MAA (Mycosporine like aminoacids) which are usually accumulated intracellularly. MAAs is well known UV-absorbing/screening compounds that provide photoprotection against UV-B radiation. Spectrophotometric analysis of the UV-B treated algae revealed the presence of MAA compounds. Further study on this line also in progress was used for qualitative screening of UV-B screening compounds. In this study, the UV absorption band for MAAs was found at 332 nm Mycocporine 2 glycine in *Chlorella vulgaris* **(Figure 8)**.

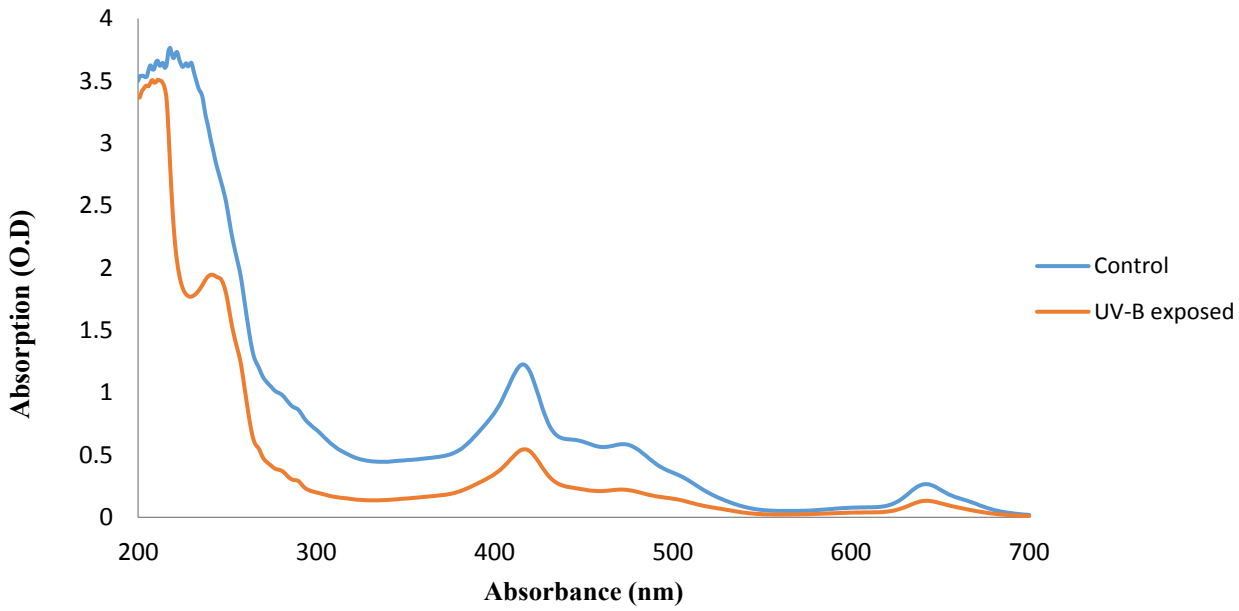

Figure 8. UV spectrum showing absorption peak for UV absorbing compounds.

DISCUSSION

Solar UV-B radiation reaching the Earth surface exerted significant changes in the ecosystem. The adverse effect of UV-B on microalgae are too as they form basis for food chains in aquatic systems [18]. As found with higher plants, the deleterious effects of UV-B radiation stress on microalgae include inhibition of photosynthesis, damage to DNA [19], proteins and lipids [19], generation of oxygen radicals [20] and inhibition on nutrient uptake [21]. UV-B induces structural changes in both algal cells [22]. In the present study, the results showed pronounced inhibitory effects of UV-B on growth and survival of *Chlorella vulgaris*, where the inhibition increased with increasing of UV-B intensity and exposure time. In agreement with our results, harmful effect of simulated UV-B radiation at irradiances ranging from 3 to 5 Wm^{-2} has been reported in several algae [23].

It is also clearly demonstrated that, UV-B irradiation at 5 Wm^{-2} significantly decreased chlorophyll a and b contents of *Chlorella vulgaris*. However, the effect of 5 Wm^{-2} was more significant than that of 3 Wm^{-2} of UV-B radiation. These results agree with those obtained on Ulva [24,25]. UV-B exposure may cause the loss of photosynthetic pigments, and reduce the expression of genes involved in photosynthesis [26]. Among the various physiological processes, photosynthesis is potentially the main target of UV radiation due to multiplicity of possible effects [27]. The damaging effect of UV-B on photosynthetic pigments may be due to the decolorizing caused by UV-B irradiation or may be attributed to the damage of the enzymes involved in chlorophyll biosynthesis [17].

The results revealed that, small doses of UV-B radiation stimulated the synthesis of extracellular carbohydrates in *Chlorella vulgaris*. However, higher doses inhibited the extracellular carbohydrate production. The previous results were in agreement with the study on the effect of UV-B irradiation on the production of extracellular polysaccharides in Nostoc commune [28].

Results showed also that, UV-B irradiation (1, 3 and 5 W m^{-2}) significantly increased carotenoids at low UV-B doses which is similar to the reports where the synthesis of pigments absorbing wavelength in the UV range is an important protective mechanism against UV-B radiation displayed by algal cells [29-31]. These include mycosporine-like amino acids (MAAs) which are mostly present at the periphery of the cell thereby preventing penetration of UV- B radiation further into the cell.

MAAs is small, water-soluble molecules of imino-carbonyl derivatives of cyclohexanone with absorption maxima between 280 and 360 nm. Scytonemin, a water-insoluble molecule, occurs in the extracellular mucilaginous sheath surrounding cyanobacterial cells and also considered to be a photoprotective compound [32]. High amounts and diversity of MAAs was also found during red tide of dinoflagellates occurred in the Argentine sea. Similarly, in Gymnodinium cf. aureolum showed *in vivo* and *in vitro* absorption in the UV region, with maxima at 334 nm was reported that was similar to this study [33].

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CONCLUSION

It is suggested that microalgae have evolved ways of protecting themselves against UV-B damage–either by producing screening compounds Results indicate that there is cumulative increase in the lipid content of *Chlorella vulgaris*. Resistance induced to UV-B radiations in microalgae of commercial significance i.e., *Chlorella vulgaris* that have the potential to serve as source of biodiesel. Considering the soaring oil price in the global market, there is an urgent need to evaluate the potential of algae in the production of energy as bio-diesel. Carbon allocation for the synthesis of molecules for biodiesel production in this algae remains to be explored.

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