Impact of ultraviolet-B radiation on growth and biochemical composition of *Botryococcus braunii* Kutz.

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The present study examines the impact of ultraviolet-B (UV-B) radiation stress on commercially significant microalga for biofuel application. Experimental alga *Botryococcus braunii* was treated under different doses of artificially enhanced UV-B radiation. The organism was treated under high dose rates of 1 and 5 Wm^{-2} with altered time durations of 15, 30, 45 and 60 min. It showed large variations in the growth characteristics analysed. The rate of whole-cell photosynthetic oxygen evolution showed steep drop in high dose compared to low dose-treated cultures. As a result, level of photosynthetic pigment chlorophyll *a* content decreased drastically while carotenoid level invariably increased. Consequently, the level of primary metabolites such as total carbohydrate, protein and lipid was drastically reduced under high dose while marginal decrement was observed at lowest dose of UV-B radiation. Overall, the impact of UV-B radiation on *B. braunii* led to a drop in protective mechanisms with associated decline in growth and cellular imbalance at high intensity studied.

Keywords: *Botryococcus braunii*, chlorophyll *a*, oxygen evolution, ultraviolet-B radiation.

As primary producers, microalgae are the basic unit of the food chain, dominating both aquatic and terrestrial ecosystems. They account for about 60% of oxygen production, which is ultimately reducing to 50% CO_2 via carbon fixation[1]. In recent years, significant changes have been observed in the aquatic ecosystem owing to increased solar ultraviolet-B (UV-B) radiation penetrating the earth's surface. This is due to increased reduction of stratospheric ozone layer as a result of adverse anthropogenic activities. The undesirable effects of UV-B radiation on photosynthetic microalgae could also affect the next trophic level and can have far-reaching consequences as they form the basis of the food chain[2]. In general, high rate of UV-B radiation is known to have a negative impact on living organisms, starting at the molecular, cellular and ultimately population level[3].

The process which is predominantly affected by UV-B radiation is photosynthetic reaction, which leads to damaging effects[4]. However, photosynthetic organisms depend on solar energy containing damaging UV radiation for their photosynthetic process. This non-ionizing UV radiation is found to be 8–9% of the total earth surface reaching radiation[5,6]. Of the total non-ionizing radiation, only 1.5% is constituted by UV-B radiation (280–320 nm) reaching the earth surface having direct damaging affects on both aquatic and terrestrial biota[7]. Decreasing stratospheric ozone layer highly increases UV-B penetration into the earth's atmosphere. This harmful abiotic stress affects all forms of life starting from the lowest-level of living organisms in trophic level, i.e. microalgae. This vulnerability is exerted in photosynthesis, growth and eventually in survival as well, due to the necessity of solar energy for photosynthetic process. UV-B radiation can alter cellular morphology, motility and DNA damages which eventually exert in reduced biomass production[8–10].

The penetration of UV-B radiation is found to be high in tropical regions than temperate regions[11,12]. Several studies have reported the impact of UV-B radiation on plant growth pattern and productivity. However, studies pertaining to the influence of UV-B radiation on growth and physiological activities of colonial green microalga *Botryococcus braunii* at high dose are lacking. Therefore, *B. braunii*, a renewable source of biomass and hydrocarbons was chosen for this study[13,14]. *B. braunii* grown under outdoor conditions in raceway ponds showed good growth with biomass yield of 1.5 g l^{-1} and 30% hydrocarbon content[15]. Besides biofuel applications, *B. braunii* contains 79–84% lutein of total carotenoids which could be exploited for pharmaceutical applications[16]. In this article, we report the effect of short-term and high-dose artificial UV-B radiation on growth and physiological activities of *B. braunii*.

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Materials and methods

Culture and growth conditions

B. braunii was obtained from Algal Culture Collection, Centre for Advanced Studies in Botany, Guindy campus, University of Madras, Chennai. It was cultured photo-autotrophically using Modified Chu-13 medium[17] and grown at $25 \pm 2°C$ with 12 : 12 h light–dark photoperiod conditions.

Different doses of UV-B intensities and durations on B. braunii

The *B. braunii* culture was treated at different intensities (1 and 5 Wm^{-2}) of UV-B radiation for different durations (15, 30, 45 and 60 min). The UV-B treated cultures were kept under dark for 15 min (ref. 18); then transferred to fresh medium and growth parameters recorded for a period of 18 days at a regular time interval of 3 days.

Photosynthetic oxygen evolution measurement

Photosynthetic oxygen evolution of the experimental culture was monitored for a period of 5 min at an interval of 30 sec at 25°C using YSI biological oxygen electrode, immediately after UV-B treatment. Saturating white light (200 Wm^{-2}) was passed through 1% $CuSO_4$ filter solution which acts as a heat filter. Two millilitre of cells containing 30–40 $\mu g\ ml^{-1}$ of chlorophyll *a* was used for estimation of oxygen evolution.

Specific growth rate analysis

The number of generations per unit time in an exponentially growing culture was determined following standard method[19].

Biomass estimation

To determine the yield of dry biomass, 30 ml culture was filtered through GF/C filter. The filtered biomass was oven-dried at 60°C till constant weight was obtained; it was then weighed gravimetrically. The dried biomass of *B. braunii* was expressed in g l^{-1} dry wt.

Photosynthetic pigments estimation

Photosynthetic pigments were estimated spectrophotometrically in 100% acetone[20].

Chlorophyll *a* (mg l^{-1}) = $11.24 \times A_{661.6} - 2.404 \times A_{644.8}$.

Total carotenoid (mg l^{-1})

$$= \frac{(1000 \times A_{470} - 1.9 \times \text{Chl } a - 63.14 \times \text{Chl } b)}{214},$$

where $A_{661.6}$ is the absorbance at 662 nm, $A_{644.8}$ the absorbance at 645 nm and A_{470} is the absorbance at 470 nm. The values are expressed in mg l^{-1}.

Total carbohydrate, protein and lipid estimation

Total carbohydrate and protein were estimated from *B. braunii* biomass according to the methods of Dubois[21] and Bradford[22] respectively. For estimation of total lipid, Folch method was followed[23].

Tolerance and LD$_{50}$ value determination

The measure of toxicity as lethal dose$_{50}$ (LD$_{50}$) value of UV-B radiation towards *B. braunii* was calculated based on 50% reduction in chlorophyll *a* content compared to control.

Statistical analysis

Data were analysed statistically and results expressed as mean ± SEM.

Results and discussion

Global warming directly influences ozone layer depletion, which eventually results in increasing UV radiation penetration having far-reaching impacts on living biota. This especially affects the primary producer microalgae that form the basic unit of food webs in aquatic ecosystems. Hence there is a need to focus research at this micro level to find the harmful effects of UV-B radiation. This has to be addressed for the benefit of mankind to reduce the negative influential activities made by human being. *B. braunii* is reported to contain maximum lipid, hydrocarbon and carbohydrate content of 19%, 11% and 33% respectively, which could be resourcefully exploited for various commercial applications[24]; especially *Botryococcus* sp. having prospective applications from food to biodiesel feed stock[25]. The hydrocarbons produced by *B. braunii* isolates were reported to be saturated hydrocarbons[26]. Hence the present study is aimed to focus on the effect of UV-B radiation stress on growth and photosynthetic activities of *B. braunii*.

UV-B radiation and photosynthetic oxygen evolution

Table 1 and Figure 1 show photosynthetic oxygen evolution recorded immediately after UV-B treatment in

Table 1. Changes in photosynthetic oxygen evolution of *Botryococcus braunii* at different intensities and durations of UV-B treatment

| Intensities (Wm^{-2}) | Control* (without UV-B treatment) | Duration (min) (μmol O_2 mg chlorophyll^{-1} h^{-1}) | | | |
		15	30	45	60
1	159 ± 2.07	155 ± 2.07	135 ± 0.69	95 ± 2.76	82 ± 2.21
5	167 ± 3.50	117 ± 0.69	69 ± 2.10	46 ± 2.00	45 ± 1.38

*Freshly sub-cultured.
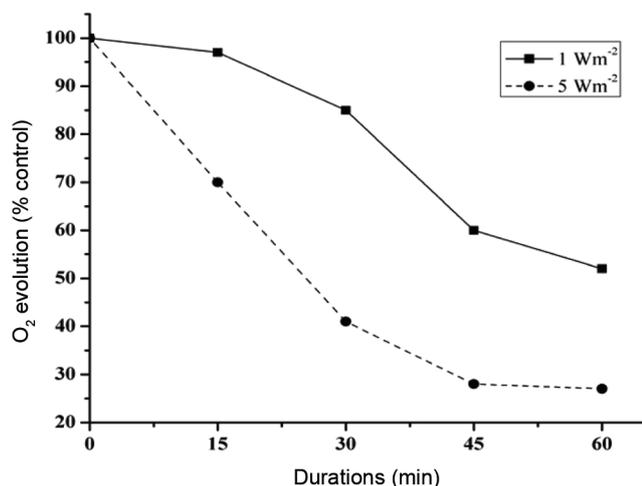
Figure 1. Changes in photosynthetic O_2 evolution of *Botryococcus braunii* after UV-B treatment with respect to percentage activity of control at different intensities and durations.

control and UV-B treated *B. braunii*. Table 1 shows that oxygen evolution reduced to a minimum of 3% at 1 Wm^{-2} in 15 min-treated cultures and maximum reduction of 49% and 73% was recorded at 60 min for 1 and 5 Wm^{-2} treatments respectively. The short duration of treatment to high UV-B dose is more damaging than long periods of exposure at low irradiance[27,28]. The present study shows that UV-B radiation produces differential rate of inhibition in photosynthetic activity. However, such loss in photosynthetic activity with a reduction of 50% shows the severity of damage. Thus, the amount of exposure to UV-B can determine the severity of damage to the microalga. This implies that UV-B primarily attacks the photosynthetic reaction centre components[29].

Growth measurements in terms of biomolecules are helpful in calculating possible changes, since growth is taken as an important factor that indicates the influence of stress in biochemical process within the primary producers. Therefore, in the present study other parameters, namely biomass, chlorophyll *a* and metabolites, including total carotenoid, protein, carbohydrate and lipid were analysed over a specified time interval.

UV-B radiation and growth rate

A study of the short-term effect of UV-B radiation stress on growth rate of *B. braunii* under different treatment conditions revealed the following results. The alga exhi-

bited highly reduced growth rate when the doubling time of *B. braunii* was studied under 5 Wm^{-2} UV-B treatment compared to 1 Wm^{-2} (Table 2). From Table 2 doubling time of 2.19 days could be observed under control growth conditions; however, in UV-B treated *B. braunii* at 1 Wm^{-2} for 15 min doubling time increased to 2.13 days. Comparatively, the doubling time has highly abridged at 5 Wm^{-2} UV-B treatment studied, except at 15 min duration having a value of 2.45 days; rather it has increased to 4.68, 5.35 and 7.62 days at successive increased durations of 30, 45 and 60 min respectively, showing severity of damage caused. Microalgae contribute up to 45% of the annual total global primary productivity and thus fix carbon dioxide into organic matter[30]. Thus the reduced growth rate will cause reduced atmospheric CO_2 fixation. From the results it is clear that increased UV-B radiation imposes reduced growth rate, thereby indirectly reducing CO_2 fixation rate. This will eventually lead to an increase in global warming and make the earth uninhabitable.

## UV-B radiation and biomass production

The biomass yield also showed gradual decrement with an increased intensity of UV-B treatment (Figure 2). On the 18th day control culture showed a high biomass content of 0.97 g l^{-1} dry wt which was comparatively similar to earlier report[31] having a biomass of 1 g l^{-1} on 25th day. UV-B treatment at 1 Wm^{-2} for 15 min showed a biomass of 0.57 ± 0.03 g l^{-1} dry wt which was 19% less than control (0.70 ± 0.07 g l^{-1} dry wt) on the 15th day. It further decreased by 56% (0.31 ± 0.04 g l^{-1} dry wt) for 60-min treated culture on 15th day at the same intensity studied (Figure 2 *a*). Similarly, treatment at a minimum duration of 15 min at 5 Wm^{-2} UV-B radiation showed a decrement of 46% (0.38 ± 0.05 g l^{-1} dry wt) on the 15th day. Likewise, treatment for 30, 45 and 60 min duration at 5 Wm^{-2} showed the rate of biomass decrement as 75% (0.18 ± 0.04 g l^{-1} dry wt), 75% (0.18 ± 0.02 g l^{-1} dry wt) and 78% (0.16 ± 0.04 g l^{-1} dry wt) respectively, on the 15th day (Figure 2 *b*). Similarly, stunted growth rate of diatom *Malosira nummoloidas* under different doses of UV-B radiation has been reported[32].

UV-B radiation and photosynthetic pigment

Immediately after 1 Wm^{-2} UV-B treatment for 60 min duration, the concentration of chlorophyll *a* decreased by

Table 2. Doubling time of *B. braunii* under UV-B treated condition

		Doubling time (day)			
UV-B intensity (Wm^{-2})	Control	15 min	30 min	45 min	60 min
1	2.19	2.13	2.33	2.55	2.29
5		2.45	4.68	5.35	7.62
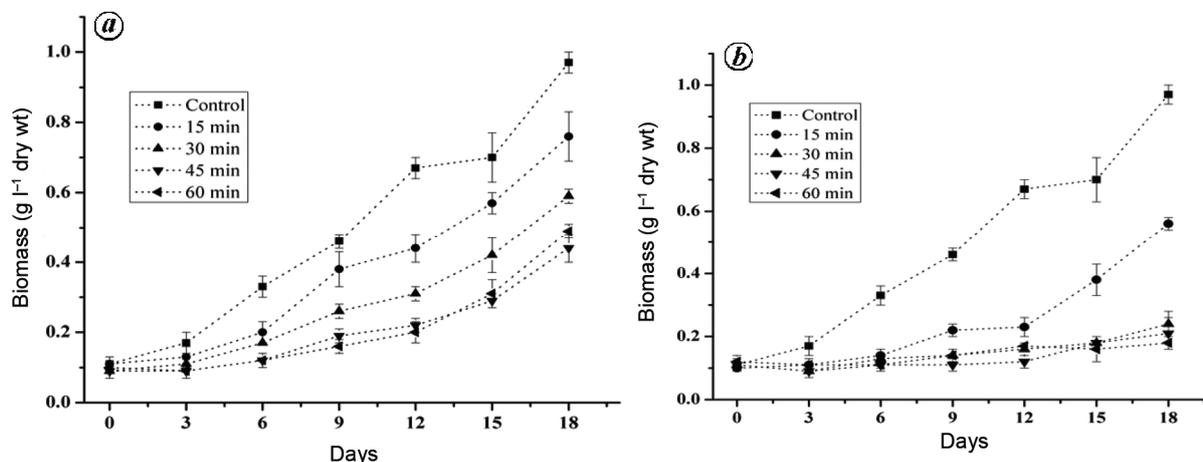
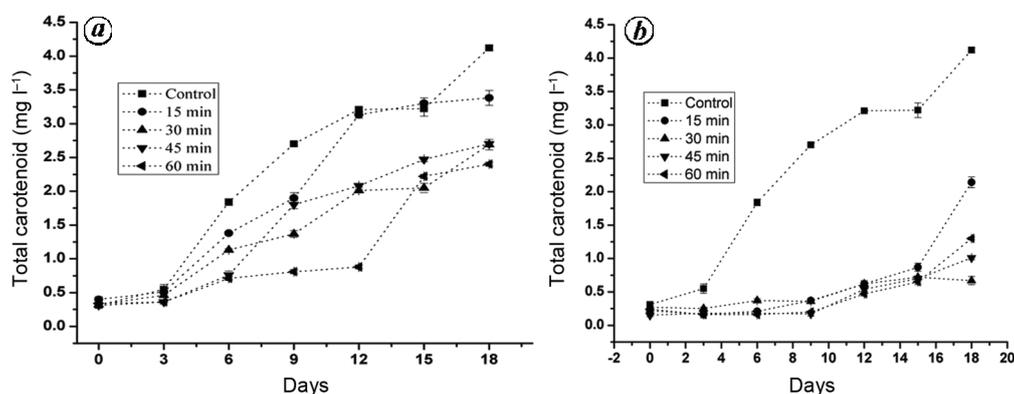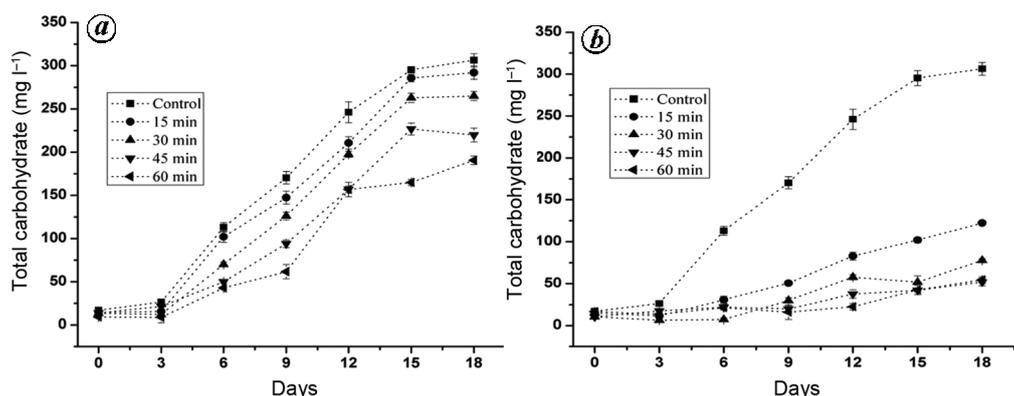**Figure 2.** Effect of (*a*) 1 Wm^{-2} and (*b*) 5 Wm^{-2} UV-B on biomass of *B. braunii*.
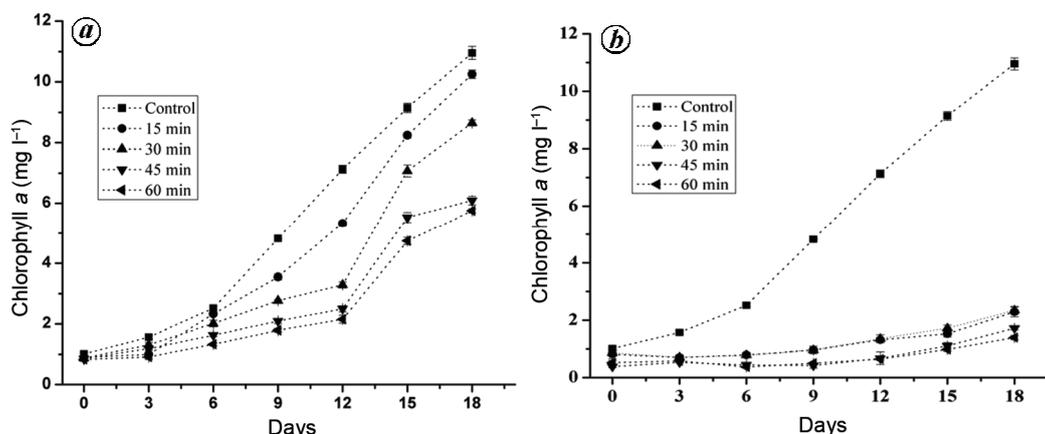
Figure 3. Effect of (*a*) 1 Wm^{-2} and (*b*) 5 Wm^{-2} UV-B on chlorophyll *a* of *B. braunii*.

18% (0.83 ± 0.03 mg l^{-1}) than control (1.01 ± 0.05 mg l^{-1}). On the 15th day, decrement raised to 49% (4.74 ± 0.12 mg l^{-1}) than control (9.14 ± 0.15 mg l^{-1}) (Figure 3 *a*). Whereas for alga treated for 15 and 60 min duration at 5 Wm^{-2}, chlorophyll *a* concentration decreased by 84% (1.52 ± 0.11 mg l^{-1}) and 90% (0.98 ± 0.07 mg l^{-1}) respectively, on the 15th day compared to control (9.14 ± 0.15 mg l^{-1}) (Figure 3 *b*). The decreased chlorophyll *a* concentration directly shows pigment bleaching effect caused due to UV-B radiation stress[33]. In contrast to chlorophyll *a*, the concentration of total carotenoid decreased by only 32% (2.22 ± 0.03 mg l^{-1}) at 1 Wm^{-2} for 60 min of UV-B-treated culture on the 15th day (Figure

4 *a*). However, the culture treated for 60 min at 5 Wm^{-2} showed a decrease of 80% (0.65 ± 0.04 mg l^{-1}) on the 15th day than control (3.22 ± 0.11 mg l^{-1}) (Figure 4 *b*). The results of photosynthetic pigment level towards UV-B radiation showed a high reduction of chlorophyll level than the other light harvesting carotenoids. As carotenoids are known to play a photoprotection role against photooxidation caused by abiotic stress, the level of carotenoids increases to protect against damage[34]. Less inhibition of carotenoids than chlorophylls by UV-B radiation was observed due to antioxidant property of the former, which neutralizes the singlet state of oxygen produced and mitigates the adverse effect of UV-B radiation.

Table 3. LD$_{50}$ percentage values of *B. braunii* under different UV-B treated condition on 15th day

	LD$_{50}$ (reduction %) Durations (min)			
UV-B intensity (Wm^{-2})	15	30	45	60
1	9	22	39	48
5	83	81	87	89

Figure 4. Effect of (*a*) 1 Wm^{-2} and (*b*) 5 Wm^{-2} UV-B on total carotenoid of *B. braunii.*

Figure 5. Effect of (*a*) 1 Wm^{-2} and (*b*) 5 Wm^{-2} UV-B on total carbohydrate of *B. braunii.*

The reduction in chlorophyll after being exposed to only 5 h per day of UV-B radiation, as reported by Wu *et al.*[35], was due to the differences in UV-B intensity[35] (which is in contrast to the present study).

UV-B radiation and primary metabolites accumulation

The primary metabolites, namely total carbohydrate, protein and lipid levels after UV-B treatment were also concurrently estimated. The total carbohydrate content was same as control (295 ± 3.54 mg l^{-1}) at 1 Wm^{-2} for 15 min UV-B-treated cultures (285 ± 4.28 mg l^{-1}) on the 15th day. However, 60 min-treated cultures at the same intensity showed 45% decrement (164.92 ± 3.57 mg l^{-1}) (Figure 5 *a*). However, UV-B treatment at 5 Wm^{-2} showed a highly reduced carbohydrate accumulation (Figure 5 *b*).

The protein level was also altered with a reduction of 57% (55.02 ± 2.11 mg l^{-1}) and 79% (28.76 ± 2.88 mg l^{-1}) at 1 and 5 Wm^{-2} on the 15th day respectively, at the same duration of 60 min for UV-B treated cultures compared to control (132.36 ± 4.53 mg l^{-1}) (Figure 6 *a* and *b*). Thus the key enzymes and proteins are reported to be the direct targets of UV-B radiation[36]. As lipid peroxidation is oxidative degradation of lipid molecules, upon UV-B stress, *B. braunii* showed reduced growth rate due to cell damages, and therefore present lipid estimation result showed reduced lipid accumulation. There was a reduction of only 2% (162 ± 4.20 mg l^{-1}) upon 1 Wm^{-2} UV-B treatment for 15 min on the 15th day, but a high reduction of 45% (91.52 ± 3.49 mg l^{-1}) was recorded at 1 Wm^{-2} on the 15th day compared to control (164.57 ± 4.07 mg l^{-1}) (Figure 7 *a*). At 5 Wm^{-2} with minimum and maximum durations of 15 and 60 min UV-B treated cultures having
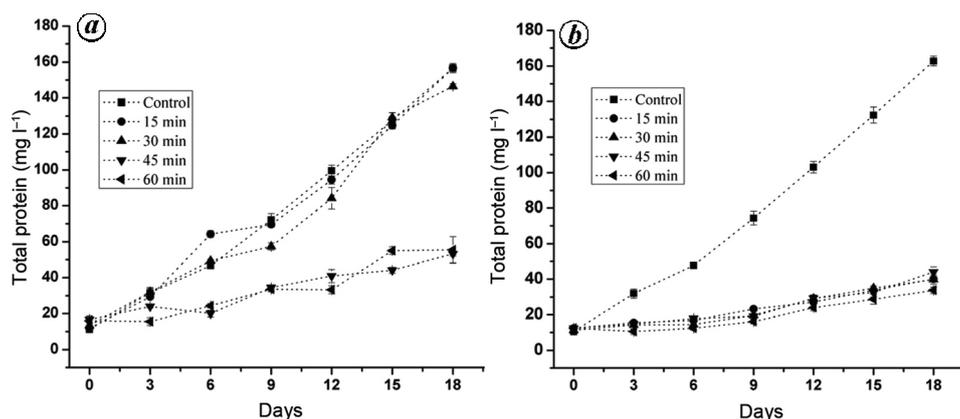
Figure 6. Effect of (*a*) 1 Wm^{-2} and (*b*) 5 Wm^{-2} UV-B on total protein of *B. braunii*.

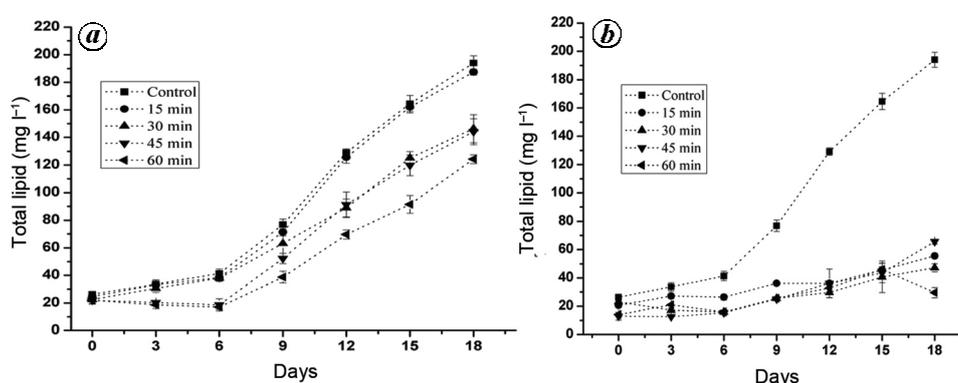**Figure 7.** Effect of (*a*) 1 Wm^{-2} and (*b*) 5 Wm^{-2} UV-B on total lipid of *B. braunii*.

lipid accumulation of 45.71 ± 2.23 and 46 ± 4.49 mg l^{-1} respectively, both showed a high reduction rate of 73% on the 15th day (Figure 7*b*). Thus, UV-B radiation directly affects growth through destruction of proteins and lipids with a reduced supply of ATP due to damages imposed on photosynthetic apparatus[37].

LD$_{50}$ percentage determination

Among the chlorophyll *a* concentrations studied under different treatment conditions, *B. braunii* treated for 15 min at 1 Wm^{-2} retained growth almost equal to control and the alga showed LD$_{50}$ value at 60 min of 1 Wm^{-2} UV-B treatment (Table 3). Treatments at and above 15 min at 5 Wm^{-2} showed a high degree of damage with declined growth (Table 3), which is calculated from the chlorophyll *a* level. The effect of UV-B radiation on algae depends upon intensity and duration of exposure[38]. This is well-depicted in the present study, as UV-B treatment showed detrimental effects upon increasing intensities and durations on *B. braunii*. Thus, increased duration of UV-B treatment has a large impact on various growth factors. A decrement in photosynthetic efficiency and biomolecules accumulation in response to UV-B treatment has been observed with increasing intensities and durations studied[39,40].

Conclusion

It is important to study the effects of UV-B radiation on commercially important and highly resistant species of microalgae. *B. braunii* is well known for its biofuel applications and can also be exploited for pharmaceutical applications. The present study showed the negative effects of UV-B radiation on *B. braunii*. The alga was found to retain its growth to a certain level; thereafter any increase seemed to be highly lethal for its survival. Hence, it is important to save our earth from the harmful effects of UV-B radiation caused by human activities, which greatly affect the low level of the ecosystem.

Conflict of interest: The authors declare that they have no conflict of interest.

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