International Journal of Agriculture and Environmental Research

ISSN: 2455-6939

Volume:02, Issue:05

EFFECT OF ULTRAVIOLET-B RADIATION ON THE GROWTH AND THE PHOTOSYNTHETIC PIGMENTS OF SOIL-ISOLATED BLUE GREEN ALGAE

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ABSTRACT

This study aimed to determine the influence of UVB irradiation on cell growth and pigments present in four cyanobacterial isolates. The changes in growth density and pigment content of cells grown under exposure to UVB radiation were detected. The results revealed that cells of all cyanobacterial strains were inhibited after prolonged duration of UVB exposure. Great reduction in the carotenoid level was found in all cyanobacterial strains during UVB irradiation exposure. Low reduction percentage in the chlorophyll a content was observed after UVB irradiation. No significant decline in phycocyanin level despite 120 min exposure of UVB irradiation. Also, there is a correlation between different UVB irradiation time and cell growth of cyanobacterial strains. These findings suggest the dramatic effects of UVB radiation on cell growth and pigment content of cyanobacterial strains. This study clearly highlights the urgent need for advanced studies on DNA repair in algae during UV exposure.

Keywords: algae; cell growth; cyanobacteria; pigment; UVB irradiation.

INTRODUCTION

Effects of ultraviolet (UV) irradiation on living organisms have become one of the most important environmental issues due to increase of UVB radiation (280-320 nm) at the Earth's surface resulted from the stratospheric ozone layer depletion, which covers and protects the earth's surface from harmful UV radiation. Also, UVB radiation can negatively impact the structure-function of aquatic and terrestrial ecosystems (Figueroa *et al*. 2009). Several studies revealed the harmful effects of UV-B radiation on growth, survival, development, pigmentation, nutrient uptake and various other metabolic processes of cyanobacteria (Häder *et al*. 2007; Babele *et al*.

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ISSN: 2455-6939

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2012). These effects resulted from direct impact on membrane proteins, photosystem II, DNA, enzymes, growth bioregulators or indirect impact from formation of reactive oxygen. Of the several dramatic UVB radiation effects, morphological and physiological changes in cyanobacteria are recognized as the most predominant processes (Xue *et al*. 2005; Singh *et al*. 2010).

Cyanobacteria are primitive group of gram-negative prokaryotes that possess higher-plant-type oxygenic photosynthesis. They are capable of living in a wide variety of habitats ranging from aquatic to terrestrial ecosystems. So, they are suffered from a wide spectrum of global environmental stresses such as heat, cold, drought, salinity, nitrogen starvation, photo-oxidation, osmotic and UV stress (Sinha *et al*. 1998). They also possess a central position in nutrient cycling due to their inherent capacity to fix atmospheric N_2 with the help of the nitrogenase enzyme directly into ammonium (NH_4), a form through which nitrogen enters the food chain (Sinha *et al*. 1996). Moreover, they significantly contribute to fertility as a natural biofertilizer (Sinha *et al*. 1998). On the other hand, different strategies of UV tolerance have been developed by cyanobacteria. UV screening compounds such as mycosporin-like amino acids (MAAs), carotenoides, or scytonemin in the outer sheath have been detected. However, other strategies such as avoidance responses of cyanobacteria by directed gliding motility for escaping diurnal high UV intensities have been investigated (Figueroa *et al*. 2009).

Considering the vital role of cyanobacteria as a biofertilizer in crop production and UV radiation impinging on them, the present study aimed to determine the effects of UVB irradiation on cell growth and photosynthetic pigments of the four investigated nitrogen-fixing cyanobacterial strains isolated from soil.

MATERIALS AND METHODS

For the isolation of cyanobacterial strains, soil samples were collected twice a month, for a period of three months giving a total of 30 samples, from various localities in Al-Kharj region, Saudi Arabia during 2015. The superficial loose soil layers were removed and the underlying soil samples were collected in sterile polythene bags using an opened soil borer (20 cm depth and 2.5 cm diameter) at depths between 10-20 cm, labeled and transported to the laboratory. Samples were then air dried, mixed thoroughly with $CaCO_3$ (10% w/w), incubated at 30°C for 14 days and screened for cyanobacteria (Suneetha and Lakshmi, 2006).

The nitrogen-fixing cyanobacterial strains *Anabaena aequalis*, *Nostoc commune*, *Nostoc carmium* and *Scytonema* sp. were isolated from soil samples. Purification of cyanobacterial strains were achieved in accordance with standard microbiological techniques described by Safferman and Morris (1964) and Sinha *et al*. (1996). Soil sample was shaken to suspend sediment, and then triplicate 10-µl aliquots were removed and diluted into 100 ml of sterile

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ISSN: 2455-6939

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distilled water which was vacuum filtered through a sterile 47-mm-diameter polycarbonate membrane filter (0.4-µm pore diameter; Nuclepore). The filters were aseptically transferred, inoculum side up, onto plates of BG-12 or BG-13 medium containing nystatin and cycloheximide. The plates were incubated for 14 days, after which the cyanobacterial colonies growing on the surface of the membrane filters were counted with the aid of a dissecting microscope (x10 to x50 magnification) as indicated by Ferris and Hirsch (1991). Cultures with an initial dry weight of about 0.15 mg/ml were used in all experiments. Cultures were routinely grown in an autoclaved liquid medium in Erlenmeyer flasks filled to 40% of their nominal volume, placed in a culture room at 20°C and illuminated with UVB radiation (280-320 nm) (Sinha *et al*. 1996). Growth density scored as specified by Ibrahim and Abd El Salam (2015).

Photosynthetic pigments (chlorophyll a, carotenoids and phycocyanin) were determined according to standards methods performed by Buckley and Houghton (1976).

Suspension cultures were growing by Beckman J2-21 M/E Centrifuge at 1500 g for 10 min at room temperature, transferred to sterile Petri dishes (75 mm in diameter) and exposed to artificial UV produced from a UV lamp (Vilber Lourmat, VL-115 M, its wavelength at 312 nm, with filter size (295 x 66 mm) to remove wavelengths less than 280 nm) (Sinha *et al*. 1996).

The data were analysed using the Statistical Package for Social Sciences (SPSS) version 11.0 computer software package (Daniel, 1995). A two-tailed p value ≤ 0.05 was considered to be statistically significant. The paired samples t-test was used to compare the means of photosynthetic pigments before and after exposed to UVB irradiation. Correlation coefficient was performed to detect the relation between the different irradiation time and growth density of cyanobacterial strains. A confidence level of 95% was used in all analyses.

RESULTS

UVB radiation is a highly reactive component of the solar spectrum, and it has the potential to influence biological processes at all levels, from biomolecules to whole ecosystems as presented in Figure 1 (Vincent and Roy 1993).

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ISSN: 2455-6939

Volume:02, Issue:05

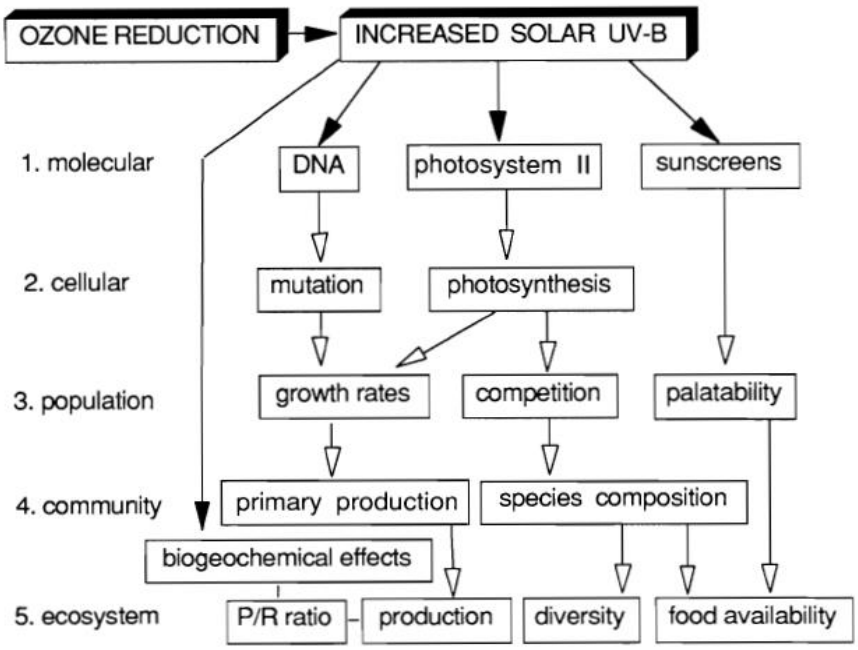

Fig. 1 Effect of UVB, from the molecular level to community effects, on photosynthetic organisms in aquatic and terrestrial environment; source: Vincent and Roy (1993)

The results revealed that growth density of *Anabaena aequalis*, *Nostoc carmium*, *Nostoc commune* and *Scytonema* sp. decreased after exposed to UVB at 25, 30, 45, 60 and 120 min, respectively as shown in Table 1. Also, our finding showed that growth inhibition in all four strains exposed to UVB radiation of 5 W m^{-2} at 120 min (Table 1). Figure 2 showed that growth and survival of all cyanobacterial strains were severely inhibited after prolonged duration of UVB exposure. Also, it is found that there is a correlation between different UVB irradiation time and cell growth of cyanobacterial strains (Figure 2).

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ISSN: 2455-6939

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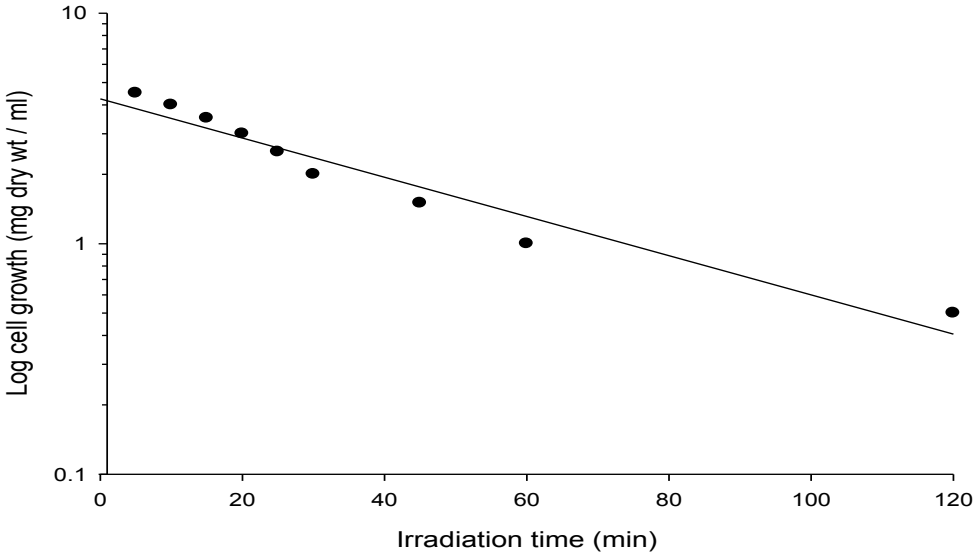

Correlation Coefficient	-0.851*
Sig.(2-tailed)	0.000
* $p \leq 0.05$	

Fig. 2 Cell growth of cyanobacteria exposed to UVB radiation at different time

Table 1 Growth density of four cyanobacterial strains formed after exposed to UVB of 5 W m $^{-2}$ at different irradiation time (min)

Species	Growth density (µl/ml) at different irradiation time (min)									
	Control	5	10	15	20	25	30	45	60	120
Anabaena aequalis	++++	+++	+++	++	+	-	-	-	-	-
Nostoc commune	++++	++++	+++	+++	++	++	+	-	-	-
Nostoc carmium	++++	+++	+++	++	++	+	-	-	-	-
Scytonema sp.	++++	++++	+++	+++	+++	++	++	+	+	-

High growth (++++); Good growth (+++); Weak growth (++); Rare growth (+); No growth (-); scored as mentioned by Ibrahim and Abd El- Salam (2015)

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ISSN: 2455-6939

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Table 2 shows characteristics of photosynthetic pigments in the four studied cyanobacterial strains. It is obvious from this table that the mean concentrations of chlorophyll a in all cyanobacterial isolates ranged from 24 to 59 µg/cm^2 and carotenoid levels have a range of 19-53 µg/cm^2. However, phycocyanin content in the four investigated strains extended from 25 to 89 µg/cm^2.

Table 2 Characteristics of photosynthetic pigments in four cyanobacterial strains

Species	**Photosynthetic pigments** (µg/cm^2)		
	Chlorophyll a	Carotenoids	Phycocyanin
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Anabaena aequalis	32 ± 4.62	37 ± 3.41	25 ± 1.87
Nostoc commune	46 ± 7.84	41 ± 5.20	76 ± 9.34
Nostoc carmium	24 ± 3.75	19 ± 2.86	35 ± 2.71
Scytonema sp.	59 ± 8.41	53 ± 8.64	89 ± 7.29

In the present study, no differences in cell growth of all cyanobacterial strains were found, however, pigmentation among them appeared to be different during UVB exposure and so the pigmentation of strains was compared. Three major groups of photosynthetic pigments (chlorophyll a, carotenoids and phycocyanin) were detected (Figure 3 a ,b, c, d). It is evident from this figure that *Scytonema* sp. had the highest carotenoid content than the other strains, however, no other pigment differences were found.

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ISSN: 2455-6939

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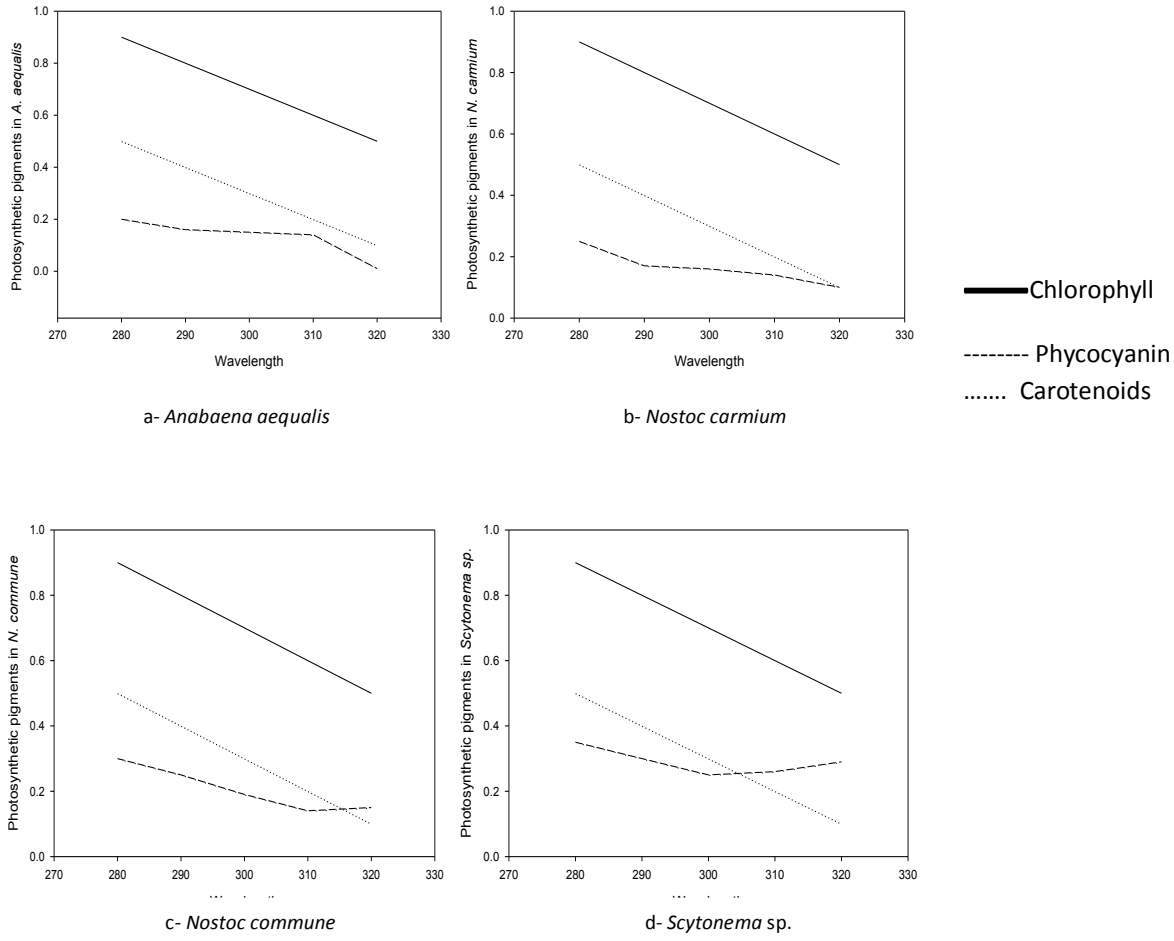

Fig. 3 a, b, c, d. Absorbance of photosynthetic pigments in cyanobacterial strains during UVB exposure

After cyanobacterial strains exposure to UVB radiation, several changes in the overall percentage of photosynthetic pigments content were observed as recorded in Table 3. It is clear from this table that the percentage of carotenoid content reduced from 87.5% to 10.7% with reduction percentage of 87.7%. It is indicated that reduction percentage of carotenoid (87.7%) compared with the pre-UVB irradiated cells was the most apparent. Low reduction percentage recorded in the chlorophyll a content (43.9%) after UVB irradiation where it is reduced from 98.4% to 55.2%. In addition, the lowest reduction percentage was observed in phycocyanin content (10.4%). It is reflected that no significant change exhibited in the phycocyanin level even when exposed to UVB irradiation for 120 min.

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ISSN: 2455-6939

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Table 3 Percentage of photosynthetic pigments content in cyanobacterial strains before and after exposed to UVB irradiation

Exposure to UVB irradiation	Percentage of photosynthetic pigments content		
	Chlorophyll a	Carotenoids	Phycocyanin
Before exposure	98.4	87.5	93.1
After exposure	55.2	10.7	83.4
***p*-value; Sig. (2-tailed)**	.001*	.000*	.071

* $p \leq 0.05$

DISCUSSION

It is evident from the present investigation that the studied cyanobacterial strains are sensitive and severely affected by UVB. Increases in the level of UVB radiation are likely to induce changes in photosynthetic pigments content and cell growth of soil-isolated cyanobacteria. However, there are great differences in susceptibility of different strains to UVB-induced damage. Similar results were obtained by Sinha *et al*. (1995) study, indicating that growth and survival of cyanobacteria are affected by UVB exposure however degree of growth inhibition and survival significantly varied in different strains. Also, they recorded that different organisms showed various changes in terms of growth and survival after UVB exposure. Complete killing of *Anabaena aequalis* and *Nostoc carmium* exhibited after 2 h of UVB exposure whereas in the case of *Nostoc commune* and *Scytonema* sp., longer exposure time is required. Growth patterns of the cells treated with UVB revealed that *Nostoc commune* and *Scytonema* sp. are comparatively more tolerant than *Anabaena aequalis* and *Nostoc carmium* (Sinha *et al*. 1995). In addition, Sinha *et al*. (1996) found that cultures of all cyanobacterial strains which exposed to UVB with 2.5, 5 and 10 W m^{-2} for defined time intervals showed different percentage of survival. UVB exposure at 2.5 W m^{-2} for 30 min had no significant effect on growth whereas 5 W m^{-2} exhibited about 50% killing. However, all strains inhibited after 30 min of exposure to 10 W m^{-2} of UVB radiation. In consistent, Matsunaga *et al*. (1993) revealed that seventy-seven isolates of cyanobacteria were found to be sensitive to UVB radiation during screening where growth inhibition was observed. Also, they reported that exposure of cyanobacteria to UVB irradiation produces a significant reduction in growth and photosynthetic pigments (Matsunaga *et al*. 1993).

Similar pigmentation results were recorded by Quesada *et al*. (1999) who found that chlorophyll a levels ranged from 17 to 45 μg cm^{-2}, and carotenoid concentrations varied over a similar range.

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ISSN: 2455-6939

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Also, the results obtained by Buckley and Houghton (1976) are consistent with our results which recorded changes in the pigmentation during UVB irradiation period, with only the chlorophyll and carotenoid levels monitored. In addition, cell growth in cyanobacteria under UVB exposure found to be reduced the survival rate. This phenomenon indicated that reduction of carotenoid level in the cell can perform an important protective function. The reduction in the carotenoid level could be due to their absorbing near UVB radiation more strongly than other photosynthetic pigments as mentioned by Buckley and Houghton (1976). De Oliveira *et al*. (2014) studied the effect of light intensity on the production of pigments in *Nostoc* sp. and found that the content of light-absorbing pigments, such as chlorophyll a and phycobiliproteins, was higher when the light availability was low. When the light availability increased, the content of these pigments decreased as a strategy for prevention of photo-oxidative damage caused by the production of free radicals. The increase in carotenoids may represent two different phenomena. At low irradiance, carotenoids function as accessory pigments, increasing the light absorption, whereas at high irradiance, carotenoids would act as dissipaters of the excess energy absorbed and as antioxidants (De Oliveira *et al*. 2014). Baalen (1968) examined the effects of UVB irradiation on cyanobacteria in terms of the survival curve and measurement of short time photosynthetic rates. Measurements of photosynthetic rate suggest that there is a correlation between decay of photosynthesis and survival after UVB exposure. The UVB induced decay in photosynthetic activity is reversed by the identical photoreactivation conditions that increase the survival level.

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