The Effect of Ultraviolet Radiation on Chlorophyll in Chlamydomonas Reinhardtii

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Abstract

The study was conducted during 2017 in the laboratories of the department of Botany at Tishreen University, Lattakia, Syria. The effect of ultraviolet radiation (UV-C and UV-B) for different periods (0.5, 1, 3, 5, 15, 15, 30, 45, 60 minutes) on the photosynthetic pigments of Chlamydomonas reinhardtii was studied. The results showed that chlorophyll pigments were more sensitive to UV-C compared to UV-B. These reductions were correlated with the increase in the exposure time, reaching the lowest value after 60 minutes exposure (96.36, 90.94% for chlorophyll a and chlorophyll b when measured after 24 hours respectively).

The usage of polyethylene films protects the chlorophyll against UV radiation. The protection rate was more evident against UV-C reaching 20.9% after 24 hours in the algae exposed for 30 minutes.

Keywords — *Chlamydomonas reinhardtii, UV-B, UV-C, Chlorophyll, polyethylene films.*

Abbreviations — *Chl.: Chlorophyll, UV-B: Ultraviolet Radiation B, UV-C: Ultraviolet Radiation C, OEC: Oxygen Evolving Complex, PS: Photosystem, LHC: Light Harvesting Complexes, ETR: Electron Transport Rate, RubisCO: Ribulose 1,5 diphosphate Carboxylase Oxygenase.*

I. INTRODUCTION

The sun emits a mixture of UV, visible and infra-red radiation. The UV wavelengths in the solar spectrum can be categorized as the UV-C (100 – 280 nm), UV-B (280 – 320 nm) and UV-A (320 – 400 nm). UV-C, and much of the solar UV-B wavelengths, are absorbed in and over the stratospheric ozone layer. Therefore, they do not reach the Earth`s surface ([1], [2], [3]). Although very small proportions of solar UV radiation contribute to the total irradiance of the Earth's surface, this portion of the solar spectrum is highly energetic. Anthropogenic activities since 1979 have contributed to the depletion of the ozone layer and, therefore, the consequent increase in solar UV radiation reaching the Earth's surface has become an important issue ([4], [5], [6]).

Aquatic ecosystems with high transparency of oligotrophic waters (marine and freshwaters) are exposed to the highest levels of ultraviolet radiation. UV irradiation in lakes can affect phytoplankton down to a depth of 10-15 m [7]. The depth of the UV penetration depends, among other factors, on the incidence of solar radiation, transparency of water and wind mixed layer effects [8].

Chlamydomonas reinhardtii is a unicellular green alga that lives in fresh water. It is characterized by two anterior flagella, a cell wall and a single cup-shaped chloroplast ([9], [10]).

There are numerous studies that link harmful UV effects to cell division, growth, development, biomass, photosynthesis, productivity and other physiological processes in algae ([6], [8], [11], [12]). UV also induces destruction of amino acids especially the aromatic ones (such as tyrosine and tryptophane), which leads to denaturation of structural protein and inactivation of enzymes. Beside, Lipid peroxidation can be detected under UV stress ([1], [8], [13]). UV radiation has negative effects on photosynthesis apparatus, pigments, thylakoids integrity, light harvesting complexes (LHC), oxygen evolving complex (OEC), electron transport rate (ETR), ribulose 1,5 diphosphate carboxylase oxygenase (RubisCO) and ATPase ([1], [3], [7], [8], [14], [15], [16], [17], [18], [19]).

In this study, we will investigate the effect of UV-C radiation, UV-B radiation on photosynthetic pigments of *Chlamydomonas reinhardtii*, and the efficiency of polyethylene filter in protecting these pigments against UV radiation.

II. MATERIAL AND METHODS

A. Cell Culture

Chlamydomonas reinhardtii was obtained from Goettingen Algal Culture Collection in Germany and cultured in a growth chamber (23°±1 degrees, 70%±1 humidity, 14 light/10 dark, with continuous aeration). The experiments were conducted during summer 2017 at the laboratories of Botany Department, Faculty of Science, Tishreen University, Syria.

B. UV treatments

The cultures were exposed to artificial UV-B (306 nm) and UV-C (254 nm) for different periods: 0, 0.5, 1, 3, 5, 15, 30, 45, 60 minutes.

C. Protection Using Polyethylene:

UV lamps were covered by blue polyethylene films (0.05 mm thick) to investigate their efficiency in protecting algae against UV radiation.

D. Chlorophyll Content

The pigments were extracted after 0, 6, 24 hours of exposure to UV radiation using methanol. The chlorophyll contents were determined using a Spectrophotometer UV/VIS (Model: SECOMAM), and calculated as follows [20]:

$$C_a = 16.72\ A_{665.2} - 9.16\ A_{652.4}$$

$$C_b = 34.09\ A_{652.4} - 15.28\ A_{665.2}$$

$$C_{a+b} = 1.44\ A_{665.2} - 24.93\ A_{652.4}$$

E. Statistical Analysis:

Data were analyzed using one-way ANOVA.

III. RESULTS AND DISCUSSION

A. The Effect of UV-B Radiation on Chlorophyll a Content:

After exposing the algae to UV-B for 0.5 minutes, chlorophyll a (Chl.a) content increased slightly after 0, 6, 24 hours (1.37, 0.89, 0.89% with protection rates 101.37, 100.89, 100.89% respectively), without any significant differences (Fig. 1).

On the other hand, Chl. a content decreased constantly with significant differences after the exposure to UV-B for different periods (5 to 60 minutes), reaching their lowest level after the exposure to 60 minutes (10.76, 79.62, 91.67% with protection rates 89.24, 20.38, 8.33% after 0, 6, 24 hours respectively). These results are similar to the findings on the cells of *Chlorella* sp. exposed to UV-B radiation ([21], [22], [23]).

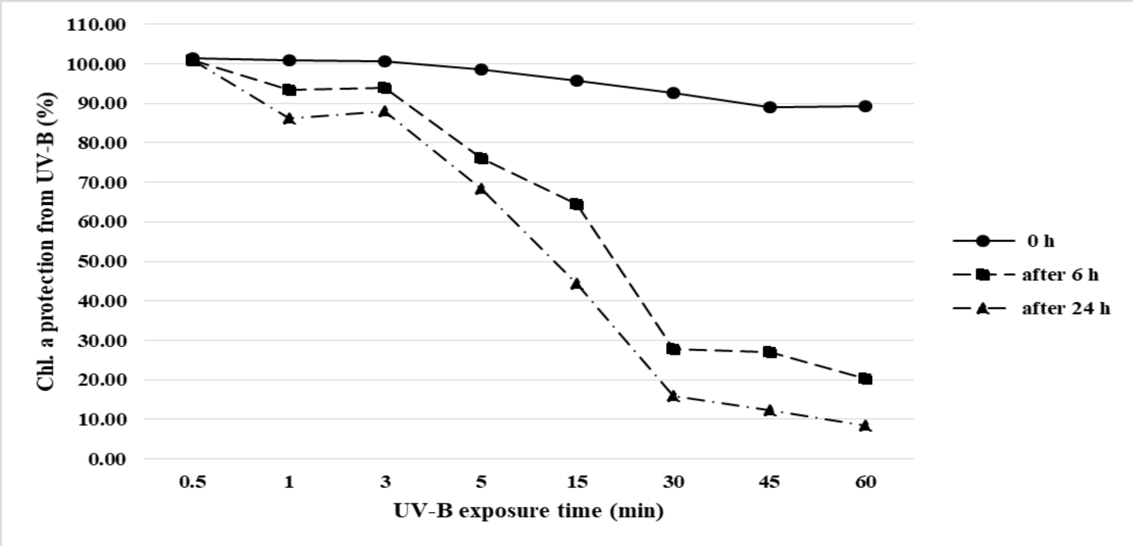

Fig 1: The Protection Rates of Chl. a Against UV-B (%)

B. The Effect of UV-C Radiation on Chlorophyll a Content:

When the samples were exposed to 0.5 minutes of UV-C, the chlorophyll a content increased slightly after 0, 6, 24 hours (5.89, 2.82, 1.2% with protection rates 105.89, 102.82, 101.2% respectively), without much significance (Fig. 2).

On the other hand, when the samples were exposed to UV-C for different periods (3 to 60 minutes), the chlorophyll a content decreased rapidly with significant differences, reaching its severe level at 60 minutes (67.21, 83.87, 96.36% with protection rates 32.79, 16.13, 3.64% after 0, 6, 24 hours respectively). These results are similar to the findings on the cells of *Chlorella* sp. and *Nostoc* sp. exposed to UV-C radiation ([24], [25], [26]).

Chl. a is the principal pigment in photosynthesis, forming the reaction centers of both photosystems (PS). It is considered the main part in the light harvesting complexes (LHC). So, Chl. a decrease causes a direct reduction in the algal productivity.

UV-C has more negative effect on Chl. a content than UV-B, especially when the algae are exposed to UV for 60 minutes and measured directly (67.21% for UV-C and 10.76% for UV-B).

The effects of UV radiation might be due to the inhibition of Protochlorophyllide oxidoreductase responsible for photoreduction of Protochlorophyllide to Chlorophyllide during early stages of chlorophyll biosynthesis. UV radiation cause a degradation of structural proteins that are bound to chlorophyll molecules in the thylakoid membranes ([23], [27], [28]). It is also reported that UV affects the orderly pattern of thylakoid's gana and stroma and causes the breakdown of structural integrity of chloroplasts ([29], [30]).

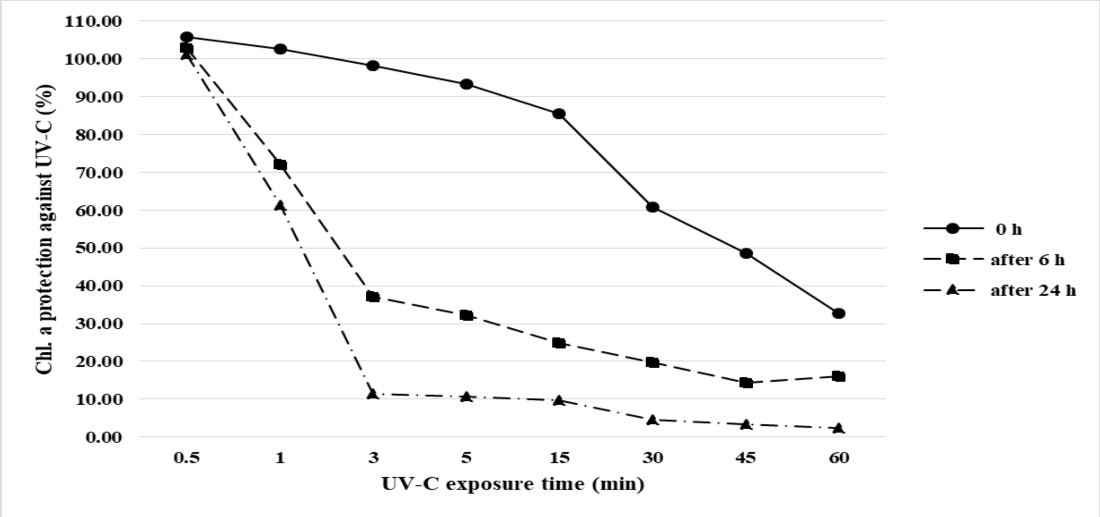

Fig 2: The Protection Rates of Chl. a Against UV-C (%)

B. The Effect of UV-B Radiation on Chlorophyll b Content:

After exposing the algae to UV-B for 0.5 minutes, the chlorophyll b (Chl. b) content increased slightly after 0, 6, 24 hours (0.5, 1.69, 2.95% with protection rates 100.5, 101.69, 102.95% respectively), without any significant differences. When the samples were exposed to UV-B for different periods (1 to 60 minutes), the chlorophyll b content increased significantly directly after the exposure, and the highest value was after exposing the samples for 15 minutes (11.46%, protection rate 111.46%), (Fig. 3).

However, Chl. b content decreased rapidly with significant differences after exposing the samples to UV-B for different periods (1 to 60 minutes), reaching their severe level after their exposure to 60 minutes (49.09, 81.44% with protection rates 50.9, 18.56% after 6, 24 hours respectively). These results are similar to the findings on the cells of *Chlorella* sp. exposed to UV-B radiation ([21], [22], [23]).

C. The Effect of UV-C Radiation on Chlorophyll b Content:

After exposing the algae to UV-C for 0.5 minutes, the Chl.b increased slightly after 0, 6, 24 hours (3.74, 5.51, 1.26% with protection rates 103.73, 105.51, 101.26% respectively), without any significant difference. When the samples were exposed to UV-C for different periods (1 to 45 minutes), the Chl. b content increased significantly directly after the exposure, and the highest value was 18.64% after exposing the samples for 15 minutes with protection rate 118.64% (Fig. 4).

However, Chl. b content decreased rapidly with significant differences after the exposure to UV-C for different periods (1 to 60 minutes). Chl. b content decreased continuously and significantly, reaching its severe level when exposed to 60 minutes (60.15, 90.94% with protection rates 39.85, 9.06% after 6, 24 hours respectively). These results are similar to the findings on the cells of *Chlorella* sp. exposed to UV-C radiation [25].

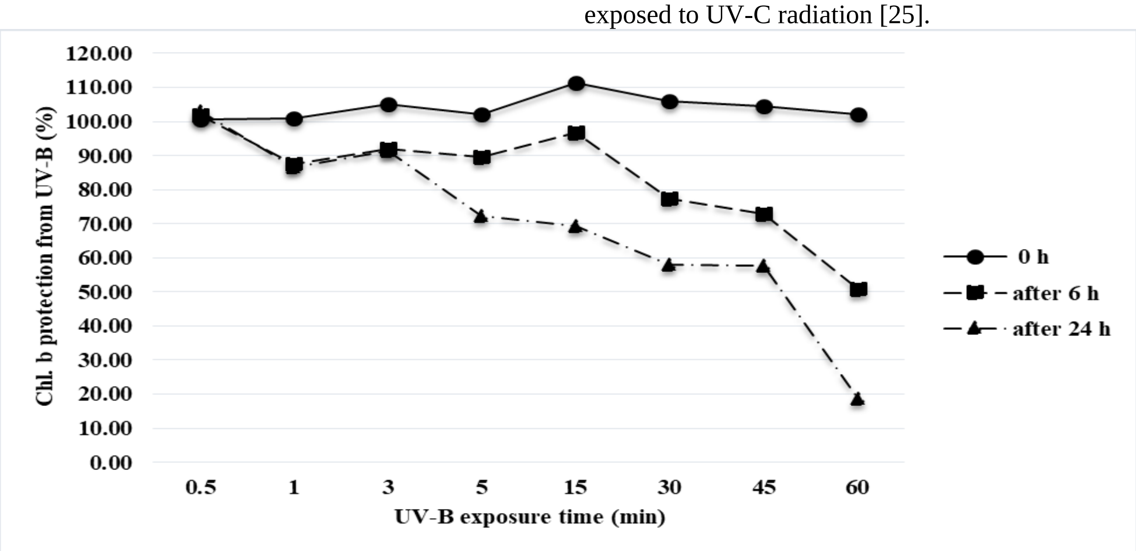

Fig 3: The Protection Rates of Chl. b Against UV-B (%)

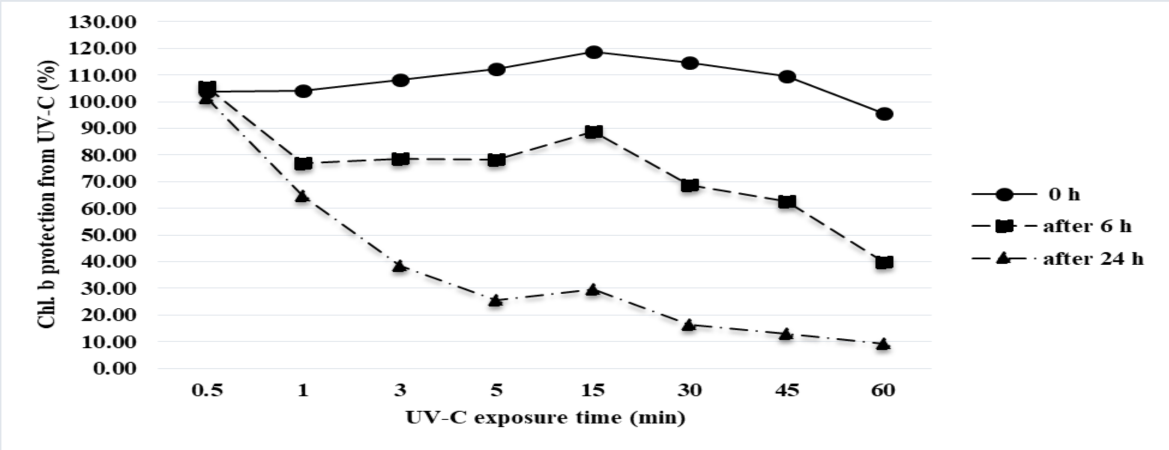

Fig 4: The protection Rates of Chl. b Against UV-C (%)

Chlorophyll b - an accessory pigment in photosynthesis- absorbs the light and transfers it to the reaction centers of photosystems (II, I). It also protects Chl. a from intensive light (including UV radiation), so the increase of its content may help in retaining algal productivity ([31], [32], [33]).

UV-C has more negative effect on Chl. b content than UV-B, especially when the algae are exposed to UV for 60 minutes and measured directly (4.27% reduction for UV-C and 2.22% increase for UV-B).

D. The Role of Polyethylene Filter in Protecting Chlorophyll Against UV Radiation:

After exposing the samples to UV radiation for 30 minutes, the total chlorophyll (Chl.a+b) protection rates from UV-B were 41.15, 23.64% after 6, 24 hours respectively. Chl.a+b protection rates became 53.83, 42.62% in the presence of blue polyethylene films, with protection improvement rates of 12.69, 18.97% after 6, 24 hours respectively (Fig. 5).

In the case of UV-C radiation, the protection rates of chlorophyll were 22.36, 5.84% after 6, 24 hours respectively, and became 38.14, 26.74% in the presence of blue polyethylene films, with protection improvement rates of 15.78, 20.9% after 6, 24 hours respectively.

The previous results indicated that polyethylene films minimize the negative effects of UV radiation. The protection role of polyethylene was more evident in the case of UV-C compared to UV-B.

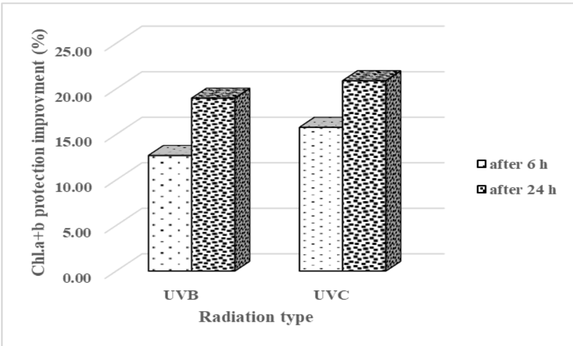

Fig. 5; The Efficiency of Polyethylene Filter in Chlorophyll Protection Against 30 min of UV

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