

Effect of Gamma Radiation on Growth and Metabolic Activities of *Arthrospira platensis*

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ABSTRACT

This work aimed to study the influence of gamma radiation on the growth and production of some active substances of *Arthrospira platensis*. Biomass production was significantly inhibited ($p \leq 0.05$) by 21 and 34%, with respect to the control at 2.0 and 2.5 kGy, respectively. Chlorophyll-a content showed 11% reduction at 2.5 kGy compared to the control. As a result of growth and Chl-a inhibition, chlorophyll productivity recorded a continuous significant decrease below the control in the cells exposed to 1, 1.5, 2 and 2.5 kGy by 8, 12, 15 and 25%, respectively after 15 days of incubation. In addition, phycobillins productivity showed significant decrease by 10 and 36% below the control at 2 and 2.5 kGy of gamma radiation, respectively. Protein production decreased significantly by 24% at 1.5 kGy; low doses of gamma irradiation (0.5, 1.0 and 1.5 kGy) induced carbohydrate production by 106, 246 and 146%, respectively. Lipid content increased significantly over the control at 0.5 kGy of gamma irradiation by 22%, which was decreased at higher doses. Interestingly, carotenoid productivity showed significant increase at all used gamma doses up to 155% over the control.

Key words: *Arthrospira platensis*, *Spirulina platensis*, Gamma radiation, Metabolic activity, Nutritional value.

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INTRODUCTION

Arthrospira, formerly known as *Spirulina*, is characterized by cylindrical, multicellular trichomes in an open left-hand helix. Many reports have mentioned that *Arthrospira* sp. was used as food in Mexico about 400 years ago during the Aztec civilization (Abdulqader et al. 2000). However, it has been produced commercially during last 20 years for food and specialty feeds (Belay et al. 1994; Belay 1997; Kumar et al. 2013; Vo et al. 2015). *A. platensis* contains unusual high amounts of protein, between 26 to 72% of the dry weight, depending on growth conditions (Coca et al. 2015). It is a complete protein containing all essential amino acids, though with reduced amounts of methionine, cysteine and lysine compared to the proteins of meat, eggs and milk. However, it is superior to typical plant proteins, such as that from legumes (Babadzhanov et al. 2004). *A. platensis* is rich in γ -linolenic acid, provides α -linolenic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid and arachidonic acid and contains vitamins B₁ (thiamine), B₂ (riboflavin), B₃ (nicotinamide), B₆ (pyridoxine), B₉ (folic acid), C (L-ascorbic acid) and E (e.g., γ -Tocopherol). It is a rich source of potassium and contains optimum amounts of calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium and zinc (Tokuşoglu and üUnal 2003; Kumar et al. 2013; Benelhadj et al. 2016).

Gamma rays are high energy electromagnetic ionizing radiation emitted in the excitation of the atomic nucleus. Ionizing radiation can be quantified in terms of absorbed dose, which is the amount of ionizing radiation energy deposited per unit mass of irradiated material. The most often unit used to quantify the biological effects of ionizing radiation is the gray (Gy). One gray is equivalent to the absorption of one joule of radiation energy per kilogram of irradiated material. Ionizing radiation, nowadays, is a very important way to create genetic variability that does not exist in nature, or that is not available to the breeder (Ahloowalia and Maluszynski 2001; Lemus et al. 2002).

Many characteristics of *A. platensis* suggest that they should be excellent organisms for the investigation of biological interaction with radiation, particularly ionizing radiation. Hu et

al. (1990) studied the effect of gamma radiation on the growth and morphology of *A. platensis*. They reported that low doses of gamma rays, less than 1 kGy, could stimulate its growth. Small changes in the morphology of the filament were found at doses less than 0.5 kGy. The LD₅₀ was 1.0 kGy, while 2.5 kGy caused 100% lethality. Wang et al. (1998) studied the effect of gamma radiation (up to 6 kGy) on the growth and morphology of four different strains of *Arthrospira* sp. and concluded that it showed resistance to gamma irradiation with stimulation of growth at low doses, while the filaments would break up or even disintegrate at high doses. Although many studies have evaluated the biological response of microalgae to high doses of gamma radiation, few studies have focused on stimulation of bioactive compounds production in *A. platensis*. The aim of the present work was to study the effect of different doses of gamma radiation on the growth and some bioactive compounds, in respect to content and productivity, in *A. platensis*.

MATERIALS AND METHODS

The Organism and Growth Conditions

Arthrospira platensis (SAG 257.80) was obtained from the Phycology Research Lab, Botany Department, Faculty of Science, Tanta University. It was cultivated in 300 mL of modified Zarrouk medium described by Aiba and Ogawa (1977). Cultures were incubated on an orbital shaker with 80 rpm and illuminated by tubular fluorescent lamps (PHILIPS Master TL-D 85 W/840). The light intensity at the surface of the culturing vessels was 70 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 30°C.

Irradiation of *Arthrospira*

Volumes of 250 mL of *A. platensis* culture grown for four days were exposed to five doses of gamma rays (0.5, 1.0, 1.5, 2.0 and 2.5 kGy) using Co⁶⁰ as gamma rays source at the Egyptian Atomic Energy Authority (EAEA), Nasr City, Egypt. After keeping overnight in the dark, a specific volume of the dark-adapted irradiated cells was used for inoculation of 750 mL of modified Zarrouk medium in 1 L Erlenmeyer flasks at an initial OD₇₅₀ of 0.06. Optical density was measured every alternate day, while dry weight and the concentration of different

compounds were estimated at late exponential phase.

Biomass Assay

A. platensis growth was monitored using the optical density of the culture at 750 nm (OD_{750}) and by the determination of cellular dry weight (CDW) according to Bhattacharya and Shivaprakash (2005).

Estimation of Carotenoids

Carotenoids were measured spectrophotometrically using the modified method of Mackinney (1941). Briefly, a known volume of *A. platensis* culture was centrifuged at 4000 g for 10 min. The supernatant was decanted and the same volume of methanol was added to the pellet. The mixture was incubated in a water bath at 55°C for 15 min, and then centrifuged at 4000 g for 10 min. The absorbance of the extract (A) was measured against blank of free methanol at 650, 665 and 452 nm. Carotenoids were estimated as $mg\ mL^{-1}$ of culture suspension using the following equation

$$\text{Carotenoids (mg mL}^{-1}\text{)} = 4.2 A_{452} - [0.0246 (10.3 A_{665} - 0.918 A_{650})]$$

Estimation of Total Soluble Proteins

After carotenoids extraction, residual cells were extracted using 1 N NaOH in a boiling water bath for 2 h as described by Payne and Stewart (1988). Protein concentration as $mg\ mL^{-1}$ was determined according to Bradford (1976) using bovine serum albumin as a standard reference.

Estimation of Total Carbohydrates

Total carbohydrates were quantitatively determined by the phenol sulphuric acid method described by Kochert (1978) using glucose as a standard reference.

Estimation of Total Phycobillins

Fifty milliliter of algal suspension were centrifuged at 4000 g for 10 min. The obtained algal cells were re-suspended in 20 mL of sterile distilled water. The quantitative extraction of phycobiliproteins was achieved by the combination of prolonged freezing and sonication, followed by centrifugation at 4000 g

for 20 min. The crude extract was completed to 50 mL and the concentration of total phycobillins was calculated by measuring the absorbance at 615 and 652 nm according to Bennett and Bogorad (1973).

Estimation of Total Lipids

Extraction of the lipids was done using chloroform: methanol (2:1). The pre-weighed glass vials containing the lipid extracts were dried at 80°C for 30 min, cooled in a desiccator and weighed (Folch et al. 1957).

Productivities Calculation

Productivities of different measured parameters (biomass, Chl-*a*, carotenoids, total soluble proteins, total carbohydrates, total phycobillins and total lipids) were calculated according to the modified method of Abomohra et al. (2013)

$$\text{Biomass productivity (g L}^{-1}\text{ d}^{-1}\text{)} = (\text{CDW}_L - \text{CDW}_0)/t$$

$$\text{Desired product productivity (mg L}^{-1}\text{ d}^{-1}\text{)} = (\text{P}_L - \text{P}_0)/t$$

Where; CDW_0 and CDW_L represent the CDW ($g\ L^{-1}$) at the start of the culture and at late exponential phase, respectively. P_0 and P_L represent the concentration of the desired product ($mg\ L^{-1}$) at the start of the culture and at late exponential phase, respectively, during time (t).

Statistical Analysis

Results are presented as the mean of three replicates \pm standard deviation (SD). The statistical analyses were carried out using SAS (v 6.12). Data obtained were analyzed statistically to determine the degree of significance using one-way analysis of variance (ANOVA) at $p \leq 0.05$. Comparison of treatment means was obtained by Tukey's analysis at $p \leq 0.05$.

RESULTS

Growth curve of un-irradiated *A. platensis* cells grown in modified Zarrouk medium showed that the end of exponential phase was reached after 15 days, which was then directly followed by the death phase. Irradiated cells showed the same behavior with slight growth inhibition, which was more pronounced (28 and 40% lower than

the corresponding control) at 2.0 and 2.5 kGy after 20 days of incubation (Fig. 1). Biomass production showed no significant decrease at low irradiation doses, while high dose of 2.5

kGy resulted in 34% inhibition of biomass productivity (Fig. 2).

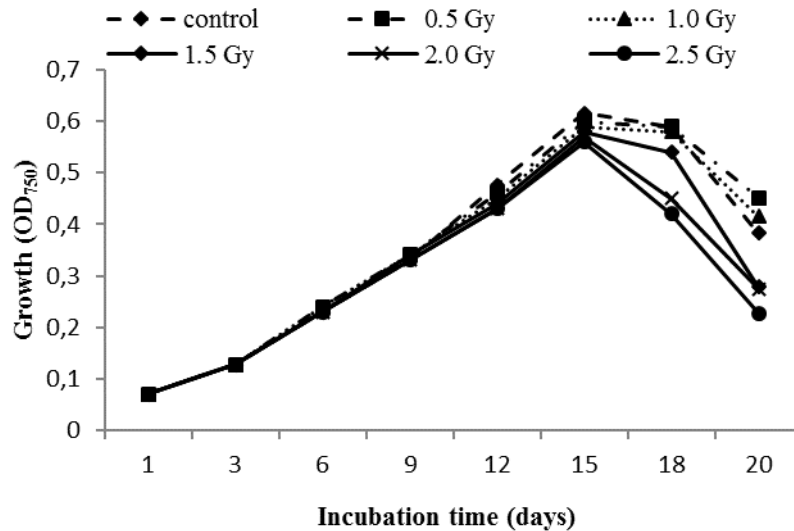


Figure 1- Effect of different doses of gamma radiation on growth of *Arthrospira platensis*.

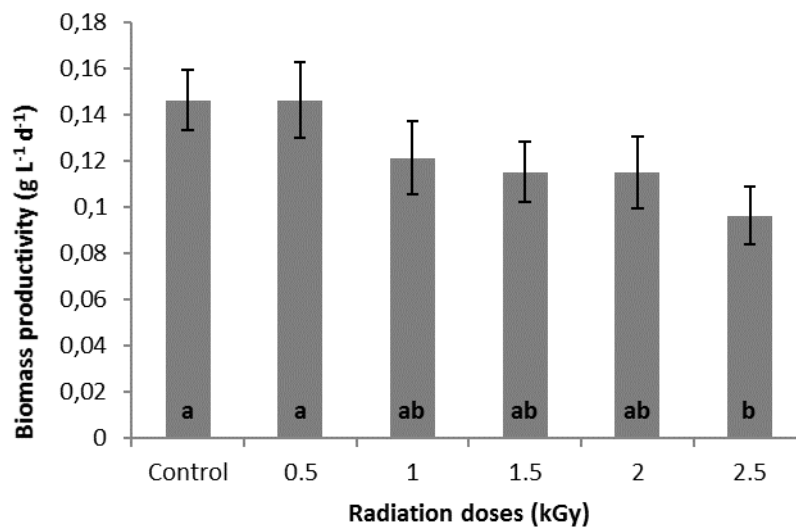


Figure 2- Effect of different doses of gamma radiation on biomass productivity of *Arthrospira platensis* after 15 days of incubation. Error bars represent the SD of three replicates. Columns with the same letter showed insignificant difference (at $p \leq 0.05$).

Figure 3 shows the effect of gamma radiation on pigments content of *A. platensis*. As shown in Figure 3A, gamma irradiation significantly enhanced carotenoids accumulation in the cells at all exposure doses ($p \leq 0.05$). The increases in carotenoid content were 78, 85, 126, 133 and 193% over the control in 0.5, 1.0, 1.5, 2.0 and 2.5 kGy irradiated cells, respectively. However, Chl-*a* and total phycobillins content showed insignificant changes as the irradiation dose increased up to 2 kGy, while a significant reduction by 11 and 23%, respectively was recorded by exposure to 2.5 kGy (Figs. 3B and 3C). Chl-*a* productivity was continuously and significantly decreased ($p \leq 0.05$) in the cells exposed to 1, 1.5, 2 and 2.5 kGy by 8, 12, 15 and 25%, respectively. In contrast, carotenoid productivity showed significant increase by 80, 110, 117 and 155%, respectively; phycobillins showed insignificant decrease (at $p \leq 0.05$) up to 1.5 kGy and significant decrease by 10 and 36%, with respect to control by exposure to 2 and 2.5 kGy, respectively (Table 1).

Exposure of *A. platensis* to 0.5 kGy significantly enhanced its lipid content by 20% over the control; however, higher doses of

gamma radiation led to significant reduction in lipid content (Fig. 4A). Exposure to low doses of gamma radiation resulted in significant decrease in protein content up to 17% over the control at 1.5 kGy. Interestingly, protein content was increased significantly over the control at 2.0 and 2.5 kGy by 19 and 20%, respectively (Fig. 4B). Carbohydrate content showed significant increase over the control up to 248% at 1.0 kGy; however it was decreased by 13 and 21% at 2.0 and 2.5 kGy of gamma irradiation, respectively (Fig. 4C). As a result of increase of lipid content, lipids productivity of *A. platensis* increased significantly by 20% compared to the control at 0.5 kGy of gamma irradiation; however lipid productivity showed significant decrease with respect to the control at the higher doses (Table 2). In addition, protein productivity decreased significantly at low doses of gamma irradiation up to 57 mg L⁻¹ d⁻¹ for the cells exposed to 1.5 kGy and then significantly increased up to 78.8 mg L⁻¹ d⁻¹ at 2.0 kGy. Moreover, gamma irradiation enhanced carbohydrate production with maximum value of 50.8 mg L⁻¹ d⁻¹ at 1 kGy (Table 2).

Table 1- Effect of different doses of gamma radiation on different pigments productivities (mg L⁻¹ d⁻¹) of *A. platensis* after 15 days of incubation.

Doses (kGy)	Carotenoids	Chl- <i>a</i>	Phycobilins
Control	0.36±0.051 ^a	0.55±0.021 ^a	7.6±0.20 ^a
0.5	0.64±0.030 ^b	0.53±0.021 ^{ab}	7.6±0.20 ^a
1.0	0.65±0.031 ^b	0.50±0.020 ^{bc}	7.6±0.20 ^a
1.5	0.76±0.031 ^c	0.48±0.019 ^{cd}	7.3±0.19 ^a
2.0	0.78±0.030 ^c	0.46±0.019 ^d	6.8±0.26 ^b
2.5	0.92±0.031 ^d	0.41±0.018 ^e	4.9±0.17 ^c
F-value	419.3	17.8	93.2
p-value	0.0001	0.0001	0.0001

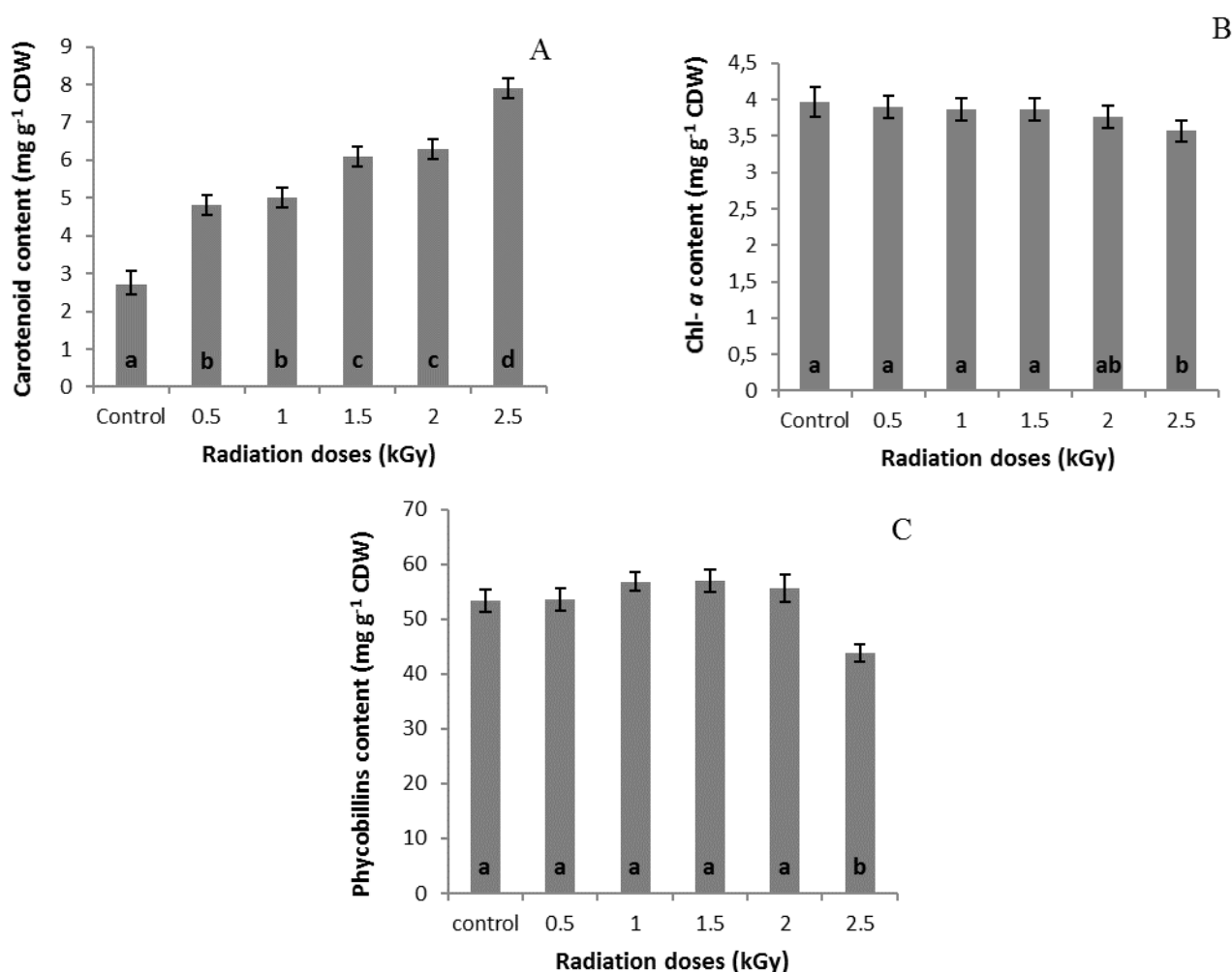
Each value is the mean of three replicates ± SD

Values with the same letter in the same column showed insignificant difference (at $p \leq 0.05$).

Table 2- Effect of different doses of gamma radiation on lipids, proteins and carbohydrates productivities ($\text{mg L}^{-1} \text{d}^{-1}$) of *A. platensis* after 15 days of incubation.

Doses (kGy)	Lipids	Proteins	Carbohydrates
Control	6.8±1.00 ^a	74.9±2.00 ^a	14.7±1.50 ^a
0.5	8.1±0.82 ^c	64.1±2.11 ^b	30.4±1.41 ^b
1.0	3.1±0.72 ^b	60.5±1.81 ^c	50.8±1.11 ^c
1.5	2.8±0.80 ^b	57.0±1.61 ^d	35.9±1.11 ^d
2.0	3.7±0.60 ^b	78.8±1.40 ^e	11.9±1.42 ^e
2.5	3.7±0.62 ^b	73.8±1.52 ^a	8.5±1.11 ^f
F-value	359.4	1644.2	5546.5
p-value	0.0001	0.0001	0.0001

Values are mean of three replicates \pm SD. Values with the same letter in the same column showed insignificant difference (at $p \leq 0.05$).

**Figure 3-** Effect of different doses of gamma radiation on carotenoids (A), Chl-*a* (B) and total phycobillins (C) contents of *A. platensis* after 15 days of incubation. Error bars represent the SD of three replicates. Columns with the same letter showed insignificant difference (at $p \leq 0.05$).

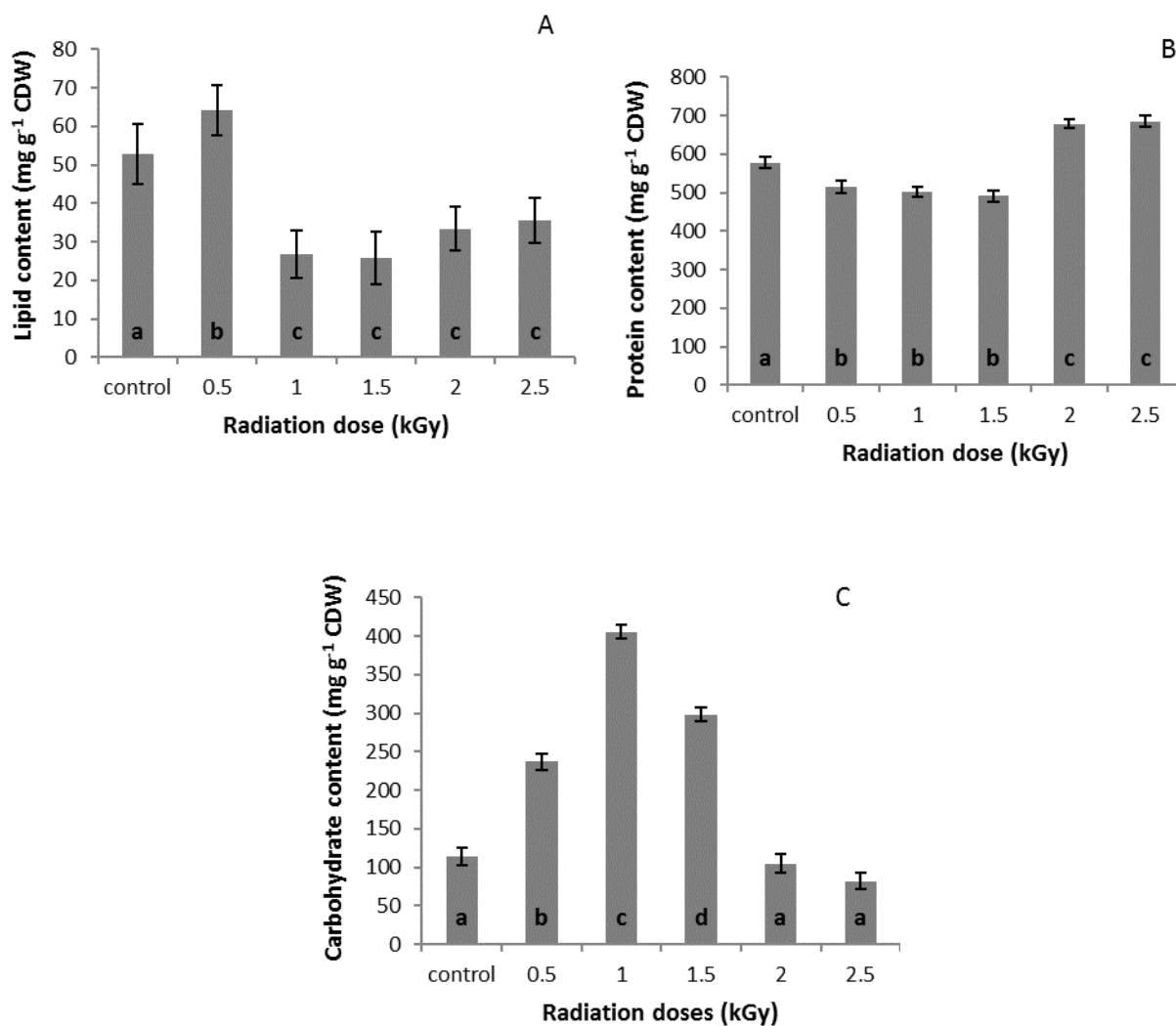
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Figure 4- Effect of different doses of gamma radiation on lipids (A), proteins (B) and carbohydrates (C) contents of *Arthrospira platensis* after 15 days of incubation. Error bars represent the SD of three replicates. Columns with the same letter showed insignificant difference (at $p \leq 0.05$).

DISCUSSION

Arthrospira sp. has gained a great importance as human food and pharmaceutical agent for its high protein, vitamins, carotenoids and essential fatty acids content (Vonshak et al. 1982; Belay et al. 2002; Vo et al. 2015; Benelhadj et al. 2016). Gamma radiation is one of ionizing radiations that react with atoms or molecules within the living cells to generate free radicals. The produced radicals are able to transfigure essential constituent of the cell (Mohajer 2014). In the present study, *A. platensis* showed a resistance to all tested gamma doses, up to 2.5 kGy. However, exposure to gamma radiation inhibited its growth up to 30% at the maximum tested dose (2.5 kGy). Radioresistance of *A. platensis* might be explained by the finding of Shevchenko et al. (1982) who reported that the repair of transforming DNA was performed with the participation of DNA polymerase and polynucleotide ligase, which were functioning in the cell free extract of the cyanobacterium *Anacystis nidulans*. An intermediate level of radioresistance has been reported in the unicellular cyanobacterium *Chroococcidiopsis* sp. isolated from desert and hypersaline environments with D₁₀ dose (dose required for 1 log cycle reduction in survival) of 3–5 kGy (Billi et al. 2000) and in the halophilic archae *Halobacterium* sp. NRC1, which exhibited a D₁₀ dose of 5 kGy (Kottemann et al. 2005). Singh et al. (2010) found that nitrogen-fixing cultures of two *Anabaena* strains tolerated a 5 kGy gamma-ray dose without loss of survival; however, exposure to 6 kGy of gamma rays resulted in genome disintegration but did not reduce viability.

Carotenoids produced from microalgae are non-hazardous colorants which are commonly used as enhancers of antibody production, anticancer and functional supplements (Ng et al. 2011). Hence, carotenoids are of increasing demand and application in various fields (Liu et al. 2016) and, therefore, more and more researches focus on enhancement of carotenoids production in microalgae (Kuo et al. 2012; Reyes et al. 2014; Liu et al. 2016). Although the results showed that gamma radiation had a negative influence on the growth of *A. platensis*, positive effects on the production of some phytochemicals were recorded. Gamma irradiation enhanced the accumulation of carotenoids, which were usually

enhanced under stress conditions to protect chlorophyll from photooxidative damage. Kovács and Keresztes (2002) reported that carotenoids protected chlorophyll from damage when photosynthesis light was saturated by directly accepting electronic excitation energy from triplet chlorophyll. Interestingly, low doses of gamma radiation stimulated carbohydrates and inhibited protein production, whereas high doses of gamma radiation inhibited carbohydrates and stimulated protein production. Farhi et al. (2008) and Choi et al. (2014) concluded that green microalgae exhibited radioresistance for high doses of gamma radiation (up to 6 kGy) with significant changes in metabolites concentrations, such as carbohydrate concentrations which decreased with increasing of gamma irradiation. This might be explained by repair mechanisms that required energy to function by burning of storage compounds for ATP production. Therefore, carbohydrates are used as cellular energy source and consumed more under stresses. The present study established that protein content of *Arthrospira* increased significantly over the control as a result of gamma irradiation. Farhi et al. (2008) reported that the pool of free amino acids increased even at low doses of irradiation. The increase in amino acid concentration was attributed to the increase in protein content, which played an important role in DNA repair mechanism (Reeves et al. 2015; Won et al. 2015; Yu et al. 2016).

The important role of protein synthesis for resistance of gamma rays, UV irradiation and H₂O₂ oxidative stress has been demonstrated by postulating newly synthesized proteins called “heat shock proteins”, which help living cells to defend against the stress (Schorpp et al. 1984; Christman et al. 1985; Abo-Shady et al. 2008). Tammam et al. (2005) studied the differences of protein pattern and number of nucleotides of four mutant strains of *Dunaliella salina* obtained by gamma irradiation and found great variations in their nucleotides, which led to their alteration in the pattern of gene expression and also peptide mapping. Rivasseau et al. (2010) reported the resistance of microalgae grown in the storage pools of a nuclear reactor and investigated the metabolic impact of irradiation using NMR and neutron spectroscopy. They revealed intense protein repair activity, called

autophagy, which resulted in stimulation of protein production. Yoon et al. (2013) found high efficiency of *Spirogyra varians* mutant induced by gamma radiation, which showed higher protein content comparing to the wild type. They reported 18 new expressed proteins that were suggested to be involved in photosynthesis, carbohydrate biosynthesis and energy metabolism. Kojima et al. (2011) studied the role of antioxidants to prevent the oxidative damage of gamma radiation and ATP released by low dose of gamma irradiated cells and the relation between gamma-radiation-induced ATP release and induction of cellular antioxidant thioredoxin-1 (Trx-1) via purinergic signaling. Irradiation with gamma rays, or exogenously adding ATP caused an increase in Trx-1 expression. It was further revealed that ATP generated intracellular reactive oxygen species (ROS), and thereby increasing Trx-1 expression as an adenosine receptor to ROS. They suggested that gamma radiation induced release of extracellular ATP, which induced the production of ROS via purinergic signaling leading to the promotion of intracellular antioxidants production such as pigments and proteins in response to the oxidative stress.

CONCLUSIONS

In conclusion, *A. platensis* showed radioresistance for high doses of ionizing gamma radiation. Gamma irradiation could be used to generate stable varieties of *A. platensis* with high ability to produce certain bioactive compounds. The results confirmed the role of gamma irradiation in stimulation of lipids, proteins, carbohydrates and carotenoids as antioxidant compounds in *A. platensis*.

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Erratum

In the 01 page, that read:

“Mai Abp-Eleneen³”

Read:

“Mai Abo-Eleneen³”

In the 01 page, that read:

“Effect of Gamma Radiation on Growth and Metabolic Activities of *Arthrospira Platensis*”

Read:

“Effect of Gamma Radiation on Growth and Metabolic Activities of *Arthrospira platensis*”