



## Effect of Gamma Irradiation on Growth and Biochemical Aspects of Some Microalgae

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### ABSTRACT

Recent years have witnessed great interest in using  $\gamma$ -irradiation to stimulate biological processes in microalgae to increase their productivity and the naturally effective ingredients. The findings demonstrated that the algae species had high-quality phytochemicals, which may either directly or indirectly support and maintain the health of living organisms. The purpose of this study was to assess the growth and some biochemical aspects of some microalgae, including *Spirulina platensis*, *Chlorella vulgaris*, and *Scenedesmus obliquus*.

These microalgae were irradiated with different doses of gamma irradiation (0.0, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 Gray). The optimum doses of gamma irradiation that significantly increased the maximum growth value and biomass productivity after two weeks in *A. platensis*, *S. obliquus*, and *C. vulgaris* were 700, 300, and 200 Gray (Gy), respectively, in contrast to the control group. The growth value and biomass productivity declined above the optimum dose. The biomass yield and growth rate decreased drastically upon exposure of cultures to a dose of 1000Gy. Gamma irradiation treatments in the three algae significantly increased the biochemical contents of malondialdehyde, free proline, nitric oxide, and total soluble protein, as compared to the untreated algae (control). The obtained data revealed high values in *S. obliquus* at 300Gy, followed by *A. platensis* at 700Gy, and *C. vulgaris* at 200Gy, respectively, as compared to the control. Therefore, treatments of *A. platensis*, *S. obliquus*, and *C. vulgaris* with the optimum dose of  $\gamma$ -irradiation significantly increased the growth rates and biomass yield of the three algae, which is of great importance in increasing income. It also increases the total content of proteins (natural, safe, cheap, available, and easy to obtain) that are important and beneficial to human and animal health for future applications, especially in countries that suffer from malnutrition.

### INTRODUCTION

Recently, there have been an increasing interest in using gamma radiation to stimulate biological processes in microalgae (Ermavitalini *et al.*, 2017; Moiescu *et al.*, 2019; Almarashi *et al.*, 2020; Amr *et al.*, 2023). Gamma irradiation causes oxidative stress by producing excessive amounts of reactive oxygen species (ROS), which react quickly with practically all structural and functional organic molecules, including proteins, lipids, and nucleic acids, disrupting cellular metabolism. ROS include superoxide radicals, hydroxyl radicals, and hydrogen peroxides (Moussa, 2001; Al-Rumaih & Al-Rumaih, 2008; Abo El-Fatah *et al.*, 2016). The generated radicals possess the ability to alter vital

components within the cell (Mohajer, 2014). Free radicals have the potential to cause damage to various substances, such as proteins, DNA, lipids, and others if their concentrations are not managed by antioxidants (Amr *et al.*, 2023). The increasing number of people on the planet has accelerated efforts to find new ecological technology and alternate food sources (Amr *et al.*, 2023). Microalgae are primary producers in the food chain and can be found in a variety of habitats, including marine systems and freshwater (Udayan *et al.*, 2021). Microalgae have been used as sources of important biochemical, which may be rare in plant and animal. The food industry is experiencing a surge in demand for a variety of algal-driven components, including carotenoids, proteins, natural pigments and vitamins, to substitute synthetic pigments and enhance the nutritional value of food for both animals and human (Moussa *et al.*, 2015). Microalgal metabolites have several health advantages, including immune system boosting actions. Especially in light of the Covid-19 pandemic, people are searching for effective immune boosters and compounds that promote health (Udayan *et al.*, 2021). The three primary nutritional components found in microalgae biomass are carbohydrates, proteins, and lipids (Priyadarshani & Rath, 2012).

The aim of this manuscript was to assess the specific growth rate, biomass productivity and some biochemical composition (malondialdehyde, free proline, nitric oxide, and total soluble protein) of *A. platensis*, *S. obliquus*, and *C. vulgaris* under various  $\gamma$ -irradiation doses as compared to the control.

## MATERIALS AND METHODS

### Strains, growth medium, and growth conditions

The algae used in this study, *Arthrospira platensis*, *Scenedesmus obliquus*, and *Chlorella vulgaris*, were obtained from the National Institute of Oceanography and Fisheries, hydrobiology laboratory, Qanater branch, Egypt. *A. platensis* was cultivated using Zarrouk's medium (Zarrouk, 1966; Effat *et al.*, 2017), while the microalgae *S. obliquus* and *C. vulgaris* were cultured in BG-11 media (Amr *et al.*, 2023; Supriya *et al.*, 2023). The chemical composition of BG-11 and Zarrouk's culture media used is illustrated in Table (1). The culture medium was autoclaved at 121°C for 20 minutes before inoculation using an autoclave (STERIF0W-1362), and the required illumination was provided by sunlight. The solution was continually mixed by an aerator at a rate of 0.5L/ min (Heidolph MR Hei-Mix S magnetic stirrer, Germany), the photoperiod was 16/ 8h of day/ night cycle, a temperature of 30± 2°C, and the pH was adjusted at 7.5 for *S. obliquus* and *C. vulgaris*, 8.5 to 9 for *A. platensis*. The harvested biomass was allowed to precipitate before being filtered using 0.45mm pore-size Whatman cellulose filter papers to get concentrated algae paste (Hamid *et al.*, 2016). Every treatment (irradiated and nonirradiated control) was carried out independently at least three times (N = 3).

**Table 1.** Chemical composition of BG-11 and Zarrouk's culture media used

Chemical (g/L)	BG-11 media	Zarrouk's media
NaNO <sub>3</sub>	1.5	2.5
K <sub>2</sub> HPO <sub>4</sub>	3.050	0.500
MgSO <sub>4</sub> .7H <sub>2</sub> O	7.500	0.200
NaCl	-	1.000
CaCl <sub>2</sub> .2H <sub>2</sub> O	3.600	0.040
Citric acid. 1H <sub>2</sub> O	0.600	-
Ammonium ferric citrate	0.600	-
FeSO <sub>4</sub> .7H <sub>2</sub> O	-	0.010
EDTA (disodium salt)	0.100	0.080
NaHCO <sub>3</sub>	-	16.800
Na <sub>2</sub> CO <sub>3</sub>	0.020	-
K <sub>2</sub> SO <sub>4</sub>	-	1.000
Trace metal	1 ml	1 ml
H <sub>3</sub> BO <sub>3</sub>	2.860	2.860
MnCl <sub>2</sub> .4H <sub>2</sub> O	1.810	1.810
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.222	0.222
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.390	0.017
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.079	0.079
Co(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	0.049	-
Distilled water	1.0 L	1.0 L
pH	7.5±0.2	9.0±0.2

### Gamma irradiation of *A. platensis*, *S. obliquus*, and *C. vulgaris*

The  $\gamma$ -irradiation is produced using a Co<sup>60</sup> source at the Egyptian Atomic Energy Authority in Nasr City, Egypt (Moussa *et al.*, 2015; Amr *et al.*, 2023). A 250mL volume of *S. obliquus*, *A. platensis*, and *C. vulgaris* of four-day-old culture grown were subjected to ten doses of  $\gamma$ -irradiation (0.0 (Control), 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000Gy). The exposure rate was 0.84Gy min<sup>-1</sup>. A specific volume of the irradiated cells, dark-adapted, was used to inoculate 750mL of Zarrouk's or BG-11 media into one-liter Erlenmeyer flasks with an initial optical density of 680nm. All treatments were performed in triplicate.

### Estimation of growth rate and biomass productivity

Batch cultures of *A. platensis*, *S. obliquus*, and *C. vulgaris* after  $\gamma$ -irradiation treatments were kept in an incubator for a maximum of 14 days, and the optical density (OD<sub>680</sub>) was used to track the growth of the microalgae at a two-day interval (Held, 2011).

The growth value ( $\mu$ ) was calculated using the following equation (1):

$$\mu = \frac{\ln N_{t_2} - \ln N_{t_1}}{t_2 - t_1} \quad (1)$$

Where,  $N_{t_1}$  and  $N_{t_2}$  are the microalgal densities at the time of  $t_1$  and  $t_2$ , and  $t_1$ ), while  $t_2$  stands for the beginning and end of the logarithmic growth phase, respectively.

The production of biomass in *A. platensis*, *S. obliquus*, and *C. vulgaris* was evaluated in accordance with the protocol of **Zhu and Lee (1997)**, created specifically for marine algae. 10mL samples of the algal cells were obtained both at the time of inoculation (T0) and upon reaching the stationary phase (Tend). A vacuum pump was used to filter the algal suspension through glass fiber filters (Whatman GF/F, 47mm) that had been previously weighed. The salts were eliminated from the filters by twice washing them with 0.5M ammonium formate. Finally, the filters were weighed again after being dried at 90°C to a consistent weight and cooled in a vacuum desiccator. The biomass production was estimated by subtracting the two dry weights and dividing the result by the volume of samples multiplied by the number of culture days. Following the drying of the algal cells at 90°C, the dry weight of the biomass was measured at the times of inoculation (T0) and harvesting (Tend). The biomass production was calculated according to **Zhu and Lee (1997)** and **Eladel *et al.* (2019)** using the following equation (2):

$$P_x = C_x / V_t \quad (2)$$

Where,  $P_x$  is the biomass production in  $\text{g L}^{-1} \text{d}^{-1}$ ;  $V$  the culture volume in L,  $C_x$  the difference of dry weights in g, and  $t$  the duration of the cultivation in days. All measurements were carried out in triplicate.

### Biochemical analysis of *A. platensis*, *S. obliquus*, and *C. vulgaris*

Biochemical composition (malondialdehyde (MDA,  $\mu\text{M g}^{-1}\text{FW}$ ), free proline ( $\mu\text{M g}^{-1}\text{FW}$ ), nitric oxide (NO,  $\mu\text{M g}^{-1}\text{FW}$ ), and total soluble protein ( $\text{mg g}^{-1}\text{DW}$ ) in *A. platensis*, *S. obliquus*, and *C. vulgaris* treated with and without gamma irradiation were carried out when the exponential growth phase came to an end (20 days).

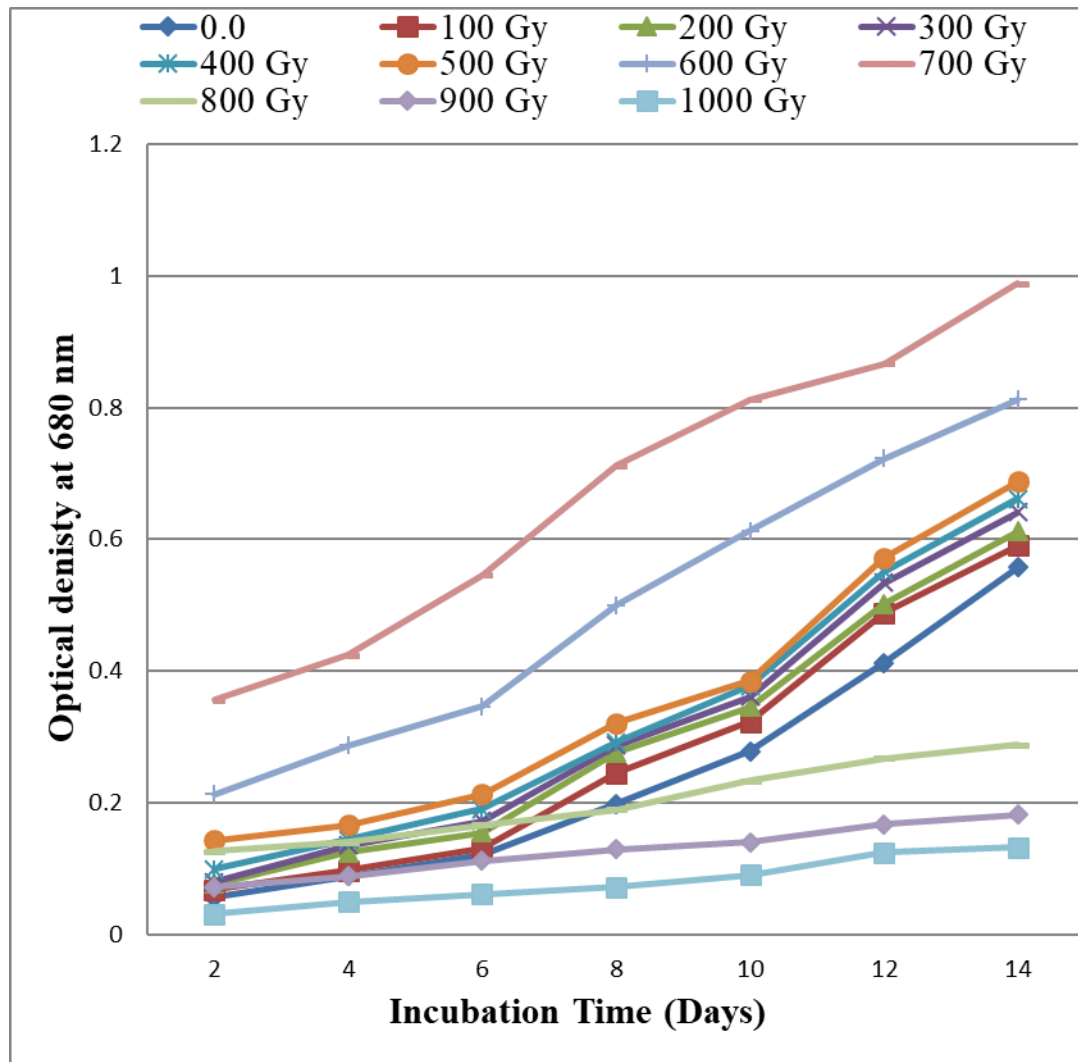
The amount of malondialdehyde (MDA) was employed as a marker to measure the rate of lipoperoxidation (**Haraguchi *et al.*, 1997**). Free proline content was calculated using the technique suggested by **Bates *et al.* (1973)**. Using Griess reagent, the activity of nitric oxide radical scavenging was determined (**Anbarasan *et al.*, 2011**). According to **Bradford (1976)**, the total soluble protein concentrations were determined using the reference standard of bovine serum albumin.

## RESULTS AND DISCUSSION

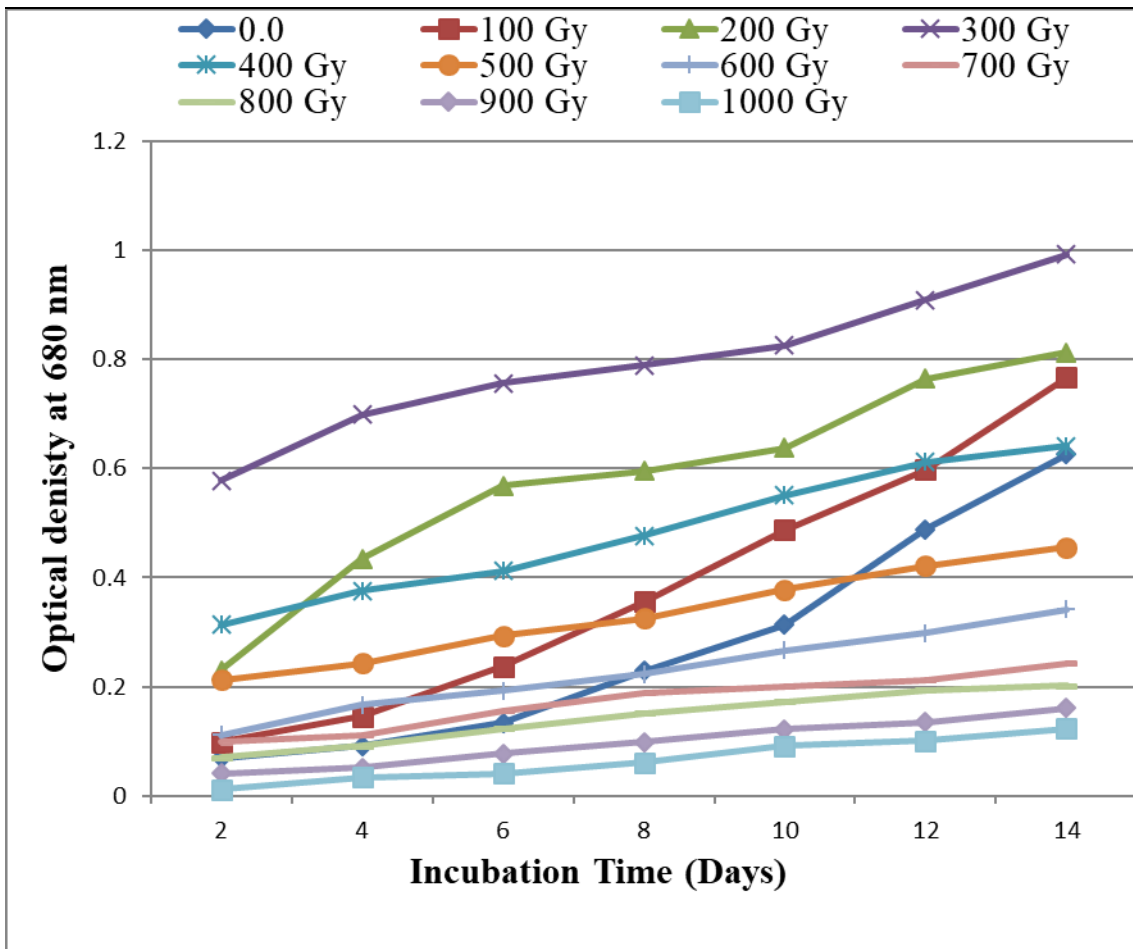
### Growth value of *Arthrospira platensis*, *Scenedesmus obliquus*, and *Chlorella vulgaris* after 14 days of growth

The data for growth values of *A. platensis*, *S. obliquus*, and *C. vulgaris* after 14 days of growth are shown in Figs (1- 3). *Arthrospira platensis*, *Scenedesmus obliquus*, and *Chlorella vulgaris* were irradiated with various doses of  $\gamma$ -irradiation (0.0, 100, 200, 300,400, 500, 600, 700, 800, 900, and 1000Gy). Afterward, the optical density at 680nm was measured every two days for two weeks to monitor the growth. The results showed that growth value increased gradually at most concentrations,

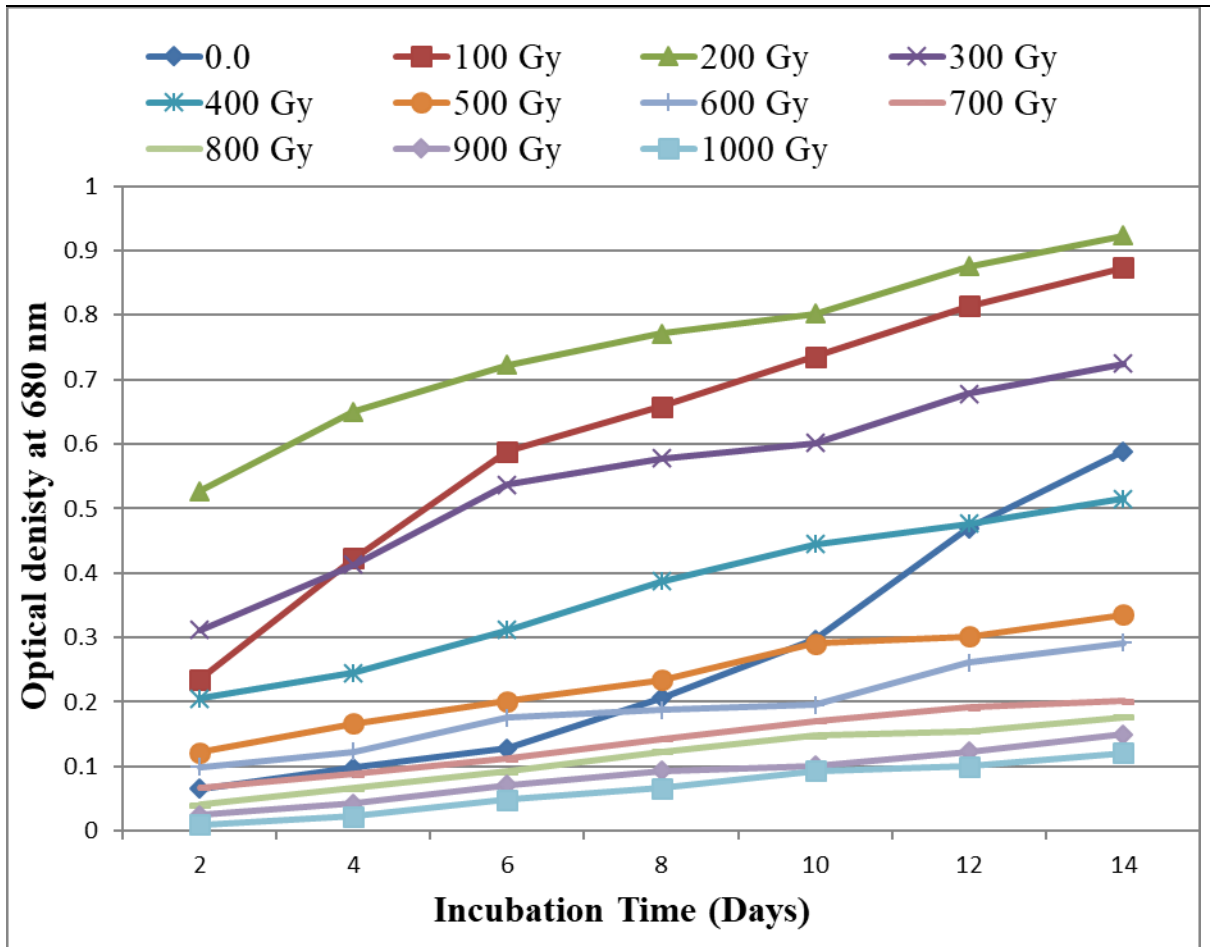
reaching its peak on the 14<sup>th</sup> day of growth. It was noted that the development of the growth value in *A. platensis*, *S. obliquus*, and *C. vulgaris* increased with an increase in time at lower and moderate gamma irradiation doses (Abo El-Fatah *et al.*, 2016). The optimum dose of gamma irradiation that induced the maximum growth value in *A. platensis*, *S. obliquus*, and *C. vulgaris* was 700, 300, and 200Gy, respectively, in contrast to the control group. The growth values of *A. platensis*, *S. obliquus*, and *C. vulgaris* declined above the optimum doses of 700, 300, and 200Gy, respectively. Algal growth parameters for the three algae were demonstrated to be negatively correlated with gamma irradiation doses after the optimum dose. Therefore, no dose beyond the optimum dose was attempted. After 20 days, there was a more noticeable reduction in biomass in irradiated algal cultures. These findings showed that microalgal cell development was considerably inhibited by the high doses of gamma irradiation. The stimulation of growth value by gamma irradiation could be explained by the increase in RNA synthesis (Moussa *et al.*, 2015; Effat *et al.*, 2017). Gamma irradiation increased lipid accumulation and the growth rate of *Chlorella vulgaris* (Mervat *et al.*, 2019). Conversely, *Chlorella pyrenoidosa* exposed to 300 and 500Gy and *C. vulgaris* at 500Gy grew more rapidly than the original strain (control); high doses of gamma irradiation caused damage to or even the death of cells (Cheng *et al.*, 2013). Furthermore, a low gamma-ray exposure (less than 1.0kGy) might promote the growth of *A. platensis* (Tianci *et al.*, 1990). The findings indicated that *C. vulgaris* vitality was significantly decreased by gamma irradiation dosages more than 1kGy (Mohammad *et al.*, 2020). Cheng *et al.* (2016) demonstrated that a mutant of *Chlorella* sp. was capable of withstanding gamma irradiation for up to 900Gy. Additionally, according to Choi *et al.* (2015), gamma irradiation above 1kGy killed and diminished the vitality of *Zygnema* sp., a type of green algae. Similarly, it was proposed by Badri *et al.* (2015) that *Arthrospira* sp. exposed to 800Gy gamma rays will exhibit an early death phase. The growth value of *Chlorella pyrenoido* increased by gamma irradiation at 500Gy by 53.1% (Cheng *et al.*, 2013). However, high doses of  $\gamma$ -irradiation caused the microalgal cells to disintegrate or break down; they lost their ability to repair themselves and were unable to fully recover (Kovacs & Keresztes, 2002; Agarwal *et al.*, 2008). In contrast, algal cells exposed to low doses of gamma irradiation were still slightly damaged, but they quickly recovered to normal levels (Fuma *et al.*, 2009). Conversely, high gamma irradiation doses damage the system of cell metabolism regulation (Agarwal *et al.*, 2008).



**Fig. 1.** Growth curve of *A. platensis* (optical density at 680nm) under different doses of gamma irradiation at a two-day interval for 14 days of growth



**Fig. 2.** Growth curve of *S. obliquus* (optical density at 680nm) under different doses of gamma irradiation at a two-day interval for 14 days of growth

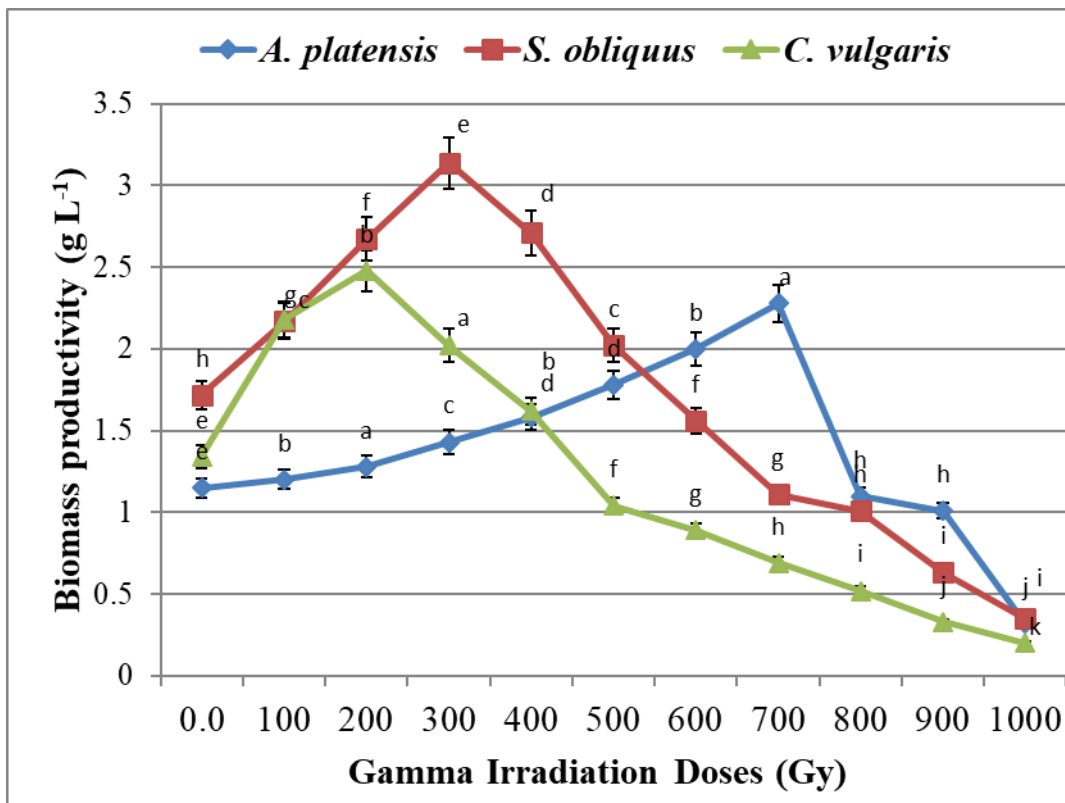


**Fig. 3.** Growth curve of *C. vulgaris* (optical density at 680nm) under different doses of gamma irradiation at a two-day interval for 14 days of growth

### **Biomass productivity of *Arthrospira platensis*, *Scenedesmus obliquus*, and *Chlorella vulgaris* after 14 days of growth**

The data for biomass productivity of *A. platensis*, *S. obliquus*, and *C. vulgaris* after 14 days of growth are shown in Fig. (4). The biomass yield decreased when a dose of 1000Gy of gamma irradiation was applied to the cultures. According to these findings, the proliferation of microalgal cells was considerably hampered by high doses of gamma irradiation (Abo El-Fatah *et al.*, 2016). Gamma irradiation increased the biomass productivity of *Chlorella vulgaris* (Mervat *et al.*, 2019). The increased photosynthetic activity in *A. platensis*, *S. obliquus*, and *C. vulgaris* by gamma radiation treatment might be due to the increased photosynthetic pigment content, potassium level, phosphoenol pyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase/oxygenase activities owing to the increased carbon fixation, which is closely related to a significant increase in microalgal cell growth and, in turn, increased total biomass (Moussa *et al.*, 2015; Abo El-Fatah *et al.*, 2016; Effat *et al.*, 2017).





**Fig. 4.** Effect of different doses of gamma irradiation on biomass productivity of *A. platensis*, *S. obliquus*, and *C. vulgaris* after 14 days of growth

#### Biochemical composition of *Arthrospira platensis*, *Scenedesmus obliquus*, and *Chlorella vulgaris* after 20 days of growth

Treatment of *A. platensis*, *S. obliquus*, and *C. vulgaris* with gamma irradiation significantly increased the malondialdehyde, free proline, nitric oxide, and total soluble protein contents as compared to the control samples, as shown in Table (2). The production of free radicals, especially hydroxyl radicals, which target the side chains of polyunsaturated fatty acids (lipid peroxidation) in the algal cells, is responsible for the elevated MDA level (Effat *et al.*, 2017). Reduced oxidative stress-induced damage to living cells could be achieved by inhibiting or delaying the lipid peroxidation process (Sreedhar *et al.*, 2013; Mohamed *et al.*, 2023a, b). Gamma irradiation treatment significantly increased malondialdehyde content in *A. platensis* (Effat *et al.*, 2017). Since proline acts as a scavenger of free radicals, it is one of the most significant antioxidant amino acids that builds up significantly in a variety of stressed plants. This protects the plants from radical oxidative stress (Smirnoff & Cumbes, 1989). Gamma irradiation is one of the environmental factors that can change the production of osmolytes like proline (Al-Rumaih & Al-Rumaih, 2008). Gamma irradiation treatment significantly increased the free proline content in *A. platensis* (Effat *et al.*, 2017). Proline and carbohydrate concentrations were created following the algae's exposure to certain stressors (Sharma & Dubey, 2005; Mishra & Dubey, 2006; Mishra & Jha, 2009). It was assessed that plants might modify their

metabolism to generate more suitable solutes, including proline, which are essential for stabilising and protecting cellular structures and macromolecules under a variety of stressful circumstances. (Szabados & Savoure, 2010). In addition to being one of the reactive nitrogen species (RNS) and a poisonous chemical, nitric oxide (NO) is a crucial redox-active signalling molecule. Nitric oxide is actually a two-edged sword; when combined with ROS, it can be poisonous or helpful, stimulating defence mechanisms in both plants and mammals. In addition to these functions, NO can function as a signalling molecule and be crucial to an organism's ability to survive. Additional findings demonstrated that NO dramatically raised the protein and proline contents (Lin *et al.*, 2006). Nitric oxide levels in *A. platensis* were considerably raised by gamma irradiation treatment (Abo El-Fatah *et al.*, 2016). Since proteins make up more than 50% of the biomass found in microalgae, biorefineries have the potential to produce products with an additional value from this high protein concentration. Nowadays, food supplements containing microalgae with high protein contents, including spirulina (60% protein on a dry basis) and *Chlorella vulgaris* (51- 58% dry basis), are being sold (Hariskos & Posten, 2014; Trivedi *et al.*, 2015). Additionally, it was shown that protein synthesis decreased as the dosage of radiation increased, suggesting that photosynthetic photoinhibition may become more pronounced (Agarwal *et al.*, 2008). According to the current study's findings, gamma irradiation caused *Arthrospira*'s protein content to rise dramatically over the control group (Abomohra *et al.*, 2016). The increase in soluble protein of *A. platensis* following irradiation could be attributed to the biosynthesis of de novo low molecular weight polypeptides that function as chaperones to shield the remaining folded proteins, or it could be the result of some insoluble proteins being de-folded to a more soluble form by the effect of gamma irradiation stress (Rajaram & Apte, 2008). According to Farhi *et al.* (2008), even at low irradiation doses, the pool of free amino acids increases. According to Reeves *et al.* (2015), Won *et al.* (2015) and Yu *et al.* (2016), the rise in protein content was responsible for the increase in amino acid concentration and was crucial to the DNA repair mechanism. The hypothesis of newly synthesised proteins known as "heat shock proteins," which assist living cells in defending against stress, has demonstrated the critical role of protein synthesis for resistance to gamma rays, UV irradiation, and H<sub>2</sub>O<sub>2</sub> oxidative stress (Schorpp *et al.*, 1984; Christman *et al.*, 1985; Abo-Shady *et al.*, 2008). Additionally, in order to deal with the active peroxides and oxygen radicals created by irradiation, the production of some proteinaceous antioxidants, such as amino acids or antioxidant enzymes, may indirectly lead to an increase in the protein content of the irradiated algal cells (Yang *et al.*, 2011). Similar investigations by Weidang *et al.* (2008) revealed that *Arthrospira*'s protein content might be enhanced by a 1.5kGy gamma-ray dosage. Abomohra *et al.* (2016) observed a decrease by 24% at 1.5kGy, while an equivalent stimulation was seen at 2kGy. Yoon *et al.* (2013) also discovered that gamma-irradiated mutant *Spirogyra varians* had a greater protein content.

**Table 2.** Changes in contents of malondialdehyde (MDA,  $\mu\text{M g}^{-1}\text{FW}$ ), free proline ( $\mu\text{M g}^{-1}\text{FW}$ ), nitric oxide (NO,  $\mu\text{M g}^{-1}\text{FW}$ ), and total soluble protein ( $\text{mg g}^{-1}\text{DW}$ ), in *A. platensis*, *S. obliquus*, and *C. vulgaris* treated with and without gamma irradiation after 20 days of growth

Algae	Dose (Gy)	MDA	Free proline	Nitric oxide	Total soluble protein
<i>A. platensis</i>	0.0	76±3.8 <sup>d</sup>	32.1±1.6 <sup>c</sup>	26.5±1.6 <sup>e</sup>	480±24 <sup>b</sup>
	700	97±5.8 <sup>b</sup>	34.6±2.2 <sup>b</sup>	28.3±1.9 <sup>c</sup>	676±63 <sup>a</sup>
<i>S. obliquus</i>	0.0	82±3.3 <sup>c</sup>	34.2±2.1 <sup>b</sup>	28.2±1.6 <sup>c</sup>	315±27 <sup>d</sup>
	300	111±9.8 <sup>a</sup>	35.8±2.0 <sup>a</sup>	30.7±1.2 <sup>a</sup>	487±29 <sup>c</sup>
<i>C. vulgaris</i>	0.0	58±6.4 <sup>e</sup>	31.8±2.4 <sup>d</sup>	27.2±2.4 <sup>d</sup>	356±19 <sup>f</sup>
	200	78±7.1 <sup>d</sup>	32.0±1.9 <sup>c</sup>	29.3±2.6 <sup>b</sup>	501±17 <sup>e</sup>

Values are represented as mean  $\pm$  SD of samples in triplicate. Means assigned the same superscript letters in each column are not-significant different ( $P > 0.05$ ), whereas others with different superscript letters are significant different ( $P < 0.05$ ).

## CONCLUSION

Utilizing the optimal dose of  $\gamma$ -irradiation on *A. platensis*, *S. obliquus*, and *C. vulgaris* at 700, 300, and 200Gy, respectively, resulted in a notable improvement in their growth rates and biomass yields; this is a crucial factor in augmenting revenue. Moreover, it raises the overall amount of proteins that are cheap, readily available, natural, safe, and easy to obtain, which are crucial and advantageous for the health of people and animals in the future, particularly in countries that suffer from malnutrition.

## ABBREVIATION

**ROS:** Reactive oxygen species

**RNS:** Reactive nitrogen species

**$\gamma$ -rays:** Gamma irradiation

**NO:** Nitric oxide

**Gy:** Gray

## REFERENCES

**Abo El-Fatah, A.; Wagih, E.; Mona, S. and Mai, A.** (2016). Effect of Gamma Radiation on Growth and Metabolic Activities of *Arthrospira platensis*. Brazilian Archives of Biology and Technology, 59: e16150476.

- Abo El-Fatah, A.; Wagih, E.; Mona, S. and Mai, A.** (2016). Effect of gamma radiation on growth and metabolic activities of *Arthrospira platensis*. *Brazilian Archives of Biology and Technology*, 59: e16150476.
- Agarwal, R.; Rane, S.S. and Sainis, J.K.** (2008). Effects of Co-60 gamma radiation on thylakoid membrane functions in *Anacystis nidulans*. *Journal of Photochemistry and Photobiology. B, Biology*, 91(1) :9–19.
- Almarashi, J.Q.M.; El-Zohary, S.E. and Ellabban, A.A.E.** (2020). Enhancement of lipid production and energy recovery from the green microalga *Chlorella vulgaris* by inoculum pretreatment with low-dose cold atmospheric pressure plasma (CAPP). *Energy Conversion and Management*, 112314.
- Al-Rumaih, M.M. and Al-Rumaih, M.M.** (2008). Influence of ionizing radiation on antioxidant enzymes in three species of *Trigonella*. *American Journal of Environmental Sciences*, 4: 151–156.
- Amr, M.H.; Mohamed, A.D.; Moussa, H.R.; Feryal, M.A.Y. and Mohamed, E.A.** (2023). Evaluation of antioxidant characterization in some microalgae exposed to gamma irradiation. *Egyptian Journal of Aquatic Biology & Fisheries*, 27(5): 1241–1252.
- Anbarasan, V.; Kishor, K.V.; Satheesh, K.P. and Venkatachalam, T.** (2011). In vitro evaluation of antioxidant activity of blue green algae *Spirulina platensis*. *International Journal of Pharmaceutical Sciences and Research*, 2(10): 2616–2618.
- Badri, H.; Monsieurs, P.; Coninx, I.; Wattiez, R. and Leys, N.** (2015). Molecular investigation of the radiation resistance of edible cyanobacterium *Arthrospira* sp. PCC 8005. *MicrobiologyOpen*, 4: 187–207.
- Bates. L.; Waldren, R.P. and Teare, I.D.** (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39: 205–207.
- Bradford, M.M.** (1976). A rapid sensitive method for the quantification of microgram quantities of protein utilising the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248–254.
- Cheng, J.; Huang, Y.; Feng, J.; Sun, J.; Zhou, J. and Cen, K.** (2013). Mutate *Chlorella* sp. by nuclear irradiation to fix high concentrations of CO<sub>2</sub>. *Bioresource Technology*, 136C: 496–501.
- Cheng, J.; Lu, H.; Huang, Y.; Li, K.; Huang, R.; Zhou, J. and Cen, K.** (2016). Enhancing growth rate and lipid yield of *Chlorella* with nuclear irradiation under high salt and CO<sub>2</sub> stress. *Bioresource Technology*, 203: 220–227.
- Choi, J.I.; Yoon, M.; Lim, S.; Kim, G.H. and Park, H.** (2015). Effect of gamma irradiation on physiological and proteomic changes of Arctic *Zygnema* sp. (Chlorophyta, Zygnematales). *Phycologia*, 54: 333–341.
- Effat, F.S.; Mahmoud, A.G.; Moussa, H.R.; Enas, A.E. and Mostafa, M.S.I.** (2017). Biochemical composition and antioxidant activities of *Arthrospira* (*Spirulina*) *platensis* in response to gamma irradiation. *Food Chemistry*, 214: 550–555.

- Eladel, H.; Abomohra, A.E.F.; Battah, M.; Mohmmmed, S.; Radwan, A. and Abdelrahim, H.** (2019). Evaluation of *Chlorella sorokiniana* isolated from local municipal wastewater for dual application in nutrient removal and biodiesel production. *Bioprocess and biosystems engineering*, 42(3): 425–433.
- Ermavitalini, D.; Yuliansari, N.; Prasetyo, E. and Saputro, T.** (2017). Effect of Gamma  $^{60}\text{Co}$  Irradiation on the growth, lipid content and fatty acid composition of *Botryococcus* sp. microalgae. *Biosaintifika: Journal of Biology & Biology Education*, 9(1): 58–65.
- Fuma, S., Ishii, N.; Takeda, H.; Miyamoto, K.; Yanagisawa, K.; Doi, K. and Polikarpov, G.G.** (2009). Effects of acute gamma-irradiation on the aquatic microbial microcosm in comparison with chemicals. *Journal of Environmental Radioactivity*, 100(12): 1027–1033.
- Hamid, S.H.A.; Lananan, F.; Khatoon, H.; Jusoh, A. and Endut, A.** (2016). A study of coagulating protein of *Moringa oleifera* in microalgae bio-flocculation. *International Biodeterioration and Biodegradation*, 113: 310–317.
- Haraguchi, H.; Ishikawa, H. and Kubo, I.** (1997). diterpenoids from *Podocarpus nagi*. *Planta Medica*, 63: 213–215.
- Held, P.** (2011). Monitoring of algal growth using their intrinsic properties: Use of a Multi-Mode Monochromator-Based Microplate Reader for Biofuel Research. Inc., Vermont.
- Kovacs, E. and Keresztes, A.** (2002). Effect of gamma and UV-B/C radiation on plant cells. *Micron*, 33(2):199–210.
- Mervat, A.M.A.; Sanaa, M.M.S. and Hamdy, E.A.A.** (2019). Effect of nutrients and gamma radiation on growth and lipid accumulation of *Chlorella vulgaris* for biodiesel production. *Journal of Radiation Research and Applied Sciences*, 12(1): 332–342.
- Mohajer, S.; Taha, R.M.; Lay, M.M.; Esmaeili, A.K. and Khalili, M.** (2014). Stimulatory effects of gamma irradiation on phytochemical properties, mitotic behaviour and nutritional composition of Sainfoin (*Onobrychis viciifolia* Scop.). *The Scientific World Journal*, 854093.
- Mohammad, A.T.; Farah, K.; Sayed, A.H.T. and Daryush, T.** (2020). Two distinct time dependent strategic mechanisms used by *Chlorella vulgaris* in response to gamma radiation. *Journal of Applied Phycology*, 32: 1677–1695.
- Moisescu, C.; Ardelean. A.; Negut. D. and Ardelean, I.** (2019). Effect of acute gamma irradiation on generation time, lipid, chlorophyll *a* and carotenes, in *Chlorella sorokiniana* UTEX 2130 and *Synechocystis* PCC 6803. *Scientific Bulletin. Series F. Biotechnologies*. Faculty of Biotechnology from Bucharest, 23: 122–127.
- Moussa, H.R.** (2001). Physiological and biochemical studies on the herbicide (Dual) by using radiolabeled technique. PhD Thesis. Faculty of Science, Ain shams university.

- Moussa, H.R.; Ismaie, M.M.S.; Shabana, E.F.; Gabr, M.A. and El-Shaer, E.A.** (2015). The Role of Gamma Irradiation on Growth and Some Metabolic Activities of *Spirulina platensis*. *Journal of Nuclear Technology in Applied Science*, 3(2): 99–107.
- Priyadarshani, I. and Rath, B.** (2012). Commercial and industrial applications of micro algae – A review. *Journal of Algal Biomass Utilization. Phycospectrum Utl.*, 3(4): 89–100.
- Supriya, P.; Ishvarya, N.; Ramesh, V.; Raja, S.; Thivaharan, V. and Arivalagan, P.** (2023). A review on the effect of blue green 11 medium and its constituents on microalgal growth and lipid production. *Journal of Environmental Chemical Engineering*, 11(3): 109984.
- Tianci, H.; Shijie, Y. and Yanlin, M.** (1990). The effect of  $\gamma$ -irradiation on *Spirulina platensis*. *Acta Agriculturae Nucleatae Sinica*, 1990–2002.
- Udayan, A.; Pandey, A.K.; Sharma, P.; Sreekumar, N. and Kumar, S.** (2021). Emerging industrial applications of microalgae: challenges and future perspectives. *Systems Microbiology and Biomanufacturing*, 1–21.
- Zarrouk, C.** (1966). Contribution à l'étude d'une cyanophycée influencée de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima* (Setch. et Gardner) Geitler, University of Paris, Paris, France. (PhD Thesis).
- Zhu, C.J. and Lee, Y.K.** (1997). Determination of biomass dry weight of marine microalgae. *Journal of Applied Phycology*, 9: 189–194.