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Stimulating Gamma-Linolenic Acid Productivity by *Arthrospira platensis* (*Spirulina platensis*) Under Different Culture Conditions (Temperatures, Light Regime, and H_2O_2 stress)

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ABSTRACT

Arthrospira platensis considered a storehouse rich in nutritionally active compounds with dietary and pharmaceutical applications. Gamma-Linolenic acid (GLA) or ώ-6, which characterized by several therapeutic properties, can be obtained safely from *A. platensis*. This study aimed to stimulate GLA productivity by *A. platensis*, through using different culturing conditions of temperature and light regime as well as hydrogen peroxide. The obtained results reflected that continuous lighting regime (24L) enhances the production of the unsaturated fatty acid especially GLA than the regime of 12 hours darkness: 12 hours lighting (12D:12L), at the same time low temperature (24ºC) enhance GLA productivity (29.98 mg. g^{-1} dry weight). Although hydrogen peroxide (H_2O_2) did not support biomass production, its low concentrations induced the GLA productivity. The cited results clarified that the maximum GLA (31.62 mg. g^{-1}) was obtained under continuous lighting regime, at 24ºC by adding 2 mM H_2O_2, forming 27.42% from the total fatty acids. Nevertheless, with the increase in population accompanied by increasing their needs, researchers will compete to obtain the maximum productivity of *A. platensis* for GLA with its numerous therapeutic properties.

INTRODUCTION

The valuable filamentous cyanobacterium, *Arthrospira platensis* or *Spirulina platensis* as it was commonly named for a long time; is a concentrated source of many active compounds, consumed by humans as a functional super-food used as a supplement/ingredient to enhance the human body performance or improve a specific function (Elshouny *et al.*, 2017; Mostolizadeh *et al.*, 2020). *Spirulina platensis* can produce beneficial compounds with therapeutic properties, characterized by fast digestion and absorption, so any of its nutritional benefits do not lose (Agustini and Wijayanto, 2020), which led some researchers to believe that *Spirulina* as an exceptional organism. Xuea *et al.*, (2011) reported that the dry weight of *Spirulina platensis* contains protein by about 50-70 %, fiber 8-10 % and lipid 5-7 %. It also contains pigments (3-10 %), includes phycoerythrin and phycocyanin, which have potential properties as anti-aging, antimicrobials, anticancer, antioxidant and anti-inflammatory (Kapoor and Huang, 2006; Mofeed and Mosleh, 2013; Elshouny *et al.*, 2017; Mofeed *et al.*, 2018; Deyab *et al.*, 2019; Mostolizadeh *et al.*, 2020).

Various vitamins: B1, B2, B3, B6, B9, B12, vitamin E, vitamin D, vitamin C (Watanabe *et al.,* 2002), β-carotene, polysaccharides (Babadzhanov *et al.,* 2004) and vital unsaturated and saturated fatty acids. Fatty acids derivates (such as Linolenic, Stearidonic, Linoleic, and Acids) are commonly used as emulsifiers in many pharmaceuticals, foods and cosmetic products (Alvarez and Rodriguez, 2000). Moreover, the unsaturated fatty acids were known as vitamin F due to their significant role as bio-regulators and antioxidants (Babadzhanov *et. al.,* 2004) and assumed to be responsible for many curative properties in the treatment of pre-menstrual tension, arthritis and as an auxiliary in weight loss (Saki, *et al.,* 2015) as well as its role in treating atherosclerosis and hypercholesterolemia (Colla *et al.,* 2008; Cingi *et. al.,* 2008). The essential unsaturated fatty acid; Gamma-Linolenic acid (C18:3 - GLA) which known as ω−6 earned the attention of researchers due to its performance in inhibiting or at least controlling several diseases such as blood pressure, cholesterol level and inflammation (Khan, *et al.,* 2005; Colla *et al.,* 2008; Ali *et al.,* 2017) and play a vital role in the regulation of immune system (Kapoor and Huang, 2006), treating eczema (Kawamura *et al.,* 2011), rheumatoid arthritis (Zurier *et al.,* 1998), premenstrual syndrome (Saki *et al.,* 1995), besides affecting the health of the hair, nails, and skin (Otles and Pire, 2001).

Production of fatty acids, especially the form of GLA from *Spirulina platensis* has recently been a fertile research area, where although GLA could be acquired from other sources such as several genera of fungi like *Mucor* sp. (Kennedy *et al.,* 1999), evening primrose' seed oils, borage, and black currant plants (Alonso and Maroto, 2000), producers and researchers have their reasons to consider *S. platensis* as a promising alternative source. Where, fungi, for example, should grow in sophisticated bioreactors with high production costs to avoid safety problems, besides the resulted product has a disagreeable taste and odor, which reduces the ability to use it as dietary supplements, however, *Spirulina* has the ability to grow in an open raceway or simple bioreactors with an economical cost. Moreover, unlike other sources of Gamma-Linolenic acid, the absence of GLA isomers in *Spirulina* facilitates its extraction and purification (Agustini and Wijayanto, 2020), although crud *Spirulina* powder can be safely used by direct consumption in the form of capsules or food additive without need to spend on separation and purification process. In addition, the ratio of GLA to total fatty acids (GLA/TFA) could be improved by cultivation under specific conditions.

As the fatty acids content considered to be affected by culture conditions, previous studies had carried out based on varying nutritional and physical conditions. Temperature (Colla *et al.,* 2004), light (Oliveira *et al.,* 1999; Ronda and Lele, 2008), pH, and even supplementation by additional nutrients (Ronda and Lele, 2008; Saki *et al.,* 2015) were influencing parameters, enhancing *Spirulina* productivity, however it is better to combine more than one influencing parameter to obtain the maximum efficiency. Recently, permanently there is an interest to get a new combination of the influencing factors producing the maximum proportions of those important active compounds. For this purpose, the overall intention of this study is to use the multilevel factorial design to evaluate the impact of three tested parameters: lighting regime, temperature, and H_2O_2 concentration on the profile of fatty acids for *S.platensis* and spot the best conditions that encourage the productivity of GLA and many other important active compounds from *Spirulina platensis*.

MATERIALS AND METHODS

Algal Strain and Culture Medium:

The used cyanobacteria; Arthrospira *platensis* (*Spirulina platensis*) was isolated

from *Spirulina*'s bloom at Wadi El Natroun, Egypt. The isolated species was morphological and molecular identified. The obtained sequence was added to the GenBank database with accession number *Arthrospira platensis* MH285264 (Mofeed *et al.,* 2018). The used medium for maintaining and cultivation of *A. platensis* in batch culture was Zarrouk's synthetic medium (Zarrouk, 1966) under light intensity 3500 µmol $m^{-2}s^{-1}$ and with initial medium, pH 9.

Experimental Design:

A multilevel factorial design was used to evaluate the impact of the three tested parameters on the productivity of *S.platensis* to GLA. Under different temperature degrees (24, 27, 30, 33 and 36 ^{o}C), the growth of *S. platensis* was studied. At the same time, two lighting regimes were used: either continuous lighting (24h) or 12 hours of darkness: 12 hours of lighting (12D:12L) for ten days. Then likewise, by enriching the medium with different concentrations (0, 2, 4, 6 and 8 mM) of H_2O_2; the influence of Hydrogen peroxide (30%, w/v at stock) was evaluated. All experiments were carried out in triplicate and the flasks were continuously shaken by orbital shaker during the experiment period.

Harvesting:

At the end of the incubation period, the algal culture of *S. platensis* was harvested by filtration through Whatman GF/C glass microfiber filter (0.47µm diameter) and then washed twice with distilled water to remove all the remaining salts from the algal surface and dried in the oven at 60^{o}C until constant weight and stored at -20°C. After that, the fatty acid composition was determined (Ali and Amber, 2010).

Extraction of Lipids for Fatty Acid Identification

The extraction of lipids from biomass samples of *A. platensis* was carried out as explained by Folch and Lees, (1957). The fatty acid methyl esters were prepared to estimate the profile of fatty acids by Gas Chromatography (GC) analysis, using EGSS-X column as described by Metcalfe and Schmitz, (1966).

Statistical Analysis:

All the recorded data were determined from the triplicates and the results are expressed as a mean ± standard deviation (SD).

RESULTS

Spirulina platensis was cultivated at different temperatures (24, 27, 30, 33 and 36^{o}C) and under two lighting regimes. It is obvious from the cited results that the dry weight (DW) of *S.platensis* under the continuous lighting regime (1.06-1.81 $g.L^{-1}$) was more than that obtained (0.83-1.62 $g.L^{-1}$) under the 12D:12L regime (Fig. 1). Concerning data pertaining to the effect of temperature, it is of interest to mention that, the maximum dry weight values of both light regimes were recorded at temperature 33^{o}C.

The fatty acids profile of *Spirulina platensis* (Table 1) gave two saturated fatty acids: Stearic acid (C18:0) and Palmitic acid (C16:0), In addition to four unsaturated fatty acids: Palmitoleic (C16:1), Oleic acid (C18:1), Linoleic acid (C18:2) and Gamma-Linolenic acid (C18:3). The illustrated results (Table 1) elucidate that, generally the 24L lighting regime supported more fatty acid production than the 12D:12L lighting regime. Taking into consideration the influence of temperature**,** a notable encouragement recorded during the entire period of the experiment at the lowest temperature (24˚C) at which, the unsaturated fatty acids obtain their maximum values under the two lighting regimes. Where, Gamma-Linolenic, Linoleic, Oleic, and Palmitoleic acids reached 29.98, 29.01, 9.34 and 4.32 $mg.g^{-1}$, respectively; under 24L lighting regime; and obtained 27.88, 26.24, 7.35 and 3.94 $mg.g^{-1}$ under the 12D:12L lighting regime, respectively. Meanwhile, the production of saturated fatty acids (Palmitic and Stearic acids) was stimulated at the higher temperature under both regimes of lighting (24L and 12D:12L).

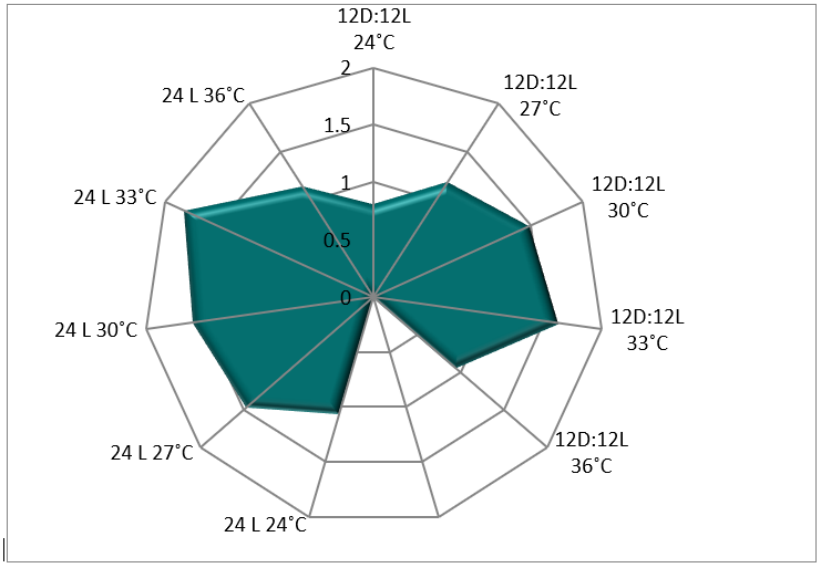

Fig. 1. Dry weight of *Spirulina platensis* (g. L^{-1}) cultivated at different temperature (˚C) under 24 L and 12D:12L lighting regimes.

Table 1. Fatty acid composition (mg. g^{-1}) of *Spirulina platensis* cultivated at different temperature under 24 L and 12D:12L lighting regimes.

Fatty acid (mg. g^{-1}DW) *							
Lighting regime	**Temp. (°C)**	**Palmitic acid (C16:0)**	**Palmitoleic acid (16:1)**	**Stearic acid (C18:0)**	**Oleic acid (C18:1)**	**Linoleic acid (C18:2)**	**Gamma-Linolenic acid (18:3)**
12D:12L	24	31.35 ± 0.91	3.94 ± 0.08	1.24 ± 0.22	7.35 ± 0.36	26.24 ± 0.66	27.88 ± 0.66
	27	31.69 ± 0.76	2.57± 0.24	1.33 ± 0.14	6.97 ± 0.16	25.64 ± 0.85	25.90 ± 0.21
	30	33.87 ± 0.27	2.33± 0.12	1.89 ± 0.07	6.86 ± 0.28	23.98 ± 0.38	24.42 ± 0.52
	33	35.55± 1.21	2.03 ± 0.18	2.11 ± 0.05	6.58 ± 0.32	20.89 ± 0.35	20.03 ± 0.34
	36	34.15 ± 0.73	1.54 ± 0.19	1.03 ± 0.23	5.57 ± 0.33	19.25 ± 1.01	18.61 ± 0.47
24 L	24	35.54 ± 0.81	4.32 ± 0.13	1.16 ± 0.02	9.34 ± 0.34	29.01 ± 0.62	29.98 ± 1.09
	27	35.97 ± 0.94	4.01 ± 0.08	1.06 ± 0.04	8.67 ± 0.51	28.26 ± 0.43	28.41 ± 0.64
	30	37.16 ± 1.04	3.96 ± 0.16	0.98 ± 0.14	8.34 ± 0.72	27.60 ± 0.48	24.38 ± 0.38
	33	37.82 ± 0.98	3.36 ± 0.11	1.35 ± 0.03	8.12 ± 0.16	24.88 ± 0.51	23.56 ± 0.74
	36	36.08 ± 0.92	3.01 ± 0.11	1.01 ± 0.05	7.32 ± 0.25	23.18 ± 0.55	20.31 ± 1.03

* Mean ± standard deviation

Gamma-Linolenic acid (C18:3) ranged from 18.61 to 27.88 mg. g^{-1} under the 12D:12L lighting regime, where the minimum value was recorded at temperature 36°C, while the maximum value was recorded at 24°C (Fig. 2). Consequently, 24°C under the continuous lighting regime is the optimum condition to obtain the maximum GLA (29.98 mg. g^{-1}), which formed 27.42% of the total fatty acids. A glance on table (2) revealed that the ratio of Gamma-Linolenic acid to the total fatty acids (GLA/TFA), unsaturated fatty acids (GLA/UFA), saturated fatty acids (GLA/SFA) and the sum of both Oleic and Linoleic acids (GLA/O+L) gave their maximum values under both 24L lighting regime (27.42, 81.69, 41.27 and 78.17%, respectively) and 12D:12 L lighting regime (28.45, 85.55, 42.62 and 83.00%, respectively) at the lowest temperature (24°C). In this connection, the ratio of unsaturated fatty acids to total fatty acids also obtained its maximum (66.74 and 66.44% under 12D:12L and 24L, respectively) at the same temperature. However, 36°C supported the maximum SFA/UFA ratio.

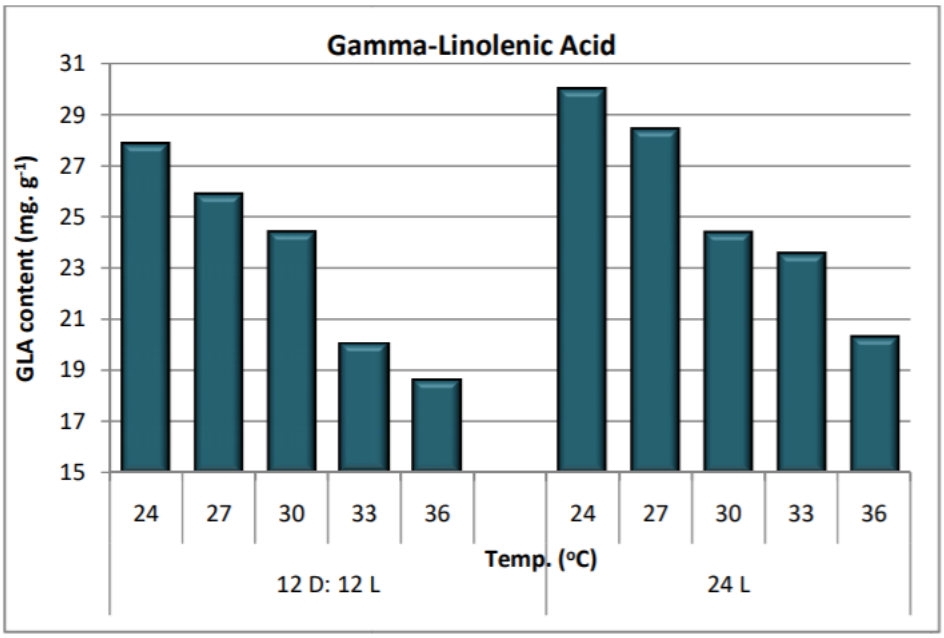

Fig. 2. Effect of both temperature and light regimes (24 L and 12D:12L) on production of GLA by *Spirulina platensis*.

Table 2. Ratio of Gamma-Linolenic acid (GLA) to total fatty acids (TFA), saturated fatty acids (SFA), unsaturated fatty acids (UFA) and Oleic + Linoleic acids (GLA/O+L) in *Spirulina platensis* cultivated at different temperature under 24 L and 12D:12L lighting regimes.

Fatty acid ratio (%)								
Lighting regime	**Temp. (°C)**	**UFA/TFA**	**SFA/TFA**	**SFA/UFA**	**GLA/TFA**	**GLA/SFA**	**GLA/UFA**	**GLA/O+L**
12D:12L	24	66.74	33.26	49.82	28.45	85.55	42.62	83.00
	27	64.91	35.09	54.06	27.52	78.44	42.40	79.42
	30	61.69	38.31	62.09	26.16	68.29	42.40	79.18
	33	56.81	43.19	76.03	22.97	53.19	40.44	72.92
	36	56.11	43.89	78.23	23.22	52.90	41.38	74.98
24 L	24	**66.44**	33.56	50.52	27.42	81.69	41.27	78.17
	27	65.19	34.81	53.40	26.71	76.72	40.97	76.93
	30	62.76	37.24	59.33	23.80	63.92	37.93	67.84
	33	60.47	39.53	65.37	23.78	60.15	39.32	71.39
	36	59.20	**40.80**	68.91	22.34	54.76	37.74	66.59

The impact of different H_2O_2 concentrations (0, 2, 4, 6 and 8 mM) on the productivity of *S.platensis*, was conducted based on the results of the previous experiment, revealing that, all concentrations of H_2O_2 suppressed the biomass of *S. platensis* and hence reduce the dry weight (Fig. 3). The response of fatty acids to the stress by H_2O_2 differs from the unsaturated to saturated fatty acids. Although, all unsaturated fatty acids obtained their maximum values at 2 mM H_2O_2 (Table 3). The saturated fatty acid; Palmitic acid gave its maximum value (37.7 mg. g^{-1}DW) under 24L lighting regime at 4 mM H_2O_2, meanwhile under 12D:12L lighting regime it gave its maximum (34.4 mg. g^{-1}DW) at 6 mM H_2O_2.

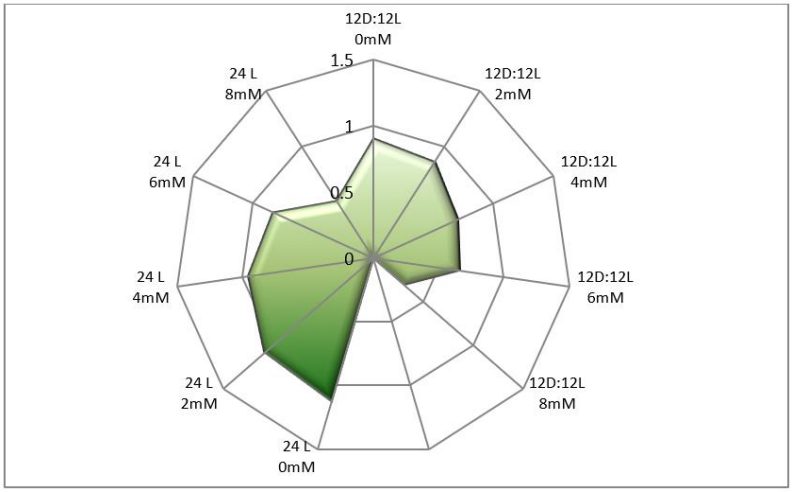

Fig 3. Dry weight of *Spirulina platensis* ($g.L^{-1}$) cultivated in different H_2O_2 concentrations under 24 L and 12D:12L lighting regimes.

Table 3. Fatty acid composition (mg. g^{-1}) of *Spirulina platensis* cultivated in different H_2O_2 concentrations (Mm) under 24 L and 12D:12L lighting regimes.

Fatty acid (mg. g^{-1}DW) *							
Lighting regime	**H_2O_2 Conc.**	**Palmitic acid (C16:0)**	**Palmitoleic acid (16:1)**	**Stearic acid (C18:0)**	**Oleic acid (C18:1)**	**Linoleic acid (C18:2)**	**Gamma Linolenic acid (18:3)**
12D:12L	0	31.35± 0.27	3.94 ± 0.12	1.24 ± 0.14	7.35 ± 0.36	26.24 ± 0.66	27.88 ± 0.66
	2	30.83 ± 1.12	5.03 ± 0.67	1.51 ± 0.14	7.96 ± 0.08	27.68 ± 0.31	29.84 ± 037
	4	32.74 ± 0.81	3.98 ± 0.07	2.44 ± 0.47	7.56 ± 0.24	27.43 ± 0.52	28.78 ± 0.76
	6	34.44 ± 0.58	3.42 ± 0.28	2.88 ± 0.21	6.35 ± 0.15	26.04 ± 0.64	26.46 ± 0.48
	8	31.02 ± 0.61	2.41 ± 0.16	1.07 ± 038	6.12 ± 0.39	25.35 ± 0.93	23.57 ± 0.19
24 L	0	35.54 ± 1.04	4.32 ± 0.16	1.16 ± 0.07	9.34 ± 0.34	29.01 ± 0.62	29.98 ± 1.09
	2	36.17 ± 0.99	5.96 ± 0.57	1.30 ± 0.64	9.89 ± 0.25	29.95 ± 0.56	31.62 ± 0.83
	4	37.7 ± 0.37	4.48 ± 0.14	1.62 ± 0.12	8.87 ± 0.61	29.44 ± 0.14	29.47 ± 1.01
	6	37.26 ± 0.13	3.86 ± 0.21	1.92 ± 0.59	7.43 ± 0.09	28.79 ± 0.25	26.08 ± .67
	8	36.47 ± 0.14	3.77 ± 0.39	0.98 ± 0.31	7.28 ± 0.68	28.47 ± 0.80	25.57 ± 0.24

* Mean ± standard deviation

However Stearic acid (C18:0) obtained its maximum values at 6 mM H_2O_2 under both lighting regimes. Among this, GLA reached its maximum (31.62 mg. g^{-1}DW) at concentration 2 mM H_2O_2 under continuous lighting regime**,** while it gave 29.84 mg. g^{-1}DW under 12D:12L lighting regime at the same H_2O_2 concentration (Fig. 4), which represents 27.52 to 29.01% of the total fatty acid, respectively. The high concentration of H_2O_2 minimized the efficiency of *S. platensis* to produce Gamma-Linolenic acid, where the lowest values of GLA were recorded at 8 mM H_2O_2.

As previously explained, it is worthwhile to discuss some mathematical relationships between Gamma-Linolenic acid and total fatty acids (GLA/TFA), saturated fatty acids (GLA/SFA) and the sum of both Oleic and Linoleic acids (GLA/O+L), besides the unsaturated to the saturated fatty acids (UFA/TFA) which obtain their maximum values under the 24L lighting regime (29.01, 92.27, 83.73 and 68.56%, respectively) and 12D:12L lighting regime (27.52, 84.39, 79.37 and 67.39%, respectively) at 2 mM H_2O_2. The maximum ratio of saturated to total fatty acids (SFA/TFA) and unsaturated fatty acids (SFA/UFA) obtained at 6 mM H_2O_2 (Table 4).

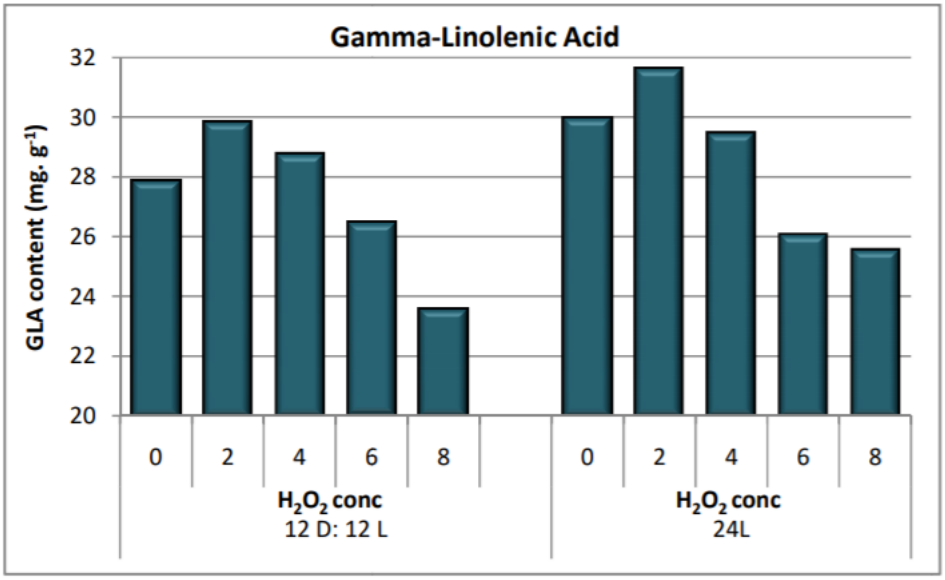

Fig. 4. Effect of both H_2O_2concentration and light regimes (24 L and 12D:12L) on production of GLA by *Spirulina platensis.*

Table 4. Ratio of Gamma-Linolenic acid (GLA) to total fatty acids (TFA), saturated fatty acids (SFA), unsaturated fatty acids (UFA) and Oleic + Linoleic acids (GLA/O+L) in *Spirulina platensis* cultivated in different H_2O_2 concentrations (mM) under 24 L and 12D:12L lighting regimes.

Fatty acid ratio (%)								
Lighting regime	**H_2O_2 Conc.**	**UFA/TFA**	**SFA/TFA**	**SFA/UFA**	**GLA/TFA**	**GLA/SFA**	**GLA/UFA**	**GLA/O+L**
12D:12L	0	66.74	33.26	49.82	28.45	85.55	42.62	83.00
	2	68.56	31.44	45.87	29.01	92.27	42.32	83.73
	4	65.82	34.18	51.93	27.96	**81.81**	42.48	82.25
	6	62.53	37.47	59.93	26.57	70.90	42.49	81.69
	8	64.16	35.84	55.86	26.32	73.45	41.03	74.90
24 L	0	66.44	33.56	50.52	27.42	81.69	41.27	78.17
	2	67.39	32.61	48.40	27.52	84.39	40.84	79.37
	4	64.19	35.81	55.80	26.18	73.09	40.78	76.93
	6	62.81	37.19	59.22	**24.76**	**66.56**	39.42	72.00
	8	63.48	36.52	57.54	24.94	68.28	39.28	71.52

DISCUSSION

Since 1974, The United Nations World Food listed *Spirulina platensis* at the forefront of the best nourishing food sources for the future, where it is rich in proteins, vitamins, fatty acids, carbohydrates, fibers as well as anti-oxidants and several other vital compounds. Generally, the growth and consequently the dry weight of *S.platensis* affected by cultivation conditions, where the 24L lighting regimes support the biomass productivity and it gave its maximum dry weight ($1.81 g.L^{-1}$) at temperature 33ºC. The results agreed with Ronda and Lele, (2008) who described that, for *S. platensis*; light significantly influenced its growth and metabolic activities; where it considered as a catalyst in cell building processes through controlling the photosynthesis (Sharoba, 2017).

The fatty acids profile of *S. platensis* in the obtained results varied obviously with the tested parameters. Whereas the continuous lighting regime supported more fatty acid production than the 12D:12L

lighting regime during the entire period of experiments. As reported by Ronda *et al.*, (2012), light has the efficiency to enhance both the productivity of unsaturated fatty acids and algal biomass; and added that the light can mutate the composition of the unsaturated fatty acids by motivating the desaturase enzyme in photosynthetic organisms, who, in turn, convert the saturated to unsaturated fatty acids.

However, Colla *et al.,* (2008) confirmed that the composition of *S. platensis*'s fatty acids was also temperature-dependent. The present study manifested the extent to which the unsaturated fatty acids composition improved by the lowest temperature (24ºC), giving their maximum values under both the two lighting regimes. Where, Gamma-Linolenic, Linoleic, Oleic, and Palmitoleic acids reached 29.98, 29.01, 9.34 and 4.32 mg. g^{-1}, respectively, under the continuous lighting regime, and obtained 27.88, 26.24, 7.35 and 3.94 mg. g^{-1}, respectively under the 12D:12L regime.

As discussed earlier, Tomaselli *et al.,* (1988) indicated that the decrease in temperature was associated with a deficiency in the C16:0 content. Similarly, Romano *et al.,* (2000) recorded an increase in the content of both palmitoleic (C16:1) and Linoleic (C18:2) acids at low temperatures (26 ºC). Also, Oliveira *et al.*, (1999) concluded an inverse relationship between temperature and Gamma-Linolenic acid. The hypothesis which agreed with the present results, where low content of the saturated fatty acids (Stearic and Palmitic acids) were recorded at high temperatures; while the maximum obtained at 36ºC.

It was obvious that the temperature takes the maestro position in controlling the composition of fatty acids in *Spirulina* cell, which responded to the decrease in temperature by de-saturating the fatty acids of the membrane, as a precautionary way to treat the decrease in the fluidity of cell membrane in order to maintain the optimal function of the membranes, where the physiological characteristics of the membrane depend on the un-saturation level of the fatty acids (Junga *et al.,* 2019), hence low temperature increases the proportion of unsaturated fatty acids. Colla *et al.,* (2004) reported a different explanation for this change in the level of saturation: at lower temperatures, more dissolved oxygen is available in the culture medium for the oxygen-dependent desaturase enzymes that accelerate the conversion from saturated to unsaturated fatty acids. In another study, Meesapyodsuk *et al.,* (2001) reported that the synthesis of unsaturated fatty acids is not conclusively understood despite numerous studies that have been undertaken to identify the mechanism of de-saturation.

Remarkable attention has been given to Gamma-Linolenic acid (C18:3) which recording high values, ranged from 29.98 to 18.61 mg. g^{-1}, obtaining its maximum at 24ºC under the continuous lighting regime, which formed 27.42 % of the total fatty acids. Ronda and Lee, (2008) demonstrated that any decrease in temperature will cause an increase in GLA productivity, where *Spirulina* gave the maximum GLA (23.2 mg. g^{-1} DW) at 25ºC, against 20.8 mg. g^{-1} at 33ºC. Sharoba, (2017) demonstrated that the GLA yield depends on light and dark cycles, time of harvesting, temperature, culture conditions.

The obtained results highlighted a positive relationship between GLA and the unsaturated Linoleic (C18:2), Palmitoleic (16:1) and Oleic acid (C18:1) as opposed to the inverse relationship with Palmitic (C16:0) and Stearic acid (C18:0). The GLA/TFA ratio in the present study showed a narrow range of variation during the experiment period. Colla *et al.,* (2008) explained that at all light intensities, the alteration in GLA/TFA ratio was almost constant despite any variation in GLA content, which can be attributed to; the cell concern to accumulate more TFA than that present during the normal condition to keep ratio GLA/TFA balanced, in order to maintain the balance in the membrane fluid. It is of interest to mention that, the ratio of Gamma-Linolenic acid to unsaturated fatty

acids (GLA/UFA), saturated fatty acids (GLA/SFA) and the sum of both Oleic and Linoleic acids (GLA/O+L) decrease with increasing culture temperature and H_2O_2 concentration. Although the treatment of *Spirulina* with H_2O_2 did not enhance the biomass, nevertheless growth with low concentrations promote the GLA content (31.62 mg. g^{-1}). This may be attributed to the fact that H_2O_2 represents a disturbing factor that induces the cell to produce defensive anti-oxidative and regulatory systems including GLA. But high concentrations of H_2O_2 reduce the ability of the cell to resist, which consequently leads to a decrease in the ratios of GLA/UFA and GLA/SFA.

CONCLUSION

Based on the obtained result, *Spirulina platensis* considered as rich resources of Gamma-Linolenic acid, which has several dietary and pharmaceutical applications. From that perspective, many researchers target to increase the efficiency of *Spirulina* to produce GLA. This study clarified that the fatty acid profile of *Spirulina* changed with changing the culture conditions. According to the cited results, the maximum GLA (31.62 mg. g^{-1}) were obtained under continuous lighting regime, at 24^{o}C by adding 2 mM H_2O_2, which formed 27.42% from the total fatty acids. However, more studies with other combinations of the influencing parameters should be conducted for improving the productivity of GLA and many other important active compounds from *S.platensis*.

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