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Impact of Culture Media Composition, Nutrients Stress and Gamma Radiation on Biomass and Lipid of the Green Microalga, *Dictyochloropsis splendida* as a Potential Feedstock for Biodiesel Production

***Sanaa Mahmoud Metwally Shanab*[1*]** ***Mervat Aly Mohamed Abo-State*[2]**
***Hamdy Elsayed Ahmed Ali*[2]**

[1]Department of Botany and Microbiology, Faculty of Science, Cairo University, 12613 Giza, Egypt.
[2]Department of Radiation Microbiology, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority, 11787, Cairo, Egypt.
[*]Corresponding author: sanaashanab@sci.cu.edu.eg[*], abostatem@yahoo.com, hamdy44044@gmail.com
[*]ORCID ID: https://orcid.org/0000-0002-3254-9618[*], https://orcid.org/0000-0002-9027-2717, https://orcid.org/0000-0001-6441-8535

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Abstract:

Biodiesel production from microalgae depends on the biomass and lipid production. Both biomass and lipid accumulation is controlled by several factors. The effect of various culture media (BG11, BBM, and Urea), nutrients stress [nitrogen (N), phosphorous (P), magnesium (Mg) and carbonate (CO_3)] and gamma (γ) radiation on the growth and lipid accumulation of *Dictyochloropsis splendida* were investigated. The highest biomass and lipid yield of *D. splendida* were achieved on BG11 medium. Cultivation of *D. splendida* in a medium containing 3000 mg L^{-1} N, or 160 mg L^{-1} P, or 113 mg L^{-1} Mg, or 20 mg L^{-1} CO_3, led to enhanced growth rate. While under the low concentrations of nutrients caused a marked increase in the lipid content. Cultures exposure to 25 Gy of γ-rays, led to an increase in lipid content up to 18.26 ± 0.81 %. Lipid profile showed the maximum presence of saturated fatty acids (SFAs, 63.33%), and unsaturated fatty acids (UFAs, 37.02%). Fatty acids (FAs) recorded the predominance of C16:0, C18:2, C15:0 and C16:1, which strongly proved *D. splendida* is a promising feedstock for biodiesel production.

Keywords: Biodiesel, *Dictyochloropsis splendida*, Gamma radiation, Lipid content, Nutrients.

Introduction:

Renewable, sustainable, and eco-friendly biofuels are development fields and attractive research that are much needed because of fossil fuels depletion and environmental pollution. Biodiesel has several advantages such as high biodegradable, absence of any aromatic compounds and 90% reduction in air toxicity may conduct to 95% decrease in the applicable cancer cases and have similar properties of fossil diesel [1,2].

Biodiesel can be classified according to their source into 1) biodiesel produced from edible oil (first generation) such as soybeans, rapeseed, and sunflower seeds [3]. About 7% of global edible vegetable oil supplies were utilized for biodiesel production in 2007. However, vast use of edible oils may cause food supplies versus fuel issue (food crisis) [4, 2] biodiesel produced from waste cooking oil, animal fats and nonedible vegetable oils (second generation) such as jatropha [5], and 3) third generation biodiesel is produced from microalgae [6].

The advantages of microalgae over higher plants as a source of biodiesel: 1) synthesize and accumulate large quantities of neutral lipids, 2) Possess a high photosynthetic efficiency and growth rate, 3) Grow on saline/brackish water and non-arable land as well as it can utilize nitrogen (N) and phosphorous (P) of wastewater, 4) Can grow in photobioreactors with higher biomass production. 5) Sequester CO_2 through photosynthesis and so reducing greenhouse gas emission [7].

Current research into increasing lipid accumulation in microalgal cells mainly focuses on the optimization of culture conditions, screening microalgae species, and the transformation of microalgae by genetic engineering. Limitation of nutrients in culture media is a commonly technique

used to increase lipid inside the microalgal cells. N and P starvation besides magnesium (Mg) and carbon supplementation can induce biosynthesis of FAs [8, 9].

Little information is available on the effect of γ- radiation on the physiological mechanism and biochemical composition of microalgae [10, 11]. The objectives of this study were to investigate the effect of culture media composition, nutrients concentration (nitrogen, phosphorous, magnesium, and carbonate) as well as the dosage of γ- radiation on both algal growth and lipid parameters of microalga, *Dictyochloropsis splendida*

Materials and Methods:

Cultivation of microalgae

The green alga, *Dictyochloropsis splendida* was provided by the algal culture collection from the Laboratory of Phycology in Botany and Microbiology Department, Faculty of Science, Cairo University, Egypt. The alga was cultivated on BG-11 medium [12] and incubated under a continuous light intensity of 40 µE m^{-2} s^{-1} (daylight fluorescent lamps, Philips, TLD18W/54-765) at 25± 1°C and aeration with constant sterilized bubbling of air (by a 0.22µm filter) for 25 days.

Influence of media composition on growth and lipid production of *D. splendida*

To evaluate the impact of media composition on the growth and lipid content, *D. splendida* was cultured in 1L glass flasks in BG11 medium [12], BBM [13] and urea medium [14]. Flasks were incubated under the same pervious conditions.

Influence of nutrients concentrations on growth and lipid production of *D. splendida*

In all experiments, *D. splendida* was grown in BG11 medium [12] under continuous illumination with aeriation rate of 1.25 L/min at 25± 1°C for 25 days. Nitrogen was used in the form of $NaNO_3$ in concentrations 0, 380, 750, 1500, and 3000 mg L^{-1}. Phosphorous (P) was used in the form of K_2HPO_4 in concentrations of 0, 40, 80, 160, and 320 mg L^{-1}. Magnesium (Mg) was used as $MgSO_4 \cdot 7H_2O$ in concentrations 19, 38, 75, 113 and 150 mg L^{-1}. Carbonate (CO_3) as Na_2CO_3 in concentrations of 0, 10, 20 40 and 80 mg L^{-1}. Growth parameters and lipid content were determined at each experiment.

Influence of γ-radiation on growth and lipid production of *D. splendida*

Cultures of *D. splendida* were irradiated by different γ-doses 0, 25, 50, 100, 200,300, 500, 1000 Gy of ^{60}Co γ-rays. Irradiation was performed by ^{60}Co γ-rays (Gamma cell 4000-A- India) at National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Egypt at a dose rate of 1.296 KGy/h. Cultures after irradiation were incubated under previous conditions and growth was determined as optical density at 680 nm. The lipid content was calculated at the end of the experiment.

Cell growth measurements

From 1L incubated algal culture (900 ml BG11 + 100 ml algal inoculum), optical density (OD at 680nm) of the microalgal sample (3ml) was determined at regular interval of 5 days (in triplicates) using spectrophotometer (UV–Vis spectrophotometer, T60, UK). Twenty ml of washed filtered culture were dried at 105°C for 24 hrs., chilled in a desiccator, and the algal dry weight was determined and expressed as g L^{-1}.

The maximum specific growth rate, μ_{max} (d^{-1}), was evaluated as:

$$\mu_{max} = \frac{1}{t} * ln\frac{x_f}{x_0} \dots\dots\dots\dots (1)$$

Where X_f and X_o are the biomass concentrations (g L^{-1}) at the final and the start of a batch run, respectively; and *t* is the time span of the run (day).

The biomass productivity (*BP*) (mg $L^{-1}d^{-1}$) and biomass yield (*BY*, g L^{-1}) were assessed as follows [15]:

$$BP = \frac{(X_f - X_0)}{(T_2 - T_1)} \dots\dots\dots\dots (2)$$

$$BY = (X_f - X_0) \dots\dots\dots\dots (3)$$

Where X_f and X_o are the biomass concentrations (g L^{-1}) at the final and the start of a batch run, respectively; and T_1 and T_2 (day) represent the incubation time of an experiment at the start time day and the final day of incubation, respectively.

Determination of lipid content

Lipids were extracted at the final incubation time by a 1:1:0.9 ratios of chloroform: methanol: deionized water mixture on volumetric basis [16] where 5 ml chloroform, 10 ml methanol, and 4 ml of deionized water were initially added to 0.3 g dried sample (0.3 g dried algal biomass/1L algal culture). Then, the mixture was shaken for 10 min, and then another 5 ml chloroform and 5 ml deionized water were added and shaken for overnight. The algal-solvent mix was refined to eliminate the algal precipitates. The chloroform layer of the filtrate was removed, solvent was volatilized at 40-45°C and the lipid was weighed. Lipid content was determined as percentage of cell dry weight:

$$LC = \frac{W_L}{W_B} * 100 \dots\dots\dots\dots (4)$$

Where *LC* is the lipid content (%), W_L and W_B are the weights of the extracted lipids and the dry biomass, respectively.

The lipid productivity (*LP*) was calculated as follows [17]:

$$LP = BP * LC \dots\dots\dots\dots (5)$$

Where *LP* is the lipid productivity (mg $L^{-1}d^{-1}$), *BP* (mg $L^{-1}d^{-1}$) and *LC* (% dry weight) are biomass productivity (BP) and lipid content, respectively.

Lipid yield was calculated as follows [18]:

$$LY = BY * LC \dots\dots\dots\dots (6)$$

Where *LY* is lipid yield (g L^{-1}), *BY* (g L^{-1}), *LC* (% dry weight) are biomass yield and lipid content, respectively.

Transesterification and Fatty acid analysis

Lipid was transesterified to produce fatty acid methyl ester (FAMEs) using 2% sulphuric acid in methanol [19]. FA analysis was achieved in Central Laboratory, Faculty of Agriculture, El-Azhar University by gas chromatography (Perkin Elmer Auto System XL) using DB5 silica gel capillary column (60 m ×0.32mm i.d.) with flame ionization detector and Helium was applied as the carrier gas (at the flow rate of 1 ml min-1).

Statistical analysis

All the experiments were conducted in 3 replicates. One-way ANOVA with 95% confidence (probability limit of $p < 0.05$ was utilized to estimate the significant difference in dependent variables, and Tukey's test at a reliability level of ($p<0.05$) was used to identify differences between each level of treatment. The statistical analyses were achieved using Minitab software (V18, Minitab Inc., State College, PA, USA).

Results and Discussion

Influence of media composition on growth and lipid accumulation

The effect of various culture media (BG11, BBM and Urea) composition on the growth of *D. splendida* were assessed as outlined in Figure1. The highest *BY* of *D. splendida* (0.90 ± 0.01 g L^{-1}) resulted in culturing on BG11 medium. With this medium, the maximum μ_{max} and *BP* were 0.097 ± 0.002 d^{-1} and 32.96 ± 0.54 mg $L^{-1}d^{-1}$, respectively. Also, highest *LC*, *LB* and *LY* were 16.92 ± 0.07 %, 5.58 ± 0.07 mg L^{-1} d^{-1} and 0.152 ± 0.001 g L^{-1}, respectively while urea medium showed the lowest *LC* (10.43 ± 0.79 %) as illustrated in (Figure 2A,B). The increase in the *LY* of *D. splendida* when cultured on the BG11 medium may be back to the high N concentration (1.5 g L^{-1}) in the BG11 medium which led to an increasing μ_{max}, where *LY* is the product of the *BY* multiplied by the *LC* [18]. This finding went parallel with Chandra et al [20] who studied the effect of different culture media (BG-11, modified CHU-13 and BBM medium) on the growth and lipid production of *Chlorella minutissima*. Maximum *BY* and *LY* were achieved by modified CHU-13 medium (970 ± 0.21 and 356.63 ± 0.51 mg L^{-1}, respectively) succeed in descending order by those produced by BG-11 medium (850 ± 0.12 mg L^{-1} and 243.65 ± 0.30 mg L^{-1}, respectively) and the minimum values were recorded by BBM medium (730 ± 0.42 mg L^{-1} and 196.83 ± 0.43 mg L^{-1}, respectively).

In another study, *Chlorella* sp. and *Scenedesmus* sp. were cultivated in media with more or less nutrients. Accumulation of lipid was higher in media deficient of nutrients whereas μ_{max} and *LP* were reduced[21]. Furthermore, micronutrients such as iron, cobalt, zinc, copper and manganese and nickel are the most essential trace metals required by algae for several metabolic functions [22]. This supports our results, where the highest μ_{max} and *LP* of *D. splendida* were recorded on BG11 medium followed in descending order by BBM and urea medium, which may be due to the availability (or not) of nutrients in the media[23]. On other hand, several studies used nitrate in source of N in culture media, whereas urea has been highly applied in large-scale algal cultivation due to its competent low cost compared to the others. Nevertheless, the manipulation of urea concentration through the cultivation is the challenge. Urea can liberate urease or be hydrolyzed to ammonia in basic conditions which lead to the growth of inhibition at high levels [24].

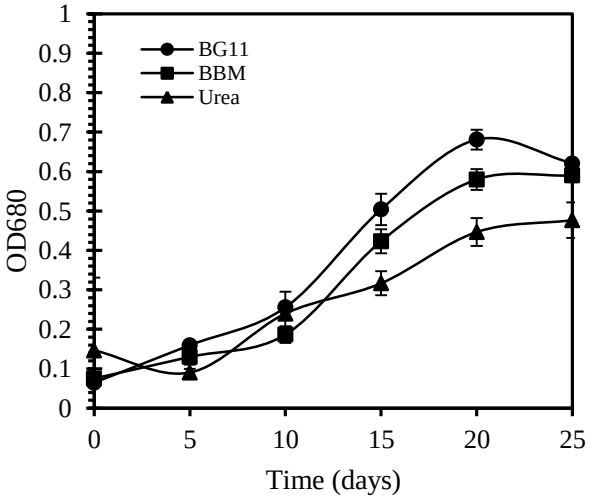

Figure1. Growth curve of *D. splendida* cultured on different culture media. Error bars represent ±SD of three replicates.

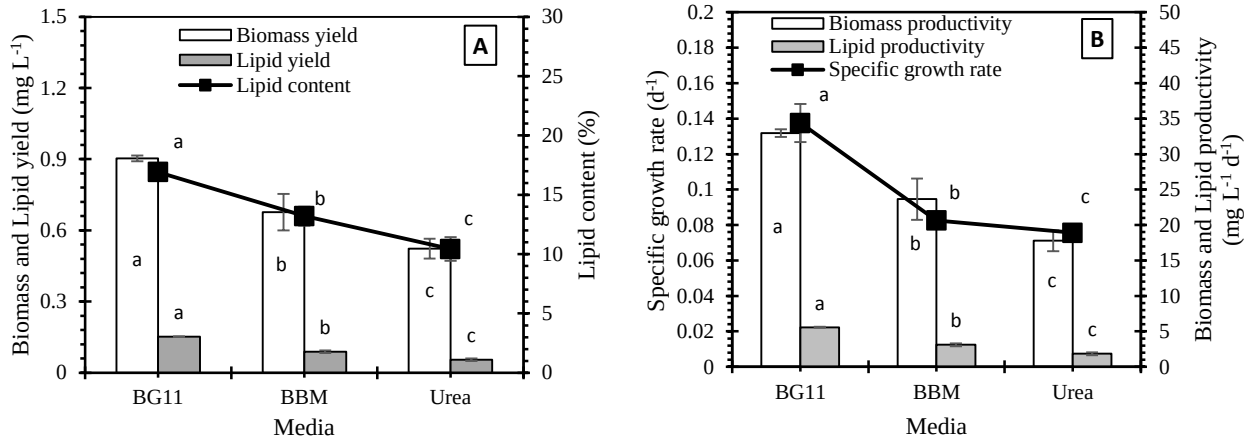

Figure 2. Growth and lipid parameters of *D. splendida* cultured on different of culture media. Different small letters on the same lines and bars indicate significant difference (p<0.05). Error bars represent ±SD of three replicates.

Impact of nutrients concentrations on growth and lipid formation

Nitrogen

The impact of initial concentrations of N on the growth of *D. splendida* was represented in Figure 3A. Increasing the P and N, was accompanied by an increase in growth. The highest *BP* and *BY* of 42.06 ± 2.25 mg $L^{-1}d^{-1}$ and 1.15 ± 0.05 g L^{-1}, respectively were obtained by cultivation with a start N concentration of 3000 mg L^{-1} (Table 1). Elevation of the N concentration from 0 to 3000 mg L^{-1} showed an obvious increment in biomass and growth rate, but a decline in lipid accumulation. The highest *LC* of 18.09 ± 0.03 % was recorded under N depletion (380 mg L^{-1}) as illustrated in Figure 4A. The *LY* of *D. splendida* was significantly influenced by the N concentration (P <0.05). The highest *LP* (5.37 ± 0.12 mg $L^{-1}d^{-1}$) and *LY* (0.0152 ± 0.001 g L^{-1}) were recorded at N concentration of 1500 mg L^{-1}.

Nitrogen is the most commonly reported nutrient-limiting factor in the growth and lipid accumulation of microalgae [24]. The obtained results agrees with Ishika et al. [25] who reported that N deficiency results in an increment in lipid and /or carbohydrate accumulation of microalgae and a decline in growth rate, photosynthetic efficacy, and protein amounts. Rehman and Anal [26] noted that the *LC* of *Chlorococcum* sp. TISTR 8583 increased by 1.7 folds when cultured on N-deficient medium and optimized light intensity. Similarly, Yodsuwan et al. [27]reported that the maximum *LC* of *P. tricornutum* (53.04 ± 3.26% %) was noted under N-deficient condition.

Phosphorous

The growth curve of *D. splendida* in the growth medium for different initial P concentrations are shown in Figure 3B. Reasonably, the maximum cell density increased with an increase in initial P concentration. From the ANOVA results, we found that P had a remarkable effect (p< 0.05) on biomass production of *D. splendida*. The maximum $\mu_{max,}$ *BP* and *BY* of 0.111 ± 0.010 d^{-1}, 41.01 ± 3.96 mg $L^{-1}d^{-1}$ and 1.10 ± 0.11 g L^{-1} were obtained at 160 mg L^{-1}, respectively. Increasing the P concentration from 40 mg L^{-1} to 320 mg L^{-1} had an insignificant effect (p> 0.05) on *BY* (Table 1). The lipid accumulation of *D. splendida* under different initial P concentrations was given in Figure 4B. While deficiency in P significantly promoted lipid accumulation (p< 0.05). The highest *LC* (18.39 ± 1.22 %), *LP* (7.06± 0.82 mg L^{-1}d-1) and *LY* (0.189 ± 0.023 g L^{-1}) were recorded at 80 mg L^{-1} P as shown in Table 1and Figure 4B.

Phosphorous is the main player in cellular metabolic processes, which are connected to photosynthesis and energy transfer. The results agreed with those of Guschina and Harwood [28] who mentioned that under P deficiency, the photosynthetic rates decreased, the cell division rates reduced, and this may lead to the accumulation of triacylglycerols. Also, under P limitation, the *LC* of *Tisochrysis lutea* [29] and *P. tricornutum* [30] were increased. In addition, the total FAs content increased over two folds under P depletion, conversely total FAs content was inversely proportional with P concentration over a factor of ten [31].

Magnesium

Figure 3C illustrates the time-course study on the effect of Mg on the growth of *D. splendida*. Increasing the Mg from 0–75 mg L^{-1} showed significant increase (p> 0.05) on the growth of *D. splendida*, while increasing Mg from 75 to 150 mg L^{-1} had insignificant effect on the *BY*. The maximum μ_{max} and *BP* of 0.120 ± 0.001 d^{-1} and 34.47 ± 0.46 mg $L^{-1}d^{-1}$ were obtained at 113 mg L^{-1}

Mg, respectively (Table 1). On the contrary, the increasing Mg concentration exhibited a negative impact on the *LC*. The maximum *LC* (20.06 ± 0.15 %) was achieved at 19 mg L^{-1} of Mg (Figure 4C). Further, the *LP* and *LY* of the tested microalga were significantly affected by alteration in the Mg concentration (P<0.05).

Mg plays a key role in the growth of microalgae, whereas it is the central atom of chlorophyll and as a co-factor of some enzymes in the metabolic pathway [32]. There are limited studies on microalgae responses during Mg limitation in terms of biomass growth and lipid accumulation [33]. The lipid yield and growth of microalgae were improved by Mg supplementation, whereas the starvation of Mg ions anticipates the decrease in mitotic division, hinder of chlorophyll formation and, so, the biomass yields [34].

In harmony with the obtained data, Gorain et al. [35] found a marked increase in the neutral lipid content of *Chlorella vulgaris* and *Scenedesmus obliquus* in Mg- and Ca-free medium. Also, Increasing the concentration of Mg exhibited positive effects on *BY* of *C. vulgaris* and *S. obliquus*, and at concentration (150 mg L^{-1}) the *BY* was elevated up to 1.5 g L^{-1} (36% rise) for *S. obliquus* and 1.6 g L^{-1} (33% rise) for *C. vulgaris* on the 18th day of incubation. While the *LC* was increased with maximum up to 27% and 26%, respectively at 100 mg L^{-1} of Mg. The function of Mg ions in switch on the enzyme Acetyl-CoA carboxylase and catalyzing the first stage of FA production was proved[36]. In addition, the productivity of microalgae is augmented when Mg^{2+} concentration is in the range of 2-8 mg/L [24].

Carbonate

High and low sodium carbonate concentration in the growth medium had significant influence (p< 0.05) on growth (Figure 3D) and lipid production of *D. splendida* (Figure 4D). Table 1 summarizes the biomass and lipid parameters of *D. splendida* under different concentrations of sodium carbonate. At 20 mg L^{-1} of CO_3, the maximum *BY* (0.90 ± 0.01 g L^{-1}) was recorded, whereas, rising the CO_3 concentration showed a significant decrease in the growth parameters (μ_{max} and *BP*) (p<0.05). The highest *LC* of 19.46 ± 0.32 % was showed at 40 mg L^{-1} as presented in Figure 4D. The *LP* and *LY* ranged between 1.66-5.37 mg $L^{-1}d^{-1}$ and 0.051-0.152 mg L^{-1}, respectively.

Most investigations that have been done on the effect of inorganic carbon supply and lipid formation in microalgae cultures have converged on the addition of CO_2 [37]. In some works, NaH_2CO_3 has been utilized as a source of carbon on experimenting growth and biochemical composition in various microalgae species [38] and induced the accumulation of triacylglycerol in microalgal species. On the contrary, Zhao et al. [39] recorded that the addition of sodium bicarbonate in the culture medium of *Scenedesmus quadricauda* had a negative influence on the lipid production and the highest *LC* was obtained under air. On the other hand, Li et al. [40] found that the maximum *LC* of 494 mg g^{-1} and *LP* of 44.5 mg L^{-1} d^{-1} of *C. vulgaris* were recorded at 160 mM $NaHCO_3$ and pH 9.5, and 10 mM $NaHCO_3$ was the optimal concentration for cell growth and elevating $NaHCO_3$ from 10 to 160 mM prosecute an inhibition to biomass.

Gamma radiation

Figure 3E shows the growth curve of *D. splendida* under different gamma radiation doses. The data exhibited that high doses of γ-ray had a negative effect on growth. The maximum μ_{max} was decreased with elevating irradiation dose (Table 2). The *BY* declined from 0.90 ± 0.01 g L^{-1} to 0.21 ± 0.02 g L^{-1} (decreased by 76.67 %) when cultures were displayed to irradiation dosage of 1000 Gy. The *LC* of *D. splendida* given in Figure 4E, the highest *LC* of 18.26 ± 0.81 % was achieved when the alga cell exposed to 25 Gy. While the higher irradiation doses had negative impact on the lipid accumulation. The maximum *LP* (5.37 ± 0.12 mg L^{-1} d^{-1} and 5.24 ± 0.43 mg L^{-1} d^{-1}) was recorded at zero and 25 Gy, respectively.

Gamma rays can generate free radicals (ROS), which have the ability to change the composition of cells in comparison with the slight penetration influence of UV-B [41]. Hence, 60 Co-γ-rays were selected for irradiation due to their powerful penetration ability. In concomitant with the obtained results, Cheng et al. [10] found that the lipid amount of *Nitzschia* sp. declined with increased irradiation dose (0-900 Gy). Agarwal et al. [42] reported that the high irradiation doses extremely injure cell metabolism regulation complex and growth cease if cells lose their self-repair potential through injury recuperation. Considering that various strains had diverse irradiation vulnerability to nuclear irradiation, whereas under low dosages of γ-ray irradiation, some microalgal cells were still slightly damaged and recuperate their normal states within a brief period [43].

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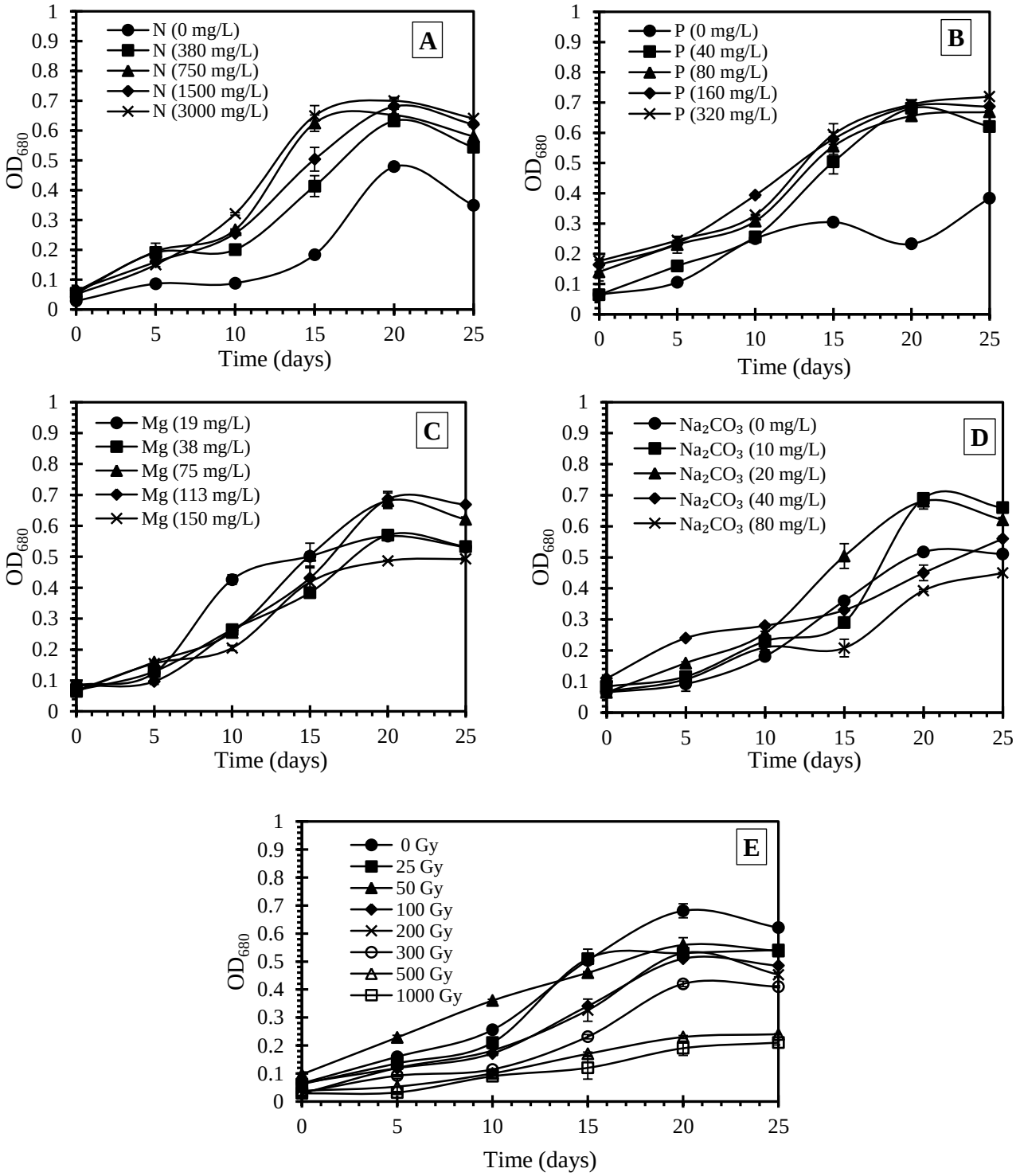

Figure 3. Growth curve of *D. splendida* cultured under different concentrations of nutrients and gamma radiation doses. (A) Nitrogen, (B) Phosphorous, (C) Magnesium, and (D) Carbonate, and (E) gamma radiation. Results represent mean ±SD of three replicates.

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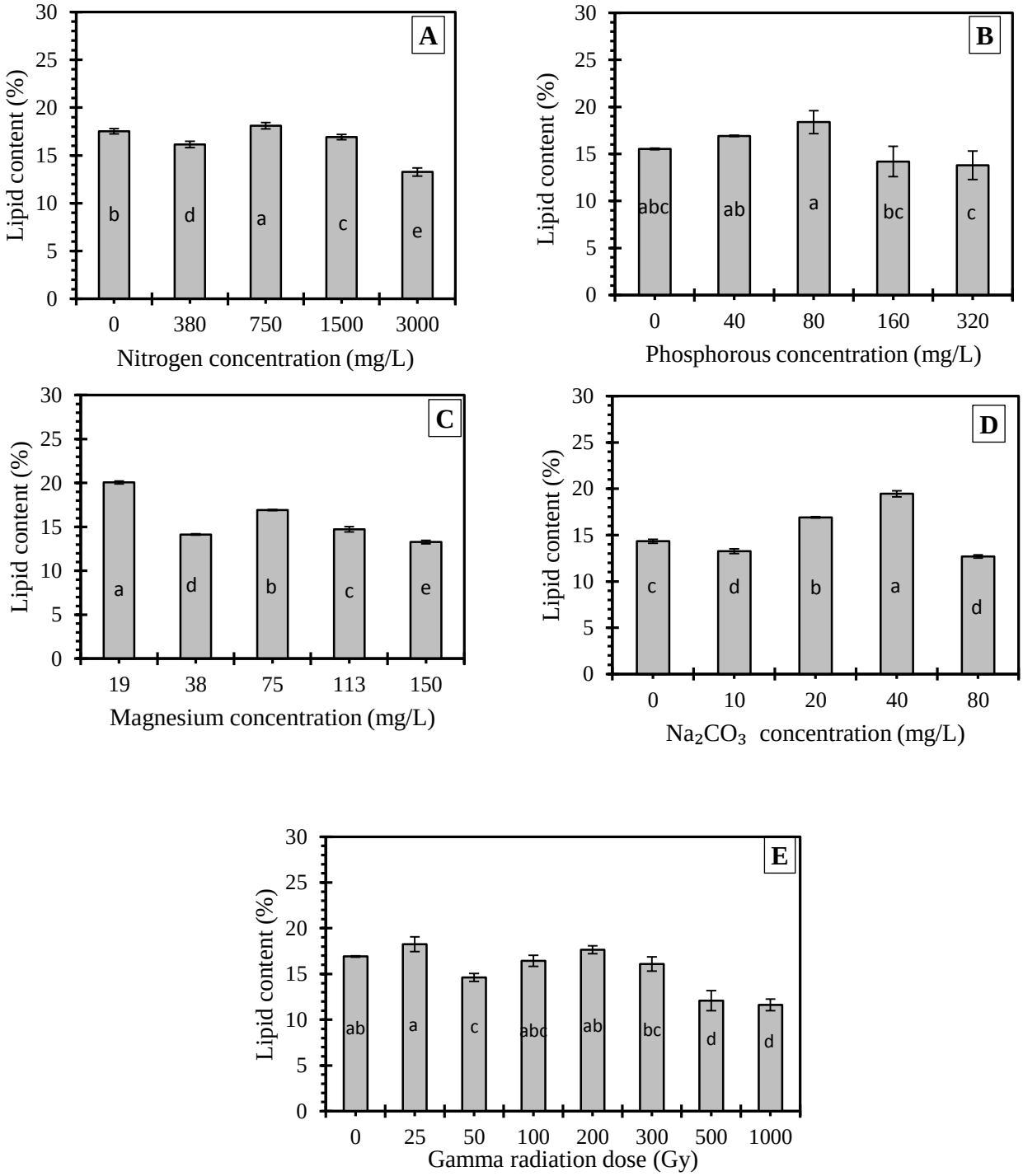

Figure 4. Lipid production of *D. splendida* cultured under different concentrations of nutrients and gamma radiation doses. (A) Nitrogen, (B) Phosphorous, (C) Magnesium, and (D) Carbonate, and (E) gamma radiation. Different small letters on the bars indicate significant difference at $p<0.05$. Results represent mean ±SD of three replicates.

Table 1. Kinetics of cell growth and lipid production of *D. splendida* under on different nutrients stress

Nutrient concentration (mg L^{-1})	Biomass productivity (*BP*) (mg $L^{-1}d^{-1}$)	Maximum specific growth rate (μ_{max}) (d^{-1})	Biomass yield (*BY*) (g L^{-1})	Lipid productivity (*LP*) (mg $L^{-1}d^{-1}$)	Lipid yield (*LY*) (g L^{-1})
$NaNO_3$					
0	16.70 ± 0.98^{c}	0.071 ± 0.004^{c}	0.50 ± 0.02^{d}	2.93 ±0.16^{bc}	0.088 ± 0.003^{c}
380	17.65 ± 1.47^{c}	0.081 ± 0.027^{c}	0.55 ± 0.04^{cd}	2.85 ± 0.23^{c}	0.088 ± 0.005^{c}
750	19.93 ± 2.13^{c}	0.082 ± 0.004^{b}	0.60 ± 0.02^{c}	3.61 ± 0.39^{b}	0.108 ± 0.003^{b}
1500	31.73 ± 0.83^{b}	0.084 ± 0.004^{b}	0.90 ± 0.01^{b}	5.37 ± 0.12^{a}	0.152 ± 0.001^{a}
3000	42.06 ± 2.25^{a}	0.099 ± 0.005^{a}	1.15 ± 0.05^{a}	5.58 ±0.31^{a}	0.152 ± 0.007^{a}
K_2HPO_4					
0	21.36 ± 1.20^{c}	0.076 ± 0.002^{b}	0.63 ± 0.04^{b}	3.31 ± 0.18^{c}	0.097 ± 0.005^{c}
40	31.73 ± 0.83^{b}	0.084 ± 0.004^{b}	0.90 ± 0.01^{a}	5.37 ± 0.12^{b}	0.152 ± 0.001^{b}
80	38.31 ± 1.92^{ab}	0.105 ± 0.006^{a}	1.03 ± 0.06^{a}	7.06± 0.82^{a}	0.189 ± 0.023^{a}
160	41.01 ± 3.96^{a}	0.111 ± 0.010^{a}	1.10 ± 0.11^{a}	5.84 ± 0.97^{b}	0.156 ± 0.026^{b}
320	40.91 ± 4.51^{a}	0.105 ± 0.003^{a}	1.09 ± 0.12^{a}	5.62 ± 0.56^{b}	0.149 ±0.015^{b}
$MgSO_4.7H_2O$					
19	17.08 ± 0.82^{d}	0.073 ± 0.001^{c}	0.51 ± 0.03^{c}	3.43 ± 0.15^{c}	0.102 ± 0.005^{d}
38	23.68 ± 0.96^{c}	0.075 ± 0.006^{bc}	0.70 ± 0.02^{b}	3.35 ± 0.13^{c}	0.098 ± 0.003^{d}
75	31.73 ± 0.83^{b}	0.084 ± 0.004^{b}	0.90 ± 0.01^{a}	5.37 ± 0.12^{a}	0.152 ± 0.001^{a}
113	34.47 ± 0.46^{a}	0.120 ± 0.001^{a}	0.91 ± 0.01^{a}	5.08 ± 0.05^{a}	0.133 ± 0.001^{b}
150	30.53 ± 0.75^{b}	0.084 ± 0.003^{b}	0.87 ± 0.03^{a}	4.06 ± 0.16^{b}	0.115 ± 0.005^{c}
Na_2CO_3					
0	20.97 ± 1.19^{c}	0.079 ± 0.002^{a}	0.61 ± 0.03^{c}	3.01 ± 0.21^{c}	0.087± 0.005^{c}
10	28.28 ± 0.55^{b}	0.085 ± 0.002^{a}	0.80 ± 0.01^{b}	3.75 ± 0.02^{b}	0.106 ± 0.001^{b}
20	31.73 ± 0.83^{a}	0.084 ± 0.004^{a}	0.90 ± 0.01^{a}	5.37 ± 0.12^{a}	0.152 ± 0.001^{a}
40	13.91 ± 0.83^{d}	0.066 ± 0.001^{b}	0.43 ± 0.01^{d}	2.70 ± 0.06^{d}	0.083 ± 0.003^{c}
80	13.07 ± 0.74^{d}	0.065 ± 0.001^{b}	0.41 ± 0.02^{d}	1.66 ± 0.11^{e}	0.051± 0.0033^{d}

All cultures were incubated under continuous illumination of 40 µE m^{-2} s^{-1} and temperature of 25± 1 ^{o}C with aeriation rate of 1.25 L/min for 25 days. Different superscript letters within the same column for each nutrient indicate significant difference at p<0.05. Results represent mean ±SD of three replicates.

Table 2. Kinetics of cell growth and lipid production of *D. splendida* exposed to different gamma radiation doses

Gamma radiation (Gy)	Biomass productivity (*BP*) (mg $L^{-1}d^{-1}$)	Maximum specific growth rate (μ_{max}) (d^{-1})	Biomass yield (*BY*) (g L^{-1})	Lipid productivity (*LP*) (mg $L^{-1}d^{-1}$)	Lipid yield (*LY*) (g L^{-1})
0	31.73 ± 0.83^{a}	0.084 ± 0.004^{a}	0.90 ± 0.01^{a}	5.37 ± 0.12^{a}	0.152 ±0.001^{a}
25	28.67 ± 1.28^{b}	0.079 ± 0.002^{b}	0.82 ± 0.04^{b}	5.24 ± 0.43^{a}	0.149 ±0.012^{a}
50	27.09 ± 1.04^{b}	0.079 ± 0.002^{b}	0.78 ± 0.03^{b}	3.96 ± 0.26^{b}	0.113 ± 0.006^{b}
100	11.76 ± 1.05^{c}	0.052 ± 0.006^{c}	0.40 ± 0.02^{c}	1.93 ± 0.19^{c}	0.066 ± 0.003^{c}
200	12.69 ± 0.25^{c}	0.057 ± 0.001^{c}	0.42 ± 0.01^{c}	2.24 ± 0.02^{c}	0.073 ±0.007^{c}
300	11.44 ± 0.14^{c}	0.057 ± 0.002^{c}	0.38 ± 0.01^{c}	1.84 ± 0.11^{c}	0.060 ±0.003^{c}
500	7.41 ± 1.27^{d}	0.046 ± 0.006^{d}	0.27 ± 0.03^{d}	0.89 ± 0.18^{d}	0.032 ± 0.005^{d}
1000	4.53 ± 0.61^{e}	0.032 ± 0.001^{e}	0.21 ± 0.02^{e}	0.53 ±0.09^{d}	0.024 ±0.003^{d}

All cultures were incubated under continuous illumination of 40 µE m^{-2} s^{-1} and temperature of 25± 1 ^{o}C with aeriation rate of 1.25 L/min for 25 days. Different superscript letters within the same column indicate significant difference at p<0.05. Results represent mean ±SD of three replicates.

Fatty acid composition

The fatty acid composition of *D. splendida* was given in Table 3. The FAME mainly contains saturated fatty acids (SFAs, 63.33 %) and unsaturated fatty acids (UFAs, 37.02%), also, the carbon chain lengths were from C12 to C24. Among the identified FAs, C16:0 was found to be present in higher concentration about 43.58 % followed by 19.22 % of C18:2, 10.72 % of C15:0 and 9.40 % of C16:1. The amounts of other FAs species were 17.08 % of the total FAs. Also, PUFAs and MUFAs values recorded 22.8 % and 14.2 %, respectively.

Table 3. Lipid profile of *D. splendida* lipid cultivated on BG11 medium

FA types	FA (%)
Lauric acid (C12:0)	1.15
Myristic acid (C14:0)	3.10
Pentadecanoic acid (C15:0)	10.72
Palmitic acid (C16:0)	43.58
Palmitoleic acid (C16:1)	9.40
Stearic acid (C18:0)	0.64
Oleic acid (C18:1)	4.80
linoleic acid (C18:2)	19.22
linolenic acid (C18:3)	3.60
Arachidic acid (C20:0)	0.46
Behenic acid (C22:0)	1.05
Lignoceric acid (C24:0)	2.28
Saturated fatty acids (SFAs)	63.33
Unsaturated fatty acids (UFAs)	37.02
Monounsaturated fatty acids (MUFAs)	14.2
Polyunsaturated fatty acids (PUFAs)	22.82

FA, fatty acid

Regarding the biodiesel formation from *D. splendida*, the green microalgal lipid usually has a FAs content of mostly C16 and C18 FAs that is alike to that of vegetable oils, and so appropriate for biodiesel formation [44]. The C16-C18 FAs of *D. splendida* were 80.55%, which can give the best relation between oxidative stability and cold flow properties[45]. MUFAs, which mainly formed of C16:1 and C18:1, are regarded as the most favorable components for forming biodiesel, and they give the best compromise between oxidative stability and cold flow properties [46].

The tested microalga had ~~a~~ distinctly higher amounts of C16 and C18 which were closer to those of *Haematococcus pluvialis* (76.6%) [47]. Also, *D. splendida* demonstrated considerable amount of C18:2 and C18:3, formed in low melting points, and are preferable for the improvement of the low temperature properties of biodiesel [48].

Conclusion:

The impact of media components, nutrients stress and γ- radiation on the biomass and lipid production of *D. splendida* was studied. The highest *BY* and *LY* were achieved when alga culturing on BG11 medium. The maximum μ_{max} was obtained at high N, P and Mg as well as low CO_3. While the highest *LC* was observed under nutrients limitation. Additionally, high γ-radiation doses expressed a negative influence on both growth and lipid production. The C16-C18 FAs of *D. splendida* were 80.55% which firmly manifested that *D. splendida* is a promising source for biodiesel formation.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in Cairo University.

Authors' contributions statement:

Conception, Shanab SMM, Ali HEA; Design, acquisition of data and analysis, Ali HEA; Interpretation, Shanab SMM; drafting the MS, SMM, Ali HEA, Revision, and proofreading, Shanab SMM, Ali HEA, Abo-State MAM

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تأثير تركيب الاوساط الغذائية و المغذيات واشعة جاما على إنتاج الكتلة الحيوية والدهون من الطحلب الدقيق الاخضر *Dictyochloropsis splendida* ، كمواد خام واعده للديزل الحيوى

ثناء محمود متولى شنب [1] **مرفت على محمد ابوستيت [2]** **حمدى السيد احمد على [2]**

[1] قسم النبات والميكروبيولوجى، كلية العلوم، جامعة القاهرة، الجيزة، مصر.
[2] قسم الميكروبيولوجيا الاشعاعيه، المركز القومى لبحوث وتكنولوجيا الاشعاع، هيئه الطاقة الذرية، القاهرة، مصر.

الخلاصة :

يعتمد إنتاج الديزل الحيوي من الطحالب الدقيقة على إنتاج الكتلة الحيوية ومحتوى الدهون .يتم التحكم في زياده انتاج الكتلة الحيوية و تراكم الدهون بواسطة عدة عوامل.فى هذا العمل تم دراسة تأثيرات ثلاث اوساط غذائيه خاصة بزراعة الطحالب (BG11, BBM, Urea media) وايضا بعض المغذيات(النيتروجين والفوسفورو المغنيسيوم والكربون) واشعة جاما على نمو وانتاج الدهون لطحالب *Dictyochloropsis splendida*. تم الحصول علي أعلي انتاج للكتله الحيويه والدهون لطحلب *Dictyochloropsis splendida*عندما تم زراعة الطحلب على الوسط الغذائي BG11 . علاوة على ذلك كان اعلى انتاجية للكتله الحيويه عند 3000 مليجرام / لتر نيتروجين او 160 مليجرام / لتر فوسفور او 113 مليجرام/لتر مغنسيوم او 20 مليجرام/ لتر كربونات. بينما عند غياب المغذيات فان تراكم الدهون زاد. من ناحية اخرى فان المحتوى الدهنى للطحلب وصل الى 18.26% عندما تعرضت خلايا الطحلب لجرعة 25 جراى من اشعة جاما. وكانت الدهون المستخلصة من الطحلب تتكون من نسبة عالية من الاحماض الدهنية المشبعة (SFAs, 63.33%) والاحماض الدهنية الغيرمشبعة (UFAs, 37.02%) وكانت السيادة لحمض البالمتيك (C16:0) ثم حمض اللينوليك (C18:2) فحمض بنتاديكانويك (C15:0) وحمض بالميتوليك (C16:1). و تمثل الاحماض الدهنية المستخلصه من طحلب *Dictyochloropsis splendida* بهذا التركيب مواد خام واعدة لانتاج الديزل الحيوي.

الكلمات المفتاحية : وقود الديزل الحيوي ، *Dictyochloropsis splendida* ، إشعاع جاما ، محتوى الدهون ، العناصر الغذائية.