Zagazig J. Agric. Res., Vol. 45 No. (6A) 2018 2087-2099

Biotechnology Research

http:/www.journals.zu.edu.eg/journalDisplay.aspx?JournalId=1&queryType=Master

MAXIMIZING LIPIDS PRODUCTION FROM SOME LOCAL CULTURES OF MICROALGAE USING PHYTOHORMONES

Ahmed M.A. Mousa*, Fatma I. El-Zamik, M.I. Hegazy and A.S.A. Salama

Agric. Microbiol. Dept., Fac. Agric., Zagazig Univ., Egypt

Received: 15/08/2018 ; Accepted: 25/09/2018

ABSTRACT: This study was performed to evaluate the impact of phytohormones indole-3- butyric acid and gibberellic acid (IBA and GA3) on 6 indigenous species of microalgae, isolated from different regions in Sharkia Governorate, Egypt, in terms of their dry weight, lipids and protein contents. Lipids were measured after ethanol-chloroform extraction, and proteins were measured by micro-Kjeldahl method. In general, all treatments of IBA gave positive effects on biomass dry weight, total lipids and protein contents compared to control except high level of IBA (20 mg/l medium). On the contrary, most concentrations of GA3 gave negative results in all microalgal cultures. The increase in biomass dry weight, total lipids and protein contents due to phytohormones treatments has reached 3.4 times in biomass, 3 times in total lipids, and 3.5 times in total protein contents. In biomass dry weight, the highest responses were found in the *Chlorella vulgaris* MG14 340% to IBA treatment of 15 mg/l, followed by *Chlorella vulgaris* MG30 329% under the same treatment. The highest value for each of total lipids and proteins for *Chlorella vulgaris* MG14 were 300% and 351%, respectively in response to 15 mg/l treatment. In general, it is most likely that phytohormones may be considered as a promising tool in maximizing the exploitation of microalgal cultures in biomass and biodiesel production.

Key words: Microalgae, phytohormones, IBA, GA3, biomass dry weight, protein content, lipid production.

INTRODUCTION

Microalgae are perceived as a potential wellspring of economical biomass feedstock for biofuel generation and are capable of multiplying under flexible ecological conditions **(Borowitzka and Moheimani, 2013)**. They are photosynthetic microorganisms with a high growth rate and a capacity to change over carbon dioxide into biomass and are a rich resource of potential molecules *e.g.*, lipids, proteins, and starches that can be changed over into fuel substitutes that are inexhaustible, non-harmful, and biodegradable; along these lines, they are perceived as a naturally safe fuel source **(Dillschneider *et al*., 2013)**.

The current generation of microalgal biofuel is expensive and renders this procedure as a financially unfeasible alternative to non-renewable energy sources **(Chisti, 2013).** The direct barrier to effectively present microalgal biofuels as a substitute for petroleum derivatives is making economically feasible mass development *i.e.,* practical biomass creation. Expanding biomass profitability and additionally lipid and starch creation of microalgae can enhance the economical possibility of algal development **(Abdelaziz *et al.,* 2014)**. To accomplish this, inorganic-natural carbon sources, light sources, supplements and saltiness have been explored **(Salama *et al.,* 2013)**, and elucidate that phytohormones have been accounted for to expand the development of microalgae by controlling inner biochemical pathways. Phytohormones compounds that impact and control plant development and formation procedures. Auxins, cytokinins, gibberellins and

* **Corresponding author: Tel. :** +201029812537
E-mail address: amagdy63@yahoo.com

abscisic acid are the main phytohormones of plants.

In plants, auxins are in charge of the lengthening of undifferentiated cells, promoting cell division, separation of phloem components, apical predominance, start of root development and postponed leaf abscission **(Galun, 2010)**, while cytokinins control senescence, chloroplast development and cell division **(Tarakhovskaya *et al.*, 2007)**. Auxins and cytokinins have been recognized in microalgae, and these phytohormones are expected to play homologous action in microalgae, as algae growth are phylogenetically identified with plants **(Raposo and Morais, 2013)**.

The empowering impact of phytohormones and their analogs on microalgal development and metabolite generation (carotenoid, lipid, starch and protein) has been investigated (**Czerpak and Bajguz, 1997; Bajguz and Piotrowska-Niczyporuk, 2013). Park *et al.* (2013)** studied morphological changes in microalgae affected by phytohormones and found that Indole-3-acetic acid (IAA) is the most plentiful and normally occurring auxin in plants, which controls essential physiological procedures including cell augmentation and division, tissue separation and reactions to light and gravity (**Leveau and Lindow, 2005**). In addition **Chen *et al.* (2013)** reported that the phytohormone, diethyl aminoethylhexanoate (DAH) has been altered to upgrade the plant development**,** and it will be fascinating to assess its impact on the microalgae development and biochemical structure. Nevertheless, the impact of phytohormones on microalgal development, sugar and protein contents, and their effects on the unsaturated fat profile must be assessed, as unsaturated fats are the significant factor for biodiesel production.

In this study, the impact of phytohormones (IBA and GA3) on microalgal development and unsaturated fat profile was studied. Adjustments in sugar content, protein substance and different developmental rates were additionally assessed.

MATERIALS AND METHODS

Microalgae and Culture Conditions

The microalgae isolates used in the present study were previously recovered from different regions of canals and spillways at Zagazig, Abo-Kabeer, Belbies, El-Husseineya, Abo-Hammad, San El-Hagar and Al-Kurein in Sharkia Governorate, Egypt. The Bold basal medium (BB) (**Bold, 1949; Bischoff and Bold, 1963)** was used for microalgal isolation, purification and lipid production. The fresh microalgae cultures were prepared by inoculating 200 ml of BB medium with 20 ml of 10- day old culture (10% *V/V*) containing 6.00×10^7 cells/ml in 500 ml Erlenmeyer flask. The flasks were incubated at 25± 2ºC on a rotary orbital shaker at 150 rpm under continuous illumination using white fluorescent light using 36W white fluorescent lamp at intensity of 500 Lux for two weeks.

Identification of the Microalgal Isolates

The morphological examination of the selected microalgal isolates was carried out at different stages of growth on BB medium and BG11medium **(Rippka *et al.*, 1979)**. The isolates were examined under 10, 40 and 100X powers of Microscope IrrMecoGmb H Germany, model IM 800 according to **Prescott (1973)**, to specify the general shape and colour of both cultures and vegetative cells. The shape, width and length of the vegetative cells, were also determined.

Determination of the Efficient Microalgal Isolates

Biomass dry weight

Dry cell weight was determined by taking 50 ml of the grown microalgal culture in a centrifuge tube, and harvesting by centrifugation at 5,000 rpm for 15 min. The supernatant was removed and the residual was re-suspended in 1 ml of distilled water and dried at 80ºC in an oven for 3 hr., using dried filter paper **(Ogbonna *et al.*, 1997)**, the biomass was cooled in a desiccator and weighted again to estimate the final dry weight**.**

Total lipid assay

Total lipids were extracted from the dry microalgal biomass using a slightly modified method of **Bligh and Dyer (1959)**. Total lipids in 0.2 g of dry biomass were extracted by mixing 1 ml of a mixture of chloroform: methanol (1:1 *V/V*), then vortexed well and incubated at 78ºC for 3 hr. The mixture was transferred into a separating funnel and shaked for 5 min, an additional 0.5 ml of chloroform

was added, and the extraction mixture was vortexed well again for 5 min. To separate the chloroform from aqueous methanol layer (aqueous top, organic bottom), the same volume of distilled water was added and then centrifuged at 5,000 rpm for 10 min. The chloroform layer was gently removed from the bottom using micropipette with gentle positive-pressure (*i.e.*, gentle bubbling) so that the upper phase does not get into the pipette tip. After performing a second extraction, the chloroform portion was collected and evaporated in an oven at 70ºC to obtain the crude lipid. Thereafter, the weight of the crude lipid was measured gravimetrically.

Total protein assay

Total nitrogen content was determined in the dry microalgale biomass by micro-Kjeldahle method **(Bremner and Mulvaney, 1982)** using H_2SO_4 digestion for the microalgae cells, followed by steam distillation and estimation of ammonia produced by titration with diluted HCl, then the total protein content was calculated.

Experimental design for lipid production using phytohormones

Among 40 indigenous microalgae isolated from Sharkia Governorate, six efficient cultures were chosen on the basis of their growth rates and lipid contents to be used in this experiment. These cultures were *Chlorella pyrenoidosa* MG11*, Chlorella vulgaris* MG14*, Chlorella vulgaris* MG30*, Chlorella vulgaris* MG37, *Scenedesmus dimorphs* MG32*,* and *Scenedesmus dimorphs* MG33. Phytohermone stock solutions were prepared according to **Fabian *et al.* (2015).** 20 mg of each of the phytohormones was dissolved in the appropriate solvent (GA3 in 5.0 ml of de-ionized water, and IBA in 0.5 ml of 95% ethanol) and then completed up to 200 ml with de-ionized water to obtain 100 mg/l of each phytohormone.

Experimental Treatments

500 ml conical flasks were used, containing 200 ml of Bold basal medium (BB) and 6.00×10^7 cells/ml, were incubated in 500 lux illumination with continuous aeration. Inoculated flasks were incubated at 25°C for 15 days, and the flasks were divided into the following ten treatments as follows:

1. Control (BBM without phytohormones)
2. BBM with 10 mg/; IBA
3. BBM with 15 mg/l IBA
4. BBM with 20 mg/l IBA
5. BBM with 10 mg/l GA3
6. BBM with 15 mg/l GA3
7. BBM with 20 mg/l GA3
8. BBM with 10 mg/l IBA and 5 mg/l GA3
9. BBM with 5 mg/l IBA and 10 mg/l GA3
10. BBM with 15 mg/l IBA and 5 mg/l GA3.

The experimental design was completely randomized with 10 treatments and 3 replicates were performed

Statistical Analyses

The data were recorded in three replicates for the parameters in various treatments, and subjected to the analysis of variance (ANOVA) in accordance with the factorial experimental design (3x3) according to **Snedecor and Cochran (1980).** SPSS statistical package was used to evaluate the sources and magnitudes of variations.

RESULTS AND DISCUSSION

Identification of Microalgal Cultures

Identification of the 6 microalgal cultures, based on morphological features like colony characteristics (colour, form, elevation and margin of colony) and also the morphology of the individual cells following microscopic examination (cell shape, cup-shape, cells number in series, dimensions (µm) and Pyrenoids) (Table 1) were conducted based on morphological characteristics and according to **Sanet *et al*. (2006)**.

Based on morphological characterizations, Table 1 show that the 6 microalgal cultures were classified, in general to 2 genera and 3 species with different morphotypes. *Chlorella* genus was the most common microalgae (4 cultures). Two species of *Chlorella* including 3 cultures of *Chlorella vulgaris* and one species of *Chlorella pyrenoidosa*. *Scenedesmus* includes 2 cultures in one species (*Scenedesmus dimorphs*).

Table 1. Morphological characteristics of the used microalgal cultures revealed by microscopic observations

Alga	Colony characteristic				Individual cell morphology				
	Colour	Form	Elevation	Margin	Shape	Cup-shape	Cells number	Dimensions (µm)	Pyrenoids
***Chlorella vulgaris* MG14**	grass-green	circular	convex	entire	spherical	+	unicellular	2-3 diameter	-
***Chlorella vulgaris* MG30**	grass-green	circular	convex	entire	spherical	+	unicellular	6-8 diameter	-
***Chlorella vulgaris* MG37**	grass-green	circular	convex	entire	spherical	+	unicellular	4-6 diameter	-
***Chlorella pyrenoidosa* MG11**	green	circular	convex	entire	spherical	+	unicellular	7-8 diameter	+
***Scenedesmus dimorphs* MG32**	olive-green	circular	convex	entire	fusiform	-	2-8 series	(2-3 width and 16-20 length)	+
***Scenedesmus dimorphs* MG33**	olive-green	circular	convex	entire	fusiform	-	4-8 series	(2-4 width and 18-20 length)	+

In details, looking to results obtained in Table 1 and pictures 1-6, results showed that the classification depended on the morphological characteristics has revealed that the microalgal cultures namely MG14, MG30 and MG37 belonged to *Chlorella vulgaris* since they were characterized by grass green colour culture, the shape of their vegetative cells spherical, unicellular, and their diameters ranged from 2.0-4.0 µm. Cup-shape was present in different cultures and pyrenoids were absent in all cultures (Pictures 1, 2 and 3). The culture *Chlorella pyrenoidosa* MG11 characterized by green colour culture, spherical shape, unicellular, and their diameters ranged from 7.0-8.0 µm. Cup-shape and pyrenoids were present (picture 4). The cultures *Scenedesmus dimorphs* MG32 and *Scenedesmus dimorphs* MG33 were characterized by olive-green colour culture, the cells fusiform shape, the cells were arranged in chains with a length of 2-8 and 4-8 cells per string, respectively, and their diameters ranged from 2.0-4.0 µm in width and from 16.0-20.0 µm in length. Cup-shape, was absent and pyrenoids were present (Pictures 5 and 6). Morphology of algae can vary with age and cultural conditions within each chemical race for same strain (**Metzger and Largeau, 2005**).

Effect of Phytohormones on Biomass Dry Weight, Lipids and Protein Contents

The response of the six microalgae cultures grown on Bold's Basal Medium (BBM), in this study, supplemented with two phytohormones (IBA) and (GA3) either alone or in different combinations was evaluated by determining biomass dry weight, total lipid and protein contents (g/l medium).

In case of *Chlorella pyrenoidosa* MG11

Results in Fig. 2 show that there were significant differences ($P \leq 0.05$) among phytohormones treatments in biomass dry weight of *Chlorella pyrenoidosa* MG11 culture. The biomass dry weight generally, ranged from 0.78 to 1.52 g/l. The biomass dry weight of microalgae can be divided statistically into four groups in terms of dry weight yield as affected

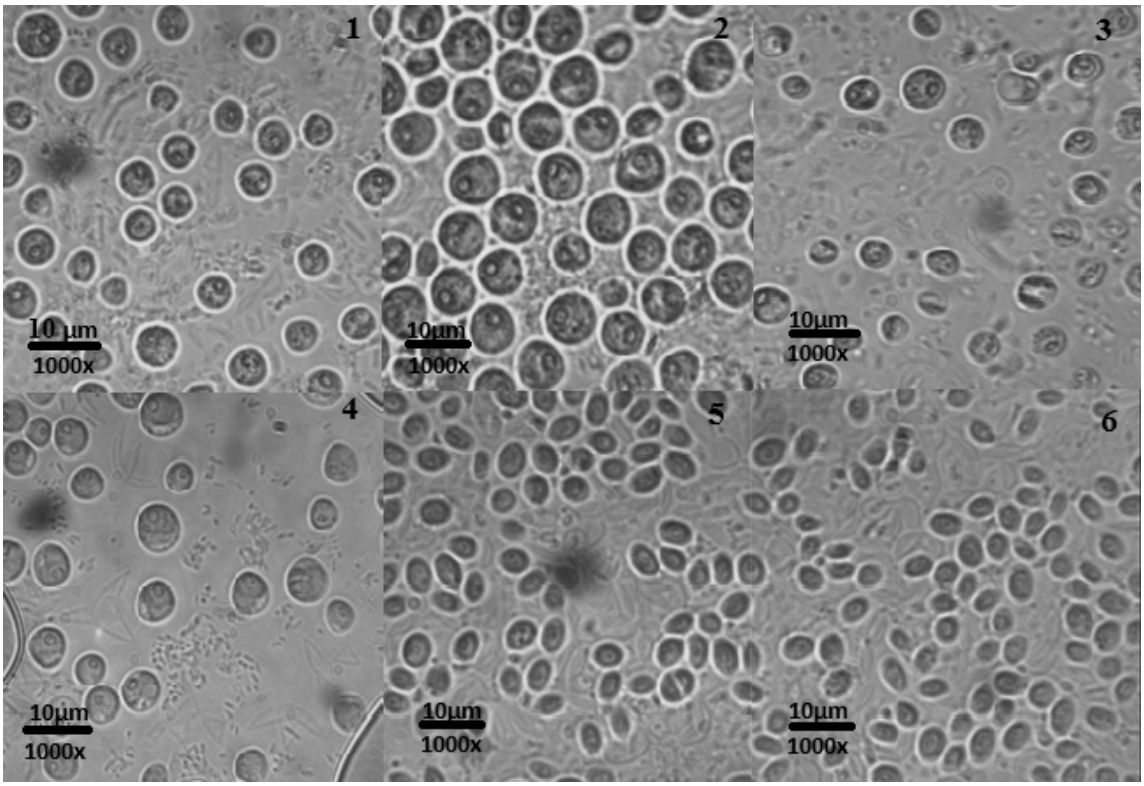

Fig. 1. Photomicrographs selected microalgal cultures

1- *Chlorella vulgaris* MG14 2- *Chlorella vulgaris* MG30 3- *Chlorella vulgaris* MG37

4- *Chlorella pyrenoidosa* MG11 5- *Scenedesmus dimorphs* MG32 6- *Scenedesmus dimorphs* MG33

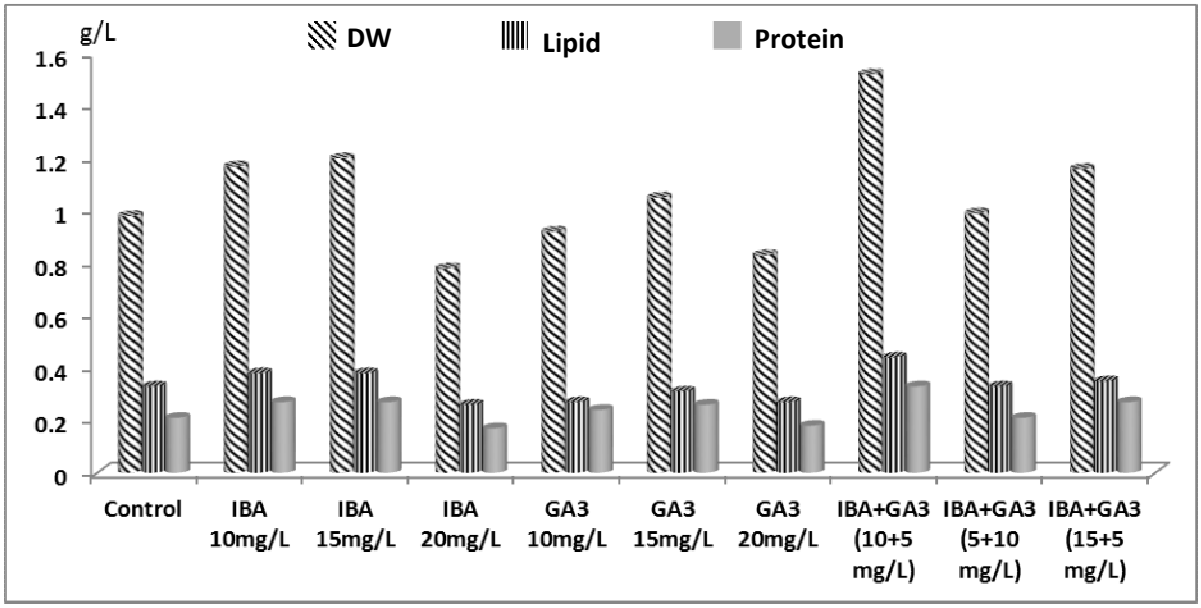

Fig. 2. Effect of phytohormones supplements (IBA and GA3) on biomass dry weight, total lipids and protein contents in *Chlorella pyrenoidosa* MG11

by the applied groups. The first group included treatment number 8, treated with 10 mg/l IBA and 5 mg/l GA3, which exhibited the highest value (1.52 g/l) dry weight of biomass. Treatments number 2, 3 and 10 came in the second group of yield without significant differences among them. The third group included treatments number 6, 9, 5 which containing 10 mg/l GA3 or more and the control, then, the last group containing the lowest values 0.78 g/l reaching as low as in treatment number 4. From the results, it could be detected that 15 mg/l of the IBA increased the biomass of microalgae, while GA3 had no effect on microalgae biomass individually.

Results in Fig. 2 indicate that addition of the two phytohormones led to significant increases in total lipid content of *Chlorella pyrenoidosa* MGA11 and ranged between 0.26 and 0.44 g/l. Also it was found that the lowest value of total lipid content was obtained in the treatment with 20 mg/l IBA, while the highest lipid content was observed after the dual treatment with10 mg/l IBA and 5 mg/l GA3. On the other hand, the total protein content in *Chlorella pyrenoidosa* MGA11 biomass showed similar pattern to that recorded in total lipid content with significant differences. In this respect, the treatment 10 mg/l IBA and 5 mg/l GA3 resulted in the highest value as compared to all treatments being 0.33 g/l protein.

Abdelaziz *et al.* (2014) reported that the increase in biomass productivity and/or lipid and carbohydrate production of microalgae can improve the economic feasibility of algal cultivation. The present results are in accordance with those of **Czerpak *et al.* (2003**) who showed that biochemical stimulants such as phytohormones had significant potential to enhance microalgal productivity. Also, IAA and DAH enhanced microalgal growth which was increased with increasing phytohormone concentrations **(Salama *et al.*, 2014)**.

In case of *Chlorella vulgaris* MG14

Application of phytohormones to BB medium inoculated with *Chlorella vulgaris* MG14 significantly increased the biomass dry weight, total lipid and protein contents compared to control (Fig. 3). The results showed that biomass dry weight of *Chlorella vulgaris* MG14 ranged from 1.40 to 5.37 g/l media. The highest value of biomass dry weight (5.37 g/l medium) was obtained when algal culture received 15 mg/l of IBA. The treatments receiving GA3 and control treatment gave low values of biomass dry weight.

Results in Fig. 3 reveal that the total lipid contents of all phytohormone treatments, generally, ranged from 0.46 to 1.59 g/l medium. In this respect, the phytohormone IBA treatments showed the highest values 1.59 and 1.49 obtained from concentrations 15 and 10 mg/l. As such, results showed similar trend for total protein contents (g/l medium). The best results of relative increases in total lipid and protein contents in biomass of *Chlorella vulgaris* MG14 were obtained by the treatment receiving 15 mg/l IBA, being 1.59 g lipid/l medium and 1.59 g protein/l media, respectively. The lowest values were obtained from treatment receiving GA3 alone or the control, without significant differences between them.

In this respect, **Hunt *et al.* (2010)** reported that the biomass productivity of *Chlorella sorokiniana* in the presence of 5 ppm naphthaleneacetic acid (NAA) is about 0.042 g l^{-1} day^{-1} compared to 0.018 g l^{-1} day^{-1} in the control sample, which means 133% biomass increase. They also concluded that naphthaleneacetic acid and indole-3- butyric acid (IBA) had the highest influence on improving the growth of microalgae. Recently **Kozlova *et al.* (2017)** stated that auxins such as indole-3- butyric acid play several roles in microalgal growth and metabolism, and even very small concentrations of auxins can stimulate each of growth, biomass production, and the biosynthesis of valuable biomolecules and IAA and IBA were used at a 50 ppm concentration supplemented with the growth medium of *Chlorella vulgaris*, it was found that cell counts per unit volume were increased by 11–19 times after 26 days of growth.

In case of *Chlorella vulgaris* MG30

Results presented in Fig. 4 show that the application of phytohormones supplemented in BB medium inoculated with *Chlorella vulgaris* MG30 significantly increased the amount of biomass dry weight, total lipid and protein contents compared to the control treatment, as

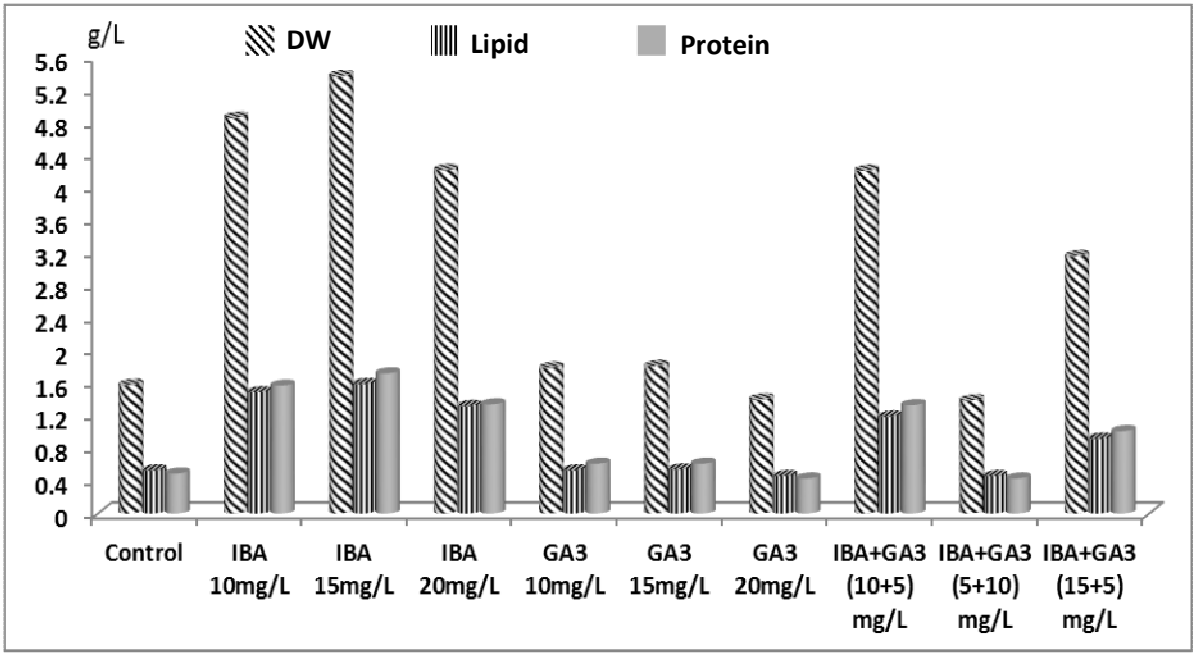

Fig. 3. Effect of phytohormone supplements (IBA and GA3) on biomass dry weight, total lipids and protein contents in *Chlorella vulgaris* MG14

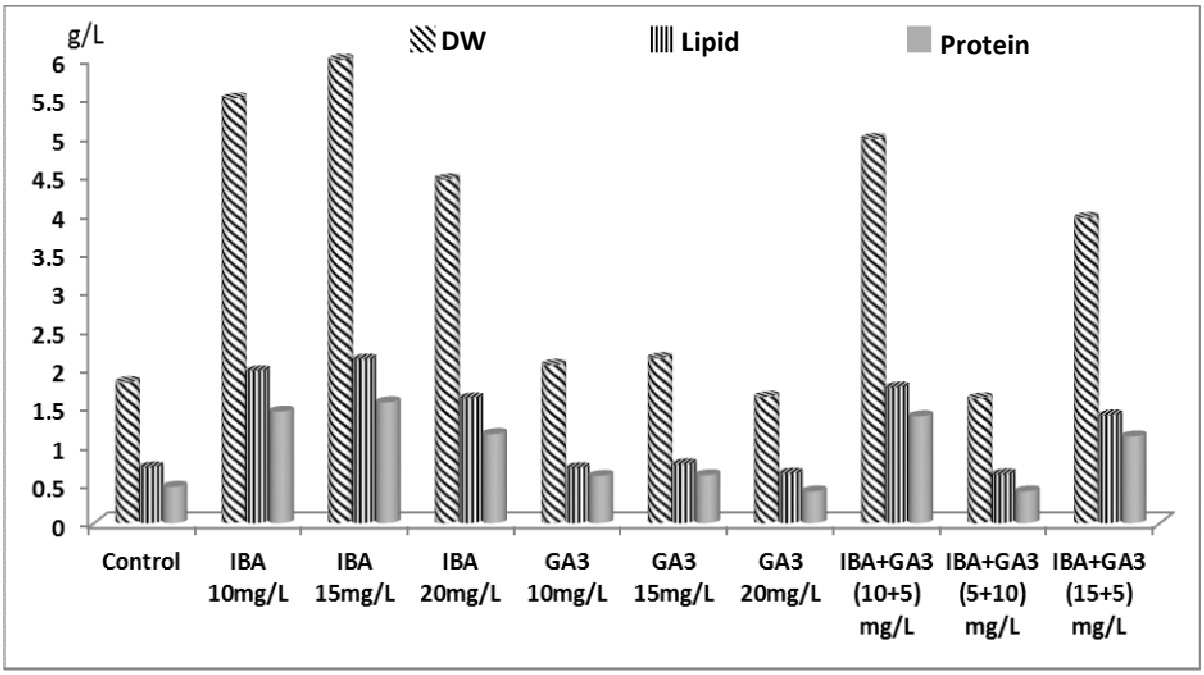

Fig. 4. Effect of phytohormone supplements (IBA and GA3) on biomass dry weight, total lipids and protein contents in *Chlorella vulgaris* MG30

they ranged from 1.63 to 5.98 g/l medium. Also, the high values of biomass dry weight (5.98 and 5.48 g/l medium) were obtained by the treatment with 15 mg/l and 10 mg/l of IBA, in that order, in contrast, the treatments receiving GA3 only or control treatment gave low values of biomass dry weights. The total lipid contents ranged between 0.64 and 2.11 g/l medium. The lowest value was found in algal culture when were single (treated) supplied with 20 mg/l GA3. The treatments 10 and 15 mg/l IBA showed high values (1.96 and 2.11 g/l lipids, respectively). The best results of relative increases in total protein contents in biomass of *Chlorella vulgaris* MG30 were obtained by treatments receiving 15 and 10 mg/l IBA (1.56 and 1.44 g/l, respectively). The lowest value was shown in treatment receiving 20 mg/l GA3 alone (0.41 g/l).

On the other hand, this microalgal culture gave the highest value of lipid percentage compared to others. The lipid percentage in biomass dry weight of *Chlorella vulgaris* MG30, generally ranged between 35.03 and 37.50%. Protein percentage in biomass dry weight ranged between 25.19 and 29.67%, and the high values (29.67and 29.13%) were obtained by treatments of 10 and 15 mg/l of GA3, respectively.

It was reported that auxins can also enhance, the biosynthesis of pigments, monosaccharides, and soluble proteins in *C. vulgaris* (**Piotrowska-Niczyporuk and Bajguz, 2014**). IBA has already promoted biomass (28.5% increase) and lipid productivity (33.5% increase) more than NAA and 2,4-dichlorophenoxyacetic acid (2,4-D), which are two synthetic auxins . Meanwhile, both 2,4-D and IBA effectively enhanced the accumulation of α-linolenic acid (ALA) with a productivity of 2.12 g/m^2/day (**Parsaeimehr *et al.*, 2017**). The functions of microalgal gibberellins are physiologically similar to those of higher plants. It has also been stated that gibberellins are mainly involved in cell elongation and expansion but not in cell division (**Romanenko *et al.*, 2016**).

In case of *Chlorella vulgaris* MG37

Addition of different concentrations (10.0, 15.0 and 20.0 mg/l) of IBA and GA3 or mixture (3:1) of them showed varying responses by *Chlorella vulgaris* MG37 (Fig. 5). The results showed that biomass dry weight of *Chlorella vulgaris* MG37, ranged from1.25 to 2.16 g/l medium. The highest value of biomass dry weight (2.16 g/l medium) was recorded in the treatment of 15 mg/l of IBA, followed by the treatments of (15 mg/l IBA and 5 mg/l GA3), respectively, while all other treatments gave proximate values of biomass dry weights. The treatment receiving 15 mg/l IBA gave the highest value of lipid content (0.70 g/l).

Similarly, the obtained results indicated that increases in total protein contents in biomass of *Chlorella vulgaris* MG37 were obtained by treatment receiving 15 mg/l IBA, being 0.67 g/l, while the lowest value was obtained from single treatment receiving 20 mg/l IBA.

In case of *Scenedesmus dimorphs* MG32

The effects of IBA and GA3 treatments on biomass dry weight, total lipids and protein contents of *Scenedesmus dimorphs* MG32 are given in Fig. 6. The application of IBA and GA3 enhanced the biomass dry weight, total lipid and protein contents compared to the control. The results showed that biomass dry weight of this algal culture ranged from 1.54 to 3.34 g/l. The high values of biomass dry weight (3.34, 3.12 and 2.94 g/l) were observed in treatments receiving 15 mg/l of IBA, 10 mg/l IBA + 5 mg/l GA3, and 10 mg/l IBA, respectively. The treatments receiving GA3 only and the control showed low values of biomass dry weights.

In this respect, **Salama *et al.* (2014)** found that IAA and IBA caused the maximum growth of *Scenedesmus obliquus* at 10 ppm with 190% increase in biomass and lipid content compared to the control. The stimulatory effect of auxins on lipid content and lipid productivity of *C. pyrenoidosa* and *Scenedesmus quadricauda* was studied by **Liu *et al.* (2016)**, since they reported a concentration of 60 mg/l indole-3-n-propionic acid (IPA) exhibited a maximum growth rate with a 3 times increase, and lipid production increased 4 times.

In case of *Scenedesmus dimorphs* MG33

Concerning the influence of both IBA and GA3 on dry weight, total lipid and protein content of *Scenedesmus dimorphs* MG33, results

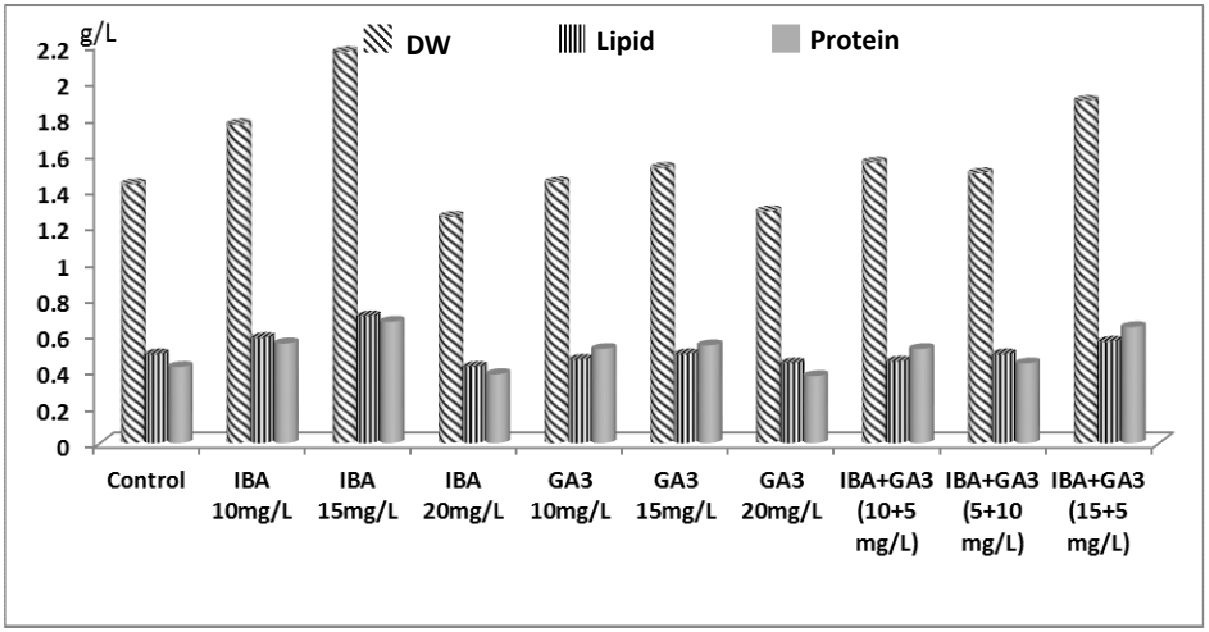

Fig. 5. Effect of phytohormone supplements (IBA and GA3) on biomass dry weight, total lipid and protein contents in *Chlorella vulgaris* MG37

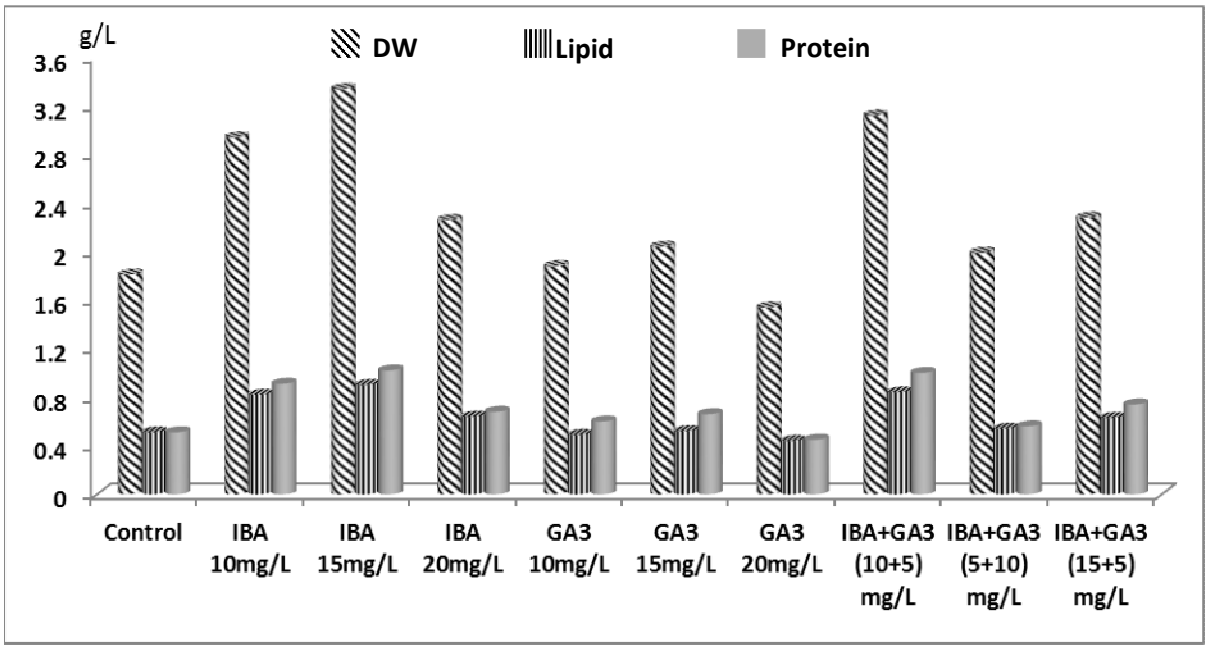

Fig. 6. Effect of phytohormone supplements (IBA and GA3) on biomass dry weight, total lipids and protein contents in *Scenedesmus dimorphs* MG32

in Fig. 7 show similar responses to that obtained with *chlorella vulgaris* MG30. For example results showed that biomass dry weight ranged from 1.15 to 2.30 g/l medium. The high values of biomass dry weight (2.30 , 2.16 and 2.10 g/l) were obtained by treatments number 3, 10 and 2, receiving 15 mg/l IBA, 15 mg/l IBA+ 5 mg/l of GA3, and10 mg/l of IBA, respectively. Total lipid contents, ranged from 0.34 to 0.72 g/l, being the highest value after the treatment of 15 mg/l of IBA, as the lipid content reached 0.72 g/l. At the same time, relative increases in total protein contents were obtained by treatments received 10 and 15 mg/l GA3, respectively.

In this regard, **Sulochana and Arumugam (2016)** reported that *Scenedesmus quadricauda* culture supplemented with 20 ppm of abscisic acid (ABA) was increased in it′s biomass production 2.1 times in nitrogen-limited conditions. So, this may be a potential strategy for efficient microalgal cultivation for biodiesel production**.** On the other hand, GA3 application led to some increase in the cell number and size, and the augmentation of dry mass of *Scenedesmus quadricauda* (**Buczek *et al.,* 1975**) and *Chlamydomonas reinhardtii* (**Park *et al.,* 2013**).

Gibberellins were involved mainly in cell elongation and expansion but not in their division. The content of the basic metabolites resulting from deactivation revealed a gradual reduction in growing cultures. Thus, under different lighting conditions, the cultivation exhibited a permanent conversion of various GA forms that promoted the maintaining of hormone homeostasis in *Chlorella minutissima* (**Stirk *et al.,* 2014**).

Comparisons Among Six Microalgal Cultures Responses in Biomass Dry Weight, Lipids And Protein Contents to Different Phytohormones Treatments

Results in Table 2 show that in general, all treatments of IBA gave positive effects in the percentages of biomass dry weight, total lipids and protein contents compared to control except high level of IBA (20 mg/l) in all tested microalgal cultures. On the contrary, most concentrations of GA3 showed negative responses in all tested microalgal cultures.

The response of biomass dry weight, total lipids and protein contents in microalgal cultures to phytohormone treatments generally, ranged from 80% to 340% in biomass, 79% to 300% in total lipids contents and 81% to 351% in total protein contents. In biomass dry weight, the highest responses were found in the *Chlorella vulgaris* MG14 culture (340%) to IBA treatment (15 mg/l), followed by *Chlorella vulgaris* MG30 (329%) under the same treatment. The same results were found in total lipids and protein contents, as the highest results were found in *Chlorella vulgaris* MG14 (300% in lipids and 351% in protein) in response to IBA treatment (15 mg/l).

In general, the lowest responses to phytohormone treatments were found in *Chlorella pyrenoidosa* MG11 followed by *Chlorella vulgaris* MG37, *Scenedesmus dimorphs* MG33 and *Scenedesmus dimorphs* MG32, while, the best responses were found in *Chlorella vulgaris* MG14 followed by *Chlorella vulgaris* MG30.

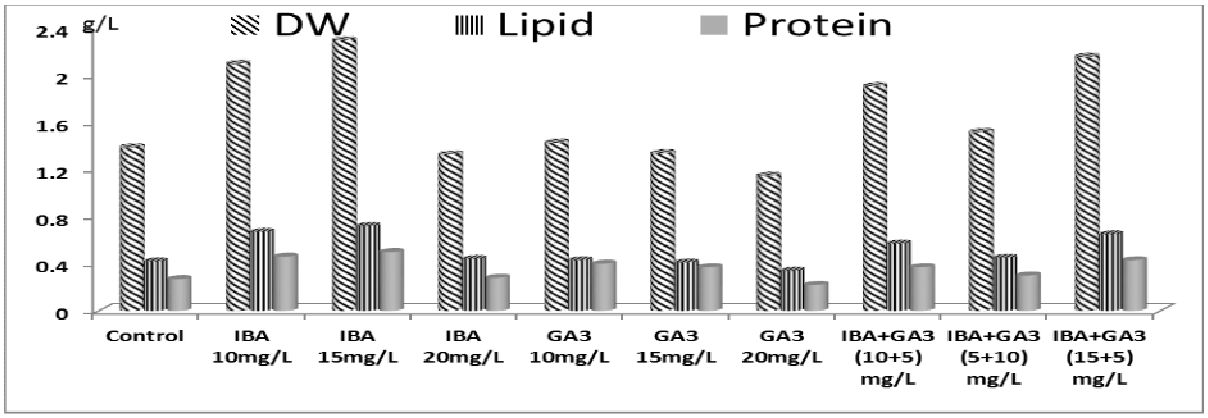

Fig. 7. Effect of phytohormone supplements (IBA and GA3) on biomass dry weight, total lipids and protein contents in *Scenedesmus dimorphs* MG33

Table 2. Variable responses of all tested microalgal cultures by means of dry weight, total lipids and proteins after treatment with IBA and GA3

Microalgae / Treatment	Responses (%)																	
	Chlorella pyrenoidosa MG11			*Chlorella vulgaris* MG14			*Chlorella vulgaris* MG30			*Chlorella vulgaris* MG37			*Scenedesmus dimorphs* MG32			*Scenedesmus dimorphs* MG33		
	DW	TL	PC	DW	TL	PC	DW	TL	PC	DW	TL	PC	DW	TL	PC	DW	TL	PC
1. Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
2. IBA 10 mg/l	119	115	129	308	281	320	301	272	306	123	118	131	162	161	178	151	160	170
3. IBA 15 mg/l	122	115	129	340	300	351	329	293	332	151	143	160	185	176	200	165	171	185
4. IBA 20 mg/l	80	79	81	267	249	273	244	224	245	87	86	90	125	125	133	96	105	104
5. GA3 10 mg/l	94	82	114	113	100	124	106	94	121	101	94	124	104	96	118	103	102	148
6. GA3 15 mg/l	107	94	124	115	102	124	117	106	132	106	100	129	113	102	129	96	98	137
7. GA3 20 mg/l	85	82	86	89	87	88	90	89	87	90	90	88	85	86	88	83	81	81
8. IBA+GA3 (10+5) mg/l	155	133	157	266	225	271	273	243	294	108	92	124	172	165	196	137	136	137
9. IBA+GA3 (5+10) mg/l	101	100	100	88	87	88	112	111	109	104	100	105	110	106	110	109	107	111
10. IBA+GA3 (15+5) mg/l	118	106	129	200	174	206	216	193	238	132	114	152	126	124	145	155	155	159

***** DW = Dry weight, TL= Total lipids, PC= Protein content

REFERENCES

Abdelaziz, A.E., D. Ghosh and P.C. Hallenbeck (2014). Characterization of growth and lipid production by *Chlorella* sp. PCH90, a microalga native to Quebec. Bioresour. Technol., 156: 20–28.

Bajguz, A. and A. Piotrowska-Niczyporuk (2013). Synergistic effect of auxins and brassinosteroids on the growth and regulation of metabolite content in the green alga *Chlorella vulgaris* (*Trebouxiophyceae*). Plant Physiol. Biochem., 71 : 290–297.

Bischoff, H.W. and H.C. Bold (1963). Phycological Studies IV. Some soil algae from enchanted rock and related algal species. Univ. Texas Publ., 6318: 195.

Bligh, E.G. and W.J. Dyer (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37 (8): 911- 917.

Bold, H.C. (1949). The morphology of *Chlamydomonas chlamydogama* sp. nov. Bull. Torrey Bot. Club., 76: 101-108.

Borowitzka, M.A. and N.R. Moheimani (2013). Algae for biofuels and energy. Springer, Dordrecht, New York, 165-185.

Bremner, J.M. and C.S. Mulvaney (1982). Total-Nitrogen. In: Methods of Soil Analysis. Agron.Monograph 9, Part 2, 2nd Ed. Ame. Soc. Agron. Madi. WI, 595-624.

Buczek, J., G. Kubik-Dorosz and E. Tatkowska (1975). The influence of gibberellic acid and kinetin on the growth of *Scenedesmus quadricauda* (Turp.) Bréb., Acta Soc. Bot. Pol., 44 (3): 415–421.

Chen, X.L., J.J. Zhang, R. Chen, Q.L. Li, Y.S. Yang and H. Wu (2013). An uncommon plant growth regulator, diethyl aminoethylhexanoate, is highly effective in tissue cultures of the important medicinal plant purple coneflower

(*Echinacea purpurea* L.). Biomed. Res. Int., 54: 03-16.

Chisti, Y. (2013). Constraints to commercialization of algal fuels. J. Biotechnol., 167:201–214.

Czerpak, R. and A. Bajguz (1997).Stimulatory effect of auxins and cytokinins on carotenes, with differential effects on xanthophylls in the green alga *Chlorella pyrenoidosa* Chick. Acta Soc. Bot. Pol., 66 : 41–46.

Czerpak, R., A. Bajguz, A. Piotrowska, R. Dobrogowska, W. Matejczyk and W. Wieslawski (2003). Biochemical activity of di- and polyamines in the green alga *Chlorella vulgaris Beijerinck* [*Chlorophyceae*]. ActaSoc. Botanic. Pol., 72: 19–24.

Dillschneider, R., C. Steinweg, R. Rosello-Sastre and C. Posten (2013). Biofuels from microalgae : Photoconversion efficiency during lipid accumulation. Bioresour. Technol., 142: 647–654.

Fabian, U., V. Ozioko, N. Chiejina, C. James and Ogbonna (2015). Effect of some phytohormones on growth characteristics of *Chlorella sorokiniana* IAM-C212 under photoautotrophic conditions. Afr. J. Biotechnol., 14 (30): 2367-2376.

Galun, E. (2010). Phytohormones and patterning: The role of hormones in plant architecture. World Scientific, Singapore, London.

Hunt, R.W., S. Chinnasamy, A. Bhatnagar and K.C. Das (2010). Effect of biochemical stimulants on biomass productivity and metabolite content of the microalga, *Chlorella sorokiniana*. Appl. Biochem. Biotechnol., 162 (8): 2400-2414.

Kozlova, T.A., B.P. Hardy, P. Krishna and D.B. Levin (2017). Effect of phytohormones on growth and accumulation of pigments and fatty acids in the microalgae *Scenedesmus quadricauda*. Algal Re., 27: 325–334.

Leveau, J.H. and S.E. Lindow (2005). Utilization of the plant hormone indole-3-acetic acid for growth by *Pseudomonas putida* strain 1290. Appl. Environ. Microbiol., 71:2365–2371.

Liu, J., W. Qiu and Y. Song (2016).Stimulatory effect of auxins on the growth and lipid productivity of *Chlorella pyrenoidosa* and *Scenedesmus quadricauda.* Algal Res., 18: 273–280.

Metzger, P. and C. Largeau (2005). *Botryococcus braunii* a rich source for hydrocarbons and related ether lipids. Spring., 486–496

Ogbonna, J.C., H. Masui and H. Tanaka (1997). Sequential heterotrophic/autotrophic cultivation: An efficient method of producing *Chlorella* biomass for health food and animal feed. J. Appl. Phycol., 9 : 359-366.

Park, W.K., G. Yoo, M. Moon, C.W. Kim, Y.E. Choi and J.W. Yang (2013). Phytohormone supplementation significantly increases growth of *Chlamydomonas reinhardtii* cultivated for biodiesel production. Appl. Biochem. Biotechnol., 171 : 1128–1142.

Parsaeimehr, A., E.I. Mancera-Andrade, F. Robledo-Padilla, H.M. Iqbal and R.A. Parra-Saldivar (2017). Chemical approach to manipulate the algal growth, lipid content and high-value alpha-linolenic acid for biodiesel production. Algal Res., 26 : 312–322.

Piotrowska-Niczyporuk, A. and A. Bajguz (2014). The effect of natural and synthetic auxins on the growth, metabolite content and antioxidant response of green alga *Chlorella vulgaris* (*Trebouxiophyceae*). Plant Growth Regul., 73: 57–66.

Prescott, G.W. (1973). Algae of the western great lakes area, 5th Ed., WMC. Brown Publishers, Dubuque, Iowa.

Raposo, M.F.J. and R.M.S.C. Morais (2013). Influence of the growth regulators kinetin and 2, 4-D on the growth of two chlorophyte microalgae, *Haematococcus pluvialis* and *Dunaliella salina*. J. Basic Appl. Sci., 9 : 302–308.

Rippka, R., J. Deruelles, J.B. Waterbury, M. Herdman and R.Y. Stanier (1979). Generic assignments strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol., 111: 1– 61.

Romanenko, K.O., I. V. Kosakovskaya and P. O. Romanenko (2016). Phytohormones of microalgae: Biological role and involvement in the regulation of physiological processes.

Pt II. Cytokinins and Gibberellins. Int. J. Algae, 18: 179–201.

Salama, E.S., H.C. Kim, R.A.I. Abou-Shanab, M.K. Ji, Y.K. Oh, S.H. Kim and B.H. Jeon (2013). Biomass, lipid content, and fatty acid composition of freshwater *Chlamydomonas mexicana* and *Scenedesmus obliquus* grown under salt stress. Bioproc. Biosyst. Eng., 36 (6): 827-833.

Salama, E.S., A.N. Kabra, M.K. Ji, J.R. Kim, B. Min and B.H. Jeon (2014). Enhancement of microalgae growth and fatty acid content under the influence of phytohormones. Bioresour. Technol., 172: 97–103.

Sanet J.V.V., T. Jonathan, V.G. Carin, G. Annelise (2006). Easy identification of the most common freshwater algae. RQS.

Snedecor, G.W. and W.G. Cochran (1980). Statistical Methods, 7th Ed. Iowa State Univ., Press, Ames, Iowa.

Stirk, W.A., D. Tarkowská, V. Turečová, M. Strnad and J. van Staden (2014). Abscisic acid, gibberellins and brassinosteroids in Kelpak®, a commercial seaweed extract made from *Ecklonia maxima*. J. Appl. Phycol., 26 : 561-567.

Sulochana, S.B. and M. Arumugam (2016). Influence of abscisic acid on growth, biomass and lipid yield of *Scenedesmus quadricaudaunder* nitrogen starved condition. Bioresour. Technol., 213 : 198–203.

Tarakhovskaya, E.R., M.I. Yu and M.F. Shishova (2007). Phytohormones in algae. Russ. J. Plant Physiol., 54: 163–170.

تعظيم إنتـــاج الـدهـــون مـــن بعـض مـــــزارع الطحالـب الـدقيقـة المحلية باستخدام منظمات النمـــو

أحمد مجدي احمد موسي – فاطمة إبراهيم الزامك – محمد إبراهيم حجازي – علي سلامه علي سلامه

قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة الزقازيق - مصر

تمت هذه الدراسة لمعرفة مدي تأثير بعض منظمات النمو (حامض الجبرلين وأندول حامض البيوتريك) على ستة سلالات من الطحالب الدقيقة المحلية (المعزولة من مدن الزقازيق، بيلبيس، القرين، أبو حماد، الحسينية، صان الحجر وأبو كبير من محافظة الشرقية - جمهورية مصر العربية) وذلك من خلال تقدير الوزن الجاف ومحتواها من الدهون والبروتين بعد تنميتها ومعاملتها بمنظمات النمو وحصادها، تم استخدام خليط الإيثانول والكلوروفورم في تقدير الدهون وطريقة ميكرو كيلداهل في تقدير البروتين، وأظهرت النتائج تفوق معاملات اندول حامض البيوتريك والتي أعطت نتيجة ايجابية على الوزن الجاف ومحتوي الدهون والبروتين مقارنة مع الكنترول ماعدا التركيز العالي منها (٢٠ مللي جرام/ لتر)، على النقيض معظم التركيزات من حامض الجبرلين أعطت نتيجة سلبية في كل الصفات المدروسة علي كل العزلات، ووصلت الزيادة في الوزن الجاف للخلايا إلى ٣,٤ ضعف الكنترول، و ٣ أضعاف الكنترول في نسبة الدهون، و٣,٥ ضعف في محتوي البروتين، وجد أن أفضل المزارع لصفة الوزن الجاف ومحتوي الدهون والبروتين هي المزرعة *Chlorella vulgaris* MG14 ثم يليها المزرعة *Chlorella vulgaris* MG30 باستخدام التركيز ١٥ مللي جرام/لتر من اندول حامض البيوتريك، من خلال النتائج نجد أن منظمات النمو تحت الدراسة كان لها دور رئيسي في زيادة كمية الوزن الجاف وبالتالي زيادة إنتاج الدهون.

المحكمـــــون:

١- أ.د. عزيز محمد غريب حجازي أستاذ الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة القاهرة.

٢- أ.د. حسن إبراهيم عبدالفتـــاح أستاذ الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة الزقازيق.