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EFFECT OF NITROGEN STARVATION ON SOME METABOLIC ACTIVITIES OF *OSCILLATORIA ACUTISSIMA* KUFFERATH AND *SCENEDESMUS OBLIQUUS* (TURP.) KÜTZ. WITH SPECIAL REFERENCE TO INDUCED CHANGES IN FATTY ACIDS PATTERN.

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Abstract

Oscillatoria acutissima (cyanobacteria) and *Scenedesmus obliquus* (green alga) were cultured under nitrogen starvation conditions and evaluated for growth and some metabolic activities. Under nitrogen starvation condition pigments, protein, saponification values and lipase activity were decreased, while carbohydrate contents were increased for tested organisms. At the same time, iodine number increased in *O. ocutissima*, and decreased in *S. obliquus*. Infera red analysis showed reduction in the concentration of amino group in lipid materials of both organisms. However, GLC analysis cleared that nitrogen was significantly reduced in the lipid materials. The intra-cellular fatty acids of *O. acutissima* showed reduction in the total saturated fatty acids and increase in the total unsaturated acids, meanwhile *S. obliquus* recorded the opposite trend.

Key words: *Oscillatoria acutissima*- *Scenedesmus obliquus*- Metabolic activity- Fatty acid contents.

Introduction

Cyanobacteria and green algae are ancient and divers groups of photosynthetic microorganisms which have evolved to inhabit many different environments and indicate a high degree of biological adaptation which has enabled these organisms to thrive and compete effectively in nature. Microalgae contain lipids and fatty acids as membrane components, storage products, metabolites and sources of energy. Algal oils posses characteristics similar to those of fish and vegetable oils, and can thus be considered as potential substitutes for the products of fossil oil (Princen, 1982). Lipid material associated with cyanobacteria and green algae is indicative of chemical diversity, which is an essential substance in the production of energy and participated in different industrial and pharmaceutical field. Some lipids are actively involved in photosynthetic process. For these reasons, the metabolism of these compounds

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has been continued to be studied in algae (Sato and Murata 1982 a, b and Emdadi and Berland 1989).

Pharmaceutical interest and limited availability of γ- linolenic acid (GLA, 18:3 w6) (Cohan *et al.*, 1987) and eicosapentoenic acid (EPA, 20:5 w3) (Bimbo, 1987) has prompted the search for genetic means for increasing the production of these fatty acids from algal sources. γ- linolenic acid is known to be useful in the treatment of various diseases. Simpoulos (1995) found that microalgae were a good source for omega-3-fatty acids, which are protective factor against chronic diseases, coronary-heart diseases, diabetes and cancer.

Lipid and fatty acid contents of microalgae vary in accordance with culture conditions in some cases. Lipid content can be enhanced by the imposition of nitrogen starvation or other stress factors. Morris and Clarke (1978) observed that *Chlorella emersonii* under sub optimal concentration of nutrients accumulated lipid and became more resistant to the damage caused by freezing and thawing. *Oscillatoria acutissima* and *Scenedesmus obliquus* were included in this study because preliminary analyses showed that they have above average levels of lipid. The present work is intended to estimate the growth and some metabolic activities of the selected two species and characterization of the different fatty acids under nitrogen starvation conditions.

Materials and Methods

Organisms and culture conditions

The tested species, *Oscillatoria acutissima* Kufferath and *Scenedesmus obliquus* (Turp.) Kütz., were isolated from Egyptian soil at Tanta city and identified according to Prescott (1962 and 1975). Cultures of *Oscillatoria acutissima* and *Scenedesmus obliquus* were grown in the media described by (Allen and Stanier, 1968 and Kuhl, 1962), respectively under nitrogen starvation condition. The cultures were incubated under continuous fluorescent light of 6000 Lux at 30^{o}C and supplied with a sterilized mixture of 97% dry air and 3% CO_2. The following determinations were carried out after 13 days of incubation for *O. acutissma* and after 8 days for *S. obliquus*.

Metabolic activity

The spectrophotometric method recommended by Mckinney (1941) was used for estimation of chlorophyll a, b and carotinoids. Phycobiliproteins were determined according to the method described by Bennett and Bogorad (1973).

Total soluble proteins were quantitatively determined according to Lowry *et al.* (1951). Total carbohydrates were quantitatively determined by the method of Phenol-Sulphoric acid described by Kochert (1973). Starch content of *Scenedesmus obliquus* was measured according to Takeda and Hirokawa (1978). Lipase activity was assayed according to Sierra (1957) and Hankin and Anagnostakis (1975). The iodine number was determined according to the method described by Said *et al.* (1964). Saponification value was estimated by the method

reported by Association Agricultural Chemists (1960). An elemental analyzer (Perkin-Elmer 2400 CHN) was used for measuring C, H and N by the thermal conductivity for each gas then the percentages of element were measured automatically. The molecular structure of the lipid material was partially identified using Perkin Elmer 1430 infrared spectrophotometer. The measurements were carried out at infrared spectra between 500- 4000 nm. Fatty acids analysis was performed on a Hewlett-Packard 5880 gas liquid chromatography (GLC) (Chman and Jangaard, 1973).

Statistic analysis

The averages were statistically analyzed by using P-value to accept the significance of the tested effect if P-value ≤ 0.05.

Results and Discussion

Data concerning changes in pigments content of *O. acutissima* and *S. obliquus* as affected by nitrogen starvation after 13 and 8 days respectively are present in Tables (1 and 2). Chlorophyll a content was reduced by about 10% and phycobiliprotein by 5% in *O. acutissma*; however carotenoids content showed significant increase (31%).

Table (1): Pigments content of *O. acutissima* under control and nitrogen starvation condition measured as µg/ml algal suspension.

Culture	Chl.a (µg/ml)	Carot. (µg/ml)	Phycobiliprotein (µg/ml)			
			PC	PE	APC	Total
Control	2.30 ±0.42	1.02 ± 0.16	$1.25 \times 10^2 \pm 3.2$	$0.086 \times 10^2 \pm 2.8$	$1.13 \times 10^2 \pm 2.8$	$2.48 \times 10^2 \pm 7.35$
N-starved	2.07 ±0.16*	1.34 ± 0.04*	$1.05 \times 10^2 \pm 4.1$	$0.266 \times 10^2 \pm 3.5$	$1.06 \times 10^2 \pm 4.1$	$2.35 \times 10^2 \pm 6.80^{NS}$

Anent *S. obliquus*, the results revealed that nitrogen starved cultures showed significant decrease in chlorophyll a (32%), chlorophyll b (14%) and significant increase in carotenoids content (34%). These observations are in agreement with the results obtained by Gordillo *et al.* (1999) who mentioned that nitrogen limitation condition caused reduction in pigment contents (decrease as a total), while one type of pigments (carotenoids) is increased. Piorreck *et al*. (1984) concluded that chlorophylls content of the cells were dropped with decreasing nitrogen levels indicating a rapid reduction or even breakdown of the whole chloroplast apparatus.

Table (2): Pigments content of *S. obliquus* under control and nitrogen starvation condition measured as µg/ml algal suspension.

Culture	Chl.a (µg/ml)	Chl.b (µg/ml)	Carot. (µg/ml)	Chl.a/b (µg/ml)	Chl a + Chl b (µg/ml)
Control	6.06 ± 0.76	4.22 ± 0.41	1.65 ± 0.11	1.44 ± 0.13	10.28 ± 0.39
N-starved	4.11 ± 0.46*	3.63 ± 0.37*	2.21 ± 0.31*	1.13 ± 0.03*	7.74 ± 0.28*

Each value is the mean of three replica ± stander deviation * Significant at P ≤ 0.05 N.S. non-significant Chl.a = Chlorophyll a Chl.b = Chlorophyll b Carot. = carotenoids PC = c-phycocyanin APC = allophycocyanin PE = c-phycoerthrin,

Also, Vergara and Niell (1993) reported that, phycobiliprotein was drastically decreased under nitrogen limitation, indicating that phycobiliprotein is mobilized towards the synthesis of non-pigmented proteins rather than pigmented ones.

As clear from data recorded in Table (3), nitrogen limitation led to the suppression of total soluble proteins by about 31% for *O. acutissima* and 33% for *S. obliquus*, compared to control. The reduction in protein synthesis could be attributed to inhibition of the protein synthesis at the initiation stage of growth as concluded by Mukamedzhanov *et al*. (1991), degradation of granules containing protein (Reed *et al.* 1984) or loss of polysomes as indicated by Rhodes and Matsuda (1976).

Table (3): Total soluble proteins, carbohydrates, iodine number (IN) and saponification value (SV) of *O. acutissima* under control and nitrogen starvation condition.

Culture	Protein (µg/ml)	Carbohydrates (µg/ml)	IN (ml/g)	SV (ml/g)
Control	271.67 ± 6.41	147.33 ± 231	119.87 ± 2.18	11.13 ± 0.65
N-starved	187.67 ± 7.51*	161.67 ± 3.51*	248.17 ± 2.35*	8.30 ± 0.70*

Nitrogen starvation condition induced significant increase in total soluble carbohydrates of *O. acutissima* and *S. obliquus* by 10 and 24%, respectively (Table 4). These results are in accordance with that obtained by Chu *et al*. (1995) who found that total carbohydrates increased in cells of *Nitzschia inconspicua* Grunow, grown under nitrogen starvation condition. Also, starch content of *S. obliquus* increased by 22% under nitrogen starvation condition.

This increase in carbohydrate content in response to nitrogen starvation could be attributed to the fact that photoassimilation of carbon was redirected towards the synthesis of carbohydrates instead of protein and chlorophyll, as indicated by Gordillo *et al*. (1999).

Iodine number of the extracted lipid material showed significant increase in case of *O. acutissima* (1.1 fold above the control value) and significant reduction with *S. obliquus* (43%) under nitrogen starvation condition (Table 3 and 4), which indicate that nitrogen starvation caused increase in the total unsaturated fatty acids of *O. acutissima* more than the control. On the other hand the reduction in the iodine number of the lipid material of *S. obliquus* may indicate that nitrogen starvation causes increase in the saturated fatty acids of this organism. These results were confirmed by gas liquid chromatography (GLC) technique.

Table (4): Total soluble proteins, carbohydrates, starch, iodine number (IN) and saponification value (SV) of *S. obliquus* under control and nitrogen starvation condition.

Culture	Protein(µg/ml)	Carbohydrates (µg/ml)	Starch(µg/ml)	IN (ml/g)	SV (ml/g)
Control	143.33 ± 7.41	108.00 ± 3.00	46.73 ± 1.17	134.53 ± 3.26	16.49 ± 1.82
N-starved	96.00 ± 8.54*	134 ± 2.08*	56.80 ± 1.25*	77.10 ± 2.14*	11.17 ± 0.91*

Results indicated that, nitrogen starved culture media caused significant reduction in saponification values of *O. acutissima* and *S. obliquus* by 25 and 32%, respectively (Table 3 and 4). The saponification value gives an indication of the nature of the fatty acids in the fat since the longer the carbon chain the less acid is liberated from the hydrolyzed fats (Plumer, 1978). Thus, it could be concluded from this result that nitrogen starvation induced the synthesis of long carbon chain fatty acids in both organisms

Lipase activity of *O. acutissima* and *S. obliquus* showed significant reduction (13 and 49%, respectively) in response to nitrogen starvation (Table 5). These results indicate inhibition of lipase activity in response to nitrogen starvation as suggested by Jonsson and Snygg (1974).

Table (5): Lipase activity of *Oscillatoria acutissima* and *Scenedesmus obliquus* grown under nitrogen starvation condition measured as µM tween 20 decomposed/ 72 h /ml algal suspension.

Culture	Lipase (µM tween 20 decomposed / 72 h / ml)	
	O. acutissima	***S. obliquus***
Control	**2.94 ± 0.025**	**2.91 ± 0.03**
N-starved	**2.60 ± 0.02***	**1.95 ± 0.03***

Each value is the mean of three replica ± stander deviation. * Significant at P ≤ 0.05

Elemental analysis was carried out to reach the nearest chemical structure of the extracted lipid of tested algae. The result showed increase in hydrogen (3.5%) and carbon (10%) of *O. acutissima* under nitrogen starvation condition, whereas, nitrogen was represented by a small percentage (0.06%) (Table 6). On the other hand, the lipid material of *S. obliquus* showed reduction in carbon (8.5%) and increase in hydrogen (2%) under nitrogen starvation condition, at the same time nitrogen was present by 0.03% in the lipid. This observation is in agreement with the results obtained by Roessler (1988), who reported that the changes in the levels of the principal nutrients such as carbon and nitrogen can result in marked changes in the biochemical composition of fatty acids of algae.

Table (6): Percentage of C, N, and H in the lipid material extracted from *Oscillatoria acutissima* and *Scenedesmus obliquus* under nitrogen starvation condition.

Species	Culture	Carbon (C %)	Nitrogen (N %)	Hydrogen (H %)
O. acutissima	**Control**	**69.83**	**0.75**	**7.21**
	N. starved	**80.93**	**0.06**	**10.73**
S. obliquus	**Control**	**62.18**	**0.38**	**8.64**
	N. starved	**53.69**	**0.03**	**10.25**

The IR spectroscopy (Table 7) indicated the presence of different classes of lipid such as: hydrocarbons, phosphorus, sulpher, ester, anhydrides, ethers,

aldehydes and amines. These compounds varied greatly under nitrogen starvation condition. Such variations were confirmed by some authors (Harvey *et al*., 1988; Cranwell *et al.*, 1990; and Mohammady 2001).

Table (7): Lipid classes of *O. acutissima* and *S. obliquus* analyses by infrared (IR) after 13 days of incubation under control and nitrogen starvation condition.

Lipid classes	Absorption mode	Frequency (cm^{-1})	*O. acutissima*		*S. obliquus*	
			C	N-starvation	C	N-starvation
Hydrocarbon						
Alkane	**Asymmetrical CHbending**	**1450 ± 20**	+	+	-	+
Alkenes	**CH stretching**	**2929 ± 10**	+	+	+	+
Phosphorus						
Phosphonates	**P-C stretch**	**750 – 650**	+	+	-	+
Phosphates	**P=O stretch (bonded)**	**1250 – 1200**	+	+	+	+
Free acids	**O=P-OH**	**2700 - 2500**	-	-	-	-
Sulphur						
Sulphonate	**S=O stretch**	**1420 – 1330**	-	-	+	+
sulfates	**S-O stretch**	**1040 - 760**	+ +	+ + +	+	+ +
Ester						
α-β unsaturated	**C-O stretch**	**1730 – 1717**	+	+	-	+
β ketoesters	**C-O stretch**	**1650 - 1540**	+	+	-	-
Anhydrides	**C-O stretch**	**1170 – 1050**	-	-	+	-
Ethers						
Aryl ether	**C-O stretching**	**1270 – 1230**	-	-	-	+
Alkyl ether	**C-O stretching**	**1150 - 1060**	-	-	-	-
Alcohols						
Primary	**O-H stretching**	**3650 – 3590**	+	+	+	+
secondary	**C-O stretching**	**1120 - 1110**	-	-	-	-
Aldehyde						
Saturated	**C=O vibrations**	**1740 – 1720**	+	+	-	-
α-β unsaturated	**C=O vibrations**	**1705 – 1680**	-	-	-	-
Amines						
Primary	**NH stretch**	**3500 – 3300**	+	-	+	+
secondary	**NH deformation**	**1650 - 1550**	+	+	+	-

- =No bands detected +=single band detected ++=two bands detected +++=three bands detected

Table (8) shows the variations of profiles of extra- and intra-cellular fatty acids of *O. acutissima* and *S. obliquus* under nitrogen starvation condition. The fatty acids composition of extra-cellular contents of *O. acutissima* were assigned to chain length varying from C14 to C20, whereas C12 to C18 were observed with *S. obliquus*. This result was coincided with Dowiadar (2002) who mentioned that the filtrate of *Anabaena oryza* contained seven fatty acids including saturated and unsaturated ones. The results cleared also that saturated fatty acids were more dominant than unsaturated one for both algae. At the same time, saturated fatty acids was dominant by C18:0 (91%). In this connection, Omar (2002) reported that the major saturated fatty acid was C18:0 in cell free media of some

Chlorococcales species. Concerning the extra-cellular mono-and polyunsaturated fatty acids of both tested algae, the results revealed that C16:1 and C18:2 were the major ones. Sriharan *et al*. (1990) indicated that the most common fatty acids synthesized in microalga *Ankistrodesmus* sp. *Botryococcus braunii, Dunaliella* sp. and *Nitzschisa* sp. were C14:0, C16:0, C18:1, C18:2 and C18:3.

Table (8): Fatty acids composition of extra- and intra-cellular lipid of *O. acutissima* and *S. obliquus* (data was expressed as percentage).

Types of fatty acid	Extra-cellular		Intra-cellular			
	O. acutissima	*S. obliquus*	*O. acutissima*		*S. obliquus*	
	Control	Control	Control	N-starvation	Control	N-starvation
Saturated						
C12:0	----	1.65	3.54	1.96	0.11	----
C14:0	0.97	1.3	3.27	2.84	1.06	7.88
C16:0	0.46	2.98	11.45	17.53	0.7	----
C18:0	95.88	91.47	78.35	61.13	70.94	72.26
C20:0	0.39	-----	0.61	2.04	0.11	3.39
C22:0	----	-----	0.52	1.31	0.07	----
Total	97.7	97.4	97.13	86.81	72.99	83.53
Mono-Unsaturated						
C16:1	0.66	0.84	0.42	2.2	25.02	8.24
C18:1	0.37	0.4	0.48	3.71	0.44	2.65
Total	1.03	1.24	0.9	5.91	25.46	10.89
Poly-Unsaturated						
C18:2	0.96	0.87	0.87	2.04	----	2.74
C18:3	0.32	0.51	0.49	5.04	2.16	3.39
Total	1.28	1.38	1.36	7.08	2.16	6.13
Total Unsaturated	2.31	2.62	2.26	12.99	27.62	17.02

---- =No fatty acid detected.

The previous investigations indicated that the values of extra-cellular fatty acid contents of both tested algae were small if compared with intra-cellular lipid contents. Therefore, this study was focused on the intra-cellular fatty acids.

The results show that the intra-cellular fatty acid profiles of *O. acutissima* and *S. obliquus* exhibited different values of C12:0-C22:0 under control and nitrogen starvation conditions. In this regard, Mendoza *et al*. (1996) studied the

composition of the fatty acids of lipid in *Dunalliella salina*, and demonstrated that C14:0 and C18:3 were detected in the lipid material. The most abundant intra-cellular saturated fatty acid was C18:0 for tested algae. C18:0 showed an increase in *S. obliquus* and a reduction in *O. acutissima* under nitrogen starvation conditions. Sriharan *et al.* (1990) suggested that stressful conditions invariably increase the accumulation of some fatty acids. The composition of *O. acutissima* saturated fatty acids was the same as control under nitrogen starvation condition, whereas *S. obliquus* recorded the disappearance of C12:0, C16:0 and C22:0. Piorreck *et al.* (1984) reported that fatty acids composition of cyanobacteria did not changed significantly when nitrogen concentrations in the nutrient medium changed.

The intra-cellular mono- and polyunsaturated fatty acids of both tested algae were represented by C16 and C18. Xu and Beardall, (1997) mentioned that *Dunalliella* species were characterized by the presence of C16 and C18 polyunsaturated fatty acids.

The major intra-cellular unsaturated fatty acids were C18:3 and C16:1 of *O. acutissima* and *S. obliquus*, respectively. Under nitrogen starvation condition the results show an increase of C18:3 in both tested algae. Chu *et al*. (1995) concluded that the changes in the relative amounts of C18:3 may be attributed to effects on the desaturation pathways of fatty acids. C18:3 are known to be useful in treatment of various diseases, so pharmaceutical interest of this acid has prompted the search for genetic means for increasing its production.

The results show that nitrogen starvation induced a reduction in intra-cellular total unsaturated fatty acids and an increase in saturated ones of *S. obliquus*. Xu and Beardall (1997) reported that the reduction in polyunsaturated fatty acid fractions and increase in the saturated ones might be due to reduction in membrane fluidity and permeability.

As described above, the metabolism of lipids and fatty acids production in *O. acutissima* and *S. obliquus* could be regulated and controlled by nitrogen starvation.

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تأثيرالحرمان من النيتروجين فى الوسط الغذائى على بعض الأنشطة الأيضية لطحلبى الأوسيلاتوريا أكيوتسما و السينيديسمس أوبليكس مع اشارة خاصة الى التغيرات المستحثة فى أنماط الأحماض الدهنية.

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قسم النبات - كلية العلوم - جامعة طنطا.

تم فى هذه الدراسة زراعة طحلبى الأوسيلاتوريا أكيوتسما (سيانوبكتريا) و السينيديسمس أوبليكس (طحلب أخضر) تحت ظروف نقص النيتروجين فى الوسط الغذائى لكليهما ، مع دراسة النمو و بعض الأنشطة الأيضية لكلا الطحلبين. و قد أثبتت الدراسة أن الوسط الغذائى الخالى من النيتروجين أدى إلى نقص فى الأصباغ ، و البروتين ، و درجة التصبن ، و نشاط أنزيم الليبيز ، و إلى زيادة فى محتوى الكربوهيدرات فى كلا الطحلبين. كما أدى نقص نقص النيتروجين فى الوسط الغذائى إلى زيادة فى الرقم اليودى لطحلب الأوسيلاتوريا أكيوتسما و نقص فى طحلب السينيديسمس أوبليكس. كما أظهر التحليل بالأشعة تحت الحمراء (IR) انخفاض تركيز مجموعة الأمين فى المواد الدهنية المستخلصة من خلايا الطحلبين.أما التحليل بوساطة جهاز كروماتوجرافى الغاز السائل (GLC) فقد أظهر نقصا ملحوظا فى النيتروجين الموجود بالمواد الدهنية لكلا الطحلبين. و فى نفس الوقت سجلت الأحماض الدهنية المنتجة خارجيا بواسطة الاوسيلاتوريا أكيوتسما – انخفاضا فى محتوى الأحماض الدهنية الكلية المشبعه ، و زيادة فى محتوى الأحماض الدهنية الكليه غير المشبعة ، و ذلك على عكس ما حدث فى طحلب السينيديسمس أوبليكس.