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USE OF SOME COMMERCIAL FERTILIZER COMPOUNDS FOR *SCENEDESMUS* CULTIVATION

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Abstract

The objective of this work was to investigate the effect of some commercial fertilizer compounds on growth yield and characteristics of the isolated alga *Scenedesmus sp.* For pre-cultivation and isolation, the sterilized drainage water was enriched by 10% N8 macronutrients solution. Tap water was used for scaling-up of indoor cultivation,. Then nutrients were added to reach the content of the different nutrients to those of the drainage water plus 10% of N8 macronutrients solution.

Out door cultures were started by Plexiglas aquarium (200 L) followed by open plate unit (1000 L); and ended by open ponds of 15000L.Nutrient solution was composed of tap water enriched by 0.3 $g.l^{-1}$ urea (46.5% N), 0.1$ml.l^{-1}$ phosphoric acid (85%), 0.05 $g.l^{-1}$ SoluPotass (mini.50.0%K_2O & 46.0% SO_3) and 0. I $ml.l^{-1}$ Nitrophoska Foliar (a fluid fertilizer suspension contains 10.0, 4.0, 7.0 and 0.2% N, P, K and Mg; respectively).

The daily measurements of dry weight and total chlorophyll on the average showed that the maximum dry weight was obtained by the 1 1 th day, which was about 0.9$g.l^{-1}$ and of 10.0 mg. l^{-1} of total chlorophyll by such time. Growth in term of dry weight was also characterized by 0.2, 0.09, 7.7, 2.53, 0.18, 0.38 and580 of,$\mu_{max.}$,$\mu_{avr.}$, doubling time, degree of multiplication, daily increment, initial increase, and percentage increase, respectively. As for total chlorophyll accumulation, it was characterized by 0.36, 0.14, 4.95, 4.12, 6.45, 13.88 and 1746 for the aforementioned parameter.

Introduction

The main concept of algae production; as a good light harvesting system; is to produce all the plantarium products except wood and fibers. Production costs were considered as the main reason limits the widespread of algae production. Of these costs, nutrients, harvesting and drying were considered. For instance, carbon represents the single largest nutrient cost in *Spirulina* production and constitutes up to 70% of the total cost of nutrients if sodium bicarbonate is used (Zaborsky, 1985). Furthermore, the relatively low yields of algal biomass obtained in large-scale production sites resulted in a high cost of production which presently prevents extensive use of microalgal biomass as food (Vonshak, 1992). The green algae produced for nutritional purposes contain up to 50% of their dry weight crude protein (El-Fouly *et al.,* 1979) and up to 5% of total pigments on dry weight basis, which composed of 55% chlorophyll a, 35% chlorophyll b and 6% of total cartenoids (El-Fouly *et al.,*1985a). In addition, such chemical composition of algae is very sensitive to both environmental and nutritional conditions (Becker, 1994 and El-Shafey *et al.,* 1999). Of the various fertilizer suspension used, Complesal (t2:4:6 - NPK), Complesal (8:8:6 NPK), Foliar Nitrophoska (10:4:7:0.2- NPKMg) and Wuxal

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(8:8:6-NPK) were traditionally used by El-Fouly *et al.*(1985b). Others of this formula were also widely used normally at 1.0 $ml.l^{-1}$ (Becker and Venkataraman 1982).

Material and Methods

1. Microorganisms and sub-culturing technique

Scenedesmus sp. was isolated from the drainage water obtained from El-Rayan Valley, El-Fayoum Governorate, Egypt. Water sample was filtrated through Whatman 50 filter paper. A part of the sterilized sample was enriched by 10% of N8 macronutrients solution (Soeder *et al.*, 1967) containing KNO_3 substituted by urea as a source of nitrogen (Tablel) with PAZ micronutrients stock solution according to Payer and Trultzsch, 1972 (Table 2) plus 6mM sodium acetate (Kobayashi *et al.*,1992,1993) and incubated under continuous light (100 μe), daily shacked several times.

Table 1. Chemical composition of N8 macronotrients solution.

Chemical	Element	Conc. ($g.l^{-1}$)
$CO(NH_3)_2$	N	0.560
$Na_2HPO_4.2H_2O$	P 0 260	
KH_2PO_4	K	o.740
$CaCl_2$	Ca	0.010
FeEDTA	Fe	0.010
$MgSO_4.7H_2O$	Mg	0.050
Tracc elements*	Al, Mn, Cu and Zn	I $ml.l^{-1}$

The remainder volume was sterilized at 120$lb.in^{-2}$ .The growing culture was microscopically tested and *Scenedesmus* cells were picked-up. Dilution technique for isolation was followed and the picked cells were incubated with the above-enriched medium after sterilization.

Table 2. Chemical composition of PAZ trace elements solution.

Chemical	Element	Conc. ($mg.l^{-1}$)
H_2BO_3	B	30.50
$Na_2MoO_2.2H_2O$	Mo	0.39
$Al_2 (SO_4)_3.18H_2O$	Al	6.70
$MnCI_2. 4H_2O$	Mn	99.00
$CuSO_4.5H_2O$	Cu	500
$ZnSO_47H_2O$	Zn	6.30
$COSO_4.7H_2O$	Co	2.80
LiCI	Li	8.50
NH_4VO_3	V	2.90
$NiSO_4.6H_2O$	Ni	26.30
$(NH_4)_6Mo_7)O_{24}.4H_2O$	Mo	1.8
Kl	I	249.0
KBr	Br	238 0

For indoor scaling-up of the sterilized medium was analyzed, by the methods described by NRC-GTZ, 1998 for both macro and micro-nutrients (Table 3),

reformulated, and, then enriched as mentioned above. When cultures reached the maximum green color, were continuously diluted till 5000 ml and aerated by compressed air.

Table 3. Mineral composition (ppm) of El-Rayan valley drainage water.

Element	N	p	K	Mg	Na	Ca	Zn	Fe	Mn	Cu
ppm	140	1.5	43.2	260	600	270	0.32	0.12	0.18	0.03

Out door cultivation ~ started by Plex-glass aquarium of 40x50xl00 cm (w, h, 1) with a final capacity of 200L. The next growth was scaled-up using an open plate system of 1000 L aerated by air left potential due to pumping system and followed by three open ponds of I5000L for each one, agitated by paddle wheal at 24 round per minute. The medium composed of tap water enriched by low priced commercial fertilizer compounds. Fertilizer compounds and their concentrations used are listed in Table 4.

Table 4. Commercial fertilizer compounds and their concentrations used.

Element	**N**	**P**	**K-S**	**Fe-S**	**NPKMg***
Source	Urea (46.5%)	H_3PO_4 (84.0%)	SoluPotass 50.0%K_2O 46.0%SO_3	$FeSO_4.7H_2O$	Nitrophoska Foliar
Conc.l^{-1}	**0.3g**	**0.1ml**	**0.05g**	**0.001g**	**0.1ml**

*Nitrophoska foliarl (10.0: 4.0: 7.0 : 0.2 %N: P: K :Mg)

2. Measurements

2.1 Dry weight determination:

Five ml of algae suspension were filtered through pre-weighed dried membrane filters (0.45µm. white grade). Filters were dried at 105°C for two hours and cooled over anhydrous calcium chloride and re-weighted. The biomass production during a particular time period *(e.g.* 24 hrs.) is the result of variation of the biomass content in alga growth.

2.2. Pigment analysis

Chlorophyll of fresh cultures was extracted by DMSO (95%) according to Burnison 1980). The extract was measured at 666 nm according to Seely *et al.* (1972).

3. Growth characterization

Growth was characterized by the equations listed by Pirt 1973.

Results and Discussion

Dry weight of laboratory grown *Scenedesmus sp.* culture (Fig. 1 a) was proportionally associated with the advancing time. Cells reached their maximum dry weight after 11days of incubation. The daily increase might be attributed to enrichment of water sample by N8 medium.

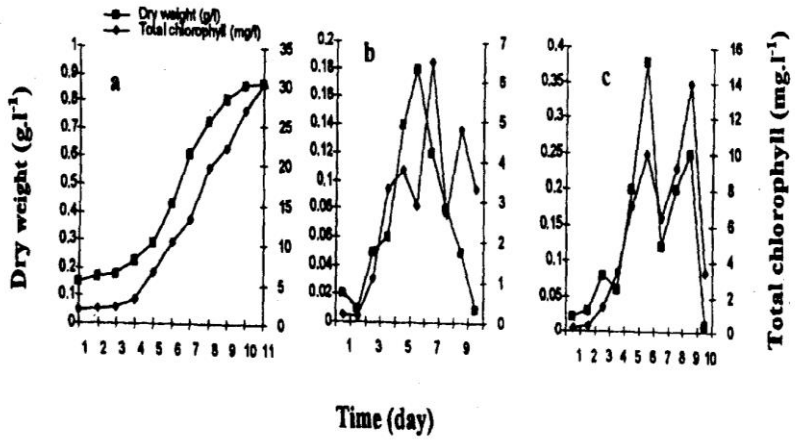

Fig.l. Growth measurements (a), daily increment (b) and initial increase (c) of laboratory grown *Scenedesmus sp.* measured as dry weight ($g.l^{-1}$) and total chlorophyll ($mg.l^{-1}$).

Concerning growth evaluation, cells reached their maximum growth rate (μmax.) as 0.2g d^{-1}compared with 0.09 $g.d^{-1}$ on the average ($\mu_{avr.}$). Doubling time (g) under these conditions was 7.7 and the degree of multiplication (n) was 2.53 suggesting 580% of percentage increase compared with the initial biomass at zero time. The maximum increment per day was found to be 0.18 $g.d^{-1}$ by the 11th day (Fig. 1 b).

Table 5. Growth characteristics of laboratory grown *Scenedesmus sp.*

$\mu_{max.}$	$\mu_{avr.}$	g	n	Xn-Xn-1	Xn-Xo	
Dry weight ($g.l^{-1}$)						
0.2	0.09	7.7	2.53	0.18	0.38	580
Total chlorophyll ($mg.l^{-1}$)						
0.36	0.14	4.95	4.12	6.45	13.88	1476

By such time, cultures reached their maximum increment (0.38 g) as compared with the initial biomass at zero time (Fig.1c). Laboratory data showed that *Scenedesmus* cells required a long adaptation time (about 4 days) to reach their normal growth. This could be attributed to the nutrient deficiency of the given media.

As for total chlorophyll, the rate of accumulation was found to be associated with the rate of dry weight accumulation. Such rate was tended to decrease after 20 days of incubation. The lack of growth might be due to consumption of the initial nutrients especially nitrogen (El-Fouly *el al.*, 1984 and 2000). However, $\mu_{max.}$ value was high when compared with those values of dry weight and resulted the same pattern of dry weight characteristics except doubling time.

During out door cultivation, the lack of growth in term of dry weight was observed within the early fourth days of incubation. This could be ascribed to the

nutritional status of the growth media with long adaptation potential to the given conditions. The next growth period possesses a linear growth curve and cultures resulted the maximum dry weight by the 11th day and it was about 0.9 g.l^{-1} (Fig.2a).

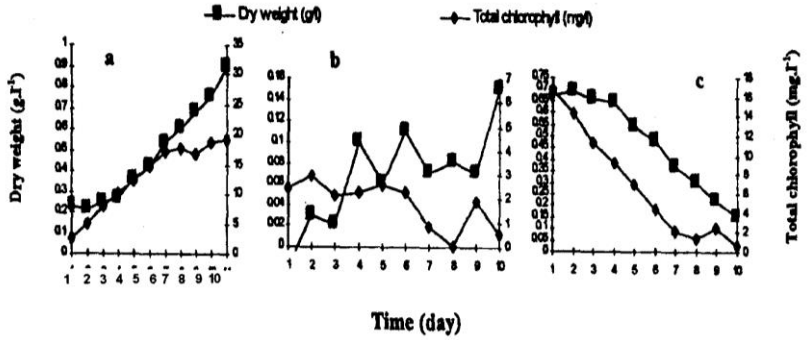

Fig.2. Growth measurements (a), daily increment (b) and initial increase (c) of outdoor grown *Scenedesmus sp.* measured as dry weight (g.l^{-1})and total chlorophyll (mg.l^{-1}).

The enhancement of growth by time (11days) as compared by indoor cultivation might be attributed to the high initial biomass at zero time parallel with high nutrient supplementation from fertilizers used. The main pattern of dry weight increment per day was found to be proportional to the advancing age except by the third day.

Growth in term of total chlorophyll presented a linear growth pattern. In all cases, the maximum value of growth was reached by the 11th day at 19.08 mg.l^{-1}. The maximum increase of chlorophyll accumulation per day was observed by the second day of cultivation, while the decline was observed by the seventh day. Cells thereafter tended to accumulate chlorophyll by a sigmoid curve (Fig.2a).

Data of growth characteristics (Table 6) as compared with such indoor data resulted in the variable pattern. In addition cells seem to successfully grow under out door conditions.

Table 6. Growth characteristics of laboratory grown *Scenedesmus sp.*

$\mu_{max.}$	$\mu_{avr.}$	g	n	Xn-Xn-1	Xn-Xo	
Dry weight (g.l^{-1})						
0.33	0.14	4.95	1.97	0.13	0.68	197
Total chlorophyll (mg.l^{-1})						
0.68	0.21	3.30	2.97	28.0	16.09	297

Here, it may be concluded that out door cultivation using commercial fertilizers could be reduce the production costs and details economic calculations are still needed.

Chemical composition of the out door produced alga as shown in Table 7, suggested the successful production by the aforementioned compounds as well as the concentrations used. Such chemical composition was found in agreement with then ex-produced alga under the same out door conditions.

Table 7. Chemical composition of the out door produced alga

Analysis %	I	II	III	Mean
Crude protein	40.81	52.09	51.10	51.00
Ether extract	7.84	7.62	6.71	7.39
Crude fiber	9.79	10.43	9.27	9.83
Ash	9.36	9.06	9.12	9.18
Moisture	4.56	4.25	4.72	4.51
Nitrogen free extract	18.64	16.55	19.08	18.09

Conclusion

It might be concluded that nutrition of higher plants by foliar application technique was advised to avoid the problems generated from different soil reactions. High pH values that enhancing the unavailability of some nutrients in the soil such as Fe, Zn and Mn. In case of algae nutrition, some fertilizer compounds could be successfully employed with the attention of the nutrients source which in turn led to resist the rise of pH values as cells were grown. Such compounds; *e.g.* urea, potassium sulfate and phosphoric acid are recommended.

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إستخدام بعض مركبات الأسمدة التجارية لزراعة طحلب السنيدزموس

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المركز القومى للبحوث – قسم النبات

استخدمت بعض مركبات الأسمدة التجارية لزراعة الطحلب الأخضر من جنس سنيدزموس المعزول من مياه وادى الريان بمحافظة الفيوم لقياس درجة النمو داخل وخارج المعمل وذلك بغرض خفض تكاليف إنتاج الطحلب تجاريا٠

استخدمت مياه وادى الريان بعد التعقيم وإضافة ١٠% من بينة N 8) كمصدر للعناصر الكبرى داخل المعمل٠ وتم إضافة العناصر الكبرى إلى مياه الشرب المعقمة للوصول إلى نفس التركيز مياه وادى الريان ٠ تمت تنمية الطحلب خارج المعمل مرورا بوحدات ٢٠٠-١٠٠٠-٢٠٠٠ لتر إلى الأحواض الأرضية سعة ١٥٠٠٠لتر وكانت بيئة النمو مكونة من٠,٣جم/لتر يوريا (٤٦,٥%) +٠,١سم٣/لتر حمض الفوسفوريك التجارى (٨٥%) +٠,٥حم/لتر مركب سولوبوتاس (٥٠%بو٢ا) +٠,١سم٣ /لتر من محلول النيتروقوسكا٠

دلت النتائج المتحصل عليها أن أقصى وزن جاف (٠,٩حم/لتر) ومحتوى صبغى (١٠ مجم/لتر) بعد ١١ يوم من الزراعة بالأحواض الأرضية (١٥٠٠٠لتر)٠ويتحليل خواص النمو للوزن الجاف خلال فترة الزراعة وجد أنه ٠,٢-٠,٠٩-٧,٧-٢,٥٣-٠,١٨-٠,٣٨-٥٨٠ وذلك لمعدل النمو النسبى والمتوسط وزمن التضاعف ودرجة التضاعف والزيادة اليومية والزيادة الكلية من زمن الزراعة والنسبة المئوي للزيادة على التوالى٠ وبالنسبة للكلوروفيل الكلى كانت٠,٣٦-٠,١٤-٤,٩٥-٤,١٢-٦,٤٥-١٣,٨٨-١٧٤٦بنفس الترتيب السابق٠