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PHYSIOLOGICAL IMPACTS OF DICHLOROPHYNYLDIMETHYL UREA ON SOME GREEN ALGAE SPECIES

Abo El-Khair B. El-Sayed, Mohamed M. El-Fouly and Abd El-Wahab A. Abdel-Maguid

Fertilization Technology Department, National Research Centre

Abstract

Three algae species belonging to *Chlorophyta; i.e Chlorella sp*., *Scenedesmus sp*. and *Haematococcus pluvialis*, were incubated under different concentrations of the photosynthetic inhibitor dichlorophynyldimethylurea (DCMU). Algae were treated with 0, 1, 2, 4 and $8x10^{-5}$M DCMU added to N8 macronutrient solution containing ammonium acetate instead of potassium nitrate. The measured parameters were optical density, cell count, medium reaction (pH), total chlorophyll, total carotenoids, crude protein and lipids as ether extract. Results showed that DCMU inhibited growth as optical density for both *Scenedesmus sp.* and *Chlorella sp. H. pluvialis* exhibited more resistance against all given concentrations. Chlorophyll reached the maximum with lower and moderate levels of DCMU with *Scenedesmus sp.* and *Chlorella sp.* As for *H. pluvialis*, all used concentrations inhibited chlorophyll accumulation. A different manner was observed on carotenoids accumulation, where the decline of chlorophyll was associated with the decomposition of crude protein and rise in carotenoids and lipids content especially with *H. pluvialis*.

Key words: Green algae, Chlorophyta, DCMU, optical density, cell count, total chlorophyll, carotenoids and biochemical metabolites.

Introduction

Unlike higher plants, algae exhibited a wide range of phototropic mode, where they capable to grow under auto, hetero and/or mixotrophic conditions. Under conditions that delay the photosynthetic process *via* CO_2 and chlorophyll, they easily shifted their trophic process to heterotrophic *via* organic carbon sources. Commercial productions of certain algal species are bicarbonate independent instead of carbon dioxide, which constitutes up to 70% of the total cost of nutrients (Zaborsky, 1985).

Since the late of 1950s, new endeavors that aiming to produce healthy food, food additives, fertilizers and an assortment of natural products have been emerged as specialized industries worldwide. The interest in Chlorella is mainly confined to Japan and Taiwan, where the products are chlorella tablets (regarded as health food) or chlorella growth factor, which might able to improve growth in lactic acid bacteria (Richmond and Grobbelaar, 1986). Many attempts were carried out to produce the natural pigments from green algae instead of other common resources such as spinach and the harmful effect from the other synthetic pigments. Dichlorophynyldimethyl urea, the photosynthetic specific and cell

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division inhibitor found to increase the chlorophyll fluorescence due to high photosynthetic pigment contents (Gerovic *et al*., 1998) and as an inhibitor of photosynthetic electron transport between Q, the primary electron transport of PSII, and plastoquinone (Duysens and Amesz, 1962; Izawa, 1968; Doring *et al*., 1969; Rosenberg *et al*., 1972; Renger, 1973; Bose and Hoch, 1981 and Kirilovsky *et al*., 1994).

The present work was conducted to evaluate the physiological impacts of DCMU on growth of three algae species belonging to *Chlorophyta*.

Materials and Methods

Algae and growth conditions:

The locally isolated green algae *Chlorella* sp., and *Scenedesmus* sp. as well as the imported green alga *Haematococcus pluvialis* were laboratory grown under the conditions of N8 macronutrients solution in the presence of PAZ; the trace element mixture (Soeder *et al*., 1967). Axenic cultures were pre-inoculated with N8 medium, exposed from two sides to 90µ.*E*. of light intensity for each one through white cool lamps and aerated by gas stream with about 1.5% of CO_2 in the presence of ammonium acetate to allow mixotrophic growth. When cultures reached their logarithmic growth phase, cultures were centrifuged at 5000 rpm/5min/5^{o}C using HERMLE cooling centrifuge and washed two times using bi-distilled water. The obtained bulk of each alga was incubated with the aforementioned medium in five treatments of DCMU (dichlorophenyldimethyl urea = Diuron – commercial herbicide) dissolved in 95% ethyl alcohol. Treatments were 0.0, 1, 2, 4 and 8x10^{-5}M of DCMU. Control cultures received the initial amount of ethyl alcohol only.

Growth measurements and analysis:

Daily measurements of the optical density, total chlorophyll, total carotenoids, cell count and acid reaction (pH) were performed. Optical density was measured by Dr. Lang photometer. Chlorophyll was extracted by hot ethanol according to Nush (1980) modified by Satory (1982) and calculated by the formula according to Wintermann and de Mots (1965). Carotenoids were determined according to Helbust and Carigie (1983). Cell count was determined by NEUBAUER IMPROVED heamocytometer. Media reaction was measured by CG-718 pH meter. Crude protein and total lipids were determined at zero time as well as at the end of incubation. Crude protein (on dry weight basis) was calculated as total-N x 6.25. Total nitrogen was determined by centrifugation of 5 ml of algal suspension using cooling centrifuge. The supernatant was discarded and the reminder bulk was received 2 ml of concentrated sulfuric acid and quantitatively transferred to micro-Kjeldahl digestion flask. The next procedure was performed as commonly used (Ma and Zauzage, 1942). Lipids were determined by filtering 5 ml of algal slurry over pre-weighted membrane filter 0.456µm. Filters were dried, weighted and raised by petroleum ether till colorless,

and then air-dried and weighted. Lipids percent (ether extract) was the variation between weights.

Results and Discussion

Optical density

Concerning control treatment of each alga, however they started with an initial biomass of about 0.1 $g.l^{-1}$. Cultures of *Scenedesmus sp.* surpass the other examined algae, where 0.3 $g.l^{-1}$ was obtained by the 8^{th} day compared to 0.21 and 0.25 with the green algae *Chlorella sp.* and *H. pluvialis* by such time (Fig. 1).

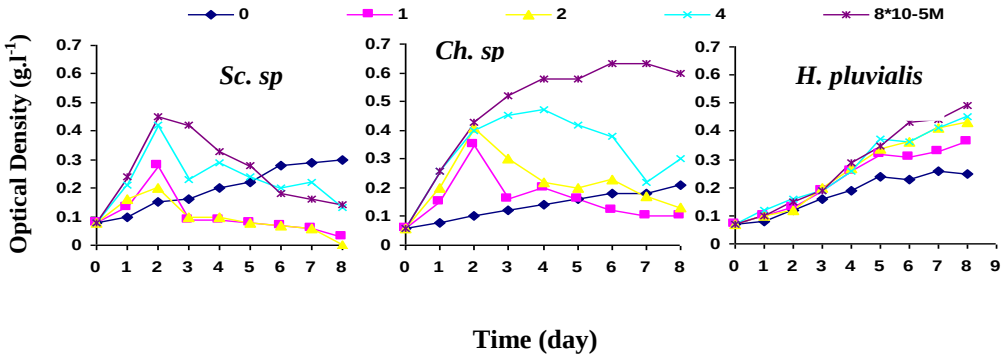

Figure (1): Effect of different DCMU concentrations on growth expressed as optical density

As cultures were fed by different concentrations of DCMU, variable results were obtained due to the examined alga and concentration used. Maximum growth expressed as optical density was obtained with the green alga *Chlorella sp.* by the 6^{th} day with a concentration of $8x10^{-5}$M DCMU which gave 0.64$g.l^{-1}$ followed by 0.55$g.l^{-1}$ of the 3^{rd} day with $4x10^{-5}$M DCMU. As for *Scenedesmus* sp., with all examined concentrations, growth enhancement was only observed during the early three days followed by a fast decline as compared with control cultures in which they also surpass the other examined algae. In case of *H. pluvialis*, liner increases in growth determined as optical density were observed. These increments were found to be associated with the rise of concentration used; meaning that *H. pluvialis* was more adapted to such treatments.

Cell count

Cell divisions of the three examined algae were increased by all the used concentrations. However, the increments of dry weight expressed as optical density could be ascribed to cell volume enlargement. It is well known that, as most herbicides used, DCMU is react as specific cell division inhibitor. Thus, due

to the blocking effect of DCMU on electron transporting chain (Gerovic *et al*., 1998), cells might be shifted to accumulate lipids on the expense of protein and carbohydrate content via heterotrophic growth (Fig.2).

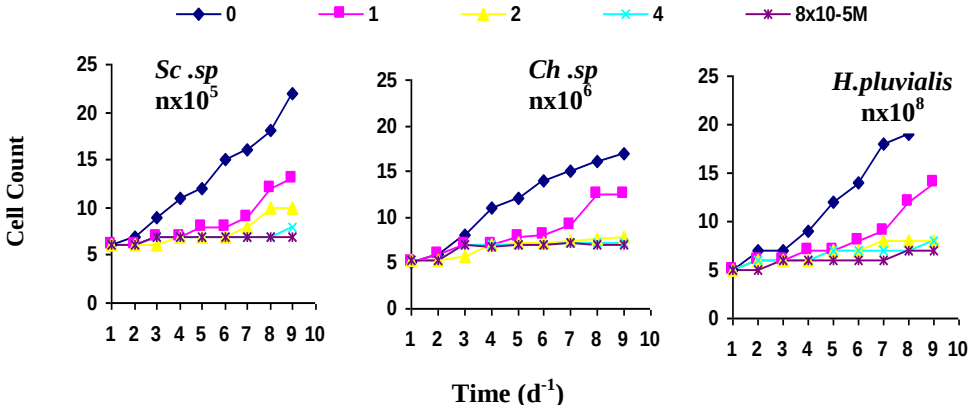

Figure (2): Effect of different DCMU concentrations on growth expressed as cell count

Acid reaction of growth medium

Acid reaction of growth medium normally monitored the growth rate and proportionally shifted to alkaline reaction as growth as progress (Venkataraman 1986). Here**,** stimulation of DCMU causing a slight increases in the media reaction which depending on the examined alga. The rise on pH values was observed with *Scenedesmus sp*. under the higher level of DCMU ($8x10^{-5}$M DCMU), whilst other minor and moderate levels represented the same result of control cultures at the end of cultivation period (pH ranged from 6.91 to 7.13). No specific trend was observed when cells of *Chlorella sp*., was treated by such concentrations of DCMU, but the slight increase of pH values was found to be proportionally with the concentration used. *Chlorella sp* was moderately responded to the different concentrations used. The resistance effect, which leds to the slight increase in pH could be ascribed to the effect of ammonium acetate and acetic acid in growth medium. *H. pluvialis* represented more sensitivity on its growth medium reaction against all concentrations of DCMU and the rise on pH was observed under all concentrations used. The obtained data illustrate the high rate of chlorophyll decomposition with an increase in both dry weight and total carotenoids. This finding is in contrast with Shiraiwa *et al*., 1993, where both of bicarbonate and carbon dioxide in air adapted *Scenedesmus* cells caused rapidly alkalized growth medium of pH over 10 as well as slower in the dark or in the light with DCMU or without dissolved inorganic carbon and O_2 evolution. Also,

the rise of pH during growth can be attributed to the consumption of CO_2 during photosynthesis, Haglund *et al*., (1992).

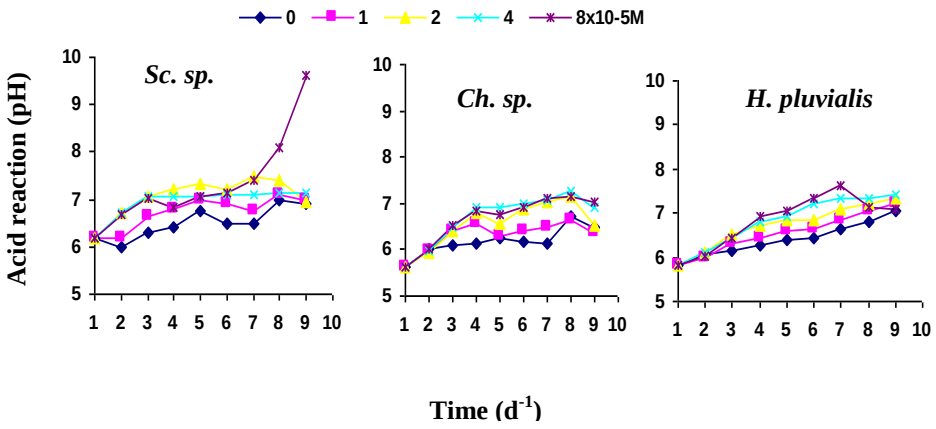

Figure (3): Effect of different DCMU concentrations on acid reaction of the examined algae

Total Chlorophyll

With *Scenedesmus sp*, all of the treated cultures represent specific increase in chlorophyll accumulation due to the given doses. Lower concentration of DCMU reached the maximum increase, while moderate and higher concentrations resulted in lower increases (Fig. 4). *Chlorella sp*., exhibited the same manner, but the initial content was found to be less in comparison with *Scenedesmus sp*. *H. pluvialis* exhibited an opposite manner, where all of the concentrations of DCMU led to drastic inhibition and/or degradation of chlorophyll content.

The results may be ascribed to the inhibitory effect of DCMU on cell division for *Chlorella sp*. and *Scenedesmus sp*. or to its direct effect on chlorophyll decomposition in *H. pluvialis*. In harmony, DCMU interrupting the ETC between PSI and PSII caused both an increase of chlorophyll a fluorescence at 680 nm and a decrease of the 460 nm blue fluorescence of about 17 to 27%. The differences resulted from a stronger re-absorption of blue-fluorescence in the more concentrated cell suspension caused by photosynthetic pigments including chlorophylls and carotenoids, Gerovic *et al*., (1998).

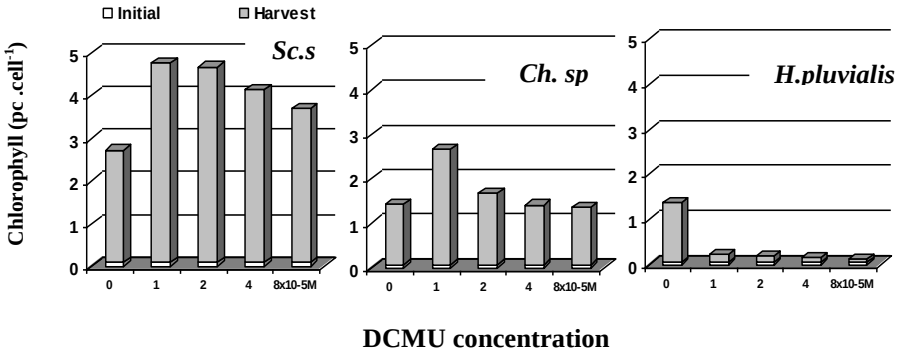

Figure (4): Effect of different DCMU concentrations on growth expressed as total chlorophyll content

Carotenoids

In contrast with chlorophyll results, an increase in carotenoids content was observed only with *H. pluvialis*. Both of *Chlorella sp*. and *Scenedesmus sp*. exhibited an inhibitory effect proportionally to DCMU increase. The stimulatory effect with *H. pluvialis* was due to the specific effect on chlorophyll decomposition and *de-novo* synthesis of specific enzyme(s) mainly phytol desaturase (Fan *et al*., 1994).

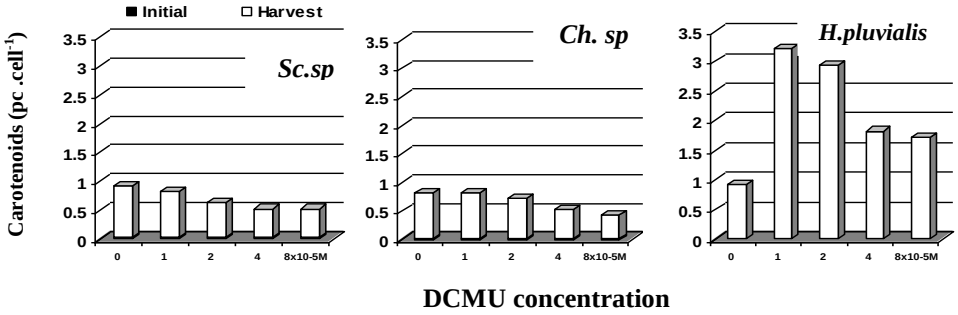

Figure (5): Effect of different DCMU concentrations on growth expressed as total carotenoids content

Bio metabolites

As shown in Table 1, crude protein and total lipids contents were severely affected by the increasing of DCMU especially with *H. pluvialis* in growth medium.

Table (1): Crude protein and total lipids (%) as affected by DCMU concentrations

Alga	0.0		$1x10^{-5}$ M		$21x10^{-5}$ M		$41x10^{-5}$ M		$8x10^{-5}$ M	
	Crude protein (%)									
	*I	**H	I	H	I	H	I	H	I	H
Sc.sp	47.2	48.3	47.2	42.3	47.2	40.5	47.2	38.3	47.2	35.1
Ch.sp.	49.5	48.7	49.5	44.7	49.5	40.2	49.5	36.4	49.5	32.1
H.pluvialis	32.1	33.4	32.1	29.5	32.1	24.2	32.1	21.3	32.1	18.6
Total lipids (%)										
Sc.sp	9.3	9.4	9.3	9.7	9.3	10.2	9.3	11.3	9.3	12.1
Ch.sp.	8.61	8.72	8.61	9.4	8.61	11.2	8.61	11.4	8.61	11.7
H.pluvialis	11.9	11.3	11.9	13.6	11.9	14.2	11.9	15.3	11.9	15.9

*I = initial **H= harvest (end of cultivation period)

The decreasing effect on crude protein was found to be associated with the rise in lipids content. This effect may be due to the general physiological effect of DCMU on weed control by direct bleaching of photosynthetic pigments.

Conclusion

All used concentrations of DCMU inhibited algal growth determined as optical density or cell division and crude protein with an increase in chlorophyll content and lipids. Such effects might be ascribed to the general physiological effect of DCMU as specific cell division inhibitors and blocking the electron transport chain in many ways. Thus, such response could be technically used in plant nutrition for the bio-regarding of residuals herbicides, water cleaning and to obligate cells to produce specific metabolites like phyto-pigments.

Acknowledgment

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التأثيرات الفسيولوجية للداى كلوروفينايل داى ميثايل يورياعلى بعض الطحالب الخضراء

أبو الخير بدوى السيد- محمد مصطفى الفولى – عبدالوهاب عبدالقصود عبدالمجيد

قسم تكنولوجيا التسميد - المركز القومى للبحوث

أضيف مبيد الحشائش (داى كلوروفينايل داى ميثايل يوريا) المعروف بالديوران إلى بيئة N8 لإنماء ثلاثة طحالب خضراء هى:

(*Scenedesmus sp*., *Chlorella sp*. and *Haematococcus pluvialis*)

بتركيزات صفر-1-2-4-8x10^{-5}مول . أضيفت خلات الأمونيوم إلى بيئة النمو لتشجيع التغذية الكربونيه المختلطه فى وجود ثانى أكسيد الكربون (Mixotrophic).

تم قياس النمو يوميا فى صورة الكثافة الضوئية وعدد الخلايا والمحتوى الكلى من الصبغات (كلوروفيل كلى – كاروتينات كلية) والحموضة

. تم قياس النسبة المئوية للدهون والبروتين الخام فى بداية ونهاية فترع التحضين.

ولقد وجد الآتى:

- لم تتأثر حموضة البيئة كثيرا على عكس المتوقع والذى قد يعزى إلى وجود خلات الأمونيوم التى تسمح بتوفر ثانى أكسيد الكربون حرا فى البيئة حيث يعمل على خفض الحموضة لبيئة نمو الطحلب.
- إنخفاض النمو فى صورة الكثافة الضوئية وعدد الخلايا
- إرتفاع نسبة الكلوروفيل الكلى فى طحلبى *Scenedesms* and *Chlorella* مع إنخفاضها فى طحلب *Haematococcus pluvialis* .
- إرتفاع نسبة الكاروتينات الكلية فى طحلب *Haematococcus pluvialis* .
- إنخفاض النسبة المئوية للبروتين فى الطحالب الثلاثة مع إرتفاع النسبة المئوية للدهون .

وعليه يمكن إرجاع هذه التأثيرات إلى الدور الفسيولوجى العام للديوران (الديوران) كمثبط عام لإنقسام الخلايا والتمثيل الضوئى. والذى يمكن الإستفادة منه فى التخلص من بقايا المبيدات وكذلك دفع خلايا الطحالب الخضراء لإنتاج الصبغات المرغوب فيها مثل الكلوروفيل والكاروتينات للأغراض الطبية والصناعية.