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ENHHANCEMENT OF LIPOID AND BIOMASS CONTENTS BY MALONATE INTERFERENCE IN THE METABOLISM OF FIVE GREEN ALGAE

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ABSTRACT:

Optical density, chlorophyll, carotenoids and oil contents were the highest at 10 mM malonate; higher concentrations were inhibitory (with few exceptions). Rates of respiratory oxygen uptake also decreased at malonate concentrations above 10 mM. Dry mass was, however, continually increasing by increased malonate concentrations. Free glycerol contents have not been generally altered by malonate concentrations, with few exceptions. The above-mentioned observations were consistent at all of the studied species (local isolates of *Chlorococcum humicola, Scenedesmus dimorphus, Scenedesmus acutiformis* and *Scenedesmus quadricauda*), except in the dry mass of Chlamydomonas (SAG isolate (Samlung für Algen kulturen).

The inhibitory action on respiration by malonate concentration above 10 mM was accompanied by lipoid decrease. Thus, the hypothesis of competitive inhibition of succinate dehydrogenase (SD) by malonate, with increased acetyl CoA and subsequently triglycerides, is ruled out at malonate concentration above 10 mM. Increased oil yield at ≤ 10 mM malonate resulted from increased culture cell density along with increased oil content per cell. Doubling oil and dry mass contents in one litre (10 mM) of the studied algae roughly costs 0.23€.

Key words: Oils; Dry mass; Malonate; Respiration; Algae

INTRODUCTION:

Malonate is a well-recognized competitive inhibitor of the Krebs cycle enzyme succinate dehydrogenase (SD). Its appreciation as such is credited back to a very long time (Thunberg, 1909). Whether malonate inhibition is strictly specific to SD was debatable throughout some decades since then (Thunberg, 1909 and Hanly *et al,* 1951). Any impact of malonate on glycolysis has not been confirmed (Hanly *et al,* 1951). However, inhibition of SD and other reactions in Krebs cycle by malonate have been proved (Hanly *et al,* 1951; Li and Copeland, 2000 and Kim, 2002); finally, it is accepted as SD inhibitor. The central hypothesis of this work was to block succinate dehydrogenation in Krebs cycle via inhibition of SD. Thus, the accumulation of succinate acts as a feedback inhibitor leading to the accumulation of Krebs cycle acids. Pyruvate concentration is subsequently elevated and the probability to be oxidized by CoA toacetyl CoA, CO_2, NADH is thus increased. Malonate, on the other side, would react with coenzyme A to give malonyl CoA, which is further carboxylated to longer chain fatty acids. Both pathways, either inhibited Krebs cycle cascade or malonyl CoA formation should, theoretically, increase fatty acid contents; with glycerol they form triglycerides in algal cells. In this respect, only few sporadic studies have been conducted and used malonate as a carbon source but without introducing any mechanistic approaches (Mojaat *et al,* 2008).

Algal oils are one of the most reliable and promising alternates for fossil fuels among other forms of renewable fuels under worldwide concentration (hydrogen, ethanol methane, etc.); upon transesterification, oils become biodiesel. Microalgae have been extensively studied for their application in biodiesel production, as they have been reported to accumulate high lipid content ranging from 1 to

85% of the dry cell weight (Chisti, 2007; Chisti, 2008 and Karemore *et al,* 2013). *Scenedesmus sp* and *Chlorella sp* produced high lipid content of 27.4±0.75% and 22.3 ± 0.6% dry weight respectively (Prabakaran and David 2012). Lipid accumulation in algae is caused by stress conditions such as sharp changes in environmental factors and also due to depletion of growth limiting substrates. Limiting the concentrations of N and P in the medium has been reported to increase the accumulation of lipids by restricting the growth of algae (Rodolfi *et al,* 2009; Scott *et al,* 2010; Chen *et al,* 2011 and Karemore *et al,* 2013). Under normal conditions, the lipid content of the *Chlorococcum sp*. was found to be 12±2% (Karemore *et al,* 2013) whereas in the modified media it increased to 15±2%. However, nitrogen starvation resulted in more than two fold increase (35±2%) in lipid content (Karemore *et al,* 2013). Algal oils certainly are cost effective, compared with other higher plants, at least from growth requirements perspective. Algae needs only water, light, carbon dioxide and mineral elements for their growth, enrichment and even blooming. Furthermore, fresh or standard clean water is not necessarily prerequisite but low quality water such as sewage or wastewater is rather better as these waters contain a lot of nutrients. Algae, in addition, have the ability to reduce oxides of carbon, nitrogen and sulphur (CO_X, N_X and S_X; respectively), thus cleaning these waters and turn them more hygienic.

The aim of this work was to shift the metabolism in of studied algae by supplemental malonate for more lipoid accumulation, and unravelling the nature of such interference whether inhibition of SD or direct incorporation into fatty acids. Comparison between the five algal species was also undertaken in respect to oil accumulation efficiency as well as economic feasibility.

MATERIALS AND METHODS:

Four chlorophycean algal species (*Chlorococcum humicola, Scenedesmus dimorphus*, *Scenedesmus acutiformis* and *Scenedesmus quadricauda*) were

isolated from local water bodies at Assiut region (Egypt) and used as the case study organisms in this investigation in addition to *Chlamydomonas reinhardtii* (a gift from SAG). For growth and enrichment, Bold Basal nutritive media (Bold 1949, Bischof and Bold 1963) were used for the growth of *Chlorococcum humicola* and *Scenedesmus* species whereas in the case of *Chlamydomonas reinhardtii* TAP media was used (Goman and Levine 1965). Bottles (500 ml) in triplicates were stoppered with rubber plugs and autoclaved at 1.5 atmospheres for 20 min. After cooling, 400 ml of the autoclaved medium was transferred to each bottle which was, then, inoculated with tested algae at the same optical density (0.2).

The culture bottles were aerated with sterile air and incubated under continuous illumination by fluorescent light of about 20 μmole. $m^{-2}.s^{-1}$ at 28°C for all cultures and let grown for 7 days. Malonic acid concentrations (0, 1, 5, 10, 15, 20 and 25 mM) were added to the algal cultures after being adjusted to pH 7 using KOH. At acidic pH, malonate was severely inhibitory and lethal to the algae, so that all malonate treatments have been conducted at pH 7. The optimum pH for succinic dehydrogenase is between 7 and 8, both substrate and inhibitors may be considered to combine with the enzyme as ions (Hanly *et al,* 1951).

Zhao *et al* (2005) used the optical density (O.D.750 nm) in following up the growth of the different algal cultures. Chlorophylls (Chl.a and b) and carotenoids were measured in methanol extracts according to Marker (1972). Dry mass was estimated in algae collected above glassfibre filters (GF/A) .Respiratory O_2 uptake (R_D) was monitored using a Clark type oxygen electrode in the dark (Oxygraph, Hansatech Instruments Inc., donation from the Alexander von Humboldt foundation, Germany to R. Abdel-Basset). Total lipids were determined gravimetrically in chloroform extracts after evaporation; oil yield was calculated as mg/ml culture whereas oil content as percentage of mg/mg dry mass %. Free glycerol was

determined as done by Chitlaru and Pick (1989).

RESULTS:

Chlorococcum humicola, Scenedesmus dimorphus, *Scenedesmus acutiformis, Scenedesmus quadricauda,* and *Chlamydomonas reinhardtii* have been studied in this work. Their growth was followed for one week under the effect of different malonate concentrations. Growth of control or malonate-supplemented *Chlorococcum humicola* cultures continued to increase for 5 days and levelled off by the 6^{th} day (data not shown). Highest growth magnitude (O.D. 750 nm) was induced at malonate concentrations ranging from 15-25 mM (Table 1). However, chlorophyll content exhibited their highest level at 15 mM mlonate concentration (20% more than the control culture) while higher concentrations of 20 mM were relatively inhibitory by about 11% (Table 1). Carotenoids content in cultures of *Chlorococcum humicola* was increasing as malonate concentration up to 10 mM (Fig 1a). The increased value accounted to about 60%, compared with the control culture (containing no malonate). Concentrations higher than 10 up to 25 mM did not exert any enhancing effect on carotenoids content relative to 10 mM, although it was higher than that of the control (Fig 1a). The yield of lipoids (mg/ml culture) in *Chlorococcum humicola* has been elevated by concentrations from 0-10 mM, attained its maximum level at 10 mM malonate-fed cultures (Fig 1a). The highest oil yield was recorded at 10 mM was 0.16 mg/ml culture accounting to 3.5 times that of the control culture (0.047 mg/ml culture). The high malonate concentrations of 20–25 mM severely inhibited the oil content where they lowered down to the control level (Fig. 1a). Dry mass of *Chlorococcum humicola* have been increased as supplemental malonate concentration was increased; threefold that of the control resulted from the highest (25 mM) concentration of malonate (Fig 1b). Rates of *C. humicola* respiration (oxygen uptake in the dark) at 10 mM malonate were quite similar to that of the control; higher concentrations obviously inhibited respiration (11.4% at 20 mM and further to 25% at 25 mM). The highest percentage of oils in

cells' dry mass (mg/mg dry mass) of *Chlorococcum humicola* was recorded 10 mM malonate while the lowest content was observed under 25 mM malonate concentration (Fig 2).

The growth of *Scenedesmus acutiformis* as optical density (O.D.750 nm) was not affected by the different (1-25 mM) malonate concentrations (Table 1). The highest chlorophyll content was recorded at 10 and 15 mM malonate (Table 1). The concentration of 10 mM induced twice as much as the carotenoids contents of the control culture (Fig 1c). Oil yield in *Scenedesmus acutiformis* cultures has been increased by increasing malonate concentration up to 10 mM at which increased oil (yield) content attained three fold that of the control; higher concentrations were relatively inhibitory (Fig 1c). The dry mass of *Scenedesmus acutiformis* was continually increasing by increasing malonate concentration (Fig 1d); the highest malonate concentration (25mM) induced threefold increase in dry mass yield. Respiration was enhanced by low concentrations of malonate (1-10 mM) while exhibited a relative inhibition at malonate concentrations above10 mM (Fig 1d). Percentage of oils in the dry mass of *Scenedesmus acutiformis* cells increased by lower concentrations of malonate (1 and 5 mM); higher concentrations decreased oil proportion to lower values than that of the control (Fig 2).

In cultures of *Scenedesmus quadricauda* malonate concentrations of 15-25 mM enhanced the final optical density (O.D. 750 nm) of the cultures (Table1); lower malonate concentrations elevated O.D. only at early stages of growth up to 5 days (data not shown). Highest chlorophyll contents in *Scenedesmus quadricauda* has been induced by 15 mM malonate (Table1); higher concentrations caused relative inhibition. Carotenoids content of *Scenedesmus quadricauda* have been elevating by up to 10 mM malonate (40% higher than the control), levelled off at 15 mM while inhibited by a value of 30% at 25 mM (Fig 1e). Lipoid and carotenoids yield of *Scenedesmus quadricauda* were

enhanced by increasing malonate concentrations up to 10 mM which induced the highest content (Fig. 1e). Higher malonate concentrations did not sustain oil levels; otherwise they were markedly inhibitory. Dry mass of *Scenedesmus quadricauda* was not affected by up to 10 mM malonate whereas almost doubled at 15 mM and higher concentrations (Fig 1f). Respiration was lowered by malonate concentrations higher than 10 mM (Fig 1f). Percentage of oils per dry mass of *Scenedesmus quadricauda* was enhanced by increasing malonate concentration that was at 10 mM almost 155% relative to that of the control (Fig 2).

Optical density of *Scenedesmus dimorphus* was slightly similar at all cultures supplemented or not with malonate (Table 1). Total chlorophyll of *Scenedesmus dimorphus*, however, was enhanced by arrange (5–20 mM) of malonate concentrations (Table 1). Carotenoids content of *Scenedesmus dimorphus* was relatively enhanced by 15 and 20 mM malonate (Fig 1g). Lipoid yield was highest in cultures of *Scenedesmus dimorphus* received 10 mM malonate, dropped at 15 mM relative to 10 mM but still was relatively increasing up to 25 mM compared with that of the control (Fig. 1g). Dry mass of *Scenedesmus dimorphus* was obviously enhanced by malonate addition; at highest malonate concentration (25 mM) it became three times that of the control (Fig 1h). Respiration of *Scenedesmus dimorphus* was slightly enhanced by concentrations up to 5 mM malonate but dropped by higher concentrations (Fig 1h). Percentage of lipoids in dry mass of *Scenedesmus dimorphus* was increasing only up to 10 mM malonate, relatively lowered at higher concentrations (Fig 2).

Growth (O.D. 750 nm) of *Chlamydomonas reinhardtii* was not enhanced by malonate; rather, it has been inhibited by malonate concentrations above 1 mM (Table 1). Elevating concentrations of malonate slightly decreased chlorophyll contents in *Chlamydomonas reinhardtii* (Table 1). Carotenoids were maximal at 5 mM malonate (Fig 1i). Lipoid yield in

Chlamydomonas reinhardtii **was highest at 5 and 10 mM malonate (Fig 1i) whereas dry mass of** ***Chlamydomonas reinhardtiii*** **was highest at 10 mM (Fig 1j). Respiration was highest at 5 mM malonate but regularly lowered by increasing malonate concentrations (Fig 1j), so was the percentage of oils on dry mass basis (Fig 2).**

Table (2) shows the percentage of enhancement of oil and dry mass induced by 10 mM malonate at all algal species. More than threefold increase (3.4 fold) in oil yield of ***S. acutiformis*** **was induced by malonate. Higher than twofold increase in** ***C. humicola, S. dimorphus*** **and** ***S. quadricauda*** **whereas only 1.6 in** ***C. reinherdtii*** **is observed. Economic feasibility shows that 10 mM malonate, costing 0.23 €, can double oil and biomass content in one liter.**

Free glycerol content of the five algal specieswas not affected by malonate concentrations except in few sporadic points (Table 1).

DISCUSSION:

Lipoid yield in an algal culture is a product of the cellular content times cell density (the number of cells in a culture) i.e. depends on the above two factors and thus can be increased by enhancing either or both of them (cell multiplication and/or oil proportion in each cell). In this work, malonate was added to five green algal cultures aiming to enhance their oil content and yield. Malonate is a known inhibitor of SD (E.C. 1.3.99.1) that leads to an inhibitory cascade in Krebs cycle reactions leading finally to increasing acetyl CoA, the primary precursor of fatty acids. Or, malonate itself would react directly with CoA giving malonyl CoA. Roessler (1988) and Karemore ***et al*** **(2013) attributed enhancement in lipid accumulation may be to the acetyl-CoA carboxylase activity due to nutrient-deprived condition. In this work, abiphasic role in oil contents and other activities (except the dry mass) has been exerted by malonate, depending on its concentration (10 mM or higher).**

The cellular oil content (percentage of oils in the algal dry mass) was mostly enhanced by 10 mM malonate indicating that most of oil yield at 10 mM comes from both increased cell multiplication as well as cellular contents. Previous studies on the nutrition of *Haematococcus pluvialis* and *Phaffia rhodozyma* have shown that acetate and malonate appear to be an interesting carbon sources, enhancing both growth and carotenogenesis (Borowitzka *et al,* 1991; Orosa *et al,* 2005 and Mojaat *et al,* 2008). This has been explained by the fact that acetic acid and mevalonic acid is key carotenoid precursors (Schneider *et al,* 1977 and Mojaat *et al,* 2008). Knowledge of malonate biosynthesis in plants is limited, although it has been suggested to be formed from malonyl-CoA, which is a biosynthetic precursor of fatty acids and other metabolites, such as flavonoids (Stumpf and Burris, 1981; Li and Copeland, 2000 and Kim, 2002). Presumably, malonate is compartmentalised in the abundant vacuoles in infected and uninfected cells of chickpea nodules (Lee and Copeland, 1994 and Li and Copeland, 2000).

Respiration (oxygen uptake in the dark) was not decreased by ≤10 mM malonate concentrations whereas inhibited by higher concentrations (15-25 mM). Inhibition of respiration might be ascribed to the coherent competitive inhibition of malonate to the Krebs cycle enzyme succinate dehydrogenase (SD) and /or other Krebs cycle reactions. The results indicated that malonate inhibition of respiration via SD or Krebs cycle may occur only at concentrations higher than 10 mM, coinciding with previous findings (Hanly *et al,* 1951; Li and Copeland, 2000 and Kim, 2002). This action (inhibition of respiration at >10 mM malonate) has been accompanied with a decrease in oil contents, which excludes the role of SD and its subsequent series of reactions (feedback inhibition of Krebs cycle, accumulation of pyruvate, oxidation of pyruvate, formation of acetyl CoA and formation of fatty acids) at >10 mM malonate).

Below or at 10 mM, respiratory oxygen uptake was not decreased indicating that enhanced oil accumulation cannot be ascribed to competitive inhibition of SD and the anticipated accumulation of acetyl CoA (precursor of fatty acids) did not occur. Malonate (10 mM) was alternatively incorporated in fatty acid synthesis via forming malonyl CoA and thus acting as a carbon source.

The increasing dry mass is not conditioned by the concentration of 10 mM; it has been continuing to increase as malonate concentration was increased. The continual increase of dry mass up to two times that of the control at the highest malonate concentrations (25 mM) was generally observed at all algal species. However, the magnitude of increase by malonate was species-dependent in the following order: *Scenedesmus acutiformis* (223%), *Chlorococcum humicola* (210%), *Scenedesmus dimorphus* (188%), *Chlamydomonas reinhardtii* (166%), *Scenedesmus quadricauda* (109%). At >10 mM malonate, the algal cells might have acquired their biomass from non-oil components (carbohydrates, proteins, etc.) as oil content decreased under such circumstances or from malonate sequestration in the vacuole. Malonate is toxic for cells at high concentrations; however, at low concentrations it may enhance growth as a carbon source (Orosa *et al,* 2005).

CONCLUSIONS:

As a quick feasibility study, doubling algal biomass with double oil content per litre culture (10 mM malonate) would roughly cost 0.23€. Slade and Bauen (2013) studied cost, energy balance and environmental impacts and future prospects in microalgae. They found that cost estimates need to be improved and this will require empirical data on the performance of systems designed specifically to produce biofuels. Significant (>50%) cost reductions may be achieved if CO_2, nutrients and water can be obtained at low cost.

Apart from the cellular oil proportion and its usefulness as a

biodiesel, algal dry mass, *per se,* is a biomass that can be incinerated, pyrolyzed, gasifiedor wet-oxidized to produce energy (these processes are briefly reviewed in El-Zohri *et al* (2013).

A negative correlation between dry mass and respiration was clearly observed in *Chlorococcum humicola, Scenedesmus acutiformis, Scenedesmus quadricauda* and *Scenedesmus dimorphus* but not in *Chlamydomonas reinhardtii.*

***C. reinhardtii* exhibited some peculiarities compared with other algae as its medium originally contains acetate and thus become acetate-malonate combination.**

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Table 1:- Optical density O.D (750 nm), total chlorophyll (µg/ml algae) and free glycerol (µg/ml culture) at the 7th day, of cultures of *C. humicola, S. acutiformis, S. quadricauda, S. dimorphus* and *C. reinhardtii* under the effect of different concentrations of malonate (0, 1, 5, 10, 15, 20 and 25 mM). Presented values are means of three replicates ±SE.

Algal species / Malonate (mM)	*C .humicola*			*S. acutiformis*			*S. quadricauda*			*S. dimorphus*			*C.reinhardtii*		
	Optical density (750nm)	Total chlorophyll	Glycerol	Optical density (750nm)	Total chlorophyll	Glycerol	Optical density (750nm)	Total chlorophyll	Glycerol	Optical density (750nm)	Total chlorophyll	Glycerol	Optical density (750nm)	Total chlorophyll	Glycerol
0	0.25	8.04 ±0.4	0.020 ±0.0002	0.46	7.05± 0.1	0.032 ±0.0001	0.32	7.63 ±0.5	0.030 ±0.001	0.41	5.97± 0.2	0.030 ±0.0001	0.59	10.28 ±0.82	0.029 ±0.00 02
1	0.25	7.03 ±0.5	0.029 ±0.00 14	0.50	6.14± 0.2	0.033 ±0.0001	0.32	6.98 ±0.4	0.029 ±0.001	0.39	6.00± 0.2	0.030 ±0.0001	0.59	7.07 ±0.45	0.030 ±0.00 04
5	0.32	7.15 ±0.6	0.033 ±0.00 23	0.49	7.14± 0.3	0.033 ±0.0001	0.34	6.57 ±0.2	0.030 ±0.001	0.38	7.07± 0.4	0.032 ±0.0001	0.45	6.41 ±0.45	0.028 ±0.00 01
10	0.38	7.91 ±0.5	0.032 ±0.00 17	0.51	9.61 ±0.2	0.035 ±0.0001	0.41	7.57 ±0.2	0.031 ±0.001	0.41	8.16± 0.4	0.028 ±0.0001	0.37	8.32 ±0.86	0.035 ±0.00 01
15	0.44	9.68 ±0.3	0.033 ±0.00 12	0.50	10.38 ±0.2	0.040 ±0.0001	0.41	9.86 ±0.3	0.030 ±0.001	0.45	7.76± 0.2	0.028 ±0.0001	0.38	7.69 ±0.12	0.030 ±0.00 1
20	0.43	7.93 ±0.3	0.030 ±0.00 064	0.45	7.91± 0.2	0.035 ±0.0001	0.44	7.48 ±0.4	0.033 ±0.001	0.43	7.86± 0.4	0.027 ±0.0001	0.36	8.12 ±0.10	0.033 ±0.00 1
25	0.42	8.43 ±0.2	0.031 ±0.00 16	0.44	8.50± 0.5	0.034 ±0.0001	0.47	7.01 ±0.3	0.029 ±0.001	0.47	7.01± 0.3	0.029 ±0.001	0.33	6.57 ±0.57	0.031 ±0.00 1

Algal specie	*C. humicola*		*S. acutiformis*		*S. quadricaudo*		*S.dimorphus*		*C. reinherdtii*	
Malonate concentrations (mM)	Dry mass	Oil yield	Dry mass	Oil yield	Dry mass	Oil yield	Dry mass	Oil yield	Dry mass	Oil yield
0	0.2 ±0.01	0.059 ±0.001	0.17 ±0.02	0.035 ±0.004	0.21 ±0.02	0.053 ±0.004	0.17 ±0.02	0.056 ±0.01	0.18 ±0.02	0.026 ±0.01
10	0.42 ±0.06	0.16 ±0.09	0.38 ±0.03	0.12 ±0.01	0.23 ±0.02	0.11 ±0.01	0.32 ±0.02	0.16 ±0.01	0.3 ±0.02	0.042 ±0.01
Enhanced level	210%	271%	223%	342%	109%	207%	188%	285%	166%	161%

Table 2:- Dry mass (mg/ml culture), oils yield (mg/ml culture), and percentage of enhanced by 10 mM malonate of *C. humicola, S. acutiformis, S. quadricauda, S. dimorphus and C. reinherdtii* relative to their respective controls. Presented values are means of three replicates ±SE.

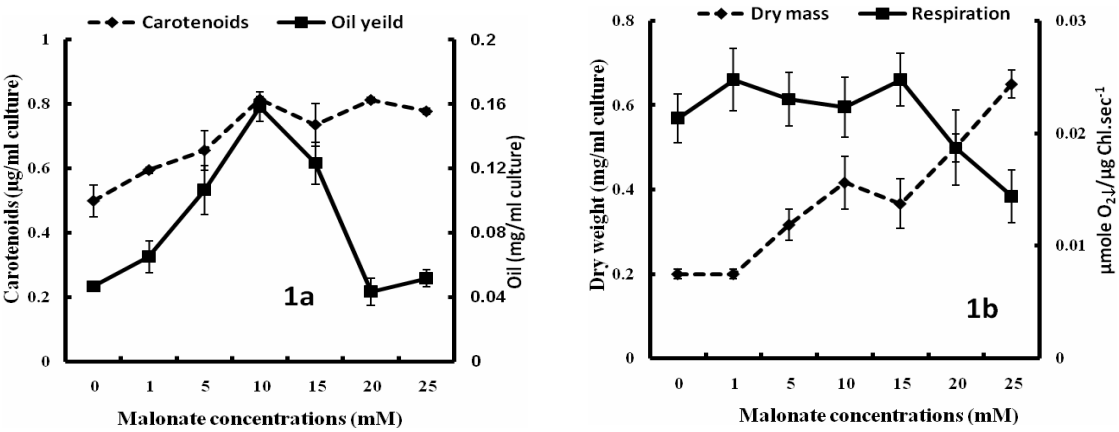

Figure (1a & 1b): Carotenoids (µg /ml culture), oil yield (mg/ml culture), dry mass (mg/ml culture) and respiratory oxygen uptake (µmole O_2↓/µg Chl.sec^{-1}), of *Chlorococcum humicola.* Presented values are means of three replicates ± SE.

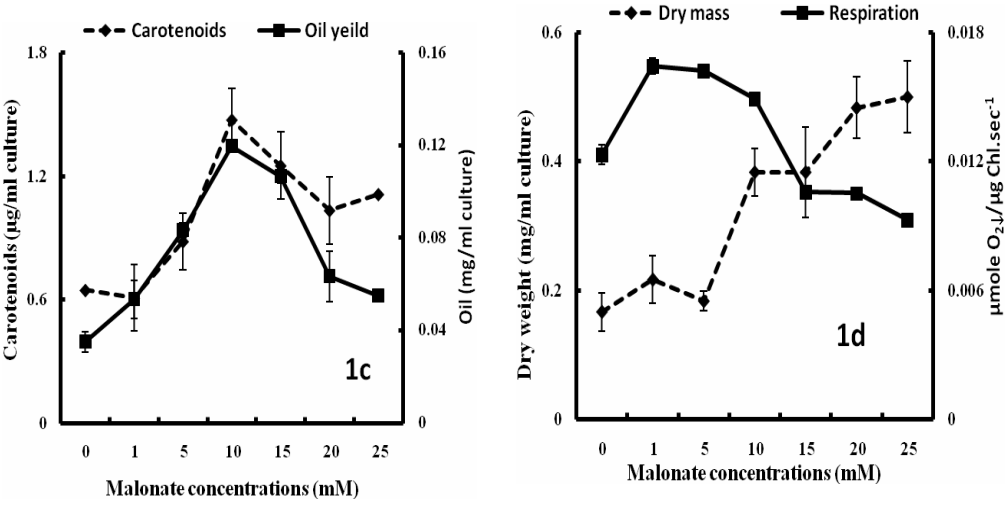

Figure (1c&1d): Carotenoids (µg /ml culture), oil yield (mg/ml culture), dry mass (mg/mlculture) and respiratory oxygen uptake (µmole O2↓/µg Chl.sec-1), of Scenedesmus acutiformis. Presented values are means of three replicates ± SE.

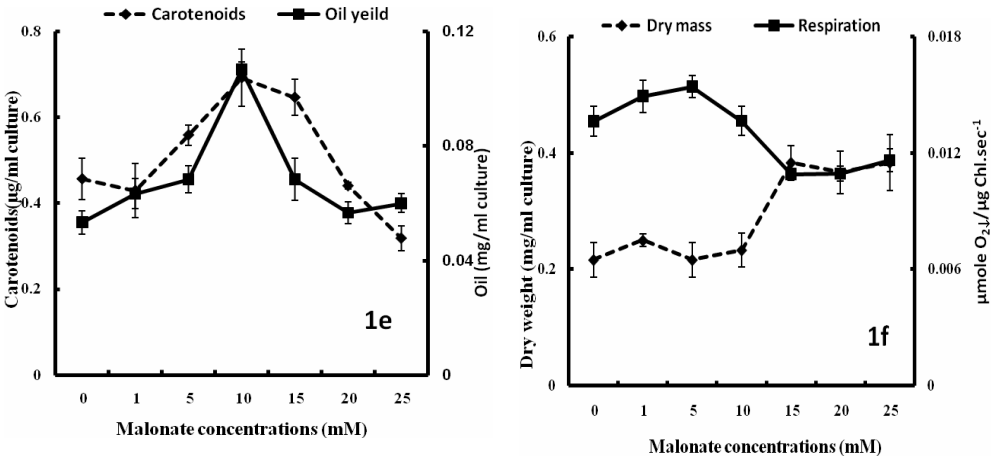

Figure (1e & 1f): Carotenoids (µg /ml culture), oil yield (mg/ml culture), dry mass (mg/ml culture) and respiratory oxygen uptake (µmole O2↓/µg Chl.sec-1), of Scenedesmus quadricauda. Presented values are means of three replicates ± SE.

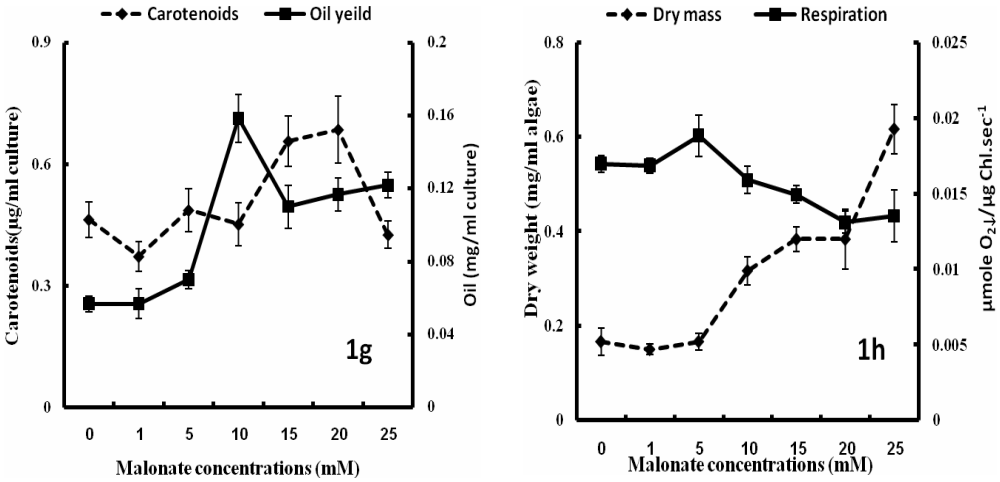

Figure (1g, 1h): Carotenoids (µg /ml culture), oil yield (mg/ml culture), dry mass (mg/ml culture) and respiratory oxygen uptake (µmole O2↓/µg Chl.sec-1), of Scenedesmus dimorphus. Presented values are means of three replicates ± SE.

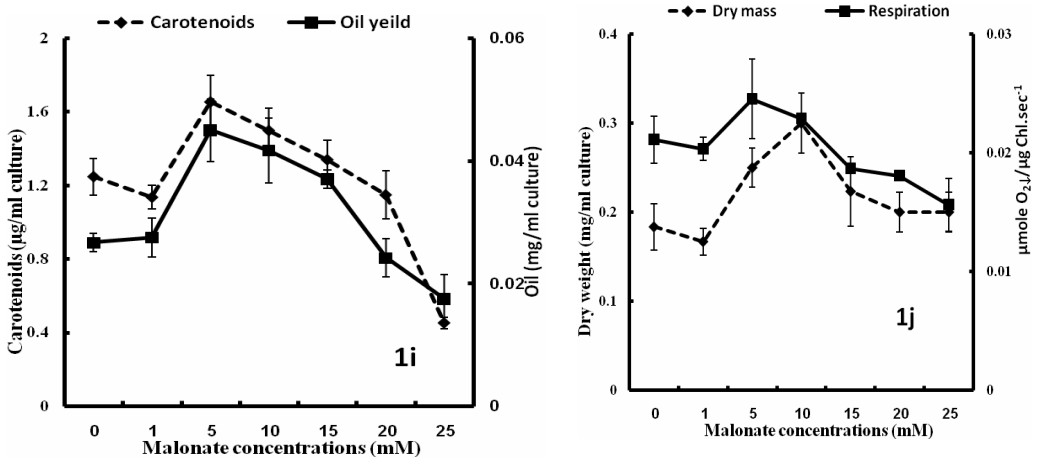

Figure (1i, 1j): Carotenoids (µg /ml culture), oil yield (mg/ml culture), dry mass (mg/ml culture) and respiratory oxygen uptake (µmole O2↓/µg Chl.sec-1), of Chlamydomonas reinhardtii. Presented values are means of three replicates ± SE.

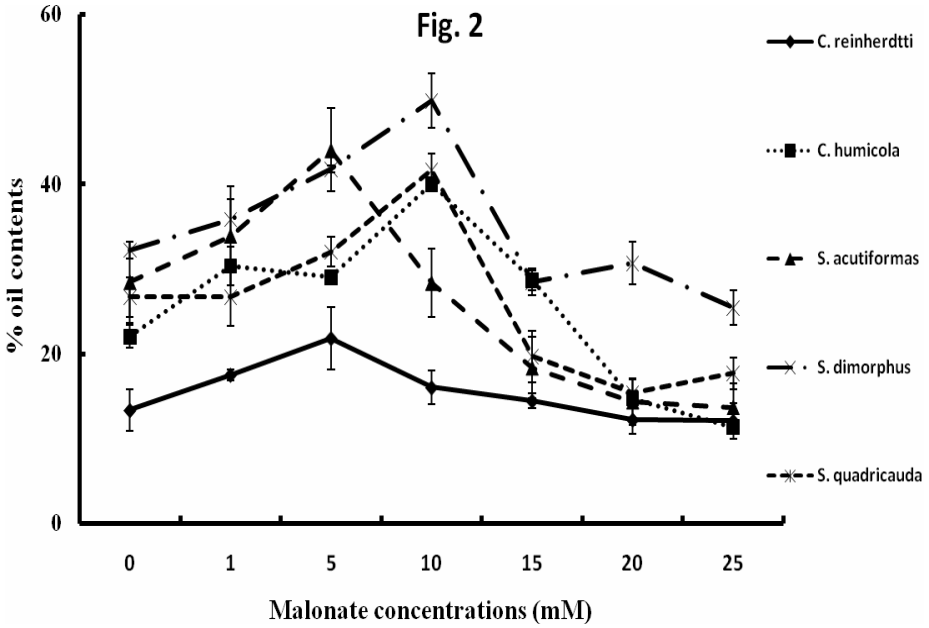

Figure 2: Percentage of oil content on dry mass basis [(mg/mg dry mass) x 100] of *C. humicola, S. acutiformis, S. quadricauda, S. dimorphus* and *C. reinhardtii* as influenced by various malonate concentrations. Presented values are means of three replicates ±SE.

تحفيز محتوى الدهون والكتله الحيوية من خلال تداخل المالونات مع العمليات الحيويه لخمسة طحالب خضراء

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أدت دراسة تأثير تركيزات مختلفه من المالونات الى زيادة كثافة الخلايا و الكلوروفيل والكاروتين و محتوى الزيوت عند التركيز ١٠ ملى مول مالونات بينما تقل مع التركيزات الأعلى (مع قليل من الإستثناءات). كما لوحظ أنخفاض معدل الأكسجين المستهلك خلال عملية التنفس مع زيادة تركيزات المالونات و في نفس السياق استمرار الوزن الجاف فى الزيادة. و على الجانب الاخرلم يتأثر محتوى الخليه من الجلسرول مع زياده تركيز المالونات (مع قليل من الإستثناءات). كل النتائج السابقة تتحقق فى كل الطحالب قيد الدراسه بإستثناء الوزن الجاف لطحلب *Chlamydomonas reinhardtti*.

حيث أن النقص فى معدل الأكسجين المستهلك خلال عمليه التنفس فى التركيزات الأعلى من ١٠ملى مول مالونات كان مصحوبا بنقص فى محتوى الخلايا من الزيوت. لذلك تستبعد نظريه التثبيط التنافسي لإنزيم الساكسينات دهيدروجينيز بواسطة المالونات و التي تؤدى الى زياده استيل كوانزيم أ (CoA) ومن ثم زياده ثلاثى الجلسرول. بينما الزيادة فى محتوى الزيوت عند التركيزات الأقل من ١٠ ملى مول مالونات ناتج من زيادة كثافة الخلايا وبالتالي زيادة محتواها من الزيوت.في ضوء نتائج هذه الدراسة تبين أن تكلفة تضاعف محتوى الخلايا من الزيوت والوزن الجاف فى لتر واحد من (١٠ ملى مول) مالونات تقدر بحوالى ٠,٢٣ يورو.