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Stimulation of biodiesel production from two algae: *Chlorella vulgaris*Berjerinckand*Nitzschiapalea* (Kütz.) Smith,and study their some growth parameters under different light intensity.

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Abstract: *Four different intensities treatments were used to stimulate the biodiesel production from two local isolated algae: Chlorella vulgarisBerjerinck and Nitzschiapalea (Kütz.) Smith. Also the study included the effect of light intensity on growth rates and primary product (carbohydrate and protein). The treatment 300µE\m^2 \sec was recorded the highest lipid content 10% of dry weight (DW), highest carbohydrate content (18% of DW) and highest protein content (58% of DW) for C.vulgaris. While the alga N. paleathe highest values were recorded for lipid content (48% of DW), carbohydrate contents (22% of DW) and 15% of DW for protein content at treatments 300, 125 and 268µE\m^2 \sec respectively. The results revealed that Stearic acid content was increased significantly at the treatments 300µE\m^2 \sec for N. palea while the Oleic acid content increased significantly at 300µE\m^2 \sec for C.vulgaris. The present study recorded Chrysocapsaplanctonica (West&West) Plscher as new recorded to Iraq algae flora.*
Key words*: light intensity, fatty acid, microalgae, Biodiesel, primary product*

I. Introduction

The major disadvantage of using petroleum based fuel is atmospheric pollution. Which are affecting the environment and causing many changes (Klass, 1998).Biodiesel from algae attracted widespread attention in recent years to overcome the expected shortage in a future(Rodolfi*et al*., 2009).

Algae have ability to fixation $CO_2$with high efficiency of 10-15 times greater than other higher plants via photosynthesis process and produced biomass for Biofuels production (Khan *et al*., 2009).Microalgae have been suggested as very good candidates for fuel production because of their advantages of higher photosynthetic efficiency, higher biomass production and faster growth compared to other energy crops and they can grow partially anywhere also use far less water than traditional oilseed crops so there will be no competition with food crops also they are the only feedstock that have the potential to replace transportation fuels (Miao and Wu, 2006).

Biofuel production has focused on terrestrial plants and ethanolproduction from starches. Terrestrial plants have been popular for biofuel production because of the low price for cultivation and production (Huntley and Redalje, 2007).

Terrestrial plants are almost limited by land availability which led to limiting the fuel which can be produced and used by humans, at this time 30-40% of worldwide primary terrestrial productivity areappropriate for food, fiber, and energy (Huesemann, 2006).

Biofuels are fuels that are derived from organic biomass, rather than minerals, it can be classified into first, second, and third generation biofuels (Koh and Ghazoul, 2008). Austria worked since 1973 after the energy crisis seriously to use biofuel for farm tractors (Pahl, 2008). Laboratory experiments using rapeseed oil for biodiesel production had been a technical success. However, this oil crop was too expensive to be used for biodiesel, since petro-diesel prices were much lower. Therefore, scientists began to look for less expensive feedstock, and they soon discovered that there was a large supply of used cooking oil available (Kulkarni and Dalai, 2006).

Light is available in different quantities and different locations. Only a part (about 45 percent) of the total light spectrum is photo synthetically active radiation thus can be used by algae to capture CO_2, during photosynthesis (Gao*et al.*, 2007). The algae uses light for photosynthesis and growth, therefore, all alga culture systems are shallow and optimized to catch more light. At night (or other dark conditions) photosynthesis cannot occur, so algae consumes stored energy for respiration depending on the temperature and other conditions (Chisti, 2007).Biodiesel can be made from any oil or lipid source such as vegetable oils and animal fats (Felizardo*et al*., 2006). The oilcontains a glycerol molecule bonded to three fatty acid chains, this structure is called a triglyceride, and it is the major component of the oil (Riekhof*et al.,* 2005). Hassan *et al*. (2013) was

studied the effect of different concentrations of nitrogen on lipids production from two microalgae, the study was revealed that nitrogen treatments affected on lipid production of the studied microalgae and produced very high values of Stearic acid and Oleic acid.

The present study aimed to examine the ability of some isolated local algae to produce lipids in different light intensities, also to determine the lipids quality that uses in biodiesel.

II. Materials and methods

Fresh water samples of algae were collected by a phytoplankton net (mesh pore 0.2 μ), from the ponds and artificial canal around University of Baghdad campus in Al-Jadriah in addition to the Tigris river at Al-Rasheed area. Samples were incubated under suitable and controlled conditions for algal growth 268 μE/m²/s, 16:8 light: dark and 25± 2 C°.

Algae Isolation and Purification:

Two methods were used for isolation and purification: The serial dilution method by using ten test tubes, each one contains 9 ml Chu-10 nutrient solution, 1ml of algal culture was added to the first tube andshake carefully then 1ml from the first tube transported to the second tube and so on then incubated for two weeks (Prescott, 1982; Hustudtt, 1930). The second method is streaking on plate agar; Chu-10 media solution solidified by 1.5 % agar-agar and sterilized by autoclave, after sterilization Chu-10 with 45-50 C° was poured into petri-dishes which left to solidify, sterile loop was used for streaking straight line. Then the plates were kept in a cooled illuminated incubator with light intensity about 268 μE/m²/s, 25± 2 C° and 16:8 light: a dark periodof 10 -14 days (Sinigalliano*et al.*, 2009).

Preparation and Sterilization of Media:

Modified Chu-10 was used for the algal growth(Kassim*et al.,* 1999). A stock solution of each salt was prepared by dissolving weight of the salt as it clearly demonstrated in Table (1), pH was set on 6.4 using (0.01N) of sodium hydroxide or hydrochloric acid.

Algae Cultivation for Biomass:

A 100 ml of isolated alga culture was prepared then transported to 1000 ml ofChu-10 media and incubated for 14 days. Finally the growth transported to glass pools 5L for mass culture (Kawaguchi, 1980)

Determination of Growth Curve:

The growth curve was determined for two alga*Chlorella vulgaris* and *NitizschiaPalau.* Microalgae concentration was determined daily by optical density (OD) measurements at 540 nm by a UV-Vis spectrophotometer all measurements of the study were triplicates.

The growth rate (K) and doubling time (G) were calculated according to the following equation:

$$K = \frac{(\log OD_t - \log OD_0}{t} \times 3.322$$ (Huang *et al*., 2002a)

$$G = \frac{0.301}{K}$$ (Huang *et al.,* 2002b)

t : time (day)
OD_t: product after (t) day
OD_0: algae at beginning of the experiment zero time

Experimental Design

The light intensity 268 μE\m^2 \sec was used for cultivation of studied isolated algae. This intensity was treated as a control in the study experiments; light intensity was measured by a light meter.Three levels of light were used 125,300 and 400 μE\m^2 \sec as treatments (Jiminez*et al*., 2003).

The growth curve was determined for the two studied isolated microalgae, in addition to the growth rate (K) and the doubling time (G) were calculated for each treatment of the study experiments (Li *et al.*, 2008).

Harvesting of Algae:

Microalgae had been harvested at the beginning of the stationary phase for*C. vulgaris* harvested on the tenth day but *N. palea* in the twelfth day.

Each culture of studied algae wascentrifuged in the cooled centrifuge at the laboratories of Center of Market Researches and Consumer Protection, for 3000 r/min for 15 min, the supernatant removed but organic precipitate had been washed with dilute water. After that it had been dried at 45 C° for two days then the dry weight collected to be extracted (Jawad, 1982)

Lipid Extraction:

One gram of dry weight was put in thimble that was carried to specific cylinders in the soxhlet then 200 ml of solvent (mixture of methanol and hexane 1:1) had been put in the flask after the processtaken three-four hours (AOAC, 1995).

Lipid analysis:

Samples were analyzed by High Performance Liquid Chromatography (HPLC) system, model SUPELCO. HPLC consists from a mobile phase which is polar and consists of a mixture of solvents such as water: methane (60:40), while the stationary phase comprises of a column which is usually stainless steel and packed with silica particles, the column is discovery HS C: 18, dimension (25cm× 4.6mm ×5µm), injection flow is 1 ml/min, the absorption at UV 210 nm, and 30 ˚C temperature.

Determination of Protein and Carbohydrate:

Algae samples were centrifuged by cooling centrifuge model Rotina 380 R. for 5000 r/min for 30 min, 4 C°. The supernatant was collected and the protein determined according to Bradford (1976) and the carbohydrate according to Dubois *et al*., (1956).

Statistical Analyses

Complete Randomized Design (C.R.D.) was used as an experimental design. Data were analyzed by using a statistical analysis system- SAS (2001) to study the effect of different concentration factors on the production of lipid. Least significant difference (LSD) was used to compare the significant difference between means at P≤ o.o5.

III. Results

Isolated Algae

Modified Chu-10 was used to cultivate isolated algae specially diatoms and green algae. Six species of microalgae were isolated from artificial waterway in University of Baghdad campus in Al-Jadriah, in addition to the Tigris River at Al-Rasheed area.

These isolated algae were identified according to references (Prescott, 1982;Hustedt, 1930 and Hassan *et al*. 2012) and to confirm samples they have been sent to the Kuwait Marine Center.The present study recorded *Chrysocapsaplanctonica* (West&West) Plscheras new record of algae flora of Iraq. Only two isolated microalgae that were cultivated for this study, they are *Chlorellavulgaris* and *Nitzschiapalea*. The isolated algae of the present study are the following:

Class: Chlorophyceae
Order: Chlorococcales
Family (1): Oocystaceae
Genus: *Ankistrodesmus*Corda 1838
Species :*Ankistrodesmus*sp.
(Bellinger and Sigee 2010, P.238 ; Prescott 1982, P.253)
Order: Chlorococcales
Family (2) :Chlorococcaceae
Genus: *Chlorella* Berjerinck 1890
Species :*Chlorella vulgaris*Berjerinck1838
(Bellinger&Sigee 2010, P.232 ; Prescott 1982, P.237, pl. 53, fig. 13)
Division (2) :Chrysophyta
Class (1) :Bacillariophyceae
Order :Pennales
Family :Bacillariaceae
Genus:*Nitzschia*Hassal
Species:*Nitzschiapalea* (Kütz.) W. Smith (pl. 1, fig. 3)
(Hustedt 1930, 416, fig. 801; Hassan*et al*. 2012, pl.3, fig.48).
Class (2) : Chrysophyceae
Order: Chrysocapsales
Family :Chrysocapsaceae

Genus: *Chrysocapsa*Plscher 1913
Species:*Chrysocapsaplanctonica* (West&West) Plscher 1913 (pl. 1, fig. 4), New Record (acoording to Maulood and Toma 2004)
(Prescott 1982, P. 385, pl. 99, fig. 8).
Division (3): Cyanophyta
Class: Cyanophyceae
Order (1): Nostocales
Family :Nostocaceae
Genus: *Anabaena* Bory 1822
Species : *Anabaena* sp. (pl. 1, fig. 5)
(Bellinger and Sigee 2010, P.164 ; Prescott 1982, P. 510).
Order (2): Oscillatoriales
Family :Oscillatoriaceae
Genus: Oscillatoria Voucher 1803
Species :*Oscillatorialimnetica*Lemmermann 1900 (pl. 1, fig. 6)
(Desikachary 1959, P. 226, pl. 37, fig.3 ; Prescott 1982, P.488, pl. 109, fig. 16)

Different growth curve and growth rate (K) were observed for both isolated algae in the treatments, and the harvesting time was also different among the treatments.

Figure (1)illustrates the effect of different levels of light intensity on*C. vulgaris*biomass growth.The lag phase of biomass growth took from the day zero to the days 5, 4, 3 and 3 at treatments 125, 268 (control), 300 and 400 (μE\m^2 \sec), while the log phase took 8, 8, 6 and 5 days at treatments (25, 268, 300 and 400(μE\m^2 \sec).

The stationary phase began in the days 13, 12, 9 and 8 for treatments 125, 268, 300 and 400 (μE\m^2 \sec) light intensity respectively. The highest K value was 0.1 in the control treatment (268 μE\m^2 \sec), while the lowest K value was 0.06 at (400μE\m^2 \sec) but at (300 μE\m^2 \sec) the K value was 0.098 as it shown in (Table2 and figure 3)

The shortest doubling time (G)was 2.7 days at a control treatment while the longest was 5 days at (400 μE\m^2 \sec) and at 125 and (300 μE\m^2 \sec), (G) was 3.3 and 3 days respectively (Table3 and figure 4).

The alga *N.palea* entered astationary phase in different days among treatments, the lag phase began from the day zero to the days 5, 4, 3 and 3 then the alga entered to log phase which was continued till the stationary phase began. The stationary phase was begun in 12, 11, 10 and 8 in treatments 125, 268, 300 and 400 μE\m^2 \sec respectively (figure 2). The highest K value was 0.13 at the treatment 125μE\m^2 \sec, while the lowest value was 0.03 at 400μE\m^2 \sec (Table 2 and figure2). The shortest doubling time (G) was 2.3 days at treatment 125μE\m^2 \sec, while the longest was 10 days at 400 μE\m^2 \sec (Table 3 and figure 6)

The lipid yield (%) for *C. vulgaris* was increased from 6% in the treatment of 125μE\m^2 \sec to 10% inthe treatment of 300 μE\m^2 \secsignificantly,while other treatments were increased comparison with control treatment but not significantly(Table4, figure 12). The lipidyield (%)for*N.palea*wasincreased from 38% in 125 (μE\m^2 \sec) to 48% in 300 (μE\m^2 \sec) significantly, also some increasing in lipid yield was noticed but not significant in comparison with control treatment expect of treatment 300μE\m^2 \sec(Table4 and 13).

Light alsoaffected the two studied fatty acids that were analyzed by HPLC, Stearic acid (18:0) and Oleic acid (18:1). In *Chlorella vulgaris*theStearic acid was recorded decreased content in all studied treatments comparison with control, while the treatment 300 μE\m^2 \sec has significantly higher content from other treatment expect with control treatment and the lowest value was recorded at the treatment 125μE\m^2 \sec (Table 5, figures 6 and 10). Oleic acid value was increased at 300μE\m^2 \secsignificantly.The lowest value was recorded at 125μE\m^2 \sec (Table 6and figures7 and 10).

In *Nitzschiapalea* Stearic acid increased from 0.8% at 125 ($ME \setminus m^2 \setminus se$) to 4.5% at 300 (μE\m^2 \sec) , then it decreased to 2% at treatment 400 μE\m^2 \sec, only the increased values of the treatment 300 μE\m^2 \sec was significant comparison with control (Table 5). The Oleic acid values in all treatment was less than control treatment, the lowest value was 0.06% at 125 μE\m^2 \ sec (figures8, 11).

Carbohydrate of *C. vulgaris* was increased from 15% to 18% when light increased from 125 μE\m^2 \sec to 300 μE\m^2 \sec, and it was 14.5% at 400 (μE\m^2 \sec), only the treatment 300μE\m^2 \sec was increase value from control significantly (Table 7, figure 12) . Carbohydrate content of *N. palea* was decreased

from 22% to 15% at treatments 125 and400 μE\m^2 \sec respectively, and it was 18% at 300μE\m^2 \sec (Table7 and figure 13).

Protein content of *C. vulgaris* increased significantly at treatment (58%) at 300 μE\m^2 \sec.the lowest value was 38% at 400 μE\m^2 \sec (Table 8 and figure 12). In *N. palea* Protein content decreased from 13% to 8.5% when light increased from 125 to 400(μE\m^2 \sec), and it was 12.6 % at 300 μE\m^2 \sec (Table 9 and figure 13).

IV. Discussion

The present study results revealed that the effect of different levels of light intensities on biomass growth for both isolated algae(*C. vulgaris* and *N. palea*) showed no significant differences among treatments and control, whichmaydue to capability of photo acclimation (Chisti, 2007).

The K value for both isolated algae was increased in low light intensity then it decreased when increased light intensity, but no significant differencesbetween treatments and control was recorded in the present study.Light abundance in low intensity will encourage photosynthesis rate which is more than photorespiration rate so the culture biomass will increase, but when the alga is exposed to high light intensity a photo-oxidative has occurredwhich leads to pigment damages (Litchman, 2000).

The doubling time was increased at high light intensity and a significant difference was recorded between zero treatment and control while there are no significant differences among other treatments comparison with control, but in *N. palea* showed a significant difference between control and treatments 300,400μE\m^2 \sec..The decreasing of growth rate led to a slowing of cell division so doubling cells took more time (Al-Saadi*et al.,* 1996).

The results of the present study is in agreement with a study by Hassan *et al.*(2004) which reported that the growth rate of *Micocystisaeruginosa* was increased from o.42 at 20μE\m^2 \sec to 0.48 at 40 μE\m^2 \sec. Other studies reported that the best light intensity for the growing of two green algae like *Scenedesmusacutus, S. quadriquada* is 380 μE\m^2 \sec(Kassim, 1998). Light limitation will increase pigment content of most species. Several studies reported that the light limitation will lead to the increase of algal pigment content and the growth was reduced at both below and above optimum light intensity in several treatments can become stressful and lead to photo inhibition by damaging the chlorophyll pigments and that may hold theculture entering to death phase (Litchman, 2000; Al- Saadi, *et al.,* 1996).

The lipid content of both isolated algae was increased especially at 300μE\m^2 \sectreatment, there was a significant difference between this treatment and other treatments, lipids show highest and fastest response to the light intensity changes than of carbohydrate and proteins (Solovchenko*et al.,* 2008).

Total lipid content increased according to growth phases, some studies reported that total fatty acids of green algae *Parietochlorisincise*increased from 43% at log phase to 77% at stationary phase, and also as in *Gymnodiniumsp* total fatty acids increased from 8% at log phase two30% at stationary phase (Mansour*et al*., 2003).

A study on *Spirulinasp* found that low light intensity (35μE\m^2 \sec) induces lipid production and high light intensity (400μE\m^2 \sec) leads to less lipid content(Solovchenko*et al.,* 2008) . Another study on the green algae *Haematococcuspluvialis* which was exposed to high light intensity (350 μE\m^2 \sec) and that stimulated high production of lipid content as an accumulate way to resist this stress, but even with this photoprotection, photoinhibition due to light damage can still occur (Zhekisheva *et al.,* 2002). Similar results were reported in the present study.

Stearic acid content in both studied microalgae recorded highest content at 300μE\m^2 \sec treatment, while lower content at 125μE\m^2 \sec. There are significant differences between the two isolated algae, and significant differences among treatments. Oleic acid content showed no significant differences between two algae at 125,300μE\m^2 \sec treatments and significant differences among treatments for each alga. This increasing of Stearic and Oleic acids followed the increasing of total lipids, similar results for other algae species were reported by other authors (Solovchenko*et al.,* 2008).

The present study results of fatty acids are in agreement with those reported byAfify*et al.* (2010). The Stearic acid (18:0) is considered as the most common fatty acid in biodiesel that is present in this study that encourage to use the studied algae to produce biodiesel in addition to increasing of Oleic acid (18:1).

The carbohydrate content of both isolated algaewas increased with the increasing of light intensity, but the treatment 400μE\m^2 \sec was decreased. A significant difference was recorded among treatments of both algae.

The increasing in carbohydrate contents may be as a response to increasing in the photosynthesis process (Clayton and King, 1990).

There are alternative pathways to convert lipids and\or carbohydrate to TAG that has recently been demonstrated in plants, bacteria and yeast, but have not yet been studied in algae, and these pathways are worth investigating for improving lipid production (Arabolaza*et al.,* 2008). The present study results of carbohydrate agreed with another study (Rafiqual*et al*., 2005) that reported increases in carbohydrate from 0.8% to 7.1% of DW when exposed to high light. Highest carbohydrate content was reported 28.8% forOscillatoriasp when exposed to high intensity of light (Nagle *et al*., 2010), Another study showed that high intensity of light effect on the second photo system which is responsible for splitting the water molecule (Adri*et al.,* 2003).

The protein content was higher in *C. vulgaris* than in *N. palea* (Convert*et al.,* 2009). Significant differences were recorded among treatments at both studied microalgae. The results of this present study can be explained on the basis of light effect on algal growth which led to increasing the organic compounds including proteins (Dybl*et al*., 2006), and also effect on the nitrogen which is the most important element that enters to the amino acids synthesis and the activities of enzymes that are responsible for protein syntheses and regulation (Sarada*et al*., 2002).

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Table (1) Thecomponent concentration of modified Chu-10 medium and the concentration of each component

Number of stock solution	Chemical formula of each salt	Concentration g/l
1	$MgSo_4.7H_2O$	10
2	K_2HPO_4	4
3	$NaNO_3$ $CaCl_2$	8 16
4	$FeCl_3$	0.32
5	EDTA-Na2	4
6	NaCl	30
7	Na_2CO_3	8
8	$MnCl_2.4H_2O$ (NH4) $6Mo_7O_{24}.4H_2O$ $ZnSO_4.7H_2O$ $CuSO_4.5H_2O$ $COCl_2.6H_2O$ H_3BO_3	0.02 0.028 0.224 0.08 0.004 0.288
9	Na_2Sio_3	5.7

Table (2). Effect of different levels of light intensities (mean ± SE) of cultivated conditions on growth rate (K) of studied microalgae

Light intensity (µE\m 2 \sec)	Microalgae		LSD value
	C. vulgaris	*N.palea*	
125	0.09±0.01A	0.13±0.02A	0.11 NS
268(control)	0.1±0.0A	0.06±0.01B	0.052 NS
300	0.098±0.01A	0.05±0.01 B	0.067 NS
400	0.06±0.01 A	0.03±0.0 B	0.045 NS
LSD Value	0.04 NS	0.041 *	----

* ($P<0.05$).

NS: non-significant.
Means having different letters at the same column are significantly different.

Table (3) Effect of different levels of light intensities (mean ± SE)of cultivated conditions on doubling time(G) of studied microalgae

Light intensity (μE\m^2 \sec)	Microalgae		LSD value
	C. vulgaris	***N. palea***	
125	3.3±0.02B	2.3±0.12B	0.784 *
268(control)	2.7±0.02 B	5±0.36 B	1.03 *
300	3±0.05 B	6±0.49 AB	1.77 *
400	5±0.07 A	10±0.86A	2.52 *
LSD Value	1.045 *	4.021 *	----

* (P<0.05).
NS: non-significant.
Means having different letters at the same column are significantly different.

Table (4) Effect of different levels of light intensities (mean ± SE) of cultivated conditions on Lipid yield(%)of studied microalgae.

Light intensity (μE\m^2 \sec)	Microalgae		LSD value
	C. vulgaris	***N. palea***	
125	6±B 0.23	38±1.52B	7.48 *
268(control)	6.5±0.28B	40±2.19B	7.81 *
300	10±1.02A	48±2.78A	6.95 *
400	7.5±B 0.57	41±1.77B	8.02 *
LSD Value	2.388*	5.39 *	----

* (P<0.05).
Means having different letters at the same column are significantly different.

Table (5) Effect of different levels of light intensities (mean ± SE) of cultivated conditions on Stearic acid (%) content of studied microalgae

Light intensity (μE\m^2 \sec)	Microalgae		LSD value
	C. vulgaris	***N. palea***	
125	0.1±B 0.00	0.8±0.02B	0.37 *
268(control)	0.7±0.04A	1.5±0.07B	0.59 *
300	0.6±0.04 A	4.5±0.14 A	1.02 *
400	0.19±0.02 B	2±0.05 B	0.66 *
LSD Value	0.171 *	1.55 *	----

* (P<0.05).
Means having different letters at the same column are significantly different.

Table (6) Effect of different levels of light intensities (mean ± SE) of cultivated conditions on Oleic acid (%) content of studied microalgae

Light intensity (μE\m^2 \sec)	Microalgae		LSD value
	C. vulgaris	***N. palea***	
125	0.03±0.0B	0.06±0.01B	0.05 NS
268(control)	0.05±0.0 B	2±0.05A	0.16 *
300	0.2±0.0A	1.6±0.03A	0.57 NS
400	0.04±0.0 B	0.20±0.0A	0.08 *
LSD Value	*0.033	0.081 *	----

* (P<0.05).
NS: non-significant.
Means having different letters at the same column are significantly different.

Table (7)Effect of different levels of light intensities(mean ± SE)of cultivated conditions on Carbohydrate (%)content of studied algae.

Light intensity (μE\m^2 \sec)	Microalgae		LSD value
	C. vulgaris	***N. palea***	
125	15±1.06C	22±1.47A	3.48 *
268(control)	16.5±1.25 B	20±0.79AB	2.39 *
300	18±1.55A	18±0.52BC	0.00 NS
400	14.5±0.85D	15±0.36 C	1.233 NS
LSD Value	1.215 *	3.91 *	----

* (P<0.05). NS: non-significant.
Means having different letters at the same column are significantly different.

Table (8) Effect of different levels of light intensities(mean ± SE) ofcultivated conditions on Protein % content of studied microalgae.

Light intensity (μE\m^2 \sec)	Microalgae		LSD value
	C. vulgaris	***N. palea***	
125	40±2.18 C	13±0.46 A	7.61 *
268(control)	50±2.51 B	15±0.02 A	11.48 *
300	58±2.88A	12.6±0.44 A	13.09 *
400	38±1.69 C	8.5±0.37B	7.68 *
LSD Value	6.48 *	2.79 *	----

* (P<0.05).
Means having different letters at the same column are significantly different.

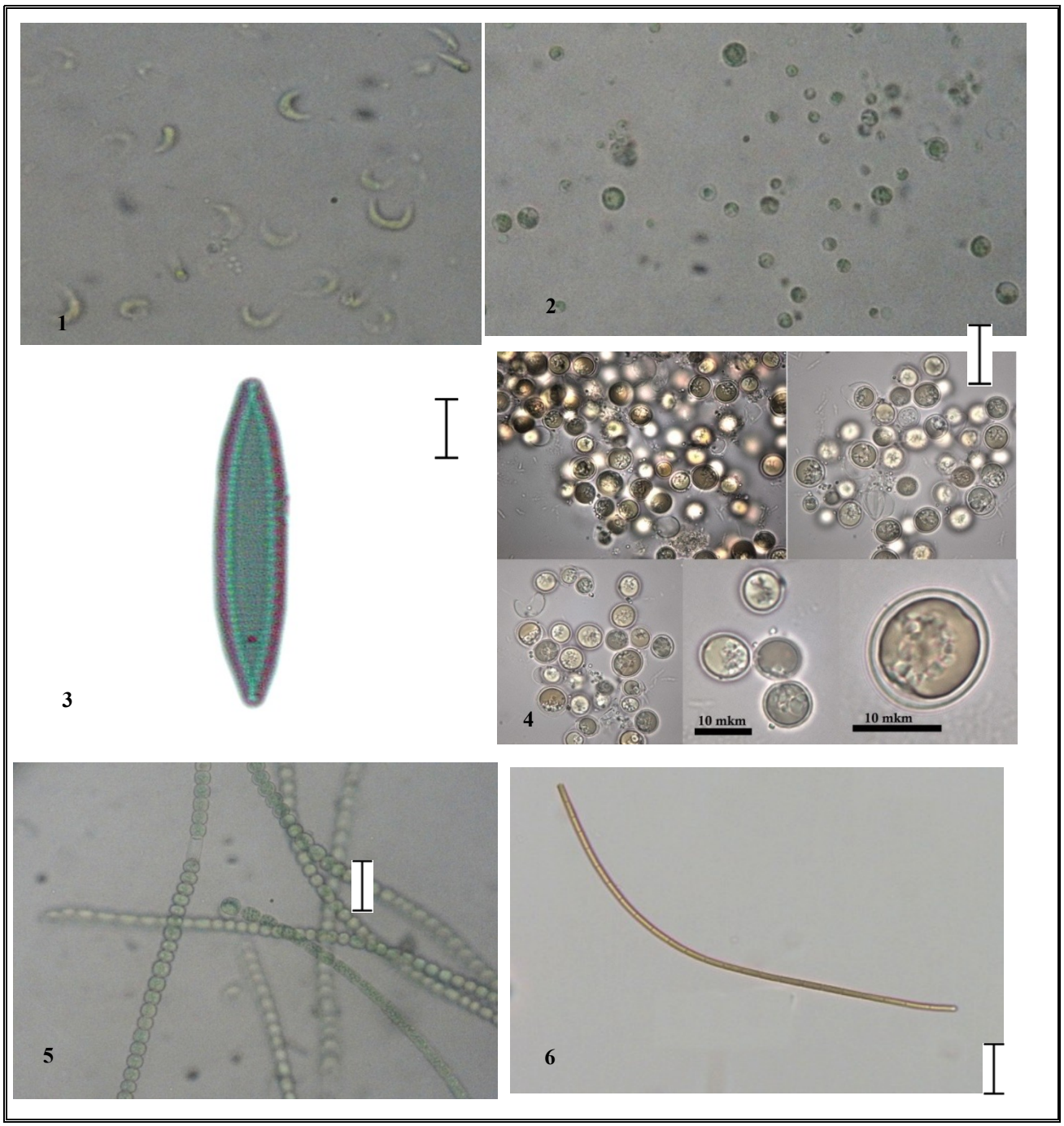


Plate (1): Isolated microalgae figures ***1.Ankistrodesmus*** **sp.** ***2. Chlorellavulgaris***
3. Nitzschiapalea 4. Chrysocapsaplanctonica 5. Anabaena sp. **6.** ***Oscillatorialimnetica***
(eachscalerepresents 10 µ otherwisementioned).

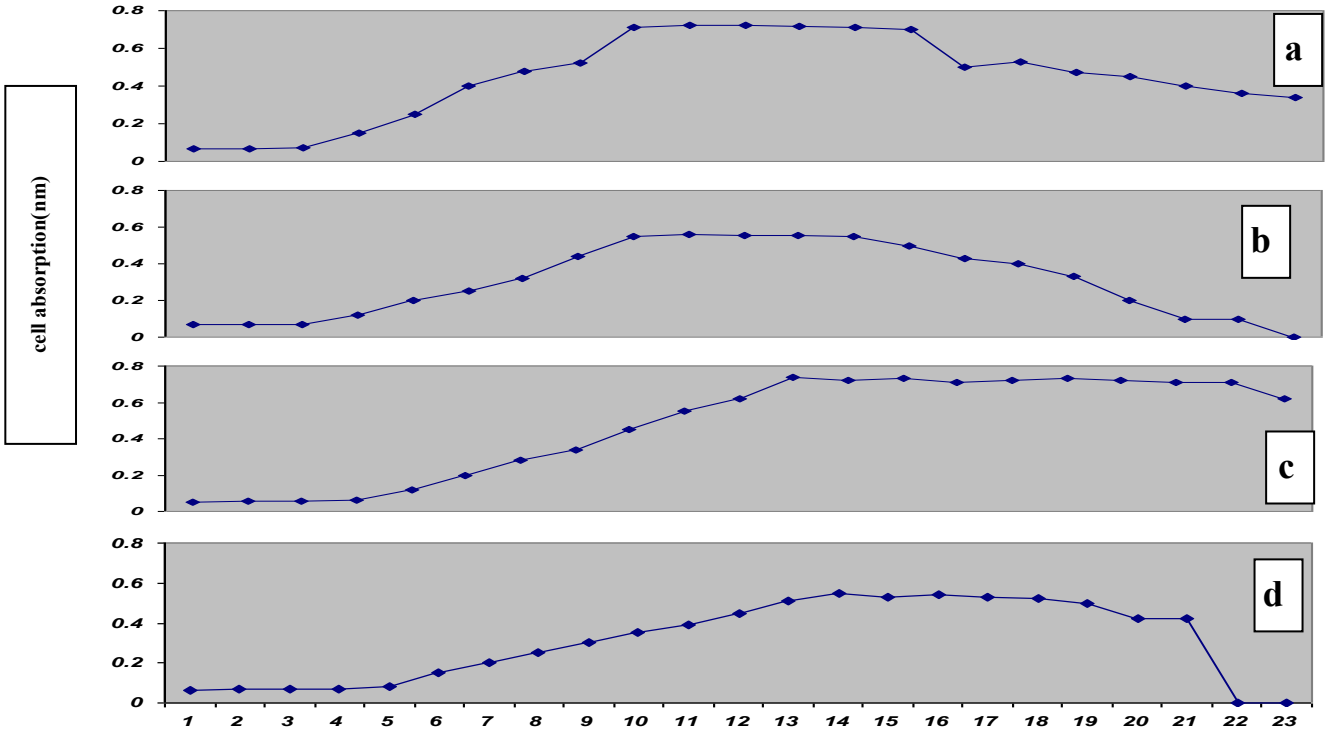


Figure (1) Growth curve of *C. vulgaris* at different levels of light intensities (µE\m ^{2} \sec) :a=400;b=300;c=268(control);d=125

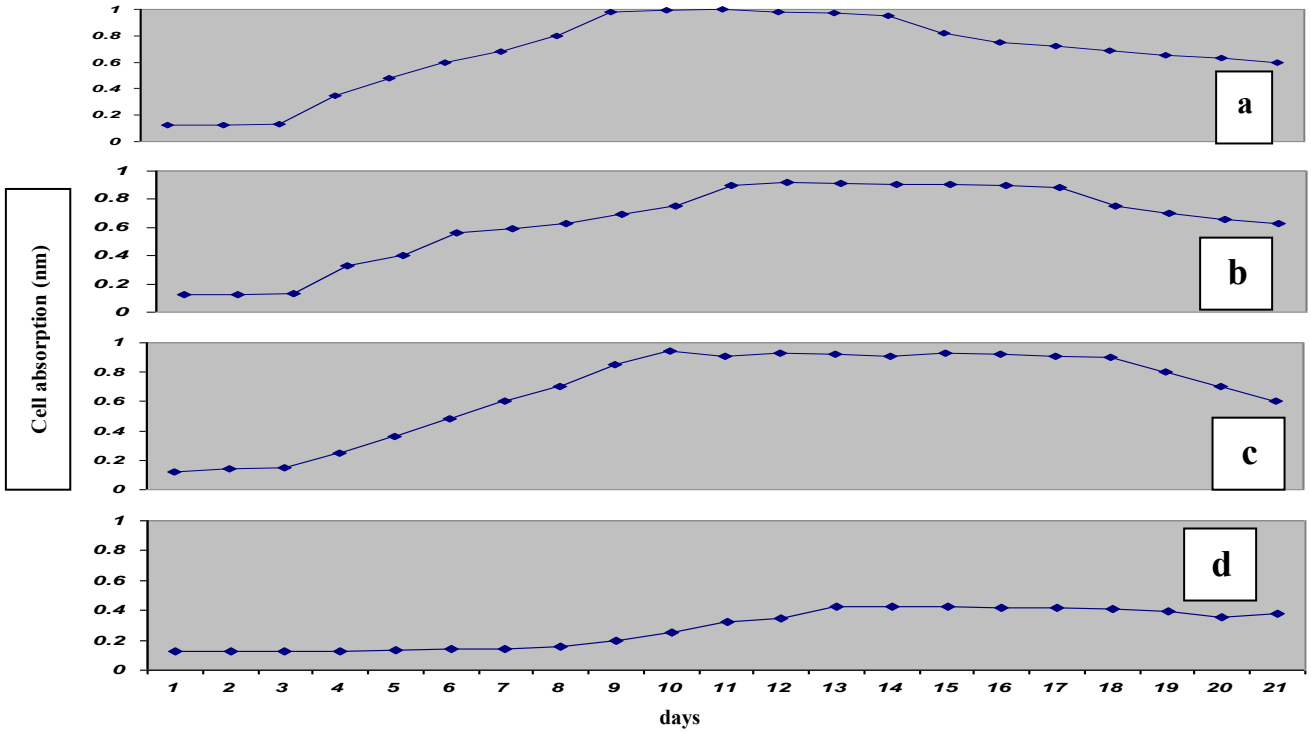
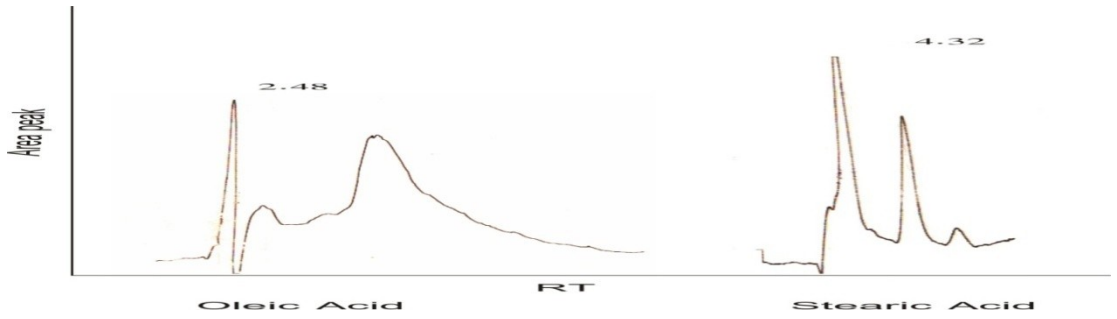
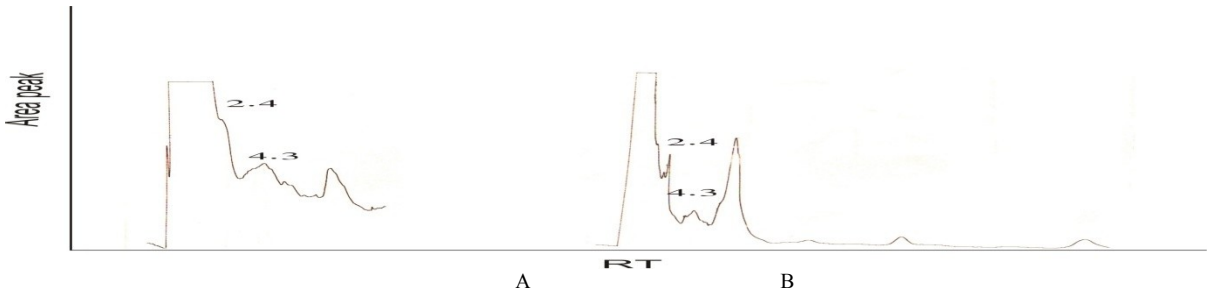


Figure (2) Growth curve of *N. palea* at different light intensities (µE\m ^{2} \sec) : a=400;b=300;c=268(control);d=125



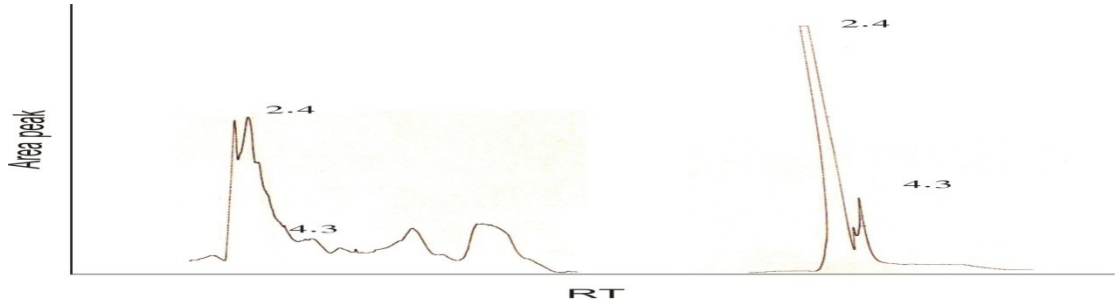
A **B**

Figure (3) Standars of fatty acids used in the study, and analyzed by HPLC .
Oleic acid , and its Retention Time (RT) is 2.4
Stearic acid, and its Retention Time (RT) is 4.3



A B

Figure (4): Chromatogram of HPLC for samples of *C. vulgaris* , the (RT) for Oleic acid is 2.4, and the (RT) for Stearic acid is 4.3
(A): the light intensity is (125 µE\m ^{2} \sec)
(B): the light intensity is (300 µE\m ^{2} \sec)



A B

Figure (5): Chromatogram of HPLC for samples of *N. palea*, the (RT) for Oleic acid is 2.4, and the (RT) for Stearic acid is 4.3
(A): the light intensity is (125 µE\m ^{2} \sec)
(B): the light intensity is (300 µE\m ^{2} \sec)