Bio-mass Production of *Chlorella vulgaris* grown on date wastes under different stress conditions

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ABSTRACT

A laboratory experiment was conducted to produce *Chlorella vulgaris* biomass grew on date wastes. The green microalgae were grown indoor on BG-11 medium in plexi glass columns with continuous fluorescent light and aeration. Date palm (*Phoenix dactylifera*) fruit wastes were washed and the flesh was oven dried at 55°C for 24h after removing the stones to obtain the flesh powder (0.9mm). Algal growth was performed under different stress conditions included (1) concentrations of 0.0, 10, 20, 30, 40 ml l^{-1} of wastes enriched growth media (2) NaCl concentration of 0.0, 0.5, 1.0,1.5, 2% and (3) nitrogen concentrations of 0, 25, 50, 75, 100%. The determinations included DW, total chlorophyll, carotenes and growth analysis. The obtained results showed that, the superior obtained net biomass was (0.215g l^{-1}) with 20 ml l^{-1} of PDWE concentration. The net obtained chlorophyll was (34.515 mg l^{-1}) with 30 ml l^{-1} of PDWE and highest carotenoid content (2.802 mg l^{-1}) was recorded with the control. The dry weight and chlorophyll content decreased with increasing salinity, on contrary, the carotene content increased up to 1.5% and then back off again. The dry weight and chlorophyll content decreased with nitrogen decreasing while, the carotene content increased with nitrogen decreasing.

Keywords: *Chlorella vulgaris*, date wastes, dry weight, chlorophyll, carotenoids.

INTRODUCTION

The yearly wasted human consumption is accounted by about one third of the food produced. The idea of food waste application as feedstock in micro-organisms cultivation allows recycling of waste matters consisting of carbon, nitrogen and phosphorous compounds (Luque and Clark, 2013). Date palm trees are grown in many parts of the world, including the Middle East, North Africa and South Asia (Selim *et al*., 2012). The worldwide production of date palm fruit has increased from 1.8 million tons in 1961 to 7.627624 million tons in 2017. Egypt is the top of the world in palm date production (1590414 metric tons) (FAO STAT., 2017). It is very rich in monosaccharides (fructose and glucose), minerals, vitamins with small amount of sucrose (Hasnaoui *et al*., 2010). It is also rich in several nutrients such as nitrogen, phosphorous, potassium, calcium, magnesium, etc. and has a high carbohydrate and fat content and is a vital source of sugar and dietary fiber (Al-Farsi *et al*., 2007). A second-grade (or low-grade) dates showed the same sugar content as dates of high quality (Besbes *et al*., 2009). Lost dates, which account for more than 2 million tones every year (30% of production) are discarded due to their inadequate texture (Besbes *et al*., 2009). These huge amounts of unused dates could be utilized for the production of fructose, ethanol, acetic acid, lactic acid and other valuable products (Zeinelabdeen *et al*., 2010). Microalgae are microscopic organisms that typically grow suspended in water and are driven by the same photosynthetic process as that of higher plants (Hanelt *et al*., 2007). *Chlorella vulgaris* is a photosynthetic microorganisms and eukaryotic from family of chlorellaceae (Ortiz Montoya *et al*., 2014). This organism is a unicellular green microalga and has spherical cells with diameter of 2 to 10 micrometers, which has asexual reproduction in which a mother cell reproduces 4 daughter cells, so that its growth rate is higher (cell mass doubling time is about 19 hrs) (Yamamoto *et al*., 2005). Rapid growth, easy and flexible terms of culture and resistance against interfering factors, are advantages that makes this microalga appropriate for use in the food industry, aquaculture, cosmetics, pharmaceutical, waste water treatment and the production of biofuel (Hultberg *et al*., 2014).

The aim of the current work is to use processed date wastes as a novel algal growth medium which in turn reduces the production costs via the utilization of initial organic carbon as well as other organic components, and to study the effect of salinity and nitrogen concentrations on *Chlorella vulgaris* growth.

MATERIALS AND METHODS

Algae and growth conditions

The green algae *Chlorella vulgaris* (fresh water) belong to *Chlorophyta,* which was obtained from Algal Bio-technology Unit, National Research Centre, Giza, Egypt. The growth was carried out indoor using BG-11

growth medium (Stainer *et al* 1971). Growth container was fully transparent Plexi Glass column with initial diameter of 7.5cm x 200cm containing 2.5 liter of the growth medium. Continuous light was provided from one side light bank supported with five white cool fluorescent lamps (40waste each). Free oil compressed air stream supported aeration from the lower hold of growth container. Inoculum was laboratory prepared after centrifugation followed by two washings using laboratory cooling centri-fuge (RUNNE HEIDELBERG Model/ RSV-200, Germany).

Waste dates

Samples of waste dates "Phoenix dactylifera" were collected from El-Wahat, Giza Governorate, Egypt. The samples were washed under running tap water for five min. to remove dust and reduce the number of contaminating microorganisms. After washing, the dates stones were separated from flesh which then dried at 55 °C for 24 hrs using heat oven (Baker *et al*., 2009). The dried flesh were powdered using a hammer mill and passed through a 0.9 mm sieve to obtain a fine powder. Samples were stored at 4°C until use.

Experiments

Based on the initial concentration of total nitrogen in waste dates and original growth medium, the algal growth was tested under concentrations of 0.0, 10, 20, 30 and 40 ml l^{-1} of wastes enriched growth medium, 0.0, 0.5, 1.0, 1.5, 2% of NaCl and 0, 25, 50, 75, 100% of nitrogen (urea) concentrations.

Growth measurements

Daily samples for determinations dry weight, total chlorophyll and carotenes were taken. Dry weight was measured by filtering a defined volume of the algal slurry (5-10 ml) over pre-weighted dried membrane filter (0.45 µm). Filters were dried at 105°C for 30 min., kept over anhydrous calcium chloride till it reached to room temperature and then re-weighted. The difference between weights monitored the net dry weight of the grown algae within defined sampling time. Chlorophyll was extracted from the pre-centrifuged algal bulk by 95% DMSO (Burnison, 1980). Chlorophyll absorbance was measured at 666 nm using spectrophotometer (PERKIN-ELEMENTAL Lambda 2 UV/VIS spectrophotometer) were the concentration was calculated (mg/ g^{-1}). To recover carotenes, saponification was performed by 5% KOH/30% MeOH and the residual was re-extracted by DMSO after the addition of 5 drops of concentrated acetic acid (Boussiba *et al*., 1992). Carotenes absorbance was measured at 468nm and the concentration was calculated (mg/ g^{-1}).

Growth analysis

Growth analysis; mainly growth rate (µ), doubling time (g), degree of multiplication(y) and increase percentage (inc.%) was performed using the methods adopted by Pirt (1973).

Statistical methodology

Statistical analyses systems were carried out using SAS Release 9.1.3 (2003) and SPSS (2011), IBM SPSS Statistics for Windows, Version 20.0. Variables having significant differences were compared using Duncan's multiple rang tests (Duncan, 1955). All experiments were replicated three times where data were presented as means of three replicates. Data obtained were analyzed statistically to determine the degree of significance using one-way analysis of variance (ANOVA) at probability level ($P \leq 0.05$).

RESULTS AND DISCUSSION

Optimizing *C. vulgaris* growth under different stress conditions

C. vulgaris grown on BG-II medium under different stress conditions including: date palm fruit waste extract (PDWE) concentration (0.0, 10, 20, 30 and 40 ml/l), salinity (0.5-2% NaCl) and nitrogen deficiency (0, 25, 50, 75 and 100%) were tested. The effect of each stress on *C. vulgaris* growth was monitored by measuring the dry weight, total chlorophyll and carotenoids content.

Effect of PDWE concentration on *C. vulgaris* dry weight

As shown in Table (1), PDWE increased the dry weight of all *C. vulgaris* grown cultures compared with control ones that grown on BG-II medium. The most effective PDWE concentration was 20 ml l^{-1} which achieved the maximum growth rate (0.552g l^{-1}), followed by 10 ml l^{-1} (0.522 g l^{-1}) and 30 ml l^{-1} of PDWE (0.512 g l^{-1}). The net obtained biomass was 0.160, 0.092, 0.215, 0.147 and 0.073 g l^{-1} with 0.0, 10, 20, 30 and 40ml l^{-1} of PDWE enriched grown culture, respectively. Significant positive correlation (r^2=0.1124) was recorded among dry biomass produced by the examined *C. vulgaris* (Fig.1). Fungal hydrolysis of commercial food residues enriched algal growth medium with trace elements and vitamins. It also contains glucose, free amino nitrogen, phosphate and most likely long chain fatty acids (Pleissner *et al*., 2013). Growth analysis confirmed that

hypothesis where the maximum growth rate of *C. vulgaris* was obtained with 20 ml/l of PDWE (0.038g l^{-1} d^{-1}) with the lowest recorded doubling time (40.94 hr) as compared with other PDWE concentrations used (Table 2). However, doubling time (DT) was less than that observed with 20ml/l PDWE concentration.

Effect of PDWE concentration on *C. vulgaris* total chlorophyll

The highest total chlorophyll content in *C. vulgaris* (62.92 mg l^{-1}) was found when the strain was grown on 30 ml l^{-1} of PDWE enriched medium (Table 3), followed by that grown on 20 ml l^{-1} of PDWE (48.209 mg l^{-1}). The net obtained chlorophyll contents were 20.461, 17.653, 20.963, 34.515 and 14.973 mg l^{-1} with 0.0, 10, 20, 30 and 40ml l^{-1} of PDWE enriched cultures, respectively, (Table 3). Significant positive correlation (r^2=0.0152) was recorded among chlorophyll produced by the examined strain (Fig. 2).

Maximum *C. vulgaris* growth rate along with superior chlorophyll content (0.0561 mg l^{-1} d^{-1}) was recorded when the strain grown on 30 ml l^{-1} of PDWE enriched medium followed by control culture (0.0538 mg l^{-1} d^{-1}) then by those grown on 20 ml l^{-1} PDWE enriched medium (0.0405 mg l^{-1} d^{-1}). Chlorophyll decline was observed with concentrations of 10 ml/l and 40 ml l^{-1} which resulted in a low growth rate corresponded with high doubling time and lower increase percentage (Table 4).

Effect of PDWE of *C. vulgaris* total carotenoids

In contrast, the opposite pattern was observed with *C. vulgaris* where lower and hyper PDWE concentrations represented the maximum carotenoids content. Moderate concentration decreased carotenoids comparing with the control culture. Data of total carotenoids accumulation by *C. vulgaris* (Table 5) was found in response with dry weight results in 40 ml l^{-1} of PDWE treatment which was the most effective in carotenoids accumulation by *C. vulgaris*, while a very slight decrease could be observed with 10, 20 and 30 ml l^{-1} PDWE which is statistically non-significant at P≤ 0.05. Highest carotenoid content was recorded for 0 ml PDWE l^{-1} (control) followed by 30 ml l^{-1} medium (2.802 and 1.907 mg l^{-1}, respectively).

C. vulgaris growth rate was maximized with the superior carotenoids content (30 ml $l^{-1}$of PDWE cultures) that reached 0.098 mg l^{-1} d^{-1}, followed by 20 ml/l of PDWE cultures (0.054 mg l^{-1} d^{-1}) then 10 ml/l of PDWE cultures (0.047 mg l^{-1} d^{-1}). Carotenoids decline was observed with the control and 40 ml/l of PDWE which resulted in a low growth rate corresponded with high doubling time and lower increase percentage (Table 6).

Effect of different NaCl concentrations on *C. vulgaris* growth

In general, salinity reduced the dry weight and productivity of *C. vulgaris*. It could be also observed that 0.5% addition of NaCl to the culture improved *C. vulgaris* growth slightly without significant difference (P≤ 0.05) compared to the control where 1.267g l^{-1} and 1.277g l^{-1} dry weight was recorded, respectively (Table 7). Addition of more than 1% NaCl (0.034 M) insignificantly decreased the dry weight. Significant positive correlation (r^2= 0.0559) was recorded between dry weight produced by the examined *C. vulgaris* and NaCl concentration (Fig. 4).

C. vulgaris growth rate was maximized in 1.5% NaCl enreched cultures (0.019 g l^{-1} d^{-1}) followed bythose enriched with 1 and 0.5% NaCl (0.007 g l^{-1} d^{-1}). Dry weight decline was observed with 0% and 2% NaCl concentrations which resulted in a low growth rate corresponded with high doubling time and lower increase percentage (Table 8).

Effect on total chlorophyll content

Total chlorophyll content affected by NaCl concentration was illustrated in Table (9). It was found that all NaCl concentrations decreased chlorophyll content. Addition of 0.5% NaCl gave 0.486 $mgl^{-1}d^{-1}$ chlorophyll which is much closed to the control without significant differences (P≤ 0.05). On other hand, all the other NaCl treatments showed significant decrease in chlorophyll productivity at (P≤ 0.05). The highest chlorophyll content was recorded by the control treatment followed by 0.5% NaCl concenration treatment (Table 9). The net increase of obtained chlorophyll content was 9.534, 6.208, 3.452, 0.625 and 0.182 mg l^{-1} with 0, 0.5, 1, 1.5 and 2% NaCl enriched *C. vulgaris* grown culture, respectively. Significant increase in biomass production by the examined strain was observed with increasing NaCl concentrations. Also, significant positive correlation (r^2= 0.955) was recorded between chlorophyll content in examined *C. vulgaris* strain and NaCl concenration (Fig.5).

C. vulgaris growth rate was maximized with the superior chlorophyll concentration at 0% NaCl cultures that reached 0.151mg l^{-1} d^{-1}, followed by 0.5% (0.082 mg l^{-1} d^{-1}) and 1% NaCl cultures (0.054 mg l^{-1} d^{-1}). Chlorophyll decline

was observed with concentrations from 0% to 2% NaCl which resulted in a low growth rate that corresponded with high doubling time and lower increase percentage (Table 10).

Effect on carotenoids

Data in Table (11) revealed that increment of carotenoids was observed in cultures grown under 0.5, 1, 1.5 and 2% added NaCl compared to control. The highest carotenoids concentration was recorded by treatment 1.5% NaCl followed by control and 2% NaCl compared with other treatments. The net obtained chlorophyll was 0.2944, 0.1335, 0.1824, 0.5942 and 0.2792 mg l^{-1} d^{-1} with 0, 0.5, 1, 1.5 and 2% NaCl enriched *C. vulgaris* grown culture, respectively. Significant increase in carotene content was found in NaCl concentrations from 0.5 to 2 %. Significant positive correlation (r^2= 0.1441) was recorded among carotenoids produced by the examined *C. vulgaris* NaCl concentrations (Fig.6).

C. vulgaris growth rate was maximized with superior carotenoids content (0.098 mg l^{-1} d^{-1}) in 2% NaCl treated cultures followed by cultures treated with 1.5% (0.093 mg l^{-1} d^{-1}) and 1% NaCl (0.040 mg l^{-1} d^{-1}). This increase in carotenoids' content resulted in a high growth rate corresponding with high doubling time and higher percentage of increase (Table 12).

This lag period is associated with the decline in chlorophyll and biomass content due to inhibition of photosynthetic and respiratory systems after exposed to high salt concentration (Vonshak and Torzillo, 2004). It has also been reported that chlorophyll is the primary target to salt toxicity limiting net assimilation rate, resulting reduced photosynthesis and reduced growth. Regarding carotenogenesis, at higher NaCl concentration the grown cells contained higher amount of total carotenoids and carotene content wich is similar to previous studies. Pisal and Lele (2005) reported that the carotene is a secondary metabolite and these molecules are produced by the cells in stress condition as cell protecting mechanism. Hence, an increase in total carotenoids and carotene content at higher saline conditions was noticed. It was stated that 0.05 M NaCl in the culture medium had practically no effect on the growth rate of *A. obliquus,* whereas a 0.3 M NaCl reduced the growth intensity by more than two times, but a 3.0–0.6 M NaCl caused the growth interruption of alga (Kaewkannetra *et al*., 2012). Growth inhibition and biomass reduction under the influence of salt stress were observed in some blue-green and green algae – *Chlorococcum* sp. (Masojídek *et al*., 2000), *Arthrospira fusiformis* (Rafiqul *et al*., 2003) and *Chlorella zofingiensis* (Del Campo et al., 2004). The content and ratio of photosynthetic pigments (chlorophylls *a* and *b*, carotenoids) belong to the indicators of the photosynthetic apparatus reactions to stressors (Babenko *et al*., 2014; Haubner *et al*., 2014; Liang *et al*., 2014). Sujatha and Nagarajan (2013) indicated that when NaCl concentrations in culture medium increased the level of chlorophylls *a* and *b* in each experiment were lower than those of the control, while the carotenoids' content increased beginning on day 12 of the experiment. A gradual increase in chlorophyll *a* and *b* content at all stages of the experiment was fixed under control conditions, whereas the total amount of carotenoids practically did not increase until the end of the experiment. Romanenko *et al*. (2017) stated that an increase in NaCl concentration in the cultural medium caused some decrease in the chlorophyll and carotenoids' content.

Effect of nitrogen deficiency on *C. vulgaris* growth

Effect on dry weight

Data in Table 13 indicated that the capacity of ***C. vulgaris*** to produce biomass was dependent on nitrogen concentration. The highest level of dry weight (1.765g l^{-1}) was formed in 100% N (urea) followed by 75% N (1.436 g l^{-1}) amended culture medium. Generally, *C. vulgaris* at 100% N had higher algal dry weight yields (0.995g l^{-1}) in comparison with the other N concentrations used. It is also evident that concentration of 0.0% N had lower algal dry weight yield (0.115 g l^{-1}) in comparison with the other concentrations used. Significant decrease in dry weight production by the examined strain was observed with nitrogen deficiency concentrations. Significant positive correlation (r^2= 0.9534) was recorded among dry weight produced by the examined *C. vulgaris* and N level in the growth medium (Fig.7). In this context, El-Sayed *et al*. (2011) reported that dry weight of *C. vulgaris* was markedly increased with culture that supported by urea as a nitrogen source comparing with nitrate supplementation under the same nitrogen content (17.6 mM N). The slightly high initial carbon content of urea (49.5% CO) with high solubility rate to form carbonic acid might be enhanced the vegetative growth of algae. Furthermore, the decomposition of urea molecule led to the fast utilization of ammonical nitrogen part by the algae. Urea degradation as a nitrogen source involves two specific enzymatic systems (urease and urea

amydolayase); which are absent in algal metabolic matrix. The degradation might be achieved by bacteria; or due to the media reaction mainly acid reaction, light, aeration and/or hydrolysis by extracellular algal excretions (El-Sayed *et al*., 2011).

The highest *C. vulgaris* growth rate was recorded with control (100%N) cultures that reached 0.0588 g l^{-1} d^{-1}, followed by 75%N cultures (0.0537 g l^{-1} d^{-1}) and 50% N cultures (0.0290 g l^{-1} d^{-1}). Dry weight decline was observed in all treatment as a result of N defficiency which resulted in a low growth rate corresponded with high doubling time and lower increase percentage (Table 14).

Lowering nitrogen concentration slightly decreased the total chlorophyll content and productivity compared to control (Table 15). The highest chlorophyll content (11.603 mg $l^{-1}d^{-1}$) was recorded in control followed by 75% N compared with other treatments**.** However, no significant differences were observed compared to control and other nitrogen concentrations (P≤ 0.05). The net obtained chlorophyll content was 9.913, 6.512, 3.452, -2.035and -1.984 mg l^{-1} with 100, 75, 50, 25 and 0% N enriched grown culture, respectively. Significant positive correlation (r^2= 0.9476) was recorded between chlorophyll produced by *C. vulgaris* and N concentration**s** (Fig. 8).

C. vulgaris growth rate was the highest with increasing chlorophyll concentration due to high N content in control cultures (0.1373 mg l^{-1} d^{-1}), followed by 75% N cultures (0.0996 mg l^{-1} d^{-1}) and 50% N cultures (0.0389 mg l^{-1} d^{-1}). Chlorophyll decline observed with concentrations of 100% N (control) to 0% N resulted in a low growth rate corresponded with high doubling time and lower increase percentage (Table 16).

Effect on carotenoids content

Significant increase in carotenoids production by the examined strain observed with decreasing nitrogen concentrations. Data of total carotenoids accumulation by *C. vulgaris* (Table 17) showed that medium with all concentrations of nitrogen recorded a significant increase in carotenoids compared to control that received full nitrogen (100%N) (P≤ 0.05). Highest carotenoid content was recorded in the medium that contained 0% N followed by 25%, 50% and 75% nitrogen (0.926, 0.935, 0.702 and 0.667 mg l^{-1} with increases of 0.529, 0.336, 0.232 and 0.152 mg $l^{-1}.d^{-1}$, respectively) compared with control medium (0.524 mg l^{-1} with increase of 0.003 mg l^{-1} d^{-1}). Significant positive correlation (r^2=0.8291) was recorded between carotenoids produced by the examined *C. vulgaris* and N content (Fig.9).

The growth rate of *C. vulgaris* was in the following descending order: 0.0 > 25% >50% >75% >100% N (Table 18). Carotenoids decline observed with increasing N concentrations resulted in a low growth rate corresponding with high doubling time and lower increase percentage (Table 18).

Urea is the major form of nitrogen commonly used for assimilation by non-nitrogen fixing cyanobacterium under laboratory conditions. The assimilatory reduction of nitrate is a fundamental biological process in which a highly oxidized form of organic form of nitrogen is reduced to nitrite and then to ammonia (Bhattacharya and Shivaprakash, 2006).

CONCLUSIONS

It can be concluded that, *C. vulgaris* have ability to produce biomass from various carbon sources.Waste dates is very rich in monosaccharides (fructose and glucose), fat content dietary fibre, minerals and vitamins with small amount of sucrose. Fructose and glucose constitute over 75% of the dry weight of pitted dates. PDWE concentration of 20 ml l^{-1} was the most suitable for the work of *C. vulgaris*. The best conecentration of NaCl and waste dates was 0.5% NaCl. *C. vulgari* was superior in production of biomass from date wastes extract and 100% N concentration.

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Table 1. Effect of PDWE concentrations on *C. vulgaris* biomass production.

PDWE concentration (ml l^{-1})	Time	Rep.			Mean ($g\ l^{-1}$)	Increasing ($g\ l^{-1}$)
Control (0)	Start	0.3014	0.3194	0.3205	0.314^{c}±0.01	0.160
	End	0.4515	0.4657	0.505	0.474^{a}±0.03	
10	Start	0.3204	0.3983	0.5719	0.430^{b}±0.129	0.092
	End	0.4381	0.515	0.6136	0.522^{a}±0.088	
20	Start	0.4313	0.2116	0.3688	0.337^{c}±0.11	0.215
	End	0.5653	0.5914	0.4986	0.552^{a}±0.048	
30	Start	0.3555	0.356	0.3861	0.366^{c}±0.018	0.147
	End	0.4531	0.4675	0.6167	0.512^{a}±0.091	
40	Start	0.4972	0.3611	0.38	0.413^{b}±0.074	0.073
	End	0.4985	0.481	0.4784	0.486^{a}±0.011	

Values are mean of 3 replicates. Means showed the same letters are not signfcantly different ($p \leq 0.05$). S.E.±0.013. Working volume = 6L.

Table 2. Dry weigh of *C. vulgaris* as affected by PDWE enriched growth medium.

	Treatments (waste extract ($ml\ l^{-1}$))				
	Control	10	20	30	40
GR ($mg\ l^{-1}\ d^{-1}$)	0.030	0.015	0.038	0.023	0.013
DT(hrs)	37.56	55.85	40.94	50.90	59.78
DM	0.59	0.31	0.77	0.47	0.25
I%	33.73	18.77	37.99	27.59	15.25

Where GR= growth rate; DT= doubling time; DM= degree of multiplication and PI= increase percentage.

Table 3. Effect of PDWE concentrations on *C. vulgaris* chlorophyll content.

PDWE concentration (ml l^{-1})	Time	Rep. ($mg\ l^{-1}$)			Mean $mg\ l^{-1}$	Dilution $mg\ l^{-1}$	Increasing $mg\ l^{-1}$
Control (0)	Start	1.478	1.671	1.630	1.593	18.159^{d}±0.102	20.461
	End	3.064	3.699	3.400	3.388	38.62^{c}±0.318	
10	Start	2.255	2.249	2.271	2.258	25.747^{d}±0.0116	17.653
	End	3.013	3.320	5.088	3.807	43.40^{bc}±1.120	
20	Start	2.045	2.783	2.341	2.390	27.245^{d}±0.37	20.963
	End	4.730	4.606	3.350	4.229	48.209^{ab}±0.763	
30	Start	2.295	2.375	2.805	2.492	28.405^{d}±0.274	34.515
	End	4.441	5.389	6.728	5.519	62.92^{a}±1.149	
40	Start	2.296	2.919	2.189	2.468	28.134^{d}±0.394	14.973
	End	3.786	3.525	4.034	3.781	43.108^{bc}±0.255	

Values are mean of 3 replicates. Means showed the same letters are not significantly different ($p \leq 0.05$). S.E.±0.111. Working volume = 6L.

Table 4. Chlorophyll content of *C. vulgaris* as affected by PDWE enriched growth medium.

	Treatments (waste extract ($ml\ l^{-1}$)				
	Control	10	20	30	40
GR ($mg\ l^{-1}\ d^{-1}$)	0.0538	0.0354	0.0405	0.0561	0.0310
DT(hrs)	20.455	37.229	30.599	19.884	45.829
DM	1.0859	0.7141	0.8173	1.1316	0.6249
PI%	52.886	37.587	42.15067	54.186	34.09

Where GR= growth rate; DT= doubling time; DM= degree of multiplication and PI= increase percentage.

Table 5. Effect of PDWE concentrations on *C. vulgaris* carotenoids content.

Treatments (ml l^{-1} of PDWE)	Time	Rep.			Mean mg l^{-1}	Dilution mg l^{-1}	Increasing mg l^{-1}
Control (0)	Start	0.4041	0.3579	0.342	0.368	1.656^{ab}±0.032	1.146
	End	0.4306	0.7483	0.6892	0.6227	2.802^{a}±0.169	
10	Start	0.3763	0.4181	0.3763	0.3902	1.756^{ab}±0.024	-0.85
	End	0.2503	0.1794	0.1716	0.2013	0.906^{d}±0.043	
20	Start	0.4356	0.3841	0.4683	0.4293	1.932^{ab}±0.042	-0.898
	End	0.1086	0.1858	0.3947	0.2297	1.034^{c}±0.148	
30	Start	0.5354	0.3625	0.3731	0.4237	1.907^{ab}±0.097	-0.686
	End	0.011	0.3468	0.4559	0.2713	1.221^{c}±0.232	
40	Start	0.3505	0.3574	0.3823	0.3634	1.635^{b}±0.017	0.028
	End	0.4908	0.2144	0.4034	0.3695	1.663^{ab}±0.141	

Values are mean of 3 replicates. Means showed the same letters are not signfcantly different ($p \leq 0.05$). S.E.±0.022. Working volume = 6L.

Table 6. Carotenoid growth analysis of *C. vulgaris* as affected by PDWE enriched growth medium.

	Treatments (waste extract (ml l^{-1}))				
	Control	10	20	30	40
GR (mg l^{-1} d^{-1})	0.036	0.047	0.054	0.098	0.022
DT(hrs)	22.526	25.163	40.735	43.138	34.748
DM	0.722	0.98	1.099	1.983	0.058
PI%	72.382	48.326	47.471	26.686	28.529

Where GR= growth rate; DT= doubling time; DM= degree of multiplication and PI= increase percentage.

Table 7. Effect of different NaCl concentrations on *C. vulgaris* dry weight production.

NaCl concentration	Time	Rep.			Mean g.l^{-1}	Increasing mg.l^{-1}
Control (0%)	Start	1.0579	1.1897	1.2559	1.168^{b}±0.101	0.109
	End	1.1565	1.3017	1.3722	1.277^{a}±0.110	
0.5%	Start	1.1505	1.1258	1.1573	1.145^{b}±0.017	0.123
	End	1.2417	1.3844	1.176	1.267^{a}±0.107	
1%	Start	1.1606	1.0555	1.1431	1.120^{b}±0.056	0.117
	End	1.1997	1.2279	1.283	1.237^{a}±0.042	
1.5%	Start	0.7546	1.0177	0.9323	0.902^{c}±0.134	0.276
	End	1.1556	1.225	1.1503	1.177^{b}±0.042	
2%	Start	1.027	0.995	0.9815	1.001^{c}±0.023	0.089
	End	1.1241	1.1176	1.0289	1.090^{b}±0.053	

Values are mean of 3 replicates. Means showed the same letters are not signfcantly different ($p \leq 0.05$). S.E.±0.014. Working volume = 2L.

Table 8. Dry weight growth analysis of *C. vulgaris* as affected by NaCl enriched growth medium.

	NaCl concentrations				
	Control	0.5%	1%	1.5	2%
GR (mg l^{-1} d^{-1})	0.006	0.007	0.0071	0.019	0.0061
DT(hrs)	108.747	95.138	97.482	36.391	113.948
DM	0.129	0.147	0.144	0.384	0.123
PI%	8.537	9.697	9.475	23.407	8.164

Where GR= growth rate; DT= doubling time; DM= degree of multiplication and PI= increase percentage.

Table 9. Effect of different NaCl concentrations on *C. vulgaris* chlorophyll content.

NaCl concentration	Time	Rep.			Mean mg l^{-1}	Dilution mg l^{-1}	Increasing mg l^{-1}
Control (0%)	Start	0.0305	0.1532	0.1609	0.1149	1.310^{d}±0.073	9.534
	End	0.7595	0.9087	1.1853	0.9512	10.843^{a}±0.216	
0.5%	Start	0.2806	0.2052	0.2762	0.254	2.896^{c}±0.042	6.208
	End	1.0053	0.5556	0.8348	0.7986	9.104^{a}±0.227	
1%	Start	0.3422	0.1672	0.2961	0.2685	3.061^{c}±0.091	3.452
	End	0.6312	0.6398	0.443	0.5713	6.513^{ab}±0.111	
1.5%	Start	0.2719	0.2243	0.3281	0.2748	3.132^{bc}±0.052	0.625
	End	0.2965	0.2617	0.4306	0.3296	3.757^{b}±0.089	
2%	Start	0.3519	0.3211	0.2604	0.311133	3.547^{b}±0.047	0.182
	End	0.3475	0.3471	0.2866	0.3271	3.729^{b}±0.035	

Values are mean of 3 replicates. Means showed the same letters are not signfcantly different ($p \leq 0.05$). S.E.±0.022. Working volume = 2L.

Table 10. Chlorophyll content in *C. vulgaris* as affected by NaCl enriched growth medium

	Treatments (NaCl concentration)				
	Control	0.5%	1%	1.5%	2%
GR (mg l^{-1} d^{-1})	0.151	0.082	0.054	0.013	0.004
DT(hrs)	4.591	8.472	12.851	53.331	194.241
DM	3.048	1.652	1.089	0.262	0.072
PI%	87.923	68.193	53.005	16.637	4.873

Where GR= growth rate; DT= doubling time; DM= degree of multiplication and PI= increase percentage.

Table 11. Effect of different NaCl concentrations on carotenoids content.

NaCl concentration	Time	Rep.			Mean mg l^{-1}	Dilution mg l^{-1}	Increasing mg l^{-1}
Control (0%)	Start	0.0929	0.0778	0.1104	0.0937	0.422^{b}±0.016	0.2944
	End	0.0773	0.1644	0.2357	0.1591	0.716^{a}±0.079	
0.5%	Start	0.1377	0.0241	0.0342	0.0653	0.294^{c}±0.063	0.1335
	End	0.1594	0.0809	0.0447	0.095	0.428^{b}±0.059	
1%	Start	0.0641	0.0742	0.0212	0.0532	0.239^{c}±0.028	0.1824
	End	0.0929	0.0778	0.1104	0.0937	0.4217^{b}±0.016	
1.5%	Start	0.0515	0.0325	0.0693	0.0511	0.223^{c}±0.018	0.5942
	End	0.0797	0.3305	0.1346	0.1816	0.817^{a}±0.132	
2%	Start	0.0192	0.0159	0.0283	0.0211	0.095^{d}±0.006	0.2792
	End	0.1163	0.0753	0.0579	0.0832	0.374^{bc}±0.030	

Values are mean of 3 replicates. Means showed the same letters are not signfcantly different ($p < 0.05$). S.E.±0.011. Working volume = 2L.

Table 12. Carotenoids growth analysis of *C. vulgaris* as affected by NaCl enriched growth medium.

	Treatments (NaCl concentration)				
	Control	0.5%	1%	1.5%	2%
GR (mg l^{-1} d^{-1})	0.038	0.027	0.040	0.093	0.098
DT(hrs)	18.326	25.921	17.128	7.472	7.083
DM	0.764	0.540	0.817	1.873	1.976
PI%	41.112	31.228	43.253	72.712	74.593

Where GR= growth rate; DT= doubling time; DM= degree of multiplication and PI= increase percentage.

Table 13. Effect of nitrogen deficiency on *C. vulgaris* dry weight production.

Treatments	Time	Rep.			Mean mg.l-1	Increasing mg.l-1
Control (100%N)	Start	0.729	0.778	0.804	0.770^{cd}±0.038	0.995
	End	1.593	1.644	2.057	1.765^{a}±0.25	
75 %N	Start	0.715	0.625	0.693	0.678^{d}±0.047	0.758
	End	1.497	1.365	1.446	1.436^{b}±0.067	
50 %N	Start	0.787	0.809	0.747	0.781^{cd}±0.031	0.391
	End	1.224	1.141	1.152	1.172^{bc}±0.045	
25 %N	Start	0.963	0.853	0.779	0.865^{c}±0.093	0.18
	End	1.092	1.059	0.983	1.045^{bc}±0.056	
0 %N	Start	0.641	0.742	0.812	0.732^{d}±0.086	0.115
	End	0.769	0.928	0.844	0.847^{c}±0.080	

Values are mean of 3 replicates. Means showed the same letters are not signfcantly different ($p \leq 0.05$). S.E.±0.018. Working volume = 2L.

Table 14. Dry weight growth analysis of *C. vulgaris* as affected by different N level enriched growth medium.

	N concentration				
	Control (100%)	75%	50%	25%	0%
GR (mg l^{-1} d^{-1})	0.0588	0.0537	0.0290	0.0137	0.0106
DT(hrs)	18.869	20.4713	38.353	86.524	183.7213
DM	1.2027	1.0637	0.6330	0.2323	0.194
PI%	56.4627	52.1837	35.5207	14.793	12.3603

Where GR= growth rate; DT= doubling time; DM= degree of multiplication and PI= increase percentage.

Table 15. Effect of nitrogen deficiency on *C. vulgaris* chlorophyll content.

Treatments	Time	Rep.			Mean mg l^{-1}	Dilution mg l^{-1}	Increasing mg l^{-1}
Control (100%N)	Start	0.1305	0.1532	0.1609	0.1482	1.690^{e}±0.016	9.913
	End	0.8595	1.0087	1.1853	1.0178	11.603^{a}±0.163	
75 %N	Start	0.1806	0.2052	0.1762	0.1873	2.136^{de}±0.016	6.512
	End	0.8053	0.6356	0.8348	0.7586	8.648^{b}±0.108	
50 %N	Start	0.4222	0.4372	0.3961	0.4185	4.771^{c}±0.021	3.452
	End	0.7312	0.7398	0.693	0.7213	8.223^{b}±0.025	
25 %N	Start	0.4719	0.5243	0.5281	0.5081	5.792^{c}±0.031	-2.035
	End	0.2965	0.2617	0.4306	0.3296	3.757^{d}±0.021	
0 %N	Start	0.4519	0.4211	0.4604	0.4445	5.067^{c}±0.089	-1.984
	End	0.2975	0.2371	0.2766	0.2704	3.083^{d}±0.031	

Values are mean of 3 replicates. Means showed the same letters are not signfcantly different ($p \leq 0.05$). S.E.±0.013. Working volume = 2L.

Table 16. Chlorophyll growth analysis of *C. vulgaris* as affected by different N levels enriched growth medium.

	N concentration				
	Control (100%)	75% N	50% N	25% N	0% N
GR (mg l^{-1} d^{-1})	0.1373	0.0996	0.0389	0.0358	0.0325
DT(hrs)	8.0077	11.2597	28.2477	31.2517	43.529
DM	2.7716	2.0094	0.7856	0.7220	0.6554
PI%	85.3515	74.728	42.0016	39.261	35.239

Where GR= growth rate; DT= doubling time; DM= degree of multiplication and PI= increase percentage.

Table. 17. Effect of nitrogen deficiency on *C. vulgaris* carotenoids content.

Treatments	Time		Rep.		Mean mg l^{-1}	Dilution mg l^{-1}	Increasing mg l^{-1}
Control (100%N)	Start	0.1129	0.1178	0.1164	0.1157	0.521^{cd}±0.003	0.003
	End	0.1134	0.1185	0.1172	0.1164	0.524^{cd}±0.003	
75 %N	Start	0.1415	0.1025	0.0993	0.1144	0.515^{d}±0.023	0.152
	End	0.1797	0.1305	0.1346	0.1483	0.667^{bc}±0.027	
50 %N	Start	0.1077	0.0909	0.1147	0.1044	0.47^{d}±0.012	0.232
	End	0.1594	0.1741	0.1342	0.1559	0.702^{bc}±0.020	
25 %N	Start	0.1163	0.1553	0.1279	0.1332	0.599^{c}±0.020	0.336
	End	0.2192	0.2159	0.1883	0.2078	0.935^{a}±0.017	
0 %N	Start	0.0841	0.0912	0.0892	0.0882	0.397^{e}±0.004	0.529
	End	0.1526	0.262	0.2027	0.2058	0.926^{ab}±0.055	

Values are mean of 3 replicates. Means showed the same letters are not signfcantly different ($p \leq 0.05$). S.E.±0.004. Working volume = 2L.

Table.18. Carotenoids growth analysis of *C. vulgaris* as affected by different N levels enriched growth medium.

	N concentration				
	Control (100%)	75% N	50% N	25% N	0% N
GR (mg l^{-1} d^{-1})	0.0005	0.0187	0.0285	0.0321	0.0589
DT(hrs)	27.7407	59.536	53.618	36.9057	19.7087
DM	0.0083	0.3771	0.5762	0.6489	1.1881
PI%	42.4437	22.9798	31.5844	35.6962	55.3578

Where GR= growth rate; DT= doubling time; DM= degree of multiplication and PI= increase percentage.

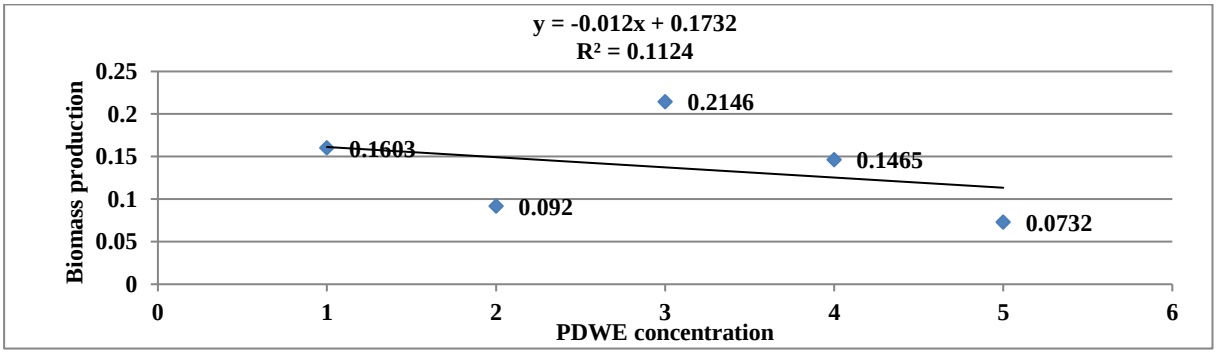

Figure 1. Correlation coefficient between biomass of *C. vulgaris* and PDWE concentration

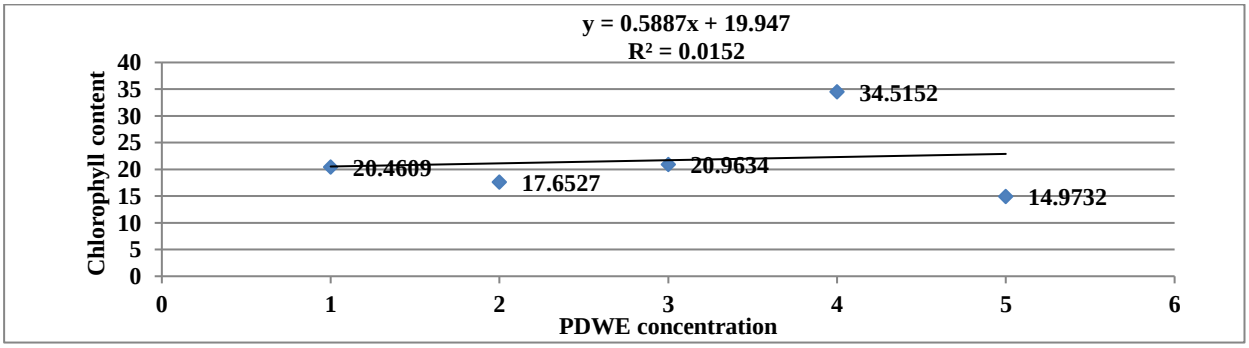

Figure 2. Correlation coefficient between chlorophyll content and PDWE concentration**.**

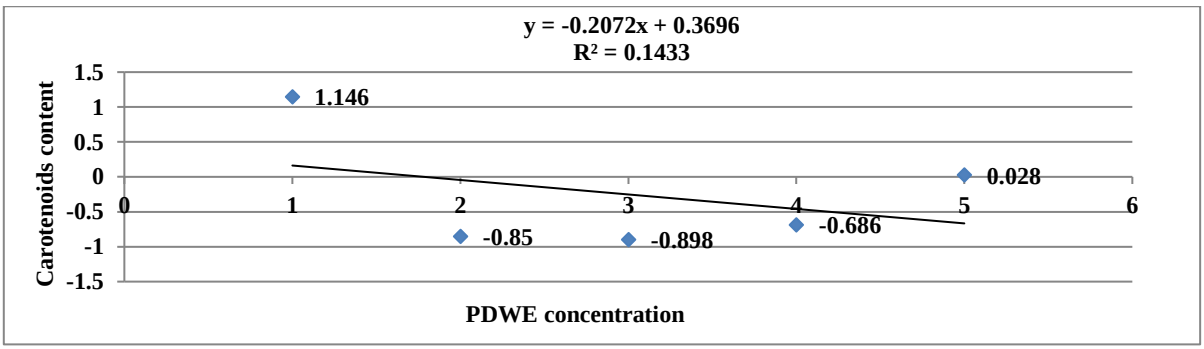

Figure 3. Correlation coefficient between carotenoids content and PDWE concentration.

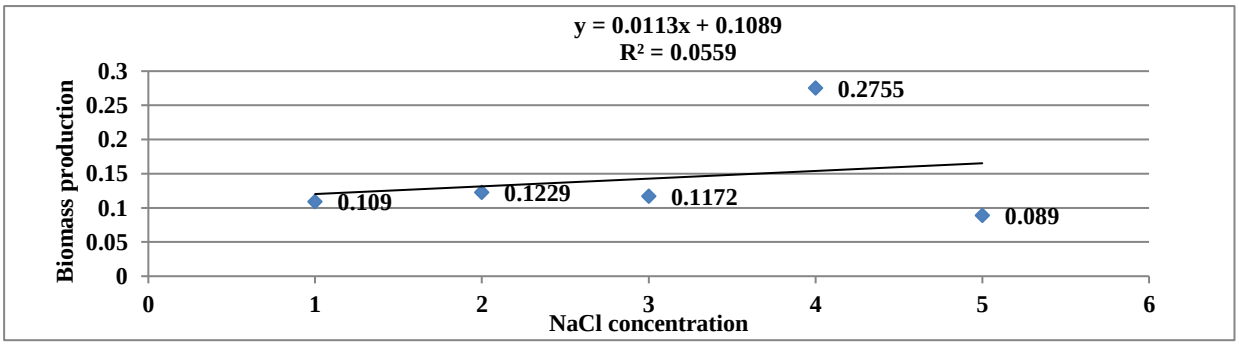

Figure 4. Correlation coefficient between biomass production and NaCl concentration.

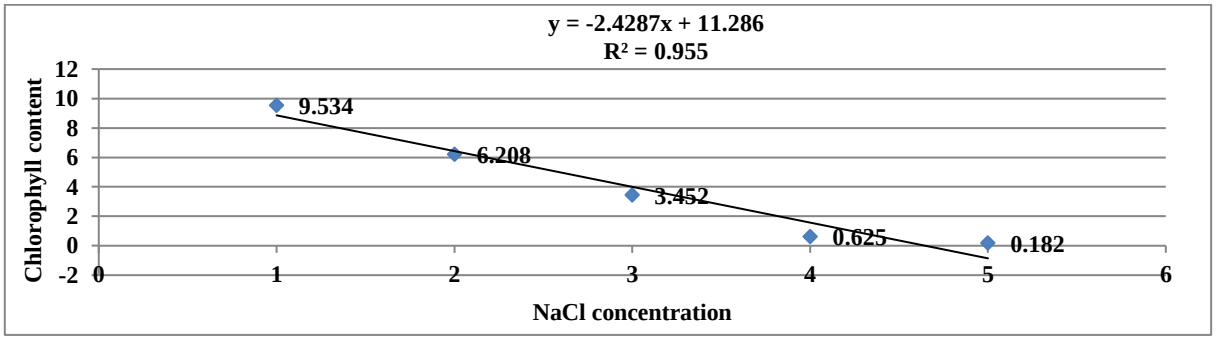

Figure 5. Correlation coefficient between chlorophyll content and NaCl concentration.

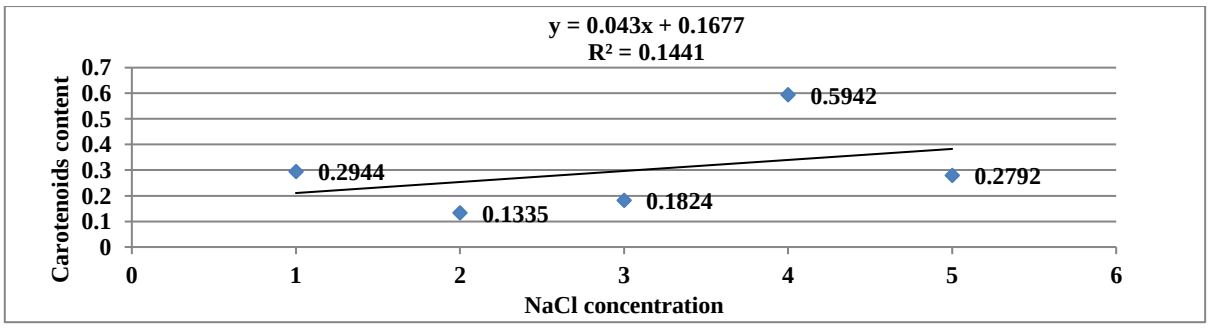

Figure 6. Correlation coefficient between carotenoids content and NaCl concentration.

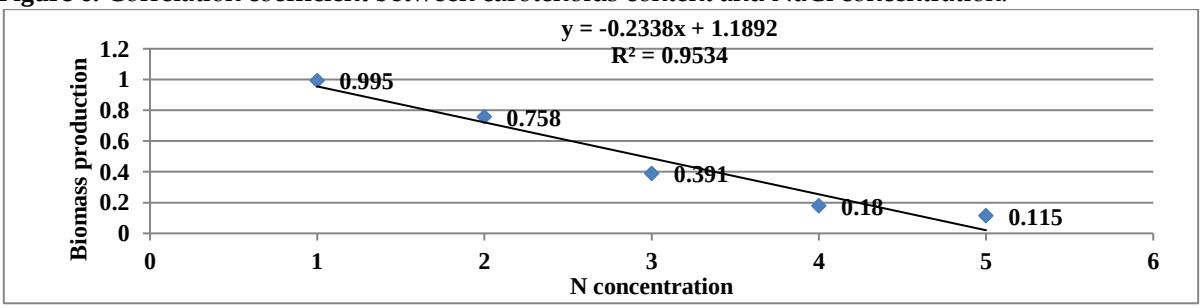

Figure 7. Correlation coefficient between biomass production and N concentration.

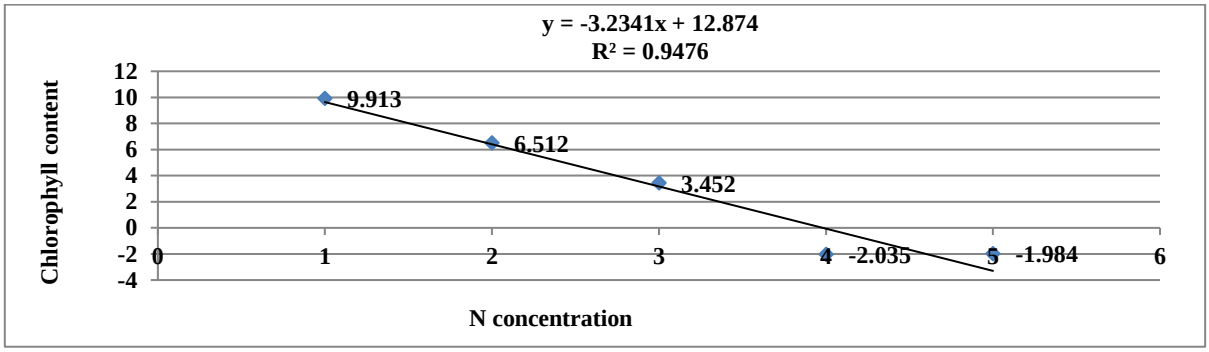

Figure8. Correlation coefficient between chlorophyll content and N concentration

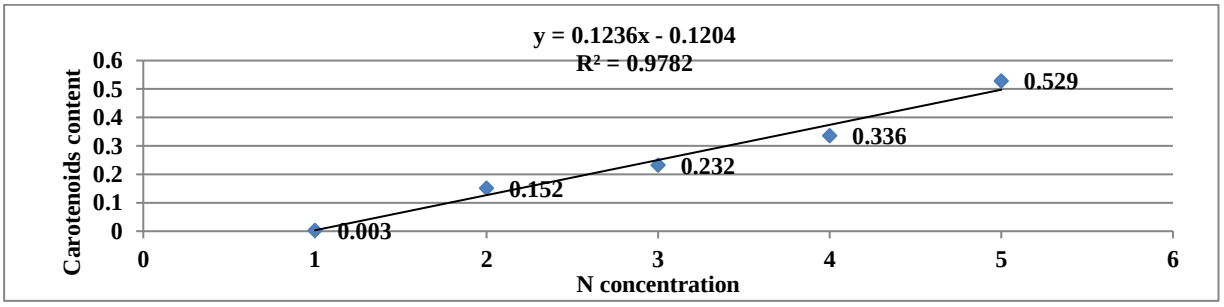

Figure 9. Correlation coefficient between carotenoids content and N concentration.

إنتاج الكتلة الحيوية من طحلب *Chlorella vulgaris* باستخدام مخلفات التمور تحت ظروف إجهاد مختلفة

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الملخص العربي

تم إجراء تجربة معملية لإنتاج الكتلة الحيوية من طحلب كلوريللا فولجارس *Chlorella vulgaris* باستخدام مخلفات التمور، وذلك بتنمية الطحلب على بيئة BG-11 في أعمدة زجاجية شفافة تحت ظروف اضاءة فلورسنتية وتهوية مستمرة، وتم غسل مخلفات التمور *Phoenix dactylifera* وتجفيفها بالفرن عند 55م^5 لمدة 24 ساعة بعد إزالة النوى للحصول على مسحوق التمر (0.9 مم). تمت تنمية الطحلب تحت ظروف إجهادات مختلفة كالتالى: تركيزات مستخلص المخلف 0,0، 10، 20، 30، 40 مل/لتر لإغناء بيئة نموالطحلب، و تركيزات كلوريد الصوديوم (0,0، 0,5، 1,0، 1,5، 2٪)، وتركيزات أزوت فى صورة (يوريا) (0، 25، 50، 75، 100٪)، وتم تقدير وتسجيل القياسات التالية: الوزن الجاف، والكلوروفيل الكلي، والكاروتينات وتحليل النمو. أظهرت النتائج المتحصل عليها فى الآتي: الحصول على أعلى صافى كتلة حيوية للطحلب (0,215 جم / لتر) بالمعاملة20 مل /لتر مستخلص مخلفات للتمورPDWE ، كما أعطت المعاملة 30 ملجم/لتر من مستخلص مخلفات التمور PDWE أعلى صافى محتوى كلوروفيلى (34,515 مجم/لتر)، أيضا أعطت أعلى محتوى كاروتينى (2,802 مجم/لتر) مقارنة بالكنترول، وأدى زيادة تركيز كلوريد الصوديوم إلى خفض الوزن الجاف ومحتوى الكلوروفيل، وعلى العكس زاد محتوى الكاروتين حتى 1.5٪ ثم إنخفض مرة أخرى، أيضا أدى إنخفاض تركيز الأزوت إلى خفض الوزن الجاف ومحتوى الكلوروفيل مع زيادة محتوى الكاروتين. من نتائج البحث المتحصل عليها نوصي بتعظيم استخدام مخلفات التمور كمصدر رخيص للكربون العضوي فى تنمية طحلب الكلوريلا والطحالب الدقيقة للحصول على الكتلة الحيوية Biomass.

الكلمات الاسترشادية: كلوريللا فولجارس، مخلفات التمور، الوزن الجاف، الكلوروفيل، الكاروتينات.