



Assessment of the Optimum Growth Medium and the Effect of Different Light Intensities on Growth and Photosynthetic Pigments of *Chlorella vulgaris* and *Scenedesmus arvernensis*

Mostafa M. El-sheekh^{(1)#}, Hanaa H. Morsi⁽²⁾, Lamiaa H.S. Hassan⁽²⁾

⁽¹⁾Botany Department, Faculty of Science, Tanta University, Tanta, Egypt; ⁽²⁾Botany and Microbiology Department, Faculty of Science, Menoufia University, Menoufia, Egypt.



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CHLORELLA *vulgaris* and *Scenedesmus arvernensis* are common eukaryotic green microalgae that have a very wide use in various applications. They can be used as raw materials in bio-energy production, in aquaculture nutrition, in pharmaceuticals, cosmetics, biofertilizers, biodegradation of wastes and dyes. So, this required several studies to test some factors that can influence the growth rate and evaluate their yield. This study investigated the effects of different media compositions: Bold's basal medium, BG-11 medium, modified Chu-10 medium, and Kuhl's medium on growth of *C. vulgaris* and *S. arvernensis*. Then, study the effect of four different light intensities on the growth rate using the optimized medium. The growth was recorded by estimating optical density (OD), cell counting (CC) and total chlorophyll contents. The results showed that the maximum growth was recorded at Modified CHU's 10 medium for *C. vulgaris* and BG-11 medium for *S. arvernensis*. The growth was compared with the initial record of *C. vulgaris* and *S. arvernensis* inocula; the OD increased by 6.85 and 5.15 times, respectively. *C. vulgaris* showed the optimum CC 9.60 times and total chlorophyll 7.96 times. CC recorded 10.42 times and total chlorophyll increased 5.90 times for *S. arvernensis*. Light intensities experiment showed that *C. vulgaris* gave the best growth rate at 60 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, on the 35th day of cultivation with increasing in OD, CC and total chlorophyll, (6.18, 5.27 and 3.58 times), of the initial record, respectively. Concerning *S. arvernensis* optimum growth rate was reached at 40 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, on the 35th day accompanied by an increment in OD, CC and total chlorophyll, (6.20, 4.55 and 10.04 times), of the initial record, respectively.

Keywords : Biomass, *Chlorella vulgaris*, Green algae, Growth media, Growth optimization, Light intensity, Light source, Microalgae, *Scenedesmus arvernensis*.

Introduction

Algae are a diverse group of photosynthetic organisms. They are classified to microalgae and macroalgae, they are present in aquatic and terrestrial ecosystems. Microalgae are microscopic organisms, including a huge variety of species. Microalgae require three main components for their growth including sunlight, nutrients from the water, and carbon source such as CO₂ from the air, they release about 50% of the atmospheric oxygen (Nigam & Singh, 2011).

Nowadays, there is a wide field of applications which depends on microalgae. They can produce valuable materials including carotenoids, lipid, natural dye, polyunsaturated fatty acid, peptide, toxin, sterols, phenolic compounds, immune modulators, agars, agarose, alginates, carrageenans and vitamin B (Wen & Chen, 2003; He et al., 2005). They can be used for aquaculture feeds, animal, human nutrition supplements, pharmaceuticals, cosmetic products, pigments and biofertilizer. They can be used for extracting high-value molecules, stable isotope

#Corresponding author email: mostafaelsheikh@science.tanta.edu.eg

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biochemicals, (Pulz & Grass, 2004; Moreno-Garcia et al., 2017). Algae are available biomass for bio-energy fuel (bioethanol and biodiesel) due to their rapid growth rate and productivity (Li et al., 2008; Abou-El-Souod et al., 2017) and high oil content (oil yield in microalgae can exceed 75% by weight of dry biomass (Christi, 2007; Hu et al., 2008). Algae are used in wastewater treatment and dyes degradation from inorganic salts, such NH_4^+ , NO_3^- , PO_4^{3-} , using them as nutrient materials (Mata et al., 2010; Makarevičienė et al., 2011; Suganya et al., 2016; Moreno-Garcia et al., 2017; El-Sheekh et al., 2018).

Several researchers studied how to maximize the growth rate (GR) of microalgae. The growth of microalgae depended on the type of microalgae, the CO_2 rate, light intensity, temperature, pH, nutrient constituents and dark-light period (Welter et al., 2013; Zhao & Su, 2014; Han et al., 2015; Cheah et al., 2018; Derakhshandeh & Tezcan Un, 2019).

Both *C. vulgaris* and *S. arvernensis* are green microalgae, in the class Chlorophyceae. They are considered to be a valuable microalgae for several applications because they have a high growth rate, (Gouveia & Oliveira, 2009; Yoo et al., 2010).

This study aimed to evaluate the growth of *C. vulgaris* and *S. arvernensis* by using different media compositions and study the effect of different light intensities on the growth at the optimum growth conditions.

Material and Methods

Algal strains

The experimental organism *Chlorella vulgaris* was obtained from Phycology laboratory, Faculty of Science, Menoufia University, Egypt. While, *S. arvernensis*, was isolated from fresh water canals in Shebin El-kom, Menoufia government, Egypt. The purified isolated microalga was identified up to species level using an inverted divert light microscope following the keys of identifications (Bold & Wynne, 1978; Prescott, 1984; Yamagishi, 1992; Vymazal, 1995).

Algae cultivation media

C. vulgaris and *S. arvernensis* were grown on four synthetic media with adjusted pH (7 ± 0.2), to check the optimized medium that gives the best growth, Bold's basal medium (Bischoff & Bold, 1963), BG-11 Medium (Ilavarasi et al., 2011),

Modified Chu-10 medium (Stein, 1973), and Kuhl's medium (Kuhl & Lorenzen, 1964).

For each species, 250ml conical flasks were prepared, each one containing 90ml of each medium. The pH for each medium was adjusted with 1M sodium hydroxide (NaOH) and 1N hydrochloric acid (HCl) by using pH meter (SCHOTT CG 843). Before experimental setup the media were sterilized in autoclave at 121°C, 1.5atm. for 20min. The autoclaved media were inoculated uniformly with 10 % (v/v) microalgal cell suspension. The flasks were attached with air pumps and incubated at $25 \pm 3^\circ\text{C}$ under continuous illumination of three standard cool white LED lamps (9W) ($60\mu\text{mol photons m}^{-2}\text{s}^{-1}$). This experiment was carried out in three replicates. The growth was observed using optical density (OD), cell count (CC) and chlorophyll contents (Chl. Cont.). The growth curve was followed up for 6 weeks.

Light intensities

According to Abou-El-Souod et al. (2016), the effect of light sources was tested to cultivate the *C. vulgaris* and *S. arvernensis*, using sunlight and artificial illumination provided from white Led lamps. Four growth sets were constructed and illuminated by white Led lamps, which were mounted in the chamber at a height of about 30cm from the bench top:

- (i) The 1st Set: Illumination by Sunlight ($125\mu\text{mol photons m}^{-2}\text{s}^{-1}$),
- (ii) The 2nd Set: Illuminated by one white Led lamp ($25\mu\text{mol photons m}^{-2}\text{s}^{-1}$),
- (iii) The 3rd Set: Illuminated by two white Led lamps ($40\mu\text{mol photons m}^{-2}\text{s}^{-1}$) and
- (iv) The 4th Set: Illuminated by three white Led lamps ($60\mu\text{mol photons m}^{-2}\text{s}^{-1}$).

Each set consisted of three 250ml Elementary flasks, each flask contained 90ml sterilized modified CHU's-10 medium for *C. vulgaris* and BG-11 medium for *S. arvernensis* which were inoculated by 10% of each algal species.

Optical density (OD)

According to (Sharma et al., 2011; Abou-El-Souod et al., 2016; Weise et al., 2019), OD is represented in terms of absorption using colorimeter at 680nm by spectrophotometry (Spectro UV-VIS DUAL BEAM 8 AUTO CELL UVS-2700, Germany).

Cell count (CC)

According to (Abou-El-Souod et al., 2016; Loftus & Johnson, 2019), depending on the size of the alga, and whether the algae are single celled and not colonial or chain-forming. Cell numbers can be determined using a Neubauer haemocytometer (Guillard & Sieracki, 2005) or Sedgwick-Rafter chamber (McAlice, 1971). The haemocytometer is the most widely used for cell counting around the world. It is suitable for cells in the size range of blood cells (i.e. less than about 100µm in diameter) and for cell densities less than 10⁵ cells.ml⁻¹. The number of cells is the summation of all the counted cells in all squares counted. The volume is the total volume of all the squares counted. The formula used when counting in the big squares. Errors in the range of 20%-30% are common in this method due to pipetting errors, statistical errors, chamber volume errors and errors from volume of sample introduced into the chamber.

$$\text{Concentration (cells/ml)} = (\text{Number of cells} \times 10.000) / (\text{Number of squares} \times \text{dilution}).$$

Extraction and estimation of photosynthetic pigment (Chlorophyll) (Mackinney, 1941)

A known volume of algal cultures (5ml) was centrifuged at 4000rpm for 10min. The pellets were washed twice with distilled water. Add 5ml of 95% methanol, then heat on water bath at 60°C for 30-60min. The extracted samples were centrifuged at 4000rpm for 10min. The extraction step may be repeated twice to get the complete extraction. The extracts of total chlorophyll were estimated by UV-VIS spectroscopy. Chlorophyll content of the algae was estimated by Spectrophotometry at 650 and 665nm.

The concentration of chlorophyll was calculated using the formula:

$$\text{Total chlorophyll (mg.ml}^{-1}\text{)} = ((2.55 \times 10^{-2} \text{ E650}) + (0.4 \times 10^{-2} \text{ E665})) \times 10^3.$$

Statistical approach

The results were presented as the mean of three replicates ± standard deviation (SD). The statistical analyses were carried out using SPSS for Windows (SPSS 16). Data obtained were analyzed statistically to determine least significant difference (LSD) at 5% probability (P ≤ 0.05) using one way analysis of variance (ANOVA).

Results

The results showed that the highest growth of *C. vulgaris* was detected at modified CHU's-10 medium, on the 35th day. As shown in Figs. 1, 2 and 3, the optical density (OD), cell count (CC) and total chlorophyll contents were increased by 6.85, 9.59 and 5.90 times compared with the initial record, respectively. While BG-11 medium recorded an enhancement of 6.69 in OD, 9.03 in CC and 5.61 times in total chlorophyll. The growth of *C. vulgaris* in Bold's basal medium and Kuhl's medium showed lower increment in all growth parameters on the 28th day.

The results of *S. arvernensis* showed that the maximum growth was detected in BG-11 medium, on the 35th day. As recorded in Figs. 1, 2 and 3, the optical density (OD), cell count (CC) and total chlorophyll contents increased by 5.15, 10.42 and 5.90 times, respectively. While modified CHU's-10 medium recorded lower values (4.51 in OD, 8.93 in CC and 5.61 times total chlorophyll). The growth in Kuhl's medium and Bold's basal medium gave the lower growth rate in all the investigated parameters.

C. vulgaris was grown in the optimized modified CHU's-10 medium and *S. arvernensis* was grown in the optimized BG-11 medium under different light sources (natural and artificial) and intensities. Figures 4, 5 and 6 showed the estimated results.

The highest growth of *C. vulgaris* was obtained at 60µmol photons m⁻²s⁻¹, on the 35th day of the growth, with increasing in OD, CC and total chlorophyll, 6.18, 5.27 and 3.58 times, of the initial record, respectively. While the growth rate showed a parallel decrease with decreasing light intensities of 40 and 25µmol photons m⁻²s⁻¹. The growth estimation at natural Sun light was 4.32 times in OD, 2.95 times in CC and 5.00 times total chlorophyll on the 42th day. The minimum growth rate was detected at 25µmol photons m⁻²s⁻¹, which was recorded (3.03, 2.38 and 1.61 times in OD, CC and total chlorophyll).

S. arvernensis maximum growth was obtained at light intensity of 40 µmol photons m⁻²s⁻¹, in the 35th day of the growth, with increasing in OD, CC and total chlorophyll, 6.20, 4.54 and 10.04 times, of the initial record, respectively. While the growth curve showed some retardation at 60µmol

photons $m^{-2}s^{-1}$ on the 28th day, (4.77 times in OD, 2.59 times in CC and 4.75 times total chlorophyll). While, the growth estimation at natural Sun light was 4.71 times in OD, 2.44 times in CC and 5.00

times total chlorophyll. The minimum growth rate was detected at the lowest intensity of 25 μmol photons $m^{-2}s^{-1}$, which recorded 3.86 in OD, 2.36 in CC and 4.81 times total chlorophyll.

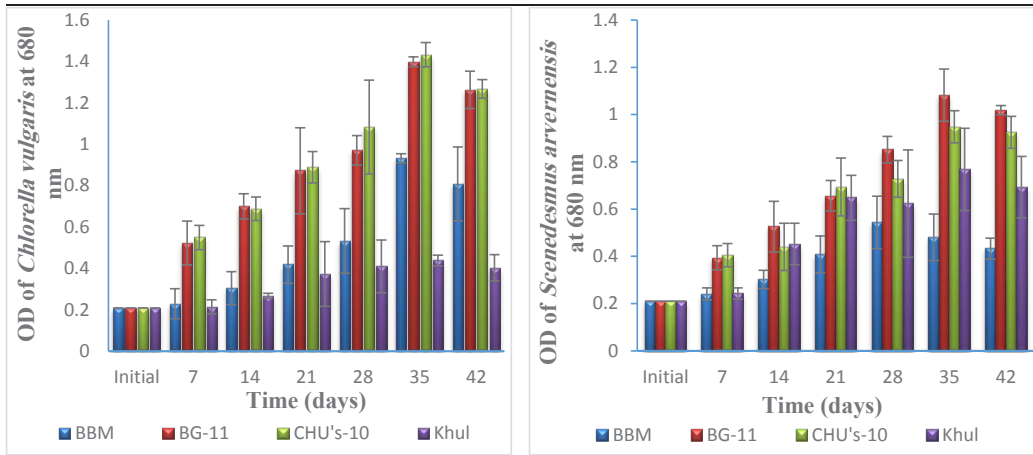


Fig. 1. Effect of different media on growth of *C. vulgaris* and *S. arvernensis* as (OD).

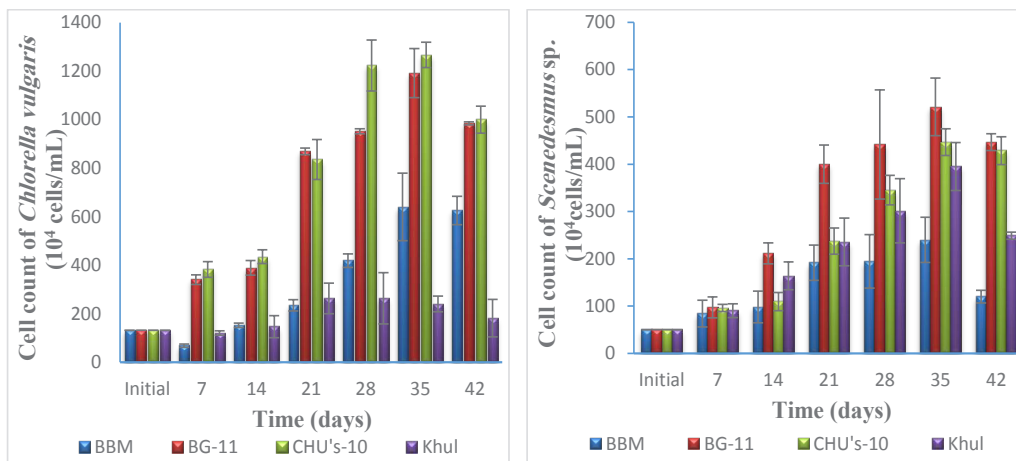


Fig. 2. Effect of different media on growth of *C. vulgaris* and *S. arvernensis* as (CC).

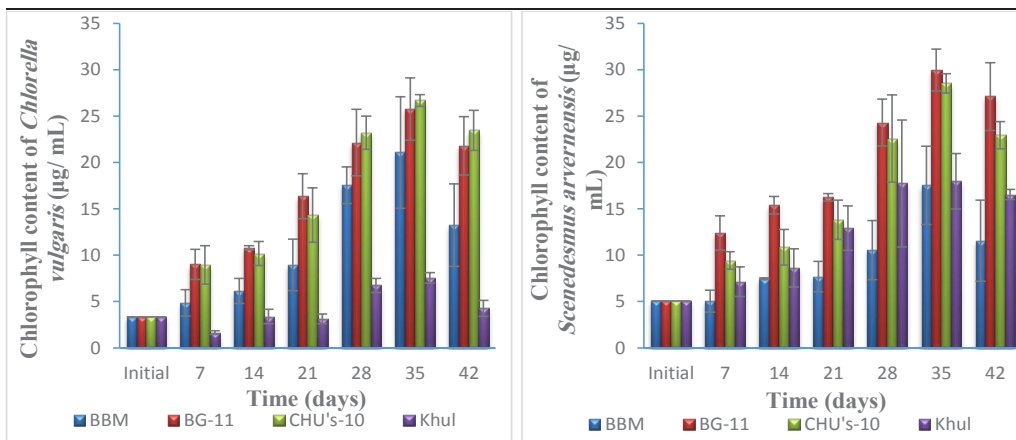


Fig. 3. Effect of different media on growth of *C. vulgaris* and *S. arvernensis* as (Chl. content).

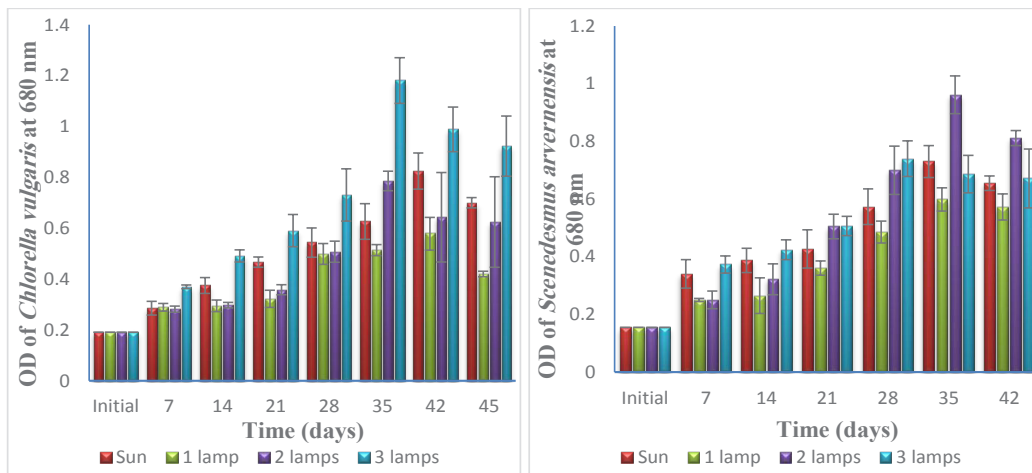


Fig. 4. Effect of different Light Sources of algal culture on growth (OD) of *C. vulgaris* and *S. arvensensis*.

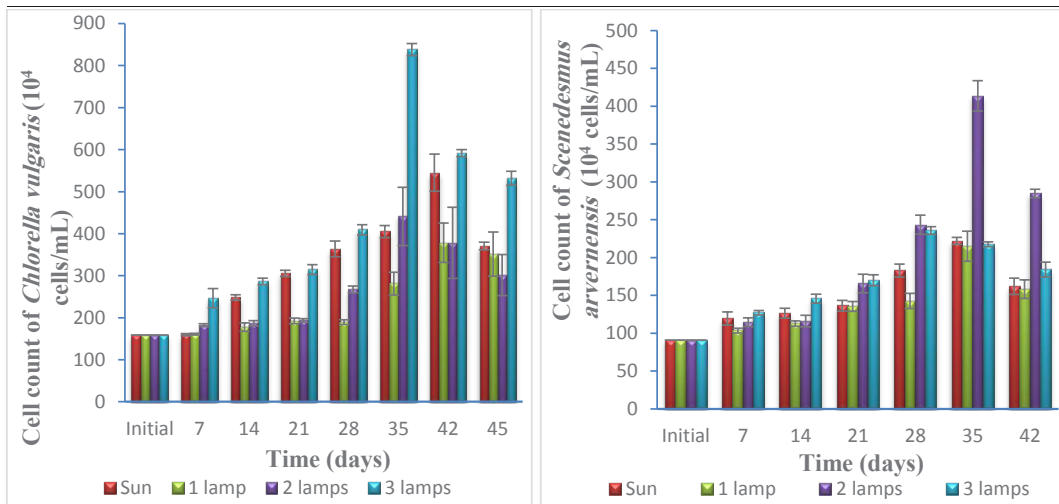


Fig. 5. Effect of different Light Sources of algal culture on growth (cell count CC) of *C. vulgaris* and *S. arvensensis*.

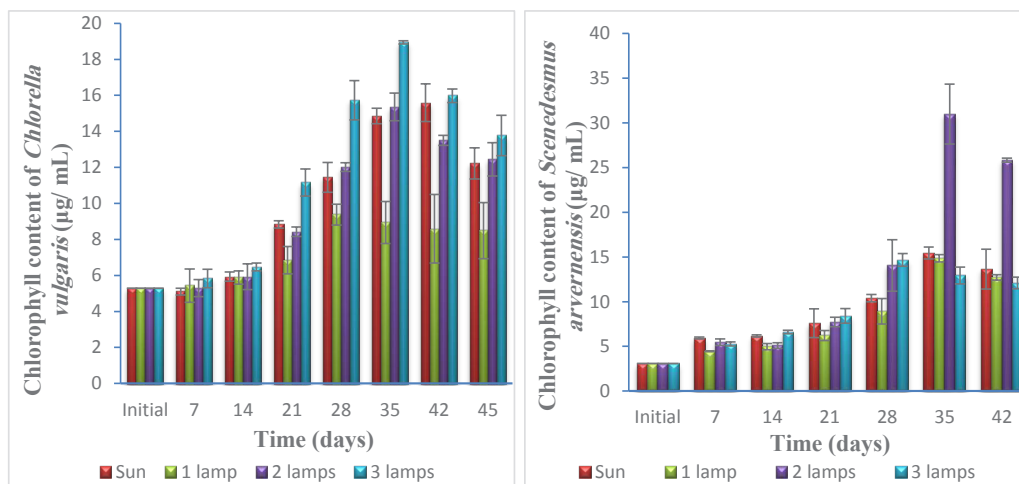


Fig. 6. Effect of different Light Sources of algal culture on growth total Chlorophyll content of *C. vulgaris* and *S. arvensensis*.

Discussion

The results showed that modified CHU's-10 medium is the optimum medium for *C. vulgaris* cultivation. This agrees with the results reported by Sharma et al. (2011) and Abou El-Soud et al. (2016) who reported that modified CHU's-10 was the best growth medium for *C. vulgaris* cultivation. Kuhl medium was the best growth medium for *C. vulgaris* cultivation according to El-Sheekh et al., 2018. While the maximum biomass productivity of *S. arvernensis* grown in BG-11 medium, compared to other tested media, this agrees with El-Naggar & El-Sheekh (1998), Hamouda et al. (2016), Ruen-ngam (2017), Derakhshandeh & Tezcan Un (2019). This disagrees with Abomohra et al. (2018) who reported that Kuhl medium gave the highest growth for *Scenedesmus* sp. All algae can do photosynthesis, as they can use natural sun light, atmospheric carbon dioxide CO₂ and nutrients. Some elements as macro-elements (N, P, and O) other microelements (Cl, S, Mg, Na, Ca and K) and trace metals from the environment. The higher biomass productivity of each microalgal species in the specific medium might be related to presence of certain components and their concentrations, comparing with the other media components, which are an essential constituents of all structural and functional components of the algal cells (Chu et al., 2007; El-Sheekh et al., 2012).

The results showed that the highest growth of *C. vulgaris* and *S. arvernensis* were obtained after cultivation under light intensity of 60 μmol photons m⁻²s⁻¹ and 40 μmol photons m⁻²s⁻¹, respectively. Sharma et al. (2012) reported that maximum biomass was observed at natural day light. This agrees with Richmond & Grobbelaar (1986), they reported that the growth of the microalgae under laboratory conditions were significantly lower than the growth under natural conditions, which could be explained as artificial illumination caused stress to the cultivated microalgae. Different light sources may cause changes in the microalgal cell properties and photosynthetic efficiency according to the light availability (Dubinsky et al., 1995). Sunlight has equal amount of all the wavelengths, while artificial light consists of a few preferred wavelengths (Rocha et al., 2003; Mercado et al., 2004). Senger & Fleischhacker (2006) observed that in the low light conditions chlorophyll content was doubled to improve the capture of more light and recover the loss of absorbable wavelengths.

However, the photosynthetic capacity increase three folds at strong light intensity, the chlorophyll contents decrease. Microalgae require light energy to convert carbon dioxide to organic compounds, Juneja et al. (2013), who reported that algal cells can do adaptation with light intensities. They may produce different types and quantities of pigments or the production of fatty acids. Photo-inhibition occurs when light intensity increase above saturating limits (You & Barnett, 2004). As the high light intensity cause damage in the chloroplast lamellae and inhibit enzymes of carbon dioxide fixation (Iqbal & Zafar, 1993), they also observed that low light intensity increased protein content while high light intensity enhanced extracellular polysaccharide content. Wahidin et al. (2013) reported that microalgae growth increased at an intensity greater than 50 μmol photons m⁻²s⁻¹ but the growth decreased at intensities higher than 200 μmol photons m⁻²s⁻¹. Bohutskyi et al. (2016) showed that intensities lower than 30 μmol photons m⁻²s⁻¹ produced inefficient growth.

Sunlight or artificial light represent the essential light source for phototrophic algal cells. Lee et al. (1999) reported that the photosynthetic activity of microalgal cells increases with light intensity until reaching the light saturation point. Intensities higher than this point cause damage of the light receptors in the chloroplasts of the cells. This leads to photo inhibition and decrease the photosynthetic rate. The high cell density culture suffers from mutual shading problem, as the cells closest to the surface of the broth culture receive the majority of the available light more than the cells below. There are other parameters affect light distribution, such as the depth of the cultivated media and mixing play key roles (Grobbelaar, 1994). Light affects the physiological process in microalgae (He et al., 2015). As light intensity can regulate the quantity and quality of the available light energy for microalgae that conduct photosynthesis, vital activities and control the metabolic pathway (Khalid et al., 2019).

Makarevičienė et al. (2011) reported that the cultivation of *Chlorella* sp. and *Scenedesmus* sp., on BG11 medium in the laboratory required 29 to 50 days. The cultivation of *Spirulina* take about 40 days in a 1000 L- photobioreactor (Delrue et al., 2017). This long growth incubation period influenced the growth by increasing the biomass. The initial density of inocula affect the duration of growth period. According to Van-

Khanh et al. (2017) the lowest initial density of the microalgal inoculum required long cultivation time comparing with the highest initial density to reach the highest growth rate.

Conclusion

The growth of microalgae can be affected by various factors, including different growth media with variable nutrient composition and different pH values. There are other environmental factors influence the growth, such as light sources, light intensities, temperature, light duration and aeration. As a result of this study; it was concluded that modified CHU's-10 medium was the best medium for *C. vulgaris* cultivation, and the best light intensity for its growth was 60 μ mol photons m⁻²s⁻¹. While, BG-11 medium at pH 7 was the optimum medium for *S. arvernensis* growth, in addition to this, light intensity of 40 μ mol photons m⁻²s⁻¹ (emitted from two white Led lamps) increased the growth and gave the highest growth rate more than the other intensities.

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تقييم وسط النمو الأمثل وتأثير شدة الإضاءة المختلفة على النمو و التمثيل الضوئي لطحلي *Scenedesmus arvernensis* و *Chlorella vulgaris*

مصطفى محمد الشيخ⁽¹⁾، هناء حسنين مرسي⁽²⁾ و لمياء حسن شحاته حسن⁽²⁾
⁽¹⁾ قسم النبات- كلية العلوم- جامعة طنطا- مصر و⁽²⁾ قسم النبات و الميكروبيولوجي - كلية العلوم- جامعة المنوفية- مصر.

Scenedesmus arvernensis و *Chlorella vulgaris* هي من الطحالب الخضراء حقيقية النواة، ذو استخدام واسع في مختلف التطبيقات. كما يمكن استخدامها كمواد خام في إنتاج الطاقة الحيوية، و في تغذية الأحياء المائية، في المستحضرات الصيدلانية، مستحضرات التجميل، الأسمدة الحيوية، التحلل الحيوي للنفايات والأصباغ. لذلك، تطلب هذا العديد من الدراسات لاختبار بعض العوامل التي يمكن أن تؤثر على معدل النمو وتقييم عائدها. خلال هذه الدراسة تم بحث تأثير الأوساط الغذائية المختلفة: Bold's basal (BBM) medium و BG-11 medium، و Chu-10 Modified medium، و Kuhl's medium على نمو *Scenedesmus arvernensis* و *Chlorella vulgaris*. و من ثم دراسة تأثير أربعة درجات مختلفة من شدة الإضاءة على معدل النمو لكل كائن باستخدام الوسط الأمثل. تم تسجيل النمو من خلال تقدير الكثافة البصرية (OD)، عد الخلايا (CC) ومحتوى الكلوروفيل. و أظهرت النتائج أن الحد الأقصى للنمو تم تسجيله في CHU's لـ *C. vulgaris* و BG-11 لـ *S. arvernensis*. و تم ملاحظة زيادة OD بنسبة 6.85 و 5.15 ضعف، على التوالي. كما أظهر *C. vulgaris* 9.60 ضعف عدد الخلايا و كذلك نسبة الكلوروفيل 7.96 ضعف. وأوضحت النتائج زيادة عدد الخلايا للـ *S. arvernensis* 10.42 ضعف وزاد الكلوروفيل 5.90 ضعف. أظهرت تجربة شدة الإضاءة أن *C. vulgaris* أعطى أفضل معدل نمو تحت تأثير ثلاثة مصابيح LED بيضاء (60 ميكرون فوتون م⁻² ثانية⁻¹)، في اليوم الخامس والثلاثين مع زيادة في OD، CC و الكلوروفيل الكلي، 6.18، 5.27 و 3.58 ضعف، على التوالي. كما بلغ معدل النمو الأمثل لـ *S. arvernensis* بعد التحضين في اثنين من مصابيح LED بيضاء (40 ميكرون فوتونات م⁻² ثانية⁻¹)، في اليوم 35 مع زيادة في OD، CC والكلوروفيل الكلي 6.20، 4.55 و 10.04 ضعف، على التوالي.