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Photobiostimulation of *Chlorella sorokiniana* Using Light Emitting Diodes (LEDs) for Increasing Lipid and Biodiesel Production

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Abstract

At present, the major body of research is focused on weaning the world from fossil fuels. The problem is that the world is running out of fossil fuel. Therefore, an alternative source must be identified. The biofuels are promising alternatives. In the case of petrodiesel, a promising alternative is biodiesel production from algae. The ability of microalgae to generate large quantities of lipids with a fast growth rate made them superior biodiesel producers. Using light-emitting diodes (LEDs) as an energy source in microalgal cultivation was recently increased owing to its large spectrum, endurance, and low-energy utilization. Changes in cultivation conditions, limited capabilities of harvesting light, and self-shading of microalgae were the most important problems. Therefore, the photobiostimulation of algae using LEDs radiation led to an increase in algal growth rate which results in increased lipid production. This research investigated the influence of monochromatic LEDs on the growth of *Chlorella sorokiniana* microalga. At the first phase, microalgae growth and algal biomass significantly increased under red LEDs [2.3 g/L], blue LEDs [1.8 g/L], green LEDs [0.7 g/L], and white LEDs (0.6) g/L as a control, respectively. At the second phase, microalgal growth and algal biomass significantly increased under red LEDs [2.9 g/L], blue LEDs 2.3 g/L, and white LEDs (1.5) g/L as a control, respectively. The percentage of extracted oil (%) or the yield of extracted oil of microalgae was 10.38 % (white LEDs), 16.94 % (blue LEDs), and 15.55 % (red LEDs) respectively. It was concluded that the photobiostimulation of algae using LEDs led to the enhanced weight of algal biomass, therefore increased of lipids and biodiesel production. The red LEDs were the best one in terms of increasing the weight of algal biomass. The blue LEDs were the best one in terms of increasing the percentage of extracted oil. However, the green LEDs were not effective.

Keywords: Biofuels; Biodiesel; Microalgae; Photobiostimulation; Photobioreactor; Light emitting diodes.

1. Introduction

Over recent years, the fast-increasing consumption and the expected depletion of fossil fuel reserves led to the classification of dependence of energy on fossil fuels as a kind of future challenge [1], and thus the increasing need for sustainable energy calls for the development of renewable and cost-effective alternative energy sources to reduce the use of fossil fuels [2]. Thus, microalgae have been widely investigated in recent years owing to their recognized benefits [3]. Algal biofuels are a renewable fuel derived from the algae as feedstock by different conversion bioprocesses. This is owing to the oil-rich structure of this substrate that can be coupled with its capability to alter metabolism under certain stress conditions. Its main advantage is the ability to convert almost all the energy from the substrate into several types of useful products apart from its large oil fraction [4]. They are recognized for CO_2 emission mitigation, fast growth rate and non-arable

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land usage for cultivation. These qualities present microalgae as beneficial over several or different other feedstocks. There is a major reason, or the main advantage of microalgae makes it an interesting alternative to the most popular feedstock of food crops is that algae do not compete with food crops [5]. To circumvent the 'food vs fuel' problem which has strongly coupled with first generation biofuel [6]. The biological treatment of lignocellulosic non crop biomass comes as the base for the improvement of second-generation biofuel techniques [7]. Especially that the lipid fraction of algal biomass comprises important fatty acids that play a vital role in anthropological nutrition [8]. Moreover, these fatty acids can be transformed into biodiesel [3].

Biofuels such as bioethanol, biohydrogen, and biodiesel are considered as alternative for petro-based fuels. Among the various biofuel options proposed, biodiesel came to be extremely promising fuel alternative [9-11]. According to research findings, biodiesel was identified as a potential resource that can satisfy the world's energy needs whereas it can be used in diesel engines (blinded by 20%) without requiring any changes to the engine as their combustion properties are nearly like the petro-based diesel [12].

The acuteness of the greenhouse effect led researchers to look up alternatives for reducing greenhouse gas emissions to the atmosphere. Energy effectiveness plays the main essential role in the problem of climate change due to emission of greenhouse gas from power consumption [13]. The amount of light produced from LEDs is the same amount of light from other energy sources, but LEDs use less energy. Besides, the generation of heat during this process is basically null, which facilitates conserve energy. In this context, in several different sectors, the LEDs topped instead of conventional light lamps owing to their low energy intake, which renders this an environmentally friendly light source [14, 15].

The research gap can be elucidated as follows: (1) the use of different wavelengths of LEDs was not thoroughly investigated, and (2) more research is needed to cover the biodiesel production from algae to fulfil the world fuel demand. The major objective of this research was to increase lipid production from algal biomass using LEDs radiation. The general objectives can be further elaborated in terms of the following specific objectives: Photobiostimulating algae using LED for enhancing lipids accumulation of algae and, therefore, increasing oil production; and cultivating the algae in photobioreactors exposed to sunlight, after being irradiated with LEDs.

2. Materials and methods

2.1. Microalgae strain

The microalgal species employed in this research was *Chlorella sorokiniana* SAG 211-8k produced by the Marine Toxin laboratory at the Egyptian Agriculture Research Institute. This oleaginous strain with low oil contents was selected to be exposed to LED lights as a photobiostimulant that could increase the lipids accumulation in the alga which have low oil contents (25 - 35%) and therefore increase biodiesel production from low-oil microalgae.

2.2. Culture medium

The medium was Blue-Green (BG-11) media composed of: $NaNO_3$ 1.5 g/l, $K_2HPO_4.3H_2O$ 0.0314 g/l, $MgSO_4.7H_2O$ 0.036 g/l, $CaCl_2.2H_2O$ 0.0367 g/l, Na_2CO_3 0.02 g/l, citric acid 0.0056 g/l, Na_2Mg (EDTA) 0.001 g/l, ferric ammonium citrate 0.0071 g/l, Trace metal mix A5+Co 1 ml was sterilized at 121°C for 15 min with pH adjusted at 7.4 [16, 17].

2.3. Experimental setup

The experimental setup can be elaborated as follows: designing an array of photobioreactors, identifying the appropriate LEDs source, and selecting the microalgae strain. Generally, there are three stages to biodiesel production from algae as illustrated in Figure 1.

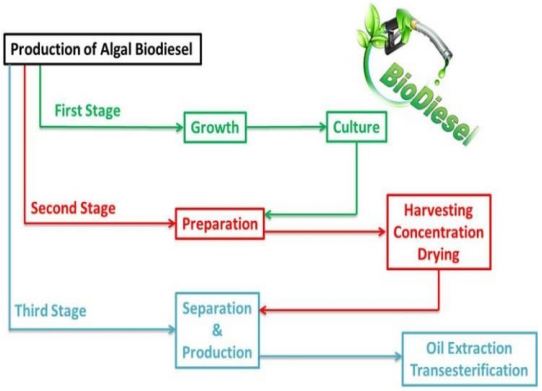

Fig. 1. Process flow chart for biodiesel production.

2.4. Culture condition

The implemented Lab-scale model is a closed photobioreactor (PBR) which consists of Erlenmeyer flask, an air pump (Shengzhe Bs-410, China), and sample purification filters (NY 0.45 µm, China). Microalga was grown in the laboratory [as shown in Figure 2] and was used as an experimental setup for *Chlorella sorokiniana* growth. Under sterilization conditions, using 2 L Erlenmeyer flask culture photobioreactor, 100 ml microalgal suspension (*Chlorella sorokiniana*) was inoculated into 900 ml of BG-11 media at 30 ± 5 °C with continuous stirring [7], pumping CO_2 and pH adjusted at 7.4. The experiments were carried out at the Department of Microbiology at the Faculty of Agriculture, Cairo University.

2.5. Irradiation or photobiostimulation setup

An important factor of determining optimal microalgal photosynthetic activity is the bioresponse to changes in light intensity and quantity [18]. The photobiostimulation was conducted at the Microbiology Laboratory at the Faculty of Agriculture, Cairo University. The light intensity of white LED was 1400 lux; blue LED was 200 lux; green LED was 3800 lux; and red LED was 600 lux, which was measured using a digital light meter (Lutron, LX-101, Taiwan).

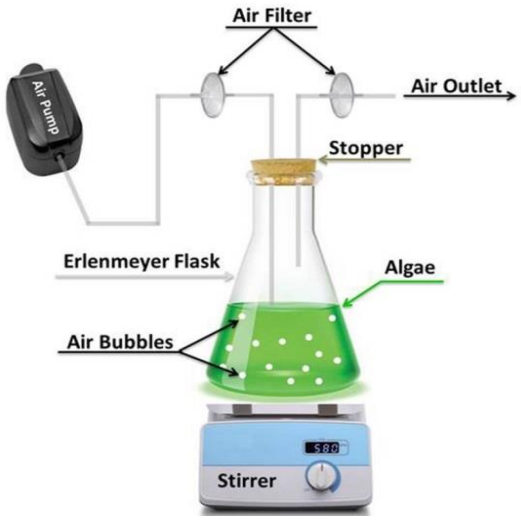

Fig. 2. Closed photobioreactor (PBR) system.

2.6. Photobiostimulation using LEDs

In this study, the experiments were performed in two phases, at the first phase algae was exposed to the following LEDs sources (Alobeidi, China): Red 635 nm; Green 525 nm; and Blue 465 nm for two hours then cultivated in the photobioreactors and exposed to white light of complete spectrum (wavelength: 400-700 nm). At the second phase algae were exposed only to the following LED*s* sources (Alobeidi, China): Red 635 nm and Blue 465 nm for the cultivation duration completely without both of white light and green LED because it was also observed that photobiostimulation of green algae using green LEDs was not effective for lipid production because the green light was reflected, therefore the green microalgae were not absorbed the green light. Thus, photosynthetic organisms in energy absorption are dependent on the chemical nature of their constitutive pigments [18, 19], thus red and blue LEDs were chosen as the wavelength for biomass production in the second phase culture. These wavelengths were selected based on previous studies [20-23]. The hydraulic retention time (HRT) of the algae in photobioreactors was twenty-one days. All experiments were conducted in triplicate.

2.7. Experimental design Algal exposure to LEDs

At the first phase, to investigate the effect of LEDs radiation on lipid production, 100 ml algal biomass were inoculated into 2L Erlenmeyer flask with continuous stirring were irradiated by both (red, blue, and green) LEDs sources (Fig. 3) compared with white LED source as a control. At the second phase algae were irradiated by both (red and blue) LEDs under the same conditions as above (Fig. 4) compared with white LED source as a control.

2.8. Oil extraction

Lipids were extracted from harvested microalgae biomass. The microalgae were harvested after twenty-one days of cultivation by centrifugation at 4500 rpm for 10 min. The algal biomasses were dried at 85 °C for 24 h before the extraction process. Total lipids were extracted using a Soxhlet Reflux Extractor with chloroform: methanol (2:1, v/v) from dried algae [24].

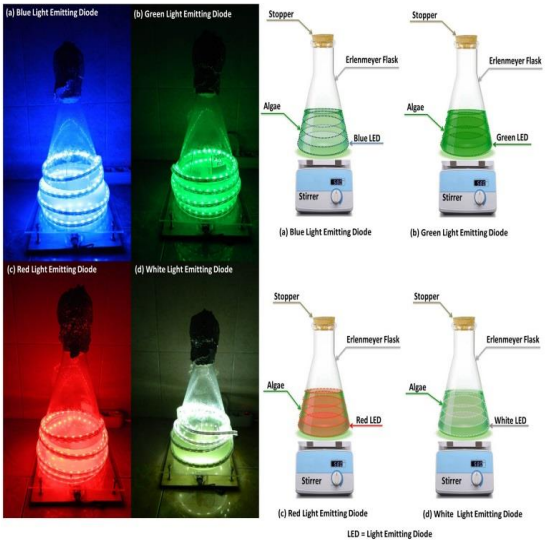

Fig. 3. Irradiation of algae using LEDs source for two hours.

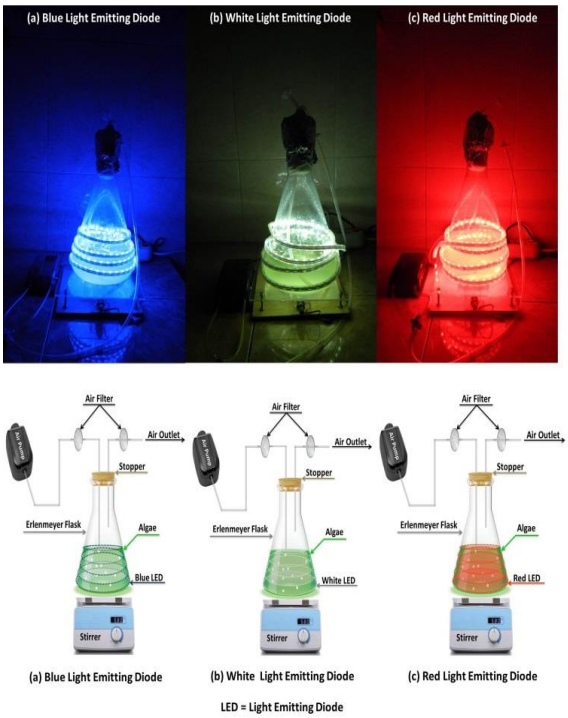

Fig. 4. Irradiation of algae using LEDs source for twenty-one days.

2.9. Statistical analysis

The aim of the statistical analysis was to evaluate the effect of LEDs irradiance selected on microalgal growth. Each experiment was carried out in triplicate. The statistical significance of diff erences in weight of algal biomass was evaluated by one-way analysis of variance and Kruskal- Wallis Test ($P \leq 0.05$) using SPSS Software (IBM, v. 20).

3. Results

3.1. Effects of LED irradiation on algal biomass

Amount of light produced from LEDs is the same amount of light produced from other energy sources, but LEDs use less energy. Further, heat generated during this process is almost null, which supports energy conservation. Accordingly, in several different sectors, the LEDs topped instead of conventional light lamps owing to their low energy requirements, which makes it an environmentally friendly light source which agrees with Duarte & Costa [20], and the implementation of LEDs in microalgal cultivation affects the quantity and quality of the produced biomass. This happens primarily owing to the light's mono-chromaticity with effective control of photosynthetic photon flux density, a property not found in sunlight that agrees with Schulze et al. [15].

3.2. Effects of using LEDs irradiation on algal biomass

The effects of different wavelengths of LEDs on the growth of microalgae were evaluated by using the monochromatic LEDs like red (635 nm), Green (525 nm), and Blue (465 nm). As a control, white LED was used in the same conditions for the microalgae conditions for the microalgal growth. As shown in Table 1 at the first phase red LED produced the highest of microalgal biomass, ranging 2.0–2.3 g/L, followed in descending order by the blue LED (1.5–1.8 g/L), the green LED (0.4–0.7 g/L), and the white LED (0.3–0.6 g/L). Also, at the second phase red LED produced the highest microalgal biomass, ranging 2.7–2.9 g/L, followed by the blue LED (2.0–2.3 g/L), and white LED (1.2–1.5 g/L). These results pointed that the weights of algal biomass at the second phase were greater than that in the first phase (Fig. 5).

Table 1. Weights of algal biomass after irradiation.

Colors of LEDs	Weights of algal biomass (g/L)	
	First phase	Second phase
White LED	0.6	1.5
Blue LED	1.8	2.3
Red LED	2.3	2.9
Green LED	0.7	-

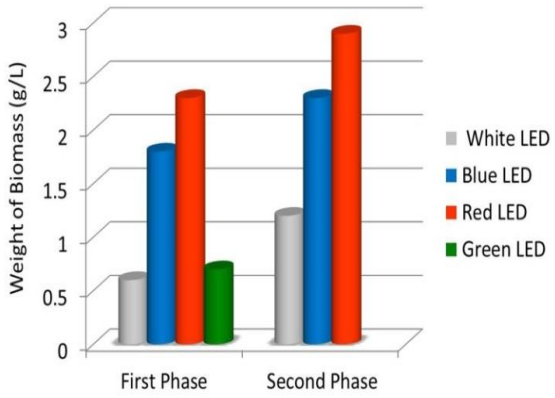

Fig. 5. Weights of algal biomass after irradiation of algae.

3.3. Yield of extracted oil

The percentage of extracted oil from algal biomass which has been irradiated with blue monochromatic light LEDs is better than that produced by red LEDs which is in agreement with Kim et al. [25] who concluded that blue light LED illumination (wavelength: 430-465 nm) led to increased cell size of some algal strains. While red light LED illumination (wavelength: 630-665 nm) led to small-sized cells with active divisions. The best color of LEDs in terms of percentage of extracted oil was the blue LEDs (465 nm), followed by red LEDs then white LEDs as a control (635 nm) as shown in Table 2.

Table 2. The percentage of extracted oil (%) from algal biomass.

Colors of LEDs	Percentage of extracted oil (%)
White LED	10.38
Blue LED	16.94
Red LED	15.55

As a result of algal irradiation by different colors of LEDs, the percentage of extracted oil (%) from 100 g of microalgae was 10.38 % (white LED) as a control, 16.94 % (blue LED), and 15.55 % (red LED) respectively as shown Figure 6.

3.4. Statistical analysis

At the first phase, Table 3 summarizes the overall mean of the weight of algal biomass affected by the irradiated monochromatic LEDs, like red (635 nm), green (525 nm), and blue (465 nm) compared with the white LED (400-700 nm) as a control. The highest significant weight of algal biomass was reached when algae were irradiated with red LED 635 nm source for 2 h which yielded 2.4683 g/L of biomass and the lowest algal biomass weight was reached obtained from the irradiance with white LED (400-700 nm) as a control for 2 h which yielded 1.6329 g/L (Fig. 7). Additionally, the irradiation with blue LED 465 nm and red LED 635 nm for 2 h, were found to be significantly different ($p \leq 0.05$) and were 3.3770 g/L and 2.4683 g/L algal biomass, respectively. Nevertheless, the irradiation with white LED 400-700 nm and green LED 525 nm for 2h, were found to be insignificantly different ($p > 0.05$) and were 1.6329 g/L and 1.7732 g/L algal biomass, respectively.

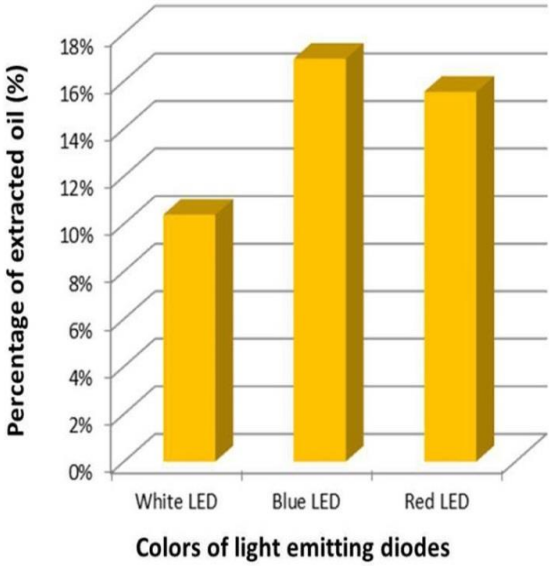

Fig. 6. The percentage of extracted oil (%) from 100 grams of microalgae.

Table 3. Mean performance of algal biomass weight influenced by di-erent irradiation with monochromatic LED sources during two hours.

Treatments	Mini-mum	Maxi-mum	Mean	Std. Deviation
White LED	1.06	2.20	1.6329	0.5711
Red LED	1.77	3.60	2.4683	0.9875
Blue LED	2.89	3.86	3.3770	0.4878
Green LED	1.69	1.85	1.7732	0.0789

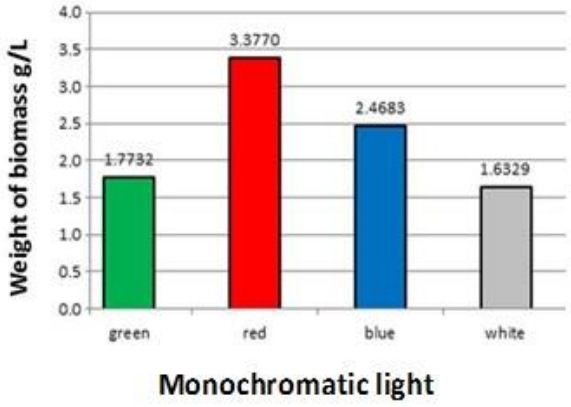

Fig.7. Mean performance of algal biomass weight influenced by di-erent irradiation with monochromatic LEDs sources for two hours.

In the second phase, Table 4 summarizes the overall mean weight of algal biomass aff ected by the irradiated monochromatic LEDs, like red (635 nm), and blue (465 nm) compared with the white LED (400-700 nm) as a control during the cultivation period. The highest significant algal biomass weight was reached when algae were irradiated with red LED 635 nm source for twenty-one days which yielded 3.911 g/L of biomass and the lowest weight of algal biomass weight was reached from the irradiance with white LED (400-700 nm) as a control for twenty-one days which yielded 2.559 g/L (Fig. 8). Additionally, the irradiation with blue LED 465 nm and red LED 635 nm for twenty-one days, were found to be significantly diff erent ($p \leq 0.05$) and were 3.411 g/L and 3.911 g/L algal biomass, respectively. Nevertheless, the irradiation with white LED 400-700 nm and both of blue LED 465 nm and red LED 635 nm for twenty-one days, were found to be significantly diff erent ($p \leq 0.05$) and were 2.559 g/L, 3.411g/L, and 3.911 g/L algal biomass respectively.

Table 4. Mean performance of algal biomass weight influenced by di-erent irradiation with monochromatic LED sources for twenty-one days.

Treatments	Minimum	Maximum	Mean	Std. Deviation
White LED	2.4384	2.6801	2.559	0.1209
Blue LED	2.8089	4.0128	3.411	0.6020
Red LED	3.1838	4.6388	3.911	0.7275

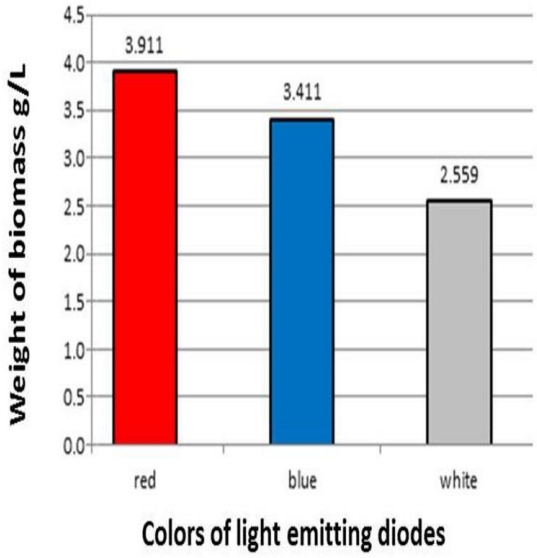

Fig. 8. Mean performance of algal biomass weight influenced by di-erent irradiation with monochromatic light LED sources for twenty-one days.

The statistical analysis of the various exposed microalgae groups revealed that all the employed treatments have resulted in significant higher production of dry weight of biomass in grams when compared to the group that just received white light ($P < 0.005$). The only exception was the green LED group as despite the dry weight of biomass was higher than the white light exposed group, but that increase was not statistically significant as shown Table 5. Nevertheless, the red LED exposed microalgae group showed the highest dry weight of biomass, but that increase was of statistical significance when compared to all the other dry weight of biomass production by other groups ($P < 0.05$) as shown Table 5. It can be concluded from Figure 9 that the light exposure of different wavelengths has increased the production of dry weight of biomass.

Table 5. Descriptive statistics and Fisher test results for dry weight of biomass.

Treatments	Sample size	Mean	Standard deviation	SE of mean
White Light	3	3.978	1.41915	0.81935
Green *LED*	3	4.322	0.1635	0.0944
Blue *LED*	3	9.43233	1.21603	0.70208
Red *LED*	3	11.56133	1.21596	0.70204

	Mean Diff	SEM	t Value	Prob	Alpha	Sig	LCL	UCL
Level 2, Level 1	0.344	0.83272	0.4131	0.68825	0.05	0	-1.51142	2.19942
Level 3, Level 1	5.45433	0.83272	6.54999	6.47295E-5	0.05	1	3.59891	7.30976
Level 3, Level 2	5.11033	0.83272	6.13689	1.10205E-4	0.05	1	3.25491	6.96576
Level 4, Level 1	7.58333	0.83272	9.10666	3.72164E-6	0.05	1	5.72791	9.43876
Level 4, Level 2	7.23933	0.83272	8.69356	5.64403E-6	0.05	1	5.38391	9.09476
Level 4, Level 3	2.129	0.83272	2.55667	0.02854	0.05	1	0.27358	3.98442

Sig equals to 1 indicates that the mean difference is significant at the 0.05 level
Sig equals to 0 indicates that the mean difference is not significant at the 0.05 level
Where Level 1 is White light exposed group
Level 2 is Green *LED* exposed group
Level 3 is Blue *LED* exposed group
Level 4 is Red *LED* exposed group

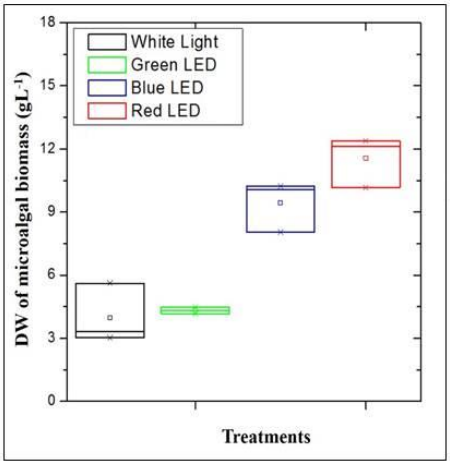

Fig. 9. Box chart for the different dry weight biomass produced by different light treatments of microalgae.

4. Discussion

In the last years, research has assessed the substitution of fluorescent LEDs sources [18, 21, 22. 23, 24, 26]. The commonly recognized viewpoint is that light is crucial for the microalgae or can be said that microalgae cultivation is strongly dependent on light availability, where the yield of biomass on the light which is an essential factor for the algal biomass yield. The quantity of light exposed to microalgae affects biomass production. Microalgal growth rises with light intensity and/or exposure time, which is associated with the light saturation of microalgae. Light exposure past the maximum light saturation could lead to photo-inhibition and therefore inhibits the growth, causing less efficient CO_2 fixation and other nutrient intake rates [27]. Sunlight is the natural energy source for algal cultivation processes but using different lights like LEDs with different colors results in an increasing challenge for efficient algal production. The use of specific narrow bands of light using LEDs is more feasible than using ordinary light sources with cost effective low-wattage irradiance, which can influence microalgae biomass [20].

The effects of LEDs on green microalgae were investigated in some literature. The different colors of LEDs led to different affections on the weight of algal biomass. The results show that the monochromatic red-light conditions red (635 nm) and blue (465 nm) were favorable for increasing or enhanced the weight of algal biomass compared to white light and green (525 nm), therefore increased of lipids and consequently biodiesel production. Our result indicated that at the first phase the irradiance of algae was for 2h but at second phase the irradiance of algae was for twenty-one days. With all colors of LEDs, the weights of algal biomass at the second phase were better than the first phase. At the first phase the highest biomass of microalgae was produced from algal irradiation with red LED,

followed by the blue LED, green LED, and the least one was white LED. Also, at the second phase highest biomass of microalgae was produced from algal irradiation with red LED, followed by the blue LED and the least one was white LED. Therefore, the best color is red then blue, green, and white respectively which agrees with Kim et al, (2014) who concluded that blue LED illumination (wavelength: 430-465 nm) resulted in enlarged cell size of some algae strains, while red LED illumination (wavelength: 630-665 nm) resulted in small-sized cell but having active divisions. Ra et al. [18] reported that the photobiostimulation of green algae using green LEDs was not effective for biodiesel production because the green light was reflected, therefore the green microalgae was not absorbed the green light.

Future research will focus on the photobiostimulation of algae using laser radiation, where this laser radiation was implemented in biogas production [28-32] but not yet in biodiesel production.

5. Conclusions

According to the results of this study, it can be concluded that the photobiostimulation of algae using LEDs led to the enhanced weight of algal biomass, therefore increased of lipids and biodiesel production. The red LEDs were the best one in terms of increasing the weight of algal biomass. On the other hand, the blue LEDs were the best one in terms of increasing the percentage of extracted oil. However, the photobiostimulation of green algae using green LEDs was not effective for biodiesel production because the green light was reflected, therefore the green microalgae did not absorb the green light. It was concluded that, the microalgal growth and biomass significantly increased under using red, blue, green, and white LEDs, respectively. The percentage of extracted oil from microalgae significantly increased when exposed to blue and red emitting diodes respectively in comparison to white LEDs. The application of blue and red LEDs for microalgae cultivation may have a potential impact on the future of feasible algal biodiesel production. Future research will focus on the photobiostimulation of algae using laser radiation.

6. Conflicts of interest

There are no conflicts to declare.

7. Funding sources

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8. Acknowledgments

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