



EFFECT OF LED AND HALOGEN LIGHTS ON THE GROWTH OF TWO LOCAL MICRO ALGAE AND SOME OF THEIR MEDICALLY ACTIVE SUBSTANCES

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Abstract

In the current study, two types of algae *Spirulina platensis* and *Coelastrella terrestris* were exposed to two different types of lighting, LED and Halogen with different light intensities, to identify the effect of lighting on the growth of algae and their production of chlorophyll-a, protein, carbohydrates and lipids, as well as investigating anti-properties. Bacterial to these algae extracts. The study showed that there are differences in the growth rates of the algae under study and exposed to the two types of lighting, as the intensity of illumination 2000 LED was the best when it recorded the highest concentration of the chlorophyll tincture for both algae while the lighting intensity was 2500 and 3000 lux for the source of halogen lighting is the best in the production of chlorophyll for algae r, Q, respectively, with respect to proteins, the highest concentration of 0.48 mg/ml was recorded by algae spirulina at the intensity of illumination of the 2000 lux LED and 0.501 mg/ml at the intensity of illumination of 3000 lux halogen. The highest concentration of carbohydrates was 0.288 mg/ml, which was recorded by the alga *C. terrestris* at the intensity of illumination of 3000 Lux LED, while the type of halogen lighting was the highest concentration of carbohydrates recorded by the same alga and the same intensity of illumination. The highest concentration of lipids was recorded by *S. platensis*, and it reached 0.196 mg/ml when exposed to the intensity of illumination of 2000 lux type LED. As for exposure to halogen lighting, the same algae gave the highest concentration of lipids and when the intensity of illumination was 3000 lux. In the current study, the inhibitory efficacy of extracts was examined Algae under study on three types of pathogenic bacteria, namely *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The results indicated that treatment with a concentration of 1000 mg/ml of algae extracts gave the highest inhibitory efficacy as the percentage of sensitivity to bacteria *E. coli* 97% and 36%, respectively.

Keywords: LED, Halogen lights, local micro algae

Introduction

Algae are present in all ecosystems, especially in aquatic ecosystems (Barsanti and Gaulieri, 2014). They are considered as cornerstone in these ecosystems due to their ability to harvest the energy of sunlight by converting light energy to chemical energy via photosynthesis (Taiz and Zeiger, 2003). *S. platensis* Geitler are planktonic cyanobacteria that form massive populations in tropical and subtropical water bodies characterized by high levels of carbonate and bicarbonate and high pH (up to 11).

The microalgae constituent of phospholipids can be altered due to changing environmental standards, growth rates, light intensity and amount by sunlight or artificial light, as lighting is a source of energy in autophototrophs as it uses light energy to convert the available nutrients and carbon dioxide into organic compounds stored inside their cells, lighting is one of the physical factors that affect the distribution and abundance of phytoplankton, including microalgae through its direct effect on photosynthesis and growth rate, as high lighting causes oxidation of cellular components and stabilizes necessary enzymes in the process of photosynthesis and this affects the content of algae from all active substances and compounds (Gerotti, 2001). Although sunlight is the most cost-effective source of microalgae production, it suffers from defects in uncontrollable biological clock rhythms, in addition to changes in weather, season, and time during the day. It decreases with depth and this is due to the absorption of water and suspended matter, including neighborhoods. Water is like planktons, in addition to the deviation of light by water molecules, the so-called dispersion of molecules, and light

scattering may occur by suspended matter also, because the blue color of the pure ocean water is caused by the dispersion of this color in the upper direction while the coastal waters have a color Green due to the presence of large quantities of suspended matter that reflect light in longer waves, and therefore artificial light may be more practical and economical in producing microalgae (Blanken, 2013). In recent years, a variety of artificial lamps, including the Halogen lamp and the second LED light-emitting lamp, have been used extensively in algae cultivation, and each is characterized by a different light intensity and wavelength that affects the biomass production of algae, that the artificial light is better in terms of Regulating the PPFD's photon flux density, light duration, wavelength and intensity of light, which can lead to an abundance of production and quality standards in algae biomass are key factors for the success of any industrial agricultural product (USDOE, 2013). The current study aimed to know the effect of two different types of lighting (LED and Halogen lamps) and different intensities used in algae culture emphasized the growth and physiology of two types of algae, one of which is the green *C. terrestris* and the blue green algae *Spirulina platensis* and the production of active substances by them.

Materials and Methods

Samples of the study

The microalgae of this study were obtained from advanced ecology and algology lab. in College of Education of university of Al-Qadisiyah. The experiments were achieved in illuminated incubator under 25°C temperature and different light intensities of LED (LOW CRI LED 6500K) and Halogen (Halogen Lamp 3000K) sources.

For testing light regimes and light intensities Chu -10 culture medium (Andersen, 2005) prepared for *C. terrestris* and Zarrok medium for *S. platensis* (Andersen, 2005). For bacterial assay bacterial isolates were cultured on Mueller Hinton agar medium. All media were autoclaved at 121°C for 20 min. All chemicals used were analytical grade.

Experiment set up

Indoor LEDs and Halogen illuminated growth cabinet was constructed to incubate studied algae triplicates to each light intensities for both tested light regimes, incubation period was 21 days for *C. terrestris* and 30 days for *S. platensis* with 18:6 L:D cycles with initial Conc. of approximately 5×10^4 cell/ml. Optical density (O.P) was used to measure the growth of tested algae at 680 nm for avg. of 720 nm for *S. platensis*, the growth was expressed as growth rate depending on the following equation of Fogg (1975):-

$$K = \log N_t - \log N_0 / t$$

The experiment designed as complete Randomized Design (CRD) with triplicates, analysis of variance (ANOVA) was applied on experiment data by using (SPSS software ver. 21) and the least significant differences (LSD) at $p > 0.05$.

Total Protein estimation:

The protein content of the total protein in the crude extract of the algae under study was estimated by Lawry *et al.* (1951).

Determination of Total Carbohydrate

The content of carbohydrates was estimated according to Dubois *et al.* (1956), where 1 ml of Phenol 5% was added to 2 ml of the algae sample in the test tube, then 5 ml of H_2SO_4 sulfuric acid was added 95% and then left the sample at room temperature, for a period of 35 minutes. Then the optical density was measured at 485 nm wavelength, and the standard orientation concentrations of glucose sugar were prepared by taking different sizes.

The total Lipid content

Lipid was extracted according to the Bligh and Dyer method, where ethanol and polar solvents were used: chloroform 1: 2 (V/V). The combination of polar and non-polar solvents strengthened Lipid extraction (Velichkova *et al.*, 2012). 10 ml of ethanol and 5 ml of Chloroform to 8 ml of algae. Vortexed was mixed for 5 minutes with a vortexed rotary mixer. Then, an additional 5 ml of chloroform was added.

After that, tubes were transferred to the centrifuge at a speed of 1000 rpm for 15 minutes, and the process is repeated at least 5 times, after which the filtrate representing ethanol is removed and 2 ml of the bottom layer representing chloroform is taken, and transferred to the Vacuum desiccator, until.

The chloroform was evaporated, until it is completely dried, and the dry Lipids are weighted and the Lipids calculated, according to the following formula:

$$\text{Total Lipid} = (\text{weight lipid in aliquot} \times \text{volume of chloroform}) / (\text{volume of aliquot})$$

Results and Discussion

Growth rate of studied algae in different light regimes and intensities

The current experiment reveals that the exponential phase for studied algae started after the first day of cultivation, while stationary phase of *C. terrestris* began at day 7 of exposing to LED light source. The best growth of *C. terrestris* was recorded at 2000 lux light intensity while the lowest was at 1000 lux Fig. (1).

The growth curve of *S. platensis* exposed to LED source illustrated that best growth occurred at 2000-3000 lux, while lowest growth was at 1000, 1500 and 5000 lux. Fig (2). From these results, we found that that the cultivated algae were in lag phase during 0-1 days in this time algae are adapting to the new environmental conditions and the population seemed to be constant, after the second day the growth increased dramatically, this reflected the typical stages of algal growth curve (Sathong *et al.*, 2019).

From another side the exposure of *C. terrestris* to Halogen light source showed that stationary phase was recorded at 9-13 days at 3000 lux, while the same phase was occurred at 7-10 days at 1000 and 5000 lux.

Hence *S. platensis* reached to the stationary phase at days 17-25 at 3000 lux, the lowest growth occurred at 1000, 1500 and 5000 lux. Our results agreed with Ben Aved (2015) who confirm that light intensities 2000 and 3000 lux were the optimum for the growth and biomass production and pigment accumulation of *S. platensis*, likewise with Minhas *et al.* (2019) in which low light intensities were better to the growth and production of biomass, with the increment of light intensity showed decreasing in the chlorophyll synthesis, while others noted that increasing the levels of illumination above the optimal may led to lowering or stopping of photosynthesis due to the damage in photosystem-II (PSII) in the algal cells as a result of exposure to high level of illumination (Lu and Zhang, 2000).

Chlorophyll content for *C. terrestris* and *S. platensis* algae with different lighting effects (LED and halogen) for different time periods

Lightening source: LED

The results presented in Figure (5) showed the statistically significant effect of the effect of different lighting intensity (P-value = 0.0010) as well as different time periods on the content of chlorophyll-a for *C. terrestris* algae, as the content of chlorophyll-a increased in The algae under study with the increase in illumination intensity from 1000 to 2000 lux at all time periods), the highest concentrations were recorded at the intensity of 2000 lux, reaching 0.101, 0.322 and 0.092 mg. MI-1 at the first, second and third weeks, respectively, with a clear significant difference between them. In addition, the target characteristic concentration began to decrease significantly when the intensity of illumination increased from 2000 lux for all weeks. On the other hand, the highest concentrations of chlorophyll-a in *C. terrestris* algae were recorded in the second week and the least in the third week, compared to the first week, and a clear moral difference between them, as in Figure (5) The data related to *S. platensis* algae presented in Figure (6) achieved the statistically significant significance of the effect of different lighting intensity (P-value = 0.00005) as well as different time periods (P-value = 0.000003) on the content of chlorophyll-a, have the same rhythm, the highest

concentrations were recorded for it at 2000 Lux, which amounted to 0.403, 0.626 and 0.385 mg. MI-1 at the first, second and third weeks, respectively, with a clear significant difference between them. Besides, the concentration of chlorophyll-a decreased significantly when the intensity of illumination increased from 2000 lux for all weeks to reach its lowest concentration above the highest intensity under study, reaching 0.56 mg. MI-1 in the first week and 0.213 mg. MI-1 in the second week and 0.91 mg. MI-1 in the third week in a row, on the other hand, the highest concentrations of chlorophyll-a recorded by *S. platensis* algae were in the second week and then the third week and first week in a row with a clear significant difference between them, as shown in Figure 6.

Lightening source: Halogen

The results in Figure (7) indicated the significant significance of the effect of different lighting intensity (P-value = 0.0028) as well as different time periods (on the content of chlorophyll-a for *C. terrestris* algae, as the chlorophyll-a content in the algae under study increased with the intensity of illumination from 1000 to 2500 lux at all time periods (weeks), for which the highest concentrations were recorded at the intensity of 2500 lux, reaching 0.132, 0.400 and 0.097 mg. MI-1 at the first, second and third weeks, respectively, with a clear significant difference between them, in addition to that the concentration of chlorophyll-a started Significant decrease when lighting intensity exceeds 2500 lux for all weeks, on the other hand, it is higher concentrations of chlorophyll in a moss *C. terrestris* was recorded in the second week and the lowest in the third week compared with the first week and the moral difference is clear between them, and as set out in Figure (7). The current study showed an increase in the concentration of chlorophyll at the light intensity of 2000 lux for both studied algae in the case of using an LED lamp. As for the halogen lamp, the highest concentration of chlorophyll was recorded at 2500 lux in the *Coelastrella* algae and 3000 in the *S. platensis* algae, and the current results are agreed with the Franklin *et al.* (2002) which shows that the ideal lighting It leads to an increase in chlorophyll and carotene as a result of a positive relationship between the pigments and photosynthesis process and it also agrees with Purves (1983) who found in his study that the intensity of lighting plays an important role in determining the amount of chlorophyll in algal cells. Algae growing in a feeble light on the quantities of each. chlorophyll is more than the one that lives in high light. The high brightness index of 5000 lux in both light sources had a negative effect on the concentration of chlorophyll in the isolates under study and the current study is consistent with the results (Brouers, 2007), which found a large amount of chlorophyll-a pigment was damaged at high levels of lighting in *S. Platensis* alga, as well. The current study is consistent with the study of Hegemann, (2008) that showed that high light intensity might lead to phot oxidation as the cell components are oxidized by the oxygen produced from the photosynthesis process as oxidized and chlorophyll destroyed. Chlorophyll is an appropriate factor to follow the physiological responses in the algae groups as they change How much The chlorophyll present in the cell depends on its physiological condition and growth. This means that the carbon level of chlorophyll changes significantly depending on the physiological state of the algae community as well as with growth conditions such as light intensity and

concentration of CO₂ (Ben-Ayed *et al.*, 2015). (Chlorophyll-a is commonly used as a tool for determining changes in light reactions, because environmental stress can reduce the ability of plants and algae to metabolize naturally, resulting in an imbalance between the absorption of light energy by chlorophyll and the use of energy in photosynthesis (Chen *et al.*, 2016).

Protein Concentration of *C. terrestris* and *S. platensis* with Lighting Intensity from Various Sources (LED and Halogen)

Lightening source: LED

The results shown in Figure (9) showed the significant significance of the light intensity of the light-emitting diode (P-value = 0.0063) on the protein concentration in the two algae under study, as well as the moral superiority of the *S. platensis* algae in its protein content over the *C. terrestris* at all the light intensity (P- value = 0.0007), as it was observed that increasing the intensity of illumination from 1000 to 2000 lux significantly increased the protein concentration at both algae which was above 0.270 and 0.480 mg. While the increase in the intensity of illumination from the previous limit (2000 lux) had a negative role on the protein concentration of both algae and steadily with an increase in the intensity of illumination to 5000 lux, when it reached the lowest protein concentration (0.055 and 0.206 mg.) For algae, respectively, compared to the protein concentration at the lowest luminous intensity (1000 lux) as it reached 0.166 and 0.262 mg. MI-1, respectively, as shown in Figure.

Light source: Halogen

The results of exposure of *C. terrestris* and *S. platensis* to different lighting intensity of halogen showed a significant effect of increasing protein concentration with increased intensity of illumination to 3000 lux (P-value = 0.0112) as the protein concentration reached 0.281 and 0.501 mg. The study, respectively, versus the negative effect of significant significance of 5000 lux in reducing protein concentration to 0.091 and 0.391 mg. MI-1 in *C. terrestris* and *S. platensis*, respectively. On the other hand, the protein concentration in *S. platensis* algae was significantly higher than in *C. terrestris* (P-value = 0.00002) and was nearly twice as strong as all lighting intensity of halogen, as shown in Figure 10.

The lipid concentration of the *C. terrestris* and *S. platensis* algae with a strong lighting effect from various sources (LED and halogen)

Illumination Source: LED

The results of the statistical analysis of the concentration of lipids in studied algae the effect of different lighting intensity of the LED light Fig. 13 indicated that the different lighting stresses were of significant significance (P-value = 0.0006) on the concentration of lipids in the algae under study as it increased with Increase the intensity of illumination from 1000 to 2000 lux in a direct and significant way, reaching an average maximum of 0.159 and 0.196 mg. MI-1 at the intensity of illumination 2000 lux for both algae respectively, while increasing the intensity of illumination over 2000 lux had a negative effect on the concentration of lipids as the concentration decreased with the increase in illumination intensity and significantly to 0.049 and 0.034 mg. MI-1, with the effect of the highest used light intensity (5000 Lux) under study at both algae respectively, and it is worth noting that the comparison between the two algae

under study in terms of Lipid concentrations was not significant (P-value = 0.0667), as shown in Figure 13.

Illuminating source: Halogen

Statistical analysis data gave the concentration of lipids in *C. terrestris* and *S. platensis*, with the effect of different lighting intensity of halogen (Figure 14), the significant significance of different lighting intensity (P-value = 0.0094), and for algae under study (P-value = 0.0368), as it was observed that the concentration Lipids with different lighting intensity increased in proportion to the increase in illumination intensity to 2500 lux when *C. terrestris* algae, where the concentration of Lipid at above 0.211 mg. While-1 in *S. platensis* algae above at light intensity reached 3000 lux, the concentration was 0.276 mg. In addition, the decrease in the concentration of lipids in *C. terrestris* algae began with the illumination intensity of 3000 lux reaching its lowest level at the highest used intensity under study (5000 lux), reaching 0.072 mg. MI-1 compared to *S. platensis* algae, whose Lipid concentration decreased only at the highest intensity (5000 Lux) to 0.099 mg. MI-1, noting that the lipid concentration in *S. platensis* was significantly lower than that of *C. terrestris*, with a luminous intensity of only 1000 lux and significantly superiority over the rest of the other lighting intensity, as shown in Figure 14.

Both algae under study and under two different types of lighting with different stresses contained but less protein and carbohydrates. A number of researchers recorded the content of Lipids in different algae, some of which agreed with the results of the current study or were slightly higher, and among the most prominent of these studies (1997, Flaquet), who found that the Lipid content of *S. platensis* was (6.5-7)% dry weight, and a study (Al-Badry, 2010) that found that the Lipid content of algae *platensis* was (6.4)% dry weight which is higher than the results The current study, and a study of Chowdhury *et al.* (1994) who recorded a Lipid content of (4 - 9)% by weight of dry moss *S. platensis*. Also, with a study (1983, Ciferri) that recorded a total Lipid content of (1.5-12%) of dry weight in the same algae, most researchers confirmed the possibility of increasing the microalgae content of Lipids using better extraction methods (Baumler *et al.*, 2010). While others have explained the reason for this variation, which may be due to different development conditions such as level, lighting period, temperature, pH, salinity, and other factors affecting the content of algal cells with Lipid and unsaturated fatty acids (UFAs) (Leonardos and Lucas, 2000). The biological activity of algae extract under study has also been tested and it has been shown that there is an inhibitory activity of algae extracts against some bacterial isolates under study and this effect may be attributed to their containment of some volatile organic compounds responsible for anti-bacterial activity such as D-limonene and Beta-Pinene as well as containing it Some acids such as Hexanoic acid, Heptafluorobutyric acid, Methoxymaleic acid, Dichloroacetic acid and Dimethylmalonic acid, and the results of the present study were consistent with other studies such as (Md Islam *et al.*, 2008) and (Sylvain Sutour *et al.*, 2008). And the anti-bacterial activity of microalgae may be due to its containment of Lipids responsible for its anti-microbial activity, as this anti-microbial property of microalgae is due to its capabilities to produce compounds such as α - and β -ionone- 55B - cyclocitral, neophyte diene, and phytol Related antimicrobial activity.

Detecting the sensitivity of bacterial isolates to antibiotics and algae extracts

It was noted from Figure (15) the non-significant variations (P <0.05) of different antibiotics despite the observed differences between them. On the other hand, bacterial isolates showed significant significance (P > 0.05) among themselves towards antibiotics (P-value = 0.0008), as the sensitivity of *Staph. aureus* isolation reached. *Staph. aureus* for amoxicillin-clavulanic acid (0%) versus sensitivity *Ps. aeruginosa* and *E. coli* increased by 19 and 25%, respectively. On the other hand, the isolation of *E. coli* recorded its sensitivity to the anti-ceftriaxone, chloramphenicol, nitrofurantoin and Tobramycin according to ratios 25, 28, 21 and 30%, respectively, compared to that of the two *Staphylococcus* isolates. *Staph. aureus* and *Ps. aeruginosa* is of total resistance to the aforementioned antagonists, in addition to that the total resistance of Levofloxacin by *Pseudomonas aeruginosa* isolation and somewhat converged sensitivity by bacterial isolates under study to other antibiotics, as shown in Figure 15, noting that all bacterial isolates sensitized to antibiotics under study did not exceed the sensitivity rate at half of the barrier (50%) towards When testing the efficacy of the same bacterial isolates against the algae extract of *Coelastrella* algae at different concentrations (100, 300, 600 and 1000 mg) it was noted that all the isolates under study showed their weak sensitivity (did not exceed the 50% barrier) for those concentrations of the algae extract and a direct relationship in which increased sensitivity increased With an increase The concentration of the user from the algae extract, indicating that there was no significant significance for the concentrations (P-value = 0.0502) as well as bacterial isolates (P-value = 0.1076) as they are shown in Figure 16 and 17.

It was found that there is an inhibitory activity of algae extracts against some bacterial isolates under study and this effect may be attributed to their containment of some volatile organic compounds responsible for anti-bacterial activity such as D-limonene and Beta-Pinene as well as containing some acids such as Hexanoic acid and Heptafluorobutyric acid, and Methoxymaleic acid, Dichloroacetic acid and Dimethylmalonic acid, and the results of the present study were consistent with other studies such as (Md Islam *et al.*, 2008), (Sylvain Sutour *et al.*, 2008), (Laura Espina *et al.*, 2013) and Volk *et al.*, 2009).

Conclusions

- The growth rates of the studied algae varied according to the type and intensity of the lighting used. The best lighting intensity was 2000-3000 Lux. This case reflected in the algal content of proteins and lipids and the *S. platensis* algae content was higher than *C. terrestris* algae.
- The high light intensity had adverse effects on the growth rate and the algae content of chlorophyll, protein, carbohydrates and lipids.
- The study showed that the algae extract had an inhibitory ability of the tested bacterial isolates to compete with the antibiotics used under study.

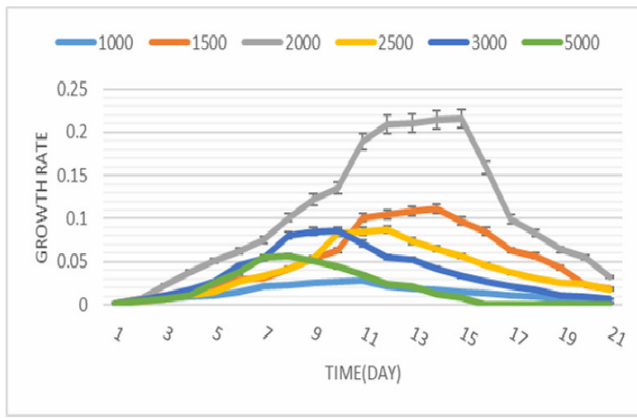


Fig. 1 : Growth curve of *C. terrestris* algae under the influence of different lighting intensity of the LED light emitting diode

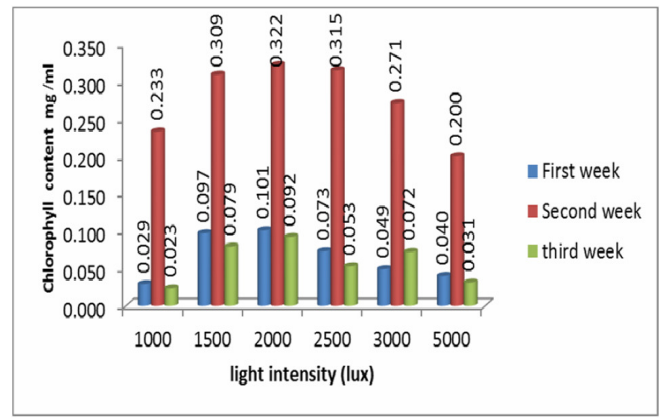


Fig. 5 : Chlorophyll-a content in *C. terrestris* with a different lighting effect of LED.

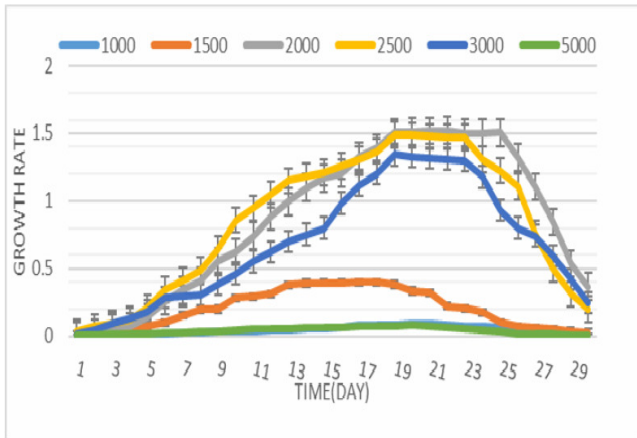


Fig. 2 : Growth curve of *S. platensis* algae under the influence of different lighting intensity of the LED light emitting diode

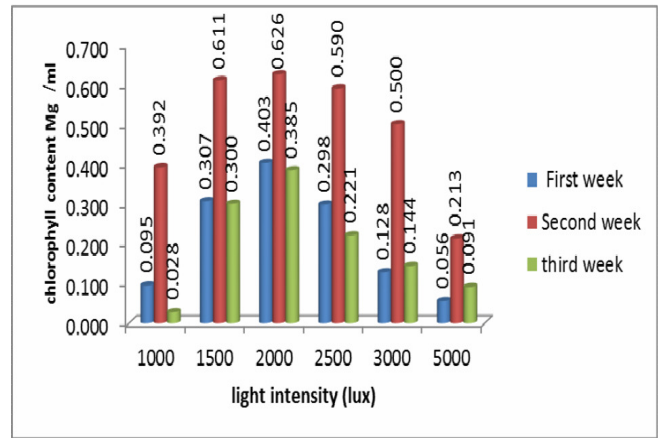


Fig. 6 : Chlorophyll-a content in *S. platensis* algae with different lighting intensity for LED lights for different time periods.

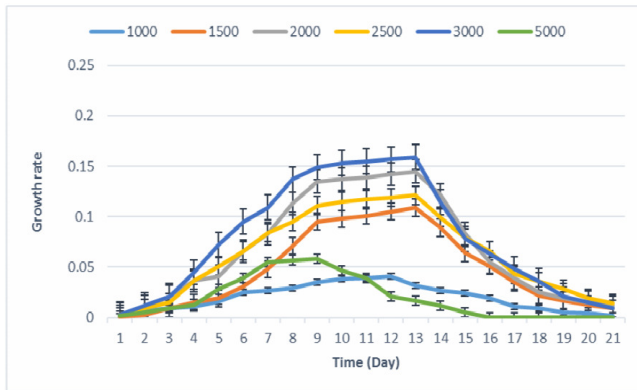


Fig. 3 : Growth curve of *C. terrestris* algae under the influence of different lighting intensity of the halogen light source

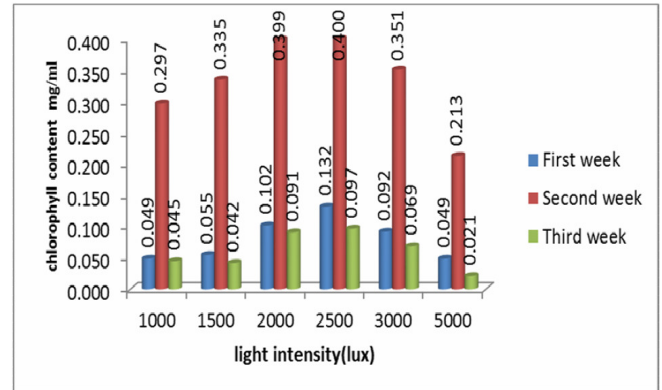


Fig. 7 : Chlorophyll-a content in *C. terrestris* with different lighting effects for halogen for different time periods.

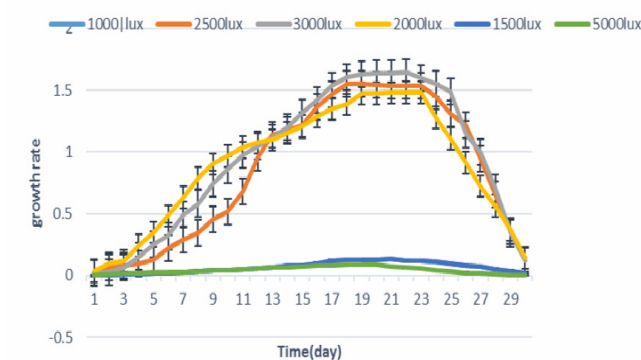


Fig. 4 : Growth curve of *S. platensis* algae under the influence of different lighting intensity of halogen

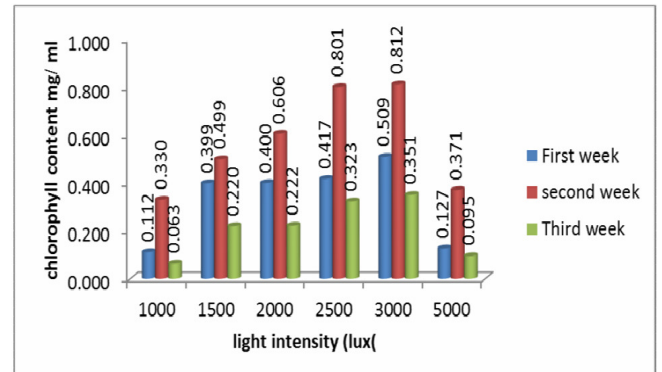


Fig. 8 : Chlorophyll-a content in *S. platensis* algae with different lighting intensities for halogens for different time periods.

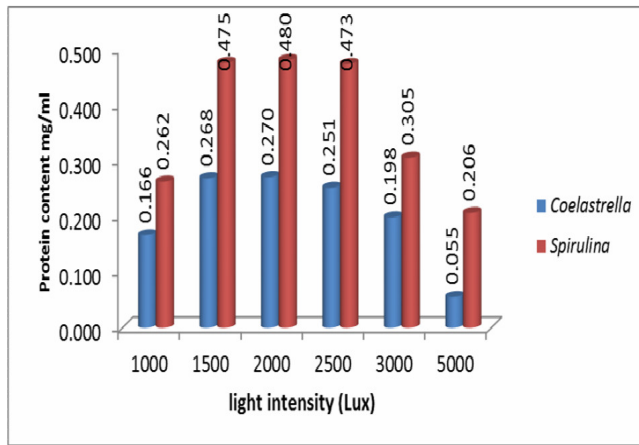


Fig. 9 : Protein Concentration in *C. terrestris* and *S. platensis* with Different Lighting Intensity of LED.

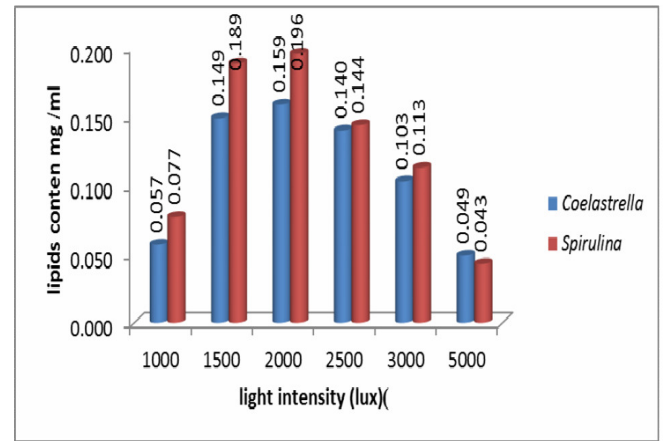


Fig. 13 : Concentration of lipids in *C. terrestris* and *S. platensis* with different lighting intensities for LED.

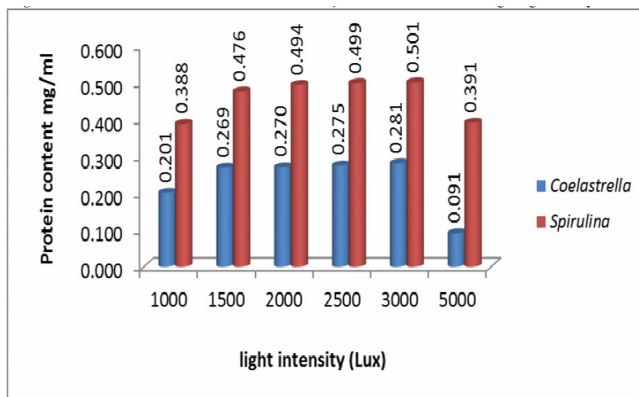


Fig. 10 : Protein Concentration in *C. terrestris* and *S. platensis* with a Different Lighting Effect of Halogen

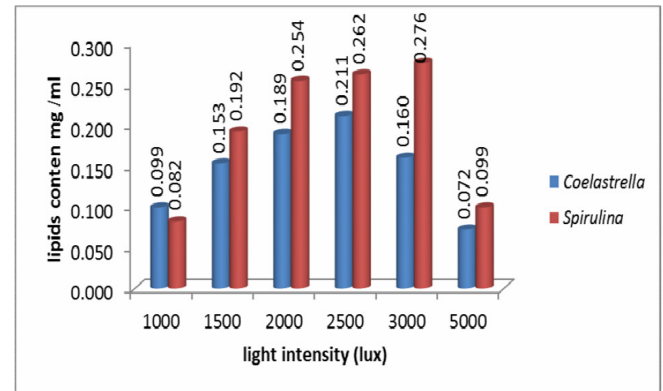


Fig. 14 : Concentration of lipids in *C. terrestris* and *S. platensis* with different lighting effects for halogen.

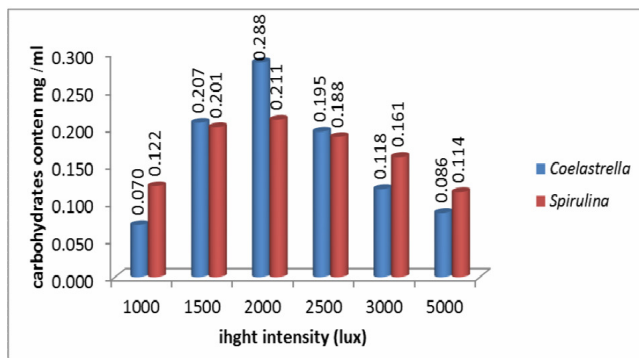


Fig. 11 : Concentration of carbohydrates in *C. terrestris* and *S. platensis* with different lighting intensity for LED

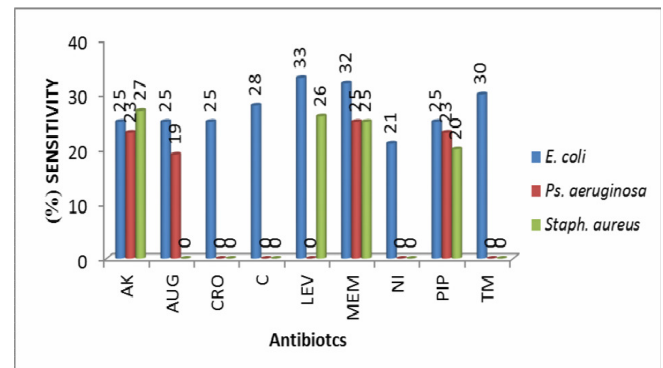


Fig. 15 : Test for sensitivity to bacterial isolates against a spectrum of antibiotics

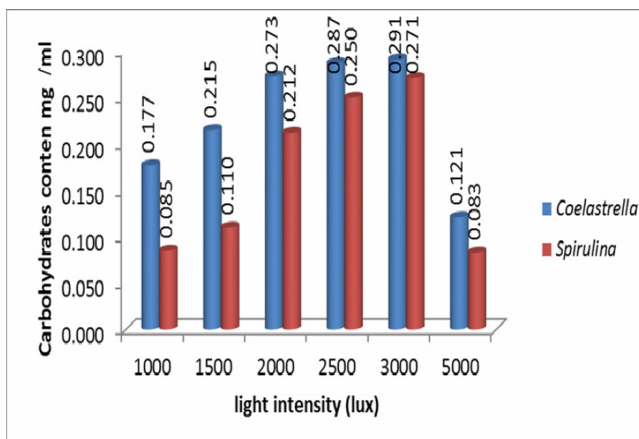


Fig. 12 : Concentration of carbohydrates in *C. terrestris* and *S. platensis* with different lighting effects for Halogen.

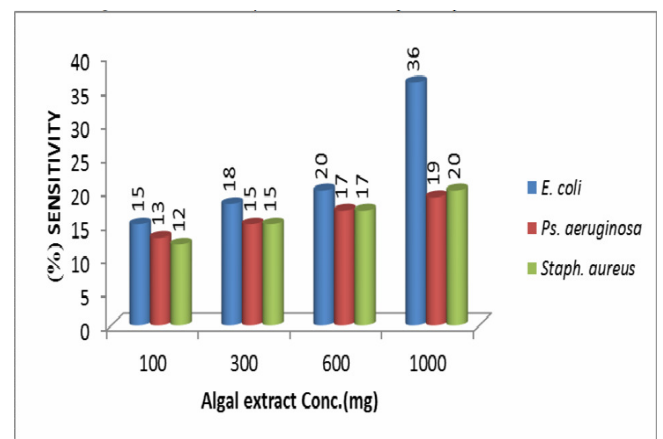


Fig. 16 : Sensitivity test for bacterial isolates against different concentrations of *C. terrestris* extract

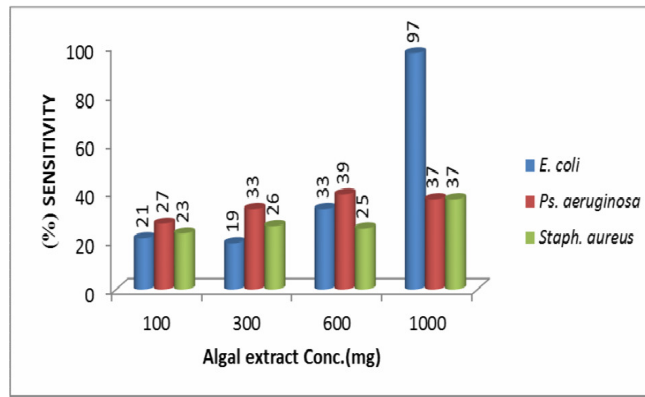
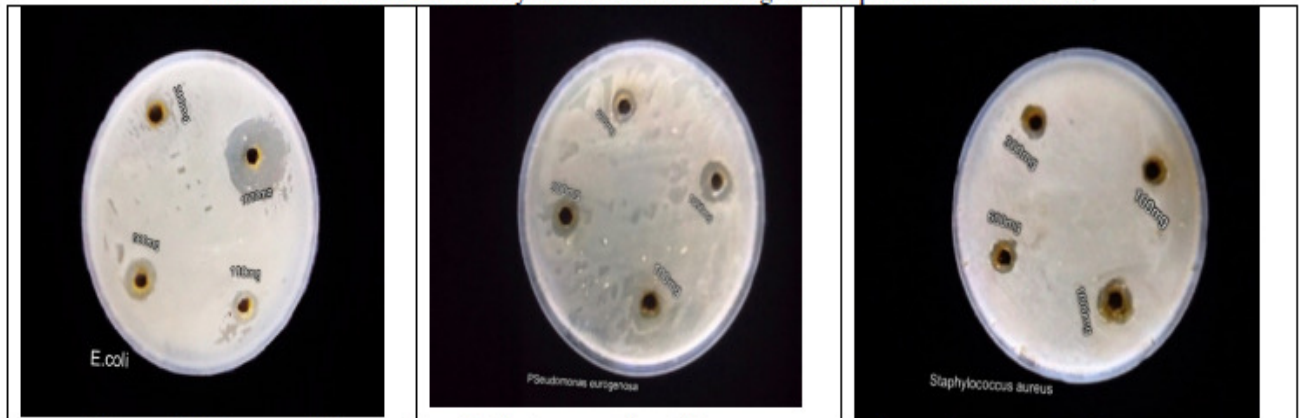


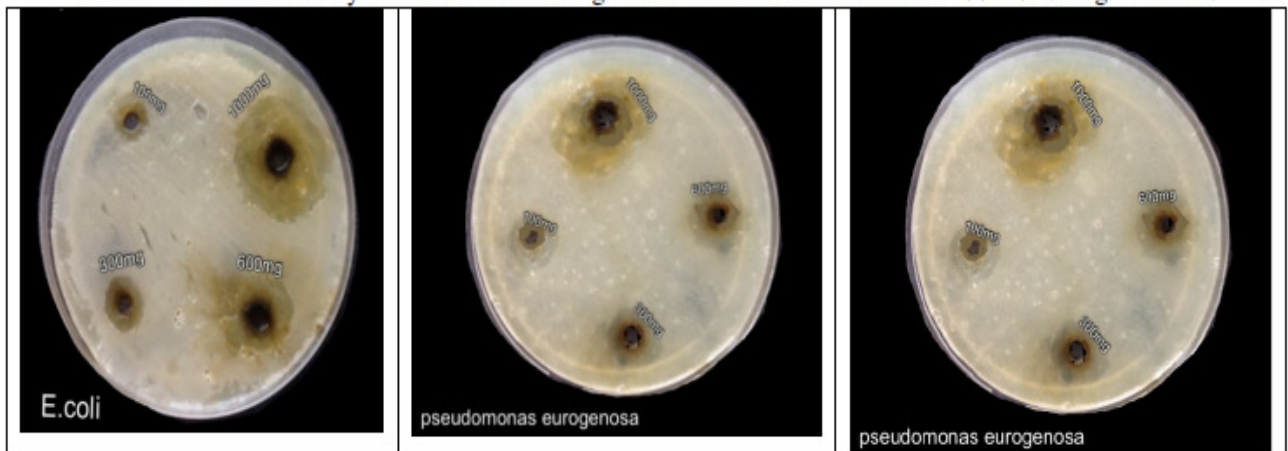
Fig. 17 : Sensitivity test for bacterial isolates against different concentrations of *S. platensis* algae extract



Picture 1 : Test for sensitivity to bacterial isolates against a spectrum of antibiotics.



Picture 2 : Test sensitivity to bacterial isolates against different concentrations of *C. terteris* algae extract.



Picture 3 : Sensitivity test for bacterial isolates towards different concentrations of *S. platensis* algae extract.

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