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# EFFECT OF CILOFUNGIN ON THE GROWTH AND METABOLITE PRODUCTION IN *SPIRULINA* SP AND *CHLORELLA* SP

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## ABSTRACT

A novel strategy of using an antifungal cilofungin to alter the algal metabolics for increased production of biomass, fat, protein and other metabolites were inferred. The effect of varying concentrations of cilofungin (0.02% to 0.1%) on unialgal cultures of *Spirulina* sp. and *Chlorella* sp. isolated from ennore estuary, Chennai, an highly polluted coastal ecosystem in India was investigated. Various parameters like cellular growth, dry mass, carbohydrates, proteins, fatty acids, chlorophyll and carotenoids were assessed with respect to time. At a given cilofungin concentration of 0.08% in *Spirulina* sp and 0.06% in *Chlorella* sp, an increase in protein content from 421mg/g to 655mg/g and 430mg/g to 646 mg/g of biomass, an increase in fat content from 57mg/g to 74 mg/g and 75 mg/g to 90mg/g of biomass was obtained in comparison to their mother cultures with a simultaneous increase in their biomass concentration from 1.09g/l to 1.5g/l and 1 g/l to 1.23g/l respectively, whereas the carbohydrates were gradually decreased in cilofungin treated cultures. This study thus analyse the effect of cilofungin in algae, inhibiting the glucan production and inducing the alternate metabolic pathways in *Spirulina* sp and *Chlorella* sp. for lipid and protein accumulation.

**KEYWORDS:** Cilofungin, Algae, *Spirulina*, *Chlorella*, Lipids, Carbohydrates, Antifungal Drug, Fungus.

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# INTRODUCTION

Algal metabolism concerns the biochemical and transport processes by which algae take up nutrients and convert them into the materials needed for their growth, reproduction and defense mechanisms. Microalgae can perform oxygenic photosynthesis and fix carbon dioxide through Calvin cycle like plant cells i.e micro algal cells can trap light energy as the energy source and assimilate $CO_2$ as the carbon source. Algae store these excess carbon in the form of carbohydrates and lipids. There are many metabolic pathways through which the excess carbon can enter, resulting in synthesis of many compounds required by the cell. These pathways consist of sequence of enzymes, each of which catalyses a specific reaction[1]. Therefore, by varying the nature of carbon and energy sources, the different underlying metabolic status of cells, especially the influence of light on the carbon and energy metabolism, can be elucidated[2]. The reactions of oxygenic photosynthesis are used to drive biosynthesis at the expense of inorganic nutrients. In darkness the intracellular carbon reserves, mainly glycogen are consumed for cell maintenance.[3] The oxidation step is obtained with the respiratory chain. In addition to being photosynthetic autotrophs, cyanobacteria possess a number of microbial characteristics including rather rapid growth rate, high valuable protein content and a variable metabolism that responds rapidly to the environmental changes.[4]During heterotrophy, the presence of a fixed carbon source (*i.e.*, sugar) can promote rapid growth, support high cell densities, and augment lipid accumulation.[5] As such, this mode of growth has been exploited for the industrial production of polyunsaturated fatty acids and bioactive pigments to serve nutritional markets. Cilofungin is the first clinically applied member of the echinocandin family of antifungal drugs, derived from the genus *Aspergillus*. It accomplishes this by interfering with an invading fungus' ability to synthesize the cell wall. Being an antifungal agent of the lipopeptide class, it targets at the 1, 3-β-D-Glucan synthase, a cell wall synthesis enzyme. It is an amphophilic antibiotic that inhibits the biosynthesis of glucan by selective blockage of glucan synthase. This property of cilofungin can be exploited beneficially to block the enzymes involved in carbohydrate metabolism and to enhance the flow of carbon towards protein and fatty acid metabolic pathways in *Spirulina* and *Chlorella* sp.

# MATERIALS AND METHODS

### *Sample collection*

Two litres of water samples were collected at Ennore estuaries in sterile plastic bottles. Samples were collected around 10cm depth in the collection site. Water temperature was measured by centigrade thermometer and pH was estimated using pH meter. Samples were preserved in 5% neutralized formalin and stored in refrigerator for further analysis. The physiochemical parameters like colour, total dissolved solids, total suspended solids, dissolved solids, biological oxygen demand, alkalnity, total hardness, calcium and nitrate were analysed using standards BIS(Bureau of Indian Standards) 2015 protocol.

### *Isolation and Identification*

Isolation and Identification of *Spirulina* and *Chlorella* sp was carried out in the laboratory in the Department of Biotechnology, School of Life Sciences, Vels University, Chennai. The collected water samples from Ennore estuaries were serially diluted and streak cultured on petri plates containing growth medium with agar (Potato Dextrose Agar). Growth colonies were isolated and sub cultured again in fresh agar plates. The isolated colonies were observed under the microscope and its morphological characteristics were studied to identify *Spirulina* and *Chlorella* sp. using the standard manuals. *Spirulina* sp were grown in Basal Zarrouk's Medium and *Chlorella* sp were grown in Bold's Basal Medium. To avoid culture contamination by bacteria, a small portion of algal culture was immersed in chlorine water at a concentration of 25mg per 100 ml for 5 minutes and the sample was centrifuged at 10,000 rpm at 30°C for 5 minutes in a cooling centrifuge. Following centrifugation, the supernatant was discarded; the pellet was washed in sterile water and transferred to freshly prepared culture medium.

### *Cilofungin treatment*

Cilofungin purchased from Sigma Aldrich was used in this study. Since cilofungin is insoluble in water, 0.5g of cilofungin was dissolved in few drops of ethanol and then added to the culture medium. Ten number of 1000ml conical flask with cotton plug with sterile growth medium with various concentration of cilofungin from 0.02% to 0.1% were prepared and 10 ml of stock *Spirulina* and *Chlorella* cultures were inoculated separately to the respective flasks. The cultures were maintained under conditions of controlled temperature and light. Algae samples were collected from these culture flasks at regular intervals for further analysis.

### *Growth measurement*

The cultures were sampled once in every 5 days and the cell numbers were measured using microscopic field counting method. Growth was measured by counting cells using microscope throughout the study period.

### *Biomass estimation*

The cultures were harvested by centrifugation at 5000 rpm and the cells were washed twice with distilled water. Then the pellet was dried. The dry weight of algal biomass was determined gravimetrically and growth was expressed in terms of dry weight (g/l).

### *Estimation of Total Proteins content*[6]

Protein was estimated as described by the method of Lowry et al. (1951). 10mg of dried and powdered alga was taken in a test tube. 10 ml hot trichloroacetic acid (TCA) (55ºC) was added to this, left for 1 min., centrifuged and the supernatant was decanted. The pellet was dissolved in IN NaOH. The test tubes were made airtight and left overnight. 0.1 ml of above extract was taken in triplicates and graded concentration of bovine serum albumin (10-100g) was taken in test tubes. 4.5 ml of alkaline reagent (G) was added to all the tubes to dissolve the TCA precipitated proteins and allowed to stand for a min. 0.5 ml of IN Folin reagent was added to all the tubes and mixed thoroughly. A blank containing 0.1 ml IN NaOH was used as control. The tubes were then kept at room temperature for half

an hour. Absorbance was read against the blank. The amount of protein present in 10 mg alga was calculated with the help of standard curve.

***Estimation of Total Carbohydrate content***[7]

The estimation of total carbohydrate was carried out by simple anthrone method. 1.0 mg algal powder taken in a test tube was added with 4.0 ml of anthrone reagent and shaken gently. The tubes were kept in boiling water bath for 15 minutes with an aluminium foil on the mouth of each test tube to prevent evaporation. The tubes were cooled under running tap water. A blank containing 1.0 ml distilled water was graded with different (10, 20, 40, 60, 80, 100, µg) concentration of glucose for standard curve. The absorbance at 620 nm was read against the blank.

***Estimation of Total lipid***[8]

About 100 mg of algal crude extract was extracted with 10 ml of chloroform: methanol (2:1) and solvent was separated and evaporated in vacuum. Briefly, lipid samples and standards are placed in a heating block set at 100°C to allow the solvent to evaporate. Once the solvent is evaporated (about 10 min), 0.1 ml of concentrated sulfuric acid is added to each tube, vortexed, and then heated at 100°C for 10 min. Samples were then removed from the heat block and allowed to cool to room temperature before adding 2.4 ml of vanillin reagent (600 mg of vanillin, 100 ml of hot water, 400 ml of 85% phosphoric acid) and vortexed. The pink colour is allowed to develop for 5 min, and then 0.2 ml of standards and samples were read at 490 nm (UV1800, UV-Vis Spectrophotometer, Shimadzu, Japan).

***Estimation of Chlorophyll***[9]

To estimate pigments, the cells were harvested by centrifugation (6000 x rpm, 10 minutes) and washed with distilled water. Chlorophyll a was extracted from the cell suspension with 90% (v/v) methanol at 4°C in dim light by repeated freezing and thawing. Centrifugation was carried out until total pigment recovery. The chlorophyll content in the biomass was calculated from the absorbance at 665nm of the methanolic extract.

***Estimation of Carotenoids***[10]

A known volume of homogenized algal suspension was centrifuged at 3000 rpm for 5 minutes. The pellet was washed with distilled water 2-3 times to remove traces of adhering salts. To the pellet, 2-3 ml of acetone (85%) was added and then subjected to repeated freezing and thawing. The suspension was centrifuged and the supernatant containing pigment was collected. The extraction was repeated till the supernatant became colourless, for complete recovery of carotenoids. The pooled fractions of supernatants were made-up to a final known volume. The absorbance was taken at 450nm using 85% acetone as blank.

## RESULT AND DISCUSSION

Algal strains obtained from the polluted site of ennore estuaries can have a greater impact on novel metabolite production owing to the stress involved that alters the metabolic pathways compared to the fresh water algal strains (REF). The analytical study of the collected water samples reveals the following facts. The concentration of total dissolved solids was 2226.45 mg/l, which was found to be beyond the permissible limit. Similar was the case with total suspended solids that was found to be 3200.25 mg/l, higher than the desirable limit. Microalgae used were all photoautotrophic which did not require for organic carbon sources; hence oxygen demand calculated would correlate more with algal respiration (Chevalier., 2000). The dissolved oxygen of the sample was found to be 2.88 mg/l. BOD of the sample was 50.7 mg/l. The PH of water sample was 9.60 and the water was found to be pale creamy colour that reveals the water sample was highly polluted. The inorganic salts analysed were also found to be exceeding the permissible limits in the collected samples. (Table: 1). Higher levels of BOD indicates the presence of high organic matter thus signifying high pollution level of the estuarine water. However, the presence of nitrates and high BOD can influence the growth of algae through oxygenic photosynthesis signifying their interrelationship involved in their growth kinetics. Studies on *Spirulina* and *Chlorella* sp growth kinetics and effect of various concentration of cilofungin on its biochemical composition were then analysed. Results shows that in *spirulina* and *Chlorella* sp the cell density, biomass production, protein and fat content are gradually increased with concentration of cilofungin. When cells were grown in a medium containing cilofungin, the carbohydrates, protein and fat content depend on the concentration of components present in the media. At a given concentration of cilofungin, an increase in protein and fatty acid content was obtained with a simultaneous increase in the cell concentration. In contrast, an increase in fat and protein concentration caused a decrease in carbohydrate content when the cilofungin concentration was fixed as 0.08% in *Spirulina* sp and 0.06% in *Chlorella* sp. These results suggest that an appropriate concentration of cilofungin can enhance the accumulation of fat and protein content in *Spirulina* and *Chlorella* sp.

***Biomass estimation***

In *Spirulina* sp the maximum biomass production shown in 0.08% treated culture was about 1.50g/l, whereas in *Chlorella* sp, 0.06% of cilofungin treated culture shown about 1.23g/l. Effect of cilofungin in biomass production was represented in Table:2 The maximum bulk density was attained on 20th day after the inoculation of culture in medium. The increase in the biomass production of *Spirulina* and *Chlorella* sp could have been due to the availability of cilofungin. The growth of *Spirulina* and *Chlorella* started to decline after 20 days of incubation. This could be attributed to the increase of death rate over the growth rate. Most of the previous works measured the biomass of *Spirulina* sp after 15 and 25 days and not much studies have recorded the time to follow the biomass depletion (Olivera et al., 1999, Volkman et al., 2008, Murugan and Radham, 2010& Kumer et al, 2011).

***Growth measurement***

0.08% of cilofungin treated *Spirulina* culture shown 3200.19 cells/ml compared to the Stock cultures

showing 2769.25 cells/ml on the harvesting day. Similarly in 0.06% of treated *Chlorella* shown about 2500.64cells/ml when compared to mother culture of 1380.18 cells/ml. Effect of cilofungin on growth rate of *Spirulina* and *Chlorella* sp were represented in Table:3 &4.

***Estimation of Total Proteins content***

In the study the highest protein content of 655.5 mg/g, 646.5mg/g respectively were obtained in 0.08% Cilofungin treated *Spirulina* culture and 0.06% of treated *Chlorella* culture. *Spirulina* protein content was gradually increased upto 0.08% of cilofungin treatment but declined at 0.10%, similarly *Chlorella* protein content was also increased upto 0.06% of cilofungin treatment then declined slowly with high concentration of cilofungin. Effect of cilofungin in protein content were represented in Table:5.

***Estimation of Carbohydrate content***

Isolation and determination of β- glucans from alga like Chlorella sp. and Spirulina sp. have been reported in few papers contributing to their composition of carbohydrates (16, 17). During the experiments, the result shows that both stock cultures show increased in Carbohydrates with respect to biomass. In contrast, the carbohydrates are gradually decreased with increased cilofungin concentrations in both *Spirulina* and *Chlorella* sp. In *Spirulina* the carbohydrate content was gradually decreased from 328.1 mg/g to 200.1 mg/g, whereas in *Chlorella* it was decreased from 389.8mg/g to 320.7mg/g. Table: 6 represents the effect of cilofungin in carbohydrate content of *Spirulina* and *Chlorella* sp. Jagannathan (2015) reported similar results, that using antifungal durgs for enhacing lipids in *Cymbella* sp. Supportive evidences are obtained in the alga I. zhangjiangensis treated with micafungin, a β-1,3-glucanase inhibitor belonging to echinocandin family demonstrating the possibility to switch from polysaccharides to lipids for energy storage by regulating or modifying the β-1,3-glucanase yielding a 30% incerase in lipid production.(18) In addition similaar studies of Beaulieu et al.,(1994) showed Maximal of about 80% inhibition activity by cilofungin against (1,3)-beta-D-glucan synthase in A.fumigatus.

***Estimation of Fat content***

On the harvesting day the fat content was 74.1mg/g in 0.08% cilofungin treated *Spirulina* cultures and 90.0mg/g was in 0.06% of cilofungin treated Chlorella cultures. In contrast to carbohydrate, fat content was gradually increased from 57.4mg/g to 74.1mg/g and 75.0mg/g to 90.0 mg/g in *spirulina* and *Chlorella* sp respectively. Fat content was constantly increased along with biomass in both treated cultures. Effect of cilofungin in fat content were described in Table:7. Similar results are obtained in alga cultures treated with micafungin with higher lipid content rather than cultures grown without micafungin.

***Estimation of Chlorophyll***

Table: 8 show the effect of cilofungin on chlorophyll content. On the harvesting day 16.1mg/g and 13.8mg/g was obtained from 0.10% cilofungin treated *Spirulina* and *Chlorella* cultures. Both treated cultures shows gradually increasing the chlorophyll content along with biomass. In contrast to protein and fat, the chlorophyll was increased even in high concentration of cilofungin.

***Estimation of Carotenoids***

Concerning the influence of Cilofungin concentrations on carotenoids content, it was found that similar trend of total chlorophyll content profile was represented in Table: 9 & Table:10. The highest total carotenoid content was recorded in 0.10% cilofungin treated *Spirulina* and *Chlorella* culture were about 8.7mg/g and 5.8mg/g respectively. This may indicate a strong relation between both chlorophyll and carotenoids contents. Such correlation could be attributed to that the carotenoids protect chlorophyll molecules against photo destruction and oxidation by molecular oxygen (Krinsky, 1979). Similarly, Vonshak (1997) reported that there was a positive correlation between chlorophyll and carotenoids content of S. platensis and the incubation period up to 30 day at 35˚C.

**Table 1**
***Physiochemical analysis of Ennore Estuary water*, Ennore**

| S.No | Parameters | Results (Mean + Sem) | P value |
|---|---|---|---|
| 1 | Colour | Pale Creamy colour | - |
| 2 | pH | 9.60 ± 0.18 | 0.108 |
| 3 | Temperature (°C) | 31 ± 2.0 | 0.124 |
| 4 | Total Suspended Solids (mg/l) | 3200.25 ± 15.81 | 0.200 |
| 5 | Total Dissolved Solids (mg/l) | 2226.45 ± 11.12 | 0.154 |
| 6 | Dissolved Oxygen (mg/l) | 2.88 ± 0.55 | 0.09 |
| 7 | BOD (mg/l) | 50.7 ± 0.78 | 0.104 |
| 8 | Alkalinity (mg/l) | 6.90 ± 0.10 | 0.350 |
| 9 | Chloride (mg/l) | 812.6 ± 6.91 | 0.122 |
| 10 | Total Hardness (mg/l) | 630.4 ± 3.49 | 0.111 |
| 11 | Calcium (mg/l) | 163.0 ± 8.10 | 0.250 |
| 12 | Nitrate (mg/l) | 49.9 ± 0.74 | 0.175 |

***From Table: 1 It is inferred that P value of Physiochemical variablesof Ennore Estuary water were >0.01. Since all variables shows P value >0.01, here is no significant differences of these variable values*.**

**Table 2**

***Effect of Cilofungin in Biomass production***

| | Biomass g/l (Mean + Sem) | | | | |
|---|---|---|---|---|---|
| Cilofungin % | **0.02** | **0.04** | **0.06** | **0.08** | **0.10** |
| Spirulina | 0.91 ± 0.039 | 1.12 ± 0.016 | 1.29 ± 0.054 | 1.50± 0.018 | 1.05 ±0.015 |
| Chlorella | 0.81 ± 0.042 | 1.01 ± 0.020 | 1.23 ± 0.036 | 1.18± 0.039 | 1.09±0.010 |

***From Table:2 It is inferred that Standard Error Mean of cilofungin treatment from 0.02% to 0.10% in Spirulina is 0.91g/L to 1.05g/L, and in Chlorella is 0.81 to 1.09g/L respectively with corresponding P value 0.008 and 0.009. It implies that there exists a significant difference between the various concentrations of Cilofungin in biomass production. This proves the efficacy of cilofungin treatment in Spirulina sp and Chlorella sp.***

**Table 3**

***Growth rate of Spirulina Sp***

| Days | Mother Culture (Mean + Sem) | | Cilofungin (0.08%) (Mean + Sem) | |
|---|---|---|---|---|
| | (Cells/ml) | Biomass (g/l) | (Cells/ml) | Biomass (g/l) |
| 5th | 230.25 ± 18.82 | 0.18 ± 0.009 | 251.25 ± 7.98 | 0.20 ± 0.051 |
| 10th | 606.6 ± 21.67 | 0.30 ± 0.040 | 643.5 ± 15.00 | 0.49 ± 0.014 |
| 15th | 1067 ± 33.36 | 0.83 ± 0.012 | 1220.5 ± 26.81 | 1.02 ± 0.016 |
| 20th | 2769.25 ± 27.16 | 1.09 ± 0.081 | 3200.19 ± 33.46 | 1.50 ± 0.010 |

***From the Table:3, It is inferred that the Standard Error mean of growth rate in Spirulina mother culture and 0.08% Cilofungin treatment is 1.09g/L and 1.50 g/L respectively, with the corresponding P value of 0.000 and 0.004. Since the P value is less than 0.01 there is a significant growth differences between Cilofungin treated and untreated Spirulina sp.***

**Table 4**

***Growth rate of Chlorella Sp***

| Days | Mother Culture (Mean + Sem) | | Cilofungin (0.06%) (Mean + Sem) | |
|---|---|---|---|---|
| | (Cells/ml) | Biomass (g/l) | (Cells/ml) | Biomass (g/l) |
| 5th | 180.15 ± 12.14 | 0.09 ± 0.005 | 190.31 ± 09.81 | 0.15 ± 0.031 |
| 10th | 410.00 ± 18.01 | 0.25 ± 0.018 | 620.64 ± 13.11 | 0.60 ± 0.051 |
| 15th | 890.50 ± 24.54 | 0.75 ± 0.040 | 980.30 ± 17.55 | 0.95 ± 0.032 |
| 20th | 1380.18 ± 27.17 | 1.00 ± 0.021 | 2500.64 ± 21.64 | 1.23 ± 0.021 |

***From the Table:4, It is inferred that the Standard Error mean of growth rate in Chlorella mother culture and 0.06% Cilofungin treatment is 1.00g/L and 1.23 g/L respectively, with the corresponding P value of 0.009 and 0.005. Since the P value is less than 0.01 there is a significant growth difference between Cilofungin treated and untreated Chlorella sp.***

**Table 5**

***Effect of Cilofungin in Protein content***

| | Protein (mg/g) (Mean + Sem) | | | | |
|---|---|---|---|---|---|
| Cilofungin % | **0.02** | **0.04** | **0.06** | **0.08** | **0.10** |
| Spirulina | 469.5 ± 6.02 | 531.25 ± 12.8 | 609.5 ± 6.42 | 655.5± 6.57 | 498.25 ± 9.78 |
| Chlorella | 440.75± 6.02 | 627.5 ± 7.65 | 646.5 ± 6.50 | 604.5± 10.5 | 505.75± 11.77 |

***From the Table:5, It is inferred that the Standard Error mean of protein content in 0.08% of cilofungin treated Spirulina sp and 0.06% of cilofungin treated Chlorella sp were 655.5 mg/g and 646.5 mg/g respectively, with the corresponding P value of 0.007 and 0.004. Since the P value is 0.01 there is a significantly increased in protein content in ciliofungin treated Spirulina and Chlorella sp.***

**Table 6**

***Effect of Cilofungin in Carbohydrate content***

| | Carbohydrate (mg/g) (Mean + Sem) | | | | |
|---|---|---|---|---|---|
| Cilofungin % | **0.02** | **0.04** | **0.06** | **0.08** | **0.10** |
| Spirulina | 303.1 ± 5.19 | 282.9 ± 8.10 | 250.4 ± 11.12 | 200.1± 8.21 | 220.7± 9.74 |
| Chlorella | 400.2 ± 4.09 | 380.0 ± 10.0 | 320.7 ± 7.11 | 275.0± 5.29 | 311.8 ± 6.19 |

***From the Table:6, It is inferred that the Standard Error mean of carbohydrate content in 0.08% of cilofungin treated Spirulina sp and 0.06% of cilofungin treated Chlorella sp were 200.1 mg/g and 320.7 mg/g respectively, with the corresponding P value of 0.009 and 0.008. Since the P value is 0.01 there is a significantly decreased in carbohydrate content of ciliofungin treated Spirulina and Chlorella sp.***

**Table 7**

***Effect of Cilofungin in Fat content***

| | Fat (mg/g) (Mean + Sem) | | | | |
|---|---|---|---|---|---|
| Cilofungin % | **0.02** | **0.04** | **0.06** | **0.08** | **0.10** |
| Spirulina | 62.1 ± 0.09 | 65.7 ± 0.18 | 68.2 ± 0.14 | 74.1 ± 0.40 | 72.5 ± 0.19 |
| Chlorella | 80.8 ± 0.12 | 81.2 ± 0.10 | 90.0 ± 0.39 | 89.4 ± 0.20 | 89.8 ± 0.34 |

***From the Table:7, It is inferred that the Standard Error mean of Fat content in 0.08% of cilofungin treated Spirulina sp and 0.06% of cilofungin treated Chlorella sp 74.1 mg/g and 90.0 mg/g respectively, with the corresponding P value of 0.002 and 0.003. Since the P value is 0.01 there is a significantly difference in fat content of ciliofungintreated Spirulina and Chlorella sp.***

**Table 8**
***Effect of Cilofungin in Chlorophyll content***

| | Chlorophyll (mg/g) (Mean + Sem) | | | | |
|---|---|---|---|---|---|
| Cilofungin % | **0.02** | **0.04** | **0.06** | **0.08** | **0.10** |
| Spirulina | 12.9 ± 0.20 | 13.5 ± 0.15 | 14.1 ± 0.09 | 15.3 ± 0.29 | 16.1 ± 0.13 |
| Chlorella | 11.8 ± 0.11 | 12.5 ± 0.19 | 12.7 ± 0.10 | 13.0 ± 0.04 | 13.8 ± 0.31 |

***From the Table: 8, It is inferred that the Standard Error mean of Chlorophyll content in 0.08% of cilofungin treated Spirulina sp and Chlorella sp 15.3mg/g and 13.0 mg/g respectively, with the corresponding P value of 0.000 and 0.001. Since the P value is 0.01 there is a significantly increased in chlorophyll content of ciliofungin treated Spirulina and Chlorella*** sp.

**Table 9**
***Effect of Cilofungin in Carotenoids content***

| | Carotenoids (mg/g) (Mean + Sem) | | | | |
|---|---|---|---|---|---|
| Cilofungin % | **0.02** | **0.04** | **0.06** | **0.08** | **0.10** |
| Spirulina | 7.1 ± 0.04 | 7.8 ± 0.10 | 8.2 ± 0.18 | 8.5 ± 0.09 | 8.7 ± 0.25 |
| Chlorella | 4.2 ± 0.09 | 5.0 ± 0.12 | 5.1 ± 0.11 | 5.5 ± 0.14 | 5.8 ± 0.19 |

***From the Table: 9, It is inferred that the Standard Error mean of carotenoids content i n 0.08% of cilofungin treated Spirulina sp and Chlorella sp 8.5 mg/g and 5.5 mg/g respectively, with the corresponding P value of 0.002 and 0.005. Since the P value is 0.01 there is a significantly difference in carotenoids content of ciliofungin treated Spirulina and Chlorella sp.***

**Table 10**
***Comparison b/w Mother and Cilofungin treated culture***

| Algae | **Spirulina** | | **Chlorella** | |
|---|---|---|---|---|
| Culture | Mother | Cilofungin (0.08%) | Mother | Cilofungin (0.06%) |
| Carbohydrates (mg/g) | 328.1±4.09 | 200.1± 8.21 | 389.8 ± 3.11 | 320.7 ± 7.11 |
| Proteins (mg/g) | 421.2± 7.81 | 655.5± 6.57 | 430.4 ± 5.48 | 646.5 ± 6.50 |
| Fats (mg/g) | 57.4 ± 0.61 | 74.1± 0.40 | 75.0 ± 0.33 | 89.4 ± 0.20 |
| Chlorophyll (mg/g) | 12.0 ± 0.41 | 15.3 ± 0.29 | 10.9 ± 0.28 | 13.0 ± 0.14 |
| Carotenoids (mg/g) | 6.5 ± 0.13 | 8.7 ± 0.25 | 4.0 ± 0.29 | 5.5 ± 0.14 |

***From the Table: 10, It is inferred that the Standard Error mean in 0.08% of cilofungin treated Spirulina sp and 0.06% of cilofungin treated Chlorella sp, shows the significance difference with mother cultures in all metabolic compounds. Since the P value is less than 0.01, metabolic compounds shows wide signification between mother and cilofungin treated Spirulina and Chlorella sp.***

## CONCLUSION

The study concludes the effect of cilofungin in enhancing the protein and fat content in Spirulina and Chlorella sp .at certain fixed concentrations of 0.08% and 0.06% respectively. . The flow of excess carbon was diverted to protein and fat metabolism instead of carbohydrates metabolism by cilofungin in both selected species. The study makes a novel insight of an antifungal agent, cilofungin in altering the metabolic pathway of alga, in particular the switch over of carbohydrate metabolism diverted towards the production of fat, protein and other metabolites. Future studies on genetic variation between the stock and cilofungin treated cultures can elucidate the phenomena behind the metabolics involved and exploiting the same in industrial production of essential metabolites.

## CONFLICT OF INTEREST

Conflict of interest declared none.

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