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Culturing of Spirulina and effect of colchicine on bioactive compounds of Spirulina (*Arthospira platensis)*

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Abstract

Spirulina is an algae belonging to the cyanophyta. Spirulina is a unicellular blue-green algae that is found almost all over the world and grows naturally in an alkaline medium with a pH range of 7.0–9.8. In this research, spirulina was cultured in a spirulina culture chamber, which shows luxuriant growth with spirulina culture medium, Zarrouk medium, and obtained 50g of spirulina biomass in a 4 litre culture. The study investigated the effect of different concentrations of colchicine on fresh-grown spirulina. Spirulina biomass was treated with colchicine of different concentrations (0.01%, 0.05%, and 0.1%) for 12 hours. The study showed that at 0.05% concentration of colchicine the amount of carotenoid and protein content increased.

Keywords: Spirulina culturing, Zarrouk medium, colchicine, protein, carotenoids, and chlorophylls etc.

Introduction

Arthrospira platensis, commonly known as Spirulina, consists of unicellular, filamentous blue-green microalgae with symbiotic bacteria that fix nitrogen from the air. The shape of spirulina is different with different species; for example, it can be a rod, spiral, or disc-like structure (Sumit Sow et al, 2021). The normal morphology of Spirulina is characterised by its frequently coiled trichomes, and the arrangement of the spirals is the principle taxonomic criteria for Spirulina (Zhi Ping Wang et al., 2005). The width of the trichomes, composed of cylindrical cells shorter than vast cells, varies from about 61 to 12 m (16 m) in a variety of structures (Sumit Sow et al., 2021). Spirulina are autotrophic organisms with a photosynthesis pigment called "Phycocyanin", which is blue in colour; also present are chlorophyll a, chlorophyll b, and carotenoids (Ahsan et al., 2008). Spirulina is naturally found in the rich alkaline waters of lakes in warm regions, marshes, freshwater, brackish water, seawater, and thermal springs with pH 8.5–11.0, which favour excellent production of spirulina (Ahsan et al., 2008). There are 35 species of Arthrospira; examples are *A. indica, A. maxima, A. platensis, A. fusiform,* etc. *A. platensis* (Spirulina) is among the richest sources of proteins. Its protein content is about 60–70% of its dry weight. In a look at that, we tried the use of spirulina as a protein supplement. Hence, they are also called "protein tablets" (Nubu, Abdulmumin A. 2013). Spirulina has a high measure of polyunsaturated fats, 1.5 to 2.0 percent of 5 to 6 percent absolute lipids (Seema Kanojia, 2019). Beta-carotene accounts for 80% of the carotenoids found in spirulina, with the remainder consisting particularly of phycoxanthin and cryptoxanthin. Each kilogramme of dry spirulina consists of between 700 and 1700 mg of beta-carotene and approximately 100mg of cryptoxanthin; these carotenoids are converted into vitamin A via mammals (JacquesFalquet and J. P. Hurni, 1997).

Colchicine is an alkaloid derived from the plant of the genus Colchicum (*Colchicum automnale*). It is pale yellow in colour and water-soluble. Colchicine is an important mutagen that works by preventing microtubule formation and doubling the number of chromosomes. It is commonly used to develop polyploidy in plants and functions as a mitotic poison by producing many mutagenic effects on plants. Colchicine, a chemical given to plants, can lead to polyploid individuals. Typical characteristics of polyploid plants are larger sizes and larger parts such as roots, stems, leaves, flowers, and fruits. A high colchicine concentration and exposure time are not sufficient to produce a polyploid individual. Most studies on polyploidy have been performed on plants such as onions, balsam, and soybeans, while the effects of colchicine treatment on microalgae are not yet known. This study was designed to evaluate the effect of colchicine treatment on the bioactive compound of *A.platensis*

Materials and Methods

The spirulina mother culture were obtained from CIFE Andheri, 500 ml of spirulina were collected.

Culturing of Spirulina.

Spirulina cultured in a culture chamber 10 litre water bottles were used as a culturing chamber with the attachment of a 240 volt AC air pump for ariation and stirring of water. The light intensity was between 5 and 7 K lux for 10 hours. 4 litres of Zarrouk medium are used as culture medium. After two months, spirulina was harvested and used for further research.

Culture medium (zarrouk medium 1966).

Chemical component	Quantity for 4Liter in gm
$NaHCO_3$	32
NaCl	4
$NaNO_3$	10
K_2HPO_4	2
K_2SO_4	2
$MgSO_4$	0.64
$FeSO_4$	0.04

Different concentration of colchicine solution.

Colchicine powder is used to make different concentrations of colchicine solution. Example for 0.01% concentration: 0.01g of colchicine powder dissolved in 100 ml of distilled water. Also, for a 0.05% concentration, 0.05g of colchicine dissolves in 100 ml of distilled water, and 0.1%–0.1 g of colchicine powder dissolves in 100 ml of distilled water.

Treatment of colchicine to fresh Spirulina biomass.

Fresh spirulina were harvested from the culture chamber, and 5g of spirulina biomass were treated with 0.01% colchicine; 5g of spirulina biomass were treated with 0.05% colchicine; and 5g of spirulina were treated with 0.1% colchicine solution for 12 hrs. One without treatment served as a control.

After 12 hours of treatment, the biomass of spirulina was air dried, and powder was collected for further work.

Estimation of proteins (Lowry method 1951)

Reagent A: 200 mg NaOH and 1 g of Na_2CO_3 were dissolved in 50 ml of distilled water.

Reagent B: (120 mg $CuSO_4$ and 250mg sodium tartrate dissolve separately in a small amount of distilled water, mix, and make a volume of 25 ml with distilled water.)

Extraction: 0.1g of sample was weighed and grinded well in a mortar and pestle in 10 ml of sodium phosphate buffer. The extract was filtered with a muslin cloth, and the filtrate was taken for protein estimation.

Estimation: 0.2, 0.4, 0.6, 0.8, 1 ml of the working standard prepared into a series of test tubes The final volume was made up to 1 ml in all test tubes. 5 ml of filtrate from each plant sample was taken in 6 test tubes. A tube with 1 ml of water was used as a blank. 5 ml of reagent C (reagent C was prepared by mixing 50 ml of reagent A and 1 ml of reagent B) into each test tube, including the blank. Mixed well and allowed it to stand for 10 minutes. Then add 0.5 ml of Folin-Ciocalteau reagent, mix well, and incubate at room temperature in the dark for 30 minutes. A blue colour developed. Absorbance was read at 620nm. A standard graph was drawn and the amount of protein in the sample calculated in percentage.

Total chlorophyll content (mg/g).

Total chlorophyll content was determined and calculated by Arnon (1949).

1 g of fresh Spirulina powder material was extracted with 10 ml of 80% acetone. The extract was centrifuged at 2500 rpm for 15 minutes. The supernatant was then collected, and the absorbance of the extract was read at 645nm and 663 nm against the solvent (80% acetone) as a blank.

The chlorophyll a, chlorophyll b, and total chlorophyll were calculated in mg/g.

Estimation of carotenoids (Chamovitz et al, 1993).

The sample is mixed with acetone in a 1:10 w/v ratio. The mixture was incubated for 3 h at 20˚C. Cell debris was removed by centrifugation at 5000 rpm for 10mins at 4˚C. The supernatant was used for further quantification.

The total carotenoid concentration was estimated by a formula (Chamovitz et al, 1993).

Carotenoids (µg ml¯1) = (A461 – (0.046 x A664)) x 4.
A461; A664: absorbance at 461 and 664 nm wavelengths, respectively.

Observations and Results

Table no 1. Harvested biomass of Spirulina.

Sr. no	Days of harvesting	Wet biomass in grams
1	30	12g
2	60	32g
3	90	**50g**

Fig 1. 30 days culture

Fig 2. 60 days culture

Fig 3. 90 days culture

Fig 4. 10g of biomass.

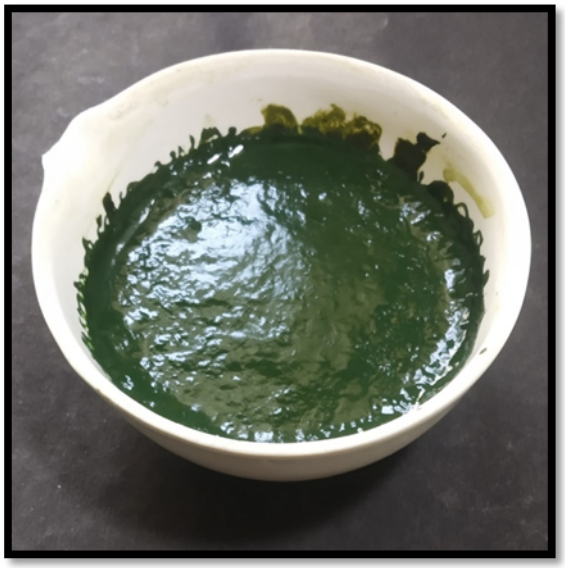

Fig 5. 50g of biomass.

Table no 2. Effect of colchicine on carotenoids. (Chamovitz et al, 1993).

Sr. no	Samples	Total carotenoids (µg/ml)
1	Control	7.20
2	0.01% treated sample	4.01
3	0.05% treated sample	**7.67**
4	0.1% treated sample	2.25

Table no 3. Effect of colchicine on chlorophyll (Arnon 1949).

Sr. no	Sample	Chlorophyll a	Chlorophyll b	Total Mg/ml
1	Control	0.08 mg/g	**0.0392 mg/g**	0.1101 mg/ml
2	0.01% treated sample	**0.0902 mg/g**	0.0225 mg/g	**0.1128 mg/ml**
3	0.05% treated sample	0.0410 mg/g	0.0161 mg/g	0.0572 mg/ml
4	0.1% treated sample	0.0185 mg/g	0.0062 mg/g	0.0247 mg/ml

Table no 4. Effect of colchicine onprotein (lowry's method 1951).

Sr. no	Sample	Protein content (%)
1	Control	54.14%
2	0.01% treated sample	52.30%
3	0.05% treated sample	**57.69%**
4	0.1% treated sample	40.76%

Zarrouk medium is a good culture medium for spirulina, and the total harvested spirulina was 50 g. From the research work, it has been found that colchicine mutagens have mutagenic activity, which is work on spirulina.

The maximum carotenoid found in a 0.05% concentration of spirulina is 7.67 mg/g, whereas the control is 7.20 mg/g.

The lowest carotenoid was recorded in 0.1%-treated spirulina at 2.25 mg/g.

The maximum chlorophyll recorded in 0.01% treated spirulina is 0.1128 mg/ml, whereas in control it is 0.1100 mg/ml.

The lowest chlorophyll levels were recorded in 0.1% treated spirulina of spirulina.

The maximum protein were recorded in 0.05% treated spirulina, which is 57.69%, and in control sample is 54.14%. Whereas the lowest protein was recorded in 0.1% of spirulina, which is 40.76%.

Conclusion

The results allow the following conclusions to be presented:

In this research, spirulina showed luxuriant growth in the culturing chamber, and the harvested biomass of spirulina was 50 g after two months of culturing.

The research results showed that the administration of colchicine at different doses affected the protein, chlorophyll, and carotenoids content of spirulina.

According to the results, colchicine treatment might be inducing polyploidy, which is a probable cause for the increase in carotenoid content in 0.05% concentration treated spirulina. And it's been concluded that a 0.05% treatment causes an increase in the carotenoids content. Also, a 0.1% concentration of colchicine can decrease the carotenoids.

The amount of chlorophyll estimated was at its maximum at 0.01% concentration in treated spirulina. Where a 0.1% concentration of colchicine can decrease the chlorophyll content in spirulina.

The treatment of colchicine can increase the protein content at a particular concentration, e.g., 0.05%, whereas at maximum concentration, it will decrease the protein content, e.g., 0.1%.

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