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"Appraisal Of Effect Of Wheat Bran Extract On The Growth Of *Spirulina* sp In Outdoor Cultivation".

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ABSTRACT:

Spirulina represents a promising future food source due to its commercially valuable by-products, such as crude protein, bio-pigments, and dietary supplements, leading to its widespread cultivation globally. *Spirulina*, a type of cyanobacterium or blue-green algae, thrives in a variety of aquatic environments, including freshwater, brackish water and saltwater habitats. It thrives especially well in warm, alkaline conditions. Numerous studies have explored various cultivation media for microalgae, yet the potential of wheat bran extract as a medium remains unexplored. This study aims to assess the suitability of wheat bran extract, in combination with cooking soda, salt lite (Tata Salt) and other additives, as a cultivation medium for *Spirulina*. In the Aquatic Biology laboratory at VNSGU, an experiment was conducted using five different concentrations (0.2 ml, 1 ml, 2 ml, 3 ml, and 4 ml per 200 ml) over a span of 18 days. As a control, *Spirulina* sp was cultivated in 200 ml of modified medium without addition of any wheat bran extract. *Spirulina* in wheat bran extract at a concentration of 4ml/200ml during present study showed dry weight of 0.307±0.0020 g, specific growth rate of 0.0167±0.00009and carotenoid of 7.869±0.016 µg/l. At a concentration of 4ml/200 ml, *Spirulina* grown in wheat bran extract contained chlorophyll-a 9.560±0.019 mg/L and chlorophyll-c 1.647±0.017 mg/L was found. At a concentration of 2ml/200 ml, *Spirulina* grown in wheat bran extract contained chlorophyll-b 3.482±0.016 mg/L was found. Which was higher than the control medium with respect to outdoor culture.

Keywords: *Spirulina* sp, Wheat bran, Growth (Dry Weight), Specific growth rate, Density, Chlorophyll and Total carotenoid content

1. INTRODUCTION:

"*Spirulina*, identified as a blue-green alga, is also recognized as the cyanobacterium *Spirulina*." "The name *Spirulina* originates from the Latin term for helix or spiral, indicating its characteristic physical shape." *Spirulina* thrives in saline and alkaline environments. "*Spirulina* was first recognized in the highly alkaline lakes of Africa and Mexico, renowned for their abundant biodiversity (Srivastava, 2017). Currently, *Spirulina* cultivation is becoming a global phenomenon owing to its remarkable nutritional attributes. "*Spirulina* is among the most abundant sources of balanced nutrition, providing both macro and micronutrients essential for maintaining overall health (Umesh, 2012). "Recent research efforts have focused on developing an efficient medium at low cost.

2. EXPERIMENTAL METHODOLOGY

2.1 CULTURE MEDIUM and MODIFIED MEDIUM:

Spirulina was cultured in a modified medium enriched with wheat bran extract. The composition of the growth medium (g/l) includes a pH of 9.5.**(Table No:1)**

Table No:1 Chemical composition of the modified medium- Outdoor culture

No	Chemical name	Concentration in stock solution (g/l)
1	Cooking soda	16
2	Sodium nitrate ($NaNO_3$)	2.5
3	Potassium sulphate (K_2SO_4)	1
4	Salt lite (Tata salt)	1
5	di-Potassium hydrogen phosphate (K_2HPO_4)	0.6
6	Ferrous sulphate heptahydrate ($FeSO_4.7H_2O$)	0.01

2.2 PREPARATION OF MEDIUM:

A detailed investigation was conducted using wheat bran extract to assess *Spirulina* growth. To create the extract, 10 grams of wheat bran were mixed with 100 ml of tap water and heated on a water bath for 30 minutes. After cooling, the mixture was filtered through filter paper until obtaining a clear solution. The resulting extract was autoclaved and stored as a 10% wheat bran stock solution (Fig. 6). A comprehensive study was conducted to assess the influence of wheat bran extract on the growth of *Spirulina* sp. Various concentrations of wheat bran, such as 0.2 ml, 1 ml, 2 ml, 3 ml, and 4 ml per 200 ml, were used. The control

group, representing 0 ml (without the addition of wheat bran extract) (Chaudhari *et al.*, 2022;2023;2024).

2.3 STERILIZATION, CULTIVATION, AND CULTURE MAINTENANCE:

The growth media were sterilized in an autoclave at a temperature of 121˚C for a duration of 20 minutes. The *Spirulina* sp culture was maintained at room temperature, with blue LED light provided for 8 hours each day. Agitation was ensured by manually shaking the culture 3-4 times daily during the experiment. All subculturing and inoculation procedures were carried out under sterile conditions (Chaudhari *et al.*, 2022;2023;2024, Pandey *et al.*, 2010; Shi *et al.*, 2016;).

2.4 MEASUREMENT OF GROWTH:

Following an 18-day period, the concentration of *Spirulina* sp biomass was assessed. Each culture medium underwent filtration using pre-weighed Whatman filter paper No. 1, followed by rinsing with acidified distilled water to eliminate salts and nutrients. The filter paper was then air-dried in an oven at 90˚C and weighed using a precision balance to determine dry weight, calculated based on the weight difference before and after drying (Fig. 9). (Chaudhari *et al.*, 2022;2023;2024, Pandey *et al.*, 2010; Palanisamy *et al.*, 2021)

2.5 SPECIFIC GROWTH RATE OF *SPIRULINA* (Abu-Razaq *et al.*,1999, Kumaresan *et al.*, 2020; Chaudhari *et al.*, 2022; 2023; 2024):

$$\mu = X2 - X1/t$$

Where,

μ =Cell weight day^{1}

μ = Specific growth rate

In X1= Initial weight of *Spirulina* biomass

In X2= Final weight of *Spirulina* biomass

2.6 DENSITY EQUATION (Chaudhari *et al.*, 2022;2023;2024):

$$p = \frac{m}{V}$$

Where,

p = Density

m= Mass

V= Volume

2.7 PIGMENTS CONTENTS: APHA (1998)

To extract chlorophyll from dried *Spirulina*, a measured amount was crushed with 10 ml of 90% acetone in a pestle-mortar. The mixture was refrigerated overnight to facilitate pigment extraction, with tubes covered by carbon paper to minimize light exposure. After centrifugation for 10 minutes at 2500 rpm, the supernatant containing extracted chlorophyll was collected (Fig. 10). Readings were then taken at specific wavelengths (630 nm, 645 nm, 665 nm, and 450 nm) using a Shimadzu-UV-1800 spectrophotometer, with 90% acetone serving as the blank. Concentrations of chlorophyll-a, chlorophyll-b, and chlorophyll-c were determined using specific formulas. (Chaudhari *et al.*, 2022;2023;2024, APHA, 1988)

$$Ca = 11.85\ (\mathrm{OD664}) - 1.54\ (\mathrm{OD647}) - 0.08(\mathrm{OD630})$$
$$\mathrm{Cb} = 21.03\ (\mathrm{OD647}) - 5.43\ (\mathrm{OD664}) - 2.66\ (\mathrm{OD630})$$
$$\mathrm{CC} = 24.52\ (\mathrm{OD630}) - 7.60(\mathrm{OD647}) - 1.67(\mathrm{OD664})$$

The total carotenoid content (Cp) was calculated using the equation proposed by Ben-Amotz and Avron in 1983, as well as by Jeffrey *et al.*, in 1997. (Kafyra*et al.*, 2018, Chaudhari *et al*., 2022; 2023; 2024)

$$CP\ (\frac{\mu g}{L}) = 7.60\ (A480) - 1.49\ (A510)$$

2.8 STATISTICAL TOOLS

The mean value of three replicates for each experimental culture flask was calculated, and represented as Mean ± SE (standard error). These values were then graphically analysed using Microsoft Excel. (Fig, 1-5)

3. RESULTS AND DISCUSSION:

Spirulina in wheat bran extract at a concentration of **4ml/200ml** during present study showed density of **0.0306±0.00004**(Fig. 1), dry weight of **0.307±0.0020 g**(Fig. 2), specific growth rate of **0.0167±0.00009**(Fig. 3)and carotenoid of **7.869±0.016 µg/l**(Fig. 4). At a concentration of **4ml/200 ml**, *Spirulina grown* in wheat bran extract contained **chlorophyll-a 9.560±0.019 mg/L** and **chlorophyll-c 1.647±0.017 mg/L** was found. At a concentration of **2ml/200 ml,** *Spirulina* grown in wheat bran extract contained **chlorophyll-b 3.482±0.016 mg/L** was found (Fig. 5).

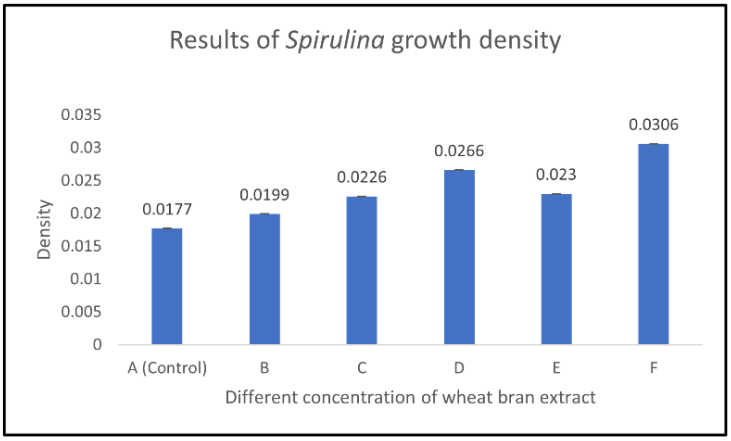

Figure 1. Results of Spirulina growth density

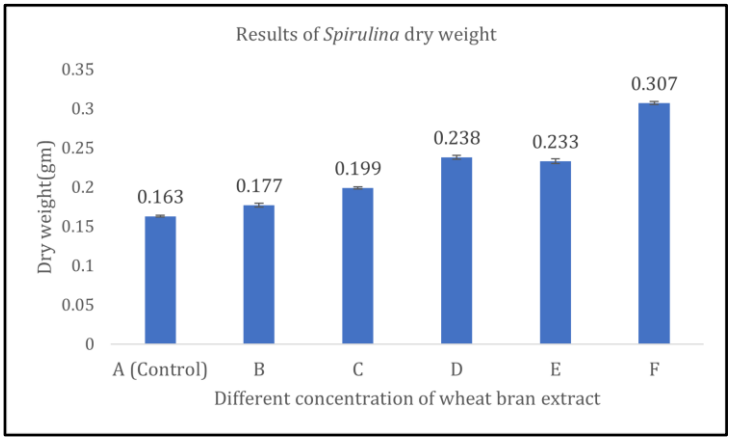

Figure 2. Results of Spirulina dry weight

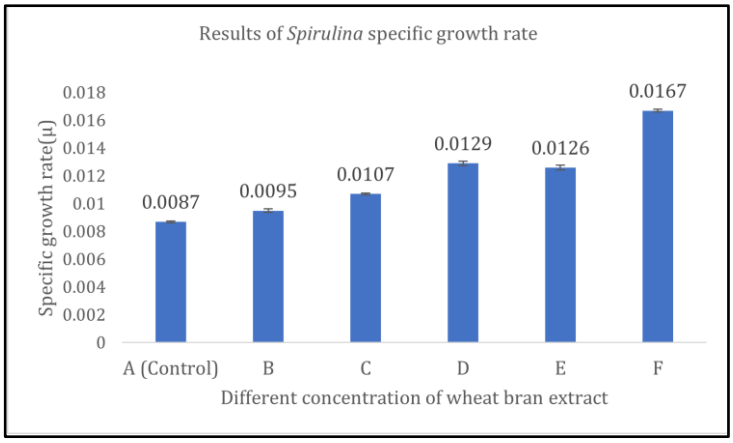

Figure 3. Results of Spirulina specific growth rate

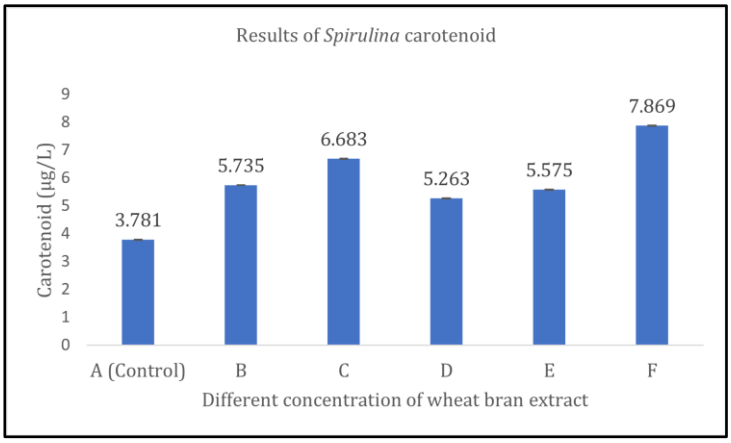

Figure 4. Results of Spirulina carotenoid

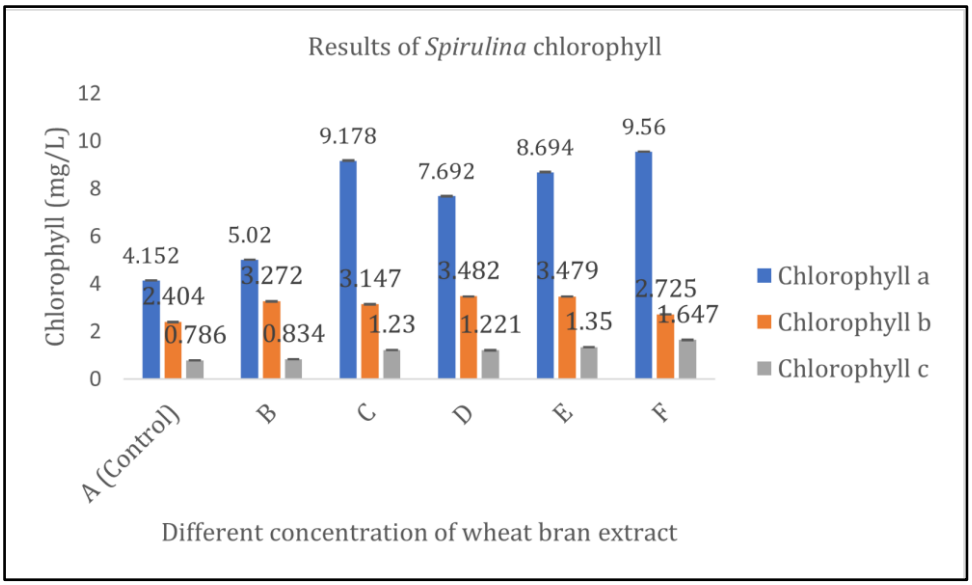

Figure 5. Results of Spirulina different chlorophyll

Figure 6. Wheat bran extract

Figure 7. 1st day incubation of Spirulina in wheat bran extract

Figure 8. 18th day after growth of Spirulina in wheat bran extract

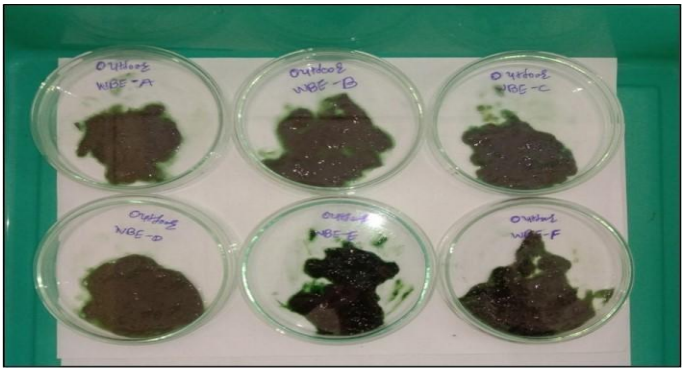

Figure 9. 18th day after growth of Spirulina in wheat bran extract

Figure 10. Result of chlorophyll (wheat bran extract)

4. CONCLUSION:

The objective of expediting the growth of *Spirulina* sp was effectively met through the addition of cost-effective wheat bran extract to the modified medium. Biomass growth was notably enhanced with a concentration of 200ml/4ml of wheat bran extract. Furthermore, levels of chlorophyll a, b, and c showed significant increases compared to the control group.

5. ACKNOWLEDGEMENTS:

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