"Investigation On the Utilization of Horse Manure Extract as an Additive in the Growth Of *Spirulina* Sp"

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Abstract
This study attempts to evaluate the feasibility of using horse manure as a cost-effective alternative nutrient source for microalgae cultivation. By harnessing its abundant minerals and cost-effectiveness, this study seeks to reduce expenses associated with nutrient mediums, ultimately enhancing the economic appeal of microalgae-derived products for commercial use. The horse manure obtained from the stables was sun-dried and then ground into a powder using a mixer grinder. To obtain horse manure extract, 10 g of powdered manure is mixed with 200 ml of water and boiled in a water bath for 30 minutes. In the cultivation process of *Spirulina* sp, horse manure extract supplements were introduced at different concentrations (0.2ml, 1ml, 2ml, 3ml, 4ml) into the indoor modified growth medium. A control group was included, utilizing an indoor modified growth medium without any addition of horse manure extract supplement. The research focused on exploring the effects of various concentrations of horse manure extract on indoor *Spirulina* cultivation, showing encouraging results. Using a 4ml/200ml concentration of horse manure extract led to *Spirulina* exhibiting a dry weight of 0.098±0.0015 g, a specific growth rate of 0.0051±0.00009, and a density of 0.0138±0.00003. Moreover, when subjected to a concentration of 0.2 ml/200 ml, *Spirulina* demonstrated 5.322±0.011 µg/l of carotenoids. Chlorophyll a, b, and c were detected in *Spirulina* across a range of horse manure extract concentrations, namely 1ml/200ml (9.770±0.018 mg/L), 3ml/200ml (3.176±0.010 mg/L), and (0.805±0.031 mg/L). These levels of chlorophyll were notably higher compared to the control medium in indoor culture settings.

Keywords: *Spirulina* sp, Horse manure extract, Growth (Dry Weight), specific growth rate, density, Chlorophyll and total carotenoid content

I. INTRODUCTION

Spirulina is comprised of approximately sixty percent complete, easily digestible protein, containing all essential amino acids. It boasts the highest beta-carotene content among all whole foods and is an exceptional source of gamma linolenic acid (GLA). Furthermore, it is abundant in B vitamins, minerals, trace elements, chlorophyll, and enzymes. Additionally, *Spirulina* contains various nutrients such as carotenoids, sulfolipids, glycolipids, phycocyanin, superoxide dismutase, RNA, and DNA.

Horse manure is rich in nutrients and commonly utilized in home gardens. Farmers highly appreciate horse dung for its ability to supercharge compost piles, enhance soil fertility, promote regeneration, and improve crop yields. It serves as a cost-effective and appropriate fertilizer for plants. For these reasons we have use try to grow *Spirulina* with the help of horse manure extract and good results was noted.

Name of Manure	N Nitrogen %	P Phosphorus %	K Potassium (Potash) %
Horse	0.7	0.3	0.6

II. RESEARCH METHODOLOGY

2.1 Culture medium

In modified medium was supplement with horse manure. (pH- 9.5)

2.2 Modified Medium

Table No:1 Chemical composition of the modified medium- Indoor 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	Sodium chloride (NaCl)	1
5	di-Potassium hydrogen phosphate (K_2HPO_4)	0.6
6	Ferrous sulphate heptahydrate ($FeSO_4.7H_2O$)	0.01

2.3 Media preparation

A detailed investigation was conducted using horse manure extract to assess *Spirulina* growth. The horse manure was dried and finely powdered by grinding and sieving through a fine cloth. Subsequently, 10 grams of the powder 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 a clear solution was obtained. The resulting extract was autoclaved and stored as a 10% horse manure stock solution.

Table No:2 Preparation of media

No	Modified medium	Horse manure (Different concentration)	Modified medium	Horse manure (Different concentration)
A	1000 ml	0 ml	200 ml	0 ml
B	1000 ml	1 ml	200 ml	0.2 ml
C	1000 ml	5 ml	200 ml	1 ml
D	1000 ml	10 ml	200 ml	2 ml
E	1000 ml	15 ml	200 ml	3 ml
F	1000 ml	20 ml	200 ml	4 ml

2.4 Sterilization

The growth media was sterilized by steam for 20 minutes at 121˚C and 15 pounds per square inch pressure in an autoclave.

2.5 Culture incubation and maintenance

Spirulina sp cultures were maintained at room temperature, exposed to blue LED light for 8 hours per day. Regular agitation was achieved by manually shaking the culture 3-4 times a day throughout the experiment. All subculturing and inoculation procedures were performed under sterile conditions.

2.6 Measurement of growth

After 18 days, 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 calculate dry weight based on the weight difference before and after drying.

2.7 Determining the specific growth rate of *Spirulina* (Abu-Razaq *et al.*, 1999)

μ (Cell weight day1) = X2-X1/t

Where,

μ = Specific growth rate

In X1= Initial weight of *Spirulina* biomass

In X2= Final weight of *Spirulina* biomass

2.8 Density equation

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

Where,

p = Density

m= Mass

V= Volume

2.9 Contents of pigments

To extract chlorophyll from dried *Spirulina*, a measured amount was crushed in a pestle-mortar with 10 ml of 90% acetone. Subsequently, the mixture was refrigerated overnight for pigment extraction, with the tubes covered using carbon paper. After centrifugation for 10 min at 2500 rpm, the supernatant was collected. Readings were obtained at wavelengths of 630 nm (A630), 645 nm (A645), 665 nm (A665), and 450 nm (A450) using a Shimadzu-UV-1800 spectrophotometer, with 90% acetone as the server blank. Specific formulas were used to determine the concentrations of chlorophyll-a, chlorophyll-B and chlorophyll-C.

C_a =11.85 (OD664) - 1.54 (OD647) - 0.08 (OD630)

C_b = 21.03 (OD647) - 5.43 (OD664) - 2.66 (OD630)

C_c = 24.52 (OD630) -7.60 (OD647) - 1.67 (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.

Cp (μg/L) = 7.60 (A480) – 1.49 (A510)

The mean value of three replicates for each experimental culture flask was calculated, and the data were presented as Mean ± SE. Graphical analysis was performed based on this format.

3. Results & Discussion

Employing a concentration of 4ml/200ml of horse manure extract resulted in *Spirulina* demonstrating a dry weight of 0.098±0.0015 g, a specific growth rate of 0.0051±0.00009, and a density of 0.0138±0.00003. Furthermore, at a concentration of 0.2 ml/200 ml, *Spirulina* exhibited 5.322±0.011 µg/l of carotenoids. Chlorophyll a, b, and c were found in *Spirulina* at various concentrations of horse manure extract, including 1ml/200ml (9.770±0.018 mg/L), 3ml/200ml (3.176±0.010 mg/L), and (0.805±0.031 mg/L).

Figures and Tables

Figure. 1

Figure. 2

Figure. 3

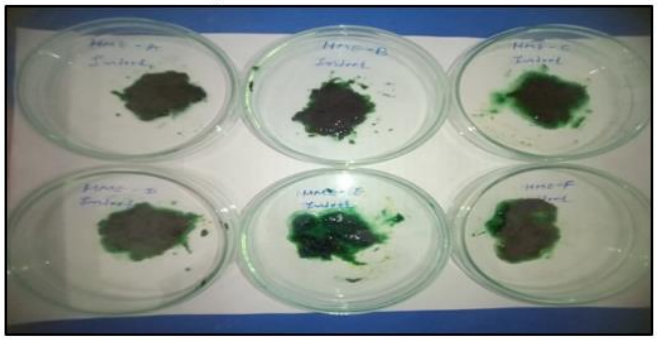

Figure. 4

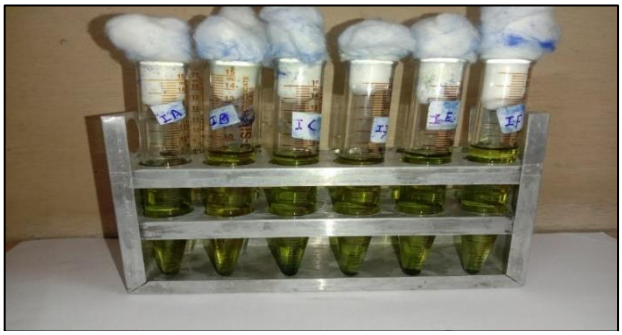

Figure. 5

Fig. 1 Horse manure extract
Fig. 2 1st day incubation of *Spirulina* in horse manure extract
Fig. 3 18th day after growth of *Spirulina* in horse manure extract
Fig. 4 18th day after growth of *Spirulina* in horse manure extract
Fig. 5 Result of chlorophyll (Horse manure extract)

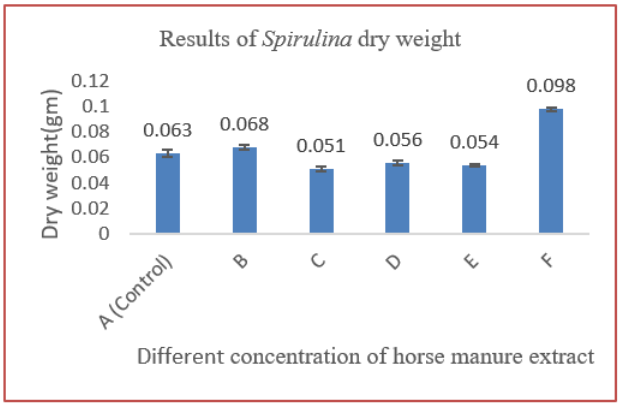

***Figure.* 6** Results of *Spirulina* dry weight

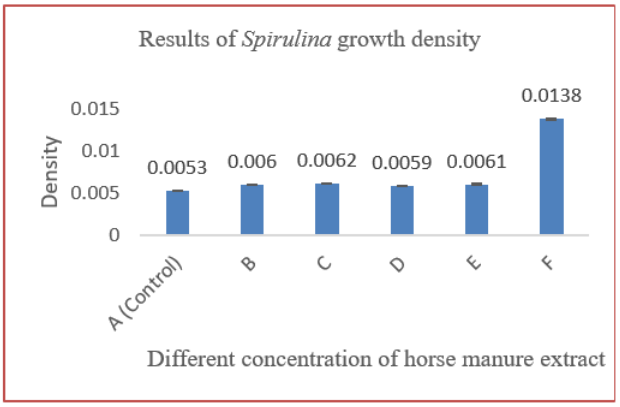

***Figure.* 7** Results of *Spirulina* growth density

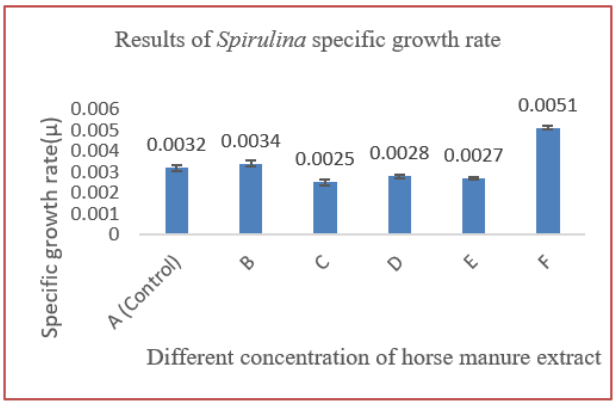

***Figure.*8** Results of *Spirulina* specific growth rate

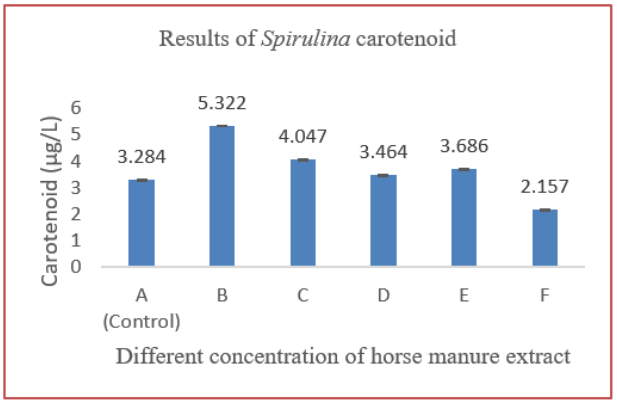

***Figure.* 9** Results of *Spirulina* carotenoid

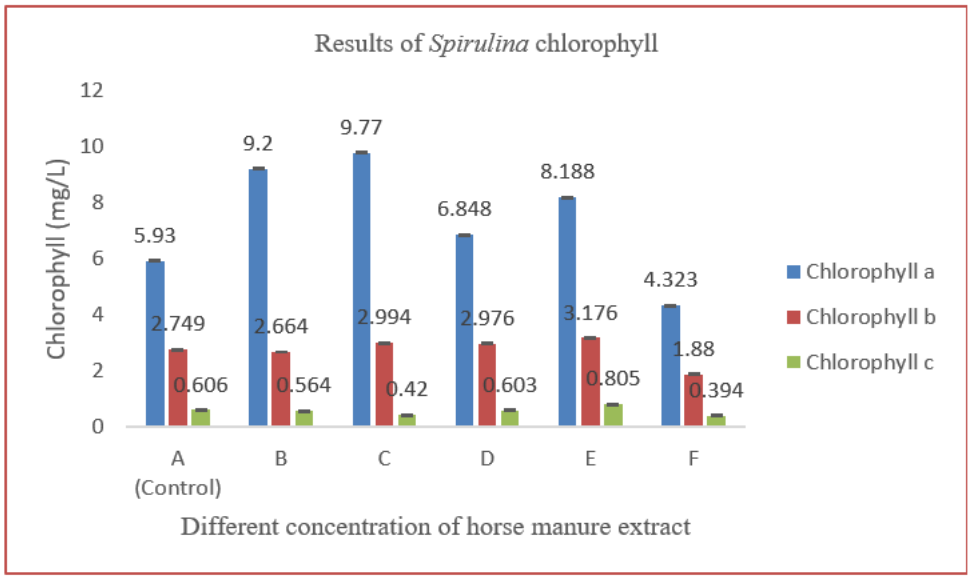

***Figure.* 10** Results of *Spirulina* differents chlorophyll

Table 4 *Spirulina* growth density Mean± Se	
HME	***Spirulina* growth density Mean± Se**
A. (Control)	0.0053±0.00002
B. 0.2 ml	0.0060±0.00002
C. 1 ml	0.0062±0.00004
D. 2 ml	0.0059±0.00004
E. 3 ml	0.0061±0.00003
F. 4 ml	**0.0138±0.00003**

Table 3 *Spirulina* dry (weight) Mean± Se	
HME	***Spirulina* dry (weight) Mean± Se**
A. (Control)	0.063±0.0029
B. 0.2 ml	0.068±0.0023
C. 1 m	0.051±0.0017
D. 2 ml	0.056±0.0020
E. 3 ml	0.054±0.0012
F. 4 ml	**0.098±0.0015**

Table 5 SSGR Mean± Se	
HME	**SSGR Mean± Se**
A. (Control)	0.0032±0.00015
B. 0.2 ml	0.0034±0.00015
C. 1 ml	0.0025±0.00012
D. 2 ml	0.0028±0.00009
E. 3 ml	0.0027±0.00006
F. 4 ml	**0.0051±0.00009**

Table 6 Carotenoid Mean± Se	
HME	**Carotenoid Mean± Se**
A. (Control)	3.284±0.015
B. 0.2 ml	**5.322±0.011**
C. 1 ml	4.047±0.020
D. 2 ml	3.464±0.016
E. 3 ml	3.686±0.012
F. 4 ml	2.157±0.007

Table 7 Chlorophyll Mean± Se			
HME	Chlorophyll a ± SE	Chlorophyll b ± SE	Chlorophyll c ± SE
A. (Control)	5.930±0.016	2.749±0.027	0.606±0.021
B. 0.2 ml	9.200±0.019	2.664±0.012	0.564±0.017
C. 1 ml	**9.770±0.018**	2.994±0.022	0.420±0.026
D. 2 ml	6.848±0.025	2.976±0.021	0.603±0.011
E. 3 ml	8.188±0.019	**3.176±0.010**	**0.805±0.031**
F. 4 ml	4.323±0.022	1.880±0.031	0.394±0.022

4. Conclusions

The goal of boosting *Spirulina* growth with an affordable horse manure extract supplement in a modified medium has been mostly accomplished. Particularly, the concentration of 4 ml of horse manure extract per 200 ml of medium proved to be successful in fostering considerable biomass growth. Across different concentrations of horse manure extract, there were notable enhancements in density, chlorophyll, and carotenoid levels compared to the control group.

5.Acknowledgment

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6. References

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