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## Study on the Use of Poultry Manure Extract in the Growth of *Spirulina maxima* with Reference to Indoor Culture.

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### Abstract

The effect of poultry manure supplementation on the development of *Spirulina maxima* was investigated for 18 days. The manure was collected from the poultry farm; after that, it was sun-dried and powdered by grinding it in a mixture grinder. 10-gram poultry manure powder was added to 100 ml tap water and it was steamed in a water bath for 30 minutes. The cultivation of *Spirulina maxima* was done by using the supplement of poultry manure extract in *Spirulina* growth medium (200ml) at different concentrations such as 0ml, 0.2ml, 1ml, 2ml, 3ml, and 4ml. *Spirulina* growth medium without any supplement of poultry manure extract was taken as a control. The experiment was done for 18 days and the culture was provided with a sufficient light source for 8 hours a day; the agitation was done by manual shaking 3-4 times a day throughout the experiment. The growth of *Spirulina* was measured as a wet weight and the dry weight of *Spirulina* by using the gravimetric method. The highest growth observed was 0.041gm in 200ml/2ml poultry manure extract. The highest specific growth rate observed was 0.0019 in 200ml/2ml poultry manure extract. Chlorophyll-a (10.6948 mg/L) and Chlorophyll-b (3.1641 mg/L) were found in higher concentrations at 200ml/1ml poultry manure extract. Chlorophyll-C (0.4142 mg/L) was high in control.

**Keywords:** *Spirulina maxima*, Modified medium, Growth (Dry Weight), Specific growth rate, Chlorophyll.

### 1. Introduction

Multicellular, filamentous blue-green algae known as *Spirulina* are becoming more and more popular in the health food sector and as a protein and vitamin addition to aquaculture diets. The cyanobacterium *Spirulina* has been commercially exploited for the production of human dietary supplements, animal feed, and pharmaceuticals. The availability of nutrients, the temperature, and the amount of light are the three most crucial variables that affect the large-scale production of *Spirulina* biomass. 35°C to 38°C is the ideal temperature range for *Spirulina* growth. Also, *Spirulina* needs a pH that is relatively high, which effectively prevents other algae from growing in the culture media.

### NPK Values

Name of Manure	N Nitrogen %	P Phosphorus %	K Potassium (Potash) %
Poultry	1.1	0.8	0.5

### 2. Materials and methods

#### 2.1 Culture medium

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

#### 2.2 Modified Medium

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No	Chemical name	Concentration in stock solution g/L
1	Cooking soda	16
2	Sodium nitrate (NaNO <sub>3</sub> )	2.5
3	Potassium sulphate (K <sub>2</sub> SO <sub>4</sub> )	1
4	Sodium chloride (NaCl)	1
5	di-Potassium hydrogen orthophosphate (K <sub>2</sub> HPO <sub>4</sub> )	0.6
6	Ferrous sulphate heptahydrate (FeSO <sub>4</sub> .7H <sub>2</sub> O)	0.01

### 2.3 Media preparation

Carried out elaborated study with Poultry manure extract on the growth of *Spirulina maxima*.

After drying poultry manure made into fine powder by grinding and sieving through a fine cloth. 10 gm of the powder of poultry manure mixed with 100 ml tap water and steamed on water bath for 30 minutes.

After cooling, filtered through filter paper, till a clear solution was obtained. Autoclaved the extract and stored as stock solution of 10% poultry manure.

### Preparation of media

NO	Modified Medium	Manures	Modified Medium	Manures
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	1.0 ml
D	1000 ml	10 ml	200 ml	2.0 ml
E	1000 ml	15 ml	200 ml	3.0 ml
F	1000 ml	20 ml	200 ml	4.0 ml



### 2.4 Sterilization

Growth media were steam-sterilized for 20 minutes at a pressure of 15 pounds per square inch and a temperature of 121°C in an autoclave.

### 2.5 Incubation and maintenance of culture

The culture of *Spirulina maxima* was maintained at room temperature.

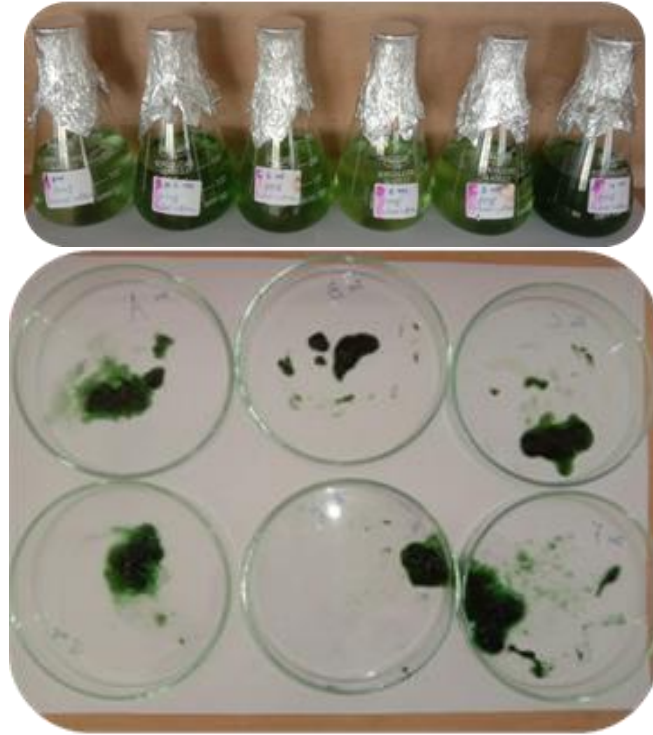
Blue light (LED) was provided for 8 hours a day. Agitation was done by manual shaking 3-4 times a day throughout the experiment.

All the subculturing and inoculation operations were carried out in aseptic condition.

### 2.6 Growth measurement

The concentration of *Spirulina maxima* biomass was estimated after 18 days. Each culture media was filtered by a pre-weighted Whatman filter paper No.1 and washed with acidified distilled water to release all salts and nutrients. After filtration, the filter papers were left to dry at 90°C in an oven for two hours and reweighed in a weight balance. Then, the dry weight was calculated.





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

$$\mu \text{ (Cell weight day}^{-1}\text{)} = \frac{X_2 - X_1}{t}$$

$\mu$  = Specific growth rate

In  $X_1$  = Initial weight of *Spirulina* biomass

In  $X_2$  = Final weight of *Spirulina* biomass

### 2.8 Pigments contents

The chlorophyll was extracted from the dried *Spirulina*. A known amount of *Spirulina* was taken in pestle -mortar and crushed with 10 ml of 90% acetone. The tubes were cover

with carbon paper and refrigerated overnight for the pigment extraction. The sample were centrifuged for 10 minutes at 2500rpm and the supernatant was collected. The reading was taken at 630 nm (A630), 645 nm (A645), 665 nm (A665), and 450nm (A450) by using shimadzu-UV-1800 spectrophotometer. 90% acetone as a blank. The concentration of Chl-a, Chl-b, and Chl-c were estimated by using the following formula:

1.  $C_a = 11.85 (OD_{664}) - 1.54 (OD_{647}) - 0.08 (OD_{630})$
2.  $C_b = 21.03 (OD_{647}) - 5.43 (OD_{664}) - 2.66 (OD_{630})$
3.  $C_c = 24.52 (OD_{630}) - 7.60 (OD_{647}) - 1.67 (OD_{664})$



### 3. Results & Discussion

Modified medium, with poultry manure supplements, in five different concentrations were tested, for the enhancement of growth of *Spirulina*. Poultry manure extract in five different concentrations (A) Control (B)0.2ml (C)1ml (D)2ml (E)3ml (F)4ml were tested for enhanced growth.

The highest growth observed was 0.041gm in 200ml/2ml poultry manure extract compared to control. The highest specific growth rates were observed ( $\mu=0.0019$ ) in 200ml/2ml compared to control. Chlorophyll-a (10.6948 mg/L) and Chlorophyll-b (3.1641 mg/L) are found in higher concentrations at 200ml/1ml poultry manure extract. Chlorophyll-C (0.4142 mg/L) is high in control.

4. Tables and Figures

Table No 1

Result of growth		
PME	<i>Spirulina</i> Wet Weight	<i>Spirulina</i> Dry (Weight)
A(Control)	0.788	0.016
B	0.629	0.029
C	0.794	0.033
D	0.807	<b>0.041</b>
E	0.641	0.018
F	1.858	0.038

Table No 2.

Result of specific growth rate	
PME	Specific growth rate ( $\mu$ )
A (Control)	0.0005
B	0.0012
C	0.0015
D	<b>0.0019</b>
E	0.0006
F	0.0017

Table No 3.

Result of Chlorophyll content			
PME	CHLOROPHYLL-a mg/L	CHLOROPHYLL-b mg/L	CHLOROPHYLL-c mg/L
A (Control)	8.6058	2.2619	<b>0.4142</b>
B	5.3754	1.5855	0.1263
C	<b>10.6948</b>	<b>3.1641</b>	0.2027
D	8.4107	2.1709	0.1356
E	3.5337	1.627	0.2486
F	8.3785	2.7277	0.1663

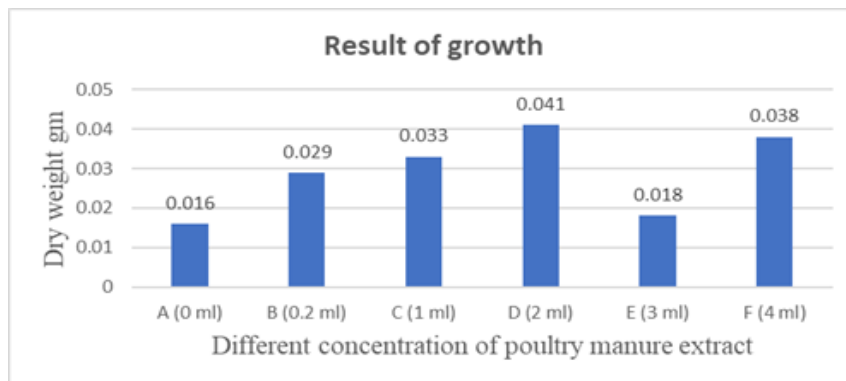


Fig. 1: Result of growth.

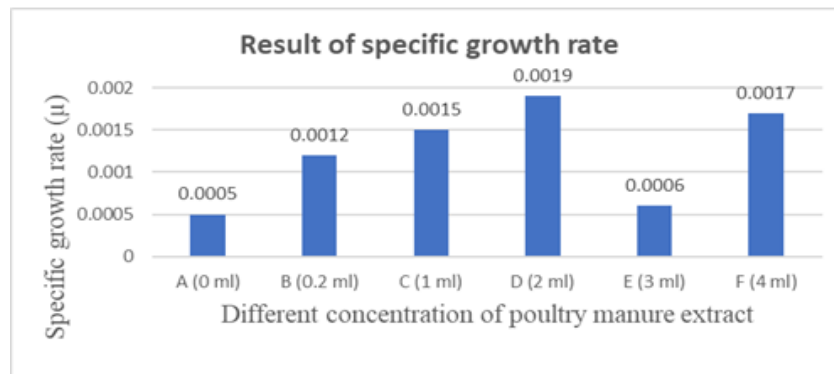


Fig. 2: Result of specific growth rate.

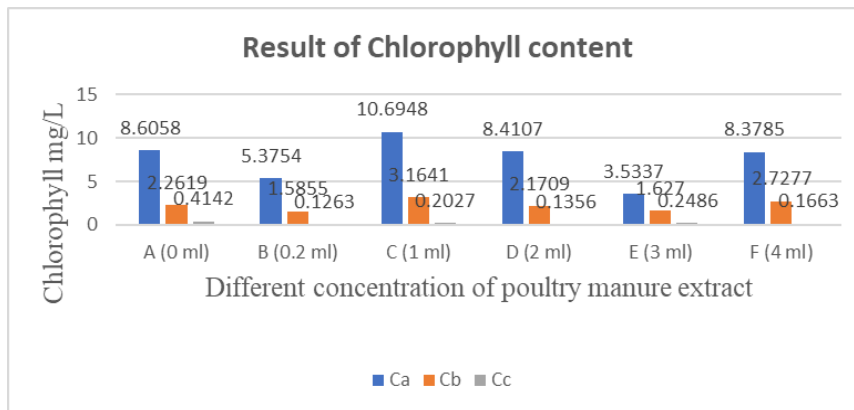


Fig. 3: Result of chlorophyll content.

## 5. Conclusions

The aim of this study to enhance the growth of *Spirulina maxima* has been fulfilled to a great extent by using low-cost poultry manure extract supplement to modified medium. 200ml/2ml concentration of poultry manure extract promoted good growth as measured by biomass. Chlorophyll a and b also increased in C flask at 200ml/1ml concentration of poultry manure extract compared to control. Chlorophyll c was highest in control.

## 6. Acknowledgments

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