A Study on Growth Performance of *Spirulina Platensis* in Different Concentrations of Rotten Apple as A Carbon Source

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***Abstract*—**An experiment was conducted on culture and growth performance of *Spirulina platensis* in various concentrations of rotten apple medium (RAM) and Kosaric Medium (KM). The observation was conducted for three months from March to May at the Live Food Culture Laboratory, Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University. Culture of *S. platensis* was performed in 1.0L glass flasks in three different media such as 2.5, 5.0 and 10% and KM with three replications under fluorescent light in light : dark (12 hr : 12 hr) condition of a period of 14 days. Growth performances of *S. platensis* varied from one medium to another. The initial cell weight of *S. platensis* was 0.0023 mg/L and a maximum cell weight of 12.44 mg/L was found in KM and 10.468 mg/L in RAM on 10^{th} day of culture. It was also observed that, the initial chlorophyll a content of *S. platensis* was 0.0015 mg/L which was attained at a highest content of 10.54 mg/L in KM and 12.35 mg/L in RAM on 10^{th} day of culture. A decreasing trend of cell weight was observed from 10^{th} day of culture. The growth of *S. platensis* was significantly ($p<0.05$) better in 5.0% Digested Rotten Apple Medium (DRAM) than other concentrations 2.5% DRAM and 10% DRAM. From the results obtained in the present study, it was summarized that the growth of *S. platensis* was better in the concentrations of 5.0% DRAM than other concentrations of RAM. Thus, the concentration of 5.0% DRAM is most suitable for *S. platensis* culture compare with standard KM. These media are easily available and most inexpensive in contrast of Bangladesh. So digested rotten apple can be used for commercially and economically viable mass culture of *S. platensis*.

Keywords*—*Spirulina platensis, rotten apple, live food

INTRODUCTION

The best potential is seen in microbial protein or single cell protein (SCP), a new source of protein independent of agriculture. The SCP are characterized by fast growth rate, high protein content (43-85%), compared to field crops, require less water, land and independent climate, grow on wastewater, can be genetically modified for desirable characters such as amino acid composition and temperature tolerance (Tri-Panji and Suharyanto, 2001). The ability of microalgae to accumulate trace elements is well documented (Sakaguchi *et al.,* 1981). The planktonic algae as well as their culture and use of cultured algae are very important for the development of fisheries and fish production. Microalgae not only play an important role in aquaculture as feed source but together with bacteria, they also have an important role in the O_2 and CO_2 balance in water (Pruder, 1983). These contain all the essential amino acids (except systine and methionine) in sufficient amount to be utilized as human and animal food (Gordon, 1970). These live foods are considered to be the best food for fishes. Fish larvae grow better on living foods than non- living diets (De Pauw, 1981). Microalgae are a potential source of minerals in fish diets, which can replace a mixture of minerals if incorporated in small amounts (Fabregas and Herrero, 1986). The number of species of microalgae is estimated to be 22,000-26,000 out of which about 50 have been studied in detail with regard to their biochemistry and echo-physiology (Clesceri, 1989).Most of microalgae species are autotrophic. Microalgae are fed to late larval juvenile fish and crustaceans in hatcheries (Renaud *et al.,* 1991) and used for rearing larvae of freshwater prawn and larvae of some marine fish like sea basses (Funjimura and Okamoto, 1972). Algae act as an ideal waste remove in nature (Rejdalje *et al.,* 1989). Some researchers used algae to remove toxic and recalcitrant compounds from the aquatic bodies to make the environment free from hazardous materials (Rejdalje *et al*., 1989). Among microalgae, *Spirulina* was the most important blue-green algae due to presence of high protein (around 60%) & lipids (11%) grown in sago waste (Phang*et al*., 2000); high amount of poly unsaturated fatty acid (PUFA) (1.5-2.0%) of 5-6% total lipid of *Spirulina* (Lu and Takeuchi, 2004); γ-linolenic acid which was about 36% of total PUFA (Ayachi*et al*., 2004); rich in carotene, phycocyanin, vitamins (Bhattacharya and Shivaprakash, 2005), essential minerals and chelating agent (Maeda and Sakaguchi, 1990); contains high crude lipids (14%) when cultured in fermented Thai rice noodle factory waste water (Veteyasuporn, 2004). Today, popular lifestyle personalities endorse *Spirulina* as a secret, potent "superfood," and a "miracle from the sea."(Gerald and Cysewski, 1983). *Spirulina platensis* was used since ancient times as a source of food because of its high nutritional value (Dillon *et al.,* 1995). It was gaining more and more attention, not only as a food aspect but also for

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the development of potential pharmaceuticals (Quoc and Pascaud, 1996). This algae was being widely studied, not only for nutritional reasons but also for its reported medicinal properties (Kim *et al.,* 1998; Subhashini*et al.*, 2004), antimicrobial activities (Demule*et al.*, 1996; Ozdemir*et al.,* 2004) as well as to inhibit the replication of several viruses, such as Herpes simplex and HIV-1 (Ayehunie *et al.*, 1998; Hernandez-Corona *et al.*, 2002). The predominant species of phytoplankton of the lake is *Spirulina platensis*. *Spirulina* grew optimally in pH range of 9-11 and there was least chance of contamination of other microbes (Supramaniyan and JeejiBai, 1992). The algae *Spirulina* was eaten in Mexico under the names 'Tecuitlatl' (Farrar,1996). *Spirulina* had also been used as a complementary dietary ingredient of feed for fish, shrimp and poultry and increasingly as a protein and vitamin supplement to aqua feeds (Ciferri and Tinoni, 1985). The amino acid composition of *Spirulina* protein was ranked among the best plant in the world, more than that of soyabean (Tanseem, 1990). Vitamins and minerals (Venkataraman and Beckar, 1986). Gamma-linolenic acid contained in this algae were reported to stimulate prostaglandinsynthesis and induction of the regulation of blood pressure, cholesterol synthesis, inflammation and cell proliferation (Venkataraman, 1993 and Borowitzka, 2010). *Spirulina* capsule was prepared to be effective in lowering blood lipid level and in decreasing white blood corpuscles after radiotherapy and chemotherapy (Ruan, *et al.,* 1988; Ruan *et al*,. 1990) as well as lowering immunological function. In addition, many experiments were carried out with *Spirulina* as feed for bees, fish, poultry, ducks and shrimps showing good results (Nguyen, 1988). One of the main reasons for this fact was the marked decrease in productivity occurring upon scaling-up laboratory conditions to outdoors, even when environmental conditions were favorable (Vonshak and Richmond, 1985). Their beneficial potential was experimentally proved *in vitro* and *in vivo* to treat some pathologies and in the prevention of the hyper cholesterol level, certain inflammatory diseases, allergies, cancer, toxicity inferred by the certain medicine, the viral infections, the cardiovascular diseases, the diabetes and other pathologies (Costa *et al*., 2007 and Assimakopoulos, 2008). *Spirulina* growth was found in a wide range of habitats, like open and closed ponds (Soletto *et al.,* 2008), photo bioreactors (Volkmann *et al.,* 2007), sewage and wastewater (Mary *et al.,* 2010), desert, marine and seawater (Hiri *et al.*,2011). In Algeria, *Spirulina platensis* is founded in Guelta (point of mountain water) in Tamanras set that resembles to the Paracas strain (Doumandji *et al.,* 2009). In Sweden low calorie bread enriched with *Spirulina* issold, and in France a vegetable pate, made of *Spirulina* is sold as bread spread (Henrikson, 1994). In Biological Research Division, BCSIR, Dhaka, *Spirulina* was cultured at pilot plant scale for over 19 years in Bangladesh (Jahan *et al*., 1994). Other media were developed in the same Laboratory for domestic scale culture of *Spirulina* in Bangladesh (Khatun *et al.,*2006). Rotten apple is one of the blooming waste in our country. About 12-15% apple becomes rotten in the country which are increasing day by day; producing huge amount of apple waste in a year. Apple contains protein, lipid, carbohydrate, vitamin, mineral and phosphorus. This phosphorus might help to produce high phospho-lipids and ultimately increased the amount of total lipids (Lu and Takeuchi, 2004). These wastes are easily available nationwide all the time and can be collected from the market. Therefore, this inexpensive waste material may be used to produce *Spirulina platensis*. The present study was conducted to observe the culture and growth performance of *Spirulina platensis* in rotten apple medium to evaluate the growth performances of *Spirulina* in rotten apple medium; and to analyze the proximate composition of *Spirulina* that grew in rotten apple medium.

MATERIALS AND METHODS

Study Area

The experiment was conducted in Live Food Aquaculture Laboratory, Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh for a period of three months from March to May, 2018.

Culture of microalgae

Selection and collection of rotten apple

The rotten apple was selected as media for *Spirulina platensis* culture. The rotten apple was collected from Kamal Ranjit (KR) Market, Bangladesh Agricultural University, Mymensingh-2202.

Analysis of proximate composition of rotten apple

The proximate composition of any media means moisture, ash, protein, lipid, crude fiber, and carbohydrate. The media was liquid and the main chemical elements of DRA as moisture, ash, protein, lipid, crude fiber, carbohydrate and nitrogen free extract (NFE) were analyzed in triplicates following the standard methods (AOAC, 2016; Rahman *et al*., 2015; Bhuiyan *et al*., 2018; Yeasmin *et al*., 2018). All of these were analyzed by using equipment's in the laboratory of the Fish Nutrition, Faculty of Fisheries, Bangladesh Agricultural University; Mymensingh (Table1).

Moisture

The moisture content of samples are determined by the methods of AOAC (2016); Rahman *et al*. (2015); Bhuiyan *et al*. (2018); Yeasmin *et al*. (2018) (Table 1).

Crude protein

The crude protein content of samples are determined by the methods of AOAC (2016); Rahman *et al*. (2015); Bhuiyan *et al*. (2018); Yeasmin *et al*. (2018) (Table 1).

Crude lipid

The crude lipid content of samples are determined by the methods of AOAC (2016); Rahman *et al*. (2015); Bhuiyan *et al*. (2018); Yeasmin *et al*. (2018) (Table 1).

Ash

The ash content of samples are determined by the methods of AOAC (2016); Rahman *et al*. (2015); Bhuiyan *et al*. (2018); Yeasmin *et al*. (2018) (Table 1).

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Table 1: *Proximate composition of Rotten Apple Media (RAM).*

Name of the RAM component	Moisture basis (%)	Dry matter basis(%)
Moisture	87.31	12.68
Ash	0.603	4.75
Protein	0.41	3.29
Lipid	0.78	6.14
Crude fiber	0.77	6.06
Carbohydrate	10.11	79.73

Analysis of physico-chemical properties of digested rotten apple

Physico-chemical properties such as pH, total suspended solids, total dissolved solids, dissolved oxygen, total alkalinity, nitrate-N (NO_3-N) and phosphate-P (PO_4-P) of digested poultry waste were analyzed in the laboratories of Live Food Culture, Nutrition and Water Quality of the Faculty of Fisheries, BAU, Mymensingh.

pH

pH of digested samples of rotten apple was determined using pH meter (Model HI 98129, HANNA).

Total suspended solids (TSS) and total dissolved solids (TDS)

This was analyzed by using the following method methods and procedure (HP Module, 1999).

Alkalinity

This was analyzed by using the following method - (APHA, 1976) and using alkalinity test kit (Thermo Fisher Scientific Company, 2008).

Nitrate-N (Available N)

This was analyzed by using Nitrite Nitrogen test kit (LR Phosphate, Model HI 93713, HANNA) through standard methods. Colorimetric Method (American Public Health Association, 1998), and in EPA method 354.1 (EMSL-Ci, 2003) to determine nitrite ion in waters as well as Nitrite nitrogen the following method - (APHA, 1976).

Phosphate-P (Available P)

This was analyzed by using Molybdenum blue method and with the help of phosphorus meter (LR Nitrate, Model HI 93713, HANNA) (WTW, 2003).

Collection of Spirulina platensis

Microalgae, *Spirulina platensis* was collected from the imported stock of Dr. Md. Ahsan Bin Habib, Professor, Department of aquaculture, Bangladesh Agricultural University, Mymensingh-2202.

Maintenance of pure stock culture of Spirulina platensis

Pure stock culture of *Spirulina platensis* was maintained in the laboratory in Kosaric medium (KM) (Modified after Zarrouk's, 1996). Growth of *Spirulina platensis* were monitored at every alternative day and was checked under microscope to confirm it's purity following some keys given by Bold and Wynne (1978), Vymazal (1995) and Phang and Chu (1999).

Preparation of digested rotten apple medium (DRAM) and Kosaric medium (KM)

70 ml/L dry rotten apple was allowed to decompose in 5.0 L glass bottle for 18 days under aerobic condition (Plate 3) in the Live Food Culture Laboratory, Department of Aquaculture, BAU, Mymensingh. Then supernatant from bottle was diluted and made three concentrations at the rate of 2.5, 5.0 and 10% digested rotten apple. Then the supernatant of three different concentrations were taken in 1.0 L flask with three replications. Simultaneously, Kosaric medium (KM) was prepared for *S. platensis* culture as a control. Compositions of rotten apple medium (RAM) and Kosaric medium (KM) were prepaired for culture of *Spirulina platensis*. Different concentrations and composition of rotten apple medium and kosaric medium are shown in the table 2 and 3 respectively.

Table 2: *Concentration of digested rotten apple medium (dram) for spirulina platensis*

Rotten apple ingredients	Concentration/dilution of RAM (%)
Digested rotten apple medium (DRAM)	2.5
Digested rotten apple medium (DRAM)	5.0
Digested rotten apple medium (DRAM)	10

For the preparation of supernatant of rotten apple collected samples were digested firstly by aerating it into a 5 liter volumetric flask under 4 liter distilled water. The concentration of rotten apple of 70g/L was maintained during digestion. After 18 day, digestion of rotten apple was completed and its supernatant was taken from the flask by filtering it with plankton net. Then the digested rotten apple was diluted according to the above direction with three replications using distilled water. Then the medium was mixed well and sterilized at 115°C for 15 minutes by high pressure bumping water autoclave. After autoclaving the media were kept 3 days to be sure about any contamination free before culture of microalgae.

Table 3: *Composition of kosaric medium (modified after zarrouk, 1996).*

Sl. No.	Chemicals/compounds	Concentration in stock solution g/l
1	$NaHCO_3$	9.0
2	K_2HPO_4	0.250
3	$NaNO_3$	1.250
4	K_2SO_4	0.50
5	NaCl	0.50
6	$MgSO_4\,7H_2O$	0.10
7	$CaCl_2$	0.02
8	$FeSO_4\,2H_2O$	0.005
9	A_5 micronutrient solution[a]	0.5ml/L
	a) A_5 micronutrient solution	G/L
	i) H_3BO_4	2.86
	ii) $MnCl_2.4H_2O$	1.81
	iii) $ZnSO_4 7H_2O$	0.22
	iv) $CuSO_4.7H_2O$	0.08
	v) MoO_3	0.01
	vi) $CoCl_2.\,6H_2O$	0.01

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For the preparation of Kosaric medium, the above mentioned amount (Table 3) of ingredients from no. 1 to 8 was weighted by the help of electric balance and took in a 1.0 L conical flask. Then 0.5 ml micronutrient solution was pipetted in the flask and distilled water was added to make the volume 1.0 L. Mixing, autoclaving and cooling were carried out pursing the procedure used during the preparation of RAM.

Experimental design of Spirulina platensis culture
Experimental design is shown in Table 4.

Table 4: *Three different doses of supernatant of digested rotten apple (DRA) through dilution to culture Spirulina*.

Types of medium	Treatments	Replications	Amounts digested rotten apple (ml/L)	Duration of culture (days)
Supernatant of DRA	T_1	3 (101, 102 and 103)	2.5	14
	T_2	3(201, 202 and 203)	5.0	
	T_3	3 (301, 302 and 303)	10	
Kosaric Medium (KM)	T_4	3(KM-1, KM-2 and KM-3)	-	14

Culture of Spirulina platensis in supernatant of digested rotten apple medium DRAM and Kosaric medium KM
Four treatments, three from supernatant of DRAM for three different concentrations (2.5, 5.0 and 10%) and one KM as control each with three replications were used to grow microalgae, *S. platensis* in 1.0 L volumetric flask. *Spirulina* was inoculated into each culture flask to produce a culture containing 10% *Spirulina* suspension (Optical density at 620 nm = 0.20) (Habib, 1998). Twenty ml of *Spirulina* suspension needed for getting the required density. All the flasks were kept under fluorescent lights (TFC, FL-40 SD/38 day light, Taiwan) in light : dark (12h : 12h) conditions in Live Food Culture Laboratory. These culture flasks was continuously aerated using electric aquarium aerator (SB-348A). Eight sub-samplings (15ml vial) were carried out at every alternative day from each flask to record dry cell weight, chlorophyll<u>a</u> content of *Spirulina*, and properties of culture media. All the glassware used in the experiment were disinfected with dry heat at 70^{o}C overnight.'

Estimation of Spirulina platensis cell weight (dry weight)
The cell weight of *Spirulina* was determined by the method of Clesceri *et al*. (1989).

Estimation of Chlorophyll <u>a</u> of Spirulina
The chlorophyll <u>a</u> of *Spirulina* was determined by the method of Clesceri *et al*. (1989).

Total biomass of Spirulina
Total biomass was calculated using the following formula given by Vonshak and Richmond (1988):
Total biomass = Chlorophyll <u>a</u> x 67

Specific growth rate (SGR) on the basis of dry weight, chlorophyll <u>a</u> content and total biomass of Spirulina (Clesceri et al., 1989)
Specific growth rate (µ/day) of cultured Spirulina on the basis of dry weight

$$SGR\ (\mu/day) = In\ (X_1\text{-}X_2)/t_1\text{-}t_2$$

Where, X_1 = Dry weight of biomass concentration of the end of selected time interval;
X_2 = Dry weight biomass concentration at beginning of selected time interval; and
and t_1-t_2 =Elapsed time between selected time in the day.

Specific growth rate (µ/day) of cultured Spirulina on the basis of chlorophyll <u>a</u>
$SGR\ (\mu/day) = In\ (X_1\text{-}X_2)/t_1\text{-}t_2$
Where, X_1 = Chlorophyll <u>a</u> at the end of selected time interval;
X_2 = Chlorophyll <u>a</u> at the beginning of selected time interval;
and t_1-t_2= Elapsed time between selected time in the day.

Specific growth rate (µ/day) of cultured Spirulina on the basis of total biomass
$SGR\ (\mu/day) = In\ (X_1\text{-}X_2)/t_1\text{-}t_2$
Where, X_1 = Total biomass at the end of selected time interval;
X_2 = Total biomass at the beginning of selected time interval;
and t_1-t_2 = Elapsed time between selected time in the day.

Analysis of physico-chemical parameters of culture media
Physical parameters
The physical parameters (temperature and light intensity) of the culture media were recorded as follows:

Temperature
Water temperature (^{o}C) of the culture media was measured and recorded during the time of sampling day by a Celsius thermometer.

Light intensity
Light intensity (lux/m^2/s) was measured during sampling day by using a lux-meter [digital instrument, Lutron (LX-101)].

Chemical parameters
The chemical parameters such as pH, alkalinity, nitrate-N and phosphate-P of the culture media were recorded following the procedures given by Clesceri *et al*., (1989) in the laboratory.

pH
pH of the culture media was measured from each sub sample by an electric pH meter (Conning pH meter 445).

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Analysis of proximate composition *Spirulina*
The chemical parameters such as moisture, crude protein, crude lipid, ash, crude fiber and NFE of the culture media were recorded following the procedures given by (AOAC, 2016; Rahman *et al*., 2015; Bhuiyan *et al*., 2018; Yeasmin *et al*., 2018) in the laboratory.

Statistical Analysis
Data of cell weight, chlorophyll a total biomass, and specific growth rates in respect to dry cell weight, chlorophyll a total biomass and proximate composition of *Spirulina* in respect to four treatments were analyzed following one ways Analysis of Variance (ANOVA) and their significant differences using Turkey's test followed Duncan's New Multiple Range (DNMR) test at 5% level of probability (Zar, 1984).

RESULTS

Physico-chemical characteristics of rotten apple
Color, Odour and Structure
The color of the rotten apple was yellow-reddish with bad smell (odour). The structure was almost semi-solid (Table 5).

Temperature (°C)
Temperature of rotten apple was little bid higher than normal ambient temperature. It was ranged from 28.30 to 28.60ºC (Table 5).

Total solid (TSS + TDS)
Total solid is the addition of total suspended solids and total dissolved solids of liquid (rotten apple) which was ranged from 1954 to 2135 mg/L (Table 5).

pH
pH of rotten apple was ranged from 6.30 to 6.45 which was alkaline in nature (Table 5).

Alkalinity
Alkalinity of digested rotten apple was quite high and ranged from 132 to 142 mg/L (Table 5).

Nitrate-N (NO_3-N)
Nitrate-N (Available N) of digested rotten apple was ranged from 1.10 to 1.15 mg/L (Table 5).

Phosphate-P (PO_4-P)
Phosphate-P (Available P) of the digested rotten apple was high and varied from 2.90 to 3.30 mg/L (Table 5).

Physico-chemical properties of supernatant of digested rotten apple
Temperature
Temperature of supernatant of digested rotten apple (DRA) used to culture *Spirulina* was varied from 28.20 to 29.50°C (Table 6).

pH
pH of supernatant of DRA used for *Spirulina* culture was found to range from 6.80 to 6.90 (Table 6).

Total solid (TSS + TDS)
Total solid (TSS + TDS) of supernatant of DRA used to culture *Spirulina* was reduced due to decomposition which was ranged from 125 to 153 mg/L (Table 6).

Alkalinity
Alkalinity of supernatant of DRA used to culture *Spirulina* was high which was ranged from 140 to 160 mg/L (Table 6).

Nitrate N (NO_3-N)
Nitrate N (Available N) of supernatant of DRA used for *Spirulina* culture was also high in amount and varied from 1.05 to 1.10 mg/L (Table 6).

Phosphate P (PO_4-P)
Phosphate P (Available P) of supernatant of DRT used to culture *Spirulina* was very high and found to vary from 2.40 to 2.70 mg/L (Table 6).

Total N
Total N of supernatant of DRAM used for *Spirulina* culture was found also high in amount and ranged from 1.20 to 1.40 mg/L (Table 6).

Table 5: *Characteristics of rotten apple just after collection*

Characteristics of past of rotten apple	Comments
Colour	Reddish white
Odour	Little bid bad
Structure	Semi-solid
Temperature	28.30-28.60ºC
pH	6.30-6.45
Total solids (TSS + TDS)	1954-2135 mg/L
Alkalinity	132-142 mg/L
Total N	1.55-1.76 mg/L
Available N (NO_3-N)	1.10-1.15 mg/L
Available P (PO_3-P)	2.90-3.30 mg/L

Table 6: *Physico-chemical properties of supernatant of digested rotten apple after digestion in aerobic condition*

Characteristics	Comments
Temperature	28.20-29.50°C
pH	6.80-6.90
Total solid (TSS + TDS)	125-153 mg/L
Alkalinity	140-160 mg/L
Total N	1.20-1.40 mg/L
Available N (NO_3-N)	1.05-1.10 mg/L
Available P (PO_3-P)	2.40-2.70 mg/L

Proximate composition of rotten apple on moisture and dry basis
Moisture
It was measured from dry apple waste 4.61% (Table 7).

Crude protein
Crude protein of rotten apple was 3.30% (Table 7).

Crude lipid
Crude protein was very high in amount in digested rotten apple 6.15% (Table 7).

Ash
Ash of rotten apple was 4.75% (Table 7).

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Crude fiber
Crude fiber in rotten apple was not high in amount but 6.07% are present (Table 7).

Nitrogen free extracts (NFE)
The NFE of rotten apple was very high in amount 75.11% (Table 7).

Table 7: *Proximate composition (%) of rotten apple on moisture and dry weight basis*

Composition	Moisture basis (%)	Dry basis (%)
Moisture	87.31	12.68
Crude protein	0.418	3.30
Crude lipids	0.780	6.15
Ash	0.603	4.75
Crude fiber	0.770	6.07
NFE*	10.11	79.73

*NFE (Nitrogen Free Extract) = 100 - (Moisture + Crude protein + Crude lipids + Ash).

Physico-chemical properties of different media contained *Spirulina* culture

Light intensity
It was varied slightly in different days in all the three culture media. However, light intensity (lux/m^2/s) was varied from 2752 ± 25 on first day to 2775 ± 29 lux/m^2/s on the last day with slight variation in other days in T_1. It was varied from 2745 ± 28 on first day to 2765 ± 26 lux /m^2/s on the last day in T_2. Similarly, it was observed 2720± 32 on the first day and 2750 ± 33 on the last day (14^{th} day) in T_3. Light intensity was found to be 2725 ± 26 lux /m^2/s on first day in T_4 and 2660 ± 15 lux /m^2/s on the last day (14^{th} day) of experiment (Fig. 1).

Temperature
The temperature of T_1 was found 30.30 ± 0.26°C (lowest) on the first day to 30.60 ± 0.28°C at the end (14^{th} day) of experiment with slight up on 2nd, 10^{th} and 12^{th} day of experiment. It was also follow the similar trend of fluctuation from first to last day T_2 and T_3. But, it was recorded 30.50 ± 0.43°C on the first day of experiment to 30.20 ± 0.35°C at the end of experiment in T_4 (Fig. 2).

pH
During the 14 days experiment, it was increased from 8.20 ± 0.19 on first day to 9.70 ± 0.24 on 8^{th} day of experiment in T_1 and then it was decreased to 8.40 ± 0.22 on last day (14^{th} day). It was found 8.20 ± 0.20 on the first day which was increased to 9.30 ± 0.33 on 8^{th} day in T_2 and then decreased and increased on 12^{th} day and again increased on the last day (14^{th} day), similar trend of fluctuation also observed in T_3. But it was increased from first day (8.20 ± 0.33) of experiment up to 12^{th}day (9.50 ± 0.30) of experiment, and then decreased on the last day (14^{th}day) on T_4 (Fig 3).

Alkalinity
It was found highest (2255 ± 105 mg/L) on first day of experiment and then gradually decreased (1935 ± 172 mg/L) up to 14^{th} day (last day) in T_1. Total alkalinity was recorded 1815 ± 165 mg/L on first day of experiment and increased up to 10^{th} day (2040 ± 187 mg/L) with decreased value on 6^{th} day (1995 ± 152 mg/L), and then decreased on 12^{th} day and again increased on 14^{th} day in T_2. It was found almost around 2130 mg/L from first day to 4^{th} day of experiment, decreased on 6^{th} day (1985 ± 152 mg/L) and again increased on 8^{th} day (2355 ± 225 mg/L), and then decreased up to 14^{th} day in T_1 (Fig 4).

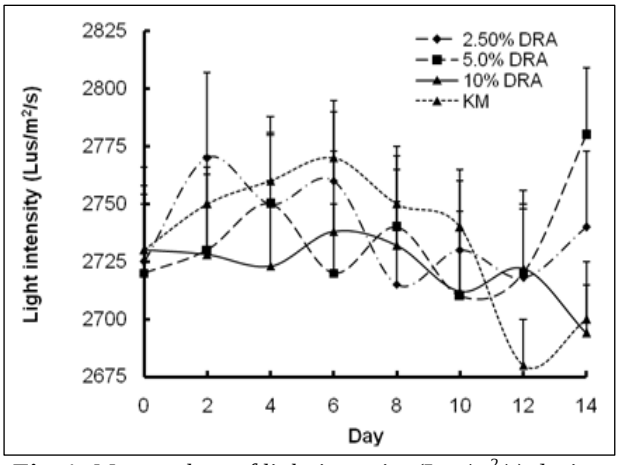

Fig. 1: *Mean values of light intensity (Lux/m^2/s) during culture of Spirulina platensis.*

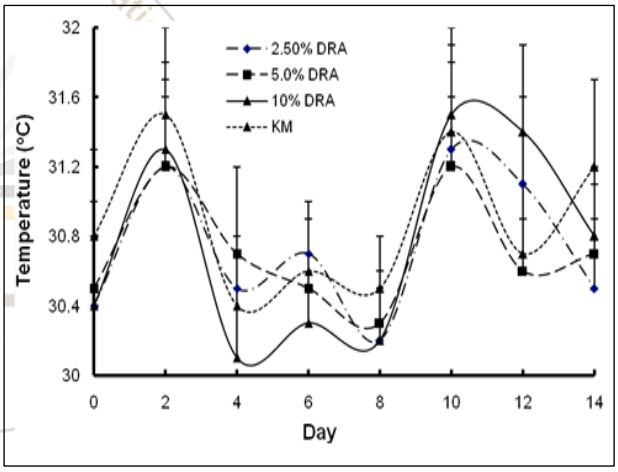

Fig. 2: *Mean values of temperature (°C) during culture of Spirulina platensis.*

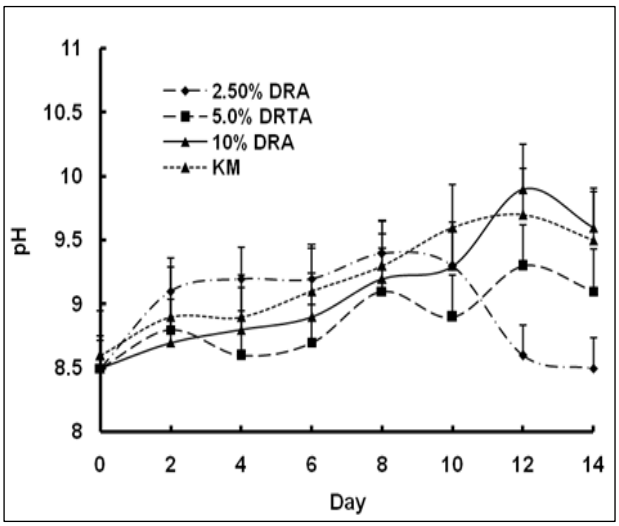

Fig. 3: *Mean values of pH of culture during Spirulina platensis.*

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Nitrate N (NO_3-N)

It was positively increased from 1.78 ± 0.16 mg/L (1st day) to 3.35 ± 0.30 mg/L (6th day) of experiment and then decreased up to 14th day in T_1. The trend of nitrate-N was found to decrease from first day (2.18 ± 0.17 mg/L) to 8th day (1.18 ± 0.15 mg/L) of culture and then increased up to 14th day in T_2. Lowest amount of nitrate-N (2.90± 0.27 mg/L) was recorded in media contained *Spirulina* and highest amount of nitrate-N (6.13 ± 0.50 mg/L) was found in T_3 on 14th day of culture. It was found lowest (2.04 ± 0.182.04 ± 0.18 g/L) in the culture of 5.0% (Fig 5).

Phosphate-P (PO_4-P)

Phosphate-P (Available P) was high in amount in the media in first day of experiment and gradually decreased in amount up to 8th day T_1, and again increased from 10th day up to 14th day of culture. But it was found to decrease from first day (46.34 ± 4.44 g/L) of experiment to 10th day (15.65 ± 1.46 g/L) but increased from 12th to 14th day of experiment in T_2. Similar trend was sharply followed T_3 and T_4 (Fig 6).

Growth parameters of *Spirulina*

Optical density of media contained Spirulina

Optical density (OD) of media contained *Spirulina* was found to increased up to 10th day of culture of all the media of digested rotten apple (DRAM) and Kosaric medium and then decreased up to 14th day of experiment (Fig 7).

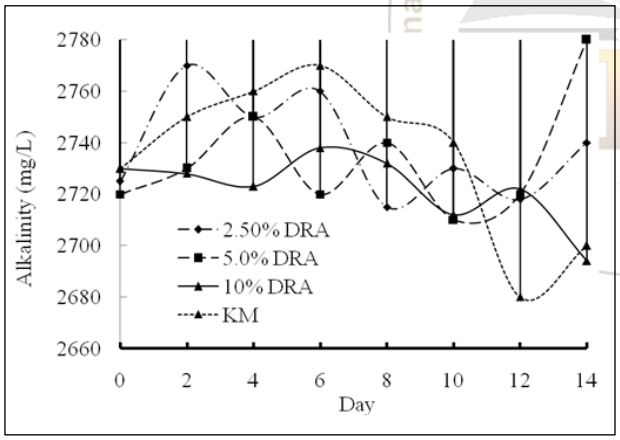

Fig. 4: *Mean values of alkalinity (mg/L) during culture of Spirulina platensis*

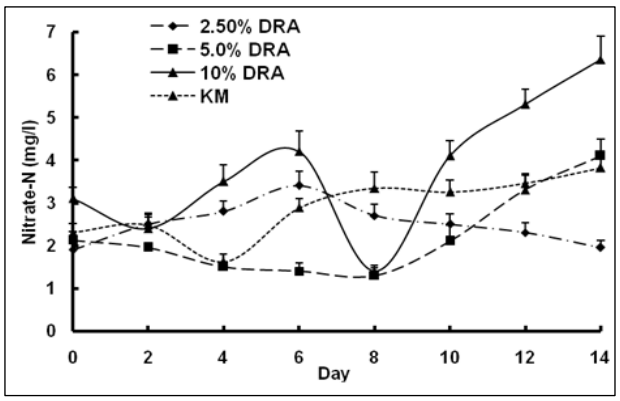

Fig. 5: *Mean values of Nitrate-N (mg/L) during culture of Spirulina platensis.*

Cell weight of Spirulina

Cell weight (mg/L) of *Spirulina* cultured in all the media was found higher on 10th day of culture than other days (Fig 8).

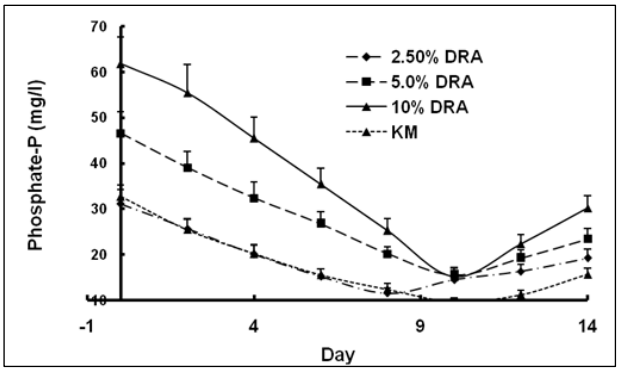

Fig 6: *Mean values of Phosphate-P (mg/L) during culture of Spirulina platensis.*

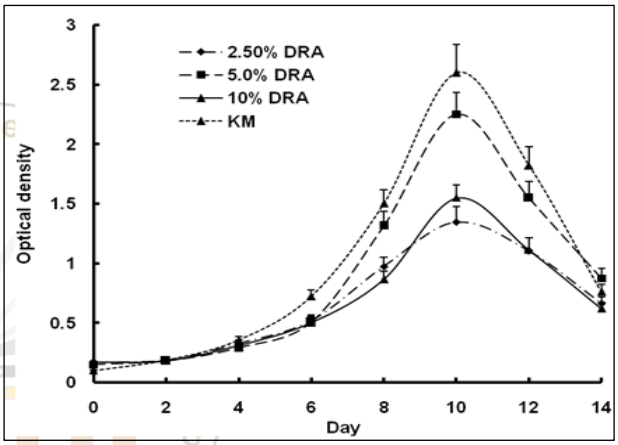

Fig. 7: *Mean values of optical density of media contained Spirulina platensis.*

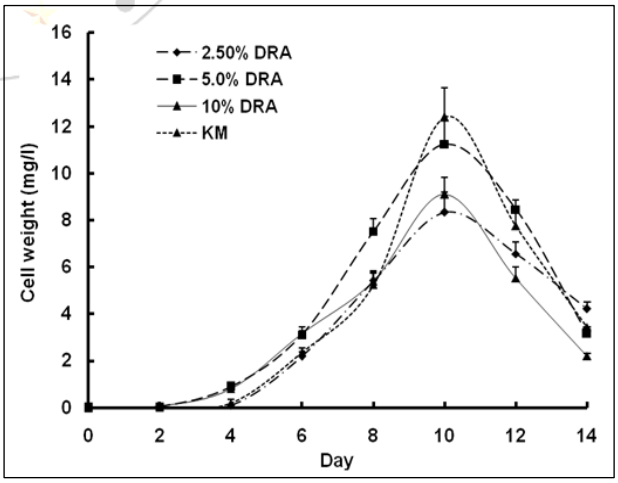

Fig. 8: *Mean values of cell weight (mg/L) of Spirulina platensis.*

Chlorophyll a of Spirulina

Chlorophyll a of *Spirulina* increased from first day up to 10th day (6.919 ± 0.14 g/L) of culture in T_1 and then decreased up to 14th day (3.127 ± 0.16g/L) of experiment. However, chlorophyll a in T_2 was 10.468 ± 0.32 g/L on 10th day and then decreased up to 14th day (3.44 ± 0.021g /L). Chlorophyll a of T_3 was 9.102 ±

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0.42g/L on 10th day and then decreased up to 14th day 2.20 ± 0.11, where the highest chlorophyll a of *Spirulina* cultured in T_4 was 12.44 ± 0.21g/L on 10th day and decreased up to 14th day 3.44 ± 0.021 of experiment (Fig 9).

Total biomass of Spirulina

Total biomass (mg/L) of *Spirulina* grown in all the media was found to be higher on 10th day of culture than other days of experiment.

Comparison of growth parameters of *Spirulina* at 10th day of culture

Optical density of media contained Spirulina

Optical density of T_2 and T_4 contained *Spirulina* (*S. platensis*) was significantly ($P < 0.01$) higher than that of two other media T_1 and T_3 (Table 8). There was no significant ($P > 0.05$) difference among optical density of T_1 and T_4, and among T_2 and T_3 during the study.

Cell weight of Spirulina

Highest cell weight (mg/L) of *Spirulina* grown in T_4 was recorded. Cell weight of *Spirulina* grown in T_4 and T_2 was varied significantly ($P < 0.01$) from that cultured in $T_1$and T_3 (Table 8). However, there was no significant ($P > 0.01$) difference of cell weight of *Spirulina* grown in T_1 and T_3.

Chlorophyll a of Spirulina

Chlorophyll a (mg/L) of *Spirulina* grown in T_4 and T_2 was significantly ($P < 0.01$) higher than that of *Spirulina* cultured in T_1 and T_3 (Table 8). There was no significant difference among the Chlorophyll a of *Spirulina* grown in T_4 and T_2 , and among the same of *Spirulina* cultured in T_1 and T_3.

Total biomass of Spirulina

Total biomass (mg/L) of *Spirulina* cultured in T_4 and T_2 was significantly ($P < 0.01$) higher than that of *Spirulina* grown in $T_1$and T_3 (Table 8). There was no significant difference found among the total biomass of *Spirulina* cultured in $T_1$and T_3.

Correlation among the growth parameters of *Spirulina*

Cell weight of *Spirulina* (*S. platensis*) had highly significant ($P < 0.01$) direct correlation with chlorophyll a ($r = 0.885$) of *Spirulina* grown in the supernatant of different digested rotten apple media and Kosaric medium during the study (Fig 11). Similarly, total biomass of *S. platensis* was highly ($P < 0.01$) and directly correlated with chlorophyll a ($r = 0.876$) of *Spirulina* cultured in the supernatant of various digested rotten apple and Kosaric medium (Fig 12). Again, total biomass of *Spirulina* was found to be highly ($P < 0.01$) and directly correlated with the cell weight ($r = 0.875$) of *Spirulina* grown in the supernatant of different digested rotten apple and Kosaric medium (Fig 13).

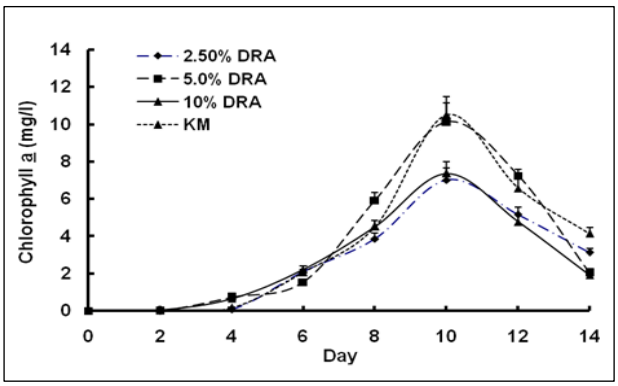

Fig. 9: *Mean values of chlorophyll a (mg/L) of Spirulina platensis.*

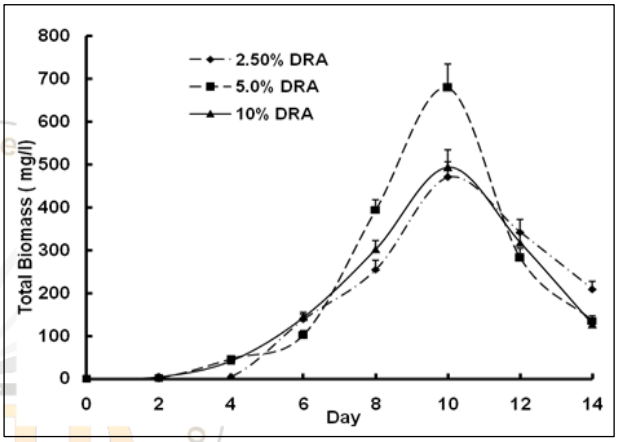

Fig. 10: *Mean values of total biomass (mg/L) of Spirulina platensis.*

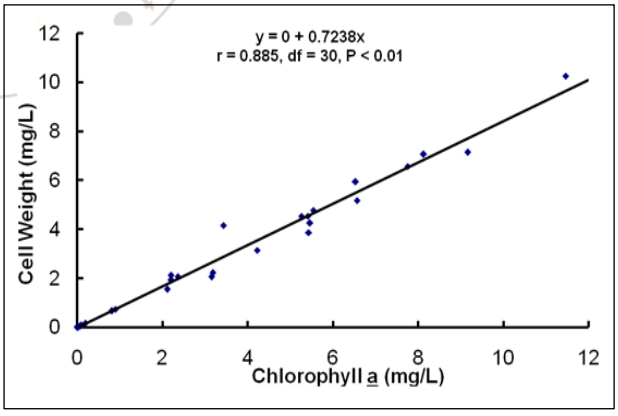

Fig. 11: *Correlation coefficient (r) of cell weight (mg/L) of Spirulina platensis With chlorophyll a (mg/L) of Spirulina grown in supernatant of three digested rotten apple, and Kosaric medium.*

Table 8: *Comparison of cell weight, chlorophyll a and total biomass of Spirulina platensis.*

Parameters	T1 (2.50% DRAM)	T2 (5.0% DRAM)	T3 (10% DRAM)	T4 (KM)
Optical density	1.331 ± 0.12^b	2.270 ± 0.15^c	1.568 ± 0.12^c	2.63 ± 0.20^a
Cell weight (mg/L)	7.679 ± 0.23^b	12.359 ± 0.52^c	9.102 ± 0.42^c	12.44 ± 0.21^a
Chlorophyll a (mg/L)	6.919 ± 0.14^b	10.468 ± 0.32^c	7.360 ± 0.20^c	10.54 ± 0.14^a
Total biomass (mg/L)*	463.57 ± 8.13^b	701.36 ± 9.28^c	493.12 ± 8.30^c	706.18 ± 9.50^a

*Total biomass = Chlorophyll a x 67 (Vonshak and Richmond, 1988). Figures in common letters do not differ significantly at 5% level of probability.

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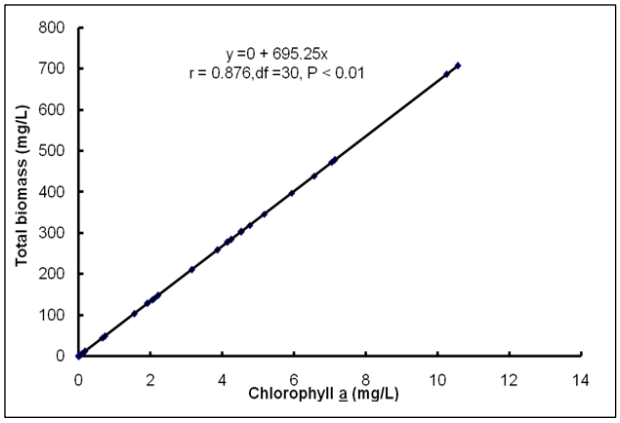

Fig. 12: *Correlation coefficient (r) of total biomass (mg/L) of Spirulina platensis with chlorophyll a (mg/L) of Spirulina grown in supernatant of three digested rotten apple, and Kosaric medium.*

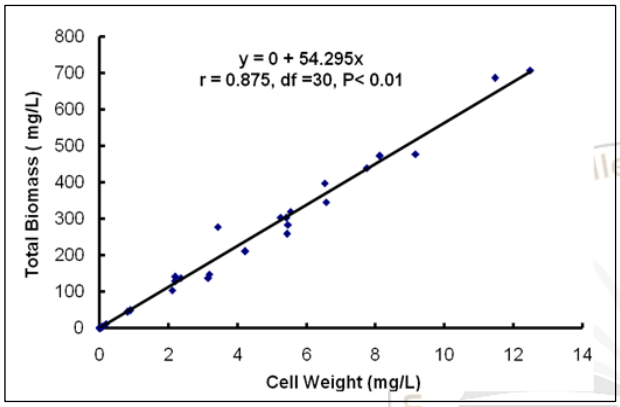

Fig. 13: *Correlation coefficient (r) of total biomass (mg/L) of Spirulina platensis with cell weight (mg/L) of Spirulina grown in supernatant of three digested rotten apple, and Kosaric medium.*

Specific growth rates (SGR) of *Spirulina*

SGR in respect to cell weight of Sprulina

Specific growth rate (SGR) in respect to cell weight of *Spirulina* grown in T_4 and T_2 was significantly ($P < 0.01$) higher than that of *Spirulina* cultured in T_1 and T_3 (Table 9). There was no significant ($P > 0.01$) difference among the SGR of cell weight of *Spirulina* grown in T_4 and T_2, and among the same of *Spirulina* cultured in $T_1$and T_3.

SGR in respect to Chlorophyll a of Spirulina

The SGR in respect to Chlorophyll a of *Spirulina* cultured in T_4 and T_2 was significantly ($P < 0.01$) varied from that of *Spirulina* grown in T_1 and T_3 (Table 9). It had no significant difference when *Spirulina* grown in T_4 and T_2, and similar thing happened when *Spirulina* cultured in T_1 and T_3.

SGR in respect to total biomass of Spirulina

The SGR in respect to total biomass of *Spirulina* cultured in T_4 and T_2 was significantly ($P < 0.01$) varied from that of *Spirulina* grown in T_1 and T_3 (Table 9). There was no significant ($P < 0.01$) difference recorded among the SGRs on the basis of total biomass of *S. platensis* grown in T_2 and T_4. Similarly, it had no significant variation among the SGR on the basis of total biomass of *Spirulina* when cultured in T_1 and T_3.

Table 9. Specific growth rates (SGRs) on the basis of cell weight, chlorophyll a and total biomass of *Spirulina platensis* grown in supernatant of three different concentrations of digested rotten apple (DRA) and Kosaric medium

Parameters	T_1 (2.50% DRA)	T_2 (5.0% DRA)	T_3 (10% DRA)	T_4 (KM)
SGR of cell weight	0.24 ± 0.021^b	0.30 ± 0.020^a	0.26 ± 0.014^b	0.31 ± 0.021^a
SGR of Chlorophyll a	0.24 ± 0.012^b	0.28 ± 0.014^a	0.25 ± 0.011^b	0.29 ± 0.014^a
SGR of total biomass	0.74 ± 0.022^b	0.82 ± 0.031^a	0.75 ± 0.018^b	0.81 ± 0.023^a

Figures in common letters in the same row do not differ significantly at 5% level of probability.

Proximate composition (%) of *Spirulina*

Moisture

Moisture of *Spirulina* grown in the supernatant of three different digested rotten apple and T_4 was varied from 8.20 to 8.21 % (Table 10).

Crude protein

Crude protein of *Spirulina* cultured in T_4 (58.52 ± 0.44%) was significantly ($P < 0.01$) higher than that of *Spirulina* grown in T_1, T_2 and T_3. The percentage of crude protein of *Spirulina* was 53.74 ± 0.38, 57.25 ± 0.42 and 54.25 ± 0.52 when grown in T_1, T_2 and T_3.

Crude lipid

Crude lipid (%) of *Spirulina* cultured in T_2 (14.62 ± 0.23%) varied significantly ($P < 0.01$) from that of *Spirulina* grown in T_1 (10.15 ± 0.28%) and 10% DRAM (11.02 ± 0.19%) followed by T_4 (6.31 ± 0.23%) (Table 10). There was no significant difference of crude lipid of *Spirulina* when cultured in $T_1$and T_3.

Ash

Ash (%) of *Spirulina* grown in T_3 (10.42 ± 0.24%) had significant ($P < 0.01$) difference from that of *Spirulina* cultured in T_2 (10.14 ± 0.19%) and 2.5% DRAM (9.20 ± 0.16%), and T_4 (13.52 ± 0.13%) (Table 10). There was no significant ($P > 0.01$) difference among the ash of *Spirulina* grown in T_1 and T_4.

Nitrogen free extract (NFE) of Spirulina

Nitrogen free extract (%) of *Spirulina* cultured in T_1 (17.99 ± 0.35%) and T_3 (15.35 ± 0.22%) varied significantly ($P < 0.01$) from that of *Spirulina* grown in T_4 (12.72 ± 0.28%) and then T_2 (19.19 ± 0.18%) (Table 10). There was no significant variation among the NFE of *Spirulina* grown in T_1 and T_3.

Crude fiber of Spirulina

Very small amount of crude fiber (%) was found in *Spirulina* grown in the supernatant of three different digested rotten apple media (DRAM), and Kosaric medium (Table 10).

Table 10. Proximate composition (% in dry matter basis) of *Spirulina platensis* cultured in supernatant of three

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different concentrations of digested rotten apple (DRA) and control as Kosaric medium

Treatments	T_1 (2.50% DRA)	T_2 (5.0% DRA)	T_3 (10.0% DRA)	T_4 (KM)
Moisture	8.20 ± 0.07	8.21 ± 0.07	8.21 ± 0.07	8.20 ± 0.07
Crude Protein	53.74 ± 0.38[b]	57.25 ± 0.42[a]	54.25 ± 0.52[b]	58.52 ± 0.44[a]
Crude Lipids	10.15 ± 0.28[b]	14.62 ± 0.23[a]	11.02 ± 0.19[b]	6.31 ± 0.23[c]
Ash	9.20 ± 0.16[b]	10.14 ± 0.19[b]	10.42 ± 0.24[b]	13.52 ± 0.13[a]
NFE*	17.99 ± 0.35[a]	19.19 ± 0.18[c]	15.35 ± 0.22[a]	12.72 ± 0.28[b]
Crude Fibre	0.71 ± 0.04	0.72 ± 0.03	0.74 ± 0.04	0.72 ± 0.03

*NFE (Nitrogen Free Extract) = 100 - (Moisture + Crude protein + Crude lipids + Ash). Figures in common letters in the same row do not differ significantly at 1% level of probability.

DISCUSSION

The most important algal species *Spirulina platensis* was cultured in supernatant of three concentrations of digested rotten apple such as 2.5, 5.0 and 10% and in Kosaric medium (KM) as control. The experiment was developed to evaluate culture and growth performance of *S. platensis* in the laboratory. The initial cell weight of *S. platensis* was 3.11 mg/L in all the treatments which finally attained maximum to 12.35 mg/L when culture in supernatant of digested 5% rotten apple (DRA), 7.69 mg/L in supernatant of digested 2.5% (DRA), 9.10 mg/L in supernatant of digested 10% rotten apple (DRA) and12.44 mg/L in Kosaric medium (KM). The growth of cell was found to vary in different media. This variation in the cell weight happened most probably due to compositions of varied media and differences in nutrient concentrations. The growth rate of *S. platensis* was found higher in Kosaric medium (KM) than other different concentrations of supernatant of digested rotten apple (DRA). Higher growth might be occurred due to presence of favorable nutrients which enhanced growth in KM than in supernatant of digested concentrations of various DRA. The higher cell weight and chlorophyll a content of *S. platensis* was observed in supernatant of digested 5.0% rotten apple than other concentrations of DRA (2.5% and 10%). It might be happened due to suitable nutrient quantity and nutrient composition for growth of cell in supernatant of digested 5.0% DRA than other concentrations of digested rotten apple during the culture. The concentrations of 2.5 and 10% DRA are not suitable and favorable for growth of *S. platensis*. During the culture period exponential phase was found up to 10th day from the beginning and then the cell weight declined i.e. stationary phase started. The physico-chemical properties such as light intensity, aeration, temperature and pH played a significant role to the whole culture system. During the culture system the climatic condition was more or less suitable and favorable for the growth of *S. platensis*. Similar type of work was carried out by Mario *et al*. (1986) where the annual yield of biomass of *Spirulina maxima* strain 4MX grown in fertilized sea water in out door system was 7.359 mg i.e. 0.39 g $L^{-1}d^{-1}$ which was higher than the present study. In the present study the cell weight of *S. platensis* in DROM and KM were lower than the findings of Mario *et al.* (1986). The variation in result probably happened because of different nutrient component of media used in culture, different culture technique and different species cultured. An experiment conducted by Becker (1984) on algal culture in a series of different horizontal ponds recorded that yield of *Spirulina* sp. was 8 to 12 g/m^2/d. The yield found from the experiment was also much higher than the present findings. Li and Qi (1997) reported that the biomass output rate in Chinese production plant was 7.0g/m^2/d which was much higher than the results of the present study. Similarly, Tanticharoen *et al.* (1990) reported that the addition of $NaHCO_3$ and nirogen fertilizer in waste water from the stabilization pond of topics starch factory raised the productivity up to 7-10 g $m^{-2}d^{-1}$ which was much higher than the findings of the present study. The variation in the above results might occur due to nutrient composition of different media and physico-chemical factors involved in the culture.

CONCLUSION

From the results obtained in the present study, it can be concluded that the growth of *Spirulina platensis* was better in the concentrations of 5.0% DRAM than other concentrations of RAM. Thus, the concentration of 5.0% DRAM is most suitable for *S. platensis* culture compare with standard Kosaric Medium (KM). These media are easily available and most inexpensive in contrast of Bangladesh. So digested rotten apple can be used for commercially and economically viable mass culture of *S. platensis*.

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