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ORIGINAL RESEARCH

Comparative study of growth, pigments and proximate composition of selected indigenous freshwater microalgae isolated from Bangladesh

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Abstract: Microalgae are considered as natural producers of protein, lipid, carotenoids, and fatty acids that are important not only in human and animal nutrition but also in medicines. Selection of the best performing locally isolated strains should be carried out to assess the nutritional properties of them as they are well adapted to local environment and exhibit better performance. Therefore, in the present study four independent primordial freshwater microalgae (*Selenastrum* sp., *Ankistrodesmus* sp., *Monoraphidium* sp., and *Scenedesmus* sp.) isolated from Bangladesh, were used to determine their growth, pigments and proximate composition. For growth determination, data were collected in terms of cell density and optical density, mass cultured in commercial Bold Basal Media and harvested at their stationary phases. Results showed that, onset of stationary phase (9-14 days) varied among the four species where *Selenastrum* sp. showed significantly highest cell density (5.66 ± 0.08cells/ml$\times10^7$). Among the four species *Monoraphidium* sp. showed significantly highest ($p< 0.05$) chlorophyll a (11.264 ± 0.065 μg/mL), b ($2.082\pm0.067\mu$g/mL) and carotenoid content ($8.05\pm0.07\mu$g/mL). Moreover, Total phycobiliproteins was highest ($p< 0.05$) in *Scenedesmus* sp. (6.105 ± 0.12mg/g). Protein (32.44 ± 2.17%dry weight) and lipids (25.28 ± 0.31 %dry weight) content were significantly ($p< 0.05$) highest in *Monoraphidium* sp. and Carbohydrates in *Scenedesmus* sp. (21.06 ± 0.19 % dry weight). Based on the obtained results, it was concluded that, *Monoraphidium* sp. can be utilized as potential pigments source and *Monoraphidium* sp. and *Selenastrum* sp. as feed source to utilize those in different commercial application.

Keywords: Stationary phase; microalgae; growth; pigments; proximate composition

1. Introduction

Microalgae are the photosynthetic organisms that found in different aquatic habitats, like freshwater, marine water even in wastewater (Khan et al., 2018). Microalgae are capable to produce a number of metabolites like proteins, lipids, carbohydrates, carotenoids, vitamins, phycobiliproteins, and asthaxanthin for health, food and feed additives, cosmetics and for energy production (Priyadarshani et al., 2012). Different microalgae can synthesize different amounts of protein and other valuable components (Ahmed et al., 2014). Moreover, blue-green and green algae, contain very high levels of protein, typically 40 to 60%, that can be used as functional food ingredients as algal protein have high nutritional value due to the presence of high quality amino acid and their nutritional acceptability (Reyes et al., 2012). Microalgae lipid content may vary from 20% to 50% of dry weight (Hu et al., 2015). High oil contribution of many microalgae

species has been exploited in biofuel production (Hussain et al., 2017). Pigments are considered as one of the most essential product from microalgae and chlorophylls, carotenoids, and phycobilins are the significant pigment group found in microalga (Koller et al., 2014). Chlorophyll a has been widely utilized as a coloring agent due to its stability (Plaza et al., 2009). Carotenoid, this is a natural, fat-soluble, yellow to red pigments, play an important role in photosynthesis in algae and photosynthetic bacteria (Lamers et al., 2012). However, the carotene production is influenced by several factors like salinity, light intensity, nutrient deprivation and temperature (Kleinegris et al., 2009). Phycobiliproteins used as natural dyes and extensively applied as nutraceuticals and in other biotechnological applications (Becker et al., 2007). Monitoring of cell growth is deliberated as a primitive portion for the usage of microalgae in aquaculture (Santos-Ballardoa et al., 2015). Although the measurement and monitoring of cell growth are an integral part for microalgal industry, its elaborate investigation has been highly ignored (Havlik et al., 2013). A deep knowledge of the behavior of a specific microalgal strain in response to various culture conditions, like nutrient supply, is important for the optimization of microalgal production (Hyka et al., 2013). Isolating and selecting of local microalgae species has a significant advantage to produce them in large scale in outdoor as they are highly adapted to the local climatic environment (Larkum et al., 2012). In Bangladesh a very few attention has been paid to the indigenous microalgae and its potentialities. In any country, to compel algal biotechnological applications, first investigation of native phycological flora and its potential is required to industrialize the sector.

Therefore, the aim of this study was to compare the growth rate, pigments content and proximate composition of four different freshwater microalgae (*Selenastrum* sp., *Ankistrodesmus* sp., *Monoraphidium* sp., and *Scenedesmus* sp.) which may contribute to select potential strains that possess fast growth, suitable pigments and high proximate profile for the sustainable use of those tropical species in aquaculture, pharmaceuticals and nutraceutical industry.

2. Materials and methods

2.1. Collection of freshwater isolates

Four different freshwater tropical microalgae pure stock were obtained from the previously isolated and preserved samples at Live Feed Research Corner, Department of Aquaculture, Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh.

2.2. Determination of growth curve

The microalgae were cultivated using the Bold Basal Media (BBM) (Stein et al., 1980). Cultures were grown at a temperature of $24 \pm 1^{\circ}$C in a 350 mL culture volume of a sterile 500 mL borosilicate Erlenmeyer flask for each species with three replicates where 2% pure culture stocks were added in each flask. Microalgae cultures were maintained at 24 hour light condition at 150 $\mu Em^{-2}s^{-1}$ intensity with continuous gentle aeration at a rate of 4.53 ± 0.53 mg/L. The experiment was continued until the death phase and finally completed the growth curve depending on cell density (cells.ml^{-1}) and optical density (absorbance).

2.3. Determination of cell density

Microalgae cell count was carried out every day by using a Neubauer hemacytometer (0.0025 mm^2, 0.1 mm deep chambers, Assistant, Germany) under the magnification of 40X. Cells were counted by using the formula of (Lavens et al., 1996).

2.4. Determination of maximum absorbance (optical density)

Optical density was measured every day for the growth curve analysis. The Bold Basal Medium without any microalgae cells were used as blank. Maximum absorbance value for each microalga was used to perform the growth curve by optical density (OD). Maximum absorbance were measured at the wavelength of 450 nm for *Selenastrum* sp., 428 nm for *Monoraphidium* sp., 363 nm for Ankistrodesmus sp. and 630 nm for *Scenedesmus* sp. as those wavelengths

gave maximum absorbance when the culture samples were scanned between 300 and 700 nm, using a NanoDrop spectrophotometer (NanoPlus, WAVE ANALYTICS D-82362 Weilheim, Germany).

2.5. Experimental Design for Pigment and Proximate composition determination

In large sterile 2L borosilicate Erlenmeyer flasks, having 1.7L pure BBM were used for this experiment. Each of the microalgae species was cultured to maintain similar environmental condition (Temperature: 24 $\pm$ 1°C; Light: 150 $\mu Em^{-2}s^{-1}$ intensity) until stationary phase. From the fresh cultured sample, carotenoid and chlorophyll were analyzed at the end of their exponential phase. As phycobiliprotein and proximate composition analysis required dry biomass, all the cultures were harvested at the end of their exponential phase by centrifugation (Hitachi* High-speed Refrigerated Centrifuge, himac CR 21g-II) depending on the growth curve experiment, and dried at 40°C temperature by using hot air oven and finally preserved at a refrigerator (4°C) for further use.

2.6. Specific growth rate:

Biomass determination is prerequisite for SGR analysis. Biomass were determined by filtering of 1ml microalgae sample from each replication of individual microalgae through a pre-weighted (after marking of filter paper rinsed with 10ml distill water and dried at 100°C for 4hour in hot air oven) glass microfiber filter paper, which was further rinsed with 10ml distill water for three times. Then the filter paper with biomass was oven dried at 100°C for 4hour. After that, final weight of filer was taken followed by 15min of desiccation and dry biomass was calculated according to (Ratha et al., 2016).

The specific growth rate (mg/day) of the cultured microalga was calculated according to the following formula (Clesceri et al., 1989):

$$\text{SGR}(\text{mg/day}) = \frac{\ln(X_1 - X_2)}{t_1 - t_2}$$

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

2.7. Determination of pigments

2.7.1. Determination of chlorophyll

For the determination of chlorophyll, microalgae were extracted according to the procedure of (Dixit et al., 2020). Chlorophyll concentration was determined according to Jenkins, (1982). Optical density at 664, 647, and 630 nm were taken for chlorophyll determination where OD at 750 nm was used as turbidity correction factor and subtracted from each of the pigments OD values before using them in the equations. Concentrations of chlorophyll a, and b in the extract were calculated by putting the optimized optical densities in the following equations (Jeffrey et al., 1975):

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

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

After the determination of the concentrations of pigments in the extract, the amount of pigments per unit volume was calculated as follows:

$$Chlorophyll\ a, mg/m^3 = \frac{\textbf{Ca} \times \textbf{extract volume,L}}{\textbf{volume of sample,m}^3}$$

2.7.2. Determination of carotenoids

Extraction of carotenoid from microalgae was done at the end of the exponential phase according to the procedure reported by (Khatoon et al., 2020). Finally, absorbance of the sepa-

rated hexane layer was determined using spectrophotometer at a wavelength of 450 nm and carotenoid was determined by multiplying the absorbance ($A_{450)}$ with 25.2 (Shaish et al., 1992).

2.7.3. Determination of phycobiliproteins

To determine the phycobiliproteins, extraction of microalgae was done by following the procedure reported by (Siegelman et al., 1978). Spectrophotometric absorbance was measured against the PBS buffer as blank at the wavelength of 562, 615, 652 and 720 nm where 720 nm measured the absorbance of the cellular debris. The amount of phycocyanin (PC), and allophycocyanin (APC) in the sample was calculated from the absorbance according to (Bennett et al., 1973) and phycoerythrin (PE) was calculated according to (Siegelman et al., 1978):

$$Phycocyanin\ (PC)\ (mg/ml)\ = \frac{A_{615} - (0.474 \times A_{652})}{5.34}$$

$$Allophycocyanin\ (APC)\ (mg/ml)\ = \frac{A_{652} - (0.208 \times A_{615})}{5.34}$$

$$Phycoerythrin\ (PE)\ (mg/ml) = \frac{A_{562} - (2.41 \times PC) - (0.849 \times APC)}{9.62}$$

Total phycocyanin, phycoerythrin, and allophycocyanin (mg/g) were calculated according to (Silveira et al., 2007):

$$P = \frac{(Pigment\ Concentration \times V)}{DB}$$

Where, V= solvent volume, DB= Dried biomass

From the sum of phycocyanin, phycoerythrin, and allophycocyanin contents, total phycobiliproteins (mg/g) were further calculated.

2.8. Determination of proximate composition

Protein and carbohydrate were analyzed according to the methods of Lowry et al. (1951) and Dubois et al. (1956), respectively, using 5–6 mg freeze-dried microalgal culture. Lipid was determined according to Bligh and Dyer (1959) and Folch et al. (1957) where 50 mg of each freeze dried sample was used.

2.9. Statistical analysis

Mean and standard error of mean were calculated using MS excel. When assumptions were met, all statistical analyses regarding the growth parameters, proximate composition, and pigments content was performed using the IBM SPSS (v. 26.0). Descriptive statistics were performed for different treatments; thereafter, a test for homogeneity of variance was done. All the collected data were analyzed using a one-way analysis of variance (ANOVA). Significant differences amongst treatments were analyzed using Tukey's multiple comparison tests at 95% confidence interval level. Post-hoc test was utilized to discern differences between groups.

3. Results

3.1. Growth phases of microalgae species

In the present study, different microalgae showed different cell concentration, pigment and proximate composition. Figure 1 illustrates the cell density and optical density versus the cultivation time of each of the four species (*Monoraphidium* sp.-A, *Selenastrum* sp.-B, *Ankistrodesmus* sp.-C, *Scenedesmus* sp.-D) during the cultivation in BBM. Observation results of growth showed that onset of stationary phase (9-14 days) varied among the four species. Based on the growth curve it is possible to determine the growth phases of those four microalgae where *Selenastrum*

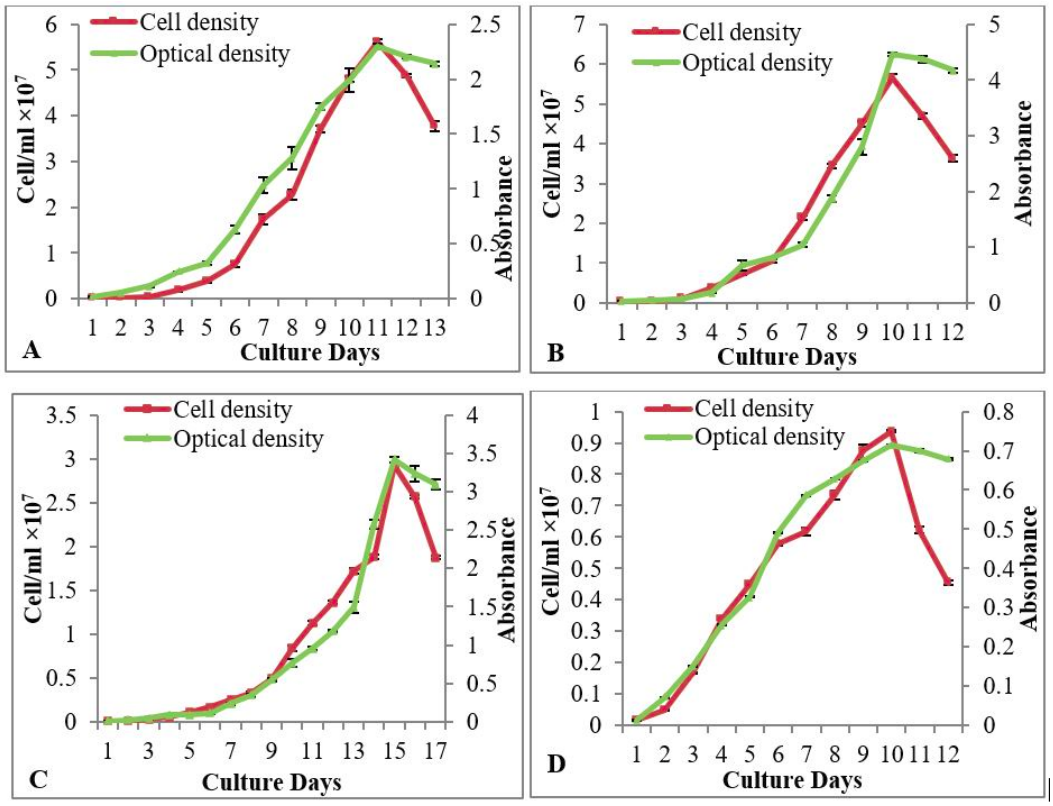

Figure 1. Growth curve in terms of cell density (cells/ml$\times10^7$) and optical density (Absorbance) of freshwater microalgae *Monoraphidium* sp. (A), *Selenastrum* sp. (B), *Ankistrodesmus* sp. (C), *Scenedesmus* sp. (D). Values are means $\pm$ standard error.

sp. showed 1 to 3 days of lag phase, 4 to 10 days of exponential phase, and 10 to 11 days of stationary phase (Figure 1.B). Along with this, *Monoraphidium* sp. resulted the lag phase, exponential phase, stationary phase and death phase on days 1 to 4, 4 to 11, 10 to 12 and from 12 days, respectively (Figure 1.A). Moreover, *Ankistrodesmus* sp. showed the lag phase on days 1 to 6, exponential phase on days 6 to 15, stationary phase on days 15 to 16 and death phase from 16 days (Figure 1.C). Furthermore, in *Scenedesmus* sp. from days 1 to 2, days 2 to 10 and days 9 to 10 was recorded as lag phase, exponential phase and stationary phase, respectively (Figure 1.D). Among the four microalgae, significantly highest and lowest cell density was detected from *Selenastrum* sp. (5.66$\pm$0.08 cells/ml$\times10^7$) and *Scenedesmus* sp. (0.94 $\pm$ 0.00408 cells/ml$\times10^7$).

A previous study done by (Lin et al., 2019) found slightly declined cells number in *Monoraphidium* sp. (HDMA-20) after 18 days in BG-11 medium, whereas in the present study highest cell density of *Monoraphidium* sp. was detected after day 11. The findings of the current study suggested that *Monoraphidium* sp. can flourish by using BBM. Similarly, *Selenastrum* sp. showed similar kind of growth phases reported by (Pugliese et al., 2020) in *Selenastrum capricornutum* cultured in $NaNO_3$, $MgCl_2$, $CaCl_2$, H_3BO_3, $MnCl_2$, $ZnCl_2$, CoCl, $CuCl_2$, Na_2MoO_4, $FeCl_3$, Na_2EDTA, $MgSO_4$, K_2HPO_4, $NaHCO_3$. Okomoda et al. (2021) earlier reported that *Ankistrodesmus falcatus* showed maximum cell density (39.00 $\pm$ 0.58 $\times10^6$ cells/ ml) at day 8 in BBM but in this study, *Ankistrodesmus* sp. showed highest (2.923$\pm$ 0.029 $\times10^7$ cells/ ml) cell density at day 14 in BBM. The observed differences can be justified as, microalgae growth characteristics vary from species to species and impacted by multiple factors, like reactor feature, culture condi-

tions (light, nutrients, temperature, pH, aeration) and the physiological need of the microalgae species (Guedes et al., 2012b). In case of *Scenedesmus* sp., current study is accompanied with the findings done by (Difusa et al., 2015) in BG-11 medium but showed lower cell densities. The variations in growth at different media are probably due to the apparent variations in the media's composition (Okomoda et al., 2021). Among the four species *Selenastrum* sp. cell density was the highest because smaller size species grow rapidly than the larger ones because of their large surface or volume ratio of smaller sized cells which simplify assimilation of nutrients at comparatively faster rate (Phatarpekar et al., 2000). Moreover, in this study, optical density showed a similar pattern like cell density as they are directly co-related and this direct relation of the cell number and optical density assure the appropriate management of the culture (Nur et al., 2008).

3.2. Specific growth rate (SGR) of microalgae species

Different microalgae showed a significant variation in SGR (Figure 2). The result showed that, SGR varied among the species where *Monoraphidium* sp., *Selenastrum* sp., *Ankistrodesmus* sp. and *Scenedesmus* sp. resulted about 0.678±0.002, 0.446±0.001, 0.497±0.001 and 0.846±0.001 mg/day of SGR respectively. Significantly highest and lowest ($p \leq 0.05$) SGR were recorded from *Scenedesmus* sp. and *Selenastrum* sp. as well as *Selenastrum* sp. and *Ankistrodesmus* sp. resulted almost similar SGR. In the present study, different microalgae species showed variation in growth rates, due to its algometric relationship between growth and cell size as well as metabolic process, also for use of different media or medium culture conditions (light, nutrients, temperature, pH, aeration) and the physiological need of the microalgae species (Guedes et al., 2012b).

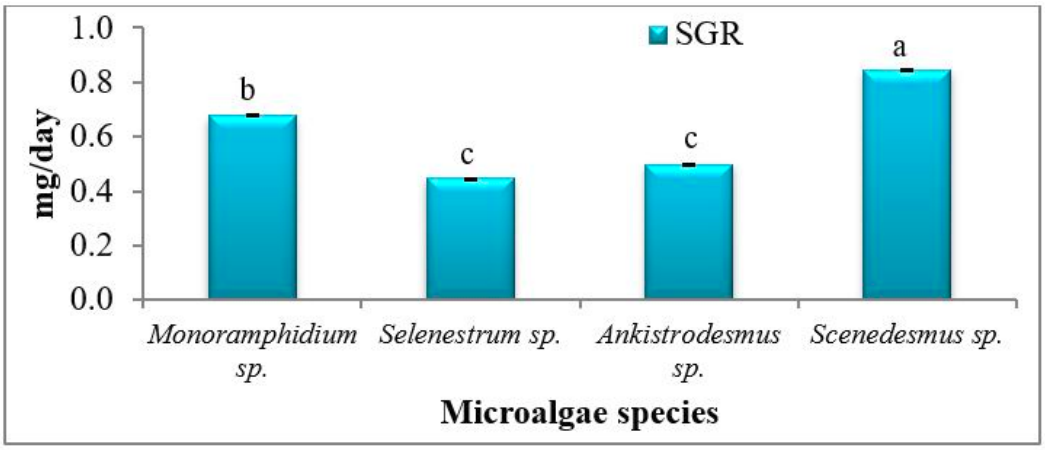

Figure 2. SGR (mean ± SE) of tropical freshwater microalgae, cultured in Bold Basal Medium. $^{a-c}$ Value under the same parameter represents the mean values with a significant difference between concentrations ($p \leq 0.05$).

3.3. Chlorophyll, carotenoid and phycobilipreoteins content in different freshwater microalgae

Chlorophyll, carotenoid and different phycoboliproteins content were investigated under this study where chlorophyll a, chlorophyll b, and carotenoid were recorded as μg/mL and phycobiliproteins content in mg/g. Different microalgae showed a considerable variation in chlorophyll, carotenoid (Figure 3) and phycobiliproteins (Table 1) content.

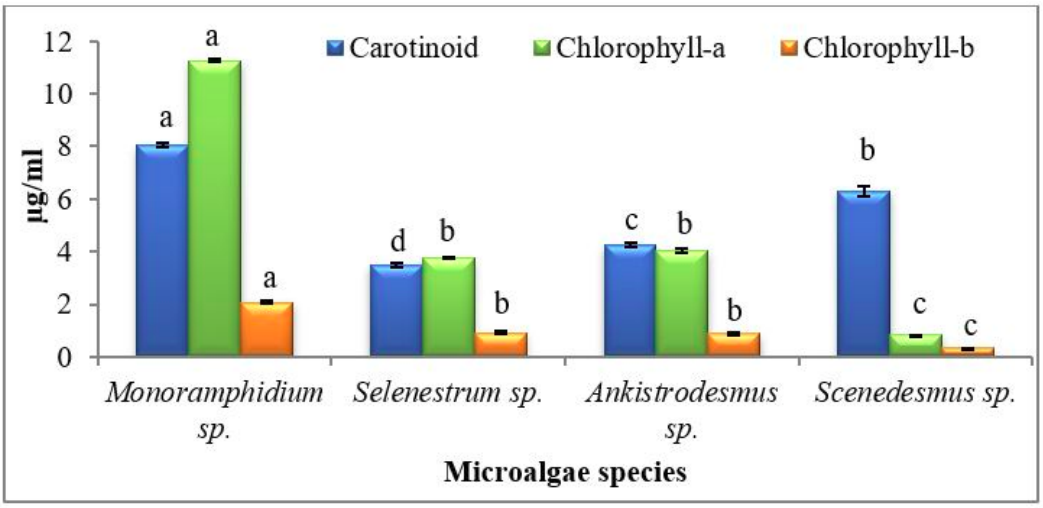

Figure 3. Chlorophyll a, b and carotenoid content of tropical freshwater microalgae, cultured in Bold Basal Medium. Values are means ± SE. $^{a-d}$ Value under the same parameter represents the mean values with a significant difference between concentrations ($p \leq 0.05$).

Significantly highest ($P < 0.05$) amount of chlorophyll a (11.264±0.065 μg/mL) and chlorophyll b (2.082±0.067 μg/mL) were recorded in *Monoraphidium* sp. on day-11. On the other hand, *Scenedesmus* sp. showed significantly minimum ($P < 0.05$) amount of chlorophyll a (0.834±0.004 μg/mL) and b (0.334±0.003 μg/mL) on day-10. Moreover, *Selenastrum* sp. (3.746±0.032 and 0.914±0.045 μg/mL) and *Ankistrodesmus* sp. (4.038±0.06 and 0.890±0.046 μg/mL) resulted about similar amount of chlorophyll a and b. In an earlier study done by (Ilavarasi et al., 2011) reported that *Monoraphidium* sp. showed 8.587 and 42.91 μg mL^{-1} of chlorophyll on 5 and 25th day respectively, in BBM. Similarly, (Oo et al., 2017) reported equivalent amount of chlorophyll a and b content from *Chlorella* sp. like *Selenastrum* sp. at 25±2°C temperature, 25 ppt salinity and 18:6 light/dark cycle photoperiod in Conway medium. Chlorophyll-a content of *Ankistrodesmus falcatus* varied under different light intensities and in different growth medium, such as 31 mg/g at 702 lux, 18 mg/g at 1307 lux and 15 mg/g at 1786 lux in BBM at 27 ± 2°C temperature (Ogbonna et al., 2021). However, finding of the current study reported lower chlorophyll-a content in *Ankistrodesmus falcatus* at 24°C temperature and 150 μE $m^{-2}s^{-1}$ intensity light condition in BBM. Although light is important for chlorophyll synthesis, very high light intensities inhibit chloroplast development (Wang et al., 2018), may be the reason for observed differences herein. Green microalgae *Scenedesmus obliquus* cultured in medium Zarrouk, medium Schlosser, and Haematococcus Provasolli Medium (PHM) showed that, chlorophyll a and b content were higher in the PHM than the others, at 25°C temperature with 2500 lux light intensity (Rinanti et al., 2013). But in the present study, *Scenedesmus* sp. showed much higher chlorophyll a and b content than the previous study reported by Rinanti et al. (2013). Chlorophyll content was increased with the increasing of light intensity (Ogbonna et al., 2021). From the present study, it can be concluded that, Monoramphidium sp. can widely be used as a great source of chlorophyll.

On the other hand, total carotenoid content was also varied among those microalgae (Fig. 3) where significantly ($P < 0.05$) highest and lowest quantity of carotenoid accumulations was detected from *Monoraphidium* sp. (8.05±0.07 μg/mL) and *Selenastrum* sp. (3.50±0.09 μg/mL). Moreover, *Ankistrodesmus* sp. and *Scenedesmus* sp. resulted about 4.25±0.08 μg/mL and 6.30±0.19 μg/mL of carotenoid, respectively. Sangapillai and Marimuthu (2019) detected almost similar amount of carotenoid content from *Asterarcys quadricellulare* (8.92 ± 0.031 mg/L) in mod-

ified BBM medium like as *Monoraphidium* sp. which concluded that, it can be considered as a good candidate for the production of chlorophyll and carotenoid. Marzorati et al. (2020) had earlier opined that *Spirulina* sp. contained about 3.5 $\pm$ 0.2 mg.g^{-1} of total carotenoid content. However, in the current study, Selenestrum sp. showed almost same amount of carotenoid content. Moreover, Ogbonna et al. (2021) also found that, *Ankistrodesmus falcatus* showed different carotenoid content in different light condition like 2.2 mg/g at 702 lux, 2.9 mg/g at 1307 lux and 7.8 mg/g at 1786 lux in BBM at 27 $\pm$ 2°C temperatures during their stationary phase on day 15. However, in the present study, carotenoid content differ from the previous study reported by (Ogbonna et al., 2021) because the time requirement of cultivating microalgae for carotenoid production differs from one microalga strain to another (Ogbonna et al., 2021). Along with this, current study reported much higher carotenoid content in *Scenedesmus* sp. than an earlier study reported by (Sanchez et al., 2008) who found about 4.9 mg/L carotenoid content in *Scenedesmus almeriensis* at 30°C. Which can be justified as, nutrient composition of culture media, temperature and light influenced the growth rate and biochemical composition in microalgae (Varshney et al., 2018). Present study also concludes that, *Monoramphidium* sp., Scenedesmus sp., and Ankistrodesmus sp. have high carotenoid content and huge potentiality to contribute in human and animal food industry.

Table 1. Phycobiliproteins (Phycocyanine, Allophycocyanin and Phycoerythrin) of tropical freshwater microalgae cultured in Bold Basal Medium. Values are means $\pm$ SE. $^{a-d}$ Value under the same parameter represents the mean values with a significant difference between concentrations($p \leq 0.05$).

Microalgal Species	Phycocyanine (mg/g)	Allophycocyanin (mg/g)	Phycoerythrin (mg/g)	Total Phycobiliproteins (mg/g)
Monoraphidium sp.	0.422±0.008c	1.339±0.013d	0.417±0.008c	2.178±0.024c
Selenastrum sp.	0.573±0.004b	1.432±0.008c	0.622±0.008b	2.627±0.010b
Ankistrodesmus sp.	0.222±0.008d	1.516±0.014b	0.391±0.007c	2.129±0.025c
Scenedesmus sp.	1.539±0.032a	2.754±0.017a	1.812±0.087a	6.105±0.12a

Phycobiliproteins analysis found that, phycocyanine, allophycocyanin, phycoerythrin and total phycobiliprotein content were significantly (P < 0.05) highest in *Scenedesmus* sp. at day 10 and lowest in *Ankistrodesmus* sp. (Table 1). In a previous study (Montero-Lobatoa et al., 2020) reported that, *Chroococcidiopsis* sp. produced about 204 mg g^{-1} of phycobiliproteins, under 10 μmol photons·m^{-2} ·s^{-1} light intensity, with a relative abundance of 40.9% for phycocyanin, 23.3% for phycoerythrin, and 35.8% for allophycocyanin in a light-dependent process. Along with this, (Arashiro et al., 2020) reported about maximum 199 mg/g DW, maximum 303 mg/g DW and 93 mg/g of total phycobiliproteins from *Nostoc* sp., *Arthrospira platensis* and *Porphyridium purpureum* in industrial wastewater. Along with this, (Silveira et al., 2007) concluded that, *Spirulina platensis* can produce about 0.0036 mg/L Phycocyanin at 25° C. But in the present study, top of that all the microalgae showed lower amount of phycobiliproteins as they belong to the Chlorophyceae class, and Chlorophyta (Green microalgae) reported to contain mostly chlorophyll a, b, b-carotene, prasinoxanthin, siphonaxanthin, astaxanthin Graham et al. (2000).

3.4. Proximate composition of different tropical microalgae species

In this study, different freshwater tropical microalgae species were cultured in BBM providing uniform parameters to determine the difference in their biochemical composition. Figure 4 shows the variation in protein, carbohydrate and lipid content for the four microalgae species. *Monoraphidium* sp. showed significantly (P < 0.05) higher amount of protein (32.44±2.17 %dry weight) and lipid content (25.28±0.31 %dry weight) as well as carbohydrate

content (21.06±0.19 %dry weight) was highest in *Scenedesmus*. On the other hand, *Selenastrum* sp., *Ankistrodesmus* sp. and *Scenedesmus* sp. resulted about 29.13±0.90, 24.26±0.78 and 21.77±2.17 %dry weight of protein, respectively. Along with this, *Selenastrum* sp. (17.47±0.14 %dry weight) and *Ankistrodesmus* sp. (16.40±0.58 %dry weight) showed almost equivalent amount of lipid whereas *Scenedesmus* sp. (13.86±0.37 %dry weight) resulted the lowest. *Selenastrum* sp., *Ankistrodesmus* sp. and *Monoraphidium* sp. resulted in 15.17±1.11, 17.71±0.97 and 20.78±0.83 % dry weight of carbohydrate, respectively.

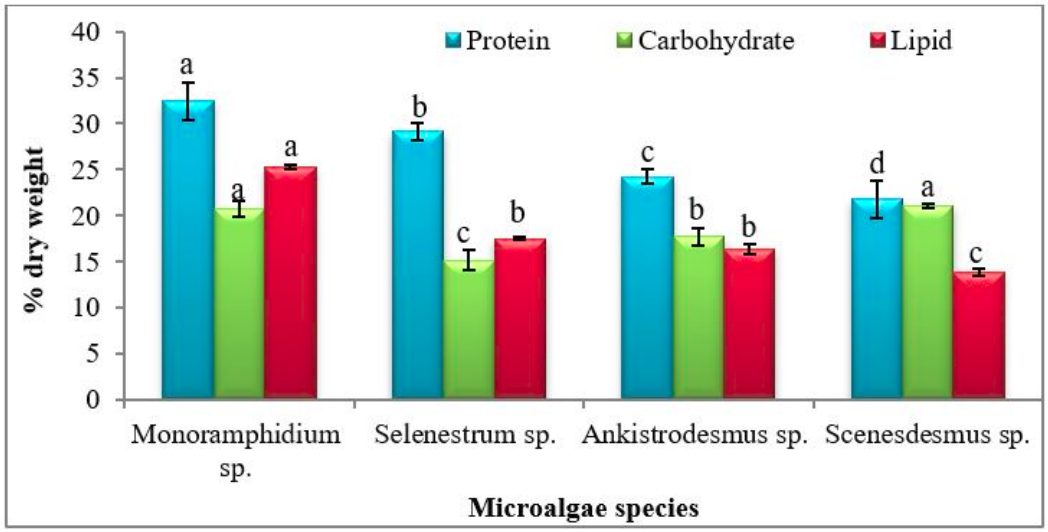

Figure 4. Proximate composition (%dry weight) (mean ± SE) of tropical freshwater microalgae cultured in BBM and isolated from freshwater sources. $^{a-c}$ Value under the same parameter represents the mean values with a significant difference between concentrations ($p \leq 0.05$).

The present study showed that the protein, lipid and carbohydrate content in *Monoraphidium* sp. was significantly higher in BBM and unanimous with the study done by (Reyes et al., 2012) opined that, *Monoraphidium* sp. contain nearly 19% - 35% lipid, 28% - 45% protein and 17% - 25% carbohydrates, in relation to the dry weight of biomass. In the present study, lipid content of *Monoraphidium* sp. is higher at stationary phase in BBM than BG11 reported by (Dhup et al., 2014). The increase in lipid content usually occurs due to different culture condition (Yoo et al., 2010); this may justify the differences detected herein for the variation in the concentration of nutrients in culture media at different culture days. Lipid content of *Selenastrum* sp. in the present study was almost same in photobioreactor reported by (Pugliese et al., 2020). Another study done by Guedes et al. (2012a) stated that, *Selenastrum* sp. cultured in 50% BG11 medium supplemented with phosphorus according to aquaculture wastewater quality resulted in 19.2% crude protein content while in the current result, protein content was significantly higher in BBM. This finding of the current study is however suggestive that protein content in *Selenastrum* sp. can ideally flourish with BBM. The findings of Okomoda et al. (2021) showed that, *Ankistrodesmus falcatus* cultured in BBM resulted in 46.41 ± 0.57% protein, 23.22 ± 0.56% lipid and 32.99 ± 0.26% carbohydrate content. However, the present study reported comparatively lower amount of protein, lipid and carbohydrate content. Along with this, Becker (2007) reported about 50-56% protein, 12-14% lipid and 10-17% carbohydrate from *Scenedesmus obliqus*. Another study done by Khatoon et al. (2017) reported that, *S. bibraianum* showed 44.7% protein when cultured in Bristol media while *S. obliquus* showed 30.7 ± 0.01% (BBM) and 31.8 ± 0.01% (Bristol) protein, together with 42.6 ± 0.01% and 38.2 ± 0.02% carbohydrate content was observed respectively in Bristol and BBM in *S. obliquus* (Khatoon et al., 2017). The present study found almost similar

lipid content that was observed by (Becker et al., 2007) in *Scenedesmus obliqus* but resulted lower amount of protein and carbohydrate content that was reported by Khatoon et al. (2017). The growth rate and biochemical composition of microalgae are highly influenced by environmental factors like light, salinity and nutrient availability (García et al., 2012) and differ from species to species (Khatoon et al., 2017).

4. Conclusion

Considering the results obtained from the study, it can be concluded that different microalgae showed considerable variation in growth, pigment production and proximate composition. Because of the higher SGR of *Scenedesmus* sp. and *Monoraphidium* sp., it can be easier to produce huge biomass from them. Along with this, elevated quantity of protein and lipid content of *Monoraphidium* sp., *Selenastrum* sp. and *Ankistrodesmus* sp. showed their importance in fish or animal feed industry and fuels production. Interestingly, *Monoraphidium* sp. showed higher chlorophyll and carotenoid contents and confirms its potentiality in pigment production. Further study will require on amino acid and fatty acid analysis of those microalgae, to boost up the feed industry and biodiesel production.

5. Data Availability

The data presented in this study are available on request from the corresponding authors.

6. Author's Contribution

Zannatul Nayma: Methodology; data curation; statistical analysis; original draft. Helena Khatoon: Conceptualization; project administration; and submission. Mohammad Redwanur Rahman: Review and editing. Fardous Ara Mukta and Razia Sultan: Formatting manuscript. Mohammad Nuruzzaman: financial support. All authors have read and agreed to the published version of the manuscript.

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Competing interests

The authors declare that they have no conflict of interest.

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References

Ahmed, et al. (2014). Profiling of carotenoids and antioxidant capacity of microalgae from subtropical coastal and brackish waters. *Food Chemistry*, *165*, 300-306.

Arashiro, et al. (2020). Natural pigments from microalgae grown in industrial wastewater. *Bioresource*

technology, 122894-122894.
Becker, et al. (2007). Micro-algae as a source of protein. *Biotechnology advances*, *25*, 207-210.
Bennett, et al. (1973). Complementary chromatic adaption in a filamentous blue-green alga. *Journal of Cell Biology*, *58*, 419-435.
Bligh, & Dyer. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911-917.
Clesceri, et al. (1989). American Water Works Association and Water Works Pollution Control Federation. *Standard Methods for the Examination of Water and Wastewater.* American Public Health Association.
Dhup, et al. (2014). Effect of nitrogen concentration on lipid productivity and fatty acid composition of Monoraphidium sp. *Bioresource technology*, *152*, 572-575.
Difusa, et al. (2015). Effect of light intensity and pH condition on the growth, biomass and lipid content of microalgae Scenedesmus species. *Biofuels*, *6*(1), 1-8.
Dixit, et al. (2020). Effect of nitrogen deficiency on the physiology and biochemical composition of microalga Scenedesmus rotundus-MG910488. *Journal of basic microbiology*, *60*(2), 158-172.
Dubois, et al. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350-356.
Folch, et al. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*, 497-509.
García, et al. (2012). Effect of salinity on growth and chemical composition of the diatom Thalassiosira weissflogii at three culture phases. *Latin american journal of aquatic research*, *40*, 435-440.
Graham, et al. (2000). Englewood Cliffs, NJ: Prentice-Hall.
Guedes, et al. (2012a). Nutritional value and uses of microalgae in aquaculture. *Aquaculture*.
Guedes, et al. (2012b). Nutritional value and uses of microalgae in aquaculture. *Aquaculture*.
Havlik, et al. (2013). On-line monitoring of large cultiva-tions of microalgae and cyanobacteria. *Trends Biotechnology*(7), 406-414.
Hu, et al. (2015). Antioxidant properties of microalgae protein hydrolysates prepared by neutral protease digestion. *Applied Mechanics and Materials*, *707*, 149-153.
Hussain, et al. (2017). Microalgae screening under CO2 stress: Growth and micro-nutrients removal efficiency. *Photochemistry and Photobiology B: Biology*, *170*, 91-98.
Hyka, et al. (2013). Flow cytometry for the development of biotechnological processes with microalgae. *Biotechnology Advances*, *31*, 2-16.
Ilavarasi, et al. (2011). Optimization of various growth media to freshwater microalgae for biomass production. *Biotechnology*, *10*, 540-545.
Jeffrey, et al. (1975). New spectrophotometric equations for determining chlorphylls a, b, and c, in higher plants, algae and natural phytoplankton. *Biochemie und Physiologie der Pflanzen*, *167*, 191-194.
Khan, et al. (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial cell factories*, *17*, 36-36.
Khatoon, et al. (2017). Growth and proximate composition of Scenedesmus obliquus and Selenastrum bibraianum cultured in different media and condition. *Proceedings of the National Academy of Sciences of the United States of America*, *89*, 251-257.
Khatoon, et al. (2020). Growth and carotenoid production of Dunaliella salina (Dunal) Teodoresco, 1905 cultured at different salinities. *Asian Fisheries Science,*, *33*, 207-212.
Kleinegris, et al. (2009). The selectivity of milking of Dunaliella salina. *Marine Biotechnology*, *2*, 14-23.
Koller, et al. (2014). Microalgae as versatile cellular factories for valued products. *Algal Research*, *6*, 52-63.
Lamers, et al. (2012). Carotenoid and fatty acid metabolism in nitrogen-starved Dunaliella salina, a unicellular green microalga. *Journal of biotechnology*, *162*, 21-27.
Larkum, et al. (2012). Selection, breeding and engineering of microalgae for bioenergy and biofuel production. *Trends in Biotechnology*, *30*(4), 198-205.
Lavens, et al. (1996). *Manual on the Production and Use of Live Food for Aquaculture*. Rome: Food and Agriculture Organization of the United Nations.
Lin, et al. (2019). Monoraphidium sp. HDMA-20 is a new potential source of α-linolenic acid and eicosatetraenoic acid. *Lipids in Health and Disease*, *18*(1), 56-56.
Lowry, et al. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265-275.
Ma, H. F., Zhuang, L. N., & Li, F. (1923). Comparison study on growth, removal of nitrogen and phosphorus, and nutritional property of two species of microalgae. *Applied Mechanics and Materials*, 209-211.
Marzorati, et al. (2020). Carotenoids, Chlorophylls and Phycocyanin from Spirulina. Supercritical CO2 and Water Extraction Methods for Added Value Products Cascade. *Green Chemistry*, *22*(1), 187-196.
Montero-Lobatoa, et al. (2020). Identification, biochemical composition and phycobiliproteins production of Chroococcidiopsis sp. from arid environment. *Process Biochemistry*, *97*, 112-120.
Nur, et al. (2008). Development of low cost medium for the culture of Chlorella ellipsoidea using poultry waste. *Journal of Agro-Environment Science*, *2*(1), 1-6.
Ogbonna, et al. (2021). Effects of light on cell growth, chlorophyll, and carotenoid contents of Chlorella sorokiniana and Ankistrodesmus falcatus in poultry dropping medium. *Journal of Applied Biology and Biotechnology*, *9*(2), 157-163.
Oo, et al. (2017). Extraction and determination of chlorophyll content from microalgae. *International journal of advanced research and publications*, *1*(5), 298-301.
Phatarpekar, et al. (2000). A comparative study on growth performance and biochemical composition of mixed culture of Isochrysis galbana and Chaetoceros calcitrans with monocultures. *Aquaculture*, *181*, 141-155.
Plaza, et al. (2009). Innovative natural functional ingredients from microalgae. *Journal of Agriculture and Food Chemistry*, *57*, 7159-7170.
Priyadarshani, et al. (2012). Commercial and industrial applications of micro algae - A review. *Journal of algal biomass utilization*, *3*(4), 89-100.
Pugliese, et al. (2020). Selenastrum Capricornutum a new strain of algae for biodiesel production. *Fermentation*, *6*(2), 46-46.
Ratha, et al. (2016). A rapid and reliable method for estimating microalgal biomass using a moisture analyser. *Journal of applied phycology*, *28*(3), 1725-1734.
Reyes, et al. (2012). Biodiesel Production by Hydroesterification of Microalgal Biomass Using Heterogeneous Catalyst. *Natural Science*, *4*, 778-783.
Rinanti, et al. (2013). Growth response and chlorophyll content of Scenedesmus obliquus cultivated in different artificial media. *Asian journal of environmental science*, *1*(1), 1-9.
Sanchez, et al. (2008). Influence of culture conditions on the productivity and lutein content of the new strain Scenedesmus almeriensis. *Process Biochemistry*, *43*, 398-405.
Sangapillai, & Marimuthu. (2019). Isolation and selection of growth medium for freshwater microalgae Asterarcys quadricellulare for maximum biomass production. *Water Science and Technology*, *80*(11), 2027-2036.
Santos-Ballardoa, et al. (2015). A simple

spectrophotometric method for biomass measurement of important microalgae species in aquaculture. *Aquaculture*, *448*, 87-92.
Shaish, et al. (1992). Biosynthesis of β-carotene in Dunaliella. Carotenoids Part A: chemistry, separation, quantitation, and antioxidation. *Methods in Enzymology*, *213*, 439-444.
Siegelman, et al. (1978). *Algal Bili-Proteins: Handbook of Psychological Method*. Cambridge: Cambridge University Press.
Silveira, et al. (2007). Optimization of phycocyanin extraction from Spirulina platensis using factorial design. *Bioresource Technology*, *98*, 1629-1634.
Stein, et al. (1980). (Vol. 448). Handbook of Phycological methods. Culture methods and growth measurements. Cambridge University Press.
Varshney, et al. (2018). Isolation and biochemical characterisation of two thermophilic green algal species- Asterarcys quadricellulare and Chlorella sorokiniana, which are tolerant to high levels of carbon dioxide and nitric oxide. *Algal Research*, *30*, 28-37.
Wang, et al. (2018). Influence of light intensity on chloroplast development and pigment accumulation in the wild-type and etiolated mutant plants of Anthurium andraeanum Sonate. *Plant Signaling and Behavior*, *13*(8), 1482174-1482174.
Yoo, et al. (2010). Selection of microalgae for lipid production under high levels carbon dioxide. *Bioresource technology*, *101*, 71-74.