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Evaluation of Banana Stem Ash Extract as a Low- Cost Culture Medium on the Growth and Pigmentation of *Spirulina platensis*

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ABSTRACT

Aquaculture diets and the health food sector can benefit from *Spirulina platensis* as a source of vital nutrients. The commonly used Kosaric medium (KM) for *S. platensis* culture is expensive and not widely accessible. Thus, the present study aimed to explore the use of banana stem ash extract (BSAE) as an alternative nutrient source and its impact on *S. platensis* growth and pigmentation. An 18-day experiment was conducted with five treatments, including control with KM (T1) and various BSAE concentrations with and without micronutrients (T2-T5). On the 15th day of the exponential phase, the control treatment (T1) exhibited significantly higher biomass concentration (1.29± 0.11g/ L) and specific growth rate (0.570µ/ day), along with higher optical density (0.87± 0.01) and chlorophyll a content (8.74± 0.06mg/ L). However, comparable results to the control were achieved in 0.5g/ L BSAE with 0.5ml micronutrient (T3) and 1.0g/ L BSAE with 0.5ml micronutrient (T5), in terms of biomass concentration, specific growth rate, and optical density. Chlorophyll a content (8.33± 0.02mg/ L) in 1.0g/ L BSAE with 0.5mL micronutrient was better than 0.5g/ L BSAE with 0.5ml micronutrient (7.75± 0.01mg/ L). Micronutrient supplementation was crucial in the BSAE medium. Both 0.5g/ L and 1.0g /L BSAE with 0.5ml micronutrient supplementation yielded comparable results to KM, suggesting their suitability as cost-effective alternatives for *S. platensis* culture without compromising growth performance and pigmentation.

INTRODUCTION

In aquaculture, microalgae play a crucial role in the aquatic species' food chain. They are essential in aquaculture as live food for the larval stages of several crab species, shrimp, and finfish (**Renaud *et al.*, 1991**). Many bioactive compounds, including carotenoids, lipids, fatty acids, hydrocarbons, proteins, carbohydrates, and amino acids, are abundant in microalgae **(Habib *et al.*, 2008)**. In recent years, carotenoids from algae have gained commercial recognition in the global market for food and cosmeceutical applications **(Ambati *et al.*, 2019)**. One of the most promising energy sources is

photosynthetic bacteria since they are CO_2 neutral and renewable. Species from the genus *Spirulina* are significant among the photosynthetic microorganisms of economic importance **(Goncalves *et al.*, 2016; Huesemann *et al.*, 2016; Salunke *et al.*, 2016)**.

Spirulina platensis is an extremely tiny cyanophyte (300– 500µm in length), filamentous, helicoidal, and widespread worldwide. It grows quite well in alkaline waters, where the pH is too high (9– 11) for most other species to live. It is now one of the most researched microalgae due to its high nutrient content and valuable bio compounds such as phycocyanin **(Moraes *et al.*, 2011)**. Due to its high nutritional value, *S. platensis* has economic significance **(Costa *et al.*, 2001; Rafiqul *et al.*, 2005)**. It has been demonstrated to be one of the richest source of proteins, polyunsaturated fatty acids, pigments, vitamins, and phenolic compounds **(Rangel-Yagui *et al.*, 2004; Colla *et al.*, 2007; Madhyastha & Vatsala, 2007; Sajilata *et al.*, 2008)**. Furthermore, it is being used in agriculture and the food sector. Additionally, *S. platensis* is used in pharmaceutics, fragrance, and medicine. Due to its high protein, polysaccharide, lipid, essential amino and fatty acid, dietary mineral, as well as vitamin content, it has numerous pharmacological activities, including antimicrobial (including antiviral and antibacterial), anticancer, metalloprotective (prevention of heavy-metal poisoning against Cd, Pb, Fe, and Hg), immunostimulant, and antioxidant effects (**Hoseini *et al.*, 2013**).

S. platensis has been consumed as food for a very long time. Mexicans used it customarily for more than a thousand years during the Aztec civilization **(Habib *et al.*, 2008)**. Due to its nutritional content, *S. platensis* has been used for a very long time in numerous regions of the world as a food supplement for humans and animals in various forms such as health drinks, tablets, powder, etc. **(Flores *et al.*, 2003)**. *S. platensis* is mostly used to extract phycocyanin, a blue photosynthetic pigment. There has been much research on the use of *S. platensis* as an additive in aquaculture feed, and it can be used as a partial replacement of fish meal to support fish growth, immunity, and viability **(Habib *et al.*, 2008)**. The absence of cellulose in the cell walls of *S. platensis*, which are composed of soft mucopolysaccharides, allows the algae to be easily digested and assimilated by fish **(Sharoba, 2014)**.

The most popular medium for *S. platensis* cultivation is the modified Zarrouk medium, often known as Kosaric medium (KM) **(Madkour *et al.*, 2012)**. However, this medium is costly due the high cost of the chemicals used in it. In order to produce *S. platensis* in large quantities, it is necessary to find a reliable, efficient, and affordable alternative medium. The mass production of *S. platensis* can be made cost- effective by reducing the input cost with cheap and readily available materials without sacrificing production efficiency. Several investigations have shown that decomposed organic and inorganic nutrient media are effective for *S. platensis* culture **(Toyub *et al.*, 2011; Jain & Singh, 2013; Habib *et al.*, 2019; Khatun *et al.*, 2019)**.

The banana plant (*Musa sapientum*) contains high amounts of base metals, and its ash solution is highly alkaline. More than 1000 varieties of bananas are produced and

consumed locally worldwide, primarily in Asia, Latin America, and Africa **(FAO, 2023)**. Therefore, it can be a rational choice for the medium of *S. platensis* culture. Banana stem ash extract (BSAE) also contains high levels of potassium, sodium and calcium **(Anhwange *et al.*, 2009)**. Moreover, it can be used as a cheap source of nutrients, increasing pH in the culture medium. Furthermore, BSAE can serve as a significant source of micronutrients for *S. platensis* culture. Therefore, the present study aimed to assess the effect of BSAE as a nutrient source on the growth and pigmentation of *S. platensis* and to formulate a low-cost medium using BSAE for *S. platensis* culture.

MATERIALS AND METHODS

Preparation of banana stem ash extract

Banana stems were selected as a potential cheap source of nutrients for the culture of *S. platensis*. The banana stems were collected from the banana farm of the Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. After collecting, the banana stems were initially sun- dried and then burned in a muffle furnace (Carbolite RHF 17/6S, Carbolite Ltd., England) at 550°C for 4 hours to collect ash. Afterward, 0.5g and 1.0g of dried banana stem ash was mixed with 7 litrers of distilled water, respectively, and left for 4 days. After 4 days, the solution was filtered twice using a fine mesh cloth and a 0.45 micrometre- sized filter paper, respectively, to get a clear solution. Subsequently, the extract was well mixed and sterilized. In this way, two concentrations of BSAE banana stem extract were obtained for the experiment.

Collection of *S. platensis*

The Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh provided the pure stock of the *S. platensis* sample. Following collection, the stock was maintained using KM in the Live Food Culture Laboratory, Department of Aquaculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University.

Preparation of KM

KM is widely used as a standard medium for *S. platensis* culture. The KM was prepared by following the chemical composition presented in Table (1) (**Madkour *et al.*, 2012)**. Chemicals from no. 1 to 8 were weighed with an electric balance and taken in a 1.0L conical flask. Subsequently, 0.5mL of micronutrient solution was pipetted into the flask and distilled water was added to make the volume 1.0L. Afterward, the medium was well mixed and sterilized at 121^{o}C for 15 minutes with moist heat by an autoclave and cooled for 24 hours.

Table 1. Composition of KM for *S. platensis* culture

Serial no.	Chemicals/ compounds	Concentration in the stock solution
1	$NaHCO_3$	9.00g/ L
2	K_2HPO_4	0.25g/ L
3	$NaNO_3$	1.25g/ L
4	K_2SO_4	0.50g /L
5	NaCl	0.50g/ L
6	$MgSO_4.7H_2O$	0.10g/ L
7	$CaCl_2$	0.02g/ L
8	$FeSO_4.2H_2O$	0.005g/ L
9	Micronutrients solution[1]	0.5ml/ L

[1] Composition of micronutrient solution (g/ L) showing: i) H_3BO_3–2.86, ii) $MnCl_2.4H_2O$– 1.81, iii) $ZnSO_4.7H_2O$– 0.22, iv) $CuSO_4.5H_2O$– 0.08, v) MoO_3– 0.01, vi) $CoCl_2.6H_2O$– 0.01.

Experimental culture of *S. platensis*

The experiment was conducted for 18 days to evaluate the growth performance of *S. platensis*. KM was used in this study as the control medium (T_1). Other treatments were prepared as 0.5g/ L BSAE without micronutrient (T_2); 0.5g/ L BSAE with micronutrient (T_3); 1.0g/ L BSAE without micronutrient (T_4), and 1.0g/ L BSAE with micronutrient (T_5). The experimental culture of *S. platensis* was conducted in 15 conical flasks (1.0L size) under 5 treatments each with 3 replications. *S. platensis* was inoculated into each flask to produce a culture containing 10% suspension (OD at 620nm= 0.20) (**Khatun *et al.*, 2019**). The flasks were kept under fluorescent light (PHILIPS-CHAMPION, 36W, 1200mm, F- 50Hz, Bangladesh) with a light: dark cycle of 12 hours light and 12 hours dark in the Wet Laboratory of the Department of Aquaculture. The culture flasks were continuously aerated using electric aerators (Sobo pump, Aquarium pump SB-348A). Samplings were performed every three days for each flask to observe the culture media's biomass production, optical density (OD), chlorophyll *a* and physico-chemical properties.

Estimation of *S. platensis* biomass production (dry basis)

The sample containing 50ml *S. platensis* suspension was filtered through a filter paper (Whatman GF/ C filter paper of 0.45µm mesh size and 47mm diameter) which was then dried in an oven for 24h at 70°C and weighed before the filtration. Subsequently, the filter papers were put in a glass petri dish and kept in the oven at 70ºC overnight. For cooling, petri dish was put into a desiccator for 20 minutes, and then filter papers were weighed. The biomass concentration was determined by using the formula specified by **Eaton *et al*. (2005)** as follows:

$$W = \frac{FFW - IFW}{\text{Amount of sample taken for filtration (ml)}} * 100$$

W= Biomass concentration in g/ L; FFW= Final filter weight in g, and IFW= Initial filter weight in g.

Estimation of chlorophyll *a*

Every three days, *S. platensis* samples were taken in order to calculate the chlorophyll *a* content. Each *S. platensis* culture flask was pipetted with a 10ml sample and filtered through Whatman GF/ C filter papers, with a 0.45µm mesh size and a diameter of 47mm, and filtration was perfomed using an electric filtration device. The filtered samples were placed in a test tube along with filter paper, which was then crushed with a glass rod before being combined with 10ml of 100% redistilled acetone. To prevent light contact, foil papers were used to wrap each test tube. After wrapping, the test tubes were chilled overnight. The chilled samples were then homogenized for 2 minutes, followed by 10 minutes of centrifugation at 4000rpm. Chlorophyll *a* content was calculated using the formula **s**pecified by **Eaton *et al.* (2005)** as follows:

Chlorophyll *a* (mg/ L) = 11.85 (OD 664) – 1.54 (OD 647) – 0.08 (OD 630)

Measurement of optical density

Using a UV spectrophotometer (DR 6000), the optical density of samples collected at various sampling dates was determined at 620nm. To conduct this analysis, a cuvette containing a sample of *S. platensis* cultivated under various conditions was inserted in a spectrophotometer. The samples' OD was then recorded.

Specific growth rate

The specific growth rate (SGR, µ/ day) of cultured microalgae was calculated using the equation **s**pecified by **Eaton *et al.* (2005)** as follows:

$$SGR\ (\mu/\ day) = \ln (X_1 - X_2)/\ t_2 - t_1$$

Where X_1 = Biomass concentration at the end of selected time interval; X_2 = Biomass concentration at the beginning of selected time interval; and $t_2 - t_1$ = Elapsed time between selected time in day.

Determination of physico-chemical properties of the culture media

The physico- chemical parameters of the culture media were measured at three days intervals up to the experiment completion following the procedures given by **Eaton *et al*. (2005)**. Water temperature (ºC), light intensity (lux/ m^2/s), dissolved oxygen (mg/ L) and pH of the culture media were measured on the sampling day by maximum-minimum thermometer (Zeal, UK), lux-meter (LX-9621, China), dissolved oxygen meter (Hach hq40d multi-analyzer, USA), and electric pH meter (SensIONTM+ PH3), respectively.

Statistical analysis

All the data were collected, recorded, and preserved on a computer spreadsheet during the experimental period. One- way analysis of variance (ANOVA) of means of all physico-chemical parameters of culture media and biomass concentration, chlorophyll *a* content, and optical density of *S. platensis* was done by using Statistix 10 software to find out whether there was any significant difference among treatment means, while LSD test was used to compare the treatment means ($P< 0.05$).

RESULTS AND DISCUSSION

Physico- chemical parameters

The values (mean ± SD) of the physico-chemical parameters in different treatments during the experimental period are presented in Table (2). No significant difference ($P> 0.05$) was observed in the physico- chemical parameters of different treatments on a sampling day.

In the present work, the temperature ranged from 31.45 ±0.05ºC to 31.57 ±0.06ºC, which is similar to the findings of **Torzillo and Vonshak (1994)**, who suggested that the optimum temperature for *S. platensis* culture is 28– 35ºC. **Fagiri *et al.* (2013)** reported that the biochemical constituents of *S. platensis* reached their maximum levels at 35^{o}C, and the optimal growth was recorded at 25 to 35°C. The range of light intensity was 2200 to 2700lux/ m^2/ s in this experiment. **Sharker *et al.* (2007)** found that the best growth of *S. platensis* is at 2110 and 2120lux/ m^2/ s, using papaya skin powder medium and KM, respectively. **Kebede and Ahlgren (1996)** found that *S. platensis* displayed a maximum specific growth rate of 1.78mg/ day when it was cultured on modified Zarrouk's medium and subjected to a range of light intensities (2000- 2500lux/ m^2/ s). The highest growth of *spirulina* was found at 2710lux/ m^2/ s and 2740lux/ m^2/ s in a 5g/ L concentration of the media and Kosaric medium (KM), respectively (**Habib *et al.*, 2019**).

The pH strongly affects the biomass production, chemical dissociation and cell physiology **(Ogbonda *et al.*, 2007a; Celekli *et al.*, 2009)**, which was found to vary from 9.00 ±0.00 to 10.47 ±0.12 in different treatments during the experimental period that is favorable for the growth of *S. platensis* **(Begum *et al.*, 1998; Toyub *et al.*, 2006)**. The initial pH (9.0) was the same in all the treatments. However, pH increased with the progress of the experiment in all treatments up to 15 days, then decreased. *S. platensis* grew effectively in the medium, leading to a progressive pH increase from 9.6 to 10.8 **(Binaghi *et al.*, 2003)**. The rise in the pH values also increased cell concentration, which was possibly due to carbonate consumption by *S. platensis* (**Pelizer *et al.*, 2003)**. **Rangel-Yagui *et al.* (2004)** reported an increase in pH with cellular growth as *S. platensis* incorporates nitrate and urea, which also led to increase the pH of the medium. They found that the bicarbonate ions in the medium were assimilated by the *S. platensis* and subsequently converted into carbon dioxide and carbonate, which can correlate with

carbon- source consumption. Concequently, the carbon dioxide was utilized in photosynthesis. The carbonate was excreted into the medium, and therefore, the pH of the system was increased due to the shift of the bicarbonate- carbonate equilibrium towards the carbonate (**Rangel-Yagui *et al.*, 2004)**. During the culture period, dissolved oxygen levels ranged from 4.21±0.15mg/ L to 4.42±0.06mg/ L. The best DO level for *S. platensis* culture is 3.5mg/ L to 4.5mg/ L **(Ogbonda *et al.*, 2007b)**. In the present experiment, since artificial aeration was continuously provided, maintaining the optimum dissolved oxygen level was never a limiting factor.

Table 2. Physico- chemical parameters of the culture media under different treatments[1]

Treatment	Parameter	Sampling time (day)					
		3	**6**	**9**	**12**	**15**	**18**
KM (Control, T_1)	Temperature (°C)	31.6 ± 0.06	31.6 ± 0.06	31.6 ± 0.06	31.6 ± 0.06	31.6 ± 0.06	31.6 ± 0.06
	DO (mg/ L)	4.21 ± 0.15	4.36 ± 0.13	4.30 ± 0.14	4.38 ± 0.12	4.40 ± 0.16	4.41 ± 0.13
	pH	9.04 ± 0.05	9.35 ± 0.01	9.53 ± 0.02	9.56± 0.02	10.5 ± 0.12	9.54± 0.04
0.5g/ L BSAE (T_2)	Temperature (°C)	31.5 ± 0.06	31.5 ± 0.06	31.5 ± 0.06	31.5 ± 0.06	31.5 ± 0.06	31.5 ± 0.06
	DO (mg/L)	4.30 ± 0.13	4.34 ± 0.11	4.32 ± 0.12	4.34 ± 0.10	4.37 ± 0.11	4.42 ± 0.12
	pH	9.00 ± 0.01	9.29 ± 0.04	9.50 ± 0.03	9.51 ± 0.03	10.4 ± 0.13	9.50 ± 0.04
0.5g/ L BSAE with micronutrient (T_3)	Temperature (°C)	31.5 ± 0.06	31.5 ± 0.06	31.6 ± 0.06	31.5 ± 0.06	31.5 ± 0.06	31.5 ± 0.06
	DO (mg/ L)	4.30 ± 0.10	4.33 ± 0.08	4.33 ± 0.09	4.39 ± 0.07	4.42 ± 0.06	4.41 ± 0.08
	pH	9.03 ± 0.02	9.34 ± 0.04	9.52 ± 0.04	9.53 ± 0.06	10.5 ± 0.12	9.54 ± 0.01
1.0g/ L BSAE (T_4)	Temperature (°C)	31.5 ± 0.05	31.5 ± 0.05	31.5 ± 0.05	31.5 ± 0.06	31.5 ± 0.05	31.5 ± 0.05
	DO (mg/ L)	4.25 ± 0.09	4.36 ± 0.10	4.30 ± 0.08	4.38 ± 0.10	4.34 ± 0.11	4.39 ± 0.06
	pH	9.02 ± 0.03	9.28 ± 0.04	9.51 ± 0.02	9.51 ± 0.04	10.4 ± 0.12	9.52 ± 0.04
1.0g/ L BSAE with micronutrient (T_5)	Temperature (°C)	31.5 ± 0.06	31.5 ± 0.06	31.5 ± 0.06	31.5 ± 0.06	31.5 ± 0.06	31.5 ± 0.06
	DO (mg/ L)	4.23 ± 0.13	4.34 ± 0.10	4.30 ± 0.12	4.31 ± 0.13	4.36 ± 0.14	4.38 ± 0.12
	pH	9.03 ± 0.04	9.35 ± 0.03	9.53 ± 0.03	9.54 ± 0.03	10.5 ± 0.12	9.51 ± 0.01

[1]Values are expressed as mean ±SD, where n = 3.

Effect of BSAE on optical density

The mean optical density (OD) of *S. platensis* in different treatments during the experimental period is presented in Fig. (1). In this study, the mean values of OD varied from 0.31± 0.06 to 0.87± 0.01 in different treatments. The variation of OD in different

treatments started from the 9th day of culture. The exponential phase was up to the 15th day from the beginning when maximum OD was found in treatment T_1, where KM was used as a culture medium and after the 15th day, OD started to decline. There was no significant difference in OD of treatments T_3 and T_5 with the control treatment (T_1), while OD in treatments T_2 and T_4 was significantly ($P< 0.05$) lower than the control treatment (T_1). **Chowdhury (2005)** found that the highest OD (1.15) was in KM and a value of 0.99 was recorded in the experimental medium (120g/ L fermented pond bottom soil) after 12 days of cultivation. OD in different treatments ranged from 0.32 to 0.98 after 12 days of culture, where different concentrations of fertilizer factory effluent were used (**Karim, 2004)**.

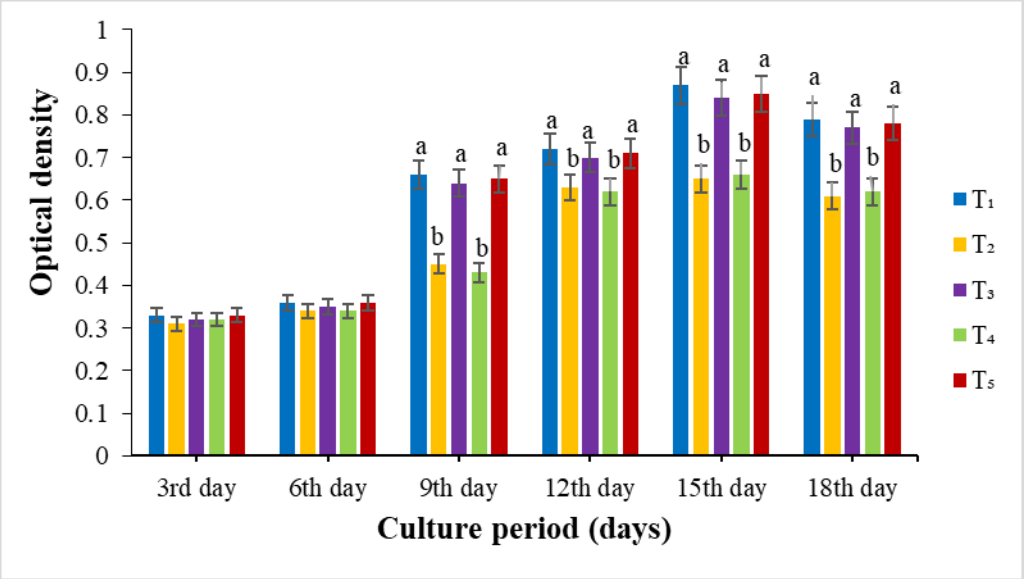

Fig. 1. Optical density of culture media under different treatments during the experimental period
Different letters on a sampling day indicate a significant difference ($P< 0.05$).

Effect of BSAE on biomass concentration

The mean biomass concentration in different treatments during the experimental period is presented in Fig. (2). In all treatments, the initial inoculum rate of *S. platensis* was 0.01± 0.01g/ L. The variation of biomass concentration in different treatments started from the 6th day of culture, and the exponential phase was found up to the 15th day from the beginning. Maximum biomass concentration (1.29g/ L) was found in KM on the 15th day of culture. At the end of the exponential phase, the highest biomass concentration was also recorded in KM. Biomass concentration in 0.5g/ L and 1.0g/ L BSAE with 0.5ml micronutrient supplementation gave comparable results to KM. Alternatively, biomass concentration in 0.5g/ L and 1.0g/ L BSAE without micronutrient supplementation was significantly ($P< 0.05$) lower than KM. **Jain and Singh (2013)** reported higher dry biomass content (1.21g/ L) of *S. platensis* in 20% formulated CDAM

(cow dung ash medium), whereas 0.52g/ L in 100% CDAM and 1.11g/ L were recorded in standard medium (CFTRI). **Toyub *et al.* (2011)** revealed that papaya skin powder medium (PSPM) at a concentration of 0.60g/ L could be successfully used as a culture medium for *S. platensis*. Biomass concentrations of 523, 485, and 307mg/ L were found at 7.2, 4.8, and 9.6mg/ L of banana leaf ash (BLA) medium for *S. platensis* culture, while 545mg/ L biomass was found in KM (**Toyub *et al.*, 2005)**.

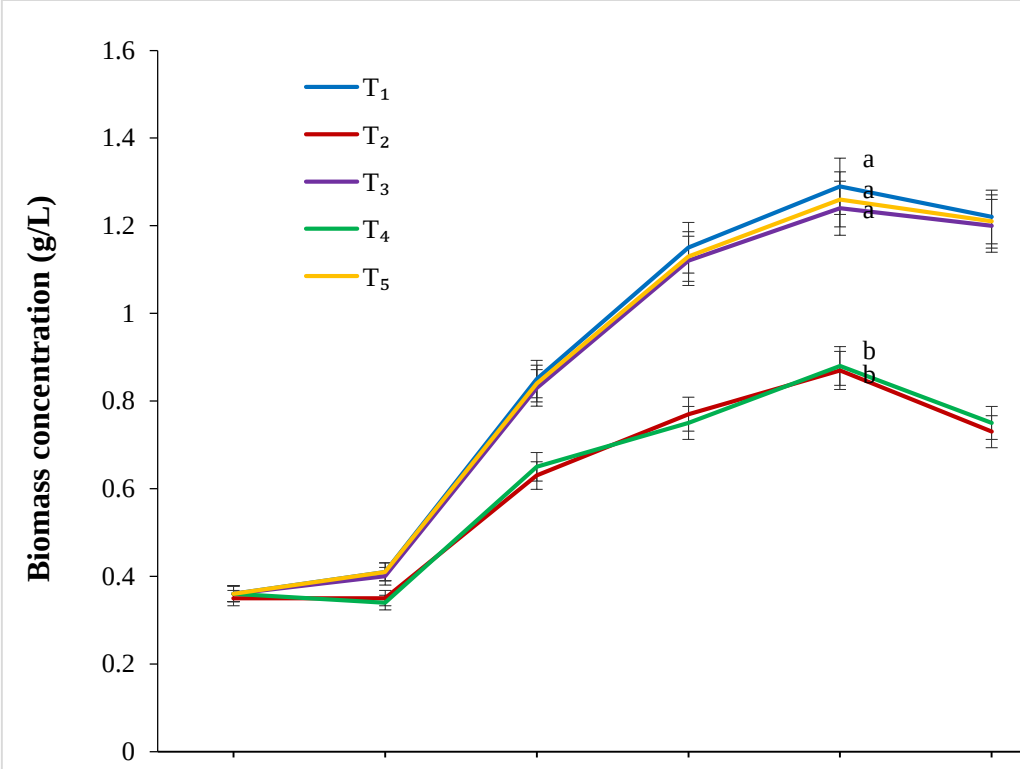

Fig. 2. Biomass concentration (g/ L) of the *S. platensis* cultured in media under different treatments during the experimental period

Different letters on a sampling day indicate a significant difference ($P< 0.05$).

Effect of BSAE on chlorophyll *a*

The mean chlorophyll *a* content of *S. platensis* in different treatments during the experimental period is presented in Fig. (3). The initial chlorophyll *a* content was 0.16± 0.01mg/ L in all treatments. The mean values of chlorophyll *a* content in different treatments ranged from 1.88 to 8.74mg/ L on the 15th day of the exponential period, where maximum chlorophyll *a* content (8.74mg/ L) was found in KM. There was no significant difference in chlorophyll *a* content of treatment T_3 and T_5 (0.5g/ L and 1.0g/ L BSAE with 0.5ml micronutrient supplementation) with the control treatment (T_1), while chlorophyll *a* content values in 0.5 and 1.0g/ L BSAE without micronutrient supplementation were significantly ($P< 0.05$) lower than KM. **Toyub *et al.* (2005)** found

maximum chlorophyll *a* content (8.12mg/ L) in *S. platensis* when cultured in KM, while 7.53mg/ L chlorophyll *a* was reported when cultured in 7.2g/ L banana leaf ash (BLA) medium.

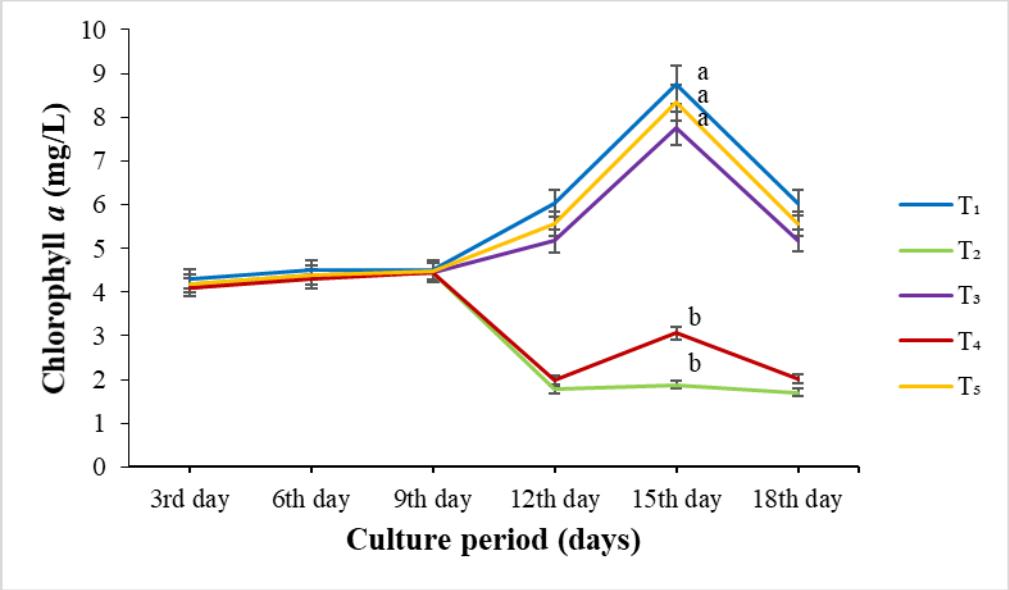

Fig. 3. Chlorophyll *a* (mg/ L) content in *S. platensis* culture in media under different treatments during the experimental period. Different letters on a sampling day indicate a significant difference ($P< 0.05$)

Effect of BSAE on specific growth rate

The specific growth rate (SGR, μ/ day) of *S. platensis* based on biomass concentration was recorded in the ranges of 0.52 to 0.57μ/day on the 15th day of the exponential phase (Fig 4). In this study, the SGR of *S. platensis* was recorded to be significantly ($P< 0.05$) higher in KM, and a similar trend of SGR was found in 0.5g/ L and 1.0g/ L BSAE with 0.5ml micronutrient, respectively. **Toyub *et al.* (2005)** recorded SGR of cultured *S. platensis* from 0.41 to 0.49μ/ day in different concentrations (4.8, 7.2, and 9.6g/ L) of banana leaf ash (BLA). **Ranjith *et al.* (2013)** found that the maximum specific growth rate (0.37μ/ day) was recorded in Zarrouk's medium.

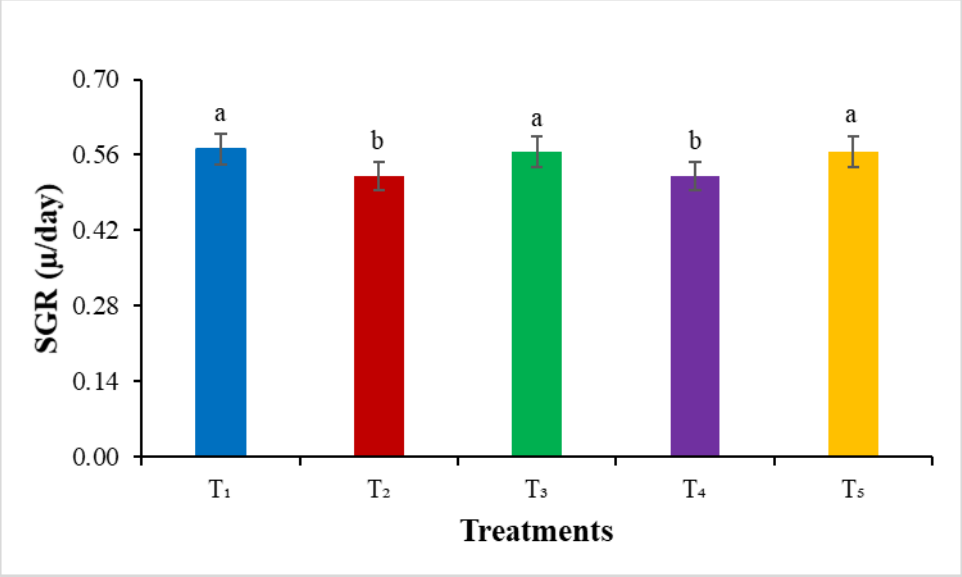

Fig. 4. Specific growth rate (SGR, µ/ day) of *S. platensis* during the exponential phase (15^{th} day)

Different letters in different treatments indicate significant difference ($P< 0.05$).

Cost of nutrient media for *S. platensis* culture

Table (3) shows a simple cost analysis of nutrient media for *S. platensis* culture. $NaHCO_3$ is the primary component of the KM and accounts for the majority of the preparation costs. It costed 370 USD to prepare 1000L KM. Alternatively, 1000L medium containing 0.5g/ L BSAE and micronutrient costed only 10.14 USD, while the same amount of medium containing 1.0g/ L BSAE and micronutrient costed 10.28 USD. **Khatun *et al.* (2019)** reported that around 300 USD is needed to prepare 1000L of KM, while the cost of banana leaf ash extract (BLAE) medium was significantly lower than KM. In the present study, it was noted that biomass concentration, chlorophyll *a* content, and specific growth rate of *S. platensis* cultured in the KM, 0.5g/ L BSAE supplemented with 0.5ml/ L micronutrient, and 1.0g/ L BSAE supplemented with 0.5ml/ L micronutrient showed comparable results. However, the cost of BSAE- supplemented medium was considerably less than KM.

Table 3. A simple cost analysis of *S. platensis* production under different culture media on the 15th day (based on biomass concentration)

Medium	Ingredient	Amount (g/ L)	Price (USD) (1 USD = 110 BDT)	Total cost of 1L medium (USD)	Cost of 1000L Medium (USD)
KM (Control, T_1)	$NaHCO_3$	9.00	0.28	0.37	370
	Other ingredients	–	0.09		
0.5g/ L BSAE (T_2)	BSAE	0.5	0.00014	0.00014	0.14
0.5g/ L BSAE with micronutrient (T_3)	BSAE	0.5	0.00014	0.01014	10.14
	Micronutrient solution	0.5 ml/L	0.01		
1.0g/ L BSAE (T_4)	BSAE	1.00	0.00028	0.00028	0.28
1.0g/ L BSAE with micronutrient (T_5)	BSAE	1.00	0.00028	0.0101	10.28
	Micronutrient solution	0.5 ml/L	0.01		

CONCLUSION

In conclusion, higher biomass concentration, specific growth rate, optical density, and chlorophyll a content of *S. platensis* were observed in KM (T1); however, these parameters were not significantly different ($P > 0.05$) with treatment T3 (0.5g/ L BSAE with 0.5ml micronutrient) and treatment T5 (1.0g/ L BSAE with 0.5ml micronutrient). BSAE proves to be a potential low- cost nutrient source for cultivating *S. platensis*. Therefore, a cost- effective culture medium containing 0.5g/ L or 1.0g/ L BSAE with 0.5ml micronutrient supplementation can be recommended for *S. platensis* culture, without compromising its growth performance and pigmentation.

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