

Preliminary study on monoculture establishment and growth assessment of three Chlorophyta microalgae from Shalimar Garden, Lahore, Pakistan

Pakistan'ın Lahor kentindeki Shalimar Bahçesinden elde edilen üç Chlorophyta mikroalg türünün monokültürü ve büyümesine ilişkin ön çalışma

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Abstract: Freshwater microalgal samples were collected from Shalimar Garden, Lahore, Pakistan, and successfully cultured to establish pure monocultures. Three Chlorophyta species *Chlorella ellipsoidea*, *Pediastrum obtusum*, and *Scenedesmus incrassatulus* var. *capitatus* were taxonomically identified under an inverted microscope and selected for monoculture studies. Monocultures were maintained on Bold's Basal Medium (BBM) and CHU-10 medium using the streaking technique. Peak biomass in fresh weight on BBM was recorded on the 18th day, reaching 2.12 g, 1.15 g, and 1.41 g for *C. ellipsoidea*, *P. obtusum*, and *S. incrassatulus* var. *capitatus*, respectively, with corresponding maximum growth rates of 0.004-0.009 g/day. In CHU-10, peak biomass and growth rates were notably lower (0.42-0.35 g; 0.001-0.003 g/day). By day 21, biomass and growth declined in both media, indicating nutrient depletion and onset of the decline phase. Net weight gains were substantially higher in BBM (1.38 g, 0.73 g, 0.94 g) compared to CHU-10 (0.34 g, 0.29 g, 0.26 g), and these differences were statistically significant ($p < 0.0001$) demonstrating BBM's superior performance in supporting faster growth and higher biomass accumulation. This study represents the first successful monoculturing of these three Chlorophyta species from the selected Shalimar Garden, Lahore, Pakistan, representing preliminary investigation thus providing baseline data that can guide future applied research in algal biotechnology and environmental studies.

Keywords: Freshwater microalgae, identification, isolation, growth, biomass

Öz: Tatlı su mikroalg örnekleri Pakistan'ın Lahor kentindeki Shalimar bahçesinden toplanmış ve saf monokültürler oluşturmak için başarıyla kültüre alınmıştır. Üç Chlorophyta türü olan *Chlorella ellipsoidea*, *Pediastrum obtusum* ve *Scenedesmus incrassatulus* var. *capitatus*, ters mikroskop altında taksonomik olarak tanımlanmış ve monokültür çalışmaları için seçilmiştir. Monokültürler, Bold's Basal Medium (BBM) ve CHU-10 besiyerinde çizgi tekniği kullanılarak muhafaza edilmiştir. BBM besi yerinde taze ağırlık olarak en yüksek biyokütle, 18. günde kaydedilmiş ve *C. ellipsoidea*, *P. obtusum* ve *S. incrassatulus* var. *capitatus* türleri için sırasıyla 2,12 g, 1,15 g ve 1,41 g'a ulaşılmış ve maksimum büyüme oranları ise 0,004-0,009 g/gün olarak tespit edilmiştir. CHU-10 besi yerinde, biyokütle ve büyüme oranlarının pik değerleri belirgin şekilde daha düşük (0,42-0,35 g; 0,001-0,003 g/gün) olduğu saptanmıştır. 21. günde, her iki ortamda da biyokütle ve büyüme azalmış, bu da besin maddelerinin tükendiğini ve buna bağlı düşüş aşamasının başladığını göstermektedir. Net ağırlık artışları, CHU-10 besiyerine (0,34 g, 0,29 g, 0,26 g) kıyasla BBM besiyerinde (1,38 g, 0,73 g, 0,94 g) önemli ölçüde daha yüksek olduğu ve bu farkların istatistiksel olarak anlamlı olduğu ($p < 0,0001$) saptanmıştır. Bu da BBM'nin daha hızlı büyümeyi ve daha yüksek biyokütle birikimini destekleme konusunda üstün performansını göstermektedir. Bu çalışma, Pakistan'ın Lahor kentindeki Shalimar bahçesinden seçilen bu üç Chlorophyta türünün ilk başarılı monokültür çalışmasını temsil etmekte olup, alg biyoteknolojisi ve çevre çalışmalarını alanlarında gelecekteki uygulamalı araştırmalara rehberlik edebilecek temel veriler sağlayan öncü bir araştırma niteliğindedir.

Anahtar kelimeler: Tatlı su mikroalgleri, tanımlama, izolasyon, büyüme, biyokütle

INTRODUCTION

Algae are diverse photoautotrophic organisms, ranging from unicellular to multicellular forms, and serve as primary producers in aquatic ecosystems. They contribute significantly to overall biogeochemical cycles by converting solar energy into organic compounds such as oils, lipids, and carbohydrates (Schenk et al., 2008). Due to their adaptability, simple morphology, and rapid growth, algae have attracted increasing attention in sectors such as food, pharmaceuticals, biofuels, aquaculture, and environmental management (Varfolomeev and Wasserman, 2010; Tippawan and Yuwadee, 2016; Zhang et al., 2018; Tan et al., 2020; Aslam et al., 2021).

Microalgae, in particular, are highly efficient, photosynthetic, microscopic organisms that form the base of aquatic food chains and have been extensively studied for their potential in biomass

production, nutraceuticals, and sustainable energy (Kais et al., 2011; Anitha and Narayanan, 2012; Mutanda, 2013; Luo et al., 2015). Globally, considerable research has focused on microalgal taxonomy, biodiversity, and cultivation methods (Allen and Stanier, 1967; Abdeshahian et al., 2010; Nagarajan et al., 2017; Won et al., 2023; Long et al., 2024; Verdev and Dolinar, 2025). Culturing practices and growth media have been optimized to enhance metabolite production and algal biomass (Hammad et al., 2017; Xu et al., 2018). Bold's Basal Medium (BBM), CHU-10, and BG-11 are among the most widely employed media for laboratory investigations (Kirrolia et al., 2014; Ilavarasi et al., 2011; Menezes et al., 2016; Mahboob et al., 2022). BBM provides an optimized balance of macro- and micronutrients suitable for fast-growing green algae, while CHU-

10 is historically employed for freshwater algal isolation (Yadav et al., 2023; Salman et al., 2023). However, growth responses are strain-specific and influenced by biotic and abiotic factors, including pH, light, temperature, and nutrient availability (Richmond, 2000; Mata et al., 2010; Munir et al., 2015).

In Pakistan, pioneering contributions to algal taxonomy were made by Faridi (1955, 1970, 1971, 1975, 1977a–c, 1978) and later by Masud-ul-Hasan and colleagues (Masud-ul-Hasan and Batool, 1987; Masud-ul-Hasan and Yunus, 1989; Masud-ul-Hasan, 1978a, 1978b, 1980), who documented a number of freshwater species from Punjab. Despite these studies, limited emphasis has been placed on establishing pure cultures and evaluating growth media for indigenous algal strains. This gap restricts the potential application of native algae in aquaculture and biotechnological fields. Although *Chlorella ellipsoidea*, *Scenedesmus incrassatulus*, and *Pediastrum obtusum* are well-studied globally, the growth characteristics of indigenous strains from our local freshwater ecosystem have not been previously reported. Understanding their growth dynamics is essential for biodiversity assessment and potential biotechnological applications.

The present study aims to address this gap by isolating and establishing monocultures of freshwater algae for the first time

from Shalimar Garden, Lahore, Pakistan, and evaluating their growth in two commonly used media, BBM and CHU-10. As a preliminary study, the results provide foundational data to support further investigations into biochemical profiling, environmental stress responses, and large-scale cultivation of these microalgae. The present study was conducted to achieve (i) to document both previously reported and unreported algal species from Shalimar Garden, Lahore, Pakistan. (ii) determine the best and suitable culturing media among Bold's Basal medium and CHU-10 medium for collected algae growth. (iii) To establish pure monocultures of identified and chosen algal species under precise laboratory settings. (iv) assess the biomass production of these algae under the experimental conditions.

MATERIALS AND METHODS

Collection

Algal samples were collected during September and November 2018 from freshwater ponds located near an urban area along a major roadway (Figure 1). Shalimar Garden, Lahore, Pakistan consists of a complex water system with approximately 410 fountains. Samples were collected in sterile plastic bottles, labeled appropriately, and transported to the laboratory for further processing.



Figure 1. Collection sites of algae at the Shalimar Garden, Lahore, Pakistan Baradari (a), Water ponds (b)

Identification

Upon arrival at the laboratory, collected samples were preserved in 4% formalin. Subsamples were examined under a compound light microscope, and taxa were identified based on morphological characteristics using standard taxonomic literature, including West, 1904; Desikachary, 1959; Prescott, 1962. Cell sizes were measured using a calibrated ocular micrometer. The systematic arrangement followed the Shameelian classification system (Shameel, 2001; 2012). Based on these identifications, three freshwater microalgal species *Chlorella ellipsoidea*, *Pediastrum obtusum*, and *Scenedesmus incrassatulus* var. *capitatus* were selected for subsequent monoculture establishment (Figure 2).

Isolation of pure cultures

Pure cultures were established by isolating individual colonies with a flame-sterilized pasteur pipette. Colonies were initially observed under a microscope, and selected ones were transferred to sterile Petri plates containing distilled water. Each species was handled separately and subsequently streaked onto nutrient agar plates prepared with the selected growth media using a sterile inoculating loop. Repeated sub-culturing of actively growing colonies ensured the establishment of stable monocultures.

Culture media and growth conditions

Bold's Basal Medium (BBM) (Bischoff and Bold, 1963) and

CHU-10 medium (Chu, 1942) were selected for algal cultivation. Both media were prepared according to standard formulations (Annexure I and II). Stock solutions of individual components were prepared using analytical-grade chemicals and stored at 4°C until use. Working media were prepared by mixing appropriate volumes of stock solutions and adjusting

the final volume to 1 L with double-distilled water. The pH was adjusted to 6.6 for BBM and 7.6 for CHU-10 using 0.01 N HCl or 0.01 N NaOH. For solid media preparation, agar (7.8g L⁻¹) was added prior to autoclaving at 121°C and 15 psi for 20 minutes. All glassware and equipment were sterilized along with the media.

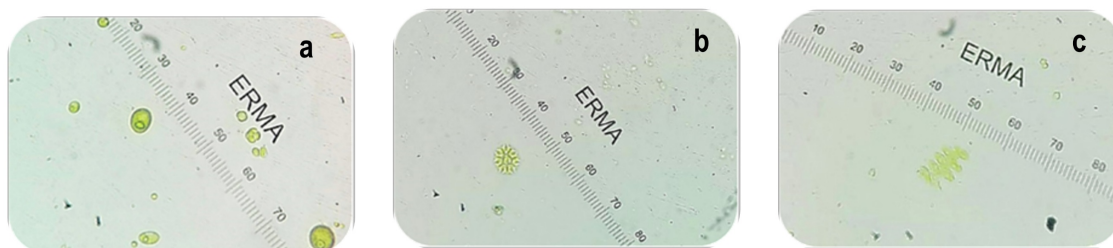


Figure 2. Microscopic images of selected micro-algal species: *Chlorella ellipsoidea* (a), *Pediastrum obtusum* (b), *Scenedesmus incrasatulus* var. *capitatus* (c)

Inoculation and incubation

Autoclaved media were poured into sterile petri plates under aseptic conditions in a laminar airflow cabinet. The weight of each empty petri plate was recorded prior to pouring. After solidification, the plates were weighed again and inoculated with isolated microalgal strains using the streak plate technique (Andersen, 2005). Following inoculation, plates were sealed with cling film and incubated at 25°C.

Culture maintenance

All cultures were maintained in a controlled growth chamber at 25°C under a 16:8 h light-dark photoperiod using cool white fluorescent tubes at 40-50 μmol photons m⁻² s⁻¹, with light intensity measured using a lux meter. Plates were monitored daily for colony development, and actively growing cultures were re-streaked before senescence to ensure purity. Cultures were established in triplicate for each micro-algal species and growth medium.

Biomass estimation

Biomass accumulation was evaluated at three-day intervals over 21-days. Biomass accumulation was measured gravimetrically by recording the weight of inoculated Petri plates before and after growth. Biomass yield was calculated as the difference between initial and final plate weights. Growth performance was assessed using both BBM and CHU-10 media under controlled conditions.

Growth rate determination

Growth rate of algal population was determined by the formula (Levasseur et al., 1993).

$$\mu = \ln \frac{g_2/g_1}{T_2 - T_1}$$

where μ is the growth rate, \ln is the natural logarithm, g_1 and g_2 are initial and final biomass, and T_1 and T_2 are initial and final time points.

Statistical analysis

All experiments were performed in triplicate (n=3), and results are expressed as mean \pm SE. Data were analyzed using one-way ANOVA to evaluate differences between BBM and CHU-10 media. Post hoc comparisons were performed using Tukey's test. Differences were considered statistically significant at $p < 0.0001$.

RESULTS

The microalgal species *C. ellipsoidea*, *P. obtusum*, and *S. incrasatulus* var. *capitatus* were identified based on morphological characteristics using standard taxonomic keys and literature, and their growth was subsequently examined.

Growth of *C. ellipsoidea*, *P. obtusum* and *S. incrasatulus* var. *capitatus* on Bold's Basal Medium (BBM) and CHU-10 Medium

All three species exhibited measurable growth on both BBM and CHU-10, with higher biomass accumulation observed in BBM (Tables 1-4). Biomass increased progressively and reached its maximum on day 18 for all species, followed by a decline by day 21 (Figure 3). Growth trends on CHU-10 also showed a gradual increase in biomass until day 18, followed by a decline on day 21 (Figure 4; Tables 2-4). However, overall biomass accumulation and growth rates were consistently lower compared to BBM. The observed decline in biomass and growth rates by day 21 in CHU-10 further indicates the onset of stationary and decline phases under the given culture conditions. Comparative biomass accumulation and growth rate trends for all species grown on BBM are presented in Figures 5 and 6.

Table 1. One-way ANOVA analysis of final day (Day 21) biomass of microalgae grown on BBM and CHU-10 media

Species	BBM (Mean \pm SE g)	CHU-10 (Mean \pm SE g)	ANOVA p-value
<i>C. ellipsoidea</i>	1.86 \pm 0.001	0.36 \pm 0.001	< 0.0001
<i>P. obtusum</i>	0.85 \pm 0.001	0.25 \pm 0.001	< 0.0001
<i>S. incrasatulus</i> var. <i>capitatus</i>	1.20 \pm 0.001	0.23 \pm 0.001	< 0.0001

Values represent mean \pm SE of three independent replicates (n=3).

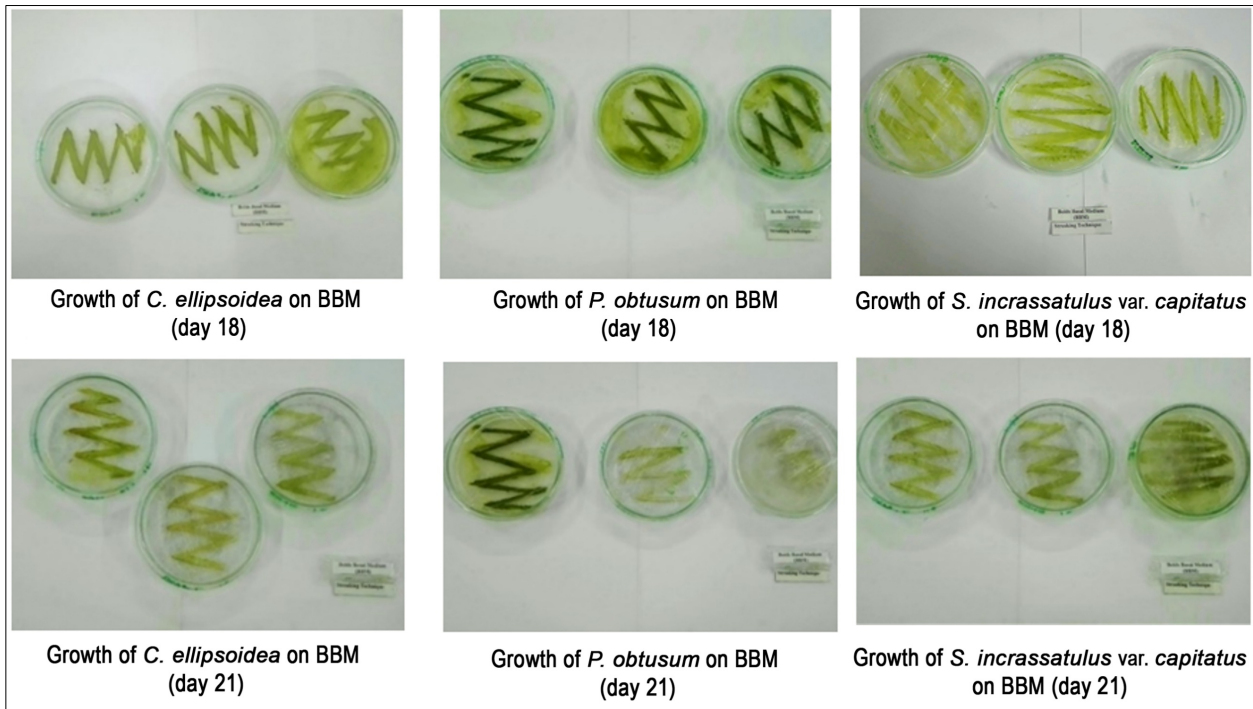


Figure 3. Growth of *C. ellipsoidea*, *P. obtusum*, and *S. incrassatus* var. *capitatus* on BBM over 18 and 21 days.

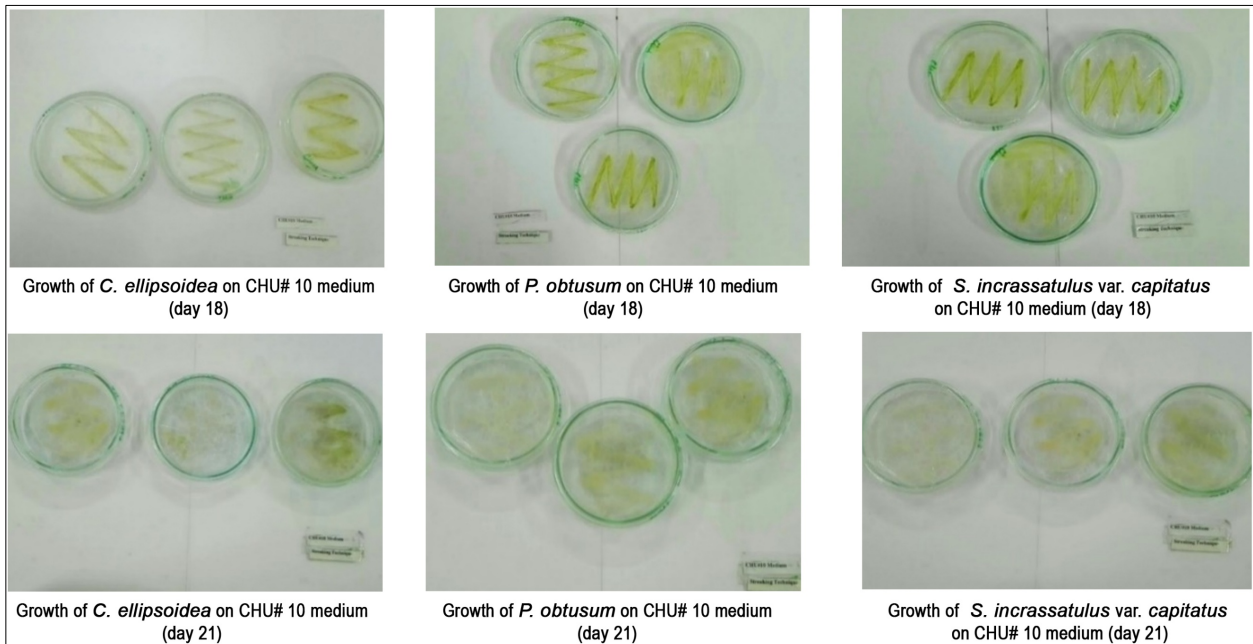


Figure 4. Growth of *C. ellipsoidea*, *P. obtusum*, and *S. incrassatus* var. *capitatus* on CHU-10 over 18 and 21 days.

Table 2. Biomass, net weight gain, and daily growth rate of *C. ellipsoidea* on BBM and CHU-10 using streaking technique

Days	Algal biomass weight (g) at different time intervals								Net weight gain (g)
	1 st	3 rd	6 th	9 th	12 th	15 th	18 th	21 st	
BBM	0.37±0.001	0.68±0.001	1.050.001	1.33±0.002	1.68±0.003	1.98±0.001	2.12±0.003	1.87±0.001	1.38
CHU-10	0.220.001	0.26±0.002	0.31±0.001	0.35±0.003	0.390.001	0.41±0.002	0.420.001	0.370.001	0.34
Algal Growth Rate (g/day)									Total Growth rate (g/day)
BBM	0	0.30	0.08	0.02	0.02	0.01	0.004	-0.006	0.053
CHU-10	0	0.08	0.03	0.01	0.009	0.003	0.001	-0.006	0.015

Values represent mean ± S.E. of three independent replicates.

Table 3. Biomass, net weight gain, and daily growth rate of *P. obtusum* on BBM and CHU-10 using streaking technique

Days	Algal biomass weight (g) at different time intervals								Net weight gain (g)
	1 st	3 rd	6 th	9 th	12 th	15 th	18 th	21 st	
BBM	0.31±0.001	0.40±0.001	0.550.001	0.67±0.002	0.84±0.003	0.98±0.001	1.15±0.003	0.85±0.001	0.73
CHU-10	0.200.001	0.27±0.002	0.29±0.001	0.31±0.003	0.330.001	0.36±0.002	0.380.001	0.250.001	0.29
Algal Growth Rate (g/day)									Total Growth rate (g/day)
BBM	0	0.12	0.06	0.02	0.02	0.01	0.009	-0.01	0.028
CHU-10	0	0.15	0.01	0.008	0.005	0.006	0.003	-0.02	0.020

Values represent mean ± S.E. of three independent replicates.

Table 4. Biomass, net weight gain, and daily growth rate of *S. incrassatulus* var. *capitatus* on BBM and CHU-10 using streaking technique

Days	Algal biomass weight (g) at different time intervals								Net weight gain (g)
	1 st	3 rd	6 th	9 th	12 th	15 th	18 th	21 st	
BBM	0.35±0.001	0.54±0.001	0.760.001	0.93±0.002	1.13±0.003	1.27±0.001	1.41±0.003	1.20±0.001	0.94
CHU-10	0.170.001	0.22±0.002	0.25±0.001	0.28±0.003	0.310.001	0.33±0.002	0.350.001	0.230.001	0.26
Algal Growth Rate (g/day)									Total Growth rate (g/day)
BBM	0	0.21	0.06	0.02	0.01	0.008	0.006	-0.008	0.038
CHU-10	0	0.12	0.02	0.01	0.009	0.004	0.003	-0.020	0.018

Values represent mean ± S.E. of three independent replicates.

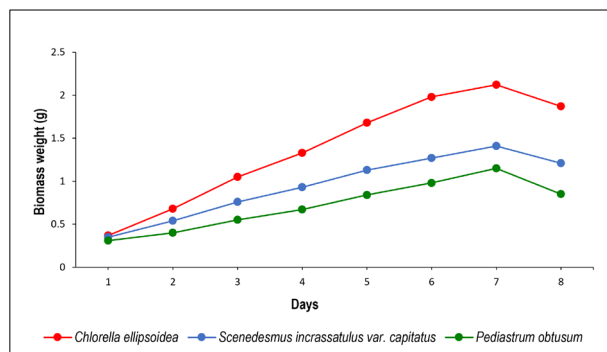


Figure 5. Comparative biomass of *C. ellipsoidea*, *P. obtusum*, and *S. incrassatulus* var. *capitatus* on BBM

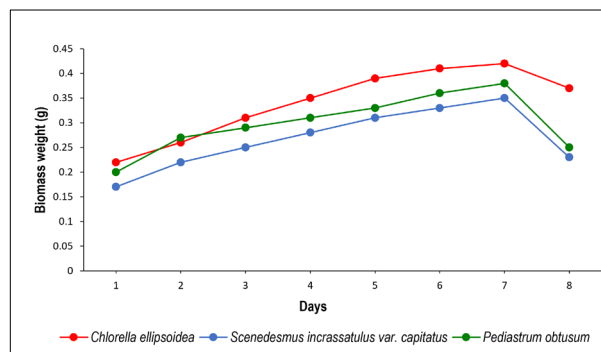


Figure 12. Comparative biomass of *C. ellipsoidea*, *P. obtusum*, and *S. incrassatulus* var. *capitatus* on CHU#10

Statistical analysis of total growth rates also confirmed that growth was significantly higher in BBM compared to CHU-10 for all tested species (Table 5), supporting the observed trend in biomass accumulation.

Table 5. One-way ANOVA analysis of total growth rates (g/day) of microalgae on BBM and CHU-10 media

Species	BBM (Mean ± SE)	CHU-10 (Mean ± SE)	ANOVA p-value
<i>C. ellipsoidea</i>	0.053±0.001	0.015±0.001	<0.001
<i>P. obtusum</i>	0.028±0.001	0.020±0.001	0.012
<i>S. incrassatulus</i> var. <i>capitatus</i>	0.038±0.001	0.018±0.001	<0.001

Values represent mean ± SE of three independent replicates (n=3).

DISCUSSION

In this study, three Chlorophyta species: *C. ellipsoidea*, *P. obtusum*, and *S. incrassatulus* var. *capitatus* were successfully isolated and established as pure monocultures using the streaking technique. Biomass accumulation and growth rate analyses demonstrated that Bold's Basal Medium (BBM) consistently supported higher growth compared to CHU-10, confirming its suitability as a growth medium for these locally sourced freshwater isolates. One-way ANOVA followed by post hoc Tukey's test indicated statistically significant differences ($p < 0.0001$) in both biomass and growth rates between the two media (Tables 1 and 5), reinforcing BBM's superior performance under controlled laboratory conditions. Peak biomass in BBM was observed on day 18, reaching 2.12g, 1.15g, and 1.41g for *C. ellipsoidea*, *P. obtusum*, and *S. incrassatulus* var. *capitatus*, respectively, followed by a slight decline by day 21. In contrast, CHU-10 exhibited lower peak biomass (0.35-0.42g) and a sharper decline by day 21, suggesting nutrient limitation and reduced culture stability. The observed decline in growth rates, shifting from positive to negative values, likely reflects nutrient depletion and the onset of the decline phase, a phenomenon commonly reported in solid-phase algal cultivation (Levasseur et al., 1993; Andersen, 2005). The noticeable differences in growth rate observed between replicates on BBM at day 21, despite using the same single colony for isolation and streaking, may be attributed to several factors. Minor variations in the initial inoculum size, slight differences in local micro-environmental conditions (e.g., light intensity, nutrient availability, or temperature gradients across the growth chamber), and small handling variations during streaking can all contribute to variability among replicates (Levasseur et al., 1993; Andersen, 2005). Additionally, slight differences in colony vigor or physiological state at the time of inoculation may influence growth performance. These factors likely explain the variations seen in the photographs and highlight the importance of maintaining strict consistency during culture handling.

The superior growth in BBM can be attributed to its higher concentrations of nitrate, phosphate, and trace metals, which are essential for chlorophyll synthesis, protein production, and enzymatic activity (Bischoff and Bold, 1963; Abou-Shanab et al., 2011). Furthermore, BBM provides a balanced carbon-to-nitrogen ratio that optimizes cellular metabolism, influencing

lipid and protein accumulation. These observations align with previous studies reporting enhanced growth of *Chlorella* and *Scenedesmus* species in nutrient-rich or balanced media (Ilavarasi et al., 2011; Bajwa et al., 2017; Simsek and Cetin, 2025).

Despite using the same single colony for inoculation, minor variability was observed between replicates, particularly during extended incubation. Such differences may be caused by localized nutrient gradients, agar heterogeneity, slight variations in inoculum size, or handling differences during streaking (Levasseur et al., 1993; Andersen, 2005). These factors explain the variation in growth rates observed among replicates in the photographs, highlighting the importance of meticulous technique during monoculture establishment.

While this study focused on solid-phase cultivation using Petri plates, it provides valuable baseline data for evaluating growth potential and medium suitability. Solid-phase systems enable controlled colony selection, contamination monitoring, and preparation for subsequent liquid-phase cultivation, which is generally preferred for large-scale biomass production due to improved nutrient homogeneity and scalability (Richmond, 2000; Tan et al., 2020). Future investigations should explore liquid-phase culture or bioreactor systems, which may yield different biomass accumulation and growth dynamics. Additionally, inclusion of more locally isolated species could expand understanding of species-specific responses to standard culture media.

Although biochemical profiling was beyond the scope of the present study, future research should focus on quantifying lipids, proteins, carbohydrates, pigments, and antioxidant compounds of these indigenous microalgae. Such analyses would provide insight into their nutritional and industrial potential, enabling evaluation for applications in biofuel production, nutraceutical development, and other biotechnological uses.

Overall, this study establishes a reliable protocol for the isolation and preliminary growth assessment of locally sourced Chlorophyta species. The results confirm that BBM supports higher biomass accumulation and more stable growth than CHU-10, providing foundational data for applied research in algal biotechnology, including biofuel production, wastewater treatment, nutraceutical development, and large-scale cultivation of indigenous freshwater microalgae (Yadav et al., 2023; Simsek and Cetin, 2025; Rautenberger et al., 2024). The findings emphasize the importance of medium selection in optimizing growth performance and provide a framework for future applied studies using locally isolated algal strains.

CONCLUSION

This study confirms that Bold's Basal Medium (BBM) is significantly more effective than CHU-10 for establishing monocultures of *Chlorella ellipsoidea*, *Pediastrum obtusum*, and *Scenedesmus incrassatulus* var. *capitatus*, as evidenced

by higher biomass accumulation ($p < 0.0001$) in all three species. To the best of our knowledge, this is the first report of successful isolation and monoculturing of these three freshwater microalgal species from a local site, providing a vital baseline for future algal biotechnology research in the region. These findings emphasize the importance of selecting an appropriate growth medium for maximizing growth performance of indigenous microalgae.

Microalgae are ecologically and industrially important, contributing to aquatic food webs and offering diverse applications in food, agriculture, pharmaceuticals, cosmetics, biofuels, and environmental remediation. By establishing reliable protocols for obtaining pure cultures of locally adapted strains, this work lays the foundation for future investigations including lipid and protein quantification, antioxidant screening, and large-scale bioreactor cultivation. These monocultures can serve as a starting point for studies on lipid production, protein profiling, and bioactive compounds in locally adapted freshwater microalgae. This study serves as a preliminary assessment, providing quantitative insights into growth performance, medium suitability, and monoculture establishment of three freshwater Chlorophyta species. These findings can guide future in-depth research on their biotechnological potential and may open new opportunities for sustainable bioenergy production, bioproduct development, and agricultural biotechnology using indigenous microalgal resources.

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AUTHORSHIP CONTRIBUTIONS

All authors contributed to the idea and design of the study. Hamna Sohail: Conceptualization, Methodology, Investigation, Resources, Funding acquisition, Data curation, Writing-Original draft preparation, Formal analysis, Visualization. Ghazala Yasmeen Butt: Supervision, Project administration, validation, review and editing.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest or competing interests.

ETHICS APPROVAL

This article does not contain any human or animal studies performed by any authors. No specific ethical approval was necessary for this study.

DECLARATION OF AI USE

The authors declare that no AI tools were used in the preparation of this manuscript.

DATA AVAILABILITY STATEMENT

All relevant data is in the article.

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ANNEXURE-I
BOLD'S BASAL MEDIUM

Component medium	Stock solutions (g. L ⁻¹ d. H ₂ O)	Quantity used(mL)	Concentration used in Final medium (M)
Macronutrients			
NaNO ₃	25.00	10	2.94×10 ⁻³
CaCl ₂ . 2H ₂ O	2.50	10	1.70×10 ⁻⁴
MgSO ₄ . 7H ₂ O	7.50	10	3.04×10 ⁻⁴
K ₂ HPO ₄	7.50	10	4.31×10 ⁻⁴
KH ₂ PO ₄	17.50	10	1.29×10 ⁻³
NaCl	2.50	10	4.28×10 ⁻⁴
Alkaline EDTA Solution			
EDTA	50.00	—	1.71×10 ⁻⁴
KOH	31.00	—	5.53×10 ⁻⁴
Acidified Iron Solution			
FeSO ₄ . 7H ₂ O	4.98	—	1.79×10 ⁻⁵
H ₂ SO ₄	—	01	—
Boron Solution			
H ₃ BO ₃	11.42	01	1.85×10 ⁻⁴
Trace Metals Solution			
ZnSO ₄ . 7H ₂ O	8.82	01	3.07×10 ⁻⁵
MnCl ₂ . 4H ₂ O	1.44	01	7.28×10 ⁻⁶
MoO ₃	0.71	01	4.93×10 ⁻⁶
CuSO ₄ . 5H ₂ O	1.57	01	6.29×10 ⁻⁶
Co(NO ₃) ₂ . 6H ₂ O	0.49	01	1.68×10 ⁻⁶

*pH of Bold's Basal medium = 6.6

ANNEXURE-II
CHU# 10 MEDIUM

Component medium	Stock solutions (g. L ⁻¹ d. H ₂ O)	Quantity used(mL)	Concentration used in Final medium (M)
Ca(NO ₃) ₂	40.0	01	2.44×10 ⁻⁴
K ₂ HPO ₄	5.0	01	2.87×10 ⁻⁴
MgSO ₄ . 7H ₂ O	25.0	01	1.01×10 ⁻⁴
Na ₂ CO ₃	20.0	01	1.89×10 ⁻⁴
Na ₂ SiO ₃	25.0	01	2.05×10 ⁻⁴
FeCl ₃	0.8	01	4.93×10 ⁻⁴

*pH of CHU# 10 medium = 7.6