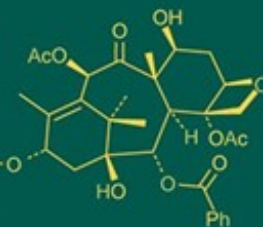
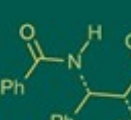


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Comparative growth performance and phycocolloids gel strength of three red marine algae during pre- and post-monsoon seasons

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ABSTRACT

Seaweed cultivation offers significant nutritional, ecological and commercial benefits, but species performance varies with seasonal and site-specific conditions. The present study evaluated the growth performance and phycocolloid quality of *Kappaphycus alvarezii*, *Gracilaria edulis* and *Gracilaria corticata* using raft culture at Mirya Bandar, Ratnagiri, Maharashtra, during pre- and post-monsoon seasons. Growth, biomass yield and environmental parameters were monitored fortnightly, while phycocolloid gel strength was assessed after harvest. During the pre-monsoon season, *G. edulis* showed the highest growth ($\text{DGR } 3.31 \pm 0.05\% \text{ day}^{-1}$) and biomass yield ($99.00 \pm 7.17 \text{ g line}^{-1}$), whereas *K. alvarezii* and *G. corticata* were affected by ice-ice disease and wave stress. During the post-monsoon season, *K. alvarezii* showed better performance, while grazing affected both *Gracilaria* species. Carrageenan gel strength in *K. alvarezii* was $53.6 \pm 3.60 \text{ g cm}^{-2}$, compared with agar gel strengths of 17.22 ± 0.43 and $13.00 \pm 0.90 \text{ g cm}^{-2}$ in *G. edulis* and *G. corticata*, respectively. The results indicate seasonal differences in species suitability and highlight the importance of environmental conditions in determining seaweed growth and phycocolloid quality.

Keywords: *Kappaphycus alvarezii*, *Gracilaria edulis*, *Gracilaria corticata*, Raft culture, Seasonal variation, Growth performance, Biomass yield, Phycocolloid

Introduction

Global fisheries and aquaculture production reached 223.2 million tonnes in 2022, comprising 185.4 million tonnes of aquatic animals and 37.8 million tonnes of algae (Cottier-Cook *et al*, 2023; FAO, 2018) [7, 8, 12, 13]. Global seaweed production has approximately tripled since 2000, reaching about 38 million tonnes in 2022, with most production originating from aquaculture. Seaweeds are increasingly valued as nutritious food resources and as sources of bioactive compounds and industrially important phycocolloids. They are rich in vitamins, minerals, amino acids, and trace elements and are widely used in food, pharmaceutical, cosmetic, and other industries (Dhargalkar and Pereira, 2005; Rajapakse and Kim, 2011) [9, 10, 32, 34]. In India, commercial seaweed utilization began during the Second World War, particularly with agar production from *Gracilaria* species (Rao and Mantri, 2006) [34, 36]. India possesses considerable marine algal diversity, with 1,153 species reported, including 442 red, 211 brown, and 212 green algae (Rao and Gupta, 2015) [33, 35]. The Maharashtra coast also supports substantial seaweed diversity, including 46 species reported from Ratnagiri waters (Redekar *et al*, 2007) [36, 39].

Seaweeds are important raw materials for commercially valuable phycocolloids such as agar, alginate and carrageenan. Increasing demand and pressure on natural resources highlight the need for sustainable cultivation and standardized production practices. Despite its potential, organized seaweed farming in India remains limited by seasonal environmental variations, site-specific constraints, and species-specific differences in growth and survival. Government initiatives such as the Pradhan Mantri Matsya Sampada Yojana (PMMSY) have further emphasized the development of seaweed cultivation, seed production, processing and marketing infrastructure.

The Konkan coast of Maharashtra, extending approximately 878 km, possesses considerable seaweed resources in intertidal and subtidal habitats (Redekar *et al*, 2007; Kavale *et al*, 2016) [23, 36, 39, 25]. However, systematic studies evaluating the cultivation potential of commercially important seaweed species under seasonal conditions in this region remain limited. Therefore, the present study was undertaken at Mirya Bandar, Ratnagiri, to compare the growth performance and biomass yield of *Kappaphycus alvarezii*, *Gracilaria edulis*, and *Gracilaria corticata* using raft culture during pre- and post-monsoon seasons. The study also evaluated the gel strength of carrageenan from *K. alvarezii* and agar from *Gracilaria spp.* to assess their phycocolloid quality.

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Materials and Methods

Site Selection: The experimental site for seaweed cultivation was selected based on the site-selection criteria suggested by Prema (2013) [31, 33] and Trono (1994) [43, 46]. Accordingly, Mirya Bandar, Ratnagiri coast, Maharashtra, India (17.039542° N, 73.269939° E), was selected as the experimental site (Fig. 1).

Raft Construction: A floating bamboo raft (1.0 × 1.0 m) was constructed using locally available bamboo poles of 3 - 4 inch diameter. The raft was covered with commercial fishnet of 10-mm mesh size on both the upper and lower sides for protection. It was anchored using 6 mm polypropylene rope attached to natural stones, following Veeragunanathan *et al.* (2015) [44].

Seedlings Material: Seed material of *Kappaphycus alvarezii* (Doty) Doty ex P.C. Silva, 1996 and *Gracilaria edulis* (S.G. Gmelin) P.C. Silva, 1952 was procured from Mandapam, Tamil Nadu. Seed material of *Gracilaria corticata* (J. Agardh) J. Agardh, 1852 was collected from natural stocks in and around the Ratnagiri region. The live material was transported in moist jute sacks to minimize desiccation and was acclimatized in natural seawater at the culture site for 2 - 3 days before deployment.

Raft Culture and Experimental Design: A 60 day raft culture experiment was conducted during the pre-monsoon (22 March - 21 May 2017) and post-monsoon (27 September - 26 November 2017) seasons. Seven monolines were initially deployed with *K. alvarezii*, *G. edulis*, and *G. corticata*. After 15 days, three monolines of *K. alvarezii* and *G. corticata* were detached due to ice-ice disease and hydrodynamic stress. Four lines (n = 4) remained intact and were subsequently used for growth assessment

- *K. alvarezii* was stocked at 2.5 kg m⁻², following Gunalan *et al.* (2010) [14, 15], with approximately 15 cm spacing between planting points.
- *G. edulis* and *G. corticata* were stocked at 1.0 kg m⁻², following Chennubhotla *et al.* (1992) [5, 6], with uniform 15 cm spacing between planting points.

Growth Assessment: Fortnightly sampling was carried out to monitor seaweed growth based on changes in biomass. Random samples were collected from the experimental lines, and wet weight was recorded using a commercial weighing balance with an accuracy of 0.5 g

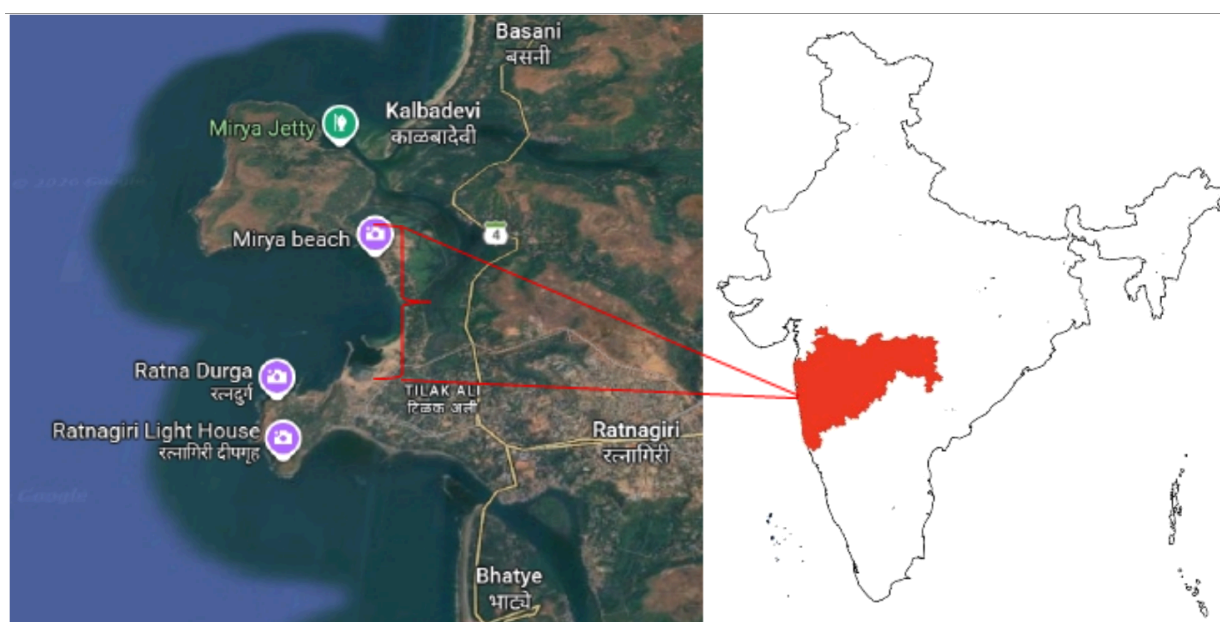


Figure 1. Experimental cultivation location - Mirya Bandar, Ratnagiri district, Maharashtra west coast of India.

a) The daily growth rate (%) was calculated using the formula given by Dawes *et al.* (1994) [8, 9]:

$$DGR (\%) = \frac{\ln(W_f - W_o)}{t} \times 100$$

b) The plant yield (Y, g⁻¹line⁻¹) was calculated using the formula of Doty (1986) [10, 11]:

$$Y = \frac{\ln(W_f - W_o)}{Lt} \times 100$$

Environmental Parameters: Water temperature, pH, and transparency were recorded weekly throughout the experimental period. Temperature was measured using a GH Zeal Ltd. thermometer (London, England) with a precision of ±1 °C, while pH was measured using an Eutech EcoTestr pH meter with a precision of ±0.1. Water transparency was determined using a 20 cm diameter Secchi disc. Water samples were collected weekly for analysis of nitrite (NO₂⁻), nitrate (NO₃⁻), phosphate (PO₄³⁻), silicate (SiO₄), dissolved oxygen (DO), and salinity following standard analytical methods

Extraction and Characterization of Phycocolloids: Phycocolloid extraction was carried out using seaweed harvested during the pre-monsoon season. Carrageenan from *Kappaphycus alvarezii* was extracted following Anggadiredja *et al.* (2009) [1]. Briefly, 50 g of dried seaweed was washed thoroughly with tap water and treated with 6% KOH for 3 h at 80 °C. The material was washed overnight under slow-running tap water and boiled for 1 h until disintegration. The extract was filtered using a pressure pump, treated with 0.2% KCl, frozen at -35 °C for 24 h, and thawed at room temperature. The extracted material was soaked in ethanol and dried at 50 °C.

Agar from *Gracilaria edulis* and *G. corticata* was extracted following Orosco *et al.* (1992) [27, 29] and Anggadiredja *et al.* (2009) [1]. A 50 g dried sample was treated with 500 mL of 5% NaOH at 90 °C for 2 h, washed with tap water for 30 min, and treated with 1,500 mL of 1.5% H₂SO₄ at room temperature. The material was subsequently washed for 12 h and extracted with 1,500 mL distilled water at 110 °C for 60 min in an autoclave. The extract was filtered, squeezed through cloth, and allowed to gel. The gel was frozen at -30 °C, thawed at room temperature, and oven-dried at 80 °C.

Determination of Gel Strength: Gel strength of the extracted phycocolloids was determined according to Thrimawithana *et al.* (2010) [45]. A 1.5 g sample of dried phycocolloid was dissolved in 100 mL distilled water and stirred continuously at 90 °C for 30 min. The solu

tion was maintained in a water bath at 90 °C for 15 min to remove air bubbles. Approximately 22 mL of the solution was transferred into three 50 mL beakers, cooled for 30 min, sealed, and stored for 24 h at 28 °C. Gel strength was measured using a TexVol TVT-300 XP (Perton Instruments) equipped with a 12.7 mm diameter probe. The probe was compressed to a depth of 15 mm at a cross-head speed of 2 mm s⁻¹. Each sample was analysed in triplicate (n = 3).

Statistical analysis: Statistical analysis of the data was carried out as per Snedecor and Cochran (1967) [39, 42] as well as statistical software SPSS-16 and SAS.

Results

Pre-monsoon growth performance

Daily Growth Rate (DGR %): The mean daily growth rate (DGR) of the three seaweed species was estimated at 15-day intervals throughout the 60-day culture period. At harvest, the highest DGR was recorded in *Gracilaria edulis* ($3.31 \pm 0.05\%$ day⁻¹), followed by *Kappaphycus alvarezii* ($3.14 \pm 0.05\%$ day⁻¹) and *Gracilaria corticata* ($2.67 \pm 0.08\%$ day⁻¹). A declining trend in DGR was observed with increasing culture duration. Linear regression analysis was performed between culture duration (15, 30, 45 and 60 days) and DGR for each species (Fig. 1). ANCOVA indicated that the regression slopes and elevations did not differ significantly among the three species ($P > 0.05$). The maximum growth rate recorded during the experimental period was % day⁻¹ in *G. edulis*.

Plant Yield: Mean plant yield of the three seaweed species was estimated fortnightly over the 60-day culture period. At harvest, the highest plant yield was recorded in *G. edulis* (99.00 ± 7.17 g line⁻¹), followed by *K. alvarezii* (77.75 ± 5.55 g line⁻¹) and *G. corticata* (41.25 ± 4.75 g line⁻¹). Plant yield increased progressively with culture duration in all three species. Linear regression relationships between culture duration and plant yield were established separately for each species (Fig. 2). ANCOVA revealed significant differences in the regression relationships among the species ($P < 0.05$). The maximum plant yield increment was 1.779 g day⁻¹ in *G. edulis*.

Environmental Parameters: Water quality parameters, including temperature (°C), pH, dissolved oxygen (mg L⁻¹), salinity (psu), transparency (cm), nitrite (mg L⁻¹), nitrate (mg L⁻¹), phosphate (mg L⁻¹), and silicate (mg L⁻¹), were monitored weekly throughout the 60-day culture period. The recorded physical and chemical parameters and nutrient concentrations are presented in Fig. 3a-c.

Gel Strength of Carrageenan and Agar: The gel strength of carrageenan extracted from *Kappaphycus alvarezii* is presented in Fig. 2a. The maximum gel strength was 53.60 ± 3.60 g cm⁻² at 2 s, with a viscosity of 839.8 cP and a gelling temperature of 43.2 °C. The agar gel strength of *Gracilaria edulis* and *G. corticata* is shown in Fig. 2b and 2c, respectively. *G. edulis* recorded a gel strength of 17.22 ± 0.43 g cm⁻² at 2 s, with a viscosity of 439.9 cP and a gelling temperature of 47.0 °C. In *G. corticata*, the gel strength was 13.00 ± 0.90 g cm⁻² at 0.5 s, with a viscosity of 314.9 cP and a gelling temperature of 64.2 °C. Overall, gel strength showed a declining trend with increasing measurement time.

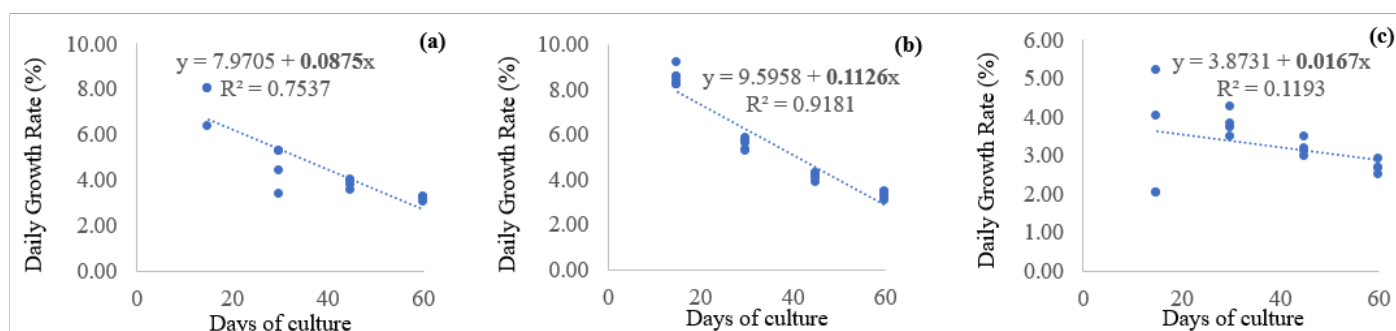


Figure 1. Relationship between days and mean daily growth rate (%) during pre-monsoon season (a) *Kappaphycus alvarezii* (b) *Gracilaria edulis* (c) *Gracilaria corticata*

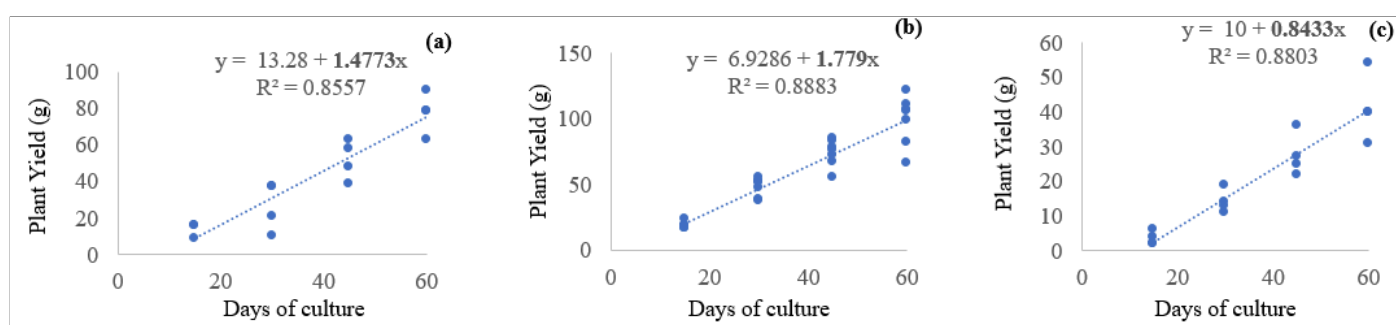


Figure 2. Relationship between days and mean plant yield (g) during pre-monsoon season (a) *Kappaphycus alvarezii* (b) *Gracilaria edulis* (c) *Gracilaria corticata*

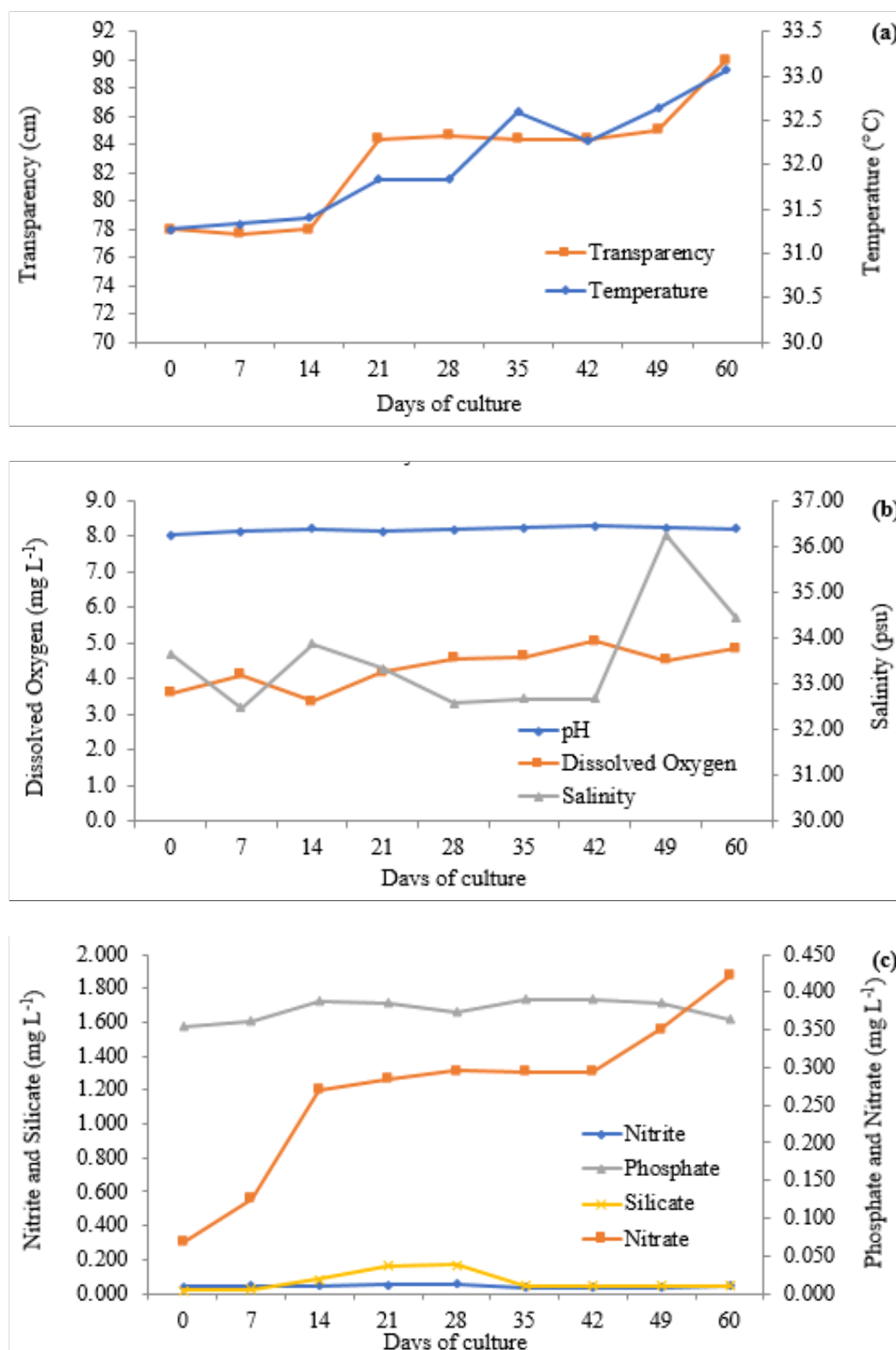
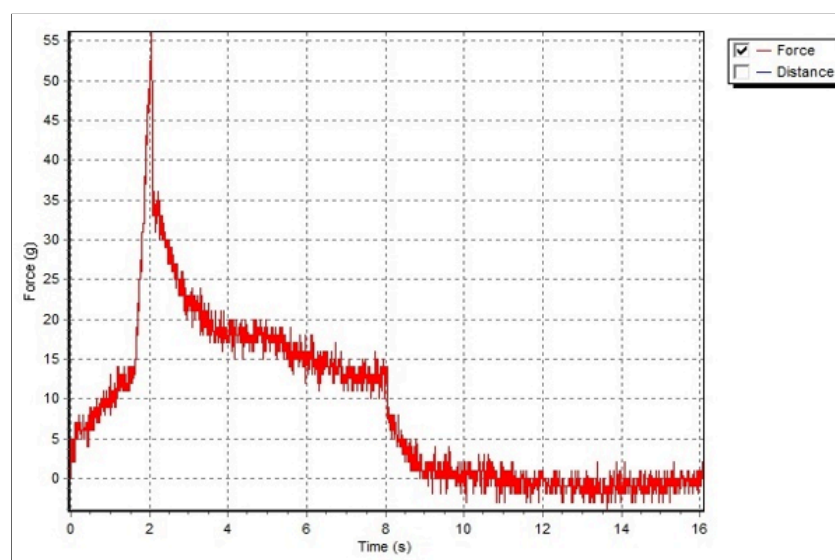
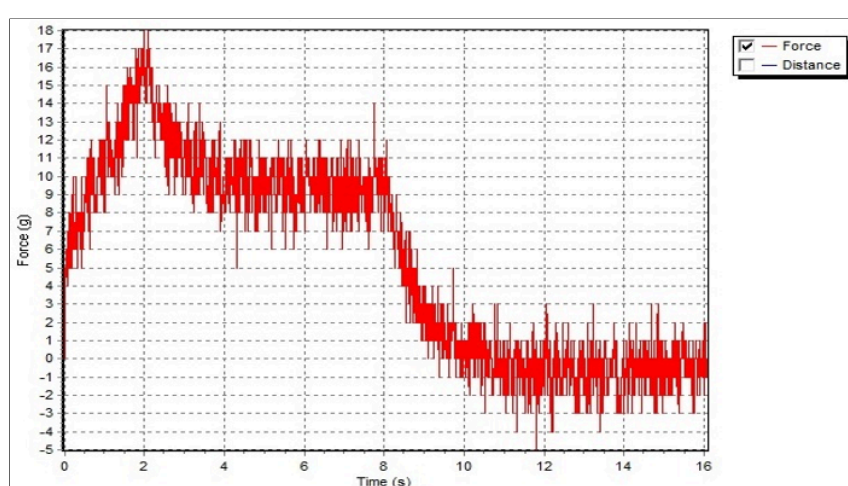


Figure 3. Variations in environmental parameters during the pre-monsoon season a) Physical parameters b) Chemical parameters c) nutrients content



(a) Mean gel strength of carrageenan (g cm^{-2}) from *Kappaphycus alvarezii*.



(b) Mean gel strength of agar-agar (g cm^{-2}) from *Gracilaria edulis*.

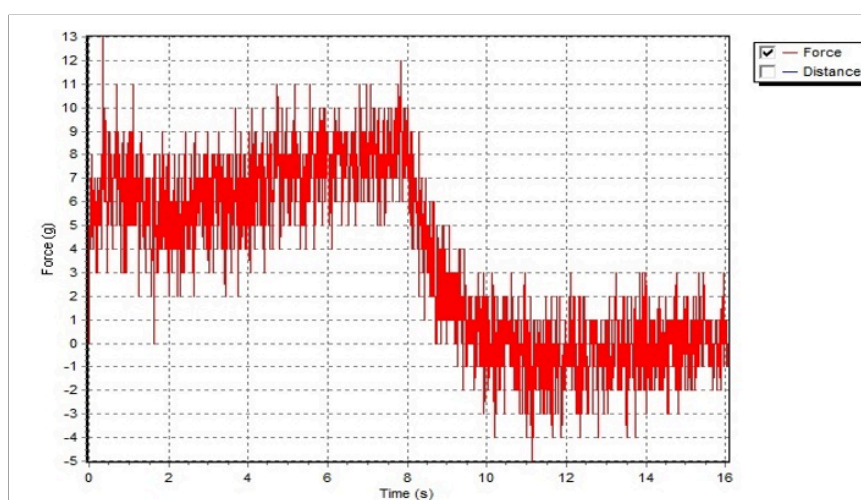


Figure 2. Mean gel strength of Phycocolloid
(c) Mean gel strength of agar-agar (g cm^{-2}) from *Gracilaria corticata*

Post-monsoon growth performance

Daily Growth Rate (DGR, % day^{-1}): At harvest, the highest DGR was recorded in *Kappaphycus alvarezii* ($4.80 \pm 0.02\% \text{ day}^{-1}$), whereas the lowest was observed in *Gracilaria edulis* ($2.60 \pm 0.45\% \text{ day}^{-1}$). DGR showed a declining trend with increasing culture duration. Linear regression was performed between culture duration (15, 30, 45 and 60 days) and DGR for each species (Fig. 4). ANCOVA indicated no significant differences in regression slopes and elevations

among the species ($P > 0.05$). The maximum growth rate obtained during the culture period was $0.1504\% \text{ day}^{-1}$ in *K. alvarezii*.

Plant Yield (Y): Mean plant yield was estimated at 15 day intervals over the 60 day culture period. The highest plant yield was recorded in *K. alvarezii* ($765.14 \pm 23.10 \text{ g line}^{-1}$), followed by *Gracilaria corticata* and *G. edulis*. Plant yield increased progressively with culture duration in all three species. Linear regression was performed between culture duration and plant yield for each species (Fig. 5). ANCOVA indicated significant differences in regression slopes and/or

elevations among the species ($P < 0.05$). The maximum plant-yield increment was $15.567 \text{ g day}^{-1}$ in *K. alvarezii*.

Environmental Parameters: During the 60 day experimental period, temperature ($^{\circ}\text{C}$), pH, dissolved oxygen (mg L^{-1}), salinity (psu), and

transparency (cm), along with nutrient concentrations of nitrite, nitrate, phosphate, and silicate (mg L^{-1}), were recorded weekly. The observed environmental parameters are presented in Fig. 6.

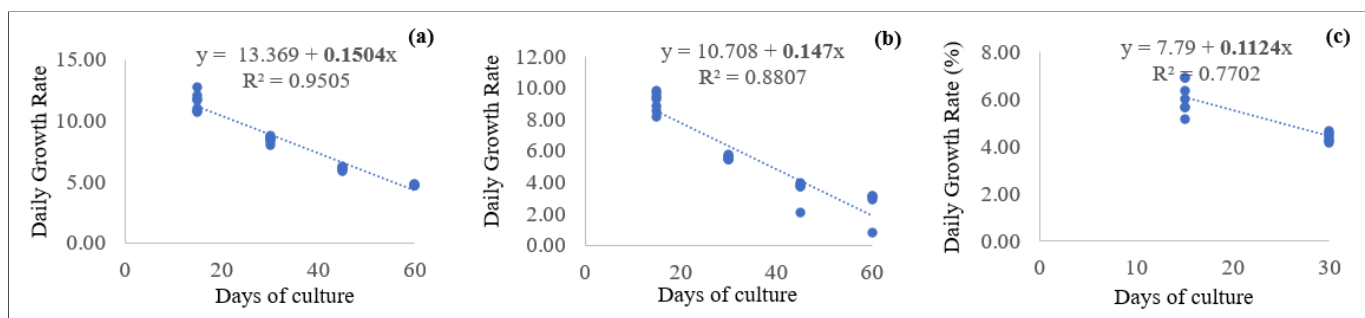


Figure 4. Relationship between days and mean daily growth rate (%) during post-monsoon season (a) *Kappaphycus alvarezii* (b) *Gracilaria edulis* (c) *Gracilaria corticata*

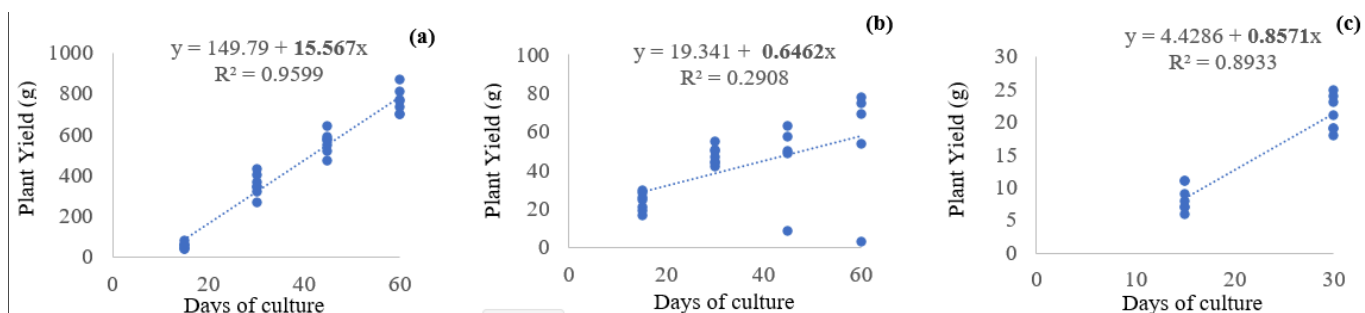
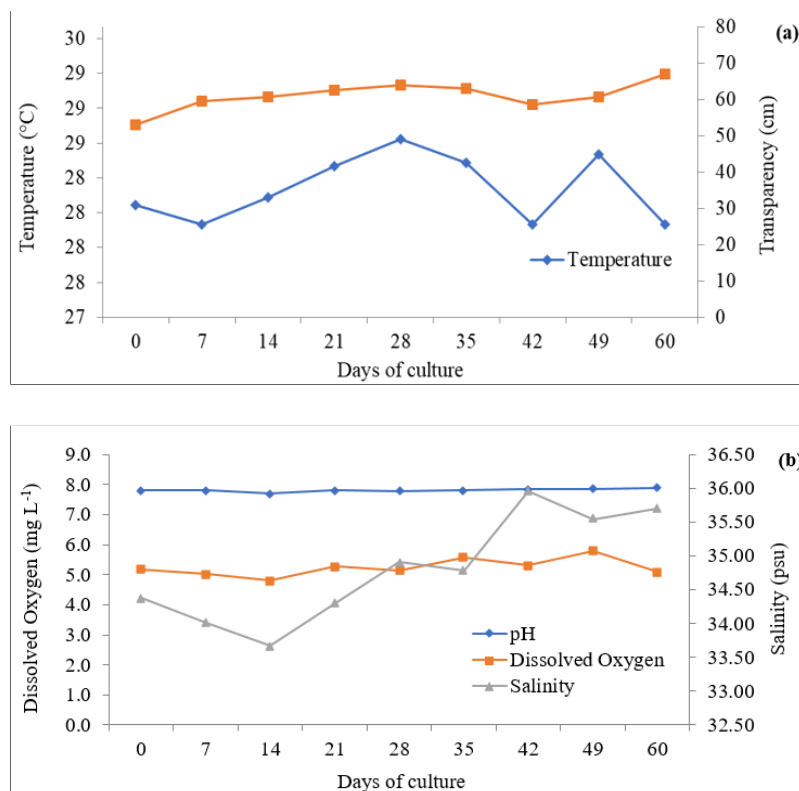


Figure 5. Relationship between days and mean plant yield (g) during post-monsoon season (a) *Kappaphycus alvarezii* (b) *Gracilaria edulis* (c) *Gracilaria corticata*



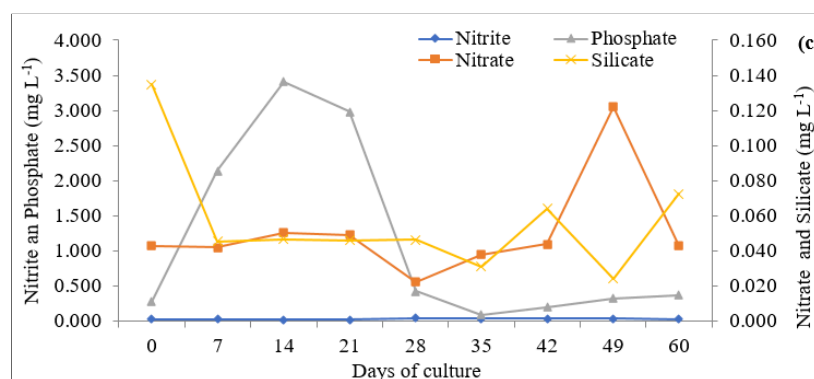


Figure 6. Variations in environmental parameters during the post-monsoon season a) physical parameters b) chemical parameters c) nutrients content

Discussion

Successful seaweed cultivation depends on suitable environmental conditions, including protection from strong waves and winds relatively calm waters, adequate water movement, and sufficient nutrient availability. The study site at Mirya Bandar was selected based on the site-selection criteria reported by Prema (2013) [31, 33] and Reddy *et al.* (2018) [35, 37]. The environmental conditions recorded during the experimental periods were generally within ranges considered suitable for seaweed cultivation and were comparable with observations reported from Indian and other tropical waters (Herliany & Meylia, 2016; Rajeki *et al.* 2018; Susilowati *et al.*, 2020; Kaliaperumal *et al.*, 1993, 2003; Subba Rao *et al.* 2008; Ganesan *et al.*, 2011; Veeragurunathan *et al.*, 2015; Meena, 2018; Periyasamy *et al.*, 2017; Warnadi *et al.*, 2018) [19,40,44,23,24,43,14,47,27,31,48].

Comparative growth performance during the pre-monsoon season

During the pre-monsoon season, *Gracilaria edulis* exhibited the highest growth performance among the three species, based on DGR and plant yield. The relatively lower performance of *Kappaphycus alvarezii* may be associated with the high water temperature recorded during the study period. Water temperature ranged from 31 to 33 °C, which may have favoured the development of ice-ice disease in *K. alvarezii*, as reported by Ohno *et al.* (1994) [26, 28], Gunalan *et al.* (2010) [14, 15], Johnson and Gopakumar (2011) [20, 22] and Arasamuthu and Edward (2018) [2]. The occurrence of disease and subsequent loss of culture lines reduced the harvested biomass of *K. alvarezii* and *G. corticata*. However, the surviving plants continued to exhibit appreciable growth, indicating their ability to tolerate the prevailing culture conditions.

The other physical and chemical parameters, including the nutrient profile, remained within ranges favourable for seaweed growth, as reported by Reddy *et al.* (2018) [35, 37]. Both *Gracilaria* species recorded DGR values exceeding 2% day⁻¹, which is considered indicative of good growth in *Gracilaria* spp. (Hendri *et al.*, 2018) [16, 17]. The comparatively higher growth and biomass production of *G. edulis* therefore suggest that this species was better adapted to the environmental conditions prevailing at Mirya Bandar during the pre-monsoon period.

Comparative growth performance during the post-monsoon season

The growth pattern changed during the post-monsoon season, with *Kappaphycus alvarezii* recording the highest DGR and plant yield. This seasonal improvement may reflect more favourable environmental conditions following the monsoon. In contrast, the growth of the *Gracilaria* species was adversely affected by grazing pressure. During the experiment, *Lutjanus argentimaculatus* was observed feeding on the cultured *Gracilaria* biomass. Herbivory can substantially reduce seaweed biomass, and biomass losses of up to 30% have

been reported in seaweed culture systems (Bindu *et al.*, 2011) [3]. Similar effects of grazing on cultured seaweeds have been documented by James *et al.* (1984) [18, 20], Chennubhotla *et al.* (1986) [6, 7], Kaliaperumal *et al.* (1993, 2003) [23, 24] and Ganesan *et al.* (2011) [13, 14].

The contrasting performance between seasons indicates that seaweed growth at Mirya Bandar was strongly influenced by seasonal environmental conditions and species-specific responses. Thus, selection of species according to season may be an important consideration for improving productivity under raft-based cultivation.

Gel strength of phycocolloids

The phycocolloid characteristics differed among the species evaluated. *Kappaphycus alvarezii* produced carrageenan with a mean gel strength of 53.60 ± 3.60 g cm⁻² and a viscosity of 839.8 cP. The observed gel strength was higher than the values reported by Eswaran *et al.* (2002) [11, 12] (244.44 ± 1.29, as reported) and Periyasamy *et al.* (2016) [30, 32] (322.70 ± 10.52), although direct comparison should be made cautiously because extraction procedures and analytical conditions can influence phycocolloid properties. Studies outside India have reported carrageenan gel strengths ranging from 42.54 – 70.41 g cm⁻² (Hasizah *et al.*, 2021) [15, 16] and 50.40 – 114.40 g cm⁻² (Bono *et al.*, 2014) [4].

Among the *Gracilaria* species, *G. edulis* produced higher agar gel strength (17.22 ± 0.43 g cm⁻²) than *G. corticata* (13.00 ± 0.90 g cm⁻²). Reported agar gel strengths for *Gracilaria* spp. vary considerably, ranging from 17–27 g cm⁻² (Oza, 1981) [28, 30], 69 – 139 g cm⁻² (Chennubhotla *et al.* 1986) [6, 7], 22 g cm⁻² (Marinho-Soriano, 2001) [24, 26], and 36 – 180 g cm⁻² (Romero *et al.* 2007) [38, 41]. Such variation may result from differences in species, geographic location, physiological condition, harvesting age, cultivation method, and extraction procedures. In particular, the extraction process and whether material is obtained from vegetative tissue or cultivated seedlings can influence phycocolloid characteristics (Yong *et al.* 2014; Jayasinghe *et al.* 2016) [49,21].

Overall, the present study demonstrated clear seasonal differences in the performance of the three seaweed species. *G. edulis* showed superior growth and biomass production during the pre-monsoon season, whereas *K. alvarezii* performed better during the post-monsoon season. Disease and grazing were important biological constraints affecting biomass production. The phycocolloid analysis further demonstrated species-specific differences, with *K. alvarezii* yielding carrageenan of higher gel strength and viscosity, while *G. edulis* produced agar with higher gel strength than *G. corticata*. These findings highlight the importance of seasonal species selection and standardized cultivation and extraction practices for optimizing seaweed biomass production and phycocolloid quality.

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