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Seasonal Biomass Dynamics and Zygote Cultivation of *Sargassum aquifolium* on Different Substrates in the Red Sea

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ABSTRACT

Sargassum aquifolium (Turner) C. Agardh is one of the most abundant and widely distributed seaweed species in the Red Sea. This study examined the seasonal fluctuations in biomass and evaluated the effectiveness of zygote cultivation on various substrates, including interlock stone, cement stone, limestone, and nylon string. The findings indicated significant seasonal variations in biomass, with peak growth observed in summer, reaching 828±70gm.m² of fresh weight. The success of zygote attachment and growth varied by substrate, with recruit density highest on cement stone, followed by interlock stone, and then limestone over a five-month observation period. Germlings exhibited a higher daily growth rate (DGR) on cement stone at 56, 112, 140, and 168 days. Young juveniles showed a consistent increase in length up to 168 days post out-planting, with some fluctuations. These insights are crucial for developing sustainable aquaculture practices for *Sargassum aquifolium*, guiding substrate selection, and improving productivity in the Red Sea.

INTRODUCTION

Sargassum is a genus of brown macroalgae (Phaeophyceae, Ochrophyta) commonly found in tropical and subtropical waters of the Indo-Pacific region, including the Red Sea. This species plays a crucial role in marine ecosystems by providing habitat and food for a wide range of marine organisms including fish, invertebrates, and microorganisms **(Omer *et al.*, 2021)**. Its complex structure offers breeding grounds and protection, supporting biodiversity and the health of marine ecosystems. In addition to its ecological roles, it is economically valuable, particularly in industries such as agriculture, pharmaceuticals, and food. It is used as a fertilizer **(Kumari *et al.*, 2013)**, a source of bioactive compounds for medicinal and nutritional purposes **(Moorthi *et al.*, 2015; Milledge *et al.*, 2016)**, and animal feed **(Schleder *et al.*, 2017)**.

The Red Sea, with its warm waters, provides an ideal habitat for various *Sargassum* species, including *Sargassum aquifolium* **(El-Manawy, 2008; Ibraheem *et al*., 2014; Galal El-Din & Rashedy, 2023)**. This species is particularly notable for forming extensive beds along rocky shores and is especially abundant in the region, reaching peak growth during the hot summer months **(Ateweberhan *et al*., 2009; Rashedy *et al*., 2022; Rashedy *et al*., 2023)**. Population fluctuations of *Sargassum* are closely tied to seasonal variations in environmental factors such as water temperature, light availability, tidal levels, and wave energy **(Low *et al*., 2019)**. Among these factors, seawater temperature is considered the most significant in regulating the seasonal growth and reproductive cycles of *Sargassum*, although its influence can vary across different regions **(Fulton *et al*., 2014)**.

Sargassum is widely cultivated in China, Japan, and Korea where it is used both as a food source (sea vegetables) and in traditional medicine **(Yu *et al.*, 2013; Kim *et al*., 2017; Amlani & Yetgin, 2022)**. In Korea, the longline method is a traditional cultivation technique where *Sargassum* seedlings, measuring 5-10cm, are inserted into ropes at intervals of 5-10cm **(Pang *et al*., 2008)**. Another approach involves seeding fertilized *Sargassum* eggs onto nylon strings in indoor tanks under semi-controlled conditions to support early development. The attached zygotes are then allowed to grow into seedlings, which, once mature enough for field cultivation, are transferred to larger culture ropes and out-planted in floating longlines **(Hwang *et al*., 2006)**. They concluded to the hypothesis that production practices for *Sargassum* in China are suited for sub-temperate and sub-tropical species and have been modified from methods originally employed for kelps, such as Saccharina and Undaria **(Hwang *et al*., 2019)**.

In the Red Sea, unique environmental conditions, such as high salinity and temperature, present both challenges and opportunities for the cultivation of *Sargassum*. This study explores the biomass seasonality of *S. aquifolium* and evaluates its zygote cultivation on various substrates, aiming to optimize growth conditions and to enhance the potential for sustainable cultivation in this region.

MATERIALS AND METHODS

Study area and seasonal dynamics of *S. aquifolium*

This study was carried out along the Red Sea coast in Hurghada, Egypt, directly in front of the National Institute of Oceanography and Fisheries (NIOF), located between 27°17'13" N latitude and 33°46'21" E longitude (Fig. 1). The site is characterized by extensive reef flats, which extend approximately 5km seaward, with depths ranging from 1.5 to 6 meters. To monitor temporal changes in *Sargassum* biomass, the intertidal zone was divided into three sections. In each section, three horizontal transects, 20 meters long and 50 meters apart, were established parallel to the coastline, spanning from the highest tidal mark to the reef edge. Along each transect, three 1m² quadrats were randomly placed. The *Sargassum aquifolium* in each quadrat was scraped off, rinsed with seawater, and weighed (fresh weight) before being shade-dried (dry weight). The size and number of quadrats were determined through trial monitoring and are consistent with methods used by **Schaffelke and Klumpp (1997)** and **El-Manawy (2008)**. Seasonal sampling visits were regularly conducted at the site during summer (August 2023), autumn (November 2023), winter (February 2024), and spring (May 2024).

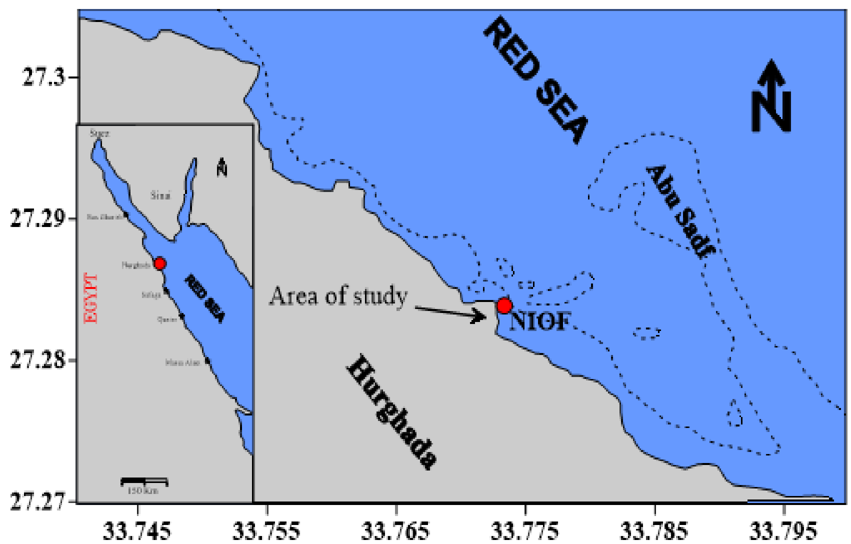

Fig. 1. Map illustrating the location of the study site

Physiochemical characterization of the *Sargassum* collection site

At the sampling site, water temperature, dissolved oxygen (DO), salinity, and pH were measured using the YSI ProODO multi-parameter instrument, based on the average of three readings. Nutrient concentrations, including dissolved nitrate (NO_3), dissolved ammonium (NH_4^+), and dissolved inorganic phosphate (PO_4), were determined spectrophotometrically in µM, following the guidelines of **APHA (2005)**.

Collection of fertile *Sargassum*

Mature parent plants of *Sargassum aquifolium* (fertile thalli, Fig. 2) were harvested from the intertidal zone at the Hurghada sampling site in Egypt during autumn (October 2023) (Fig. 1). The collected samples were promptly transported to the NIOF laboratory, where the thalli were thoroughly cleaned of epiphytes using a brush and rinsed with sterile, filtered seawater upon arrival.

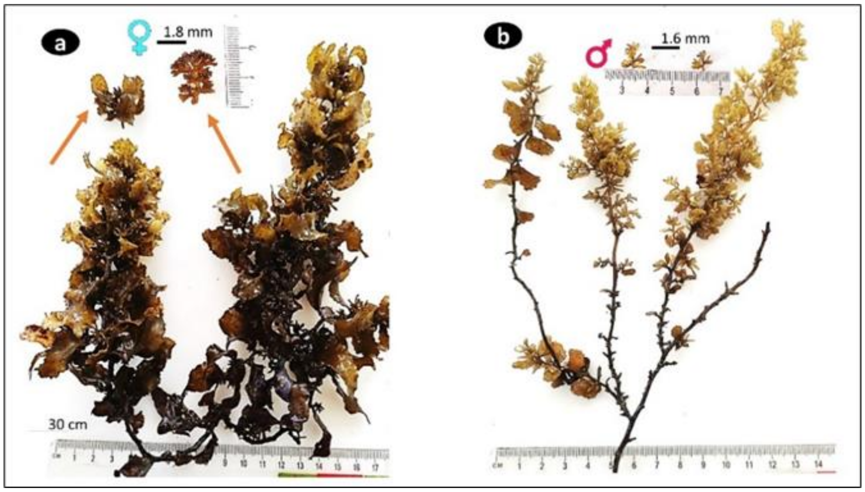

Fig. 2. Mature parent plants of *Sargassum aquifolium*. (**a**) Fertile thalli with receptacles containing female conceptacles. (**b**) Fertile thalli with receptacles containing male conceptacles

Cultivation of zygotes on different substrates under laboratory-controlled conditions

Recruitment tanks or boxes (capacity: 90L, dimensions: 35 × 25.5 × 20cm) were filled with filtered seawater 24 hours before starting the experiment. The tanks were then incubated in six replicates, maintaining a water temperature of 20-23°C, salinity of 40-41ppt, irradiance levels of 80−100 µmol photons m^{-2} s^{-1}, and a photoperiod of 12 hours. The seawater used in the culture was pumped from the sea in front of the NIOF laboratory and was filtered through sand and bag filters. Substrate panels made of interlock stone, cement stone, limestone, and nylon string were placed at the bottom of the recruitment tanks to serve as surfaces for zygote recruitment.

Mature male and female thalli were mixed in a 1:1 ratio and floated on the surface of the tanks. The reproductive thalli were positioned directly over the substrate to allow zygote to naturally drop onto it, following the method described by **Xie *et al*. (2013)**. According to **Redmond *et al*. (2014)**, the simultaneous release of gametes generally takes place within one week under ideal conditions. Once mature eggs are released from the conceptacle, they remain attached to the exterior of the receptacle, enveloped in a mucilage layer that safeguards the eggs and zygotes for several days before detaching from the parent plant. The progression of zygotes into embryos during settlement was observed over the first seven days using glass slides placed in each tank next to the substrates. Additionally, the density of settled zygotes on the substrates was assessed 24 hours after seeding. Fourteen days after recruitment, six recruits from each substrate type were randomly selected and tagged. Photos of the tagged recruits were taken on the same day, and then every seven days for a total of 168 days using a Nikon waterproof digital camera. The images were processed in the ImageJ software to measure growth by calculating the average length of the leaflet (from the tip to the base, excluding rhizoids).

The daily growth rate (DGR) was determined using the formula described by **Yong *et al.* (2013)**:

$$\text{DGR}=\left[\left(\frac{L_t}{L_o}\right)^{1/t}-1\right]\times 100\%$$

In this formula, DGR represents the mean daily growth rate, where L_t is the length at time t_2, L_o is the initial length, and t is the time in days.

Statistical analysis

All data were presented as mean of three replicates for each parameter ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA, followed by Fisher's grouping test. The number of replicate experiments was n=3. The seasonal variation of *S. aquifolium* are showed by Boxplots graph. All statistical analyses were performed using Minitab® software (Version 17).

RESULTS

1. Seasonal variation in environmental parameters

The tested physico-chemical parameters of the seawater exhibited significant seasonal differences ($P< 0.05$) during the study period (Table 1). Surface water temperature exhibited seasonal variation, ranging from a winter low of 20.9°C to a summer high of 30.46°C. Salinity reached its peak in summer at 43.23ppt, while the lowest value, 41.05ppt, was observed in autumn. pH values remained within a narrow range, fluctuating between 7.63 and 8.2.

Nutrient concentrations followed the pattern NH_4 > NO_3 > PO_4. The highest ammonium concentration, 4.78µM, was recorded in winter, while the lowest, 1.03µM, occurred in spring. Nitrate levels peaked at 1.75µM in winter and dropped to a minimum of 0.78µM in spring. Dissolved phosphate levels varied, with the highest concentration (0.19µM) observed in winter and the lowest (0.06µM) in summer.

Table 1. Seasonal variations in the physico-chemical parameters at the sampling site

Physical and Chemical Parameters of Seawater	**Summer 2023**	**Autumn 2023**	**Winter 2024**	**Spring 2024**
Temperature (ºC)	30.46 ± 0.12	21.38 ± 0.10	20.91 ± 0.03	29.03 ± 0.20
Salinity (ppt)	43.23 ± 0.05	41.05 ± 0.13	42.31 ± 0.35	41.12 ± 0.01
pH	7.89 ± 0.01	7.97 ± 0.01	8.2 ± 0.04	7.63 ± 0.05
Dissolved nitrate (µM)	1.52 ± 0.09	1.03 ± 0.14	1.75 ± 0.18	0.78 ± 0.14
Dissolved ammonium (µM)	2.69 ± 0.39	2.12 ± 0.45	4.78 ± 0.86	1.03 ±0.11
Dissolved inorganic phosphate (µM)	0.06 ±0.02	0.16 ± 0.01	0.19 ± 0.02	0.17 ± 0.06

2. Temporal dynamics of *S. aquifolium*

Box plots for *Sargassum aquifolium* cover and biomass (Fig. 3) reveal that the highest median values occurred in summer (59% cover and 865 gm. m^2 of fresh wt. biomass), followed by autumn (35% and 105 gm. m^2 of fresh wt.), then winter (10% and 75 gm. m^2 of fresh wt.). The lowest medians were recorded in spring (2% and 15 gm. m^2 of fresh wt.). The length of each box plot reflects the variability among quadrates within the same season, with summer displaying the greatest variability and spring the least. ANOVA results further indicated a significant difference in cover and biomass between seasons ($P = 0.000$, F = 68.11).

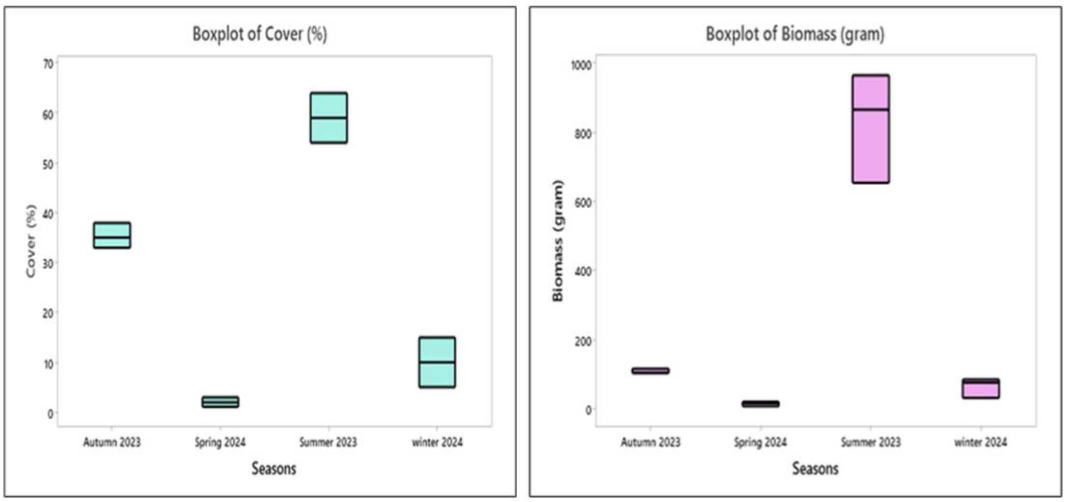

Fig. 3. Boxplots showing the variability of cover (%) and biomass of *S. aquifolium* during the four seasons at the studying site

3. The process of zygote development into juveniles

Initial trials revealed that liberated zygotes transformed into pear-shaped embryos, as observed on glass slides (Fig. 4c). During the first week of culture, the embryos began to elongate into club-shaped germlings, with an average diameter of 0.035±0.01mm and a length of 0.048mm, developing rhizoids after 48 hours (Fig. 4e). By day 28, the germlings had elongated further, reaching an average length of 0.280±0.03mm, with meristematic tissues and a protuberance becoming more prominent (Fig. 4f). The germlings continued to grow, with the development of a leaflet initial, appearing as a small protuberance, becoming visible after 140 days. By day 168, the first and second leaflets had undulated edges and reached their maximum average length (Fig. 4).

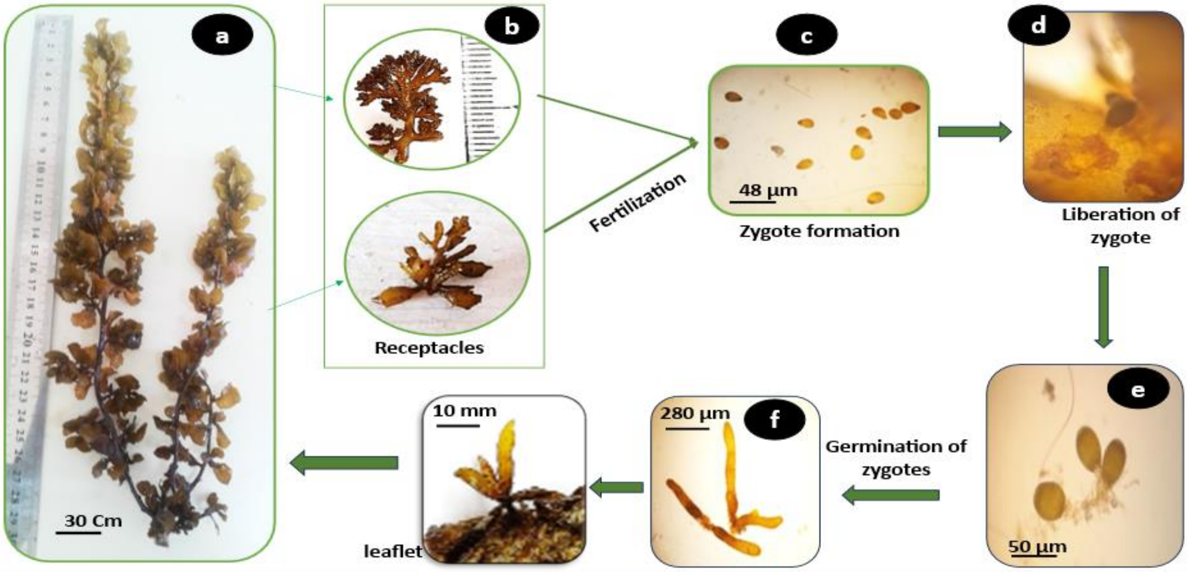

Fig. 4. Zygote development of *S. aquifolium*: a: Mature thallus (whole plant), b: Male and female receptacles, c, d, e: Zygote liberation from female, f: Zygote growth

4. Settlement of *Sargassum* zygotes on selected substrates

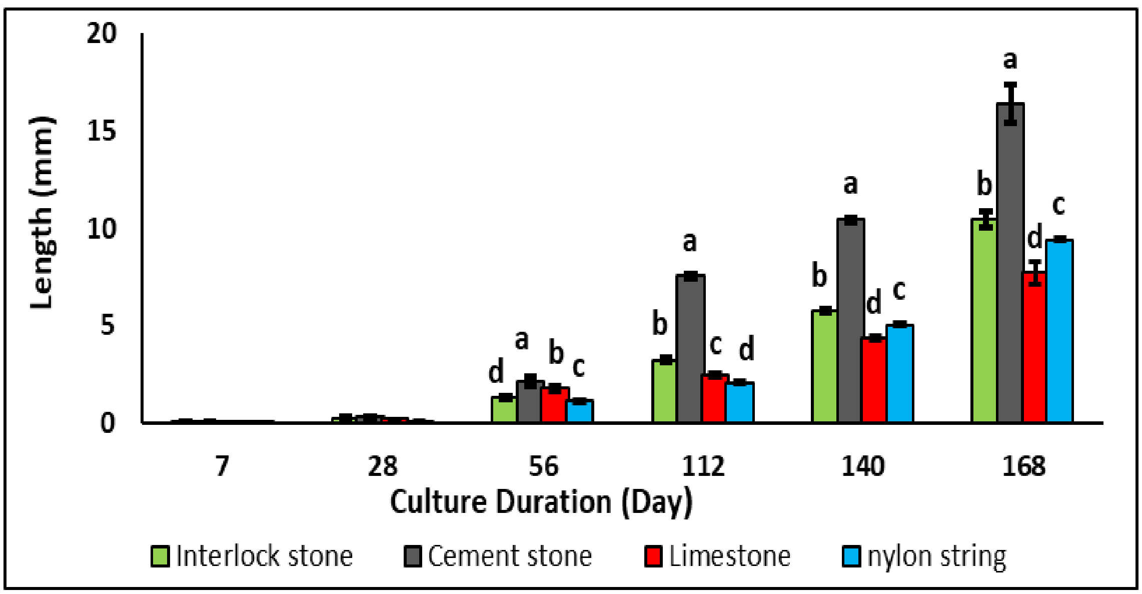

Fig. 5. Recruitment of *S. aquifolium* zygotes on selected substrates over a 168-day culture period. Each value represents the mean of three replicates. Different letters (a, b, c, and d) mean significant differences at $P \leq 0.05$

A notable interaction effect was observed among the various substrates tested, including interlock stone, cement stone, limestone, and nylon string. Cement stone demonstrated the highest effectiveness in supporting zygote recruitment and their development into juveniles. Zygote length on cement stone was 0.048mm at 7 days, reaching 16.73mm by day 168 (Figs. 5, 6). While zygote recruitment on limestone and interlock stone was excellent during the first 30 days of cultivation (Figs. 5, 6), the growth of germlings on these substrates at 140 and 168 days was lower compared to those on cement stone (Figs. 5, 6). Germlings on interlock stone, limestone, and nylon string exhibited fewer protuberances in their initial leaflets. By day 140, the number of leaflets had increased to five on both cement and interlock stone, and to four on nylon string. At this stage, all leaflets continued to elongate but retained a compressed shape, except for the newly formed ones. The first and second leaflets developed undulating edges, with the longest leaflets observed on the cement stone substrate (Figs. 5, 6).

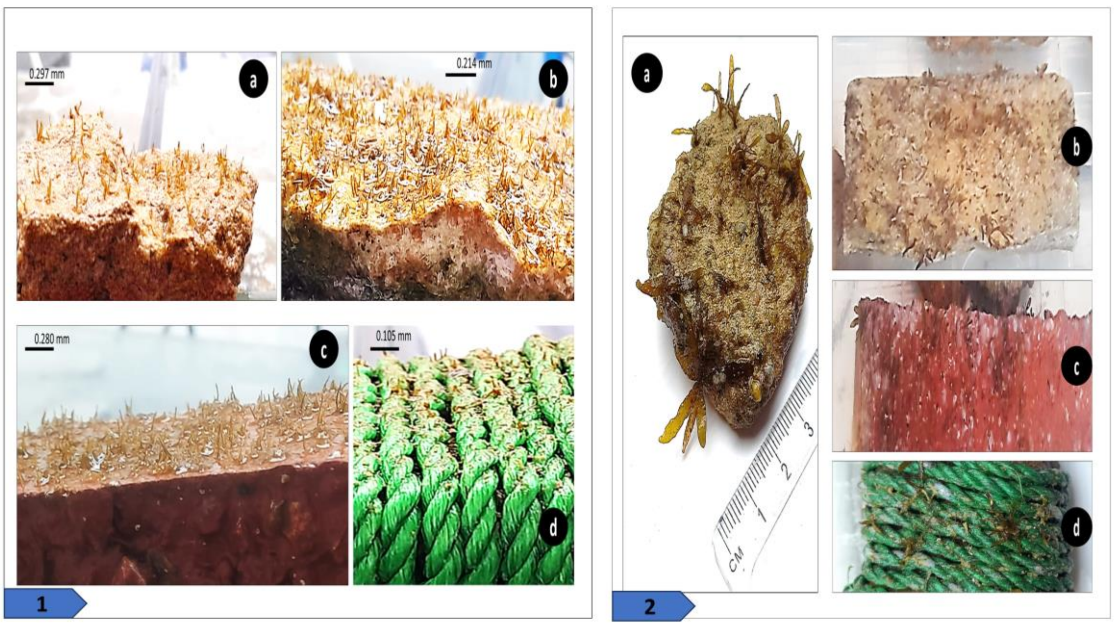

Fig. 6. *S. aquifolium* recruits on various substrates: **1-** After 30 days of cultivation, **2-** After 140 days of cultivation, (Cement stone: **(a)**, Limestone; **(b)** Interlock stone; and **(c)** Nylon string **(d)**)

5. Growth rate of zygotes cultivated under laboratory conditions

The daily growth rate (DGR) of *Sargassum aquifolium* germlings exhibited significant variation across the four substrate types and throughout the culture duration. Additionally, growth patterns differed across the various culture durations for each substrate type (Fig. 7). Rapid growth is observed during the initial phase (Day 7–28), with cement stone showing the highest growth rate (~60% day^{-1}), followed by limestone and interlock stone (~40–50% day^{-1}), while nylon string exhibits the lowest (~10% day^{-1}). Growth peaks at Day 28 and then steadily declines for all substrates. Cement stone maintains a higher growth rate than the others throughout the cultivation period, although it also decreases gradually. Interlock stone and limestone experience a faster decline, with their growth rates converging to low levels after day 112. Nylon string consistently displays the lowest growth performance, with minimal growth beyond day 56. By the final stage (Days 140–168), growth rates stabilize at low levels for all substrates, with cement stone showing a slight advantage over the others. These results highlight cement stone as the most favorable substrate for *S. aquifolium* cultivation, while nylon string is the least effective.

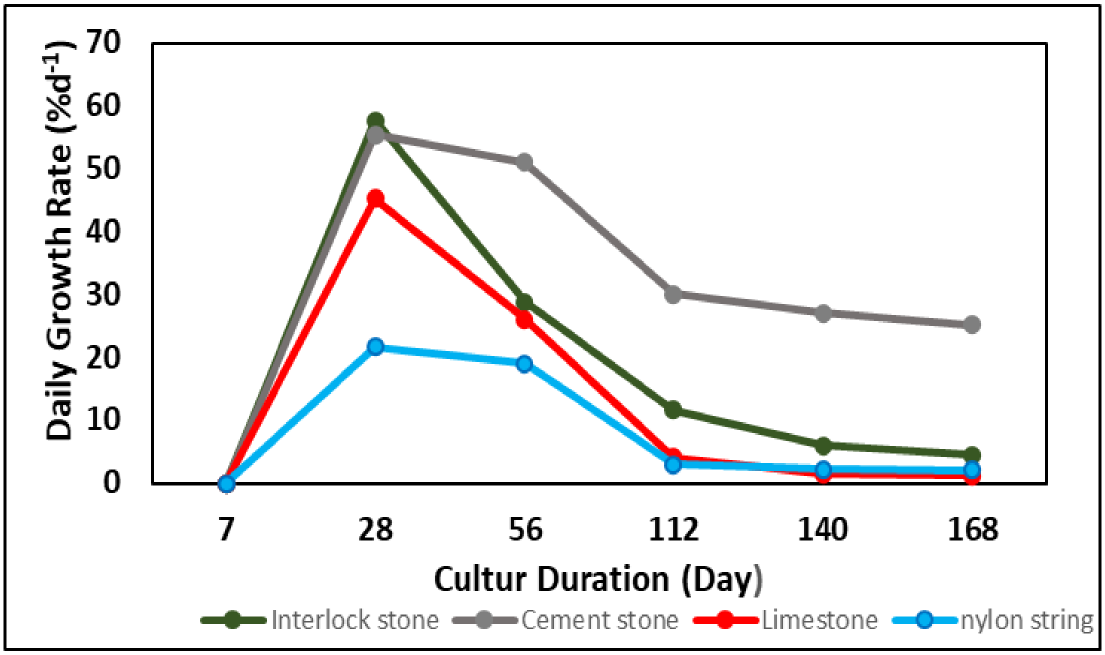

Fig. 7. Daily growth rate of *S. aquifolium* germlings on four different substrates from day 7 to day 168 of cultivation

DISCUSSION

The variation in five physicochemical parameters was examined across four seasons. Water temperature exhibited a strong association with seasonal fluctuations, with the lowest value recorded in winter (20.9 °C), and the highest in summer (30.46 °C). These findings align with **Shaltout (2019)**, who reported that water temperatures peak during summer and reach their minimum in winter. The seawater pH showed slight seasonal variation, ranging from 7.63 to 8.2, which may be attributed to the interaction among the decomposition of organic matter and buffering by carbonates and bicarbonates in seawater **(Rashedy *et al.*, 2022)**. Additionally, the high salinity of the Red Sea is a result of raised temperatures, the absence of river inflows, and high evaporation rates **(Fahmy *et al.*, 2016)**. Nutrients play a vital role in coastal environments, profoundly influencing the growth, reproduction, and metabolic activities of macroalgal species **(Hanelt &**

Figueroa, 2012). Although essential for seaweed growth, nutrients are generally present in low concentrations in marine waters, especially in the Red Sea **(Rashedy *et al.*, 2022)**. In this study, nutrient concentrations followed the pattern $NH_4 > NO_3 > PO_4$. The highest nutrient levels were observed during winter, while the lowest were recorded in spring and summer.

The temporal dynamics of *Sargassum aquifolium* in the Red Sea exhibit significant seasonal variation, with the highest cover (59%) and biomass (865g/m² fresh weight) recorded in summer, while the lowest values were observed in spring (2% cover and 15 g/m² fresh weight). **Rashedy *et al.* (2022)** reported that *Sargassum* was the dominant canopy species on the reef flat at NIOF, persisting through winter via its basal parts. Similarly, **Ateweberhan *et al.* (2009)** found a positive correlation between the abundance of *S. aquifolium* and sea surface temperature in the Red Sea reefs. The reproductive phenology of *S. aquifolium* in this study follows a summer–autumn (August–October) growth period, with reproduction occurring in late autumn (November–December), followed by senescence in winter. However, these patterns may not apply to temperate populations. Observations of reproductive phenology in subtropical *Sargassum* species suggest that winter is typically a period of senescence and low biomass following reproduction **(Fulton *et al.*, 2014)**.

Research on *Sargassum* in the Red Sea has primarily focused on its ecological distribution **(Galal El-Din & Rashedy, 2023)** and various applications, including biofuel production **(Ali & Bahadar, 2017)**, pharmacology and natural products **(Rushdi *et al.*, 2020)**, and alginate evaluation **(Rashedy *et al.*, 2021)**. However, these studies may not provide a sufficient scientific foundation for developing a comprehensive management plan for this seaweed. To address this gap, this study evaluated zygote cultivation on different substrates, aiming to identify optimal growth conditions and to enhance the prospects for sustainable cultivation in this region.

In this study, the development of zygotes of *S. aquifolium* followed a sequential process, progressing from pear-shaped embryos to fully developed germlings over 168 days. The initial transformation of zygotes into pear-shaped embryos aligns with previous observations in macroalgal species, where early developmental stages are critical for successful settlement **(Aaron-Amper *et al.*, 2020)**. The subsequent elongation into club-shaped germlings within the first week and the emergence of rhizoids after 48 hours suggest that early rhizoid development is essential for secure attachment, supporting findings in other *Sargassum* species **(Liu *et al.*, 2016)**.

A notable difference observed in this study was the delayed mucilage production by rhizoids, occurring 4–7 days post-seeding. This contrasts with species like *Sargassum thunbergii*, where mucilage is secreted within six hours **(Liu *et al.*, 2016)**. Since mucilage is known to facilitate adhesion and enhance compatibility with the substrate **(Aaron-Amper *et al.*, 2020)**, delayed secretion might influence initial settlement rates. However, our results indicate that once rhizoids were established, germlings successfully adhered to the substrate, supporting previous studies on *S. vachellianum* **(Yan & Zhang, 2014)**.

The rapid sinking of pear-shaped embryos following rhizoid development ensures large-scale settlement, a strategy also observed by **Aaron-Amper *et al.* (2020)** for the same species. By day 28, germlings exhibited significant elongation (0.280 ± 0.03mm), with meristematic tissue formation and the appearance of a protuberance, indicating the transition toward more complex morphological structures. Over an extended culture period, leaflet initials emerged after 140 days, with full leaflet development observed by day 168. These findings suggest that while initial attachment may be slightly delayed, subsequent growth and differentiation proceed comparably to other species with similar developmental patterns **(Fletcher & Fletcher, 1975)**.

Cement stone demonstrated the highest effectiveness in supporting zygote recruitment and their development into juveniles. It could be that its surface roughness played an important role in the successful attachment and settlement of *S. aquifolium*, as proposed by **Lobban & Harrison (1995)**. Although all the substrates used here were rugose, cement stone had deeper troughs that were higher than the heights of the *S. aquifolium* zygotes. The more rugose the surface, the more favorable it is for algal settlement **(Malm *et al.*, 2003)**. Surface roughness increases the surface area of the substrates, which allows physical interlocking and provides a micro-environment that prevents recruits from detaching **(Le *et al.*, 2018)**.

Germlings that survived until days 140 and 168 reached a maximum length of 1.5–2 mm across all substrates. This growth pattern was comparable to *Sargassum muticum*, which attained a similar length after 56 days **(Le *et al.*, 2018)**. Additionally, *S. thunbergii*, reached 3mm within the same culture duration **(Zhao *et al.*, 2008)**. However, the growth rate observed for *S. aquifolium* in this study is lower than that of *S. vachellianum* and *S. fulvellum*, which achieved 5mm within approximately 30 days **(Hwang *et al.*, 2006; Chai *et al.*, 2014)**. Several environmental factors may have contributed to this relatively slower growth rate. Strong light intensity, elevated water temperature, and low nutrient availability are known to influence the development of *Sargassum* species **(Aaron-Amper *et al.*, 2020)**.

CONCLUSION

This study illustrates the seasonal variations and zygote cultivation of *Sargassum aquifolium* in the Red Sea, contributing valuable insights into its growth patterns and optimal cultivation methods. The seasonal biomass results showed that *S. aquifolium* exhibits peak growth during the summer, influenced by temperature fluctuations. The results further emphasize the significance of substrate selection for zygote recruitment and juvenile growth, with cement stone developing as the most effective surface for settlement and growth. The findings provide a basis for sustainable cultivation strategies in the Red Sea, contributing to conservation efforts and the potential development of commercial seaweed farming in the region.

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