RESEARCH PAPER

Effect of Ascophyllum nodosum marine plant extract powder and Provasoli's enriched seawater media on in vitro propagation and growth performance of the commercially important carrageenophyte *Kappaphycus alvarezii* in the Gulf of Mannar, Tamil Nadu, Southeast coast of India

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ABSTRACT

This study evaluated the growth performance of Kappaphycus alvarezii under three different conditions, i.e., indoor laboratory, outdoor hatchery, and open seawater, using *Ascophyllum nodosum* marine plant extract powder (AMPEP) and Provasoli's enriched seawater (PES) as growth media at different concentrations. In the laboratory, different media with concentrations of AMPEP (i.e., 0.5, 1.0, 1.5, and 2.0 mg L^{-1}) and PES (i.e., 5, 10, 15, and 20 mL L^{-1}) were tested along with plant growth regulators (IAA + Kinetin) following micropropagation techniques. For this, seaweed sections (1 cm length) were inoculated into different culture media concentrations at three replicates and compared with a control group (UV-filtered seawater). However, the concentrations of 1.5 mg L^{-1} AMPEP and 20 mL L^{-1} PES both supplemented with PGRs displayed the better mean growth performance of *K. alvarezii* in terms of longer shoot length (4.68 ± 0.90 mm and 7.53 ± 0.20 mm), a higher number of direct axes shoots formed per segment (1.96 ± 0.20 shoots and 1.86 ± 0.05 shoots), and the percentage of segment with direct axes shoots (91.33 ± 3.78% and 92.66 ± 2.41%), respectively. Based on the micropropagation results, the better-performing media concentrations were tested in the outdoor hatchery conditions. The study found no significant differences in growth performance of the treated plantlets between AMPEP and PES. Similarly, in open-sea conditions, there was no significant difference in the survival rate of treated seedlings, which was in the range of 88.26 ± 2.01 to 80.53 ± 1.66% survival between AMPEP- and PES-treated thalli. At the end of the open culture trial, AMPEP media showed significantly higher mean daily growth rate (3.16 ± 0.35%) and mean weight gain (3.58 ± 0.90 g) compared to PES. These findings suggested that AMPEP-treated seedlings outperformed PES-treated seedlings in promoting growth under open seawater conditions, supporting large-scale plantlet production for the commercial culture of *Kappaphycus* using AMPEP as a culture medium in the nursery.

Keywords: *Kappaphycus alvarezii*, micropropagation, culture media, seaweed culture, Ascophyllum extract

How to cite this article: Joshiba P, Anand C, Thirumurugan A, Rani V, Lingam RSS, Logesh K. Effect of Ascophyllum nodosum Marine Plant Extract Powder and Provasoli's Enriched Seawater Media on In Vitro Propagation and Growth Performance of the Commercially Important Carrageenophyte Kappaphycus alvarezii in the

Gulf of Mannar, Tamil Nadu, Southeast Coast of India. Int J Drug Deliv Technol. 2026;16(19s): 470-486. DOI: 10.25258/ijddt.16.19s.55
Source of support: Nil
Conflict of interest: None

1. INTRODUCTION

Seaweeds are gaining more attention around the world in the 21st century. Mostly, it is used to produce food, thickeners, animal feed, chemicals, plant growth enhancers, functional ingredients, biofuel, and biodegradable alternatives to petroleum-based plastics (Yong et al. 2024). The world's seaweed and microalgae production was 36.5 million tonnes (wet weight) in 2022, with an estimated marketing value of USD 17 billion (FAO 2024). Innovations in seaweed processing technologies have significantly enhanced the global sustainability of the seaweed industry while simultaneously creating new entrepreneurial opportunities for coastal communities (World Bank, 2023). *Kappaphycus alvarezii,* one of the most farmed red seaweeds, is used to produce carrageenan. Recently, carrageenan has acquired global significance due to its variety of biological activities, such as antitumor, antiproliferation, antiviral, anticoagulant, *etc*. (Guo et al. 2019; Cotas et al. 2020). Initially, *K. alvarezii* farming was started in the Philippines during 1960s (Doty and Alvarez 1975). In India, the cultivation of *K. alvarezii* was first initiated at Mandapam between 1995 and 1997 (Eswaran et al. 2002). Generally, red seaweeds are farmed using thallus fragments as asexual propagules, and their continuous usage reduces seedling vigor (Dawes et al. 1993; Hurtado et al. 2006). The availability of seed is one of the most common problems found in seaweed farming (Prabowo et al. 2021).

Micropropagation, an adaptable method for multiplying certain strains of seaweeds to increase seedling production for cultivation, is a popular method used to produce a wide variety of uniform specimens with proper characteristics in a shorter duration (Hayashi et al. 2008; Yong et al. 2014). Multiplication of seaweed using an in vitro culture method offers several benefits, including the ability to produce high-quality seed, production of plants free of pathogens, and it is not reliant on the natural environmental conditions or any particular season (Purita et al. 2018). The continuous availability and easy access to various strains of *Kappaphycus* throughout the year make micropropagation a more practical, cost-effective, and dependable approach (Ali et al. 2020). Micropropagation, followed by acclimatization of propagated seaweeds to open-water conditions, offers a promising solution for producing healthier, epiphyte-free seedlings (Bixler and Porse 2011). Moreover, research indicates that micropropagation serves as an effective strategy for the sustainable production of quality seedlings suitable for commercial aquaculture operations (Reddy et al. 2017).

In general, culture media plays a vital role in micropropagation by promoting rapid propagation of seaweed shoot formation, better thallus development, and biomass accumulation (Yong et al. 2011; Yong et al. 2016). *Ascophyllum* (Acadian) marine plant extract powder (AMPEP), derived from the brown seaweed of *Ascophyllum nodosum,* has been explored for its potential in enhancing both micropropagation and field cultivation of seaweeds, especially in *Kappaphycus* species due to their bioactive materials (Di Stasio et al. 2018; Hurtado and Critchley 2018; Shukla et al. 2019; Silva et al. 2019). AMPEP was mentioned to have a "vaccine-like" effect on *K. alvarezii* and decrease the oxidative burst impact (bleaching) on the thallus (Loureiro et al. 2012). Moreover, AMPEP has positively promoted the growth of various commercially important seaweeds such as *K. striatum* (Ali et al. 2018), *Gracilaria caudata* and *Laurencia catarinensis* (Souza et al. 2019), *Saccharina latissima* and *S. angustissima* (Umanzor et al. 2019), *G. corticata* (Dawange and Jaiswar 2020), *G. salicornia* (Jaiswar et al. 2021), *Eucheuma denticulatum* (Borlongan et al. 2023), and *K. alvarezii* (Capacio et al. 2024). Similarly, application of AMPEP in the cultivation of *K. alvarezii* reduced the epiphytic infestations and improved both growth and heat tolerance (Hurtado and Critchley 2018). Additionally, AMPEP treatment has been linked to increased carrageenan production, including improvements in yield, viscosity, and gel strength (Ali et al. 2018). Similar to AMPEP, Provasoli's Enriched Seawater (PES), a natural seawater enriched with various nutrients, is widely used as a nutrient medium for macroalgal cultivation (Yuliana 2013). It has been reported that PES enhanced both algal biomass and the production of valuable metabolites (James 2012). PES as a seaweed culture media has been explored in various species such as *Laurencia* sp. (Robaina et al. 1990,1992), *Grateloupia filicina* (Baweja and Sahoo, 2009), *Sargassum polycystum* (Muhamad et al. 2018), *S. fusiforme* (Ahmed et al. 2025), *K. alvareii* (Yong et al. 2014; Hlaing and Jarukamjorn 2024; Abd Latipa et al. 2025; Selvaraj et al.,2025; Dhanasundaram et al.,2025; Perumal et al.,2023). However, the type of medium and their dose levels may significantly affect the production of seaweed at micropropagation level. Therefore, different media and their various concentrations have to be tested using

micropropagation methods for standardizing the techniques.

Seaweed tissue culture involves the axenic cultivation of explants, in laboratory conditions, typically thallus fragments, in media supplemented with plant growth regulators and nutrient-rich additives that promote regeneration (Astuti 2021). In contrast, seaweed cultivation in open sea environments faces several challenges, including environmental variations and the availability of nutrients, which significantly hamper the grow-out performance of micro-propagated plantlets. Similarly, micro-propagated plantlets of seaweed after the acclimatization phase enter the grow-out stage. Traditionally, this phase has been carried out in marine environments using open-water farming methods such as floating rafts, off-bottom monolines, multiple raft long lines, tube nets, PVC pipe rafts, cage systems, and spider web systems (Behera et al. 2022; Jaikumar et al. 2022). Transferring *Kappaphycus* microplantlets to sea-based nurseries using cylindrical net cages offered a more efficient management approach compared to extending their growth in land-based systems. This method allowed the microplantlets to develop under natural environmental conditions, promoting better adaptation and performance in field cultivations (Hurtado and Critchley 2019). Similarly, tissue cultured seaweed were tested in an open-sea conditions following various cultivation methods using plantlets of *K. striatus* (Luhan and Sollesta, 2010), *G. edulis* (Ganesan et al. 2011a; Ashok et al. 2016), and *K. alvarezii* (Kasim and Mustafa 2017; Ali et al. 2020; Faisan et al. 2024) and they found variations in production performance. Overall, the ultimate goal of micropropagation was to produce vigorous and healthy plantlets suitable for open sea water cultivation. Therefore, a field level experiment using the micropropagated plantlets of *K. alvarezii* is needed for understanding their growth performance and biomass yield. Keeping this in the mind, the present study was aimed to optimize the production parameters, including the concentration of media, and their effect on the growth, of *K. alvarezii* using AMPEP and PES media under a controlled environment and open sea conditions.

2. MATERIALS AND METHODS

2.1. Collection of seaweed

Kappaphycus alvarezii (brown strain, vegetative gametophyte, 2-4 Kg fresh weight) was collected from a seaweed farming site at Munaikadu coast (9°17′ 22.39° N; 79° 07′56.84° E) of Palk-Bay waters, southeast coast of India. At the collection site seaweeds were cleaned to remove attached organisms and then it was packed in a cool polythene cover under wet conditions and transported to the MCeSA Hatchery. The packs were immediately transported to the Laboratory unit of Mandapam Centre for Sustainable Aquaculture (MCeSA), research and extension activity centre, functioning under Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Tamil Nadu, India, following standard protocols. In the experimental site, seaweeds were kept in a glass tank (100 L capacity) filled with UV-treated seawater and mild aeration for 5-7 days. During this acclimatization phase, the water was exchanged on a daily basis (Hurtado and Cheney 2003).

2.2. Preparation of explants

The ideal seaweed thallus for micropropagation were selected based on the absence of disease symptoms, vibrant pigmentation, a well-developed thallus, an intact holdfast, multiple branches, and youthful developmental stages (Yong et al. 2011). Healthy and vigorous individuals from the algal stock were selected for further processing. Then the apical portions of the thalli were gently cleaned on the surface using a soft artist's brush and sterilized with germanium dioxide (0.01 mg L-1), followed by povidone iodine (1%). Thereafter, the apical portions were rinsed three times with autoclaved seawater to obtain clean, healthy, and diatom-free explants. The thalli were then cut into 1 cm thick cross-sections using a sterile surgical blade under the laminar airflow conditions. The seaweed sections as explants (each with 1 cm length) were prepared and rinsed three times with UV-treated seawater.

2.3. Preparation of media for tissue culture

To find out the efficiency of two important culture media AMPEP and PES media at various concentrations. AMPEP media procured in a powder form was directly mixed with the distilled water and stored in a dark bottle. PES medium was prepared following the procedure mentioned by Provasoli (1968). In the micropropagation technique, both media were used in a liquid form. AMPEP media was tested at four different conditions such as 0.5, 1.0, 1.5, and 2.0 mg L-1. Similarly, PES media was evaluated at 5, 10, 15, and 20 mL L-1 concentrations. All treatments were supplemented with Plant Growth Regulators (PGRs serve as priming that enhance cellular division; Tibubos et al. 2017; Ali et al. 2018a) each containing 0.25 mg L-1 of IAA and kinetin to induce the formation of direct axes shoots under laboratory conditions. A set of seaweed explants cultured in UV-treated seawater acted as a control.

A total of thirty plastic jars (each 5L capacity) were used for the study. Each jar was filled with 4 L (25 explants per Litre) of sterile UV-treated seawater containing the respective concentrations of growth media and PGRs. All treatments and controls were maintained in three replicates. After media preparation, one hundred (100) explants were introduced into each plastic jar. Both media were same laboratory conditions. The jars were kept in a walk-in culture room at 25 ± 2°C, under cool white fluorescent tube light providing an irradiance of 20-30 µmol photons m^{-2} s^{-1} under a 12L:12D photoperiod, with moderate aeration for 45 days. Media were changed every four days. Water quality parameters such as dissolved

oxygen, pH, temperature, salinity, ammonia, and nitrate were monitored every five days and maintained within optimal levels throughout the experimental period.

2.4. The observation of explants

After 45 days of culture under controlled laboratory conditions, the following measurements were calculated. Firstly, the overall length (mm) of the shoot per propagules was measured individually after 15 days using a Zeiss Stemi 508 Light Microscopy to ensure accurate measurement for their small size. However, at the end of 45 days the shoot length was determined by placing the explants over a white tissue paper and the length was measured using a plastic ruler (Ali et al. 2020). Then the number of direct shoots per explant was counted manually, and the percentage of new direct axes formed was also calculated. The percentage of number of explants with direct axes formed was calculated as follows: The percentage (%) of direct axes formed = (number of segments with direct shoots formed at day 45 / total number of segments incubated at day 1) x 100 (Tibubos et al. 2017).

2.5. Acclimatization of tissue-cultured seaweeds to outdoor hatchery conditions

Followed by micropropagation under laboratory conditions, acclimatization of seaweed micropropagules to the outdoor hatchery environment was carried out for hardening the micropropagules. The micropropagules with new shoots (1.86 ± 0.05 to 1.96 ± 0.20 shoots) and length (4.69 ± 0.90 to 7.53 ± 0.20 mm) were selected from the treatments with significance (1.5 mg/L of AMPEP and 20 ml/L of PES), and moved to aquarium tanks (100 L capacity) kept in a hatchery at room temperature. Each of the micropropagules from the treatments were hardened separately in a 100 L glass aquarium tank, which contained only sterilized seawater (by chlorination) without any media and PGRs (IAA and kinetin). Two aquarium tanks were used to culture the microplantlets, and the culture was carried out for a duration of 45 days. Moderate aeration was provided, and the sterilized seawater was replaced once in a week to maintain water quality. Water quality parameters such as dissolved oxygen, pH, temperature, salinity, ammonia, nitrate, and growth performance of the plantlets were monitored and documented once in a week.

2.6. Outdoor sea-based raft culture

After acclimatization of seaweed microplantlets from hardening phase were transferred to the open sea field for cultivation using raft method. The outdoor trial was carried out in intertidal areas of Thonithurai (N 09° 16.905'; E 079° 11.293') in the Gulf of Mannar, southeast coast of India. Two bamboo rafts, each measuring 1 x 1 m square and without any cracks or holes, were used and secured with 4 mm ropes. Plantation ropes were prepared by cutting 3 mm twisted polypropylene rope; a minimum of 10 monoline ropes were used for cultivation. Seaweed seedlings (125 seedlings from each treatment group), with mean length of 1.86 ± 0.15 to 2.29 ± 0.38 cm, were tied to the ropes at 5-10 cm intervals, with 10-15 tie points per rope line. To protect *K. alvarezii* from predators (fish), the rafts were covered with mosquito nets. The seedlings were cultivated during May-2025 to August-2025. Each group was tied separately and managed by cleaning every 3-4 days by brushing the netting and culture ropes to remove attached debris and ensure proper water flow. Growth performance was monitored fortnightly during the culture period. The daily growth rate (DGR) and weight gain were measured following the formula given by Dawes et al. (1994), DGR (%) = Ln final weight (g) – Ln initial weight (g) / observation duration (days) x 100 and Weight gain (g) = Final weight (g) – Initial weight (g). The survival rate (SR) was calculated using the formula of SR (%) = Number of surviving explants / Total number of explants stocked x 100 (Yustianti et al. 2013).

2.7. Data analysis

The data were statistically analyzed in SPSS 17.0.0 using a one-way analysis of variance (ANOVA) to test the significant difference among the different concentrations of each medium. Tukey post-hoc tests were used to identify significant differences among the mean values. All the data presented in text, tables, and figures were expressed as the mean ± standard deviation The significance level for the test was set at $P<0.05$.

3. RESULTS

3.1. Growth performance of Kappaphycus alvarezii propagules in the indoor culture unit

At the end of the experiment, the study found a significant difference ($P<0.05$) in growth performance between AMPEP treatments and control in terms of shoot length (mm), number of shoots per segment, and percentage of new direct axes formed (Table 1). The first shoot was formed in the explant on 11th day of the experiment. On 45th day of trial, the study recorded a significantly longer direct shoot (4.68 ± 0.90 mm), a higher number of direct shoots formed per segment (1.96 ± 0.20 shoots) (Fig. 1, 1a), and the percentage of explants with direct shoots (91.33 ± 3.78%) in 1.5 mg L-1 of AMPEP compared to the control group.

Similarly, in PES media significant difference ($P<0.05$) in growth performance among the treatments was recorded. In PES, the shoot formation was started in the 10th day of the culture. A significantly longer direct shoot (7.53 ± 0.20 mm), a higher number of direct shoots formed per segment (1.86 ± 0.05 shoots) (Fig. 2), and the percentage of explants with direct shoots (92.66 ± 2.41%) were observed in 20 mL L-1 of PES compared to the control at the end of the experiment (Table 1).

Table 1 Growth performance of *K. alvarezii* (brown strain) propagules in indoor conditions for 45 days (n=30)

Treatments	Shoot length (mm)	Number of shoots per segment	Percentage of explants with shoots per segment (%)
AMPEP			
Control (0 mg L^{-1})	3.91 ± 0.81^{b}	1.56 ± 0.15^{b}	67.33 ± 4.93^{b}
0.5 mg L^{-1}	4.16 ± 0.85^{ab}	1.73 ± 0.15^{ab}	87.00 ± 6.55^{a}
1.0 mg L^{-1}	4.35 ± 0.81^{ab}	1.66 ± 0.05^{ab}	79.33 ± 10.50^{ab}
1.5 mg L^{-1}	4.68 ± 0.90^{a}	1.96 ± 0.20^{a}	91.33 ± 3.78^{a}
2.0 mg L^{-1}	4.06 ± 0.90^{b}	1.60 ± 0.10^{ab}	74.00 ± 7.81^{ab}
p-value	0.009	0.044	0.013
PES			
Control (0 mL L^{-1})	4.20 ± 0.43^{b}	1.43 ± 0.11^{b}	57.33 ± 3.05^{c}
5 mL L^{-1}	7.01 ± 1.03^{a}	1.73 ± 0.15^{ab}	72.00 ± 6.55^{b}
10 mL L^{-1}	7.01 ± 0.28^{a}	1.73 ± 0.15^{ab}	84.33 ± 4.50^{a}
15 mL L^{-1}	4.73 ± 0.70^{b}	1.50 ± 0.10^{b}	70.00 ± 5.00^{b}
PES 20 mL L^{-1}	7.53 ± 0.20^{a}	1.86 ± 0.05^{a}	92.66 ± 2.51^{a}
p-value	0.000	0.007	0.000

Values are expressed as the mean ± SD; Under each medium, mean values of each parameter mentioned in the same column with different superscripts differ significantly ($P<0.05$)

3.2. Growth performance of K. alvarezii propagules in the outdoor hatchery unit

The study did not find any significant difference between growth performance of AMPEP and PES treated seaweed micropropagules in the student's t-test. Overall, the shoot length, direct shoots formed per segment, and the percentage of explants with direct shoots were found in the ranges of 6.82 to 10.82 mm, 2.25 to 2.55 shoots, and 88.88 to 92.96% respectively, in the outdoor hatchery unit (Table 2).

Table 2 Growth performance of *K. alvarezii* (brown strain) microplantlets in the outdoor hatchery unit for 45 days (n=20)

Growth parameters	AMPEP (1.5 mg L^{-1})	PES (20 mL L^{-1})	p-value
Shoot length (mm)	6.82 ± 1.04	10.82 ± 2.76	0.783
Number of shoots per segment	2.55 ± 0.68	2.25 ± 0.44	0.196

Percentage of explants with shoots per segment (%)	92.96 ± 1.69	88.88 ± 1.11	0.119

3.3. Growth performance of K. alvarezii propagules in the open seawater condition

At the end of the culture, the study did not find any significant difference in survival rate (%) between AMPEP and PES media treated seedlings. The survival rate was the range of 80.53 to 88.26% (Fig. 4). However, the study found significant difference ($P<0.05$) in final weight, weight gain and daily growth rate between AMPEP and PES treated seedlings in the student's t-test. The significantly higher final weight (3.77 ± 0.90 g), weight gain (3.58 ± 0.90 g) and daily growth rate (3.16 ± 0.35%) (Fig. 3, 5) were noticed in AMPEP-treated seaweed seedlings raised at the end of the open sea culture (Table 3).

Table 3 Growth performance of *K. alvarezii* (brown strain) seedlings in the open seawater conditions for 90 days (n=20)

Growth parameters	AMPEP (1.5 mg L^{-1})	PES (20 mL L^{-1})	p-value
Initial weight (g)	0.19 ± 0.04	0.22 ± 0.06	0.216
Final weight (g)	3.77 ± 0.90^{a}	2.93 ± 0.72^{b}	0.009
Weight gain (g)	3.58 ± 0.90^{a}	2.71 ± 0.72^{b}	0.006
Survival rate (%)	88.26 ± 2.01	80.53 ± 1.66	0.415
Daily growth rate (%)	3.16 ± 0.35^{a}	2.76 ± 0.34^{b}	0.012

Values are expressed as the mean ± SD; In each row, mean values with different superscripts differ significantly ($P<0.05$)

3.4. Water quality parameters of K. alvarezii (brown strain) propagules in the indoor, outdoor, and open seawater conditions

In the present study, the water temperature in the indoor culture unit, outdoor hatchery unit, and open seawater conditions were recorded in the ranges of 25.60 ± 0.69°C, 28.66 ± 0.81°C, and 31.20 ± 1.03°C, respectively. The water salinity ranged from 33.90 ± 0.87 to 34.10 ± 0.87 ppt throughout the experiment, and nitrate was not detected. The water pH in the indoor culture unit, outdoor hatchery unit, and open seawater conditions were observed in the ranges of 7.87 ± 0.17 to 7.99 ± 0.10, 7.82 ± 0.23 to 7.88 ± 0.16, and 7.96 ± 0.05, respectively. Dissolved oxygen was in the range of 5.78 ± 0.09 to 7.41 ± 0.24 mg L-1 among the three culture conditions. Ammonia were observed throughout the culture in the ranges of 0.008 ± 0.002 to 0.032 ± 0.005 ppm and light intensity ranged between 22.01 ± 0.28 to 196.06 ± 2.32 µmol photons m^{-2} s^{-1} (Table 4).

Table 4 Water quality parameters observed throughout the culture period.

Parameters	Tissue culture conditions		Outdoor hatchery conditions		Open seawater conditions
	AMPEP	PES	AMPEP	PES	AMPEP and PES
Temperature (°C)	25.60 ± 0.69	25.60 ± 0.69	28.66 ± 0.81	28.66 ± 0.81	31.20 ± 1.03
Salinity (ppt)	33.90 ± 0.87	33.90 ± 0.87	33.83 ± 0.75	33.83 ± 0.75	34.10 ± 0.87
Dissolved oxygen (mg L^{-1})	7.21 ± 0.55-7.38 ± 0.42	7.35 ± 0.20-7.41 ± 0.24	5.95 ± 0.53	6.05 ± 0.44	5.78 ± 0.09

pH	7.87 ± 0.17-7.99 ± 0.10	7.87 ± 0.19-7.98 ± 0.06	7.82 ± 0.23	7.88 ± 0.16	7.96 ± 0.05
Ammonia (ppm)	0.0235 ± 0.0032-0.0256 ± 0.0035	0.0245 ± 0.0032-0.0258 ± 0.0025	0.029 ± 0.006	0.032 ± 0.005	0.008 ± 0.002
Nitrate (ppm)	ND	ND	ND	ND	ND
Light intensity (µmol photons m^{-2} s^{-1})	22.01 ± 0.28	22.01 ± 0.28	37.16 ± 0.28	37.16 ± 0.28	196.06 ± 2.32

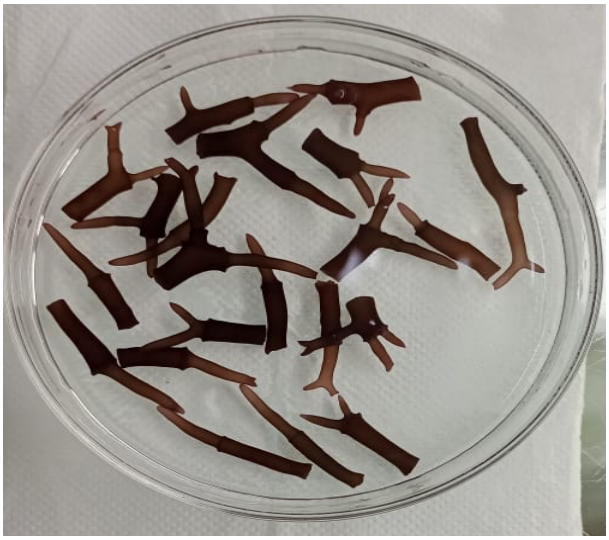

Fig. 1 Explants with shoots at the 45th day under controlled conditions

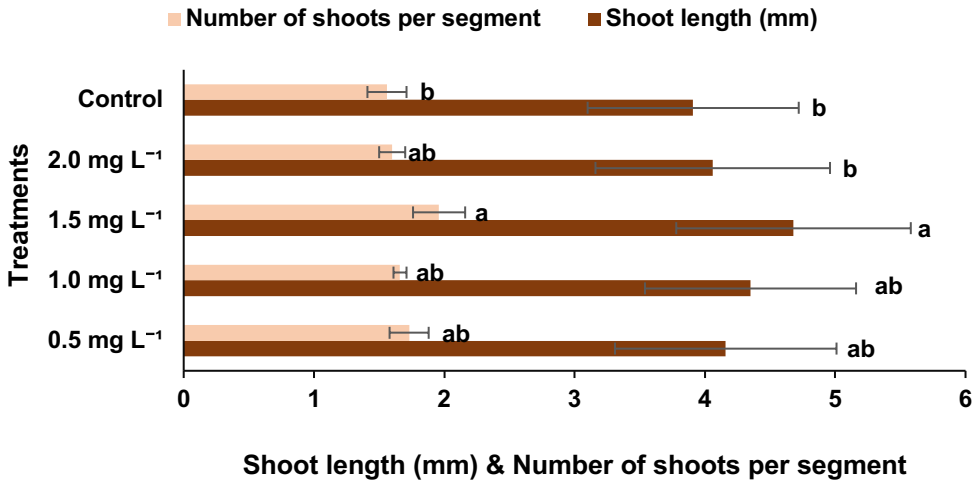

Fig. 1a Shoot length (mm) and Number of shoots per segment (n=30) in *Kappaphycus alvarezii* after 45 days of culture at different concentrations of AMPEP supplemented with fixed concentration of PGRs in indoor laboratory

conditions. Values are represented as mean ± SD. In graph, for each treatment bars with different alphabet letters indicate significant (P<0.05) difference on post-hoc Tukey test

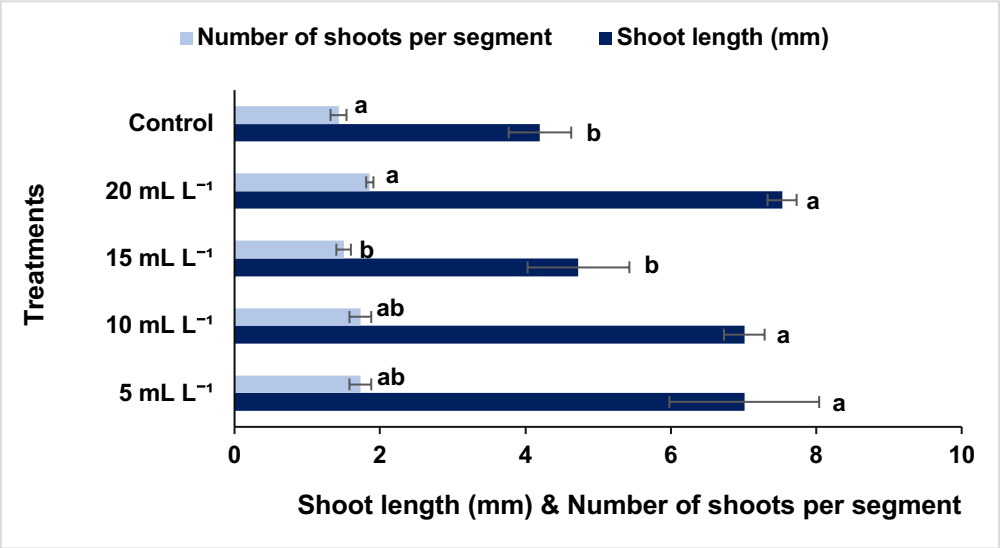

Fig. 2 Shoot length (mm) and Number of shoots per segment (n=30) for *Kappaphycus alvarezii* obtained with different concentration of PES supplemented with fixed concentration of PGRs in indoor laboratory conditions for 45 days. Values are represented as mean ± SD. In graph, for each treatment bars with different alphabet letters indicate significant (P<0.05) difference on post-hoc Tukey test

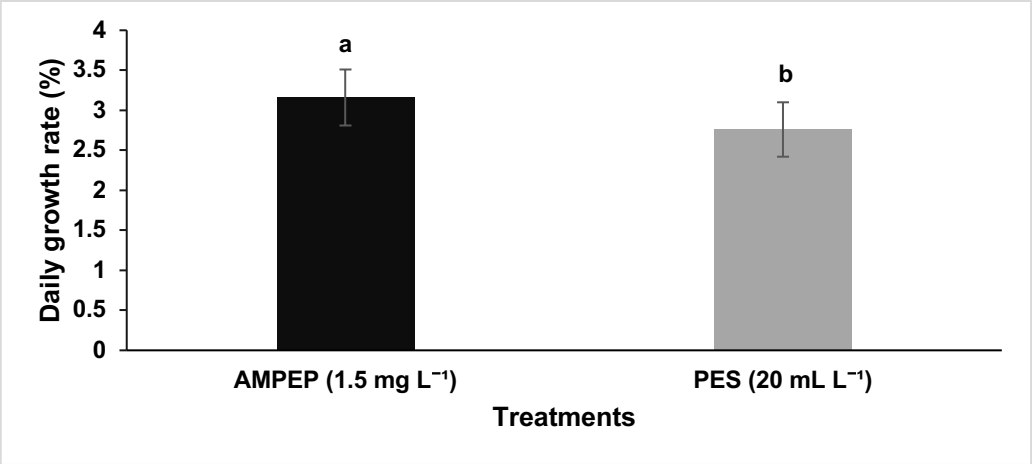

Fig. 3 Daily growth rate (%; n=20) in *Kappaphycus alvarezii* after 90 days of culture at different media of AMPEP and PES treated seedlings in field conditions. Values are represented as mean ± SD. In graph, for each media bars with different alphabet letters indicate significant (P<0.05) difference

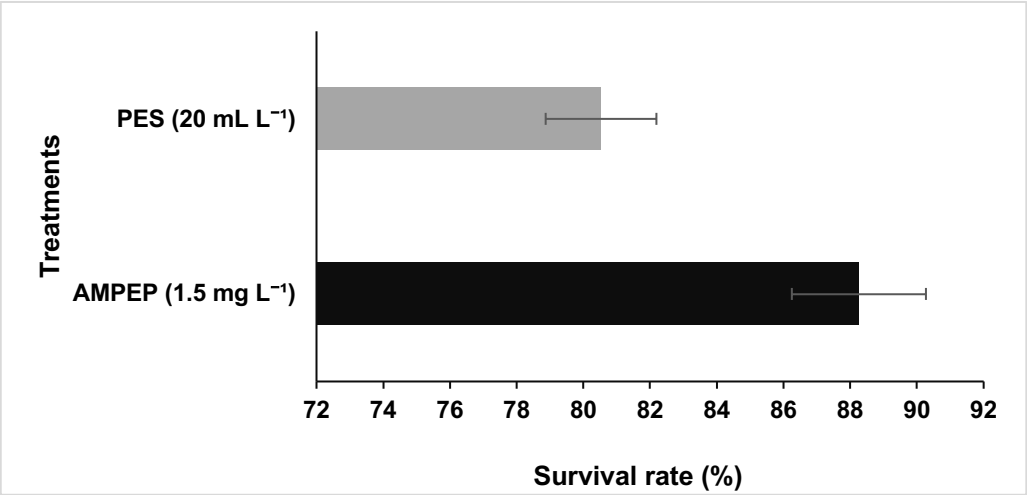

Fig. 4 Survival rate (%; n=20) for *Kappaphycus alvarezii* of AMPEP and PES treated seedlings in field conditions for 90 days. Values are represented as mean ± SD.

Figure .5 Growth performance of treated seedlings under open sea conditions. (A) Initial weight of AMPEP-treated seedlings at the start of the experiment; (B) Final weight of AMPEP-treated seedlings after the experimental period, showing growth enhancement; (C) Initial weight of PES-treated seedlings at the beginning of exposure; (D) Final weight of PES-treated seedlings after treatment, indicating changes in biomass accumulation.

4. DISCUSSION

The optimal temperature required for *K. alvarezii* in a controlled environment is 25 ± 2°C (Ali et al. 2020; Fitri et al. 2023). On the other hand, *K. alvarezii* is capable of surviving and growing well within a temperature range of 24°C to 35°C (Mairh et al. 1986; Periyasamy et al. 2014). In the present study, the recorded temperature in open seawater was 30 to 32°C which is within the optimal temperature range of *K. alvarezii* farming. The optimal pH required for *K. alvarezii* in a controlled environment and open sea ranges from 6-8 and 7-9, respectively (Semedi et al. 2016; Preisig and Andersen 2005). Similarly, the optimal DO range for ideal growth is between 5 and 8 mg L-1 (Sahoo and Yarish 2005; Preisig and Andersen 2005; Semedi et al. 2016), which supports the optimal DO concentrations recorded in the present study. The salinity suitable for cultivating *K. alvarezii* seaweed ranges from 28-37 ppt (Agustina et al. 2017). The average concentrations of ammonia in treatment tanks were found to be below 0.1 mg L-1, indicating conducive water quality conditions for ideal growth of seaweed (Luhan et al. 2015). According to Hidup (2004), the recommended level of nitrate is 0.008 mg L-1. Nitrate level should be below 1.0 mg L-1 (Periyasamy et al. 2016). Reddy et al. (2003) recommended culturing *K. alvarezii* explants under light intensities ranging from 5 to 70 μmol photons m^{-2} s^{-1} promote in vitro somatic embryogenesis and embryo regeneration.

AMPEP is extensively applied to improve the growth and productivity of crops and horticultural plants, as well as to promote the development of macroalgae such as seaweeds (Silva et al. 2019; Umanzor et al. 2019; Tahiluddin et al. 2022). The results of the present study revealed that AMPEP at 1.5 mg L-1 and PES in 20 mL L-1 with PGRs achieved significantly higher ($P<0.05$) shoot length, number of direct axes shoots per segment, and percentage of explants with direct shoots formed after 45 days compared to the control and other medium concentrations in indoor laboratory conditions. In general, the extract of *Ascophyllum nodosum* contains essential macro and micronutrients that enhance the growth of *K. alvarezii* (Hurtado et al. 2009). Similar growth-promoting effects of AMPEP have also been documented in *Gracilaria corticata* and *G. salicornia* (Dawange and Jaiswar 2020; Jaiswar et al. 2021; Thirumurugan et al., 2020; Meenaloshini et al.,2025), *Eucheuma denticulatum* (Borlongan et al. 2023), and *K. alvarezii* (Capacio et al. 2024) at various concentrations. On the other side, Ali et al. (2018) recorded the shoot formation on 10th day of culture, which was similar to the observation noticed in the present study. A study by Tibubos et al. (2017) found the longest direct axis shoots (9.6 mm ± 0.33) in *K. alvarezii* when treated with slightly higher dose of 5 mg L-1 AMPEP K^+ combined with plant growth regulators (PGR). Similarly, Ali et al. (2018, 2020) reported longest shoots, highest number of direct shoots formed per segment, and the highest percentage of direct shoots formed in *K. alvarezii*, measuring 7.6 mm, were obtained at 3 mg L-1 AMPEP K+ with PGR (IAA + Kinetin). Supporting to our study, Yunque et al. (2011) reported the successful regeneration in *K. alvarezii* (brown strain) using much lower concentrations of AMPEP combined with PGR, especially in 0.001-1.0 mg L-1 with PGR (PAA and zeatin). Yong et al. (2014) and Luhan and Mateo (2017) also suggested the use of organic and inorganic media supplemented with *Ascophyllum* seaweed extract, along with additional PGRs, for positive effects on the clonal propagation of *Kappaphycus,* leading to improved seedling production. Research indicates that adding AMPEP along with PGRs to the culture medium enhances thallus growth rates in various *Kappaphycus* species during micropropagation under laboratory conditions (Tibubos et al. 2017; Ali et al. 2018). This improvement has been linked to higher productivity and desirable traits, including better carrageenan quality and reduced biotic stress from endophytes in field conditions (Ali et al. 2018, 2020).

The PES nutrient medium contains a combination of macronutrients, micronutrients, trace elements, and vitamins essential for the growth of various seaweed species. According to Mansilla et al. (2008), cultivating seaweed seeds in the laboratory with fertilizers enriched with both macronutrients (nitrogen, phosphorus, potassium) and micronutrients (Mo, Ni, Mn, B, Co, Cl, Cu, Zn, and Na, S) resulted in higher growth rates compared to fertilizers that contain only macronutrients. Wahyudi et al. (2023) report that a 20 mL dose of PES yielded a better growth rate in *K. alvarezii* plantlets, which supports the finding of the PES in present study. In contrast, many studies have reported favourable or better growth of *Kappaphycus* in lower concentrations of PES. Rosyida et al. (2019) found that applying PES fertilizer at low doses between 0 mL to 11 mL, with 8.5 mL producing the most favorable growth results in *K. alvarezii*. Yuniarti et al. (2018) observed that a 10 mL dose of PES enhanced the weight gain of *K. alvarezii* propagules, while Supriyono et al. (2022) suggested that even a small addition of 0.5 mL could boost seaweed biomass. Mo et al. (2020) demonstrated that somatic embryos of *K. striatus* can be successfully developed by culturing callus tissue on semi-solid PES medium supplemented with 1 mg L-1 1-naphthaleneacetic acid (NAA) and 2 mg L-1 6-benzylaminopurine (BAP). Direct regeneration has been effectively used to produce uniform seedlings in species like G*rateloupia filicina* (Baweja and Sahoo 2009; Ayyadurai et al.,2026; Jeyaram et al.,2026; Thirumurugan et al.,2026; G.Jeevanantham et al.,2026), *K. alvarezii* (Yong et al. 2014), and *Sargassum polycystum* (Muhamad et al. 2018), using PES or f/2 liquid media for different purposes. However, the ideal concentration of PES for

maximizing growth can differ depending on the species and culture conditions (Yuniarti et al. 2018), which may be the reason for better performance of *K. alvarezii* at 20 mL L-1 of PES with PGRs.

The daily growth rate of algal species is a key factor influencing the economic viability of seaweed farming. Both growth rate and chemical composition of seaweeds are greatly affected by salinity and nutrient levels in the culture medium (Baghel et al. 2024). Salinity is essential for growth as it affects osmotic balance (Reis et al. 2011; Macler et al. 1988), while nutrient concentration also act as a limiting factor for seaweed development (Wijayanto et al. 2020). Moreover, Borlongan et al. (2011) suggested that incorporating AMPEP into seaweed culture can enhance growth which also helps in preventing the epiphytic infections. The present study recorded DGR of 3.16% of seaweed under open sea conditions which is similar to the DGR reported as by Hayashi et al. (2011) in *K. alvarezii* (3-4%) and Ali et al. (2014) in *K. striatum* (1.75-3.5%). On the other side, the DGR value in PES media (2.76%), recorded in the present study, was higher than the previously reported values. The values were higher than the field-cultivated *Gelidium pussilum* (0.56-1.05%) by Veeragurunathan et al. (2018) and *Gelidiella acerosa* (1.11-1.13%) by Ganesan et al. (2011b). According to Suryati et al. (2010), cultivating *K. alvarezii* on media supplemented with PES fertilizer that meets its nutrient requirements can enhance survival rates up to 40-80%, accompanied by a high specific growth rate. When explants are maintained in the laboratory and then directly transferred to field nurseries without acclimation, the survival rate in sea-based nurseries is only about 30-50%. Direct transplantation of micro propagules into the open sea induces stress and shock in seaweeds due to abrupt shifts in environmental conditions (Sollesta-Pitogo et al. 2023). Acclimating tissue-cultured seedlings in the outdoor conditions has been suggested as a strategy to improve survival and growth rates (Jiksing et al. 2022). Supporting our study, Yong et al. (2016) reported that the acclimatization protocol enhanced the growth performance of *K. alvarezii* seedlings, resulting in a daily growth rate of 3.91 ± 0.16% day-1 and a survival rate of 88.26 ± 2.01% at open sea conditions.

5. CONCLUSION

The present study found that the culture conditions for *K. alvarezii* achieved optimal growth in 1.5 mg L-1 AMPEP medium supplemented with 0.25 mg L-1 IAA and kinetin. Another, 20 mL L-1 PES medium supplemented with 0.25 mg L-1 IAA and kinetin. However, under field conditions, AMPEP displays significantly higher weight gain, daily growth rate, and survival rate compared to PES media. These findings suggest that AMPEP treated seedlings is more effective than PES treated seedlings in promoting growth under open seawater conditions. This study indicates that employing micropropagation for producing *K. alvarezii* plantlets can serve as an effective method to support large-scale commercial cultivation along the Tamil Nadu coast.

Author contributions statement

Joshiba - Conceptualization, Investigation, Sampling, Analysis & Writing; **Anand -** Conceptualization, Project administration, Investigation, Supervision & Validation; **Rani -** Supervision and guidance; **Somu Sunder Lingam R, A.Thirumurugan & K. Logesh -** Data curation, Writing, Review & Editing.

Declaration of competing interest None

Funding No funding

Data availability

Sufficient data are provided in the form of Table and Figures. Further, additional data will be shared based on request.

Acknowledgements

The authors are thankful to the Vice-Chancellor of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India, for providing necessary support and encouragement.

Declarations

Ethical approval

This study was approved by ethical committee of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India.

Consent to participate Not applicable

Consent for publication Not applicable

Conflict of Interest The authors declare that they have no conflict of interest.

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