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Immersion-based application of *Origanum vulgare* extract improves growth and pigmentation in *Kappaphycus alvarezii* under field conditions

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Environmental stress-associated tissue deterioration remains an important challenge in the cultivation of *Kappaphycus alvarezii*. This study evaluated the effects of immersion-based application of Origanum vulgare extract on the growth performance and pigmentation of *K. alvarezii* cultivated under field conditions. *In vitro* antibacterial assays showed inhibitory activity of the extract against *Staphylococcus aureus* (21 mm inhibition zone) and *Escherichia coli* (19 mm inhibition zone). An *in vivo* experiment was conducted using four immersion treatments: control, 5 min, 15 min, and 25 min exposure durations. Growth performance differed significantly among treatments, with the 15-min immersion treatment producing the highest final weight (259.67 ± 13.05 g). Pigmentation scores were also significantly higher in the 15- and 25-min treatments compared with the control. Regression analysis suggested that intermediate immersion durations between approximately 15 and 18 min may provide favorable growth responses, although this estimate should be considered preliminary because only four treatment durations were evaluated. Overall, immersion-based application of O. vulgare extract improved growth performance and maintained better thallus pigmentation under field conditions. These findings support the potential use of plant-derived bioactive treatments as functional approaches for improving thallus condition in *K. alvarezii* aquaculture systems. However, the present study did not directly quantify disease incidence, pathogen abundance, or tissue mortality; therefore, interpretations regarding ice-ice disease mitigation should be considered preliminary.

KEYWORDS
dose–response modeling, ice-ice disease, immersion treatment, *Kappaphycus alvarezii*, *Origanum vulgare*

1 Introduction

Seaweed aquaculture is a major component of global mariculture, with the Philippines consistently ranking among the leading producers of carrageenan-bearing red algae. *Kappaphycus alvarezii* is particularly valued for its high carrageenan yield and wide industrial applications in food, pharmaceutical, and cosmetic sectors (Bureau of Fisheries and Aquatic Resources [BFAR], 2022; Dumilag et al., 2022; Solorzano-Chavez et al., 2019). However, the long-term productivity and stability of *K. alvarezii* farming systems are increasingly constrained by disease outbreaks, particularly ice-ice disease, which remains one of the most significant bottlenecks in tropical seaweed cultivation (BFAR, 2022).

Ice-ice disease is characterized by localized tissue whitening, thallus fragmentation, and progressive biomass loss. Its occurrence is strongly associated with environmental stress, including elevated temperature, salinity fluctuations, and nutrient imbalance, which collectively weaken host physiology and promote opportunistic infections (Valderrama et al., 2015; Hurtado et al., 2019; Largo et al., 2020). Pathogenic bacteria such as *Vibrio* spp. and *Pseudoalteromonas carrageenovora*, along with marine fungi, are known to colonize stressed thalli and degrade carrageenan polymers, accelerating tissue deterioration (Largo et al., 1995, 1998, 1999; Riyaz et al., 2020). The increasing frequency of climate-driven stress events has intensified disease outbreaks, contributing to fluctuations and declines in seaweed production in recent years (BFAR, 2022).

Current management strategies for ice-ice disease rely primarily on farm-level interventions, such as site selection, stock rotation, and environmental monitoring, with limited use of targeted treatments. The development of sustainable and scalable disease control approaches remains a critical priority. In this context, plant-derived bioactive compounds have gained attention as eco-friendly alternatives due to their antimicrobial and antioxidant properties. Previous studies have demonstrated that extracts from mangrove species, such as *Avicennia marina* and *Sonneratia alba*, can inhibit ice-ice-associated bacteria through bioactive compounds including alkaloids, flavonoids, and tannins (Rahman et al., 2020; Syafitri et al., 2017). Similarly, macroalgal extracts (e.g., *Sargassum* spp.) have been explored as biostimulants to enhance growth and resilience (Tahiluddin et al., 2022). However, their application in operational seaweed farming systems, particularly through practical delivery methods, remains limited.

Origanum vulgare (oregano) is a well-documented medicinal plant with potent antimicrobial activity attributed primarily to phenolic compounds such as thymol and carvacrol. These compounds disrupt microbial membranes, interfere with metabolic pathways, and inhibit the growth of a broad spectrum of Gram-positive and Gram-negative bacteria (Nazzaro et al., 2013; Marchese et al., 2016). In aquaculture, oregano-derived additives have been shown to enhance immune responses, reduce oxidative stress, and improve disease resistance in fish and crustaceans (Reverter et al., 2021; Zheng et al., 2021). Despite this established efficacy, the potential application of O. vulgare in macroalgal systems has not been investigated, particularly in the context of disease prevention.

Immersion treatment represents a practical and scalable strategy for delivering bioactive compounds in seaweed aquaculture, allowing direct exposure of thalli to plant-derived bioactive treatments while maintaining operational feasibility for both smallholder and commercial farming systems (Lee et al., 2022). Despite its potential applicability, empirical investigations on immersion-based phytochemical treatments for mitigating ice-ice disease in *K. alvarezii* remain notably scarce.

Although numerous plant-derived extracts have demonstrated antimicrobial activity against marine-associated bacterial pathogens, studies assessing their application as immersion treatments under actual seaweed farming conditions are still limited. In particular, the use of *O. vulgare* extract as an immersion-based phytochemical treatment for carrageenan-producing seaweeds has not yet been evaluated under open-sea cultivation systems. Moreover, existing research has predominantly emphasized *in vitro* antibacterial assays, with comparatively little attention given to the physiological and production-related responses of *K. alvarezii* following phytochemical treatment. Specifically, limited information is available regarding the effects of immersion-based phytochemical applications on thallus condition, pigmentation, and biomass performance under field cultivation environments where multiple ecological stressors interact.

The present study focuses primarily on growth performance and thallus pigmentation responses following oregano extract immersion under field conditions. Although visual signs of tissue deterioration were monitored qualitatively, direct measurements of disease incidence, pathogen abundance, or mortality were not performed.

Accordingly, this study aimed to evaluate the effects of immersion-based application of *O. vulgare* extract on the growth performance and pigmentation of *K. alvarezii* cultivated under field conditions. Specifically, the study assessed: (1) the antibacterial activity of the extract against bacterial isolates recovered from diseased thalli, and (2) the influence of immersion duration on growth and pigmentation responses. While the study does not directly quantify disease incidence or pathogen suppression, it examines whether oregano extract immersion is associated with improved thallus condition and biomass performance under operational cultivation conditions.

2 Materials and methods

2.1 Study site and experimental duration

The experiment was conducted in Wakat, Barobo, Surigao del Sur, Philippines (Figure 1), over a 45-day cultivation period from November 2025 to January 2026. Seaweed seedlings were cultured in an open-sea floating long-line system under natural environmental conditions. The culture system was exposed to ambient photoperiod (~12 h light:12 h dark) and prevailing physicochemical conditions typical of coastal farming environments.

2.2 Preparation of *Origanum vulgare* extract

Fresh aerial parts of *Origanum vulgare* were collected from Wakat (Barobo) and Liatimco (Lianga), Surigao del Sur. Plant

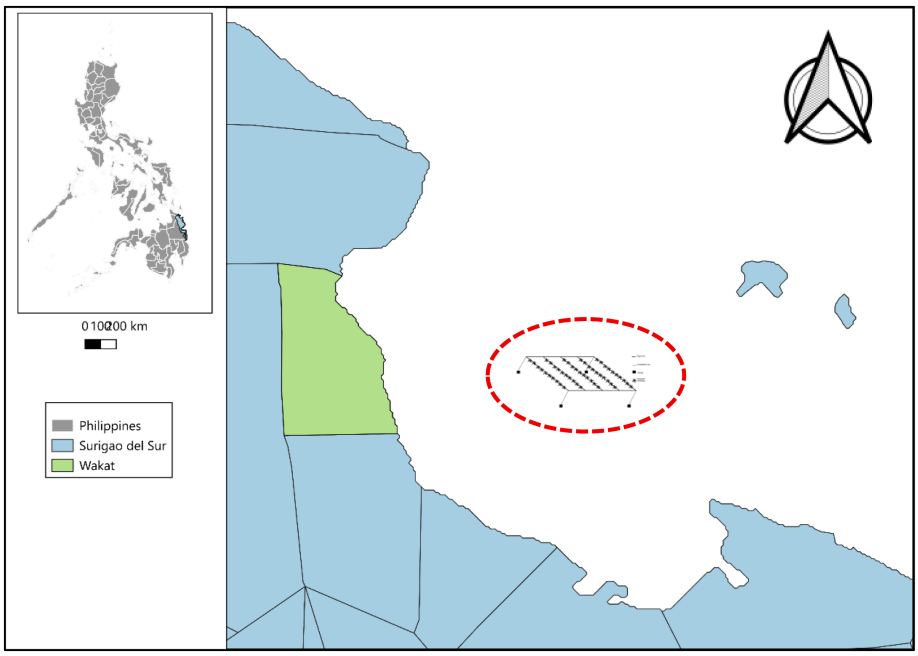


FIGURE 1
Geographic location of the study area in Surigao del Sur, Philippines, showing the experimental site for *K. alvarezii* cultivation. The map highlights the coastal environment where immersion treatments and growth assessments were conducted.

materials were thoroughly washed, shade-dried for 10 days until constant weight was achieved, and ground into fine powder.

Extraction was carried out using a percolation method with 80% ethanol for 72 h. The extract was filtered through Whatman No. 1 filter paper and centrifuged at 5000 rpm for 5 min. The solvent was subsequently removed via rotary evaporation, and the resulting crude extract was stored at 2 °C until further use (Pérez-Delgado et al., 2021; Pourmoghim et al., 2015). Prior to immersion treatment, the crude extract was diluted in sterile seawater to obtain a working concentration of 10 mg mL^{-1} (1% w/v). The same concentration was used for all immersion treatments.

2.3 Phytochemical screening

Preliminary phytochemical analysis of the crude extract was conducted using standard qualitative assays to detect major secondary metabolites. The presence of alkaloids, flavonoids, saponins, steroids, and condensed tannins was confirmed, while cardiac glycosides were absent (Table 1). Specific tests included the froth test for saponins, ferric chloride test for tannins, Liebermann–Burchard test for steroids and Bate-Smith and Metcalf method for flavonoids. These compounds are widely recognized for their antimicrobial and bioactive properties. The phytochemical screening conducted in the present study was qualitative only, and no quantitative determination of thymol, carvacrol, total phenolic content, or other major oregano-derived bioactive compounds was performed.

2.4 *In vitro* antibacterial assay

2.4.1 Bacterial isolation and identification

Symptomatic thalli of *K. alvarezii* exhibiting signs of ice-ice disease were collected and processed under aseptic conditions. Samples were initially rinsed with sterile 0.9% saline solution, surface-sterilized with 70% ethanol for 30 s, and subsequently rinsed with sterile distilled water to minimize surface contaminants. Tissue samples were then macerated, serially diluted, and inoculated onto Marine Agar plates.

The inoculated plates were incubated at 37 °C for 24 h to facilitate the recovery of culturable opportunistic bacteria detectable under standard laboratory conditions. Following incubation, bacterial colonies were enumerated and submitted to an accredited external laboratory at the Department of Science and Technology for identification using standard culture-based biochemical procedures. The bacterial isolates recovered from diseased thalli included *Escherichia coli* (40 CFU g^{-1}) and *Staphylococcus aureus* (330 CFU g^{-1}).

These isolates were regarded as opportunistic bacteria associated with physiologically stressed thalli rather than confirmed primary etiological agents of ice-ice disease. Accordingly, they were utilized as indicator organisms for evaluating the antibacterial activity of *Origanum vulgare* extract against bacteria associated with diseased seaweed tissues.

Although incubation at 37 °C enabled efficient recovery of culturable bacteria, the selected temperature may have favored non-marine or human-associated microorganisms while potentially underrepresenting marine-specific bacterial communities naturally associated with diseased seaweed thalli. This limitation should therefore be considered when interpreting the bacterial profile recovered in the present study.

2.4.2 Disk diffusion assay

The antibacterial activity of *O. vulgare* extract was evaluated using the disk diffusion method. Sterile agar plates were inoculated with bacterial isolates, and paper disks impregnated with the extract were placed on the agar surface. Plates were incubated at 30 °C for 16–18 h, and zones of inhibition were measured in millimeters.

TABLE 1 Phytochemical analysis of *O. vulgare*.

Parameter	Result
Alkaloids Confirmatory Test (+) primary alkaloid (++) secondary alkaloid (+++) tertiary alkaloid	++
Test for Quaternary Bases & Amine Oxide	+
Steroids Keller-Killiani Test: For 2-deoxysugars Liebermann-Burchard Test: For Unsaturated Steroids	- +
Flavonoids Bate-Smith & Metcalf Method: For Leucoanthocyanins	+
Saponins Froth Test	+
Tannins Ferric Chloride Test *Brownish-green color indicates the presence of condensed tannins *Blue-black color indicates the presence of hydrolysable tannins	+ Brownish-green

Bold and underlined text indicates the confirmatory phytochemical test used for the qualitative detection of each secondary metabolite.
The symbol (*) denotes explanatory notes for the Ferric Chloride Test, where a brownish-green color indicates the presence of condensed tannins and a blue-black color indicates the presence of hydrolysable tannins.

Antibacterial activity was classified as follows: <10 mm (inactive), 10–13 mm (partially active), 14–19 mm (active), and >19 mm (very active). All assays were performed in triplicate.

2.5 *In vivo* immersion experiment

2.5.1 Experimental design

Healthy *K. alvarezii* thalli were collected from a disease-free farm and prepared into uniform 50 g segments. The experiment followed a completely randomized design (CRD) with four treatments: (1) control (no immersion), (2) 5-min immersion, (3) 15-min immersion, and (4) 25-min immersion in *O. vulgare* extract. Each treatment consisted of three replicate floating long lines, with 10 thalli per replicate. Following treatment, seaweeds were cultured under open-sea conditions for 45 days. Each floating long line served as one experimental replicate, with 10 thalli attached per replicate line. Statistical analyses were performed using replicate long lines as independent experimental units to avoid pseudoreplication.

2.5.2 Growth performance and pigmentation assessment

Monitoring was conducted weekly throughout the experimental period. Growth performance was assessed using final weight (g), biomass gain (g), average daily weight gain (ADWG, g day^{-1}), and specific growth rate (SGR, % day^{-1}).

SGR was calculated as:

$$\mathrm{SGR}\ (\%\ \mathrm{day}^{-1}) = \frac{\ln W_t - \ln W_0}{t} \times 100$$

where W_t is final weight (g), W_0 is initial weight (g), and t is culture duration (days).

Pigmentation was evaluated using a semi-quantitative scoring system ranging from 1 to 4:

- 1 = faded green with extensive whitening
- 2 = moderate green with limited whitening
- 3 = moderate green pigmentation
- 4 = intense green pigmentation

Scores were recorded weekly for each replicate.

Visual observations of tissue whitening, fragmentation, and structural degradation were conducted qualitatively throughout the experiment, but disease incidence and severity were not quantitatively measured. Pigmentation was assessed using semi-quantitative visual scoring by a single observer, without direct pigment quantification or inter-rater reliability testing.

2.5.3 Water quality monitoring

Environmental parameters were monitored weekly to ensure stable culture conditions. Water temperature (°C), salinity (ppt), turbidity (cm), and pH were measured using a digital thermometer, refractometer, Secchi disk, and digital pH meter, respectively (Phillips, 1990). All values were reported as mean ± standard deviation.

2.6 Statistical analysis

All statistical analyses were performed using JASP (Version 0.19.3) and R software (Version 4.5.0). Data were initially assessed for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test prior to parametric analysis. Growth performance variables (final weight, biomass gain, average daily weight gain, and specific growth rate) were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test for pairwise comparisons. The magnitude of treatment effects was quantified using eta squared (η^2).

To characterize the relationship between immersion duration and growth performance, model-based approaches were employed. A quadratic regression model was fitted to describe the curvilinear dose–response pattern:

$$y = \beta_0 + \beta_1 x + \beta_2 x^2$$

where y represents the response variable and x denotes immersion duration. A preliminary estimate of immersion duration associated with maximum growth response was derived:

$$x_{\mathrm{opt}} = -\frac{\beta_1}{2\beta_2}$$

In addition, a broken-line (piecewise linear) regression model was applied to estimate the practical threshold (breakpoint, τ) beyond which further increases in immersion duration did not result in additional growth improvement. This complementary modeling approach enabled differentiation between theoretical

optimum and operational threshold. Pigmentation data, being ordinal in nature, were analyzed using non-parametric statistical methods. Differences among treatments were evaluated using the Kruskal–Wallis test, followed by Dunn's *post hoc* test with Bonferroni correction. Effect size was calculated using epsilon squared (ε^2). Temporal variation in pigmentation within treatments was assessed using the Friedman test, with Kendall's coefficient of concordance (W) used to estimate effect size. Weekly differences among treatments were further analyzed using Kruskal–Wallis tests. All statistical tests were conducted at a significance level of $p < .05$. Parametric data are presented as mean ± standard deviation (SD), while non-parametric data are expressed as median and interquartile range (IQR).

Longitudinal growth data were analyzed to evaluate temporal changes in biomass across treatments over the 8-week monitoring period. Weekly weight measurements from individual replicates were treated as repeated observations.

Descriptive statistics were computed for each treatment at each time point, including mean, standard deviation (SD), and standard error (SE). The 95% confidence intervals (CI) for mean weight were calculated as:

$$CI_{95\%} = \bar{x} \pm 1.96 \times SE$$

where $\bar{x}$ is the sample mean and $SE = \frac{SD}{\sqrt{n}}$.

Growth trajectories were visualized using line plots of mean weight over time, with shaded regions representing 95% confidence intervals. This approach allowed simultaneous evaluation of central tendency and variability in growth response.

To assess differences in growth dynamics among treatments, longitudinal data were analyzed using generalized estimating equations (GEE) with a Gaussian distribution and exchangeable correlation structure to account for repeated measurements within replicates. Treatment, time (week), and treatment × time interaction were included as fixed effects.

Cumulative productivity was quantified using the area under the growth curve (AUC), calculated for each replicate using the trapezoidal rule:

$$AUC = \sum_{i=1}^{n-1} \frac{(W_i + W_{i+1})}{2} \times (t_{i+1} - t_i)$$

where W_i and W_{i+1} represent consecutive weight measurements at times t_i and t_{i+1}, respectively.

Differences in AUC among treatments were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test for pairwise comparisons.

Additionally, linear regression was applied to estimate growth rates (slope, g week^{-1}) for each treatment using mean weekly weights. These slopes were used to compare relative growth velocity among treatments.

To evaluate the potential influence of environmental variables on growth performance, correlation analysis was conducted between water quality parameters and growth metrics across the experimental period.

Weekly mean values of growth (final weight per treatment) and water quality parameters (temperature, salinity, pH, and turbidity) were computed by averaging A.M. and P.M. measurements. Pearson's correlation coefficient (r) was then used to quantify the strength and direction of linear relationships among variables.

The correlation matrix was constructed using paired weekly observations and visualized through a heatmap to facilitate interpretation of multivariate relationships. Correlation coefficients were interpreted based on their magnitude (weak: $|r| < 0.30$; moderate: 0.30–0.70; strong: >0.70).

This analysis aimed to determine whether environmental fluctuations contributed to variation in growth performance and to verify whether observed treatment effects were independent of water quality conditions.

All statistical analyses were performed at a significance level of $p < .05$.

3 Results

3.1 Bacterial identification in ice-ice–Affected *Kappaphycus alvarezii*

Microbial analysis of diseased *K. alvarezii* thalli revealed the presence of two opportunistic bacterial indicators associated with diseased thalli, *Escherichia coli* and *Staphylococcus aureus* (Table 2). The quantified microbial loads showed a clear disparity between the two species. *E. coli* was detected at a relatively low density (40 CFU g^{-1}), whereas *S. aureus* exhibited a substantially higher load (330 CFU g^{-1}), indicating greater prevalence in ice-ice–affected samples.

The dominance of *S. aureus* suggests a stronger association with diseased thalli, although both bacterial species likely contribute to opportunistic colonization under stress conditions. These findings indicate the presence of opportunistic bacterial colonization in diseased thalli. Although *E. coli* and *S. aureus* are not recognized as primary ice-ice disease pathogens, their occurrence in diseased tissues may reflect opportunistic colonization associated with tissue degradation and environmental stress. Therefore, these isolates were used as indicator organisms for evaluating the antibacterial activity of the extract.

3.2 *In vitro* antibacterial activity of *Origanum vulgare* extract

The antibacterial activity of *Origanum vulgare* extract was evaluated using the disk diffusion method (Table 3). The extract exhibited strong and consistent inhibitory activity against *Staphylococcus aureus*, with a mean inhibition zone of 21 mm, classified as very active (>19 mm).

Against *Escherichia coli*, inhibition zones were consistently 19 mm across replicates, corresponding to an active classification

TABLE 2 Microbial load of bacteria isolated from ice-ice–affected *K. alvarezii*.

Sample	Parameter	Result
K. alvarezii (ice-ice–affected)	E. coli count	40 CFU g^{-1}
	Staphylococcus aureus count	330 CFU g^{-1}

(14–19 mm). This indicates lower susceptibility relative to *S. aureus*, consistent with differences in cell wall structure between Gram-positive and Gram-negative bacteria. Overall, these results demonstrate antibacterial activity of O. vulgare extract against the two tested bacterial isolates, with greater efficacy against the Gram-positive isolate.

3.3 Growth performance

Immersion duration of *Origanum vulgare* extract significantly influenced the growth performance of *K. alvarezii*. One-way ANOVA revealed a strong treatment effect on final weight, F (3, 8) = 171.27, p <.001, with a statistically detectable treatment effect ($\eta^2 = 0.985$), although the unusually high effect-size estimate should be interpreted cautiously because of the limited sample size and strong separation among treatment means.

Final weight increased markedly with immersion duration (Table 4), with the highest values observed in the 15-min treatment (259.67 ± 13.05 g), followed by the 25-min (231.67 ± 12.74 g) and 5-min (168.67 ± 18.56 g) treatments. In contrast, the control group exhibited negative growth (33.00 ± 6.08 g), indicating severe biomass loss under untreated conditions. *Post hoc* comparisons showed that the 15-min treatment did not differ significantly from the 25-min treatment ($p = .123$), but both were significantly higher than the 5-min treatment and control ($p < .01$). The 5-min treatment also significantly outperformed the control ($p < .001$).

3.4 Growth indices and biomass accumulation

Biomass accumulation and derived growth indices (final biomass, total weight gain, ADWG, and SGR) followed trends consistent with final weight (Table 4). The 15-min treatment consistently produced the highest values, followed by the 25-min and 5-min treatments, while the control exhibited minimal biomass and negative growth indices. These results confirm that immersion treatment enhances overall growth efficiency and prevents biomass decline under stress conditions.

Weekly monitoring showed clear treatment-dependent divergence in growth trajectories over time (Figure 2). All groups began with the same mean initial weight at Week 0, but differences became more pronounced from Week 2 onward and were especially evident after Week 4. The 15-min treatment exhibited the strongest and most sustained biomass accumulation, reaching the highest mean weight by the end of the monitoring period. The 25-min treatment followed a similar trajectory but remained consistently below the 15-min treatment during the later weeks. The 5-min treatment showed moderate growth improvement, whereas the control displayed unstable growth and eventual decline.

The confidence intervals indicate increasing variability at later stages, particularly in the 15-min treatment, reflecting heterogeneity among replicates. Despite this variability, the overall pattern remained consistent with the cumulative productivity analysis, in which the 15-min and 25-min treatments outperformed the 5-min treatment and control. These results indicate that immersion duration influenced not only final biomass but also the stability and trajectory of growth over time.

3.5 Relationship between water quality and growth

Correlation analysis revealed strong positive associations among growth variables across treatments (r = 0.94–0.99), indicating consistent patterns in biomass accumulation under different immersion durations (Figure 3). In contrast, water quality parameters exhibited moderate but inconsistent correlations with growth performance ($|r| < 0.70$), with no clear pattern suggesting a dominant environmental influence.

Temperature showed moderate negative correlations with growth variables (r = −0.65 to −0.70), while salinity and turbidity exhibited weak to moderate negative associations. In contrast, pH displayed moderate positive correlations with growth; however, these relationships were not consistent across treatments. Overall, the lack of strong and consistent correlations between water quality parameters and growth suggests that environmental variability was not strongly associated with the observed differences in biomass accumulation during the study period.

3.6 Dose–response relationship

Quadratic regression analysis revealed a significant curvilinear relationship between immersion duration and final weight ($R^2 = 0.974$, $p < .001$; Figure 4A). Growth increased with immersion duration up to an intermediate level and declined slightly at higher exposure, producing a clear inverted U-shaped response. The model provided a preliminary estimate of immersion duration associated with maximum growth response within the intermediate immersion-duration range evaluated in the present study.

To further refine this relationship, a broken-line regression model was applied (Figure 4B). The analysis suggested a possible saturation response at intermediate immersion durations, beyond which additional immersion did not yield further increases in final weight, indicating a saturation threshold.

The combined modeling approaches provide complementary interpretations of the dose–response pattern. While the quadratic model defines the theoretical optimum, the broken-line model identifies the practical threshold for maximizing growth. Together, these results indicate that intermediate immersion

TABLE 3 Antibacterial activity of *Origanum vulgare* extract based on zone of inhibition (mm).

Bacterial isolate	Trial 1	Trial 2	Trial 3	Mean (mm)	Activity classification
Staphylococcus aureus	22	22	20	21	Very active
Escherichia coli	19	19	19	19	Active

TABLE 4 Growth performance of *K. alvarezii* under different immersion durations of *O. vulgare* extract.

Parameter	Treatments			
	Control	5 min	15 min	25 min
Final Weight (g)	33.00 ± 6.08^{c}	168.67 ± 18.56^{b}	259.67 ± 13.05^{a}	231.67 ± 12.74^{ab}
Final Biomass (g)	165.00 ± 30.41^{c}	843.33 ± 92.78^{b}	1298.33 ± 65.26^{a}	1158.33 ± 63.71^{ab}
Total Weight Gain (g)	−85.00 ± 30.41^{c}	593.33 ± 92.78^{b}	1048.33 ± 65.26^{a}	908.33 ± 63.71^{ab}
ADWG (g day^{-1})	−0.38 ± 0.14^{c}	2.64 ± 0.41^{b}	4.66 ± 0.29^{a}	4.04 ± 0.28^{ab}
SGR (%)	−37.78 ± 13.52^{c}	263.70 ± 41.24^{b}	465.93 ± 29.00^{a}	403.70 ± 28.31^{ab}

Values are presented as mean ± standard deviation (n = 3). Different superscript letters within a row indicate significant differences among treatments based on Tukey's honestly significant difference (HSD) test at p <.05.

durations optimize growth performance, whereas prolonged exposure offers no additional benefit and may slightly reduce efficiency.

3.7 Pigmentation dynamics

Pigmentation scores differed significantly among treatments (Kruskal–Wallis H = 57.00, p <.001), with a very large effect size ($\varepsilon^2 \approx 0.95$). The control group exhibited the lowest pigmentation (median = 1; IQR: 1–2), reflecting pronounced depigmentation and poor thallus condition.

The 5-min treatment showed moderate pigmentation (median = 2; IQR: 2–3), indicating partial recovery. In contrast, the 15- and 25-min treatments achieved significantly higher pigmentation scores (median = 3; IQR: 3–4), corresponding to improved thallus coloration and structural integrity, consistent with the visual observations (Figure 5). No significant difference was observed between these treatments, indicating comparable effectiveness at longer immersion durations (Table 5).

3.8 Temporal pattern of pigmentation response

While overall pigmentation differences are summarized in Table 6, temporal analysis revealed distinct treatment-specific trajectories (Figure 5). Pigmentation changed significantly over time within all treatments (Table 6; all p <.001; Kendall's W = 1.00), indicating highly uniform directional trends in pigmentation responses across replicate observations. However, the unusually high Kendall's W values may partly reflect the limited ordinal scoring scale and relatively small number of replicate observations used in the present study. The control group exhibited a rapid and sustained decline in pigmentation after Week 0, stabilizing at low scores (1–2), indicative of progressive tissue deterioration. The 5-min treatment partially mitigated this decline but remained at intermediate levels (2–3).

In contrast, the 15- and 25-min treatments demonstrated sustained improvement, maintaining moderate pigmentation

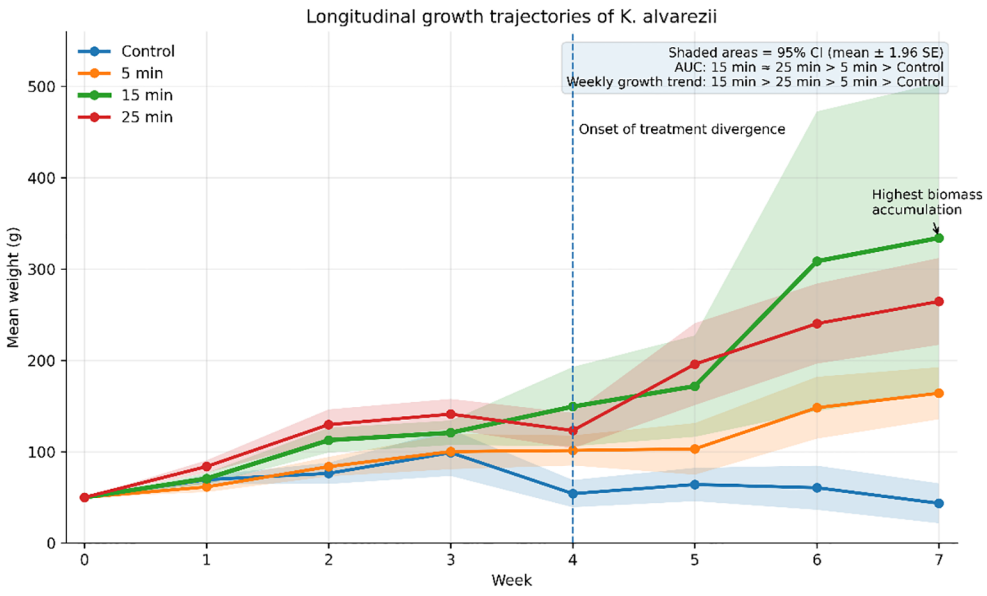


FIGURE 2
Longitudinal growth trajectories of *Kappaphycus alvarezii* under different immersion durations of *Origanum vulgare* extract. Points represent mean weight, and shaded regions indicate 95% confidence intervals (mean ± 1.96 SE). Growth trajectories diverged after Week 4, with the 15-min treatment showing the highest biomass accumulation. The 25-min treatment showed strong but slightly lower growth, the 5-min treatment showed moderate improvement, and the control exhibited unstable growth and decline. In-figure annotations summarize the pattern of cumulative productivity and weekly growth trend.

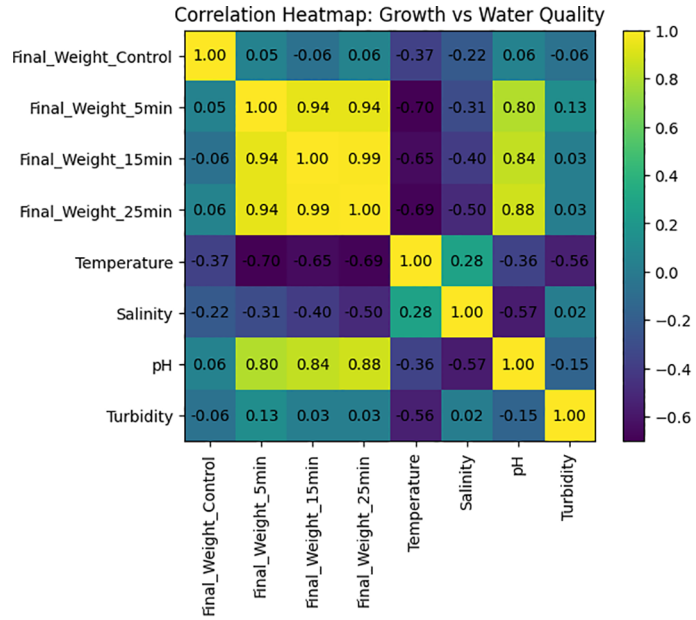


FIGURE 3
Correlation heatmap showing relationships between growth performance (final weight across treatments) and water quality parameters during the experimental period. Values represent Pearson correlation coefficients. Strong positive correlations were observed among growth variables across treatments, while water quality parameters showed moderate but inconsistent associations.

during early weeks and reaching intense green pigmentation (score = 4) from Week 5 onward (Figure 6). Between-treatment comparisons confirmed significant differences from Week 1 through Week 7 (Table 7; all p <.001), while no differences were observed at Week 0. These findings indicate that oregano immersion rapidly altered pigmentation trajectories, preventing depigmentation and promoting recovery.

Collectively, immersion duration influenced not only the magnitude of pigmentation response but also the temporal progression of thallus health, with longer durations producing more stable and sustained improvements.

4 Discussion

Immersion treatment with *O. vulgare* extract improved growth performance and pigmentation of *K. alvarezii* cultivated under field conditions. Treated thalli exhibited greater biomass accumulation, improved coloration, and more stable growth trajectories compared with untreated controls. These findings suggest that oregano-derived bioactive compounds may contribute to improved thallus condition under cultivation conditions.

Microbial analysis detected *Escherichia coli* and *Staphylococcus aureus* in diseased thalli, with *S. aureus* occurring at substantially

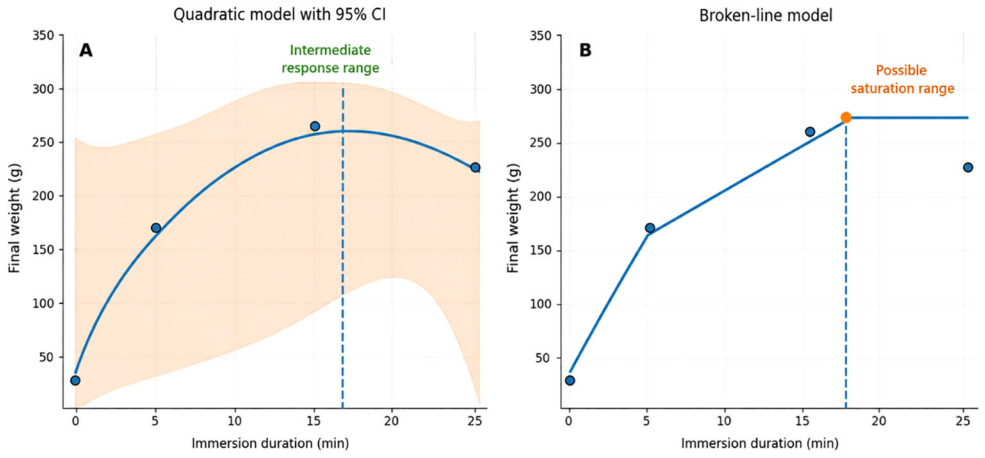


FIGURE 4
Dose–response relationship between immersion duration of *O. vulgare* extract and final weight of *K. alvarezii*. **(A)** Quadratic regression model showing a curvilinear response pattern, with growth increasing at intermediate immersion durations and declining slightly at prolonged exposure. The shaded region represents the 95% confidence interval, indicating model uncertainty. **(B)** Broken-line regression model suggesting a possible saturation response at intermediate immersion durations. Points represent treatment means (n = 3). Because only four treatment levels were evaluated, the modeled response patterns should be interpreted as exploratory rather than definitive estimates of optimal immersion duration.

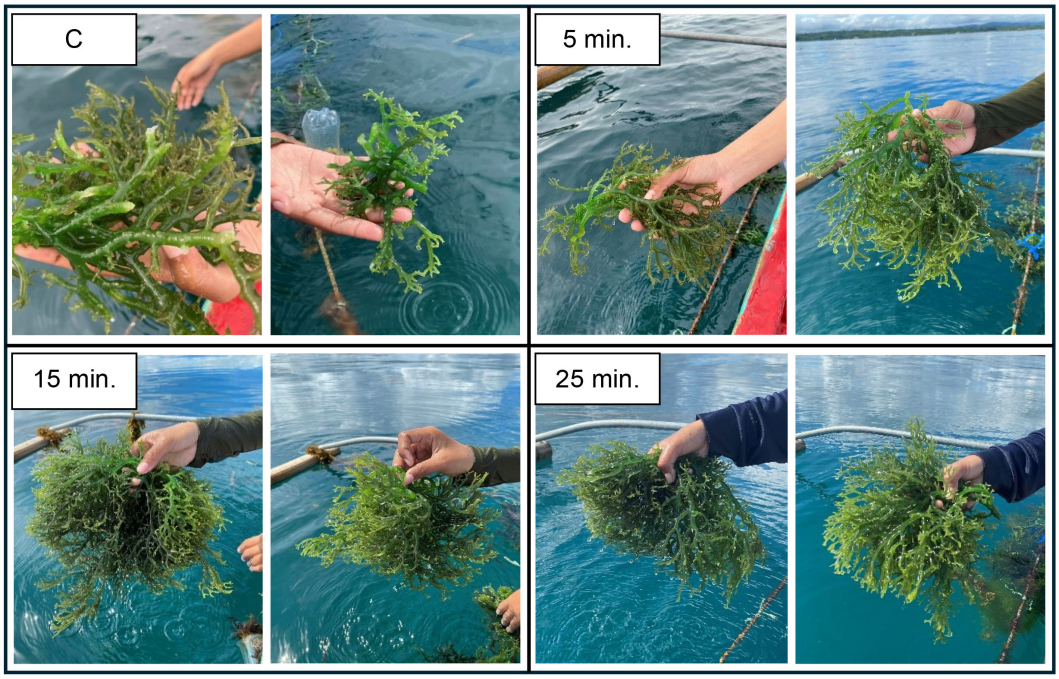


FIGURE 5
Representative visual condition of *Kappaphycus alvarezii* thalli under different immersion durations of *Origanum vulgare* extract. The control (C) exhibits pronounced depigmentation and signs of tissue deterioration associated with ice-ice disease. The 5-min treatment shows partial improvement in coloration, while the 15- and 25-min treatments display enhanced pigmentation, structural integrity, and overall thallus health.

higher densities. Although these bacteria are not commonly identified as primary causative agents of ice-ice disease, their occurrence may reflect secondary colonization associated with stressed or deteriorating tissues (Arasamuthu and Edward, 2018). The presence of these isolates therefore likely represents opportunistic bacterial association with tissue degradation under environmental stress conditions rather than direct evidence of disease causation.

The antibacterial assay demonstrated that oregano extract inhibited both bacterial isolates, with stronger activity against *S. aureus* than *E. coli*. This pattern is consistent with previous reports describing the antimicrobial properties of oregano-derived compounds such as carvacrol, thymol, flavonoids, and phenolics (Fidan et al., 2019; da Silva et al., 2021; Marchese et al., 2016; Nazzaro et al., 2013; Nurzyńska-Wierdak and Walasek-Janusz, 2025). The lower susceptibility of *E. coli* may be associated with the protective outer membrane characteristic of Gram-negative bacteria. Comparable antibacterial activity of oregano and other plant-derived extracts has also been reported in previous studies involving aquatic pathogens and opportunistic bacteria (Pérez-Delgado et al., 2021; Amri et al., 2023; Rahman et al., 2020; Sulistijowati and Karim, 2020). However, the ecological relevance of the tested isolates to ice-ice disease remains uncertain, and the antibacterial results should therefore be interpreted cautiously.

TABLE 5 Summary of pigmentation scores of *K. alvarezii*.

Parameter	Treatments			
	Control	5 min	15 min	25 min
n	40	40	40	40
Median pigmentation score	1[a]	2[b]	3[c]	3[c]
IQR	1–2	2–3	3–4	3–4

Immersion duration significantly influenced growth performance, with the 15-min treatment producing the highest final weight, biomass accumulation, average daily weight gain, and specific growth rate. In contrast, untreated thalli exhibited biomass loss and poor overall condition. These findings indicate that moderate immersion durations may be sufficient to maximize the beneficial effects of oregano extract, while longer exposure provides little additional improvement. Similar threshold responses have been observed in aquaculture applications involving phytogenic compounds and bioactive plant extracts (Fadilah et al., 2016; Reverter et al., 2021; Zheng et al., 2021). Comparable physiological effects of plant-derived bioactive compounds have also been reported in animal production systems. Chen et al. (2023) demonstrated that *Litsea cubeba* essential oil improved growth performance, antioxidant capacity, immune function, and microbial balance in livestock, suggesting that phytogenic compounds may exert multiple complementary physiological effects beyond direct antimicrobial activity. Similar multifunctional mechanisms may partly explain the improved biomass accumulation and pigmentation stability observed in oregano-treated *K. alvarezii*.

The dose–response analyses further supported this interpretation. Quadratic regression provided a preliminary estimate of immersion duration associated with maximum growth response at approximately 17.41 min, whereas broken-line regression indicated a practical threshold between 15 and 18 min beyond which growth gains plateaued. Together, these results suggest that intermediate immersion durations may optimize growth performance,

TABLE 6 Within-treatment temporal variation in semi-quantitative pigmentation scores of *K. alvarezii* across the 8-week observation period.

Treatment	χ^2 (Friedman)	df	P-value	Kendall's W	Interpretation
Control	35	7	<.001	1	Significant decline in pigmentation
5 min	35	7	<.001	1	Moderate but consistent improvement
15 min	35	7	<.001	1	Strong and sustained improvement
25 min	35	7	<.001	1	Strong and sustained improvement

Values were analyzed using the Friedman test. Kendall's W represents effect size.

whereas prolonged exposure may reduce efficiency due to diminishing biological returns. Nevertheless, these optimization estimates should be considered preliminary because only four immersion durations were evaluated. Because only four immersion durations were evaluated, the regression-derived optimization estimates should be interpreted cautiously and regarded as exploratory rather than definitive.

Longitudinal growth monitoring showed that treatment effects became more pronounced after Week 4. The 15-min treatment maintained the highest and most consistent biomass accumulation throughout the cultivation period, whereas the control group exhibited unstable growth and progressive decline. The 25-min treatment followed a similar but slightly lower trajectory, while the 5-min treatment produced only moderate improvement. These findings indicate that oregano immersion influenced not only final biomass accumulation but also the stability of growth performance over time.

The pronounced biomass decline observed in the untreated control may have been associated with cumulative environmental stress, tissue deterioration, or opportunistic microbial colonization during the cultivation period. Similar biomass reductions under stressful environmental conditions have been reported in previous *Kappaphycus* cultivation studies (Largo et al., 1995; Hurtado et al., 2019; Largo et al., 2020).

Pigmentation analysis demonstrated that oregano immersion improved the visual condition of the thalli. Treated seaweeds, particularly those exposed for 15 and 25 min, maintained higher pigmentation scores and better structural integrity than untreated controls. Because pigmentation may reflect overall thallus condition, the observed improvements may indicate reduced visible stress and better tissue maintenance. However, direct physiological measurements such as chlorophyll concentration or photosynthetic efficiency were not conducted. Previous studies have shown that stressed macroalgae commonly exhibit pigment degradation and bleaching, whereas healthy thalli maintain stronger pigment synthesis and photosynthetic activity (Wu et al., 2015; Chen et al., 2023).

Temporal pigmentation patterns further showed that oregano treatment altered the progression of thallus deterioration. The control group exhibited rapid depigmentation beginning after Week 0, whereas the 15- and 25-min treatments maintained progressively improved pigmentation throughout the monitoring period. Significant weekly differences among treatments suggest that immersion duration influenced both the magnitude and stability of pigmentation response.

Correlation analysis indicated strong positive relationships among growth variables across treatments, whereas water quality parameters showed moderate but inconsistent associations with biomass accumulation. Although temperature and pH displayed moderate correlations with growth, these relationships were insufficient to explain the observed treatment effects. These findings suggest that immersion treatment may have contributed more

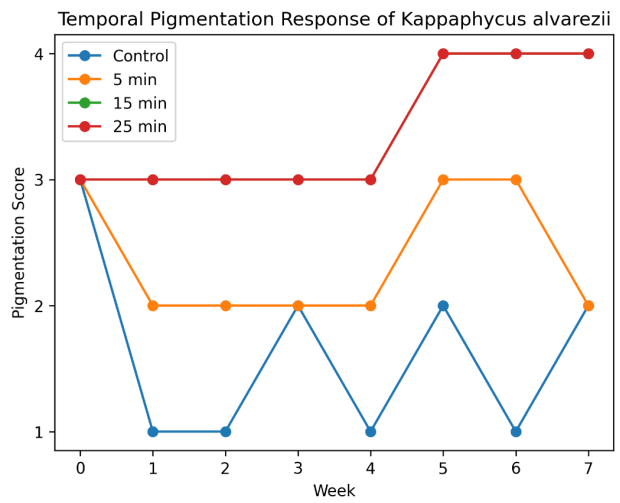


FIGURE 6
Temporal changes in semi-quantitative pigmentation scores of *K. alvarezii* under different immersion durations of *O. vulgare* extract over 8 weeks. Values represent mean pigmentation scores (n = 5). The control group shows rapid depigmentation, while the 5-min treatment maintains moderate pigmentation. In contrast, the 15- and 25-min treatments exhibit sustained improvement, reaching intense green pigmentation (score = 4) from Week 5 onward.

TABLE 7 Weekly comparison of semi-quantitative pigmentation scores of *Kappaphycus alvarezii* among treatments using the Kruskal–Wallis test.

Week	Kruskal–Wallis H	P-value	Significance
Week 0	—	—	Not tested (no variation)
Week 1	19	0.000273	***
Week 2	19	0.000273	***
Week 3	19	0.000273	***
Week 4	19	0.000273	***
Week 5	19	0.000273	***
Week 6	19	0.000273	***
Week 7	19	0.000273	***

*** indicates highly significant differences (p <.001).

strongly to growth differences than the measured environmental variables during the study period.

Environmental conditions remained within suitable ranges for *K. alvarezii* cultivation throughout the experiment, which likely minimized additional physiological stress. Previous studies have emphasized the importance of maintaining optimal temperature and salinity conditions for efficient metabolic activity and growth in carrageenan-producing seaweeds (Saguid et al., 2022; Rupert et al., 2022; Mulyono et al., 2025). Stable turbidity conditions may also have supported adequate light penetration and overall cultivation performance (Cáceres-Farias et al., 2025).

Overall, the findings suggest that the immersion-based application of O. vulgare extract may offer a practical and environmentally sustainable approach to improving growth and thallus condition in *K. alvarezii*. However, further studies incorporating pathogen-specific assays, microbial community analyses, quantitative pigment determination, and direct measurements of disease incidence are needed to clarify the mechanistic role of oregano extract in seaweed health management. The use of short immersion durations may nevertheless offer a feasible strategy for seaweed farming systems facing increasing environmental stress associated with climate variability (Masson-Delmotte et al., 2018; Tahiluddin et al., 2023).

Several limitations should be considered when interpreting the present findings. The study did not directly quantify disease incidence, pathogen abundance, microbial community composition, or tissue mortality. In addition, only four immersion durations were evaluated, and no seawater-only immersion control was included. Consequently, the observed responses cannot be attributed exclusively to oregano extract exposure because immersion handling, osmotic effects, or temporary manipulation stress may also have contributed to treatment-related variation. Therefore, the findings should be interpreted as responses associated with immersion-based oregano treatment rather than definitive evidence of extract-specific physiological effects.

Pigmentation assessment relied on semi-quantitative visual scoring rather than direct physiological measurements such as chlorophyll quantification or photosynthetic analysis, making interpretations regarding stress tolerance, physiological resilience, and tissue condition preliminary. Furthermore, the phytochemical screening conducted in the present study was qualitative in nature and did not identify or quantify the specific oregano-derived compounds responsible for the observed biological responses.

Future studies should incorporate additional immersion-control treatments, expanded treatment gradients, larger replicate numbers, quantitative physiological measurements, chromatographic phytochemical profiling, and bioactivity-guided compound analyses to establish clearer mechanistic relationships between phytochemical composition and biological activity. Structure–function characterization approaches similar to those employed by Gong et al. (2023) may provide useful frameworks for linking phytochemical composition with functional bioactivity, while the robust experimental and analytical strategies demonstrated by Pan et al. (2023) may improve the rigor of future stress-response investigations involving *K. alvarezii* phytochemical treatments.

5 Conclusion

Immersion treatment with *O. vulgare* extract improved growth performance, pigmentation, and overall thallus condition of *K. alvarezii* cultivated under field conditions. Treated seaweeds exhibited greater biomass accumulation and more stable pigmentation responses than untreated controls. The extract also demonstrated antibacterial activity against the two tested bacterial isolates recovered from diseased thalli. Regression analyses suggested that intermediate immersion durations between approximately 15 and 18 min may provide favorable growth responses, although this estimate should be considered preliminary because only four treatment levels were evaluated. While the findings support the potential application of plant-derived bioactive compounds in seaweed aquaculture, the present study does not provide direct evidence of ice-ice disease prevention or pathogen suppression. Future studies incorporating pathogen-specific analyses, quantitative disease assessment, and physiological measurements are needed to clarify the mechanistic role of *O. vulgare* extract in seaweed health management.

Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request and without undue reservation.

Author contributions

AC: Writing – original draft, Visualization, Investigation, Formal analysis, Writing – review & editing. AC: Investigation, Writing – original draft, Formal analysis. JT: Investigation, Writing – review & editing, Validation, Methodology. KG: Writing – review & editing, Methodology, Investigation, Validation. KD: Methodology, Investigation, Validation, Writing – review & editing. JC: Formal analysis, Visualization, Resources, Validation, Software, Writing – review & editing, Methodology, Supervision, Writing – original draft, Investigation, Conceptualization.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was used in the creation of this manuscript. Generative AI was used in the preparation of this manuscript for language refinement, grammatical editing, formatting assistance, and improvement of scientific clarity. The authors reviewed, verified, and take full responsibility for the accuracy, originality, interpretation, and integrity of all content presented in the manuscript.

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References

Amri, I. A., Ramadani, N. F., Hamidah, F., Dameanti, F. N. A. E. P., and Adrenalin, S. L. (2023). Potential antibacterial effects of ethanol extract and essential oil of *Origanum vulgare* on *Klebsiella pneumoniae* and *Staphylococcus aureus*. *World Veterinary J.* 13, 486–491. doi: 10.54203/scil.2023.wvj51

Arasamuthu, A., and Edward, J. K. P. (2018). Occurrence of ice-ice disease in seaweed Kappaphycus alvarezii at Gulf of Mannar and Palk Bay, Southeastern India. *Indian J. Geo Mar. Sci.* 47, 1208–1216.

BFAR (2022). *National Seaweed (Kappaphycus) Industry Roadmap 2022–2026* (Quezon City: Bureau of Fisheries and Aquatic Resources, Department of Agriculture, Philippines). Available online at: https://www.bfar.da.gov.ph/wp-content/uploads/2022/11/Seaweed-Industry-Roadmap.pdf (Accessed March 16, 2025).

Cáceres-Farias, L., Montúfar-Romero, M., Lodeiros, C., Espinoza-Vera, M. M., Cáceres-Farias, L. B., Avendaño, U., et al. (2025). Growth rate comparison of three *Kappaphycus alvarezii* colour strains cultivated using tubular netting and tie-tie methods in the waters of Ecuador. *J. Appl. Phycol.* 37, 1–14. doi: 10.1007/s10811-025-03465-5

Chen, Z., Wu, W., Wen, Y., Zhang, L., Wu, Y., Farid, M. S., et al. (2023). Recent advances of natural pigments from algae. *Food Prod. Process. Nutr.* 5, 39. doi: 10.1186/s43014-023-00164-0

da Silva, L. R. R., Ferreira, O. O., Cruz, J. N., Franco, C. D. J. P., Dos Anjos, T. O., Cascaes, M. M., et al. (2021). Lamiaceae essential oils: Phytochemical profile, antioxidant, and biological activities. *Evidence-Based Complementary Altern. Med.* 2021, 6748052. doi: 10.1155/2021/6748052

Dumilag, R. V., Crisostomo, B. A., Aguinaldo, Z. Z. A., Hinaloc, L. A. R., Liao, L. M., Roa-Quiaoit, H. A., et al. (2022). The diversity of eucheumatoid seaweed cultivars in the Philippines. *Rev. Fisheries Sci. Aquaculture* 31, 47–65. doi: 10.1080/23308249.2022.2060038

Fadilah, S., Pong-Masak, P. R., Santoso, J., and Parenrengi, A. (2016). Growth, morphology and growth related hormone level in Kappaphycus alvarezii produced by mass selection in Gorontalo Waters, Indonesia. *HAYATI J. Biosci.* 23, 29–34. doi: 10.1016/j.hjb.2015.09.004

Fidan, H., Stankov, S., Ivanova, T., Stoyanova, A., Damyanova, S., and Ercisli, S. (2019). Characterization of aromatic compounds and antimicrobial properties of four spice essential oils from family Lamiaceae. *Ukr. Food J.* 8, 227–238. doi: 10.24263/2304-974x-2019-8-2-3

Gong, Z., Liu, M., Liu, H., Deng, Z., Qin, X., Nie, J., et al. (2023). Structural features and *in vitro* antitumor activity of a water-extracted polysaccharide from *Ganoderma applanatum*. *New J. Chem.* 47, 13205–13217. doi: 10.1039/D3NJ01903A

Hurtado, A. Q., Neish, I. C., and Critchley, A. T. (2019). Phyconomy: The extensive cultivation of seaweeds, their sustainability and economic value, with particular reference to important lessons to be learned and transferred from the practice of eucheumatoid farming. *Phycologia* 58, 472–483. doi: 10.1080/00318884.2019.1625632

Largo, D. B., Fukami, K., Nishijima, T., and Ohno, M. (1995). Laboratory-induced development of the ice-ice disease of the farmed red algae *Kappaphycus alvarezii* and *Eucheuma denticulatum* (Solieriaceae, Gigartinales, Rhodophyta). *J. Appl. Phycol.* 7, 539–543. doi: 10.1007/BF00003948

Largo, D. B., Fukami, K., Adachi, M., and Nishijima, T. (1998). Immunofluorescent detection of ice-ice disease-promoting bacterial strain Vibrio sp. P11 of the farmed macroalga Kappaphycus alvarezii. *J. Mar. Biotechnol.* 6 (3), 178–182.

Largo, D. B., Fukami, K., and Nishijima, T. (1999). Time-dependent attachment mechanism of bacterial pathogen during ice-ice infection in Kappaphycus alvarezii. *J. Appl. Phycol.* 11, 129–136. doi10.1023/A:1008081513451

Largo, D. B., Msuya, F. E., and Menezes, A. (2020). *Understanding Disease and Control in Seaweed Farming in Zanzibar (FAO Fisheries and Aquaculture Technical Paper No. 662)* (Rome: FAO).

Lee, H. G., Jeon, Y. J., and Kim, J. K. (2022). Environmental impacts of antibiotic use in aquaculture and alternative approaches: A review. *J. Mar. Sci. Eng.* 10, 327. doi: 10.3390/jmse10030327

Marchese, A., Orhan, I. E., Daglia, M., Barbieri, R., Di Lorenzo, A., Nabavi, S. F., et al. (2016). Antibacterial and antifungal activities of thymol: A brief review of the literature. *Food Chem.* 210, 402–414. doi: 10.1016/j.foodchem.2016.04.122

Masson-Delmotte, V., Zhai, P., Pörtner, H. O., Roberts, D., Skea, J., Shukla, P. R., et al. (2018). "Global warming of 1.5°C: An IPCC special report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways," in *World Meteorological Organization*. Geneva: World Meteorological Organization (WMO).

Mulyono, S., Susanto, G. N., Riantini, M., Setyawan, A., and Abidin, Z. (2025). Water quality analysis on the growth of seaweed (*Kappaphycus alvarezii*) using the off-bottom method in Ketapang District, South Lampung. *Akuatikisle: Jurnal Akuakultur Pesisir Dan. Pulau-Pulau Kecil* 9, 67–71.

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., and De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals* 6, 1451–1474. doi: 10.3390/ph6121451

Nurzyńska-Wierdak, R., and Walasek-Janusz, M. (2025). Chemical composition, biological activity, and potential uses of oregano (*Origanum vulgare* L.) and oregano essential oil. *Pharmaceuticals* 18, 267. doi: 10.3390/ph18020267

Pan, Y.-H., Nong, B.-X., Chen, L., Yang, X.-H., Xia, X.-Z., Zhang, Z.-Q., et al. (2023). QTL mapping and identification of candidate genes for cold tolerance at the germination stage in wild rice. *Genes Genomics* 45, 867–885. doi: 10.1007/s13258-023-01394-6

Pérez-Delgado, O., Alvarado-Pineda, R. L., and Yacarini-Martínez, A. E. (2021). *In vitro* antibacterial activity of crude ethanolic extract from the leaves of *Origanum vulgare* against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and Escherichia coli ATCC 25922. *J. Selva Andina Res. Soc.* 12, 21–29.

Pourmoghim, H., Haghighi, M., and Rohani, M. S. (2015). Effect of dietary inclusion of Origanum vulgare extract on non-specific immune responses and hematological parameters of rainbow trout (*Oncorhynchus mykiss*). *Bull. Environment Pharmacol. Life. Sci.* 4, 33–39.

Rahman, S. A., Mutalib, Y., Sangkia, F. D., Athirah, A., Kadir, M., and Pattirane, C. P. (2020). "Evaluation of inhibitory potential of mangrove leaves extract Avicennia marina for bacteria causing ice-ice diseases in seaweed *Kappaphycus alvarezii*," in *IOP Conference Series: Earth and Environmental Science*, vol. 564. Bristol: IOP Publishing, 012056. doi: 10.1088/1755-1315/564/1/012056

Reverter, M., Tapissier-Bontemps, N., Lecchini, D., Banaigs, B., and Sasal, P. (2021). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. *Aquaculture* 541, 736795. doi: 10.1016/j.aquaculture.2021.736795

Riyaz, S. U. M., Nalini, S., Kavitha, G., Sutha, S. A. D., and Inbakandan, D. (2020). Characterization and identification of isolated bacteria from ice-ice disease infected seaweed Kappaphycus alvarezii. *Indian J. Geo-Mar. Sci.* 48 (8), 1286–1290.

Rupert, R., Rodrigues, K. F., Thien, V. Y., and Yong, W. T. L. (2022). Carrageenan from Kappaphycus alvarezii (Rhodophyta, Solieriaceae): Metabolism, structure, production, and application. *Front. Plant Sci.* 13, 859635. doi: 10.3389/fpls.2022.859635

Saguid, D. M. S., Cuevas, D. I. L., Villajin, J. A., Cawaling, J. M., and Saco, J. A. (2022). Growth rate and carrageenan yield of *Kappaphycus alvarezii* in a land-based setup using artificial seawater. *Int. Res. J. Innov. Engineering Sci. Technol.* 8, 5–9.

Solórzano-Chávez, E. G., Paz-Cedeno, F. R., and Collaborators (2019). Evaluation of the Kappaphycus alvarezii growth under different environmental conditions and

efficiency of the enzymatic hydrolysis of the residue generated in the carrageenan processing. *Biomass Bioenergy* 127, 105254.

Sulistijowati, R., and Karim, Z. (2020). The inhibition of Vibrio alginolyticus by the flavonoid extract of *Sonneratia alba* fruit. *Aquaculture Aquarium Conserv. Legislation* 13, 28–35.

Syafitri, E., Prayitno, S. B., Radjasa, O. K., and Ma'ruf, W. F. (2017). The performance of mangrove leaf extract (Sonneratia alba) in combating bacteria associated with ice–ice disease of seaweed (Kappaphycus alvarezii). *Adv. Sci. Lett.* 23 (7), 6413–6415. doi: 10.1166/asl.2017.9639

Tahiluddin, A. B., Imbuk, E. S., Sarri, J. H., Mohammad, H. S., Ensano, F. N. T., Maddan, M. M., et al. (2023). Eucheumatoid seaweed farming in the southern Philippines. *Aquat. Bot.* 189, 103697. doi: 10.1016/j.aquabot.2023.103697

Tahiluddin, A., Irin, S. S., Jumadil, K., Muddihil, R., and Terzi, E. (2022). Use of brown seaweed extracts as bio-fertilizers and their effects on carrageenan yield, ice-ice disease occurrence, and growth rate of the red seaweed *Kappaphycus striatus*. *Yuzuncu Yıl Univ. J. Agric. Sci.* 32, 436–447. doi: 10.29133/yyutbd.1071446

Valderrama, D., Cai, J., Hishamunda, N., Ridler, N., Neish, I. C., Hurtado, A. Q., et al. (2015). The economics of Kappaphycus seaweed cultivation in developing countries: A comparative analysis of farming systems. *Aquaculture Econ Manage.* 19, 251–277. doi: 10.1080/13657305.2015.1024348

Wu, H., Jiang, H., Liu, C., and Deng, Y. (2015). Growth, pigment composition, chlorophyll fluorescence and antioxidant defenses in the red alga *Gracilaria lemaneiformis* (Gracilariales, Rhodophyta) under light stress. *S. Afr. J. Bot.* 100, 27–32. doi: 10.1016/j.sajb.2015.04.010

Zheng, Y., Xie, J., Chen, Y., He, S., and Sun, C. (2021). Effect of dietary oregano (*Origanum vulgare*) essential oil on growth performance, immune response and intestinal health of Nile tilapia (*Oreochromis niloticus*). *Aquacult. Rep.* 20, 100678. doi: 10.1016/j.aqrep.2021.100678