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# Growth and epiphytic responses of *Gracilaria fisheri* to *Ascophyllum* seaweed extract under controlled culture conditions

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## Abstract

*Gracilaria fisheri* is an important red seaweed on the sea coast of Thailand. Cultivation of this seaweed has brought economic benefits to the farmers in this country. However, its low growth and quality are problematic due to high contamination and epiphyte outbreaks. This study was performed to examine the growth and epiphytic responses of *G. fisheri* to *Ascophyllum* seaweed extract (SE). The algal samples were treated with SE at different concentrations (0, 0.1, 0.5, and 1 g SE $L^{-1}$). Three sets of experiments were conducted in the laboratory under controlled culture conditions of salinity of 30‰, temperature of 25–26°C, and light intensity of 200 µmol photons $m^{-2}$ $s^{-1}$. The algal samples were soaked for 30 min in SE alone (Experiment 1), in Provasoli Enriched Seawater (PES)+SE (Experiment 2), and in PES+SE with a 5% $CO_2$ supplement (Experiment 3). The results showed a significant reduction in epiphytes (>90%) in the sample after one week of treatment with 1 g SE $L^{-1}$. The use of SE significantly stimulated the branching of *G. fisheri* ($p < 0.05$). In comparison to the control plant (PES), the growth rate of the samples treated with PES+0.1 g SE $L^{-1}$ was 3.40 ± 0.51% $day^{-1}$ in the first week of culture, and this was increased to 3.84 ± 0.63% $day^{-1}$ in the samples treated with PES+1 g SE $L^{-1}$. The growth rate was significantly increased to 5.46 ± 1.05% $day^{-1}$ in the samples treated with PES+1 g SE $L^{-1}$ with a 5% $CO_2$ supplement. This study suggested that the use of the *Ascophyllum* seaweed extract could inhibit epiphytic attachment and that supplementation with 5% $CO_2$ resulted in enhanced growth of *G. fisheri* under controlled culture conditions.

**Keyword** Epiphyte · Rhodophyta · Brown seaweed extract · Seaweed cultivation

## Introduction

The red seaweed *Gracilaria* is an important source for the global agar industry, comprising more than 91% of the raw material supply (Porse and Rudolph 2017). The world supply of seaweed is produced mainly in East and Southeast Asia from aquaculture. In 2019, the production of seaweed in Asia constituted 97.4% of the world's production, of which 99.1% was from cultivation (Cai et al. 2021). In Thailand, *Gracilaria* is a commercially available red seaweed and twenty species have been identified (Chirapart 2008; Muangmai et al. 2014). The species *Gracilaria fisheri* is commonly used for water treatment in marine aquaculture pond systems. Cultivation is mostly performed in shrimp effluent ponds, which has brought economic benefits to local farmers each year. The total production is approximately 200 t dry weight per year (Lewmanomont and Chirapart 2022). The harvested *Gracilaria* is mainly sold for human consumption and exported to neighboring countries. However, the growth and production of algae are unstable, especially the quality of the algae. The major problem in *Gracilaria* farming is the outbreak of epiphytes, particularly in the farming of *G. fisheri*, in which this seaweed has been reported to be susceptible to epiphytes (Chirapart et al. 2018).

Recently several studies have used seaweed extracts as biostimulants to enhance plant growth and development (Khan et al. 2009; Borlongan et al. 2011; Di Stasio et al. 2017). The beneficial effects of seaweed extracts on

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cultured plants are well documented (Rayirath et al. 2008; Fan et al. 2011; Mansori et al. 2016; Ali et al. 2019, 2021). The extracts of seaweeds are highly organic; therefore, they are ideal for organic farming and the production of environmentally sensitive crops (Ali et al. 2021). Seaweed extracts have been noted to enhance vegetative propagation and root vigor, increase leaf chlorophyll content and the number of leaves, improve fruit yield, and enhance the flavonoid content of treated plants (van Staden et al. 1995; Blunden et al. 1996; Rayirath et al. 2008; Fan et al. 2011; Mansori et al. 2016). The seaweed liquid extract of *Ulva rigida* has been used to enhance the antioxidant potential and drought tolerance of a medicinal plant, *Salvia officinalis* (Mansori et al. 2016). Furthermore, the seaweed extract of the brown alga *Ecklonia maxima* has been reported to influenc growth, photosynthetic activity, and mineral composition of *Brassica rapa* L. subsp. *sylvestris* under nutrient stress conditions (Di Stasio et al. 2017).

The variability in algal growth and production is influenced by ambient environmental factors, such as light, temperature, and salinity variation (Choi et al. 2010; Gorman et al. 2017). In addition, it is difficult to control growth and epiphytes under non-unialgal culture conditions. Therefore, several studies have attempted to enhance the growth and production in seaweed farming (Bidwell et al. 1985; Ask and Azanza 2002), such as through the cultivation of seaweeds in the sea or in land-based tanks with high densities of *Chondrus crispus* (Bidwell et al. 1985) and *Gracilaria domingensis* (Salles et al. 2010). Several studies have applied seaweed extracts in seaweed cultivation to enhance growth and production. The extract of the brown seaweed *Ascophyllum nodosum* has been used in laboratory and field cultivation of the red seaweeds *Kappaphycus alvarezii* and *K. striatum* in the Philippines and Malaysia (Hurtado et al. 2009; Loureiro et al. 2010; Borlongan et al. 2011; Zuldin and Shapawi 2015). However, there have been no reports of the use of seaweed extract for enhancing growth and production in the cultivation of Thai seaweeds. Hence, the current study was initiated to examine the effects of the *Ascophyllum* seaweed extract on the growth and epiphytes of *G. fisheri*. The aim of this study was to examine the responses of algal branching, growth and yield and the reduction of epiphytic attachment to the *Ascophyllum* seaweed extract under controlled culture conditions. Our results will be useful for improving the growth, yield and quality in *Gracilaria* farming.

## Materials and Methods

### Sample preparation

Fresh samples of *Gracilaria fisheri* were gathered from shrimp effluent ponds in Phang Nga and Pattani Provinces, southern Thailand. Samples were cleaned in fresh seawater to remove sand, mud and other contaminants. The algal thalli were cultured using Provasoli Enriched Seawater (PES) medium (20 mL $L^{-1}$) (Bold and Wynnw 1978). The algal samples were acclimated in the laboratory at a salinity of 30‰, a temperature of 25–26°C and 200 µmol photons $m^{-2}$ $s^{-1}$ for approximately 3 months. Then, epiphyte-infected thalli of the algal samples were selected for further experiments.

Three sets of experiments were conducted under controlled culture conditions in the laboratory (outlined above). In each experiment, the pH levels of the treatment combination were examined.

The first experiment was carried out to evaluate the effects of the *Ascophyllum* seaweed extract (SE) on the epiphytic attachment and growth characteristics of *G. fisheri*. Two gram each of the epiphyte-infected samples (n=18) was soaked daily in SE at different concentrations of 0, 0.1, 0.5, and 1 g SE $L^{-1}$ for 30 min. The algal samples were transferred to 300 mL filtered seawater (0.45 µm) in flasks (n=6) to grow the algae without additional PES medium. The culture conditions were controlled at a salinity of 30‰, temperature of 25–26°C and light intensity of 200 µmol photons $m^{-2}s^{-1}$, with a light:dark period of 12:12 h. The responses of algal branching and epiphyte attachment to SE were observed daily.

The attachment of epiphytes was determined as percent coverage per $cm^2$. The infected algal samples were selected and surface area of the sample was divided into grid area with 10×10 spaces inside. The coverage of epiphytes was estimated (3 replicates) inside the grid spaces under a microscope. The percent cover of epiphytes was calculated as to the following formula:

$$\text{Coverage } (\%\ \text{cm}^{-2}) = \frac{\text{Surface area cover with epiphytic algae}}{\text{Total surface area of algal sample}} \times 100$$

The branching of the algal sample was determined and the percent of new algal branching was calculated according to the following formula:

$$\text{New branching } (\%) = \frac{\text{Number of sample with new branching}}{\text{Total number of sample}} \times 100$$

The fresh weight of the algal sample was also determined at weekly intervals. The growth rate of the algae was calculated according to the following formula: [$\mu = [(\text{In}(N_t/N_0))\ t^{-1}]\times 100\%$], where $\mu$ = specific growth rate (% $day^{-1}$), $N_0$ = initial weight (g fresh weight), $N_t$ = weight after $t$ days (g fresh weight), and $t$ = time (day) (Lobban et al. 1985).

In the second experiment, the growth response of the alga to SE with additional PES medium was examined. Healthy thalli of the algae were selected from the above culture in laboratory. One gram of each algal sample (n=18) was soaked in PES medium (20 ml $L^{-1}$) mixed with different

concentrations of SE (PES+0 g SE $L^{-1}$ (control), PES+0.1 g SE $L^{-1}$, PES+0.5 g SE $L^{-1}$, PES+1 g SE $L^{-1}$) for 30 min. Then the samples were transferred to 300 mL filtered seawater (0.45 µm) in flasks (n=6) to grow the algae under controlled conditions at a salinity of 30‰, a temperature of 25–26°C, and a light intensity of 200 µmol photons $m^{-2}$ $s^{-1}$ with a light:dark period of 12:12 h. The growth rate of the algae was determined and calculated according to the formula described above.

The third experiment examined algal growth under controlled culture conditions with 5% $CO_2$ supplementation. One gram of each healthy sample (n=18) was soaked in a mixed solution of PES and SE for 30 min and then transferred to 300 mL of filtered seawater (0.45 µm) as in the second experiment. The algal samples were cultured under controlled conditions as described above and 5% $CO_2$ was added to the culture for 30 min every day. The concentration of $CO_2$ flowing into the culturing flask was manipulated through an air–$CO_2$ gas mixing system with a flow rate of about 0.4 L $L^{-1}$ $min^{-1}$ (vvm). The fresh weight of the algal sample was determined and the growth rate was calculated according to the formula described above. The algal yield was also determined and expressed as the percentage of algal fresh weight increased.

### Statistical analysis

The data are presented as the means ± standard deviation (SD). The statistical significance of differences was analyzed using the analysis of variance (ANOVA) and post hoc Duncan's tests at a confidence level of 95%.

## Results

The pH levels ranged from 7.80–7.90 for the non-SE treatment, and those of the SE treatment and the mixed solution of PES and SE ranged from 8.00–8.32 and 7.90–8.15, respectively (Table 1).

In the first experiment (treatment with SE alone), the *Ascophyllum* seaweed extract showed significant effects on the attachment of epiphytes and enhanced the growth of *G. fisheri*. Figure 1a–b shows the epiphytic-infected thalli of *G. fisheri* before treatment with SE, with many epiphytes attached to the surface of the alga. However, epiphyte attachment was markedly decreased after 1 week of treatment with 0.1, 0.5, and 1 g SE $L^{-1}$ (Fig. 1c–h). The epiphyte coverage in the non-SE treatment (control) was slightly decreased from week 1 (98.3% $cm^{-2}$) to week 4 (87.3% $cm^{-2}$). In contrast, the samples treated with SE showed significant decreases as the concentration increased from 0.1 to 1 g SE $L^{-1}$. The epiphyte coverage rapidly decreased to 50, 40, and 34% $cm^{-2}$ after one-week treatments with 0.1, 0.5, and 1 g SE $L^{-1}$, respectively (Fig. 2). The percent coverage of the epiphytes was significantly decreased to 26.7, 13.3, and 11.7% $cm^{-2}$ after four weeks of the treatments when compared to the non-SE treatment ($p < 0.05$).

**Table 1** Different in the pH levels of the PES medium at different concentrations of *Ascophyllum* seaweed extract (SE) solution

| Solutions | pH |
|---|---|
| SE 0 g $L^{-1}$ | 7.90 |
| SE 0.1 g $L^{-1}$ | 8.00 |
| SE 0.5 g $L^{-1}$ | 8.16 |
| SE 1 g $L^{-1}$ | 8.32 |
| PES 20 mL $L^{-1}$ + SE 0 g $L^{-1}$ | 7.82 |
| PES 20 mL $L^{-1}$ + SE 0.1 g $L^{-1}$ | 7.90 |
| PES 20 mL $L^{-1}$ + SE 0.5 g $L^{-1}$ | 8.04 |
| PES 20 mL $L^{-1}$ + SE 1 g $L^{-1}$ | 8.15 |

In addition, the alga thalli treated with SE also showed an increase in branching in comparison to the nontreated thalli (Fig. 3). The percent of branching (Fig. 4a) increased to 41.67 ± 2.58%, 46.67 ± 2.58%, and 50.00 ± 0.00% in week 4 when the thalli were treated with 0.1, 0.5, and 1 g SE $L^{-1}$, respectively, while that of the nontreated thalli was 23.33 ± 2.58%. The branching was significantly different between the non-SE treatment and the SE treatment ($p < 0.05$). The thalli treated with SE also had a higher growth rate than thalli not treated with SE. The alga had the highest growth rate in week 3, with values of 0.25 ± 0.12, 0.64 ± 0.40, 0.50 ± 0.13, and 0.95 ± 0.11% $day^{-1}$ in the treatments with 0, 0.1, 0.5, and 1 g SE $L^{-1}$, respectively (Fig. 4b). The growth rates were significantly different between the non-SE-treated thalli and the 0.1 g SE $L^{-1}$-treated thalli ($p < 0.05$), but had no differences with the 0.5 and 1 g SE $L^{-1}$-treated thalli ($p > 0.05$) after four weeks of culture.

In the second experiment (PES+SE treatment), *G. fisheri* showed a significantly higher growth rate ($p < 0.05$) than in the first experiment (Fig. 5). In week 1 of the treatment without $CO_2$, growth rates of 3.49 ± 0.83% $day^{-1}$, 3.40 ± 0.51% $day^{-1}$, and 3.72 ± 1.02% $day^{-1}$ were obtained from the thalli treated with PES, PES+0.1 g SE $L^{-1}$, and PES+0.5 g SE $L^{-1}$, respectively (Fig. 5a). The growth rate was not significantly different among the treatments ($p > 0.05$). The growth rate increased to 3.84 ± 0.63% $day^{-1}$ when the thalli were treated with PES+1 g SE $L^{-1}$, but the algal growth was markedly decreased after week 2 of cultivation in all treatments.

In the PES+SE treatment with 5% $CO_2$ supplementation, growth of the alga increased with increasing concentrations of SE from 0 to 1 g SE $L^{-1}$. In the first week of cultivation, the growth rate was lower (4.81 ± 1.00% $day^{-1}$) in the PES without SE treatment (control). However, the growth rates increased to 4.84 ± 1.52% $day^{-1}$, 5.04 ± 1.48% $day^{-1}$, and 5.46 ± 1.05% $day^{-1}$ when the thalli were treated with

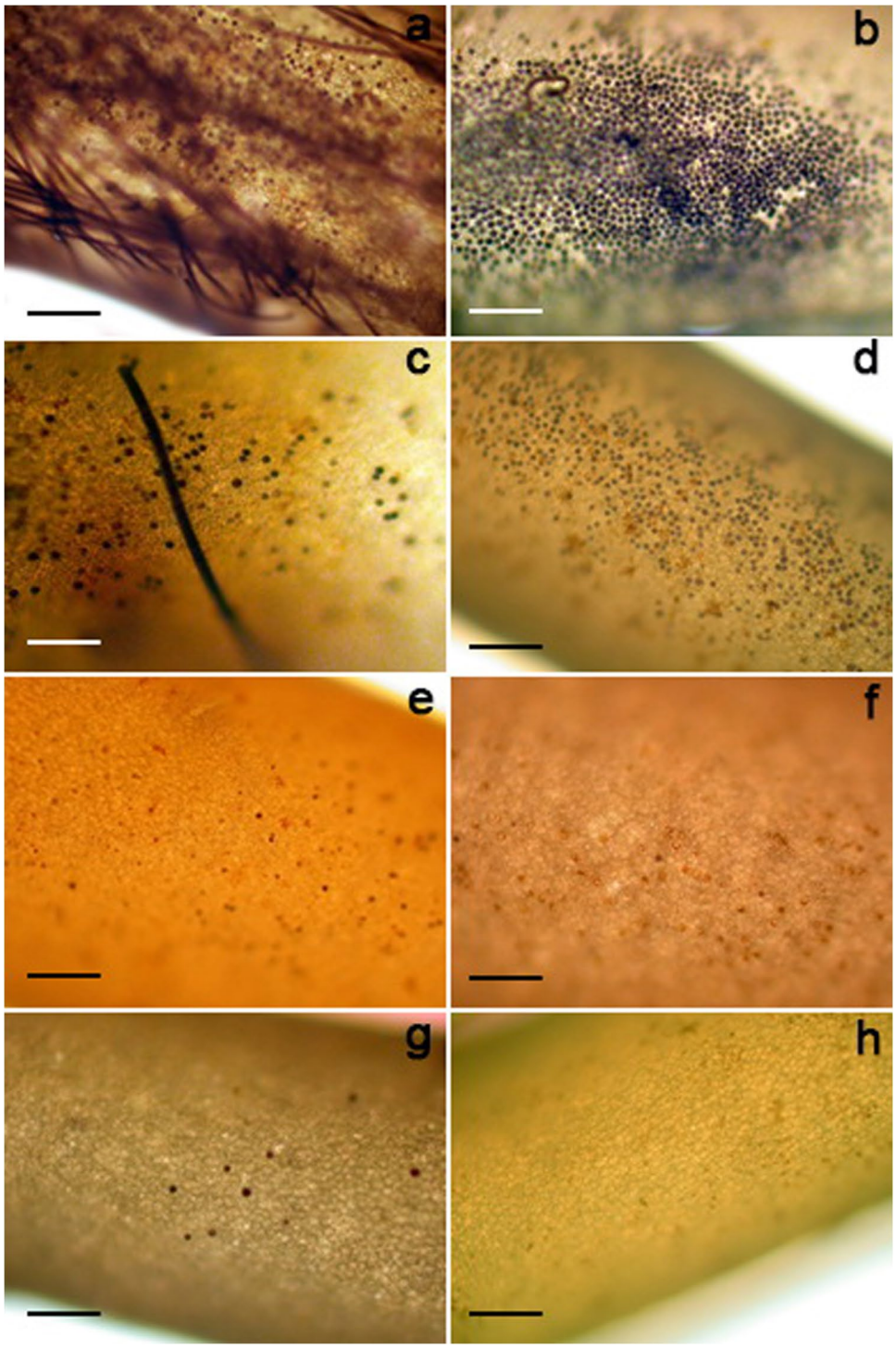


**Fig. 1** Epiphyte-infected thalli of *G. fisheri* before treatment with the *Ascophyllum* seaweed extract (**a**-**b**), and the epiphyte attachment decreased after treatment with seaweed extract at concentration of 0.1 g SE $L^{-1}$ (**c**-**d**), 0.5 g SE $L^{-1}$ (**e**-**f**), and 1 g SE $L^{-1}$ (**g**-**h**). Scale bar = 100 µm

PES+0.1 g SE $L^{-1}$, PES+0.5 g SE $L^{-1}$, and PES+1 g SE $L^{-1}$, respectively (Fig. 5b). Similar to the treatment without $CO_2$ supplementation (Fig. 5a), algal growth decreased after week 2 of culture in all treatments.

In comparison, the growth rates of the alga grown for 1–3 weeks were not significantly different in all treatments with or without $CO_2$ supplementation ($p > 0.05$). However, in week 4, the growth rate was significantly different when the SE concentration increased to 1 g SE $L^{-1}$ ($p < 0.05$). This study also showed that the growth of the alga was significantly different in all treatments between the first and the second experiments ($p < 0.05$).

In addition, under $CO_2$-free conditions, the thalli treated with PES+0 g SE $L^{-1}$ to 1 g SE $L^{-1}$ showed the highest

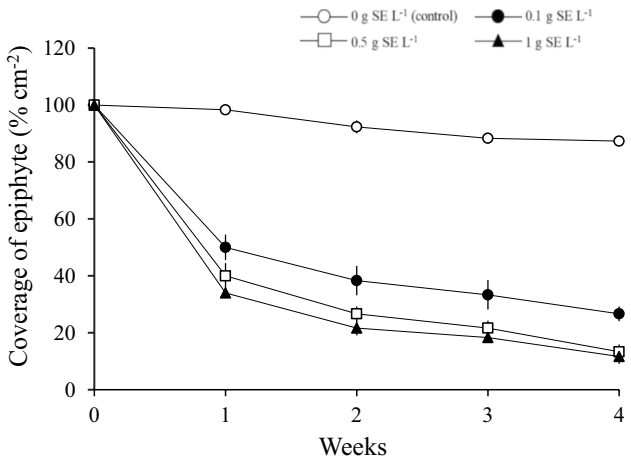


**Fig. 2** Percent coverage of epiphytes after treatment with seaweed extract at concentration of 0 (control), 0.1, 0.5, and 1 g SE $L^{-1}$. Vertical lines represent standard deviations

yields, ranging from 26.94 ± 4.97% fw to 30.93 ± 6.37% fw in week 1 of culture (Fig. 6a). However, the algal yields of all treatments gradually decreased to the lowest values at week 4 of culture. The yield of the alga was significantly different between the control (PES alone) and the PES+SE-treated alga ($p < 0.05$).

This study showed the yield of the alga response to 5% $CO_2$ supplementation (Fig. 6b), with an increasing yield from 40.37 ± 10.68% fw in the alga treated with PES alone to 46.94 ± 11.43% fw in the alga treated with PES+1 g SE $L^{-1}$. Similar to the treatment without $CO_2$, the algal yields of all treatments gradually decreased to the lowest values in week 4 of culture. The yield of algae was significantly different between the treatments with 5% $CO_2$ supplementation ($p < 0.05$). This study revealed that the treatments with 5% $CO_2$ supplementation had higher yields than those without 5% $CO_2$ supplementation.

## Discussion

Seaweed extracts have been reported to have positive effects on plant growth, yield and quality, pest and disease resistance, and environmental stress tolerance (Khan et al. 2009; Fan et al. 2011; Danesh et al. 2012; Lakshmi and Sheeja 2021). In the present study, the use of the *Ascophyllum* seaweed extract resulted in significant decreases in the percent coverage of the epiphytes in *G. fisheri*. After four weeks of


**Fig. 3** Branching of the thalli of *G. fisheri* increased after treatment with seaweed extract at concentration of 0 (T1, control), 0.1 g SE $L^{-1}$ (T2), 0.5 g SE $L^{-1}$ (T3), and 1 g SE $L^{-1}$ (T4)

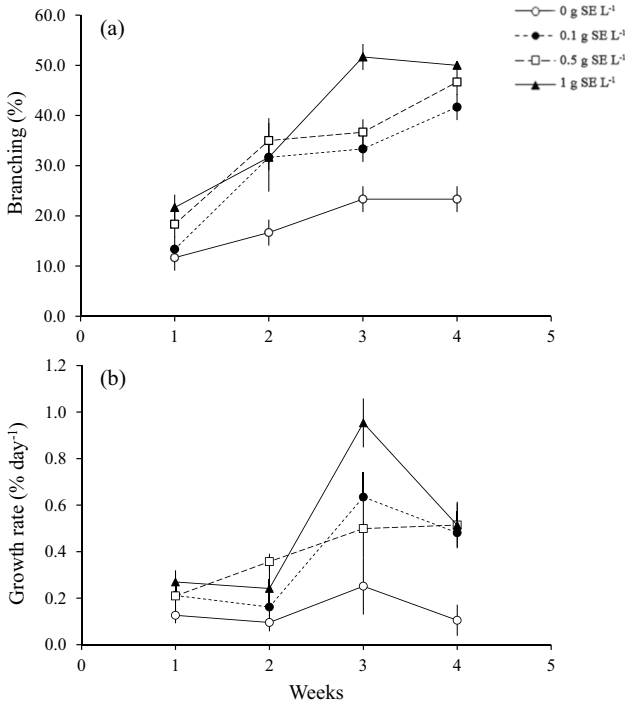


**Fig. 4** Percent of new branching (**a**) and growth rate of *G. fisheri* after treatment with seaweed extracts at concentration of 0 (control), 0.1, 0.5, 1 g SE $L^{-1}$ (**b**). Vertical lines represent standard deviations

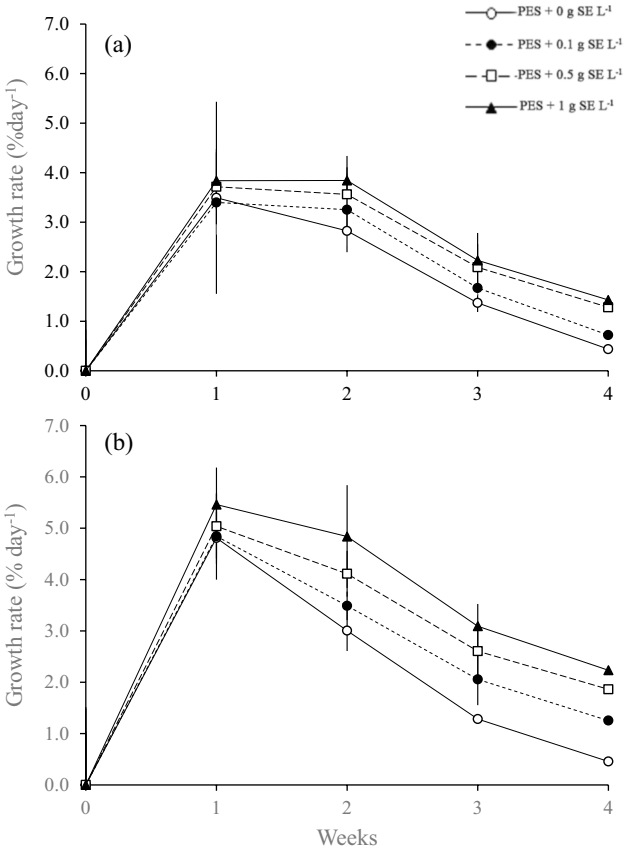


**Fig. 5** Growth responses of *G. fisheri* to the treatment with PES (control), PES + 0.1 g SE $L^{-1}$, PES + 0.5 g SE $L^{-1}$, PES + 1 g SE $L^{-1}$, **a** without $CO_2$ and **b** with $CO_2$ supplementation. Vertical lines represent standard deviations

culture, the occurrence of the epiphytes in the treatment with SE alone at 0.1, 0.5, and 1 g SE $L^{-1}$ was decreased to 50, 40, and 34% $cm^{-2}$, respectively which accounted to 3.3, 6.6, and 7.5-fold decreases in comparison to the control treatment, respectively. Similarly, Borlongan et al. (2011) found that the use of *Ascophyllum* seaweed extract resulted in a decrease in the percent occurrence of epiphytic *Neosiphonia* in field cultivation of *Kappaphycus alvarezii*. The authors reported a percent occurrence of *Neosiphonia* sp. infection on *Kappaphycus* of 6–50% (with a 0.1 g $L^{-1}$ AMPEP dipping), which was lower than the percentage of 10–75% in the controls (without AMPEP dipping). Additionally, an extract of *A. nodosum* (5–30 g $L^{-1}$) resulted in a decrease in the epiphytic *Cladophora* and *Ulva* on the cultivated *K. alvarezii* after treatment for 2 weeks but not for the treated thalli of *Polysiphonia subtilissima* which formed small red patches along the thalli (Loureiro et al. 2010). However, cyanobacteria represented the main epiphytes found in the present study, which were mostly found to be in coccoid and filamentous forms and have not been identified to species level. This group of cyanobacteria had significant responses to the seaweed extract at concentrations of 0.1–1 g SE $L^{-1}$. Thus, it is noted that the effect of seaweed extract on the occurrence of epiphytes depends on the epiphytic organisms. In addition, the laminarin found in the *Ascophyllum* seaweed extract may result from the defense responses in this seaweed. Laminarin exhibits a wide range of biological activities and has been shown to stimulate natural defense responses in plants (Fritig et al. 1998). Furthermore, it has been reported to be involved in the induction of genes encoding various pathogenesis-related proteins with antimicrobial properties in the algal cells (Fritig et al. 1998; van Loon and van Strien 1999).

The use of the *Ascophyllum* seaweed extract has shown a significant increase in the growth rate of *K. alvarezii* grown at different depths in the Philippines (Borlongan et al. 2011). These authors reported that the growth rates of *K. alvarezii* var. giant tambalang and var. tungawan dipped in *Ascophyllum* seaweed extract (AMPEP) were 3.1% $day^{-1}$ and 4.1% $day^{-1}$, respectively, which were higher than that of the control. In the current study, although the growth rate of the *G. fisheri* thalli treated with SE alone was overall not different from that of the control thalli (without SE), there was an increase in the branching of the treated thalli. The seaweed extract significantly enhanced the percent branching of this seaweed species. This was similar to the results of a report on the use of the seaweed extract from *A. nodosum* in the

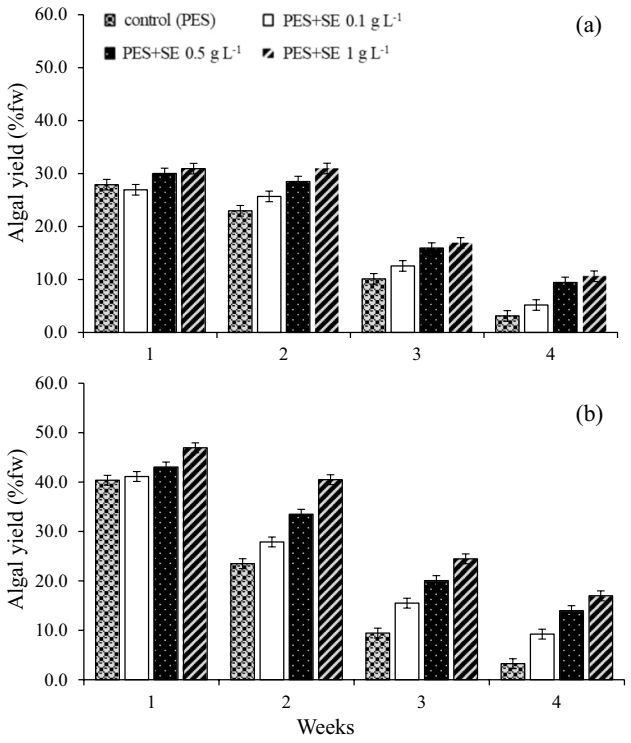


**Fig. 6** Yield (%fw) of *G. fisheri* cultured under laboratory conditions using PES + seaweed extract, **a** without $CO_2$ and **b** with $CO_2$ supplementation. Vertical lines represent standard deviations

tissue culture of *K. alvarezii*, which increased the regeneration of young plants of the alga (Hurtado et al. 2009). The addition of the AMPEP seaweed extract enhanced the growth of *K. alvarezii* and *K. striatum* in tank culture (Zuldin and Shapawi 2015). In addition, the use of seaweed extracts from *Gracilaria corticata* and *Grateloupia lithophila* enhanced the growth of the microalgae *Chlorella vulgaris*; this was due to the influence of growth hormones contained in the seaweed (Lakshmi and Sheeja 2021). In this study, the increase in growth and branching of *G. fisheri* was thought to be influenced by various chemical components (macro- and micronutrients, growth hormones, etc.) of the seaweed extract (Hurtado et al. 2009; Khan et al. 2009; Rafiee et al. 2016; Lakshmi and Sheeja 2021). The seaweed extract from *A. nodosum* has been reported to contain macro- and micro-elements and have a total amino acid content of 4.4% (Hurtado et al. 2009), which are essential for plant growth. The positive effects of seaweed extract have been reported in cultivated plants, such as watermelon (Abdel-Mawgoud et al. 2010), cucumber (Danesh et al. 2012), tomato (Ali et al. 2016, 2019), and sweet pepper (Ali et al. 2019). *Ascophyllum* seaweed extract has been shown to result in a significant increase in tomato plant height and fruit yield compared to the control plants (Ali et al. 2016). Abdel-Mawgoud et al. (2010) stated that the effect of seaweed extract application was positive and correlated with the applied concentrations. Corresponding to the results of the current study, the seaweed extract was found to have a positive effect on algal growth and branching and was correlated with an increase in the concentrations used. There are reports that the use of the brown seaweed extracts resulted to an enhanced number of branches in agricultural crop (Abdel-Mawgoud et al. 2010; Kumar and Sahoo 2011). In addition, the seaweed extract of *Ulva rigida* enhanced vegetative growth in sage plants under drought stress conditions; the shoot length, total leaf area, and number were significantly reduced under water stress treatment (Mansori et al. 2016). These authors stated that water stress increased the organic osmolyte glycine betaine (GB) content, which was significantly reduced by the applied seaweed extract. This may explain why, in our study, algal growth and branching were higher in the treatment with seaweed extract than in the control treatment.

Although the growth rate of *G. fisheri* treated with SE was not different from that of the control thalli, the growth rate and yield of the alga were found to be increased when treated with the combination of PES+SE medium and higher when supplemented with 5% $CO_2$. Similar to a previous study, the use of the seaweed extract in combination with a plant growth regulator enhanced the initiation of shoots in tissue culture of *Kappaphycus* (Hurtado et al. 2009). The seaweed extract may also improve the nutrient uptake of the algal thalli (Rathore et al. 2009). In this study, the increase in the growth rate and yield of *G. fisheri* was thought to be due to the synergistic activity of SE with PES and $CO_2$, as previously reported by Vernieri et al. (2005). Previous studies reported that elevated $CO_2$ resulted in increased growth in algae (Kang et al. 2017; Liu et al. 2018; Gao 2021). Changes in $CO_2$ concentrations are related to the pH level of the culture medium (Kang et al. 2017). Differences in pH affect the uptake of nutrients, photosynthesis and growth of algae (Nor Salamah et al. 2015; Reidenbach et al. 2017). The highest growth rate of *Gracilaria manilaensis* has been reported at pH 7.6 (Nor Salamah et al. 2015). In the present study, the PES and SE solutions had a pH of 7.9–8.3, which may affect nutrient absorption and algae growth during the short period used for soaking the algae. The thalli of *Gracilaria lemaneiformis* grown at a high level of $CO_2$ have been reported to have a lower carbon utilization capacity and a higher nitrogen uptake rate than those grown under ambient $CO_2$ (Xu et al. 2010). Additionally, these authors reported that $CO_2$ enrichment inhibited nitrate uptake by the alga by 28% compared to normal $CO_2$ in culture. The concentration of 5% $CO_2$ used in this study has been reported as the normal concentration used for algal culture (Pooja and Himabindu 2012; Yoshimura et al. 2013; Ruangsomboon and Dimak 2020). The supplementary $CO_2$ after immersion of the algae in PES and SE extracts was able to enhance the yield of the algae and lead to vigorous growth.

Based on our results it can be concluded that the *Ascophyllum* seaweed extract is effective in enhancing the growth (in term of branching) and yield of the seaweed *G. fisheri.* Treatment with SE alone reduced epiphyte attachment 3.3- to 7.5-fold compared to the control. The use of 1 g SE $L^{-1}$ could significantly reduce epiphytes (>90%) in the alga after 30 min of treatment for one week. The seaweed extract used at a concentration of 0.1–1 g SE $L^{-1}$ enhanced the branching of this seaweed (~42–50%) compared to the non-treated samples (~23%). The algal growth rates of the SE thalli treatments (except 0.1 g SE $L^{-1}$ treatment) were not significantly different from those of the non-SE-treated thalli. However, the cultivation of *G. fisheri* after soaking in the PES medium mixed with the seaweed extract and 5% $CO_2$ supplementation resulted in enhanced algae growth. Further work is required to test the feasibility of the seaweed extract for larger-scale cultivation of *Gracilaria*.

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**Authors' contributions** A. Chirapart designed all experiment, prepared figures 2 and 4, and wrote the main manuscript text. S. Khreauthong performed experiment and preparing figures 5, 6. J. Praiboon contributed the research work and discussion. S. Rattanasaensri performed experiment and preparing figures 1, 3. R. Ruangchuay provided all materials of *Gracilaria fisheri* and discussion.

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**Data availability** All data generated or analysed during this study are included in this published article. Requests for material should be made to the corresponding authors.

## Declarations

**Competing interests** The authors have no competing interests to declare that are relevant to the content of this article.

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