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Weight gain and carrageenan content of *Kappaphycus alvarezii* (Rhodophyta, *Solierisceae*) polycultured with *Sargassum polycystum* (Paeophyta, Sargassaceae)

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Abstract. The objectives of this study were to analyse the weight gain and carrageenan content of the seaweed *Kappaphycus alvarezii* polycultured with *Sargassum polycystum* seed. The study was conducted in the coastal waters of Aeng Batu-Batu Village, Takalar Regency, South Sulawesi, Indonesia from July 13, 2016 to September 7, 2016. A Complete Randomized Design (CRD) was applied with 4 treatments and 3 replicates. The treatments were a control (*K. alvarezii* with no *S. polycystum*); the combination of 25 g *K. alvarezii* with 25 g *S. polycystum*; 25 g *K. alvarezii* with 30 g *S. polycystum;* and 25 g *K. alvarezii* with 35 g *S. polycystum*. High weight gain and carrageenan content of *K. alvarezii* in the presence of *S. polycystum* could be due to the shading effect of thallus morphology and the chemical content of *S. polycystum* that might protect the thallus of *K. alvarezii* from herbivorous animals, biofouling, and UV radiation. The antibiotic compounds, hormones, and *phlorotannins* content of *S. polycystum* may synergistically protect the red seaweed from ice-ice bacteria while promoting growth and carrageenan synthesis.

1. Introduction.

Seaweed culture in the intertidal zone has developed significantly in Indonesia. *Eucheuma cottonii (Kappaphycus alvarezii) has* been cultured in waters across the Indonesian Archipelago since it was first introduced [1]. Seaweed farming is a viable alternative source of income for small-scale fishermen and coastal communities. The red alga *K. alvarezii* is an important source of raw material for carrageenan (a colloidal substance used as gelling agent, stabilizer or emulsifier in food, cosmetics and other products). This species is generally cultured in shallow waters at a depth of about 10 meters, mostly using a longline technique [2].

Several environmental problems such as predation by herbivores, the disease *ice-ice*, and solar radiation can affect the growth, production, and carrageenan content of *K. alvarezii.* One environmentally friendly way to attempt to address these problems is to use a biological approach. The brown alga *Sargassum,* which is also widely distributed throughout the coastal waters of tropical and temperate countries, has a high commercial value. This alga could potentially be cultivated and processed for alginate production, providing a source income for coastal communities and for the country.

The brown alga *Sargassum* contains *phlorotannis*, a family of compounds which have secondary roles as chemical defences such as herbivore deterrents, antibacterial agents, and UV screens. This

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alga could be polycultured with *K. alvarezii*. The objectives of the study were to analyse the influence of the presence of *S. polycystum* on the weight gain and carrageenan content of *K. alvarezii* when grown in a mixed culture (polyculture).

2. Materials and method

2.1. Study Site and Time.

The study was conducted in the waters of Jonggoa, Batu-batu Village, North Galesong District, Takalar Regency, 15 miles south of Makassar City in South Sulawesi Province, Indonesia. The study was conducted from July 13 to September 7, 2016. The seaweeds *K. alvarezii* and *Sargassum polycystum* were grown in polyculture with an intercropping pattern for 6 weeks. Carrageenan and alginate content of *K. alvarezii* and *Sargassum polycystum*, respectively were analysed at the Water Quality Laboratory of the Faculty of Marine Sciences and Fisheries, Hasanuddin University, Makassar, Indonesia

2.2. Culture Experiment.

Kappaphycus alvarezii and *Sargassum polycystum* (Figure 1) seeds were obtained locally in the study area. The long-line culture method was used. Depend on the treatments, 25 g *K. alvarezii seeds* were tied to the long line ropes, alternating with 25, 30, or 35 g S. *polycystum* seeds, or without *S. polycystum*. The long line ropes were tied to a bamboo raft (Figure 2).

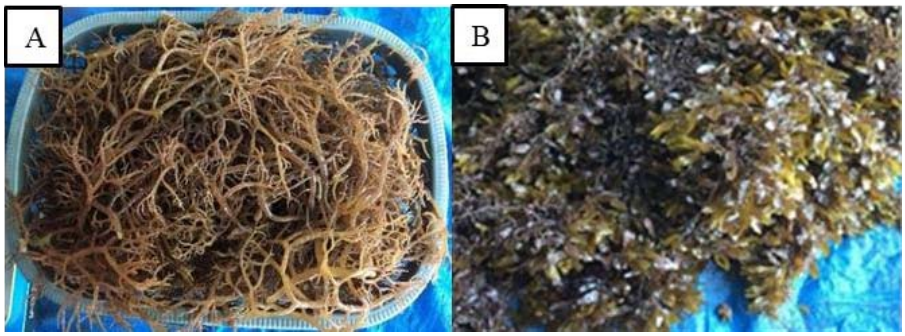

Figure 1. *Kappaphycus alvarezii* (A) and *Sargassum polycystum* (B)

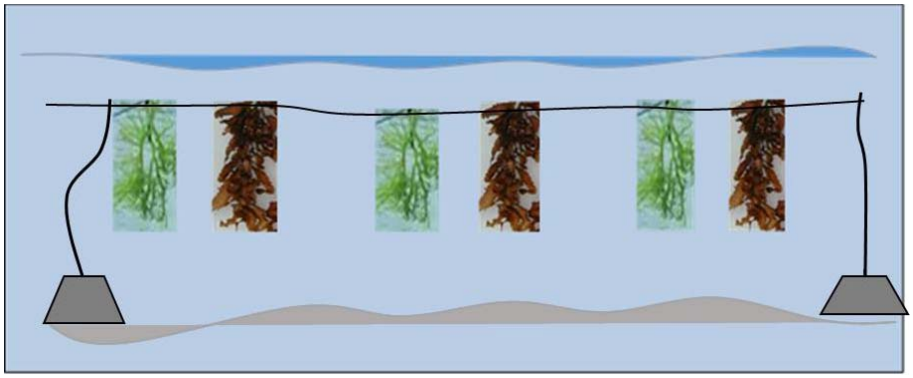

Figure 2. From frontage view of long line method applied

2.3. Treatments and Experimental Design.

Randomized Complete Design (CRD) was applied with the following 4 (four) treatments, each with three replicates:

25 g *K. alvarezii* + 25 g *S. polycystum*
25 g *K. alvarezii* + 30 g *S. polycystum*
25 g *K. alvarezii* + 35 g *S. polycystum*

25 g *K. alvarezii* + 0 g *S. polycystum* (control)

2.4. *Biological Parameters.*
Weight gain of *K. alvarezii* and *S. polycystum* was computed with the following formula:

W = Wt - Wo
W = weight gain (g)
Wt = final weight of seaweed (g)
Wo = initial weight of seaweed (g)

Carrageenan content of *Kappaphycus alvarezii* was computed based on the formula of [3,4,5] as follows:

$YC = WC.Wdw^{-1}.100;$
YC = carrageenan content (%)
WC = weight of carrageenan extract (g)
Wdw = dry weight of analysed thallus (g)

2.5. *Analysis.*
The weight gain data was statistically analysed using tan Analysis of Variance (ANOVA) followed by a W-Tukey test when significant differences were found (at the 95% confidence level). Carrageenan content was analysed descriptively, supported by references.

3. Results.
Weight gain of *K. alvarezii* was significantly different with different initial weights of *S. polycystum* seeds. Growth of *K. alvarezii* was inversely proportional to the initial weight of *S. polycystum* seed (Figure 3). The highest weight gain of K. alvarezii (1387.33±48.09g) occurred without *S. polycystum* seeds (0 g), followed by with *25 g S. polycystum* seeds.

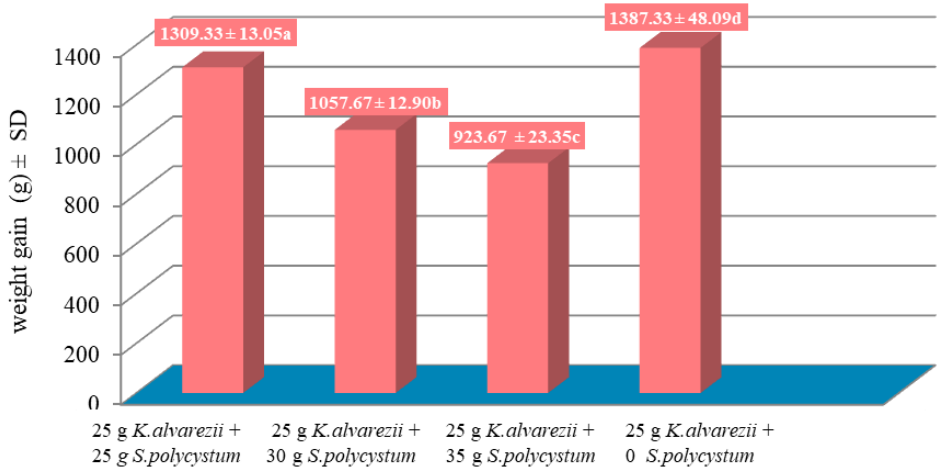

Figure 3. Weight gain of polycultured K. *alvarezii* at different initial weights of S. *polycystum* seed

The weight gain ranges of *K. alvarezii* in this experiment were higher compared to the range of 186.23-340.80 g [6] and 114 – 192 g [7] reported from the monoculture of *K. alvarezii* at the same location.

The carrageenan content of *K. alvarezii* cultured singly (without *S. polycystum* seed) and polycultured with lowest initial size (25 g) of *S. polycystum* were relatively similar, at 33.70% and 34.27% respectively. These figures were lower compared to the values of 37.57% and 43.98% obtained when *K. alvarezii* was polycultured with 30 to 35 g *S. polycystum* seed (Figure 3).

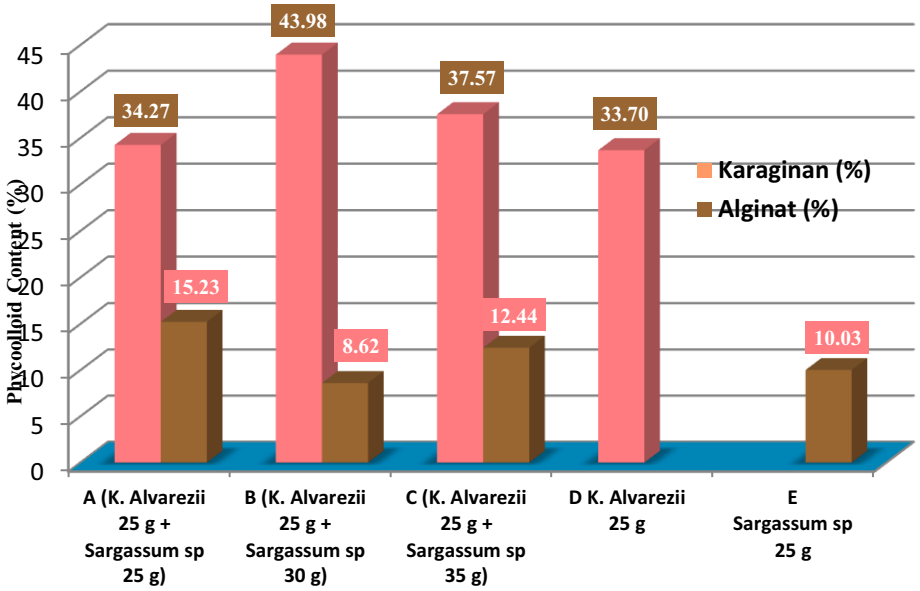

Figure 4. Carrageenan content of *K. alvarezii* polycultured with *S. polycystum* at different initial *S. polycystum* seed weights

Recorded carrageenan content of *K. alvarezii* in this study when K. alvarezii seed with initial weight of 25 g was polycultured with 30 g of *S. polycystum* was 43.98%; this value meets the FAO standard (suitable) of 40% carrageenan content for export (Doty,1985). Carrageenan content obtained from *K. alvarezii* mixed with *S. polycystum* seed of 25 -35 g initial weight (34.27 – 43.98%) was comparable to the 31-43% carrageenan content of *K. alvarezii* in [3] and relatively higher than other previous studies that obtained 40.7% from a green strain (Munoz et.al., 2004), 14.73 – 30.79%, for SRC (semi refined carrageenan) and 25 – 28% for RC (refined carrageenan) [2], all of which used monocultured *K. alvarezii*.

4. Discussion

The high weight gain of *K. alvarezii* when it was grown singly and when it was mix-cultured with smallest (25 g) *S. polycystum* seed could be related to better water motion between the plants, since water motion influences nutrient uptake kinetics and is an environmental factor regulating the growth of algae [8]. Water motion is reported as accounting for 81– 98% of the variation in weight gain of *K. alvarezii* [9], and compensating for a decline of nutrients in the growth media [10].

Higher growth rates of *K. alvarezii* in this polycultured system compared to the same species when previously cultured as a monoculture by several researchers could be due to the shading effect of the leafy morphology of *S. polycystum*. This morphology might provide some protection to the thallus of *K. alvarezii* from predation by herbivorous fishes and from biofouling (Figure 5).

In addition, the phlorotannins content of brown algae S. polycystum [11], which was exist in soluble form [12] and can form a complex with alginic acid present in the cell wall [13], could become diffused into the surrounding water by passing through the cell wall [14, 15, 16, 17, 18]. These compounds could rapidly react with both proteinaceous and carbohydrate substances of the seaweed thallus [19] and then be absorbed by the *K. alvarezii* thallus.

Figure 5. *S. polycystum* thallus covering *K. alvarezii* thallus

These compounds have secondary roles as chemical defences (herbivore deterrents, antibacterial agents, and UV screens) [19, 11, 20, 21] which might drive the rabbit fish and other herbivores out from the lush canopy of *K. alvarezii*, protect the thallus from UV radiation [11, 13, 22], help the plant to avoid bacterial infection [21, 13]. In addition to the compounds may help protect the algae against predators and epiphytes [23], acting as antifouling substances [24], and even help protect the red algae from stress conditions [21]. All these functions could promote higher weight of the red seaweed species. Together with the antibiotics and hormones contained in the brown seaweed thallus, it is likely that these compounds synergistically protected the red seaweed from *ice-ice* bacteria and promoted growth.

Influence of the thallus morphology on carrageenan content might be related to effects on access to resources [25], such as nutrient uptake from the water column ("functional form", surface/volume ratios) [26]. Higher carrageenan content of *K. alvarezii* mix-cultured with larger *S. polycystum* seed (30 – 35 g) compared to the lower (0 – 25 g) *S. polycystum* seed, as well as higher content compared to monocultured seaweeds of this species in several previous studies, could also be related to the shading effect of thallus morphology and the effects of the chemical content of *S. polycystum* on *K. alvarezii.* Carrageenan content is known to be influenced by temperature [27, 28] and light intensity [28]. When the thallus of the seaweed is directly exposed to UV, chlorophyll and other cell components can be damaged or destroyed. The leafy morphology of *S. polycystum* may shade the thallus of *K. alvarezii* from high (above optimum) temperatures and light intensity and from the negative effect of ultra violet (UV) radiation that falls on the water surface.

Acknowledgements
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