Influence of Seaweed Liquid Fertilizer on the Growth of Red Algae *Gracillaria verrucosa* under controlled conditions

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Abstract*—The use of pesticides in agriculture has a negative effect on environment. Thus, environment-friendly fertilizers are needed to minimize the use of pesticides. The present study used seaweed liquid fertilizer (SLF) extracted from Sargassum sp., Gelidum sp., and Padina sp. to support the coastal agricultural activity, especially in the production of red algae (Gracilaria verrucosa). SLF extracted from Gelidium sp. exhibited a higher nutrient content in Ca, K, and P than other seaweeds. SLF extracted from Gelidium sp. also demonstrated a higher absolute growth and daily growth rate of G. verrucosa than other treatment. The highest growth of G. verrucosa was observed on the SLF from Gelidium sp. with the optimum concentration of 2.0 – 2.5 ml/L. The results suggested that Gelidium sp. might provide a prospective future in developing seaweed SLF for Gracilaria verrucosa production.

***Keywords*— Agar, cultivation, marine algae, production**

I. Introduction

Many inorganic fertilizers, pesticides, and other agrochemicals used in agricultural areas have been used worldwide due to increased food demand. [1]. Consequently, it could contaminate the environment, human health, and crops due to the biomagnification and bioaccumulation process [2]. This practice is considered unsuitable for crop cultivation. Hence, alternative biofertilizers, that are more environment-friendly is needed in the future [3]. Seaweed or marine macroalgae are considered to be a critical renewable resource that has gained much attention due to their multiple applications such as pharmaceutical, cosmeceutical, and nutritional aspects [4]; [5]. This may represent an alternative option to conventional fertilizer. Seaweeds contain a highly nutritious source that could initiate faster germination, yield, growth and resistance ability required for the plant [6]; [7]; [8]. Many studies in the last decades have found that modern agriculture is leaning towards the use of marine macroalgae as biofertilizers [9]; [10] manifested in extract and liquid form.

Seaweed liquid fertilizer (SLF) has gained interest due to its popularity over chemical fertilizer. As previously reported, SLF extracted from Ulva rigida and Fucus spiralis have been proven to increase the growth of bean plants under the hydroponic system [11]. SLF extracted from Gracillaria textorii, and Hypnea musciformis effectively increased the growth and yield of several crops [12]. Moreover, the SLF extracted from Rosenvige intricata increased the growth of Abelmoschus esculentus [13]; [14]. This evidence shows that SLF can be considered as a prospective alternative to conventional fertilizer, for eco-sustainable agriculture and cultivation efforts.

Red algae *Gracilaria*, which grows mainly in tropical and subtropical zones [15], has been considered one of the important seaweed undergoing rapid cultivation growth since the early 20th [16]. It is a major commodity processed for agar ingredients, feedstuffs, cosmetics, and pharmaceuticals industries [17]; [18]. However, there is limited information about SLF utilization in aquatic plants, especially Gracilaria verrucosa. Another study revealed that SLF extracted from Ulva sp. can enhance the growth and quality of G. verrucosa [19], yet other noneconomic seaweed as SLF materials in the growth of G. verrucosa is lacking.

The use of inexpensive and environment-friendly fertilizers may have a potential effect on stimulating G. verrucosa production. Therefore, this research aimed to evaluate the effect of several SLF extracted from Padina sp., Gelidium sp., and Sargassum sp., based on the growth of G. verrucosa under controlled conditions, along with their overall water quality. The use of SLF can be considered as alternative fertilizer to support the growth of G. verrucose. A further experiment, using SLF extracted from Gelidium sp., was conducted to evaluate the growth of G. verrucosa.

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II. Methods

A. *Preparation of seaweed liquid fertilizer*

Seaweed liquid fertilizer (SLF) was extracted from noneconomic seaweed, i.e., Sargassum sp., Gelidum sp., and Padina sp. The raw materials were rinsed to eliminate debris attached to the thallus. The materials were finely chopped and grounded together with clear water (water: materials = 1 litre: 1 kg) and were put into a plastic container. An effective microorganism (EM4) was put into a container to accelerate the decomposing process (ratio of EM4: prepped materials = 900 mL: 10 kg), together with sugar (500 gr), to increase the microbial activity. The lid was tightly closed and fermented for 45 days until it became liquid. The SLF was further tested for the nutrient content for the plant growth, i.e. Calcium (C), Potassium (K), Sodium (Na), Phosphorus (P), Nitrogen (N), and Nitrate (NO3).

B. *Testing the seaweed liquid fertilizer on Gracillaria verrucosa*

The experiment on the growth of G. verrucosa was estimated by using 100 gr of its total weight for each treatment for 2 replications. The treatment includes SLF extracted from Padina sp., Gelidium sp., Sargassum sp., triple superphosphate (TSP) fertilizer + urea fertilizer, and non-fertilizer treatment.

C. *Testing the seaweed liquid fertilizer extracted from Gelidium sp. on Gracillaria verrucosa*

The experiment on the growth of G. verrucosa was estimated by using SLF extracted from Gelidium sp., using randomized block design for 5 different concentrations and 4 replications i.e., 0 ml/L, 1.5 ml/L, 2 ml/L, 2.5 ml/L, and 3 ml/L.

D. *Observed variables*

The colour enhancement (C) over the study period was

1. Daily growth

The daily growth was estimated in each period (4 weeks) using an analytical balance and analyzed by the following equation [20]:

$$G=[(W_t/W_i)^{(1/t)}-1]\times 100\%$$

where G is defined as the daily growth rate (%), Wt as the final weight (g), Wi as the initial weight, and t as the time of estimation.

2. Absolute growth

The method to reporting the absolute growth is by an absolute increase in weight by the following equation [20]:

$$W=W_t-W_i$$

where W is defined as the absolute weight growth (g), Wt as the final weight (g), and Wi as the initial weight.

3. Water quality parameter

As one of the important factors that may influence the growth of Gracillaria verrucosa, several water quality parameters were estimated, i.e., Salinity (ppt), temperature (°C), dissolved oxygen (ppm), acidity (pH), nitrate (ppm), and phosphate (ppm).

E. *Statistical analysis*

The data obtained from experiment 1 were analyzed using a t-test. Experiment 2 was checked for normality and homogeneity and variance test and further tested using One-Way analysis of variance (ANOVA) and posthoc Tukey test using Minitab 16 and Microsoft Excel 2012.

III. Results and Discussion

A. *Screening of nutrient content of seaweed liquid fertilizer*

The nutrient element of three noneconomic seaweeds (*Padina* sp., *Gelidium* sp., and *Sargassum* sp.), including Ca, K, Na, Mg, P, N-total, and N-NO$_3$, is presented in Figure 1. SLF extracted from *Padina* sp. seaweed showed a higher nutrient content than other seaweeds based on N-total and N-NO$_3$ (0.44 and 0.48; Figure 1). However, the measurement of the Na percentage of the SLF extracted from *Padina* sp. seaweed might show an error since it has reached an unusually high rate compared to other SLF extracted from seaweed (0.70; Figure 1). SLF extracted from *Gelidium* sp. exhibited a higher nutrient content in Ca, K, and P than other seaweeds (0.51, 0.37, and 0.16; Figure 1).

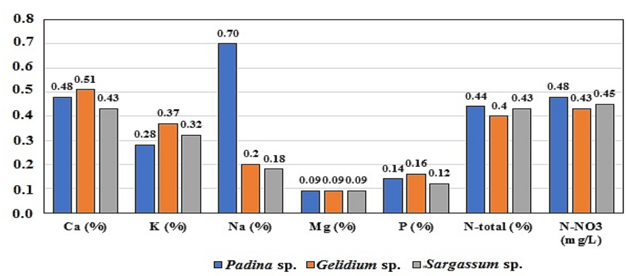

Figure 1. Comparison of the nutrient content of the 45-days fermented SLF extracted from the noneconomic seaweed (*Padina* sp., *Gelidium* sp., and *Sargassum* sp.)

Effect of SLF on the growth of G. verrucosa and water quality

The absolute growth of *G. verrucosa* after being treated with SLF extracted from all seaweed, TSP & Urea, and non-treatment R2 were not significantly different (Figure 2). However, the first replication of non-treatment (Non-treatment R1) showed a higher absolute growth level than all treatments (130.72 g; Figure 2). The treatment of SLF extracted from *Gelidium* sp. (*Gelidium* sp. R2) showed a slightly higher absolute growth rate of *G. verrucosa* than other SLF extracted from seaweeds (125.75; Figure 2).

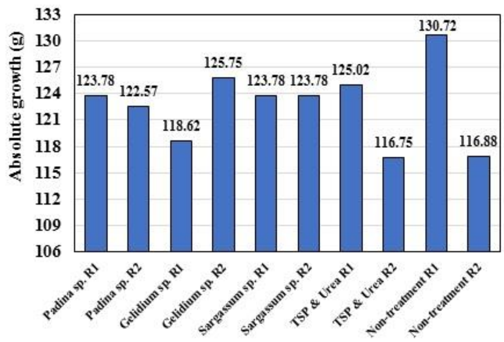

Figure 2. The effect of all treatments on the absolute growth of *G. verrucosa*. Notes: R1-R2 indicated the replications.

The treatment of the SLF extracted from seaweed, TSP & Urea, and non-treatment of 2 replications shows the insignificant value on the daily growth rate of *G. verrucosa* (Figure 3). The first and second non-treatment (Non-treatment R1 and Non-treatment R2) showed a higher daily growth rate than all treatments (0.97 and 0.98; Figure 3). The treatment of three SLF extracted from the seaweed showed that *Gelidium* sp. (*Gelidium* sp. R2) has a higher daily growth rate than other SLF extracted from seaweed (0.83). Furthermore, *Sargassum* sp. (*Sargassum* sp. R1 and *Sargassum* sp. R2) showed the lowest daily growth rate (0.29 and 0.21; Figure 3).

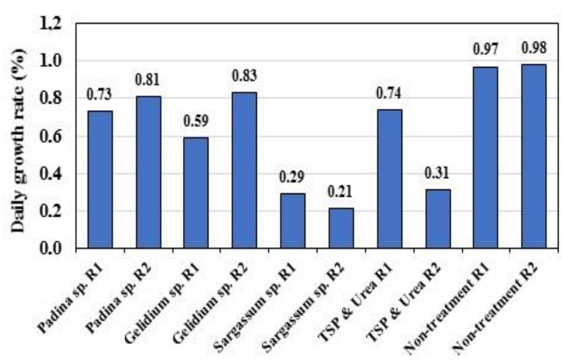

Figure 3. The effect of all treatments on the daily growth rate of *G. verrucosa*. Notes: R1-R2 indicated the replications.

Water quality parameters based on 5 treatments of SLF extracted from seaweed in *G. verrucosa* observed in 4 weeks showed a variety of threshold that still fall within their optimum ranges. However, the measurement of the phosphate parameter showed an uncertain value between the first and second replication, similar throughout all treatments (Figure 4).

Effect of SLF extracted from Gelidium sp. on the growth of G. verrucosa and water quality

The five different concentrations of SLF from *Gelidium* sp. showed significant differences in the absolute growth of *G. verrucosa*. The absolute growth of *G. verrucosa* after being treated with 2.0 and 2.5 ml/L of SLF from *Gelidium* sp. was significantly higher than other concentrations (Figure 5). The daily growth rate of *G. verrucosa* in the concentration of 2.0 and 2.5 ml/L was markedly higher than in other concentrations (Figure 6).

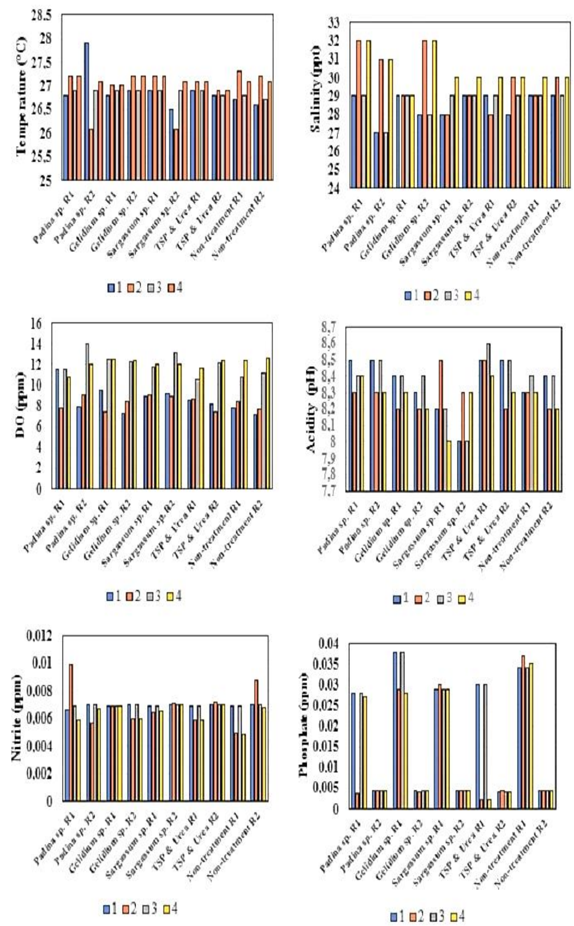

Figure 4. Water quality parameters in *G. verrucosa* after being treated by five different types of treatments (i.e., SLF extracted from seaweed, TSP & Urea, and Non-treatment). Notes: R1-R2 indicated the replications; 1, 2, 3, 4 depicting the observation period (in weeks).

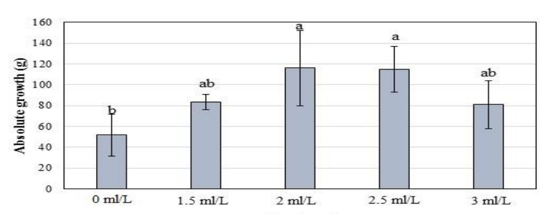

Figure 5. The effect of different concentrations of SLF extracted from *Gelidium* sp. on the absolute growth (mean ± SEM) of *Gracillaria verrucose.*

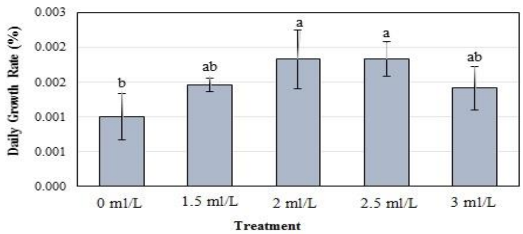

Figure 6. Effect of different concentrations of SLF extracted from *Gelidium* sp. on the daily growth rate (mean ± SEM) of *Gracillaria verrucose*

Water quality parameters of *G. verrucosa* after being treated by different concentrations of SLF from *Gelidium* sp. observed over 4 weeks period showed a variety of thresholds and fell within their optimum ranges (Table 1).

Table 1. Water quality parameters of *G. verrucosa* after being treated with different concentrations of SLF extracted from *Gelidium* sp.

Parameter	Range
Salinity (ppt)	20–24
Temperature (°C)	26–30
Acidity (pH	6–8
Nitrate (ppm),	0.0069–0.0085
Phosphate (ppm)	0.0028–0.0048

Discussion

Many species of seaweed have been used for SLF implemented in a wide variety of plants and crops, i.e. *Rosenvigea intricata* [13], *Ulva rigida* [21], *Ulva lactuca* [22]; *Sargassum wightii* [6]; [23], *Grateloupia lithophila* and *Chaetomorpha linum* [23], *Gracillaria textorii* [12], and more. Specifically, the use of SLF extracted from *Codium* sp., *Ulva* sp., *Padina* sp., and *Amphiroa* sp. on the *Gracillaria verrucosa* showed a significant increase in growth hormone [19].

Seaweed has a long history of being used as SLF throughout coastal agricultural activity [24] due to its nutrient content. Previous studies demonstrated that seaweed could enhance growth and nutritional status, also the productivity and quality of crops [25]; [26]. Apart from that, seaweed also contains essential sea minerals that are important for plant growth and cultivation [27]; [28]. Seaweed also acts as a natural chelating agent, as previously studied in *Gelidium abbottorium* [29]. This chelation process is highly associated with mannitol, a natural chelating agent. This chelating agent could provide micronutrients within the soil, revitalise the soil structure, and increase water reservoir capacity suitable for plants and crops [30].

Water quality parameters for the growth of *Gracillaria verrucosa* showed various thresholds and fell within their optimum ranges. Temperature and salinity are influential parameters that could affect the seaweed metabolism process. For instance, the optimum temperature for the growth of seaweed range between 25–33 °C [31], which was also observed in both the first and second experiments. *Gracillaria verrucosa* can live in the salinity range between 5–35 ppt, with their optimum range between 15–25 ppt [32]. The optimum salinity range was observed in the second experiment (Table 1) but not in the first experiment. Dissolved oxygen (DO) plays a vital role in seaweed's basic oxygen needs. It was previously reported that the optimum range of DO range between 4–14 ppm [33], which is observed in the first experiment. The acidity condition will also affect their suitable living conditions. The extreme value of the pH (acidic–alkaline) will cause mobility of various heavy metal substances, causing the imbalance of ammonium and nitrate that are toxic to seaweed [34]. The pH level suitable for seaweed cultivation ranges between 7.0-8.5 [35], observed in both the first and second experiments. Seaweed growth is influenced by nitrate and phosphate in maintaining seaweed fertility and the reproductive stage [36]. The optimum range for seaweed cultivation is in the range of 0.02–0.04. However, nitrate and phosphate were detected to be very low both in the first and second experiments.

In this study, *Gelidium* sp. is proven to be effective in concentrations of 2.0 ml/L and 2.5 ml/L based on the absolute growth and daily growth rate. As previously studied, *Gelidium crinale* exhibited the highest plant height and growth in maize (*Zea mays* L.) plants compared to other seaweed fertilizers [37]. A previous study of *Gelidium crinale* as biofertilizer in soil treatment also showed a higher mean leaf area/plant (cm^2) of Canola (*Brassica napus* L.) than other fertilizer, i.e., *Ulva lactuca* L. and *Cytoseria* spp. [38].

IV. Conclusion

There are insignificant differences between the SLF extracted from *Padina sp., Sargassum sp*., and *Gelidium sp*. The highest growth of *G. verrucosa* among the SLF extracted from seaweed was observed on the Gelidium sp. with the optimum concentration of 2.0–2.5 ml/L. *Gelidium sp*. might provide a prospective future in developing seaweed SLF for *G.verrucosa* production.

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