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# Productivity Improvement Of Seaweed (*Gracilaria verrucosa*) Fertilized With Vermicompost Made From Different Organic Wastes

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**The development of profitable *Gracilaria verrucosa* seaweed cultivation is possible because of the high market demand for gelatine (agarose). These advantages make *Gracilaria verrucosa* cultivation attractive but problems of decreasing productivity are encountered, due to the use of chemical/inorganic fertilizers. In this study, we investigated the use of vermicompost fertilizer, from different organic wastes feed waste, reed waste (Imperata) and banana stem waste and their effect on the productivity of *Gracilaria verrucosa* seaweed. The data were analysed using ANOVA and the Tukey test. The results showed that the lengths of the short and long axes of the seaweed cells were 165.0-227.3 μm and 170.3-253.7 μm, respectively. The daily growth rate was 0.95-1.61% per day. The agar yield quality on day 0 was 12.4-16.0% and on day 42, it was 24.6-30.6%. The nitrogen content of *Gracilaria verrucosa* seaweed on day 0 was 1.50-1.86% and 2.93-3.60% on day 42 while the phosphorus content on day 0 was 0.13-0.22% and 0.41-0.61% on day 42. Treated banana stem waste is the best waste to increase the growth and quality of seaweed.**

**KEYWORDS**

*Gracilaria verrucosa,* Mineral content, Growth, Cell size, Vermicompost

## 1. INTRODUCTION

*Gracilaria verrucosa* seaweed can be cultivated by marine aquaculture and has a high selling price. The development of profitable *Gracilaria verrucosa* businesses in Indonesia could considerably increase people's income due to the high market demand for this seaweed, as it can be processed into agarose [1]. Gracilaria is a highly favoured seaweed species for development by aquaculturists because it has a low seedstock price and can be processed into agarose, which sells at a price three times higher than the seedstock price. Another advantage is that it can be cultivated in ponds with a harvest time of just 42 days. In seaweed cultivation, there is often a decrease in productivity caused by a lack of nutrients. This problem can be overcome by fertilization. Organic fertilizers are found in natural and artificial waters and can be used by aquatic flora, directly or indirectly, to obtain the macro-elements needed to increase the weight of the plants [2].

The use of organic fertilizers is expected to reduce the use of inorganic fertilizers that can negatively affect the environment. Vermicompost fertilizer is an environmentally benign fertilizer that uses natural ingredients (waste from earthworm cultivation) and contains nutrients needed by *Gracilaria verrucosa* seaweed to increase its weight. In vermicompost fertilizer, earthworms change the activity of microorganisms so that the mineralization rate of organic materials increases rapidly [3,4]. The use of wastes, such as feed waste, reed waste (Imperata) and banana stem waste, to produce organic fertilizer by vermicomposting, is expected to improve the productivity of *Gracilaria verrucosa* seaweed. The aim of this research was to investigate the effect of vermicompost fertilizer, made from different organic wastes, on the cell size, daily growth rate, agar yield quality, nitrogen and phosphorus contents and water quality for *Gracilaria verrucosa.*

## 2. MATERIAL AND METHOD

### 2.1 Materials used

This research was conducted over 42 days in November and December 2019 on the embankments of brackish water ponds in Banyu Urip village (Ujungpangkah district, Gresik Regency, East Java Province, Indonesia), in the Fisheries Laboratory of Muhammadiyah University of Gresik and in the Soil Laboratory of Brawijaya University, Malang. Materials used during the study are given in table 1.

Table 1. *Materials or instruments used in the present study*

| Material/instrument | Description (used for) |
|---|---|
| Styrofoam (45 × 30 × 30 $cm^3$) | Research containers |
| pH meter | Measuring the pH and temperature of the water |
| Refractometer | Measuring water salinity |
| Ocular microscope | Observing short and long axis cell sizes |
| Ruler (0.0 mm) | Measuring the short and long axis cell |
| UV-visible spectrophotometer | Calculate the wavelength of nitrogen and phosphorus seaweed (nm) then convert to (%) |
| Analytical balance (0.00 g) | Weigh research test material |
| Brackish water | Cultivation media |
| Pond mud | Equalize the conditions of the original environment |
| Vermicompost | Organic fertilizer |
| Seaweed (*Gracilaria verrucosa*) | Research object |

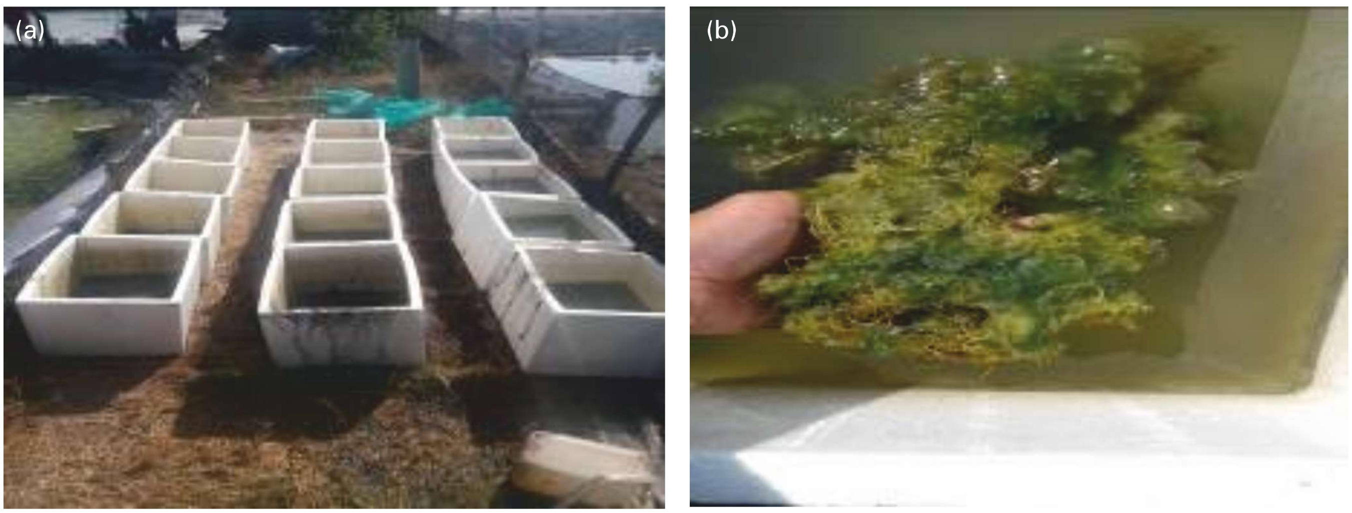


Figure 1. *(a) The layout of Gracilaria verrucosa and (b) seaweed cultivation media*

### 2.2 Fertilizer production

Experimental containers for making vermicompost fertilizer were in the form of five plastic boxes (35 × 35 × 15 cm), with nine holes (diameter 3 mm) in the bottom and covered with plastic. The starter medium contained cow dung (1.6 kg) and soil (600 g). The cow dung and soil were mixed and placed in the base of each container. Organic wastes, in the form of reeds (Imperata) and banana stems, were chopped roughly to a thickness of 2–3 cm and then put into each container (300 g for each organic waste). Meanwhile, the feed waste was aerated until the waste had been moist for seven days and then also weighed as much as 300 g. Adult earthworms (26 g/kg of vermicompost medium) was added and the resulting vermicompost medium stirred and sprayed with water every day. The fertilizer production process was complete after two months and the earthworms were then removed manually. In this study vermicompost, fertilizer (no waste) used contained 20% carbon, 2% nitrogen and 1% phosphorus [5].

### 2.3 Seedstock preparation

The *G. verrucosa* seedstock was produced by tissue culture. The seedstock used was fresh, free from impurities and had many branches, a slightly dark brownish colour, a young hard thallus, no white spots, peeled off and was atleast two weeks old when collected. The weight of seaweed seeds for each treatment was 50 gm.

### 2.4 Container preparation

Fifteen cultivation containers made of styrofoam (45 cm × 30 cm × 30 cm) were used (Figure 1). The containers were washed and dried before use and then filled with mud (5 cm depth) taken from the bottom of the pond. The mud in this study originated from the

**Table 2.** *Water quality of Gracilaria verrucosa seaweed*

| Treatment | Replication | Water quality | | |
|---|---|---|---|---|
| | | Temp. ($^{o}$C) | pH | Salinity (ppt) |
| Without addition of waste | 1 | 28.5–33.1 | 9.2–9.8 | 16–24 |
| | 2 | 28.4–33.3 | 9.0–9.9 | 15–23 |
| | 3 | 28.8–33.5 | 9.5–10.3 | 17–22 |
| Waste from feed | 1 | 27.6–33.5 | 9.0–9.1 | 18–24 |
| | 2 | 27.3–33.9 | 9.0–9.2 | 15–22 |
| | 3 | 27.0–33.4 | 9.0–9.1 | 15–23 |
| Waste from Imperata | 1 | 27.4–32.0 | 9.2–9.8 | 16–24 |
| | 2 | 27.9–33.0 | 9.1–9.7 | 16–22 |
| | 3 | 27.6–33.8 | 9.5–9.9 | 15–21 |
| Waste from banana stems | 1 | 28.0–33.3 | 9.2–9.5 | 17–24 |
| | 2 | 28.3–33.4 | 9.3–9.9 | 17–25 |
| | 3 | 28.2–33.5 | 9.1–9.4 | 18–25 |
| Combined waste | 1 | 27.8–34.0 | 9.5–9.8 | 19–23 |
| | 2 | 27.9–34.0 | 9.2–9.6 | 18–22 |
| | 3 | 28.0–34.0 | 9.8–10.5 | 15–23 |

Treatments: Water quality measurements for each treatment were carried out every week for 42 days in brackish water, using pH meter and refractometer

seaweed farming ponds which were used to equate the condition of the cultivation media with the original ponds. The containers were then filled to the brim with water.

### 2.5 Seedstock distribution

*G. verrucosa* seedstock (50 g) was spread in each of the containers, which were placed on the embankment of brackish water ponds for 42 days [6].

### 2.6 Fertilization

Fertilization involved dissolving vermicompost fertilizer in the water (0.45 g/L of seawater) [5,7].

### 2.7 Parameters analyzed

Size (length) of *Gracilaria verrucosa* seaweed cells (cell growth) were observed horizontally and vertically using a 0.0 mm ruler and an ocular microscope (80X magnification). The daily specific growth rate and agar yield quality are given in equations 1 and 2, respectively.

Daily specific growth rate (%/day) = ((Ln final weight - Ln initial weight) / days) x 100 ...(1)

Agar yield quality (%) = (Agar flour weight / Dry weight) x 100 ...(2)

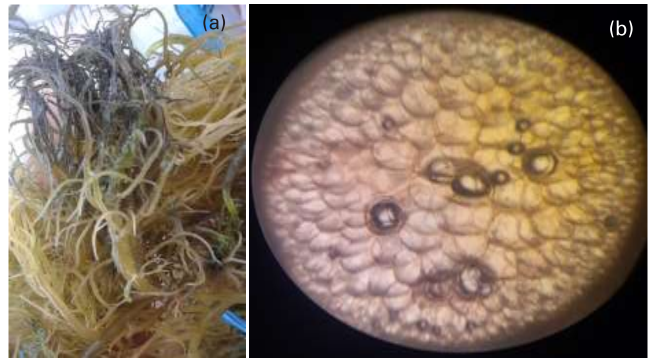


**Figure 2.** *(a) Morphology and (b) cells of Gracilaria verrucosa seaweed*

Agar yield quality was measured at the Fisheries Laboratory of Muhammadiyah University of Gresik. The nitrogen and phosphorus contents of the *Gracilaria verrucosa* seaweed were determined using a UV-visible spectrophotometer method and the water quality (temperature, pH and salinity) of the *Gracilaria verrucosa* seaweed were determined using a pH meter and refractometer [7].

### 2.8 Research design

This research used a completely randomized design with five treatments and three repetitions. The five treatments were - treatment A: vermicompost fertilizer (no waste) (2% nitrogen and 1% phosphorus); treatment B: vermicompost fertilizer (feed waste) (1.5% nitrogen and 0.8% phosphorus); treatment C: vermicompost fertilizer (imperata) (2.5% nitrogen and 1% phosphorus); treatment D: vermicompost fertilizer (banana stem waste) (3.5% nitrogen and 2% phosphorus) and treatment E: vermicompost fertilizer (combined waste) (2% nitrogen and 1.5% phosphorus).

### 2.9 Data analysis

The data were analyzed using ANOVA to determine the effect of using vermicompost fertilizer made from different organic wastes on the productivity of *Gracilaria verrucosa* seaweed. Significance was determined with Tukey's (HSD) test with a 95% confidence level ($p < 0.05$).

## 3. RESULT AND DISCUSSION

### 3.1 Morphology and cells of *Gracilaria verrucosa* seaweed

The colour of seaweed can be an indicator of the amount of chlorophyll contained in its cells. A thallus with a more concentrated colour indicates a higher chlorophyll content. The difference in the colour of the seaweed thallus is one of its morphological characteristics be-

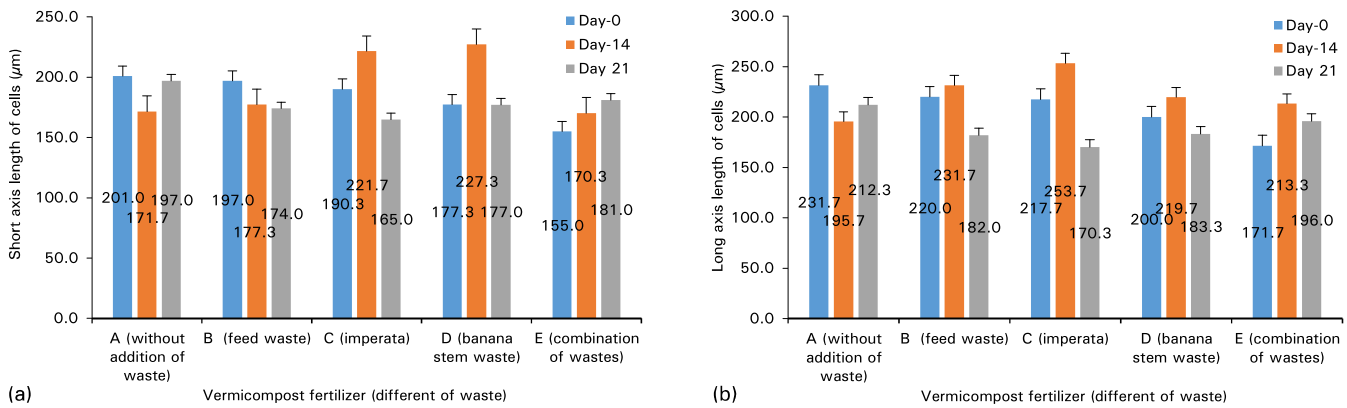


**Figure 3.** *(a) Short axis length and (b) long axis length of Gracilaria verrucosa seaweed cell*

cause seaweeds are able to synthesize colour pigments (Figure 2). Rahim stated that red seaweeds, such as *Gracilaria* sp., contain the dominant pigments phycoerythrin and ficocyanine, which are responsible for the red colour [8].

### 3.2 Cell size

With the use of vermicompost made from different organic wastes, the length of the short axis of *Gracilaria verrucosa* seaweed cells ranged from 165–227.3 m and the length of the long axis ranged from 170.3–253.7 m (Figure 3). Analysis of variance (ANOVA) for the cell sizes of *G. verrucossa* seaweed showed that the administration of vermicompost fertilizer made from different organic wastes had a significant effect on the increase in the length of the short axis ($p<0.05$). In contrast, vermicompost fertilization had no significant effect on the increase in the length of the long axis ($p>0.05$). Furthermore, the results of the Tukey (HSD) test suggested that the short axis length on day 21 in treatment D (banana stem waste) was significantly different from that in treatment A (without the addition of waste), treatment B (feed waste) and treatment E (combination of waste) ($p<0.05$). Treatment D (banana stem waste) did not show any difference with treatment C (reed waste or Imperata) ($p>0.05$). However, treatment A (without the addition of waste), treatment B (feed waste), treatment C (reed waste or Imperata) and treatment E (combined waste) did not show any difference from each other on days 0, 21 and 42 ($p>0.05$).

The long axis length of *G. verrucosa* seaweed cells was not further analyzed using the Tukey (HSD) test because the ANOVA results showed no significant effect ($p>0.05$). In one field of view 80X magnification, to get the short axis length by looking at the shortest size of an observed cell, while the long axis length is obtained from the longest size of an observed cell [7]. The best treatment was D (banana stem waste) (3.5% nitrogen and 2% phosphorus). Nitrogen and phosphorus are needed in small but very important quantities. The element is used for the formation of proteins that function to multiply the number and strengthen cell walls to produce optimal growth. According to Rahim, the short axis length of *Gracilaria verrucossa* seaweed cells ranged from 28.261–387.725 µm [8]. We found that fertilizing *Gracilaria verrucosa* seaweed with vermicompost accelerated the growth and the formation of new shoots because the vermicompost fertilizer contained nutrients needed by the seaweed in the form of macro and microelements, which accelerate cell division. Rahim also reported that the long axis length of *Gracilaria verrucossa* seaweed cells was 30.296–432.426 µm. In general, changes in the cell size are caused by environmental factors, both abiotic (light, salinity, temperature and nutrient availability) and biotic (marine organism) [9,10].

### 3.3 Daily growth rate

The daily specific growth rate (%/day) of *G. verrucosa* ranged from 0.95–1.61% (per day) (Figure 4). Analysis of variance (ANOVA) showed that the administration of vermicompost fertilizer, made from different organic wastes, had a significant effect on the increase in the daily specific growth rates ($p<0.05$). Furthermore, the Tukey (HSD) test revealed that all the treatments – treatment A (without the addition of waste), treatment B (waste from the feed), treatment C (reed waste or Imperata), treatment D (waste from banana stems) and treatment E (combined waste) were significantly different from one another ($p<0.05$) (Figure 4). Hasseltrom argued that a seaweed cultivation activity is categorized as good if the average daily growth rate is atleast 3% [11]. It has been suggested that the low daily growth rate of *G. verrucosa* is due to the less favourable environmental conditions in seaweed

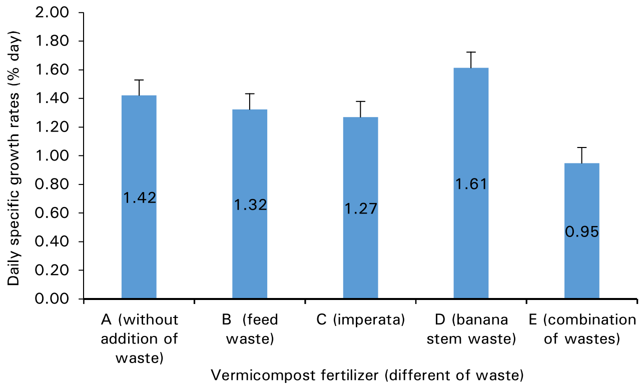


**Figure 4.** *Daily specific growth rates of G. verrucosa seaweed*

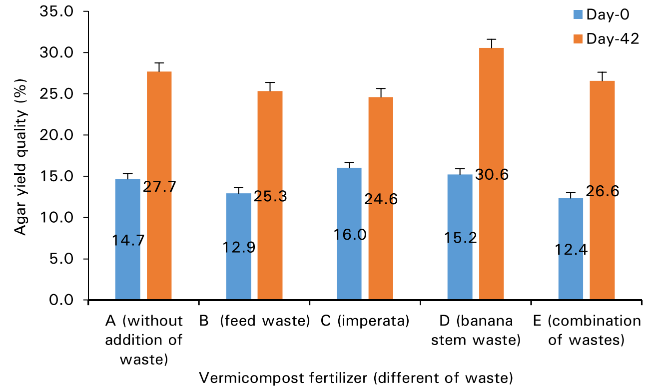


**Figure 5.** *Agar yield quality of Gracilaria verrucosa seaweed*

cultivation, leading to below optimal growth [12]. Another factor was that the rainfall was high during the weeks of this study, so the seaweed did not get enough sunlight. In addition to environmental and weather factors, the increasing weight and length of the seaweed thallus can also affect the daily growth of *Gracilaria* species.

According to Kasim, the decline in the growth rate of the seaweed was due to different rates of photosynthesis in a clump of seaweed [13]. He explained that stress on seaweed can also lead to tissue damage, resulting in lower growth rates. The results of this study indicated that the administration of fertilizer from banana stem waste influenced the growth of *Gracilaria verrucosa* seaweed. Onwu found that banana stems contain microorganisms that can store nitrogen and phosphate [14]. Bacteria that can store nitrogen can increase and improve the nitrogen content of water or soil and produce substances that can enhance plant growth. According to Liu, phosphate solubilizing bacteria on banana stems can convert insoluble phosphate into soluble phosphate by secreting organic acids, such as formic, acetic, propionic, lactic, glycolic, fumaric and succinic acids [15]. Phosphate solubilizing bacteria play an important role by increasing the availability of phosphorus for plants by upto 50%.

### 3.4 Agar yield quality

Based on the results of this research, the agar yield quality on day 0 ranged from 12.4–16.0% while on day 42 this ranged from 24.6–30.6% (Figure 5). Analysis of variance (ANOVA) found that the administration of vermicompost fertilizer made from different organic wastes had a significant effect on improving the agar yield quality on day 42 ($p < 0.05$). Furthermore, the results of the Tukey (HSD) test on the agar yield quality on day 42 indicated that treatment D (banana stem waste) was significantly different from treatment C (reed waste or Imperata) ($p < 0.05$). Treatment A (without the addition of waste), treatment B (feed waste), treatment C (reed waste or *Imperata*), and E (combination of wastes) were not significantly different from each other ($p > 0.05$). According to Beaumont, the best agar yield quality from *G. verrucosa* seaweed, based on the Indonesian National Standards on agar quality, was in the range of 20–26% [16]. Thus, the agar yield quality in this research was categorized as the best. Venugopal stated that the amount of seaweed cultivation yield varies according to ecological factors, such as light, nutrition, temperature and water content after being dried [17].

### 3.5 Nitrogen and phosphorus contents of *G. verrucosa* seaweed

The nitrogen content of *Gracilaria verrucosa* seaweed on day 0 ranged from 1.50–1.86% and on day 42 ranged from 2.93–3.60% (Figure 6a). The phosphorus content of *Gracilaria verrucosa* seaweed on day 0 ranged from 0.13–0.22% and on day 42 ranged 0.41–0.61% (Figure 6b). Analysis of variance (ANOVA) showed that the administration of vermicompost fertilizer made from different organic wastes had no significant effect on the increase in the phosphorus contents of *G. verrucosa* seaweed ($p > 0.05$), so the Tukey (HSD) test was not carried out. The application of vermicompost fertilizer from different wastes significantly increased the nitrogen content of *Gracilaria verrucosa* seaweed after 42 days ($p < 0.05$). Tukey's tests showed that treatment D (banana stem waste) was significantly different from treatment B (feed waste) ($p < 0.05$), but there was no significant difference with treatments A (without the addition of waste), C (reed waste or Imperata) and E (combination of wastes) ($p > 0.05$) in increasing the nitrogen content of *Gracilaria verrucosa* seaweed. Based on the results of this research, the nitrogen content of *G. verrucosa*

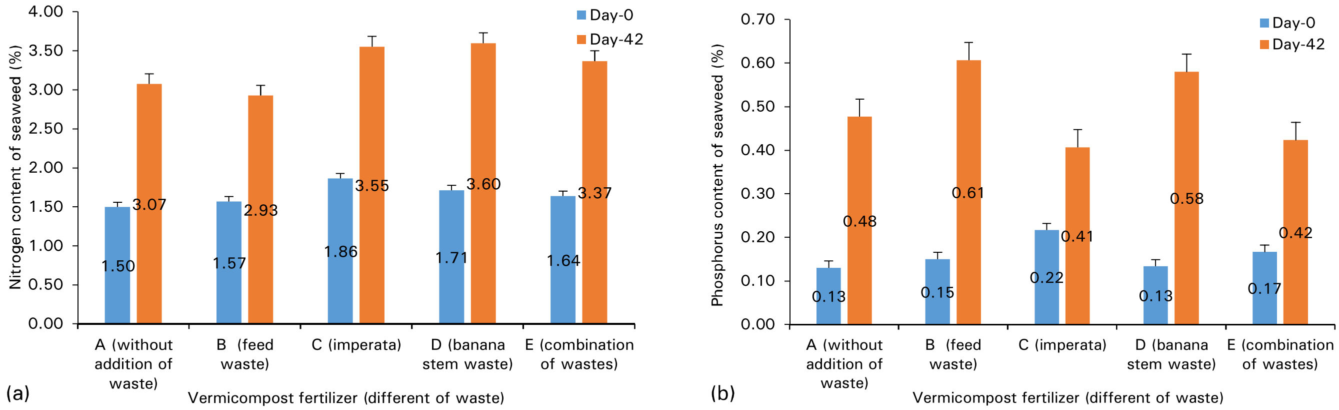


**Figure 6.** *(a) Nitrogen content and (b) phosphorus content of Gracilaria verrucosa seaweed*

seaweed ranged from 1.50–3.60% (Figure 6a). Roleda found that the nitrogen content of seaweed cultivated offshore ranged from 0.44–4.73% [18]. In a study by Boedi, the nitrogen content of *G. verrucosa* seaweed cultivated on a laboratory scale was 1.72–2.31% [19]. Nitrogen is fully utilized by *G. verrucosa* seaweed to encourage high growth rates and good seaweed quality. According to Balmori, vermicompost fertilizer is an important source of nitrogen, but the nitrogen in fertilizers mostly cannot be mixed and cannot be available immediately for plant use [20].

In our study, the phosphorus content of *G. verrucosa* was in the range of 0.13–0.61% (Figure 6b). According to Boedi, the phosphorus content of *G. verrucosa* seaweed cultivated on a laboratory scale ranged from 0.03-0.10% [19]. The optimal growth of seaweed was obtained with a phosphorus content of 0.3–0.5%. The role of phosphorus is very important for plants, as it is a constituent component of proteins, cell nuclei, cell walls, the formation of high-energy compounds and is a component of RNA and DNA [21]. However, the quantity of phosphorus in plants is smaller than the quantities of nitrogen, potassium and calcium [22]. In this study, the phosphorus content of *Gracilaria verrucosa* seaweed did not have a significant effect on its growth rate, because phosphorus is not needed in large quantities, but its presence can optimize the growth and quality of the seaweed, especially in *Gracilaria* species [5,23]. According to Weil, among the nutrients elements, phosphorus is second only to nitrogen in its impact on the productivity of aquatic ecosystems [24].

### 3.5 Water quality

It can be seen in table 2 that the temperature of the water for all treatments, ranged from 27.30–34$^{o}$C. This range is consistent with the findings of Jang, that seaweeds reproduce and grow very well in aqueous media with a temperature range of 26–33$^{o}$C [25]. The pH of the water in all treatments ranged from 9.0–10.5. According to Radulovich, seaweed growth requires an optimal pH of seawater that ranges from 6–9 [26]. The salinity was found to be 15–25 ppt for all treatments. Radulovich stated that the salinity levels for a good increase in seaweed weights are in the range of 14–35 ppt [26].

## 4. CONCLUSION

The research results above led us to conclude that for all vermicompost fertilizer treatments*, Gracilaria verrucosa* seaweed has a red colour due to the presence of phycoerythrin and ficocyanine pigments. The use of vermicompost fertilizer made from different organic wastes can increase the productivity of brackish water ponds, in terms of the cell size, daily growth rate, agar yield quality, phosphorus content and nitrogen content of *Gracilaria verrucosa* seaweed. Water quality in the form of pH, temperature and salinity and the use of vermicompost fertilizer made from different organic wastes, provides the best conditions to increase the thallus weight of *Gracilaria verrucosa* seaweed, especially treatment D.

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