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The Effect of Gracilaria Liquid Organic Fertilizer Fermented with Different Starters on the Growth of Pakcoy

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ABSTRACT This research aimed to determine the effect of adding three different starters, such as *Lactobacillus plantarum, Azospirillum* sp., and *Trichoderma* sp., on gracilaria seaweed for bok choy growth. The starter optimized fermentation and improved fertilizer quality in growing bok choy. The seaweed was reduced in size, soaked in 1% phosphoric acid solution, then homogenized and heated with 3% molasses to provide microbial nutrition and 1% KOH to maximize mineral content. Fermentation was carried out for 14 days in the microbial exponential phase for decomposition. The results showed that the addition of *L. plantarum* and *Trichoderma* sp. to gracilaria seaweed liquid fertilizer produces the highest values of gibberellin (147 ppm), auxin (140 ppm), and cytokinin (96 ppm). Next, the fertilizer was applied to bok choy to determine the effect on its growth. The highest fresh yield of bok choy was 64.0±1.41 g, the highest chlorophyll A content was 6.70±0.01%, and chlorophyll B was 2.30±0.03%. Even though the control treatment had the highest C/N ratio compared to the other treatments, the highest yields of bok choy freshness, height, and leaf area were found when applying liquid fertilizer with *L. plantarum* and *Trichoderma* sp. starter. The study concluded that bok choy growth was significantly encouraged by fertilizer-seaweed with high levels of phytohormones compared to mineral content. Adding *L. plantarum* and *Trichoderma* sp. is a good starter for increasing the growth-promoting properties of liquid seaweed fertilizer.

Keywords: Fermentation; *Gracilaria*; pakcoy; seaweed fertilizer; starters

INTRODUCTION

Organic fertilizer is an environmentally friendly alternative that can prevent land degradation. This type of fertilizer is available in two forms: solid and liquid. The advantages of liquid organic fertilizer are numerous, as it can be easily applied by pouring it directly onto the roots and plants. This application method helps keep the soil moist and improves nutrient uptake by plants (Purbajanti & Setyawati, 2020). Furthermore, the 100% solubility of liquid organic fertilizer allows for the effective distribution and utilization of nutrients during fertilization. As such, liquid organic fertilizer presents a promising option for sustainable agriculture practices.

Seaweed is a rich source of minerals, organic compounds, and growth-promoting hormones, known as phytohormones. *Gracilaria* sp. is one type of seaweed often used as a raw material for organic fertilizers due to its biostimulant properties. The seaweed contains phytohormones, including auxins, cytokinins, and gibberellins. *Gracilaria* contains 10-47% protein, which increases organic compounds and promotes plant growth (Raj *et al.*, 2018). Due to its high nutrient content and plant growth hormones, *Gracilaria* sp. has the potential to be a valuable raw material for liquid organic fertilizer (Basmal *et al.*, 2020). It is confirmed by several studies regarding the manufacture of liquid fertilizer from Gracilaria sp. (Alamsjah *et al.*, 2013; Rao & Chatterjee, 2014; Tsaniya *et al.*, 2021). The growth hormone values found in *Gracilaria* sp. were higher than those in commercial organic fertilizers, with auxin values of 144 ppm, gibberellin 1.552 ppm, and cytokinins consisting of kinetin 65 ppm and zeatin 81 ppm (Sedayu *et al.*, 2014).

The liquid fertilizer requires bacteria for the fermentation process. Liquid fertilizer from *Gracilaria* sp. has been made using *Lactobacillus* sp. (Alamsjah *et al.*, 2013). Some bacteria used to ferment organic materials include *L. plantarum, Trichoderma* sp., and *Azospirillum* sp. *L. plantarun* has been used to produce high-nitrogen fertilizer (Wang *et al.*, 2014) and organic fertilizer from tilapia waste (Abdulgawad, 2016). Trichoderma sp. and Azospirillum sp. have been used to produce organic fertilizer from Gracilaria sp. and Sargassum sp. (Kusumawati *et al.*, 2021; Tsaniya *et al.*, 2021) as well as from fish waste (Mangmang *et al.*, 2015; Patil *et al.*, 2023). *L. plantarum* acts as a decomposer of organic compounds, *Trichoderma* sp. is a group of fungi that decompose organic matter and control pests and plant diseases by producing phytohormones (Kusumawati *et al.*, 2021; Tsaniya *et al.*, 2021), and *Azospirillum* sp. is an aerobic bacterium that increases nitrogen levels and produces phytohormones (Zayed, 2018). *L. plantarum* can hydrolyze seaweed, particularly *Gracilaria* sp., to produce nutrients used in the fermentation process (Lin *et al.*, 2020).

The limited research regarding manufacturing liquid organic fertilizer from *Gracilaria* sp. uses three different bacterial species, the first in this research. In this research, molasses was added to manufacture liquid organic fertilizer. Molasses is used as a carbohydrate supply, which can change the C:N ratio in soil nutrients, influence soil microbial ecology, and prevent the growth of plant parasites (Pyakurel *et al.*, 2019; Nurhayati *et al.*, 2021). This study aimed to determine the characteristics of organic fertilizer from different combinations of bioactivators, specifically *L. plantarum*, *Trichoderma* sp., and

Azospirillum sp. Different formulations of liquid organic fertilizer with various bioactivator treatments were evaluated for their effects on N, P, K, C-Organic, pH, and growth hormone levels. The liquid organic fertilizer with the best characteristics was applied to pakcoy (Bok Choy) to evaluate its effectiveness.

MATERIALS AND METHODS

Materials

Gracilaria sp. was collected from Tambak Harja, a brackish water pond located northwest of Semarang City, Central Java, Indonesia, after being cultured for 60 days.

Methods

Liquid organic fertilizer formulations

This study involved three types of treatment for liquid organic fertilizer formulations with different seaweed concentrations as raw materials. The treatments for the liquid organic fertilizer were as follows:

A: 100% *Gracilaria* sp. + L. plantarum 1 CFU/ml + Azospirillum sp. 1 CFU/ml + 3% Molasses

B: 100% *Gracilaria* sp. + L. plantarum 1 CFU/ml + Trichoderma sp. 1 CFU/ml + 3% Molasses

C: 100% *Gracilaria* sp. + L. plantarum 1 CFU/ml + Trichoderma sp. 1 CFU/ml + Azospirillum sp. 1 CFU/ml + 3% Molasses

K: 100% *Gracilaria* sp. + 3% Molasses

Preparation of raw material

The raw material was prepared by Tsaniya *et al*. (2021) with modification. Seaweed was treated by washing and cleaning it to remove any dirt using flowing water. Then, the seaweed was reduced in size by cutting it into small pieces measuring 1-2 cm. Next, the seaweed was immersed in a 1% solution of phosphoric acid with a ratio of 1:10, and the immersion process lasted for 18-24 hours.

Preparation of microbes

The starter bioactivators used in this study were *L. plantarum FNCC 0027, Azospirillum* sp., and *Trichoderma* sp. *L. plantarum* sp. was obtained from the Center for Food and Nutrition Study at Universitas Gadjah Mada, Yogyakarta, Indonesia. *Azospirillum* sp. was obtained from the Agricultural Microbial Laboratory at the Agriculture Faculty of Gadjah Mada University, Yogyakarta, Indonesia, while *Trichoderma* sp. was obtained from the Pest and Plant Disease Laboratory in Ungaran, Semarang, Indonesia. The cultures of *L. plantarum, Azospirillum* sp., and *Trichoderma* sp. were activated or refreshed with liquid media according to their microbial specifications. *L. plantarum* was activated in de Man Rogosa Sharpe Broth (Silitonga *et al*., 2022), *Azospirillum* sp. in Nutrient Broth, and *Trichoderma* sp. in Potato Dextrose Broth. A colony of each bioactivator was activated in 9 ml of liquid media for 24 h for *L. plantarum* and *Azospirillum* sp. (Suhameena *et al*., 2020), while *Trichoderma* sp. was activated for 3x24 h (Uruilal *et al*., 2017).

Liquid fertilizer production

The liquid fertilizer was prepared by Tsaniya *et al*. (2021) with modification. Seaweed was soaking in phosphoric acid based on the treatment in the study, the seaweed was weighed again. Subsequently, the seaweed was cut into small pieces measuring 1-2 cm. A 1% solution of phosphoric acid was added to the sample in a ratio of 1:10, and then the mixture was refined and homogenized. Once the fertilizer formulation for each treatment was completely dissolved, 3% molasses was added, and heating was carried out again until the temperature reached 100 °C.

Microbial induction in liquid fertilizer

For each treatment, a bacterial inoculant was prepared by mixing 10 ml of bacterial colonies with liquid organic fertilizer medium. The adaptation process for *L. plantarum* and *Azospirillum* sp. was conducted for 2×24 h (Galindo *et al*., 2022), while for *Trichoderma* sp., it was carried out for 4×24 h (Harlis *et al*., 2019).

Fermentation process

The fermentation process was conducted for 14 days to obtain liquid organic fertilizer from different types of seaweed. Aeration was provided during the aerobic fermentation process through an aerator to ensure an oxygen supply.

Determination of C-organic

The measurement of C-Organic concentration was carried out using the Walkey and Black method (AOAC, 2012). This involved sample digestion with $K_2Cr_2O_7$ 1N and H_2SO_4, followed by dilution, homogenization, and incubation for 12 h. Subsequently, the measurement was taken using a spectrophotometer at a wavelength of 561 nm.

Determination of nitrogen

Nitrogen concentration was measured based on the Kjeldahl method, titrimetry, and Devarda method (AOAC, 2012).

Determination of phosphorus

Phosphorus concentration measurement was conducted using the Spectrophotometric method at a wavelength of 650 nm (AOAC, 2012).

Determination of potassium

Potassium concentration measurement was carried out based on the Flame Photometry method (AOAC, 2012).

Determination of pH

pH testing was carried out using a pH meter (AOAC, 2012).

Growth Hormone Analysis

The analysis of growth hormones (auxin, gibberellin, cytokinin) was performed in the Laboratory of Agriculture, Agricultural Science, Bogor. The spectrophotometric analysis, as described by Dewi *et al*. (2015), involved using 100 mL of 50% (half-strength) Tryptic Soy Broth (TSB) medium, supplemented with 200 μL of L-Tryptophan precursor. After centrifuging the sample at 8,000 rpm for 10 minutes, 1 mL of supernatant was mixed with 2 mL of Salkowski reagent and left to incubate in the dark for 30 minutes. Absorbance was then measured at 530 nm using a spectrophotometer to determine growth hormones concentration based on a standard curve. For HPLC analysis, a 5 mL sample was centrifuged at 10,000 rpm for 15 minutes, and the supernatant's pH was adjusted to 2.8. After three extractions using ethyl acetate in a 1:1 volume ratio, the resulting extraction was evaporated and analyzed using HPLC. The mobile phase consisted of methanol: acetic acid: aquabides (30:1:70 v/v/v), with pure growth hormone standards used for calibration.

Application in Pakcoy Plant

The method of liquid organic fertilizer application on plants was based on a modified method by Mutryarny & Lidar (2018). The first stage was seedling production of pakcoy using the Cap Panah Merah F1 variety. This stage was carried out for two weeks after planting, using a mixture of soil, sand/rice husk/sawdust, and manure in a ratio of 1:1:1 as the growing medium. The liquid fertilizer was applied twice a week with a concentration of approximately 2% of the total water used.

After two weeks of planting, the pakcoy seedlings were transplanted into large polybags using the same growing medium as the seedling production stage. The liquid fertilizer was applied twice a week with a concentration of approximately 2% of the total water used.

Observations and measurements of plant height, leaf number, leaf width, and leaf length were conducted every week. The height of the plant was measured using a ruler, starting from the base of the stem to the tip of the longest leaf in an upright position. The number of leaves counted included all fully opened leaves. Leaf width (cm) was measured from the widest point of the leaf, perpendicular to the midrib. The consumption weight (g) was measured at the end of the study by removing the roots and weighing the plants.

Chlorophyll Analysis

Chlorophyll content was measured in fresh leaf samples using the method described by Purbajanti & Setyawati (2020). A total of 0.5 g of leaf samples was homogenized with 90% v/v acetone, filtered, and made up to a final volume of 50 ml. The chlorophyll concentration was then calculated based on the absorbance of the extract measured using a spectrophotometer at 645 nm (UV/Vis) and 666 nm (UV/Vis) wavelengths.

Statistical analysis

The data were analyzed using parametric and non-parametric statistical tests. For parametric analysis, ANOVA was used, followed by the Tukey test. For non-parametric analysis, the Kruskal-Wallis's method was used, followed by the Mann-Whitney test.

RESULTS AND DISCUSSION

C-organic, nitrogen, phosphorus, and potassium

The nutrient elements in the fertilizer included organic carbon, nitrogen, phosphorus, and potassium.

Table 1 shows that the nutrient content in liquid organic fertilizer from *Gracilaria* sp. differed between fertilizer with added microorganisms as a fermentation inoculum and fertilizer without the addition of microorganisms as a control, except for nitrogen. Some types of seaweed, especially *Gracilaria* sp., contained high protein content, which caused the occurrence of ammonification during the fermentation process and led to non-significant differences in nitrogen content for each treatment. However, the resulting nitrogen had different characteristics; in liquid organic fertilizer without bacteria, it produced a foul smell like ammonia (Bhaskoro *et al*., 2020).

The organic carbon content in *Gracilaria* sp. liquid organic fertilizer samples added with different types of microorganisms was lower than those without the addition of bacteria. The condition was due to the presence of lactic acid bacteria as a starter in the fermentation process, which required carbon as an energy source. In addition to carbon, other nutrient elements, such as phosphorus and potassium, also played a role in strengthening plant roots and stems. The phosphorus and potassium content in seaweed, including *Gracilaria* sp., was low, so other additional materials were needed to increase the amount of phosphorus and potassium in liquid organic fertilizer (Bhaskoro *et al*., 2020). Therefore, in this study, three different types of bacteria were added with different variations for each treatment to determine the nutrient content during the fermentation process. The results showed that the potassium produced in the control liquid organic fertilizer and that added with bacteria were significantly different, but the phosphorus content did not differ significantly.

It should be noted that while seaweed contained only a small amount of potassium, it contained many other minerals, vitamins, and enzymes not found in other plants that acted as natural growth stimulants. This made it different from NPK fertilizer compounds in general. The use of a combination of three bacteria, namely *L. plantarum, Trichoderma* sp., and *Azospirillum* sp., in the study gave higher results compared to Tsaniya *et al*. (2021) who produced liquid organic fertilizer from *Gracilaria* sp. using *Trichoderma* sp. and *Azospirilium* sp. Higher results were

Table 1. C-organic, nitrogen, phosphorus, and potassium in *Gracilaria* liquid fertilizer.

No	Treatments	C-Organic (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)
1	A	0.60 ± 0.05[a]	0.25 ± 0.02[a]	0.45 ± 0.04[a]	0.35 ± 0.03[a]
2	B	0.67 ± 0.06[a]	0.26 ± 0.01[a]	0.54 ± 0.07[b]	0.33 ± 0.02[a]
3	C	0.85 ± 0.10[b]	0.27 ± 0.03[a]	0.57 ± 0.05[c]	0.47 ± 0.06[b]
4	K	1.09 ± 0.04[c]	0.30 ± 0.02[a]	0.39 ± 0.03[a]	0.83 ± 0.04[c]

Note:
Data followed by the same lowercase letter in the same column shows no significant difference (P>0.05).
Data followed by different lowercase letters in the same column are significantly different (P<0.05).
A : *L. plantarum* and *Azospirillum* sp.
B : *L. plantarum* and *Trichoderma* sp.
C : *L. plantarum*, *Trichoderma* sp., and *Azospirillum* sp.
K : Control

shown in C-organic, phosphorus, and potassium values. However, the N value in this study was lower. The results of this research need to meet the standards for liquid organic fertilizer based on the regulations of the Ministry of Agriculture (2019).

pH

The pH values are presented in Figure 1, where the lowest pH of 4.5 was observed in the liquid fertilizer treatments inoculated with three different microorganisms, namely *L. plantarum*, *Trichoderma* sp., and *Azospirillum* sp. In contrast, the control treatment had the highest pH of 7.3.

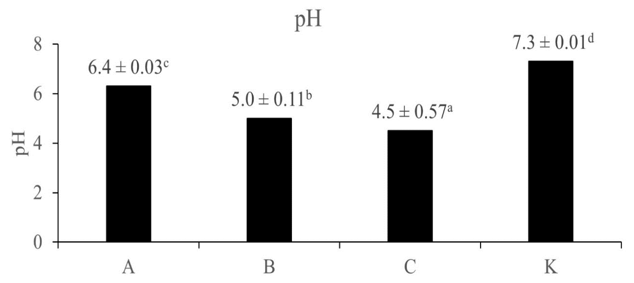

Figure 1. pH of *Gracilaria* liquid fertilizer.

Note:
Data followed by different lowercase letters are significantly different (P<0.05).
A : *L. plantarum* and *Azospirillum* sp.
B : *L. plantarum* and *Trichoderma* sp.
C : *L. plantarum*, *Trichoderma* sp., and *Azospirillum* sp.
K : Control

The pH values in organic liquid fertilizers are influenced by the decomposition process of organic carbon by microorganisms, with higher organic carbon values leading to a lower pH (Tsaniya *et al*., 2021). The pH of fermented products, including organic liquid fertilizers, depends on the activity of microorganisms and the materials used as nutrients for these microorganisms. The activity of three bacteria, namely *L. plantarum, Trichoderma* sp., and *Azospirillum* sp., produces acid, resulting in a higher pH in the control treatment compared to other treatments (Bhaskoro *et al*., 2020). Additionally, the presence of sulfated polysaccharides in seaweed is believed to influence the pH. Ammar *et al*. (2022) researched optimal pH for enhanced soil fertilizer reported pH values of 5–7.5. Figure 1 illustrates pH values ranging from 4.5 to 7.3, which fall within the standards set by the Ministry of Agriculture (2019) for organic fertilizer, biological fertilizer, and soil improvement, which should be in the range of 4 to 9.

Growth hormone

Plant growth is influenced by several factors, one of which is the nutrients provided to the plant. Among these nutrients, phytohormones can accelerate plant growth by assisting in the plant's metabolic processes and minimizing the impact of abiotic stress. Phytohormones consist of gibberellins, auxins, and cytokinins, each playing a distinct role. Gibberellins are involved in the development of seeds into flowers and shoots, auxins facilitate the growth process by supporting cell division, elongation, and maturation, while cytokinins help maintain cell division to prevent aging in plant organs (Godlewska *et al*., 2016; Egamberdieva *et al*., 2017; Dewi *et al*., 2019; Dewi *et al*., 2020).

Gracilaria sp. is a type of red seaweed that boasts high nutrient content, including phytohormones. According to research conducted by Nasmia *et al*. (2021), the liquid organic fertilizer derived from seaweed contains a higher concentration of phytohormones compared to urea liquid organic fertilizer. Moreover, the phytohormone levels in the *Gracilaria* sp. fertilizer were found to be higher than those in Sargassum sp. liquid fertilizer (Putra *et al*., 2022). This higher phytohormone content in the liquid fertilizer correlates with a faster growth rate observed in plants treated with seaweed-based liquid organic fertilizer, as opposed to those treated with urea liquid organic fertilizer.

Table 2. Phytohormone of *Gracilaria* liquid fertilizer (ppm).

No	Treatments	Gibberellin	Auxins	Cytokinin
1	A	136	136	92
2	B	147	140	96
3	C	116	128	96
4	K	130	127	92

Note:
A : *L. plantarum* and *Azospirillum* sp.
B : L. *plantarum* and *Trichoderma* sp.
C : *L. plantarum*, *Trichoderma* sp., and *Azospirillum* sp.
K : Control

The results of the phytohormone analysis of *Gracilaria* liquid organic fertilizer in this study, considering the difference in types of microorganisms, are presented in Table 2. The most significant disparity in phytohormone content, including gibberellins, auxins, and cytokinins, was observed between the liquid organic fertilizer enriched with *L. plantarum* and *Trichoderma* sp. (code B) and the microorganism-free liquid organic fertilizer used as the control (code K). The difference in gibberellin content was because Trichoderma can stimulate phytohormone production during the fermentation process (Kusumawati *et al*., 2021). Additionally, the synergy between *L. plantarum* and Trichoderma sp. also contributed to the higher production of phytohormones compared to other treatments. Seaweed serves as an excellent source of soil nutrients, encompassing nitrogen, phosphorus, magnesium, potassium, iodine, calcium, amino acids, macro and micro-nutrients, vitamins, auxins, and cytokinins (Putra *et al*., 2022).

Application in pakcoy

Figure 2 illustrates that the height of pakcoy and the quantity, width, and length of leaves from the second week to the sixth week of observation in sample code B were higher compared to other treatments. Additionally, the harvested weight of pakcoy vegetables treated with liquid organic fertilizer coded B had the highest weight and chlorophyll content, as presented in Table 3. Furthermore, the phytohormone parameters revealed that the content of gibberellin, auxin, and cytokinin in liquid

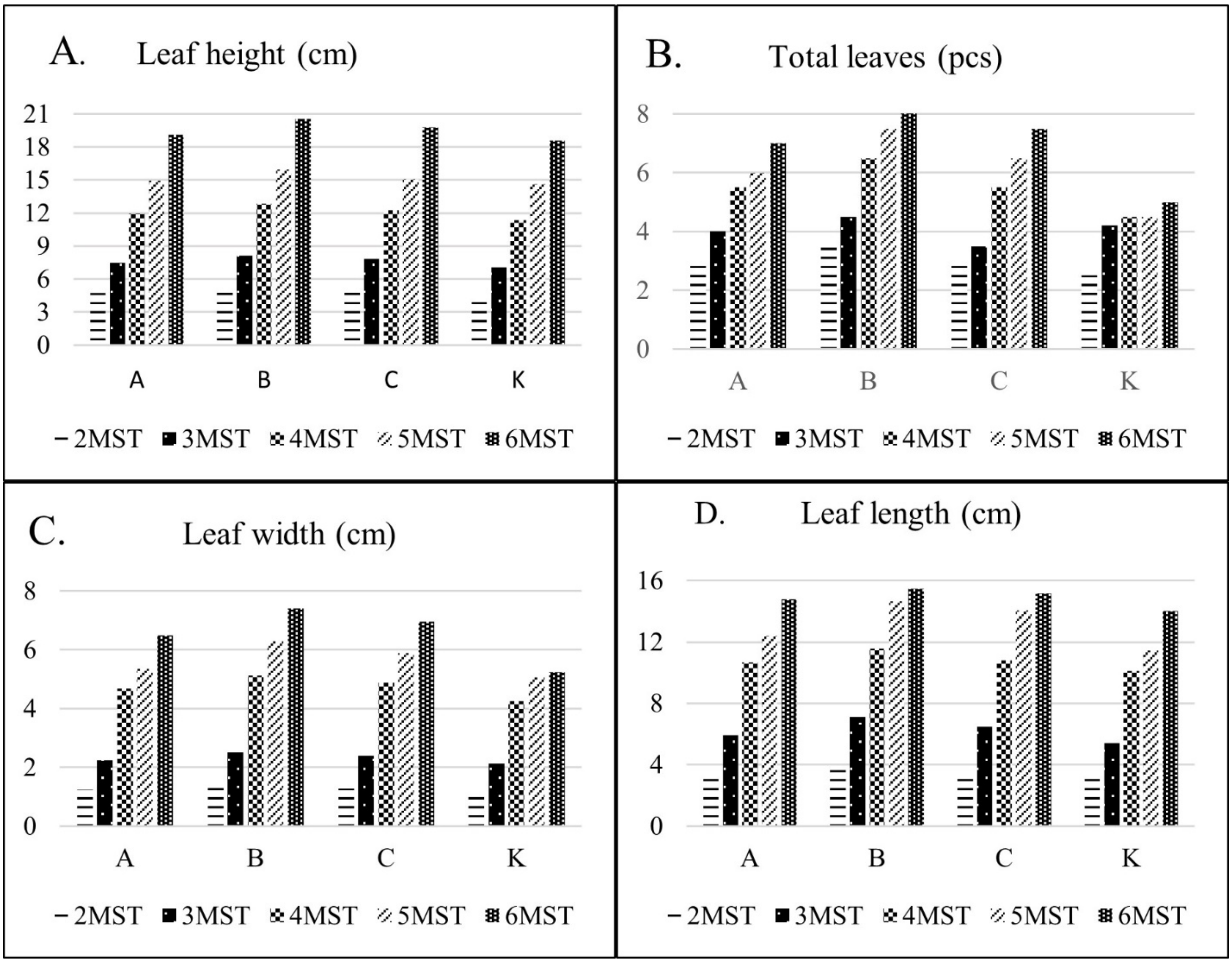

Figure 2. Pakcoy plant height and leaf quantity, width, and length.
Note:
A : *L. plantarum* and *Azospirillum* sp.
B : L. *plantarum* and *Trichoderma* sp.
C : *L. plantarum*, *Trichoderma* sp., and *Azospirillum* sp.
K : Control

organic fertilizer coded B was higher than in other treatments.

The results of applying this fertilizer to pakcoy plants demonstrated that high phytohormone levels positively influence the speed and quality of plant growth compared to high urea levels. The combination of gibberellin, auxin, and cytokinin in the phytohormones stimulated plant metabolism, leading to faster growth and improved quality. Moreover, the fermentation process by microorganisms also played a crucial role in producing liquid organic fertilizer with varying qualities. The quality of the leaves relates to the synergy and outcomes of the fermentation process, involving primary and secondary metabolites. For example, the synergy of *L. plantarum* acting as a de-

Table 3. Total harvested, chlorophyll A, chlorophyll B and total chlorophyll of pakcoy samples

No	Treatments	Total Harvested (g)	Chlorophyll A (%)	Chlorophyll B (%)	Total Chlorophyll (%)
1.	A	56.0 ± 2.83^{c}	6.70 ± 0.10^{b}	2.30 ± 0.03^{a}	9.00 ± 0.02^{b}
2.	B	64.0 ± 1.41^{d}	7.50 ± 0.09^{d}	2.50 ± 0.02^{a}	10.00 ± 0.02^{d}
3.	C	54.5 ± 2.12^{ab}	7.00 ± 0.09^{c}	2.30 ± 0.02^{a}	9.30 ± 0.01^{c}
4.	K	47.5 ± 0.71^{a}	5.30 ± 0.10^{a}	1.50 ± 0.03^{a}	6.80 ± 0.02^{a}

Note:
Data followed by the same lowercase letter in the same column shows no significant difference (P>0.05).
Data followed by different lowercase letters in the same column are significantly different (P<0.05).
A : *L. plantarum* and *Azospirillum* sp.
B : *L. plantarum* and *Trichoderma* sp.
C : L. *plantarum*, *Trichoderma* sp., and *Azospirillum* sp.
K : Control

composer in *Gracilaria* sp. (Raman *et al.*, 2022) and *Trichoderma* sp. stimulating the production of growth hormones with nutrients from the decomposition of *Gracilaria* sp. by *L. plantarum* (Kusumawati *et al.*, 2021).

Table 3 shows that the total chlorophyll in pak choy was highest when treated with liquid organic fertilizer containing *L.plantarum* and *Trichoderma*. The composition of both starter cultures in liquid organic fertilizer haVE synergy in breaking down organic materials, triggering growth hormones and preventing the presence of plant pathogens.The liquid fertilizer offered advantages, such as providing a defense mechanism and immunity for the plants to resist viruses, pathogens, or fungal attacks. It was observed that the leaves were less susceptible to damage from insect attacks. Furthermore, the liquid organic fertilizer enhanced chlorophyll synthesis, thereby improving the nutrient quality of the leaves (Raghunandan *et al.*, 2019; Prasedya *et al.*, 2022).

CONCLUSION

The characteristics of liquid organic fertilizer derived from *Gracilaria* sp. varied depending on the types of microorganisms used in urea content, pH, and phytohormone concentration. Among the treatments, the liquid organic fertilizer enriched with *L. plantarum* and *Trichoderma* sp. as bioactivators exhibited the most favorable characteristics and the highest concentration of phytohormones compared to both the control and other treatments. Moreover, when this specific liquid organic fertilizer with *L. plantarum* and *Trichoderma* sp. was applied to pakcoy plants, it resulted in rapid growth, a higher harvest weight, and increased chlorophyll content. Liquid organic fertilizer B was the best result in this study.

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AUTHORS' CONTRIBUTIONS

END is doing idea and written manuscript; ART is doing research and data analyze; and L.P is doing manuscript preparation.

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