

**ORIGINAL RESEARCH**

## Effect of Agar Industry Waste-Based Liquid Fertilizer on Growth and Nutrient Uptake in Red Lettuce

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**ABSTRACT:** The processing of *Gracilaria* sp. seaweed in the agar industry generates solid waste, which can contribute to environmental pollution. However, this waste contains valuable nutrients such as organic carbon, nitrogen, phosphorus, and potassium, which can be used to produce organic fertilizers for plant growth optimization. This study aims to determine characteristics and optimal proportion of agar industry waste for formulating liquid organic fertilizer. A quantitative experiment was conducted with four treatments, using 0%, 8%, 16%, and 24% agar waste, mixed with 8% eggshell powder, 5% molasses, water, and 4% Plant Growth Promoting Rhizobacteria (PGPR) as a bioactivator. Fermentation was carried out using an anaerobic method for 14 days. The effect of the fertilizer on red lettuce growth was evaluated. The results from ANOVA and Tukey analysis showed that different proportions of agar waste resulted in varying outcomes. The highest proportion produced the best fertilizer, with 0.51% organic carbon, 0.22% nitrogen, 0.11% phosphorus, 0.19% potassium, and a pH of 6.81. The fertilizer also resulted in a 48% yield, it appeared dark brown with a slightly pungent fermentation odor. The fertilizer application produced the best growth rate, with red lettuce plants reaching a height of 14.03 cm, 9.67 leaves, a leaf width of 8.80 cm, a stem diameter of 6.90 mm, root length of 12.23 cm, and a fresh weight of 14.67 grams. This study highlights the potential of using agar industry waste as a liquid organic fertilizer, supporting sustainable agriculture by promoting ecofriendly waste management and reducing dependence on synthetic fertilizers.

**KEYWORDS:** Agar processing waste, plant growth promoting rhizobacteria, red lettuce, seaweed

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### 1. Introduction

According to the Indonesian Ministry of Maritime Affairs and Fisheries (2022), the cultivation of *Gracilaria* sp. seaweed has been increasing to meet the growing demand for raw materials in the agar processing industry (Basyuni et al., 2024). However, this industry generates both liquid and solid by-

products, which, if not managed properly, can lead to environmental pollution and health hazards. Notably, solid waste constitutes the largest portion of the by-products, accounting for approximately 60–70% of the total raw material. This waste is particularly rich in cellulose (Muryanto et al., 2023).

The solid waste from agar processing contains valuable macronutrients, including nitrogen (0.20%), phosphorus (0.12%), potassium (0.17%), organic carbon (10.96%), sodium (0.66%), calcium (0.61%), and magnesium (0.09%) (Basmal et al., 2020). It also contains micronutrients such as copper (4.80 ppm), iron (0.24 ppm), zinc (8.42 ppm), manganese (57.58 ppm), and boron (32.32 ppm) (Basmal et al., 2020). These nutrient properties indicate the potential of agar industry solid waste as a raw material for organic fertilizer production, contributing to plant growth optimization.

In addition to industrial waste, household organic waste such as eggshells can be a valuable additive in fertilizer formulations. Eggshells are commonly used to neutralize pH and enhance nutrient availability in soil. The high calcium content in eggshells plays a significant role in cell division and apical meristem formation. Chicken eggshells contain several minerals beneficial for plants, including calcium (231.67 g kg<sup>-1</sup>), nitrogen (8.47 g kg<sup>-1</sup>), potassium (0.66 g kg<sup>-1</sup>), and phosphorus (1.00 g kg<sup>-1</sup>) (Tombarkiewicz et al., 2022). In this study, solid waste from the agar industry was combined with eggshell powder to enhance the nutrient composition of liquid organic fertilizer.

Compared to solid organic fertilizers, liquid organic fertilizers are generally more effective because they are more readily absorbed by plants (Deng et al., 2023). Liquid organic fertilizers can be produced through fermentation processes, often with the addition of bioactivators to accelerate decomposition and nutrient availability. Among these bioactivators, Plant Growth Promoting Rhizobacteria (PGPR) play a vital

role. PGPR are beneficial bacteria that colonize the rhizosphere and promote plant growth. Common strains include *Paenibacillus polymyxa* and *Pseudomonas fluorescens*, which are also found in bamboo roots (Kang et al., 2010). *Paenibacillus polymyxa* contributes to organic matter decomposition, nitrogen fixation, and phytohormone production (Huang et al., 2024), while *Pseudomonas fluorescens* aids in root phototrophy and pathogen suppression (Panpatte et al., 2016).

This study applied the formulated fertilizer to red lettuce (*Lactuca sativa* L. var. crispus), which was selected as a test plant due to its relatively short growth cycle of 30–60 days, allowing for efficient monitoring of plant response and growth performance (Yu et al., 2024). The objective of this study was to evaluate the effects of incorporating agar industry waste into liquid organic fertilizer on its nutrient composition and to assess its effectiveness in promoting plant growth. Compared to other liquid organic fertilizers such as those derived from tilapia viscera and papaya peel, which contain relatively low levels of nutrients including nitrogen (0.143%), phosphorus (0.030%), and potassium (0.070%) (Wicaksono and Rachmawati, 2022), fertilizers made from agar industry waste demonstrate significantly greater potential for supporting sustainable plant cultivation.

## 2. Materials and methods

The primary materials utilized were solid waste agar (*Gracilaria sp.*) from Bestagar Pureindo International Company in Malang, East Java. Additional materials included chicken eggshell powder, PGPR isolates and molasses come from Nusantara Organic Farm.

The liquid organic fertilizer was produced at Nusantara Organic Farm, Bantul, Yogyakarta Regency. Laboratory analysis of yield, pH, C-organic, Nitrogen, Phosphorus, Potassium and observation of the effectiveness fertilizers on the growth of red lettuce (*Lactuca sativa* var. *crispa*) plant were conducted at Central Java Agricultural Instrument Standards Implementation Center in East Ungaran, Semarang Regency. The testing analyzed include of plant height, number of leaves, leaf width, stem diameter, root length and fresh weight.

### 2.1 Material preparation

Solid waste industrial agar samples were crushed by wooden stirrers until they became fine fibers, while eggshell wastes were washed with clean water and crushed into pieces before being dried in the sun for 12 hours and roasted for approximately 5 minutes. The dried eggshells were pulverized using a blender until they became powder, then sieved using a 70 mesh sieve and packed in thin walled packaging to be stored at room temperature.

### 2.2 Preparation of PGPR isolates

The methodology for preparing Plant Growth Promoting Rhizobacteria (PGPR) from bamboo roots referred to the study by Prabewi et al. (2022), with a modification in the bacterial growth medium using coconut water instead of well water due to the presence of essential nutrients in coconut water that can support PGPR growth and development. The bamboo roots were obtained from soil at a depth of approximately 10 cm and cleaned without water. Molasses (40 mL) was mixed with 4 L of coconut water and stirred until homogeneous. Bamboo roots (maximum 100

g) were placed in a fermentation container and combined with the solution of molasses and coconut water. The PGPR mixture was fermented for 14 days, during which isolates formed, marked by the emergence of a characteristic sour odor and air bubbles in the jerry can. .

### 2.3 Preparation of liquid organic fertilizer

The production process of liquid organic fertilizer was based on the research of Ramamoorthy et al. (2024), with modifications to the organic materials used. The treatments are as follows, treatment A (0% or no addition of agar industry solid waste), treatment B (8% or 120 grams), treatment C (16% or 240 grams) and treatment D (24% or 360 grams). Eggshell powder was added to each jerry can in quantities up to 120 grams. Molasses was dissolved in water in different volumes for each treatment, with the following quantities of water, treatment A (1.245 mL), treatment B (1.125 mL), treatment C (1.005 mL) and treatment D (885 mL). The molasses solution was added to the jerry can and shaken. PGPR isolate (4%) was inoculated into each treatment, after which the jerry can were sealed. The fermentation process was carried out anaerobically for 14 days. The results were filtered, and the liquid component was collected. The liquid organic fertilizer was then sterilized in autoclave (121°C, 15 minutes).

### 2.4 Yield analysis

The yield value was calculated by determining the ratio of the final volume of fertilizer (B) to the initial volume of material (A) (AOAC, 2025). The value of yield was calculated by using equation 1.

$$\text{Yield (\%)} = \frac{B}{A} \times 100\% \quad \text{Equation (1)}$$

## 2.5 pH analysis

The pH analysis was conducted using a digital pH meter (PH-009, China). A sample of liquid fertilizer (30 mL) was placed in a beaker glass and pH was measured using a pH meter that had been previously calibrated with a buffer solution of pH 4.0 and 7.0 (AOAC, 2019).

## 2.6 C-organic analysis

C-organic analysis was conducted in accordance with specifications set forth in SNI 19-7030-2004, employing the Walkley and Black method 2.7 (BSN, 2004). The analysis procedure was conducted via the deconstruction method. The sample was then added with a solution of  $K_2Cr_2O_7$  and concentrated  $H_2SO_4$ , then mixture was shaken and left for 30 minutes. It was then diluted with distilled water. The sample was then homogenized by swirling and allowed to stand for 12 hours. The C-organic content was then quantified by spectrophotometry at

a wavelength of 587 nm. The formula for C-organic was calculating using equation 2.

## 2.7 Nitrogen analysis

Total Nitrogen analysis was conducted by measuring the levels of N-organic,  $N-NH_4$ , and  $N-NO_3$ , which refers to the Kjeldahl method through the stages of destruction, distillation and titration (AOAC, 2019). The distillate formed was titrated. Nitrogen in the form of nitrate was extracted with water, reduced with a devarda alloy, distilled and titrated once more. The calculation of the Nitrogen was as followed by equation 3.

## 2.8 Phosphorus analysis

Sample (1 mL) was placed in a digestion tube, to which concentrated  $HNO_3$  and  $HClO$  were added, and then heated at a high temperature. The mixture was allowed to stand for 30 minutes, after which it was observed on a spectrophotometer at a wavelength of 889 nm for comparison with the standard solution.

$$\text{C – organic (\%)} = \frac{\text{ppm curve} \times 100}{\text{mg sample}} \times \frac{100\text{ml}}{1000\text{ml}} \times \text{CF} \quad \text{Equation (2)}$$

$$\text{Total N (\%)} = \text{N-organic} + \text{N-}NH_4^+ + \text{N-}NO_3^- \quad \text{Equation (3)}$$

$$P_2O_5 = \frac{\text{ppm (SC)} \times \text{EV(ml)}}{1000} \times \frac{100}{S(\text{wt})} \times \text{DF} \times \frac{142}{62} \times \text{CF} \quad \text{Equation (4)}$$

Where  $P_2O_5$  indicates phosphorus content (%), ppm(SC) indicates ppm from the standard curve, EV (mL) indicates extract volume in milliliters, S(wt) indicates sample weight in milligrams, DF indicates dilution factor, CF indicates correction factor, 1000 converts milliliters of extract into liters (since ppm is in mg/L), 100 converts the phosphorus content into a percentage of the original sample, 142 is the molecular weight of diphosphorus pentoxide ( $P_2O_5$ ), and 62 is the combined atomic weight of two phosphorus atoms ( $2 \times 31$ ), representing elemental phosphorus (P).

The phosphorus content was calculated based on the concentration obtained from the standard curve (in ppm), adjusted for the extract volume, sample weight, dilution factor, and converted to  $P_2O_5$  using molecular weight ratios. The final phosphorus content was expressed as a percentage of  $P_2O_5$  using equation 4 (AOAC, 2019).

## 2.9 Potassium analysis

The Potassium analysis was conducted using concentrated  $HNO_3$  and  $HClO_4$ , which were heated to a high temperature. The clear extract formed was collected and added to distilled water, 2N  $HNO_3$  and vanadate solution, then observed on the flamephotometer and compared with the standard solution (AOAC, 2019). To calculate potassium content we employed equation 5.

$$K (\%) = \text{ppm curve} \times \text{mL extract} / 1000 \text{ mL} \times 100 / \text{mg sample} \times \text{dilution factor} \times \text{correction factor} \text{-----Equation (5)}$$

## 2.10 Planting and applying fertilizer

The seedling containers are filled with the planting medium (soil and humus in a 1:1 ratio) to a depth of 6 cm. The seeds are then scattered and covered with a layer of seedling medium, approximately 0.5 cm thick. The medium was then watered until the surface was completely saturated. The seedlings were subsequently transplanted into polybags (25 cm x 25 cm) containing a medium comprising rice hull and soil in a 1:2 ratio. Fertilization was done by watering with a fertilizer dilution ratio of 1:25. The liquid organic fertilizer was dissolved in water to a concentration of 4 mL/100 mL, with watering intervals occurring every two days in the afternoon. It was recommended that the plants be watered twice daily, in the morning

and evening. The planting period lasts for 35 days, after which the plants can be harvested by carefully pulling them up from the planting medium, ensuring that the roots are not damaged.

## 2.11 Plant growth observations

The plant height, number of leaves, leaf width and stem diameter were observed on a weekly basis, with measurements taken on the 0th, 7th, 14th, 21st, 28th and 35th days after planting. The root length and fresh weight of the red lettuce were recorded after harvesting. The height of the plants was measured from the base of the stem to the top of the highest leaf using a ruler. The number of leaves was determined by counting the number of leaflets that are growing on the plant. The diameter of the stem was measured using a caliper gauge, with the measurement taken at a point 1 cm from the base of the stem. The width of leaves was determined by measuring the largest leaf on each plant with a ruler. The fresh weight was determined by weighing the sample on a digital scale. The length of roots was measured using a ruler, with the measurement taken from the base to the end of the longest root.

## 2.12 Data analysis

The data analysis employed a Completely Randomized Design (CRD) with the treatment of adding industrial solid waste agar at proportions of 0%, 8%, 16% and 24%. The data were analyzed using the statistical software package SPSS, version 16 (SPSS Inc., Chicago, IL, USA). Analysis was done with a series of normality test, homogeneity test, ANOVA and the subsequent Tukey analysis.

### 3. Results and discussion

#### 3.1 Yield and pH value

The water content of the material significantly affects the formation of liquid component (Mohammed et al., 2017). Yield value of liquid organic fertilizer was due to the fermentation process, it causes microbes will absorb water and O<sub>2</sub>, thereby converting carbohydrates, fats and waxes into water and CO<sub>2</sub> (Table 1). The use of PGPR and an optimal fermentation time will maximize the decomposition of solid waste agar, thus reducing the solids and increasing water content of the material, which will dissolve into the yield of fertilizer.

Table 1. Yield and pH value of liquid Organic fertilizer.

Treatments	Yield (%)	pH
A	89.33 ± 1.53d	6.93 ± 0.14b
B	76.33 ± 1.53c	6.18 ± 0.05a
C	61.00 ± 1.73b	6.25 ± 0.03a
D	48.00 ± 1.73a	6.81 ± 0.37b

Note: A (0% agar solid waste), B (8% agar solid waste), C (16% agar solid waste) and D (24% agar solid waste). Data is the average result of three replicates ± standard deviation. Data followed by different superscript letters indicate significant differences.

The fermentation process converts organic matter into humic substances through the C-organic and Nitrogen decomposition pathways, which in turn triggers the formation of a stable fertilizer with a darker appearance and a high concentration of nutrients and soil improvers (Gao et al., 2024). The aroma of liquid organic fertilizer was relatively consistent across all treatments, exhibiting a specific sour and sweet fermentation profile. The sour aroma was

formed as a result of conversion of sugar and protein substances into organic acids and acidic aroma compounds during fermentation. The sweet aroma was derived from the addition of molasses. According to Wang et al. (2023), the use of microorganisms accelerates the decomposition of materials by converting sugar into alcohol, which contributes to the occurrence of flavor changes in product.

The pH value of liquid organic fertilizer with addition of agar industry solid waste ranges from 6.18 - 6.93 and was in accordance with the minimum standard of liquid organic fertilizer based on the Decree of the Minister of Agriculture of the Republic of Indonesia Number 261 of 2019, which was the pH value ranging from 4 to 9. The pH value was safe enough to be applied to plants. In comparison, liquid organic fertilizer derived from vegetable waste fermented with EM4 bioactivator for the same duration of 14 days had a lower pH value of 4.5, which is considered quite acidic. According to Nurfitri et al. (2020), such acidic conditions require the addition of neutralizing agents like Calcium Carbonate or Calcium Hydroxide before application to plants to avoid negative effects on nutrient absorption. As stated by Rahman et al. (2018), the availability of nutrients in plants was in the pH range of 6.5 - 8.0. The liquid organic fertilizer with the highest pH value was shown in the control treatment, where only eggshell powder was added. The use of eggshell powder can help to neutralize pH. The eggshells contain calcium, which can elevate the pH of fertilizers and facilitate the growth of acid tolerant plants (Jing et al., 2024). The lowest pH value was observed in

fertilizers with the lowest proportion of solid waste agar. This phenomenon can be attributed to the fermentation process, which results in protein degradation, thereby triggering an increase in pH. Protein degradation will further elevate the pH, as bacteria convert organic acids to Methane,  $\text{NH}_3$ , and  $\text{CO}_2$  (Jantti et al., 2022).

### 3.2 Nutrient content of liquid organic fertilizer

The highest proportion of solid waste agar industry (24%) was found to produce liquid organic fertilizer with the highest C-organic content (Table 2). C-organic is the primary component of organic matter (Liu et al., 2023). The greater quantity of material added, the greater extent of carbon decomposition. Furthermore, the C-organic content was also influenced by the carbon organic content from the material. The solid waste agar industry contains 10.96% C-organic (Basmal et al., 2020). This level was relatively high, but following fermentation, the carbon element will decrease due to its utilization as an energy source for microorganisms. The bacteria utilize carbon as a food and energy source during the decomposition, which

occurs continuously (Zhu et al., 2022). In comparison, liquid organic fertilizer derived from *Gracilaria sp.* seaweed, as reported by Tsaniya et al. (2021), only contains 0.65% C-organic, which is relatively low and does not meet the minimum standard for liquid organic fertilizer. This suggests that liquid organic fertilizer produced from agar industry solid waste has a higher potential in terms of C-organic content.

The addition of 24% solid waste agar to liquid organic fertilizer resulted in the highest Nitrogen content, reaching 0.22% (Table 2). Organic wastes generally possess high moisture and nutrient content, including Carbon and Nitrogen, which facilitate microorganism growth (Dar et al., 2024). Furthermore, the Nitrogen content was influenced by the use of a PGPR starter containing *Paenibacillus polymyxa*, a bacterium that plays a role in Nitrogen fixation (Huang et al., 2024). However, despite this improvement, the nitrogen content still falls below the minimum standard for liquid organic fertilizer established by the Indonesian Ministry of Agriculture (2019), which requires at least 0.5% organic nitrogen.

Table 2. Content of C-organic, nitrogen, C/N ratio, phosphorus and potassium in liquid organic fertilizer.

Treatments	C-Organic (%)	Nitrogen (%)	Ratio C/N (%)	Phosphorus (%)	Potassium (%)
A	$0.21 \pm 0.03^a$	$0.02 \pm 0.01^a$	$11.75 \pm 6.55^a$	$0.01 \pm 0.01^a$	$0.14 \pm 0.01^a$
B	$0.30 \pm 0.05^b$	$0.09 \pm 0.02^b$	$3.41 \pm 0.27^b$	$0.05 \pm 0.01^b$	$0.16 \pm 0.01^b$
C	$0.42 \pm 0.03^c$	$0.16 \pm 0.02^c$	$2.68 \pm 0.21^b$	$0.08 \pm 0.01^c$	$0.18 \pm 0.01^c$
D	$0.51 \pm 0.02^d$	$0.22 \pm 0.01^d$	$2.34 \pm 0.15^b$	$0.11 \pm 0.02^d$	$0.20 \pm 0.01^d$

Note: A (no addition of agar solid waste), B (8% agar solid waste), C (16% agar solid waste) and D (24% agar solid waste). Data is the average result of three replicates  $\pm$  standard deviation. Data followed by different superscript letters indicate significant differences

Table 3. Plant height, number of leaves, leaf width and stem diameter of red lettuce with liquid organic fertilizer

Parameter	Days after planting (DAP)	Treatments			
		A	B	C	D
Plant Height (cm)	0	4.00±0.00 <sup>A,a</sup>	4.00±0.00 <sup>A,a</sup>	4.00±0.00 <sup>A,a</sup>	4.00±0.00 <sup>A,a</sup>
	7	4.97±0.21 <sup>B,a</sup>	5.37±0.15 <sup>B,ab</sup>	5.63±0.25 <sup>B,bc</sup>	6.03±0.21 <sup>B,c</sup>
	14	6.30±0.26 <sup>C,a</sup>	7.17±0.29 <sup>C,b</sup>	7.87±0.15 <sup>C,c</sup>	8.63±0.12 <sup>C,d</sup>
	21	7.53±0.25 <sup>D,a</sup>	8.17±0.21 <sup>D,bc</sup>	8.83±0.15 <sup>D,c</sup>	10.23±0.40 <sup>D,d</sup>
	28	8.50±0.10 <sup>E,a</sup>	9.23±0.25 <sup>E,b</sup>	10.53±0.25 <sup>E,c</sup>	12.00±0.30 <sup>E,d</sup>
	35	10.07±0.31 <sup>F,a</sup>	11.20±0.40 <sup>F,b</sup>	12.47±0.45 <sup>F,c</sup>	14.03±0.42 <sup>F,d</sup>
Number of Leaves (strands)	0	3.00±0.00 <sup>A,a</sup>	3.00±0.00 <sup>A,a</sup>	3.00±0.00 <sup>A,a</sup>	3.00±0.00 <sup>A,a</sup>
	7	4.00±0.00 <sup>AB,a</sup>	4.00±0.00 <sup>A,a</sup>	4.33±1.00 <sup>AB,ab</sup>	5.00±0.00 <sup>B,b</sup>
	14	5.00±0.00 <sup>BC,a</sup>	5.33±0.58 <sup>B,a</sup>	5.67±0.58 <sup>BC,a</sup>	6.00±0.00 <sup>C,a</sup>
	21	5.33±0.58 <sup>CD,a</sup>	6.00±0.00 <sup>B,ab</sup>	6.67±0.58 <sup>CD,bc</sup>	7.33±0.58 <sup>D,c</sup>
	28	6.33±0.58 <sup>DE,a</sup>	7.33±0.58 <sup>C,ab</sup>	8.00±1.00 <sup>DE,ab</sup>	9.00±0.00 <sup>E,b</sup>
	35	7.33±0.58 <sup>E,a</sup>	8.33±0.58 <sup>C,ab</sup>	9.00±1.00 <sup>E,ab</sup>	9.67±0.58 <sup>E,b</sup>
Leaf Width (cm)	0	2.20±0.00 <sup>A,a</sup>	2.20±0.00 <sup>A,a</sup>	2.20±0.00 <sup>A,a</sup>	2.20±0.00 <sup>A,a</sup>
	7	2.80±0.15 <sup>AB,a</sup>	3.23±0.15 <sup>B,a</sup>	3.75±0.20 <sup>B,b</sup>	4.00±0.20 <sup>B,c</sup>
	14	3.23±0.35 <sup>B,a</sup>	3.87±0.15 <sup>C,ab</sup>	4.40±0.20 <sup>C,b</sup>	5.33±0.25 <sup>C,c</sup>
	21	4.27±0.21 <sup>C,a</sup>	4.80±0.17 <sup>D,a</sup>	5.43±0.25 <sup>D,b</sup>	6.27±0.25 <sup>D,c</sup>
	28	4.93±0.25 <sup>D,a</sup>	5.60±0.20 <sup>E,b</sup>	6.33±0.21 <sup>E,c</sup>	7.57±0.32 <sup>E,d</sup>
	35	5.70±0.20 <sup>E,a</sup>	6.43±0.21 <sup>F,b</sup>	7.47±0.15 <sup>F,c</sup>	8.80±0.20 <sup>F,d</sup>
Stem Diameter (mm)	0	1.20±0.00 <sup>A,a</sup>	1.20±0.00 <sup>A,a</sup>	1.20±0.00 <sup>A,a</sup>	1.20±0.00 <sup>A,a</sup>
	7	1.57±0.12 <sup>B,a</sup>	1.90±0.10 <sup>B,b</sup>	2.17±0.15 <sup>B,b</sup>	2.60±0.10 <sup>B,c</sup>
	14	2.10±0.10 <sup>C,a</sup>	2.70±0.10 <sup>C,b</sup>	3.03±0.15 <sup>C,c</sup>	3.90±0.10 <sup>C,d</sup>
	21	3.07±0.12 <sup>D,a</sup>	3.53±0.15 <sup>D,b</sup>	4.10±0.10 <sup>D,c</sup>	5.03±0.15 <sup>D,d</sup>
	28	3.87±0.15 <sup>E,a</sup>	4.37±0.15 <sup>E,b</sup>	5.13±0.21 <sup>E,c</sup>	5.97±0.21 <sup>E,d</sup>
	35	4.93±0.15 <sup>F,a</sup>	5.50±0.20 <sup>F,b</sup>	6.03±0.21 <sup>F,c</sup>	6.90±0.20 <sup>F,d</sup>

Notes :A (no addition of agar solid waste), B (8% agar solid waste), C (16% agar solid waste) and D (24% agar solid waste). ± standard deviation. Data followed by a superscript with different capital letters and small letters indicate a significant difference ( $P < 5\%$ ) between liquid organic fertilizer treatments and between the observation time for each liquid organic fertilizer application treatment.

In contrast, liquid organic fertilizer produced from red seaweed (*Laurencia papillosa*) through anaerobic fermentation

has been reported to contain nitrogen levels ranging from 1.95% to 4.45% (Henggu et al., 2022). The relatively low Nitrogen content in



the agar industry solid waste-based fertilizer is likely due to the initial low Nitrogen levels in the raw material. Despite the low Nitrogen content, this fertilizer can still be applied to plants, particularly when combined with other nutrient sources or when targeting crops that do not have high Nitrogen demands. Moreover, the presence of beneficial microbes from the PGPR starter can improve nutrient availability and promote plant health, making the fertilizer potentially effective in enhancing plant growth in an integrated nutrient management approach.

The Nitrogen and Carbon organic content of liquid organic fertilizer correlates with its success in fermentation, this is expressed in the C/N ratio (Wan et al., 2024). The higher C/N ratio in fertilizer indicates the less optimal decomposition of organic matter. C/N ratio in solid waste agar fertilizer indicates that material has undergone complete decomposition. The organic fertilizer with a C/N ratio < 20 indicates that the organic substrate was maximal decomposition (Phibunwatthanawong and Riddech, 2019). The highest Phosphorus (P) content in the liquid organic fertilizer made from agar industry solid waste reached 0.11%. Phosphorus available to plants is formed from complex compounds in organic matter (Spohn et al., 2024), and its content is also influenced by the use of PGPR bioactivators such as *Pseudomonas fluorescens*, which produce phosphatase enzymes to help solubilize phosphate during fermentation (Wafiqah et al., 2024). However, this Phosphorus level remains relatively low compared to liquid organic fertilizer made from *Gracilaria sp.* seaweed, which was fermented using *Azospirillum sp.* and

*Trichoderma sp.* and recorded a Phosphorus content of 0.55% (Tsaniya et al., 2021). The low Phosphorus content in the agar-based fertilizer is likely due to the suboptimal fermentation duration. Organic matter contains Potassium in complex forms that require decomposition to become available for plant absorption (Kuang et al., 2020). The use of PGPR contributes to increasing Potassium availability due to the presence of Potassium solubilizing bacteria such as *Paenibacillus sp.* and *Bacillus edaphicus*, which can convert Potassium minerals into available Potassium ions (Sattar et al., 2019). In this study, the highest Potassium content in liquid organic fertilizer made from agar industry solid waste was 0.19%. However, this value is still relatively low compared to liquid organic fertilizer derived from *Gracilaria sp.* seaweed, which was fermented using *Azospirillum sp.* and *Trichoderma sp.* and has a Potassium content of 0.33% (Tsaniya et al., 2021). The low Potassium content in the agar waste-based fertilizer may be due to precipitation during the fermentation process, where the concentration of compounds exceeds their solubility limits, leading to incomplete Potassium decomposition (Swetha et al., 2023).

### 3.3 Growth rate of red lettuce plants

Observation of red lettuce plant height revealed a significant increase following the application of liquid organic fertilizer derived from solid waste agar over a 35-day period (Figure 1A). The greatest average height was observed in plants treated with liquid organic fertilizer D, which showed a 251% increase from the initial height, with an average weekly increase of 29.33%. This increase in

plant height can be attributed to treatment D containing the highest proportion of agar industrial solid waste and the most nutrients, which provided optimal conditions for plant growth. Fertilizers with sufficient nutrient content help balance soil nutrients and support healthy plant development (Jakhar et al., 2022). Nitrogen in liquid organic fertilizers plays a critical role in cell elongation, particularly in the activity of the apical meristem. This finding aligns with the conclusion of Deng et al. (2023), who stated

that adequate nitrogen application enhances plant height and promotes basal internode elongation.

The results of the red lettuce leaf count following the application of liquid organic fertilizer at each observation time indicated a relatively insignificant increase (Figure 1B). This limited increase was likely due to some leaf drop observed during the growth phase, which may have resulted from potassium deficiency or suboptimal potassium absorption.

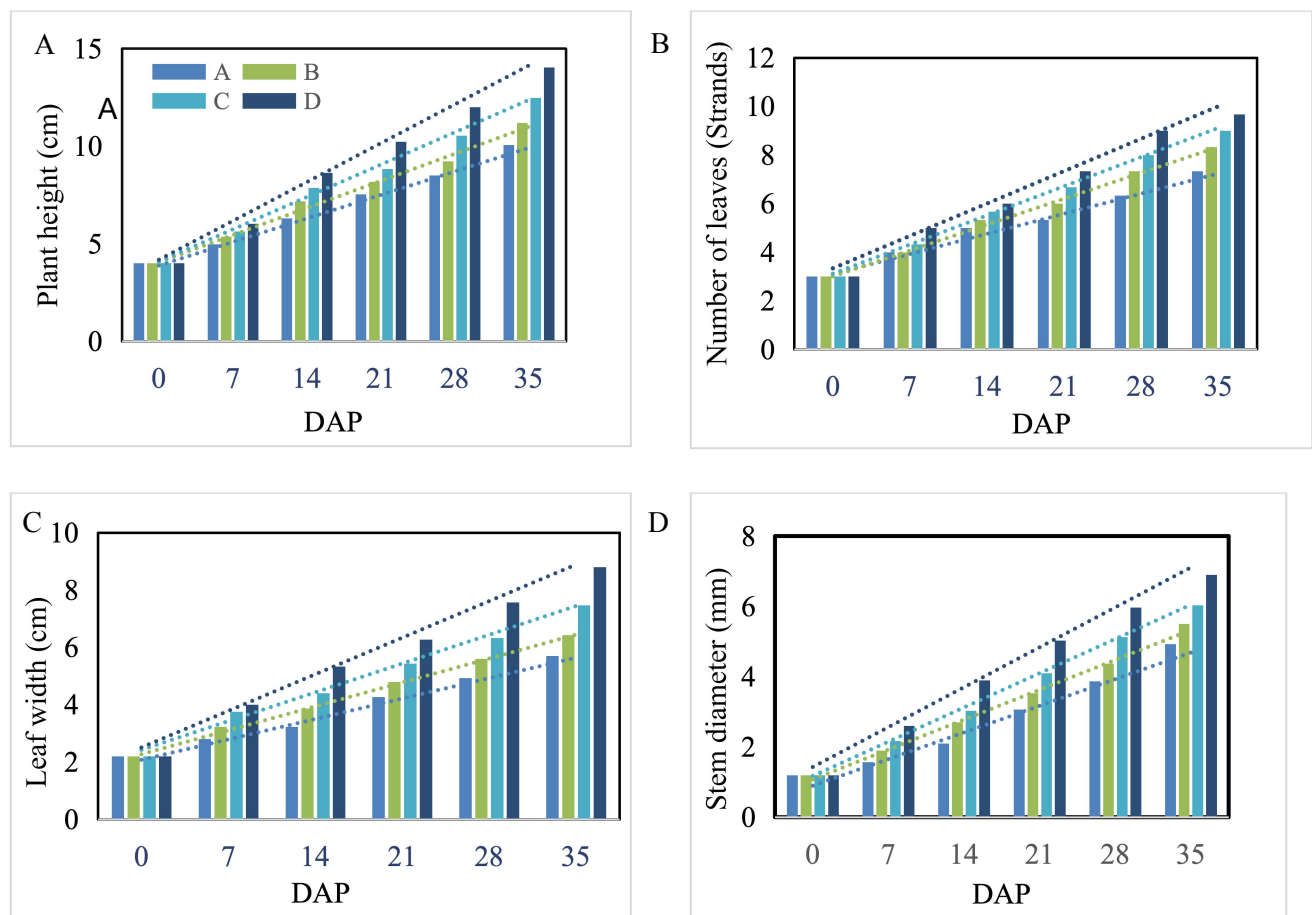


Figure 1. Effects of liquid organic fertilizer derived from agar industry solid waste red lettuce (*Lactuca sativa* var. *crispa*) (A) plant height, (B) number of leaves, (C) leaf width and (D) stem diameter. DAP-days after planting.

The highest average number of leaves was recorded in plants treated with fertilizer D, which showed a 222% increase from the initial leaf count, with an average weekly increase of 27.81%. Leaf growth in red lettuce was consistent with the nitrogen content of the applied liquid organic fertilizer—higher nitrogen levels promoted the development of more leaves. This is because nitrogen plays a key role in leaf formation and chlorophyll synthesis. Increased nitrogen availability enhances plant growth, including leaf production, as it supports the photosynthetic process (Liu et al., 2024). Stem growth also contributed to the number of leaves, as the stem serves as the attachment point for leaf development; therefore, increased stem height is typically accompanied by more leaf growth points.

Regarding leaf width, no significant difference was observed in treatment A between days 0, 7, and 14 (Figure 1C). This likely reflects an early adaptation phase during which the plants required time to adjust, resulting in suboptimal and nonsignificant changes in leaf width. As noted by Minoli et al. (2022), plants often undergo a slower growth rate during this initial vegetative adjustment period. Once past this phase, plants typically exhibit rapid vegetative growth due to more effective nutrient uptake.

The largest red lettuce leaves were found in plants treated with fertilizer D, which exhibited a 300% increase in leaf width from the initial measurement, with an average weekly increase of 33.94%. This result is likely due to the high phosphorus content in fertilizer D, which met the nutritional needs of the plants. Adequate phosphorus supply

enhances photosynthesis, a process that primarily occurs in the leaves (Chen et al., 2024).

The stem diameter of red lettuce increased with plant age and the application of liquid organic fertilizer (Figure 1D). This increase is likely due to the plants entering an active growth phase, during which the provision of adequate nutrients can optimize growth. Supplying essential nutrients, particularly nitrogen (N), phosphorus (P), and potassium (K)—is crucial, as they support vital metabolic and developmental processes in plants (Wang et al., 2020).

Table 4. Root length and fresh weight of Red Lettuce.

Treatments	Root length (cm)	Fresh weight (g)
A	7.23±0.25 <sup>a</sup>	5.00 ± 1.00 <sup>a</sup>
B	9.60±0.20 <sup>b</sup>	8.33 ± 0.58 <sup>b</sup>
C	10.23±0.31 <sup>b</sup>	10.33 ± 1.53 <sup>b</sup>
D	11.10±1.30 <sup>c</sup>	14.67 ± 1.53 <sup>c</sup>

Note: A (0% agar solid waste), B (8% agar solid waste), C (16% agar solid waste) and D (24% agar solid waste). Data is the average result of three replicates ± standard deviation. Data followed by different superscript letters indicate significant differences.

Among the treatments, liquid organic fertilizer D yielded the most promising results, with a 4.75% increase in initial stem diameter and an average weekly increase of 45.98%. This performance can be attributed to the superior nutrient composition of fertilizer D compared to the other treatments. The application of such fertilizers enhances stem growth in conjunction with soil nutrients (Rigi et al., 2023). NPK nutrients, in

particular, are vital for stem enlargement during the growth phase.

Variations in the plants' nutrient absorption capabilities also influenced root length. Enhanced uptake of water and nutrients promotes photosynthesis and the production of essential compounds such as proteins (Gul et al., 2023). Increased protein synthesis supports cell division, facilitating the growth and development of plant tissues, including roots. The longest roots were observed in plants treated with fertilizer D, likely due to its optimal nutrient composition, particularly potassium. Adequate potassium enhances auxin production, a hormone that plays a critical role in stimulating adventitious root formation and elongation (Tahir et al., 2023).

Liquid organic fertilizer treatment D also produced the greatest increase in red lettuce biomass. The availability of key nutrients such as nitrogen, phosphorus, and potassium, promotes overall plant growth. Additionally, the organic matter in the fertilizer improves nutrient absorption, thereby boosting the fresh weight of the plant. An increase in soil organic matter enhances nutrient availability, which in turn stimulates biomass accumulation (Wardhana et al., 2016). Fresh weight is a direct indicator of plant mass and productivity. As such, the morphological components of the plant height, number of leaves, leaf width, stem diameter, and root length, all contributed to the observed differences in fresh weight among treatments. Plants treated with fertilizer D showed the most favorable growth in all these parameters, resulting in a corresponding increase in fresh weight. This suggests that the enlargement of plant organs such as leaves, stems, and roots

directly correlates with an increase in overall biomass.

#### 4. Conclusion

The addition of 24% agar industrial solid waste to liquid organic fertilizer produced the best formulation, indicated by favorable nutrient contents—C-organic (0.51%), Nitrogen (0.22%), Phosphorus (0.11%), Potassium (0.19%), pH (6.81) and yield (48%). This treatment supported optimal growth of red lettuce, as shown by plant height (14.03 cm), number of leaves (9.67), leaf width (8.80 cm), stem diameter (6.90 mm), root length (12.23 cm), and fresh weight (14.67 g). The effectiveness of the 24% treatment may be attributed to improved nutrient availability and enhanced microbial activity, particularly from PGPR. These findings suggest that this formulation holds potential for practical use in sustainable agriculture. Future studies are recommended to test this formulation on different crops, evaluate its long-term effects on soil health, and explore scalability for broader application.

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