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Growth Response and Yield of Pak Choi (Brassica Rapa L. Chinensis) in Response to the Application of EM-4-Based Liquid Organic Fertilizer Derived from Tofu Waste in the Agroecosystem of Timor-Leste

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ABSTRACT

The use of tofu industry wastewater as liquid organic fertilizer is one alternative for waste management and the sustainable supply of nutrients in tropical agricultural systems. This study aims to analyze the chemical quality of liquid organic fertilizer derived from tofu wastewater based on Effective Microorganisms-4 (EM-4), analyze the growth response and yield of pakcoy (Brassica rapa L. Chinensis) in response to its application, and determine the most effective fertilizer concentration for improving crop agronomic performance under the agroecosystem conditions of Timor-Leste. The study was designed as a completely randomized design with fertilizer concentration levels of 5%, 10%, 20%, 30%, and a 0% control. The results indicate that liquid organic fertilizer derived from the fermentation of tofu wastewater using EM-4 contains macronutrients that support plant growth and is suitable for application as an alternative nutrient source. Application of the fertilizer at various concentrations statistically showed significant effects on all growth parameters, but did not yield significant effects on all yield parameters. In general, increasing fertilizer concentration tended to increase plant height, leaf number, canopy width, fresh weight, dry weight, and chlorophyll content, although not all variables showed statistically significant differences. These findings indicate that EM-4-based liquid organic fertilizer derived from tofu wastewater has the potential to serve as an environmentally friendly alternative nutrient source to support pakcoy cultivation in the agroecosystem of Timor-Leste.

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INTRODUCTION

The tofu industry is a rapidly growing plant-based agroindustry in many developing countries, driven by increasing public demand for affordable, high-nutritional-value protein sources. The tofu production process generates large amounts of organic waste, consisting of solid and liquid waste (Kurniawan et al., 2024). Solid waste in the form of tofu pulp has been utilized to a limited extent as animal feed or raw material for fermented products, whereas liquid tofu waste remains a major environmental issue because its management has not been carried out optimally and sustainably (Kusumaningtyas et al., 2020; Mualim et al., 2025). These conditions indicate that the primary focus in the management of tofu industry waste should be directed toward the treatment of liquid waste, which has the potential to pollute the environment.

The high organic content of tofu wastewater makes it a significant source of pollution with a high risk level if discharged directly into the environment. The protein, carbohydrate, fat, and nitrogen compound content in tofu wastewater can undergo decomposition processes that produce ammonia and hydrogen sulfide gases, thereby causing unpleasant odors and increasing the BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) values of water bodies (Mualim et al., 2025; Putro et al., 2021). The accumulation of organic pollutants exceeding the environment's carrying capacity has the potential to degrade water quality and disrupt the balance of aquatic ecosystems (Widyaningrum & Widyastuti, 2021). The impacts of such pollution underscore the urgency of developing an approach to tofu wastewater management that is not only focused on control but also on reuse.

Pollution issues caused by tofu wastewater have been reported to occur repeatedly in various central areas of small- and medium-scale tofu industries, particularly in regions with limited wastewater treatment technology (Herdiana et al., 2022). The practice of directly discharging soybean curd wastewater into water bodies is still common due to low technology adoption rates and a lack of locally-based studies that can serve as technical references (Mufarida & Setiawan, 2020; Rojali et al., 2024). This situation indicates that conventional approaches to tofu wastewater management have not been able to comprehensively address environmental challenges, necessitating more practical and sustainable alternative strategies.

The pollution potential of tofu wastewater is directly proportional to its content of organic compounds and nutrients. Tofu wastewater is known to contain 10% fat, 20–50% carbohydrates, 40–60% protein, as well as macro- and micronutrients such as nitrogen, phosphorus, potassium, calcium, and iron, which play a crucial role in supporting plant growth (Kurniawan et al., 2024; Mualim et al., 2025). The identified total nitrogen, phosphorus, and potassium content in soybean curd wastewater indicates that this waste has great potential to be utilized as a raw material for liquid organic fertilizer (Burhanuddin et al., 2024; Gustiari, 2023). This potential opens up opportunities to transform soybean curd wastewater from a source of pollution into a valuable agricultural input.

The use of tofu wastewater as liquid organic fertilizer requires proper processing to convert the organic compounds it contains into a stable form that is easily absorbed by plants. Fermentation using EM-4 is a widely adopted method because it accelerates the decomposition of organic matter and improves the quality of the resulting liquid organic fertilizer. EM-4 contains functional microorganisms such as Lactobacillus, Saccharomyces, nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and organic matter-degrading microorganisms that play a role in improving the physical, chemical, and biological properties of soil (Demir et al., 2024; Kurniawan et al., 2024; Mandasari et al., 2025). These advantages make EM-4 technology relevant for further study in the context of the sustainable utilization of tofu wastewater.

The application of liquid organic fertilizer derived from tofu wastewater with the addition of EM-4 is closely linked to the development of the horticultural sector. Pakcoy (Brassica rapa L. Chinensis) is a leafy vegetable crop with a short harvest cycle, adaptable to various environmental conditions, and rich in nutrients essential for meeting public nutritional needs (Noriko et al., 2024; Tan et al., 2020; Zou et al., 2021). Pak choi productivity is highly influenced by the availability and balance of nutrients in the soil (Choudhary et al., 2024; Mohamad et al., 2022), making the proper use of fertilizers a key factor in increasing crop yields.

Improper fertilization practices and the continuous use of inorganic fertilizers can degrade soil quality and reduce the efficiency of nutrient uptake by plants (Debele, 2021; Saha et al., 2024). These conditions often result in suboptimal plant growth and low crop yields, particularly on land with limited fertility. Liquid organic fertilizers derived from tofu wastewater have the potential to serve as a solution for improving soil fertility while enhancing the productivity of horticultural crops (Gustiari, 2023; Kurniawan et al., 2024). The relevance of this solution becomes increasingly important when considered in the context of agricultural conditions in developing countries, one of which is Timor-Leste.

Timor-Leste has shown that small-scale food processing industries, including tofu production, are beginning to develop in line with increasing local food demand; however, industrial wastewater management systems remain very limited. The use of EM-4 technology as an activator for liquid organic fertilizer has not yet been widely adopted by farmers, while horticultural crops such as pak choi have great potential to be developed as a source of food and income for the community. These conditions indicate an opportunity for integration between the management of tofu industrial wastewater, the application of EM-4 technology, and the development of pak choi cultivation oriented toward sustainable agriculture in Timor-Leste. Research on the utilization of tofu wastewater as liquid organic fertilizer with the addition of EM-4 has been extensively conducted; however, most studies were carried out under Indonesian agroecosystem conditions and with diverse test crops. The available research results remain contextual and have not specifically examined the response of pak choi plants to the soil and climate conditions of Timor-Leste. This lack of information indicates that the scientific basis for the application of this technology in Timor-Leste is still insufficient.

LITERATURE REVIEW

A clear research gap exists due to the lack of empirical studies evaluating the effect of liquid organic fertilizer derived from the fermentation of tofu wastewater with the addition of EM-4 on the growth and yield of pak choi (Brassica rapa L. Chinensis) and determining the optimal fertilizer concentration under the agroecosystem conditions of Timor-Leste. Differences in soil characteristics, climate, and cultivation systems have the potential to result in varying plant responses to the application of liquid organic fertilizer. This study aims to analyze the chemical quality of EM-4-based liquid organic fertilizer derived from tofu wastewater, analyze the growth and yield responses of pak choi (Brassica rapa L. Chinensis) to its application, and determine the most effective fertilizer concentration for improving crop agronomic performance under the agroecosystem conditions of Timor-Leste. The research results are expected to serve as a basis for the development of sustainable tofu industry wastewater management technology while supporting the improvement of environmentally friendly agricultural productivity in Timor-Leste.

METHODOLOGY

This study was conducted in Aldea Karungulau, Suco Metiaut, Dili, Timor-Leste. All crop cultivation activities and the application of liquid organic fertilizer were carried out at that location, while the chemical analysis of the liquid organic fertilizer was conducted at the Biology Laboratory, Universidade Nacional Timor Lorosa'e (UNTL). The study employed an experimental design using a one-factor randomized block design (RBD). The treatment factor was the concentration of liquid organic fertilizer derived from the fermentation of tofu wastewater. The treatments consisted of five levels: no liquid organic fertilizer application (control) and liquid organic fertilizer application at concentrations of 5%, 10%, 20%, and 30%. The control treatment used only water without the addition of liquid organic fertilizer. Each treatment was repeated five times, resulting in 25 experimental units that were randomly placed at the research site.

Liquid organic fertilizer is made from tofu wastewater obtained from tofu-making facilities in Mercado Comoro and Hera, Dili, which has been homogenized. The homogenized tofu wastewater is first filtered to separate any remaining solid particles that have settled. For the fermentation process, 500 ml of tofu wastewater is mixed with 20 ml of EM-4 and 100 ml of molasses as an energy source for the microorganisms. The molasses is prepared by dissolving 500 g of brown sugar in 500 ml of hot water until homogeneous, then cooled before use. The mixture of tofu wastewater, EM-4, and molasses is placed in a sealed jerrycan as the fermentation container. During the fermentation process, the container is connected via a hose to a bottle filled with water to channel the fermentation gas, ensuring the pressure inside the container remains stable. The fermentation process lasts for 15 days until the liquid organic fertilizer is ready for use.

The quality of the fermented liquid organic fertilizer was analyzed before it was applied to the plants. The analysis was conducted at the UNTL Biology Laboratory to determine the content of total nitrogen, phosphorus, potassium, and organic carbon. Total nitrogen was analyzed using the Kjeldahl method (1883), phosphorus was analyzed using spectrophotometry at a wavelength of 420 nm (Barton, 1948), potassium was analyzed using a flame photometer (Hesse, 1971), and organic carbon was analyzed using the Walkley & Black method (1934). The physical parameters of the fertilizer, namely pH, were measured using a pH meter (Kome et al., 2018), while temperature was measured using a thermometer (Hartini et al., 2018).

The test plants used were pak choi (Brassica rapa L. Chinensis), which were first sown in a germination medium for seven days until uniformly developed seedlings were obtained. The seedlings, once ready for transplanting, were then planted into 20 cm × 30 cm polybags containing topsoil weighing approximately 2 kg. Each polybag was planted with one pak choi seedling. The polybags were arranged with a spacing of 50 cm × 50 cm between plants and labeled according to the established treatments. After transplanting, the growing medium was watered to field capacity.

Plant maintenance was carried out throughout the study period by watering with clean water every morning and evening. Liquid organic fertilizer produced through fermentation was applied to the growing medium every three days in the morning according to the respective treatment concentrations. Fertilizer application was carried out by spraying the fertilizer solution onto the growing medium using a sprayer. Weeding was performed manually by pulling out weeds growing around the plants to prevent competition for nutrient uptake and sunlight.

Plant growth observations were conducted at 10, 20, 30, and 40 days after planting. The growth parameters observed included the number of leaves, plant height, and canopy width. The number of leaves was counted based on fully expanded leaves. Plant height was measured using a ruler from the surface of the growing medium to the highest point of the plant. Canopy width was measured using a ruler by measuring the widest distance between the outermost leaves of the plant. In addition to morphological parameters, leaf chlorophyll content was also measured as a physiological indicator of plant health. Chlorophyll content measurements were taken at 40 days after planting using the uppermost fully expanded leaves. Leaves were extracted using dimethyl sulfoxide (DMSO) according to Ritchie et al. (2021), then analyzed using a spectrophotometer at wavelengths of 649 nm and 665 nm to determine chlorophyll content.

Crop yield parameters were measured at harvest, which occurred 40 days after planting. Harvesting was performed by cutting the base of the stem with a knife; the plants were then weighed to determine their fresh weight and dry weight using an analytical balance.

The observational data were analyzed using analysis of variance (ANOVA) to determine the effect of the treatments on each observed parameter. If the analysis results indicated a significant effect, Duncan's Multiple Range Test (DMRT) was conducted at a 5% confidence level to determine the differences among treatments.

RESULTS AND DISCUSSION

Chemical Characteristics of EM-4-Based Liquid Organic Fertilizer from Tofu Waste and Growing Media

An analysis of the chemical characteristics of liquid organic fertilizer produced from tofu wastewater with the addition of EM-4 was conducted to evaluate the quality of the resulting fertilizer and its compliance with liquid organic fertilizer quality standards. The parameters analyzed included the macro-nutrient content of total N, P_2O_5, K_2O, organic C, and pH. In addition, analyses of the initial and final growing media were conducted to assess the dynamics of changes in soil chemical properties resulting from the application of liquid organic fertilizer at various treatment concentrations. This approach is important for understanding the relationship between the quality of the applied liquid organic fertilizer and the response of the soil as a plant growth medium. The results of the chemical analysis of EM-4-based liquid organic fertilizer from tofu wastewater are presented in Table 1 below.

Table 1. Nutrient Content and pH of Liquid Organic Fertilizer made from Tofu Wastewater with the Addition of EM-4

Parameter	Value	Unit	POC Standard
N_{-Total}	5,52	%	2-6
P_20_5	5,95	%	2-6
K_20	4,74	%	2-6
$C_{-Organik}$	10,48	%	>10
pH H_20	5,6	-	4-9

Based on Table 1, the total nitrogen content of 5.52% falls within the quality standards for liquid organic fertilizer. This value indicates that the fermentation process of tofu wastewater using EM-4 effectively increases nitrogen availability. Nitrogen is a primary macronutrient that plays a role in the synthesis of amino acids, proteins, and enzymes, as well as in chlorophyll formation, which determines a plant's photosynthetic capacity. During the fermentation process, the effective microorganisms contained in EM-4, such as lactic acid bacteria and photosynthetic bacteria, play a role in accelerating the decomposition of complex organic matter into simpler, more available forms of nitrogen. The activity of protease and deaminase enzymes during fermentation enables the mineralization of organic nitrogen into ammonium, which can then be oxidized into nitrate. This phenomenon aligns with the findings of Kolin et al. (2025), who stated that inoculating liquid organic waste with fermentative microbes can increase total nitrogen content by accelerating the mineralization process and stabilizing nitrogen compounds. The high nitrogen content in liquid organic fertilizer has the potential to optimally support the vegetative growth phase of plants, particularly in the formation of leaves and stems.

The P_2O_5 content in liquid organic fertilizer, at 5.95%, also falls within the standard quality range (2–6%), indicating that the fermentation process is effective in enhancing phosphorus solubility. Naturally, phosphorus in organic matter is often bound in complex forms that are less available to plants. During fermentation, microorganisms produce organic acids such as citric acid and lactic acid, which can solubilize bound phosphates through chelation and local pH reduction. Rahman et al. (2023) explain that the activity of phosphate-solubilizing microorganisms significantly contributes to increasing the available phosphorus fraction in liquid organic fertilizers derived from agro-industrial waste. Phosphorus plays a crucial role in energy transfer via ATP molecules, cell division, and root system development. The high P_2O_5 content in liquid organic fertilizers indicates that these fertilizers have the potential to enhance root growth and accelerate the early growth phase of plants following transplanting.

A K_2O content of 4.74% falls within the quality standards for liquid organic fertilizers, indicating that the potassium content in tofu wastewater remains relatively stable during the fermentation process. Potassium does not form complex organic structures like nitrogen and phosphorus, so it undergoes fewer chemical transformations during fermentation. Sari et al. (2024) state that potassium in liquid organic fertilizer generally originates directly from the raw materials and tends to remain in a readily soluble ionic form. Potassium plays a crucial role in regulating cellular osmotic pressure, the opening and closing of stomata, and the activation of more than 60 types of plant enzymes. Adequate potassium availability also improves water use efficiency and plant resistance to abiotic stress. Therefore, the K_2O content in liquid organic fertilizers enhances the fertilizer's potential as a balanced nutrient source to support vegetative growth and physiological resilience in plants.

The organic carbon content of 10.48% meets the minimum standard (>10%). This value indicates that the carbon fraction in the fertilizer has reached the minimum quality standard for liquid organic fertilizer. The organic carbon content in liquid fertilizer reflects the degree of organic matter decomposition and the stability of the fermentation products. Organic carbon serves as an energy source for soil microorganisms and plays a role in improving the physical and chemical properties of the soil following application. Widodo et al. (2022) explain that the application of liquid organic fertilizer with adequate organic carbon content can increase cation exchange capacity, soil aggregation, and water retention. The presence of humic and fulvic compounds resulting from decomposition also contributes to increasing nutrient availability through chelation mechanisms. Thus, the organic carbon content in this liquid organic fertilizer not only serves as a nutrient source but also as a soil conditioner that supports long-term soil quality improvement.

A pH value of 5.6 is considered slightly acidic but still falls within the safe range for most horticultural crops. A slightly acidic reaction can increase the solubility of certain micronutrients such as Fe, Mn, and Zn, although it must be controlled to prevent the pH from becoming too low, which would inhibit phosphorus availability. According to Rahman et al. (2023), liquid organic fertilizers with a pH close to neutral or slightly acidic tend to be more stable and do not cause drastic changes in soil reaction after application. This pH stability is important for maintaining the balance of soil microorganisms and preventing nutrient imbalances. A pH value of 5.6 indicates that fermentation is proceeding optimally without producing excessive acid accumulation that could reduce fertilizer quality.

The chemical characteristics of the EM-4-based organic liquid fertilizer derived from tofu wastewater indicate that the resulting fertilizer meets quality standards and has the potential to serve as an alternative nutrient source for plants. However, the effectiveness of liquid organic fertilizer is determined not only by its nutrient content but also by the initial condition of the growing medium that will receive the fertilizer application. Therefore, evaluating the nutrient status and pH of the growing medium before treatment is crucial to understanding the extent of changes occurring after the application of the liquid organic fertilizer. The nutrient content and pH of the initial growing medium are presented in Table 2 below.

Table 2. Nutrient Content and pH of the Initial Growing Medium

Parameter	Research Findings	Unit
N-Total	1.05	%
P_20_5	0.07	%
K_20	0,23	%
$C_{-Organik}$	6,61	%
pH H_20	5.5	-

The initial growing medium analysis in Table 2 shows that the soil used as the growing medium has a relatively low content of macronutrients, particularly K_2O and P_2O_5, as well as a pH value that tends to be slightly acidic. These initial conditions indicate limited soil fertility, which could potentially restrict plant growth if no external nutrient inputs are added. The application of organic liquid fertilizer is expected to improve soil nutrient status while stabilizing soil pH toward a more neutral condition. Furthermore, to determine the extent to which the application of organic liquid fertilizer made from soybean curd wastewater with the addition of EM-4 can increase nutrient content and improve soil reaction, an analysis was conducted on the growing medium after treatment at various concentration levels. This evaluation is crucial as a basis for assessing the effectiveness of organic liquid fertilizer in improving soil chemical quality compared to initial conditions. The results of the analysis of nutrient content and final growing medium pH following the application of tofu wastewater-based organic liquid fertilizer with EM-4 are presented in Table 3 below.

Table 3. Nutrient Content and pH of the Final Growing Medium after Application of Tofu Wastewater POC with EM-4

POC Concentration in Tofu Wastewater Treated with EM-4 (%)	N-Total (%)	P_2O_5 (%)	K_2O (%)	C-Organic (%)	pH
control	2.26 (s)	2.72 (s)	2.14 (s)	6,55 (r)	6,11 (am)
5	3,57 (t)	4,58 (t)	3,67 (t)	8,68 (s)	6,00 (am)
10	4,21 (t)	5,45 (t)	4,52 (t)	9.36, (s)	6,32 (n)
20	4,82 (t)	5,79 (t)	4,97 (t)	10,24 (t)	6.25 (n)
30	5,79 (.t)	5,88 (t)	5,68 (t)	10,68 (t)	6.38 (n)

Information:
Nutrients (%):
sr = very low
r = Low
s = currently
t = tall
soil pH:
sm = very sour (pH < 4,5)
m = sour (4,5-5,5)
am = a bit sour (5,6–6,5)
n = neutral (6,6-7,5)
ak = slightly alkaline
a = alkaline

Soil Research Institute, Bogor. (2009). Appendix: Criteria for Assessing Soil Chemical Properties and Soil Fertility Status. Bogor: Soil Research Institute.

The total N value in the control group was 2.26%, which is classified as moderate (m). Increasing the concentration of liquid organic fertilizer from 5% to 30% resulted in a gradual increase, reaching 5.79% at a 30% concentration, which falls into the high category (h). The increase in total N content following the application of liquid organic fertilizer indicates that liquid organic fertilizer serves as an effective source of nitrogen. Nitrogen in liquid organic fertilizer is generally present in the form of organic and inorganic compounds that decompose easily in the soil. The activity of microorganisms derived from EM-4 plays a role in accelerating the mineralization of nitrogen into ammonium and nitrate forms available to plants. This phenomenon aligns with the findings of Kolin et al. (2025), who stated that the application of fermentation-based liquid organic fertilizer can increase soil nitrogen content through the stimulation of microbial activity and the acceleration of the nitrogen cycle.

The P_2O_5 content in the control group was 2.72% (moderate) and increased consistently to 5.88% (high) at a 30% concentration. This significant increase in P_2O_5 indicates that liquid organic fertilizer also contributes to an increase in available phosphorus. Phosphorus in acidic soils is often fixed by Al and Fe, making it difficult for plants to absorb. Organic matter from liquid organic fertilizer can produce organic acids that play a role in releasing bound phosphorus through chelation. Rahman et al. (2023) explain that increasing the organic matter content in soil can enhance phosphate solubility and reduce phosphorus fixation in acidic soils. Increases in P_2O_5 at concentrations of 5–30% indicate that higher concentrations contribute more significantly to improving soil phosphorus status.

K_2O also showed a similar increase, from 2.14% (moderate) in the control to 5.68% (high) at the highest concentration. This increase in K_2O indicates that liquid organic fertilizer also serves as an additional source of potassium for the soil. Potassium in liquid organic fertilizer tends to be in a soluble form, making it relatively quickly available after application. Potassium plays a role in regulating osmotic pressure, stomatal opening, and the translocation of photosynthetic products. Sari et al. (2024) state that increased potassium in the soil following the application of liquid organic fertilizer can improve water use efficiency and plant resilience to environmental stress. The 40% concentration showed the highest K_2O content, indicating a positive relationship between liquid organic fertilizer concentration and soil potassium accumulation.

Organic carbon in the control group was 6.55%, which is classified as low (r), and then increased to 10.68% (high) at a concentration of 30%. The increase in organic carbon from the low to the high category indicates that POC functions as an effective source of organic matter. The addition of organic matter increases cation exchange capacity, improves soil structure, and enhances soil microorganism activity. Widodo et al. (2022) explain that an increase in soil organic carbon content contributes to improved aggregate stability and water retention, which ultimately supports increased nutrient availability. The consistent rise in organic carbon with increasing concentration indicates that the application of liquid organic fertilizer plays a role in improving soil quality both chemically and biologically.

The soil pH in the control group was 6.11, which is classified as slightly acidic (am). The application of POC increased the pH to 6.32–6.38, which falls into the neutral category (n), particularly at concentrations of 10–30%. The shift in pH from slightly acidic to neutral indicates a buffering effect from the organic matter. Humic and fulvic compounds resulting from decomposition are capable of neutralizing excess H^+ ions in the soil. Rahman et al. (2023) state that repeated application of organic matter can increase soil buffering capacity and stabilize soil pH within a range approaching neutral. The neutral pH condition at a concentration of 10–30% indicates that liquid organic fertilizer not only adds nutrients but also improves the soil's chemical environment to make it more conducive to plant growth.

The overall increase in nutrient content in the final growing medium indicates that the applied POC serves as a source of nutrients for the soil. This finding provides an important basis for further investigation into how these improvements in soil chemical properties affect the growth and yield of pak choi.

The Effect of Liquid Organic Fertilizer Made from Tofu Waste Treated with EM-4 on the Growth of Pak Choi (Brassica rapa L. Chinensis)

The growth response of pak choi plants to the application of EM-4-based liquid organic fertilizer derived from tofu wastewater was analyzed using several vegetative growth parameters, namely plant height, number of leaves, canopy width, and total chlorophyll. These parameters were selected because they represent the plant's ability to utilize nutrients to support biomass growth during the vegetative phase. Analysis of variance (ANOVA) was conducted to determine the effect of the treatment on each parameter. The results of the ANOVA test on the effect of liquid organic fertilizer application on growth parameters are presented in Table 4 below.

Table 4. ANOVA Test of the Effect of Liquid Organic Fertilizer Derived from Tofu Wastewater on Growth Parameters

Parameter	F-value	KV %	Sig. (p-value)	Interpretation
Plant Height (cm)	16.56	16	0.0001	Very real
Number of Leaves (pieces)	17.32	11	0.0001	Very real
Crown Width (cm)	18.84	13	0.0001	Very real

The calculated F-value, which is greater than the table F-value, and the significance level ($p < 0.01$) in Table 4 indicate that the application of liquid organic fertilizer has a highly significant effect on plant height, number of leaves, and canopy width. The relatively low coefficient of variation (CV) indicates that the data exhibit an acceptable level of variability for agronomic research. These results indicate that the POC concentration treatments play a significant role in influencing the vegetative growth of pak choi. The mean plant height, number of leaves, and canopy width for the various treatment concentrations are presented in Table 5 below.

Table 5. Average Plant Height, Number of Leaves, and Canopy Width of Pak Choi at Various Treatment Concentrations

Concentration of Tofu Wastewater Treated with EM-4 (%)	Plant Height (cm)	Number of Leaves (pieces)	Crown Width (cm)
Control	8.67c	9,23c	13.45d
5	10.56b	10,45cb	18.74c
10	12.48b	11.78ab	19.67c
20	15.36a	13.31a	22.24b
30	18.86a	13.46a	26.52a

Note: Different letters in the same column indicate a statistically significant difference at the 5% level.

The table shows an increase in plant height, number of leaves, and canopy width as the concentration of EM-4-based liquid organic fertilizer derived from tofu wastewater increased. Plant height in the control treatment was 8.67 cm and differed significantly from all liquid organic fertilizer treatments. Application of 5% liquid organic fertilizer increased plant height to 10.56 cm, while a 10% concentration resulted in a height of 12.48 cm. A greater increase was observed at 20% and 30% concentrations, at 15.36 cm and 18.86 cm, respectively. The significant increase in plant height at the 20% and 30% concentrations indicates that the availability of nutrients from the liquid organic fertilizer was in optimal condition to support stem elongation. The nitrogen contained in the liquid organic fertilizer plays a crucial role in protein synthesis and cell division in the apical meristem tissue. Sufficient nitrogen availability accelerates growth activity, causing the stems to elongate more rapidly compared to untreated plants. Kolin et al. (2025) explain that increased available nitrogen in the soil is directly associated with an increase in the vegetative growth rate of leafy plants. Rahman et al. (2023) also state that adequate nitrogen supply enhances new tissue formation through increased enzymatic activity. Sari et al. (2024) add that a high plant response to liquid organic fertilizer typically exhibits a positive concentration-response pattern up to a physiological optimum.

The number of leaves showed a relatively consistent pattern of increase. The control produced 9.23 leaves, which then increased to 10.45 leaves at 5%, 11.78 leaves at 10%, 13.31 leaves at 20%, and 13.46 leaves at 30%. The 20% and 30% treatments did not differ significantly from each other, but both differed significantly from the control. The increase in the number of leaves at concentrations of 10–30% indicates that the plants were able to utilize nutrients to form photosynthetic organs more intensively. A greater number of leaves increases the photosynthetic surface area, thereby increasing assimilate production. Nitrogen and phosphorus play a crucial role in leaf primordia formation and cell division at the growing point. Kolin et al. (2025) state that an increase in soil nitrogen significantly enhances the formation of new leaves in leafy vegetable crops. Rahman et al. (2023) emphasize that the balance of nitrogen and phosphorus strongly determines the rate of vegetative tissue formation. Sari et al. (2024) report that horticultural crops receiving liquid organic fertilizer show an increase in leaf number that correlates with an increase in soil nitrogen content.

Canopy width showed the most significant increase compared to other parameters. The value in the control group, which was 13.45 cm, increased to 18.74 cm (5%), 19.67 cm (10%), 22.24 cm (20%), and reached 26.52 cm at a 30% concentration. The consistent increase in canopy width up to a 30% concentration indicates that the combination of nitrogen and potassium in liquid organic fertilizer supports leaf tissue expansion and cell enlargement. Potassium plays a role in regulating turgor pressure and cellular water balance, which are crucial for cell enlargement. A wider canopy indicates an increase in photosynthetic area and greater potential for biomass production. Kolin et al. (2025) state that an optimal combination of nitrogen and potassium results in a significant increase in plant canopy area. Rahman et al. (2023) explain that potassium improves water use efficiency and supports leaf cell expansion. Sari et al. (2024) also confirm that increased canopy width is a strong indicator of the success of liquid organic fertilization in supporting vegetative growth.

Total Chlorophyll

Measurements of total chlorophyll following the application of liquid organic fertilizer derived from tofu wastewater with the addition of EM-4 showed no significant effect on the applied concentration; the average total chlorophyll measurements are presented in Figure 1.

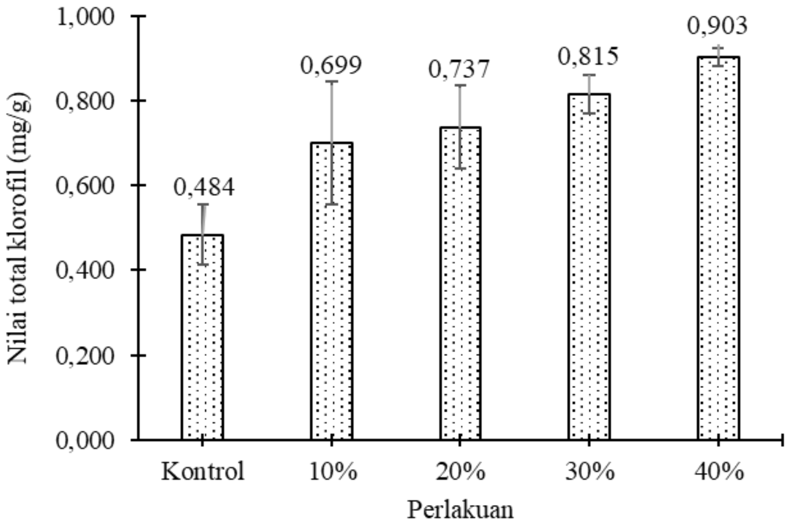

Figure 1. Graph of Total Chlorophyll Measurements Based on Average Values

The results of treating with varying concentrations of liquid organic fertilizer derived from tofu wastewater supplemented with EM-4 for total chlorophyll (Figure 1) showed no significant effect in the analysis of variance (ANOVA) conducted; however, total chlorophyll values increased gradually with increasing concentrations of the liquid organic fertilizer derived from tofu wastewater supplemented with EM-4. The chlorophyll value in the control was 0.484 mg/g, then increased at concentrations of 5% (0.699 mg/g), 10% (0.737 mg/g), 20% (0.815 mg/g), and reached a maximum of 0.903 mg/g at 30%. Descriptively, there was a trend of increasing chlorophyll content in line with increasing treatment concentrations.

ANOVA results showed that this increase was not statistically significant; this may have occurred because the variation between replicates was relatively large, so the differences between treatments did not exceed the threshold for statistical significance. Biological variability in plants is often influenced by genetic factors, microclimatic conditions, and the heterogeneity of the growing medium, leading to physiological responses that are not entirely uniform (Rahman et al., 2023). Sari et al. (2024) explain that chlorophyll content is highly sensitive to fluctuations in light intensity and nitrogen availability; thus, even though an increase occurred, the differences between treatments may not necessarily be statistically significant.

Chlorophyll content still increased due to the liquid organic fertilizer derived from tofu wastewater with EM-4, as the nitrogen content serves as the primary component in chlorophyll molecule synthesis. Nitrogen enhances chlorophyll synthesis by activating chlorophyll-forming enzymes within leaf tissue (Kolin et al., 2025). Rahman et al. (2023) stated that the gradual supply of organic nitrogen is capable of increasing chlorophyll concentration, although it does not always show a clear difference at a specific test level. This condition indicates that the plant’s physiological response still occurs, but it is not yet statistically strong enough to be categorized as significant.

Response of Pak Choi (Brassica rapa L. Chinensis) Yield to the Application of Liquid Organic Fertilizer from Tofu Waste with the Addition of EM-4

The effect of applying different concentrations of liquid organic fertilizer derived from tofu wastewater with the addition of EM-4 to Pakcoy (Brassica rapa L. Chinensis) plants on crop yield, consisting of fresh weight and dry weight, as determined by analysis of variance (ANOVA), showed no significant effect for any of the concentrations applied. Figures 3 and 4 are graphs based on the average values of fresh weight and dry weight according to the concentrations applied.

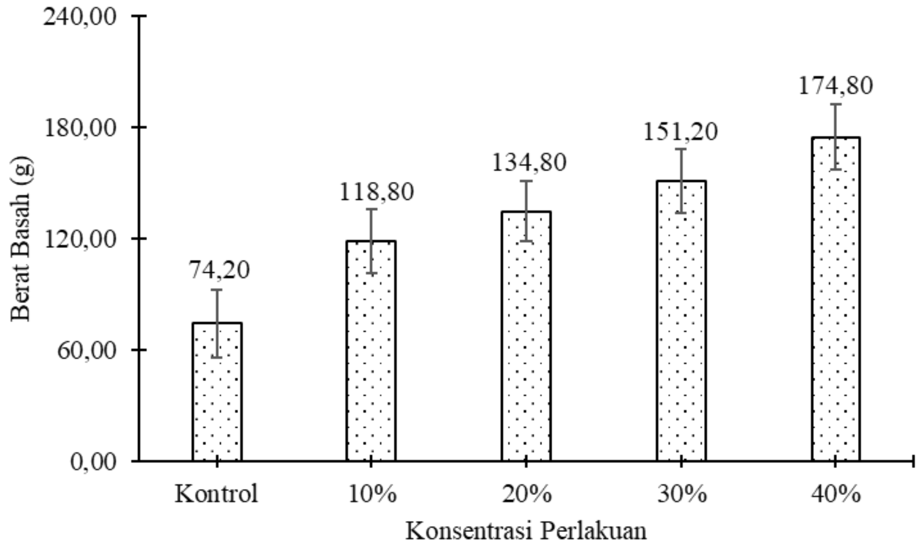

Figure 2. Graph of Fresh Weight Measurements of Plants Across Treatments

The fresh weight of plants in Figure 2 shows a consistent increasing trend from the control to a 30% concentration. The average fresh weight in the control was 74.20 g, increasing to 118.80 g at 5%, 134.80 g at 10%, 151.20 g at 20%, and reaching a peak of 174.80 g at 30%. Although there was a fairly noticeable increase in the mean values, the results of the statistical analysis showed no significant effect. This may be due to high variability among samples within a single treatment, resulting in a large standard deviation and reducing the statistical power of the test (Sari et al., 2024). Rahman et al. (2023) explained that fresh weight is highly influenced by the water content of plant tissues; thus, fluctuations in water content among plants can cause numerical differences without yielding statistical significance. An increase in fresh weight still occurred because the application of liquid organic fertilizer increased the availability of macronutrients such as nitrogen, phosphorus, and potassium, which support vegetative tissue formation and enhance photosynthetic capacity (Kolin et al., 2025). Nitrogen accelerates leaf formation, phosphorus supports energy metabolism, while potassium plays a role in regulating cell turgor pressure. The combination of these elements increases fresh biomass accumulation, although biological variation results in no statistically significant differences in yield (Rahman et al., 2023).

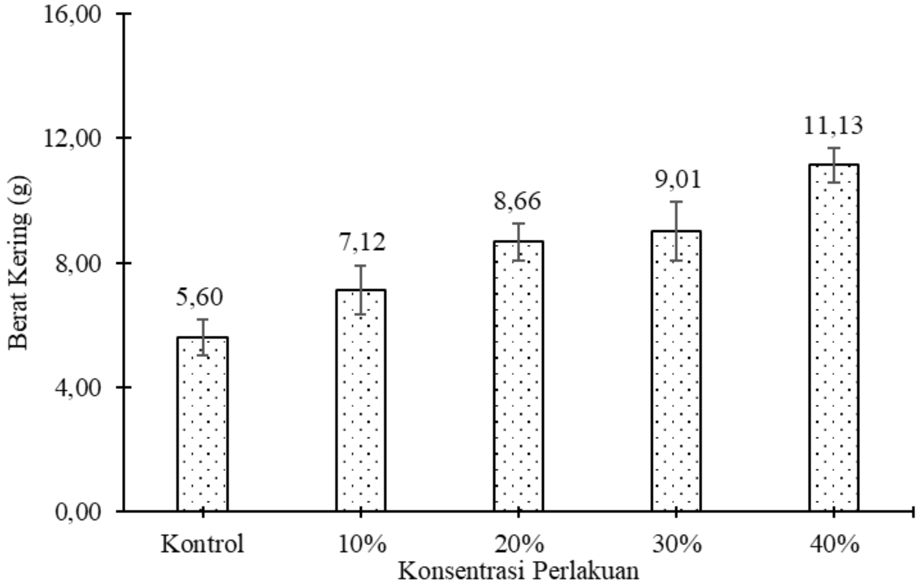

Figure 3. Graph of Dry Weight Measurements of Plants Across Treatments

Plant dry weight also showed a relatively consistent increasing trend (Figure 5). The dry weight value in the control was 5.60 g, increasing to 5% (7.12 g), 10% (8.66 g), 20% (9.01 g), and peaking at 30% at 11.13 g. However, the results of the analysis of variance indicated that these differences were not statistically significant. Dry weight serves as an indicator of the accumulation of photosynthetic products that have undergone metabolic and respiratory processes. Variations in photosynthetic efficiency, respiration, and the distribution of assimilates among plants can result in differences in average values that are not large enough to reach the level of statistical significance (Sari et al., 2024). Rahman et al. (2023) noted that in several studies on liquid organic fertilizers, increases in dry weight often show a positive trend without significant differences because the physiological response of plants occurs gradually. The increase in dry weight still occurs because the available nutrients from the liquid organic fertilizer enhance photosynthetic activity and the formation of structural organic compounds such as cellulose and lignin (Kolin et al., 2025). EM-4 also contains microorganisms that aid in the mineralization of organic matter, making nutrients more readily available to plants. This condition enhances biomass accumulation, although statistically, no significant differences have yet been observed between treatments (Rahman et al., 2023).

CONCLUSIONS AND RECOMMENDATIONS

This study shows that liquid organic fertilizer produced by fermenting tofu wastewater with the addition of EM-4 has chemical properties that meet the quality standards for liquid organic fertilizer, with total N, P_2O_5, K_2O, and organic C content within acceptable application ranges, as well as a relatively stable pH ranging from slightly acidic to near-neutral. The application of this liquid organic fertilizer was proven to increase nutrient content and improve the chemical properties of the growing medium, as evidenced by increases in total N, P_2O_5, K_2O, and organic C, as well as a shift in soil pH toward neutral at higher concentrations. The application of liquid organic fertilizer had a very significant effect on the vegetative growth parameters of pakcoy (Brassica rapa L. Chinensis), namely plant height, number of leaves, and canopy width, with the best response generally observed at a 30% concentration. Meanwhile, physiological parameters (total chlorophyll) and yield parameters (fresh weight and dry weight) showed an increasing trend as fertilizer concentration increased, although statistically, no significant differences were observed. A 30% concentration is recommended as the most effective for supporting sustainable pakcoy cultivation under the agroecosystem conditions of Timor-Leste.

A recommendation for future research is the need for further study on optimizing the application frequency of EM-4-based liquid organic fertilizer derived from tofu wastewater, given that the application interval has the potential to influence nutrient availability dynamics and nutrient uptake efficiency by plants. An evaluation of the combination of liquid organic fertilizer with solid organic fertilizer or low-concentration inorganic fertilizer is also important to assess the possibility of synergistic effects in enhancing plant growth and yield. Such a combinative approach has the potential to yield a more balanced, efficient, and sustainable fertilization system, while simultaneously reducing reliance on inorganic fertilizers entirely. A comprehensive study covering agronomic aspects, soil chemistry, and nutrient use efficiency will provide a stronger scientific basis for the development of integrated fertilization strategies in pak choi cultivation in Timor-Leste.

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