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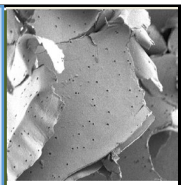

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Liquid Tempeh Wastewater and EM4: A Combined Approach to Enhance Chilli Seed Germination

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ABSTRACT

Tempeh liquid wastewater, a by-product of tempeh production, is increasingly recognized as a potential liquid fertilizer. However, research on its fermentation into fertilizer is limited. This study aimed to enhance tempeh wastewater-derived fertilizer using effective microorganisms 4 (EM4) and evaluate its impact on chili seed germination. Two sets of organic fertilizers were prepared, including three controls and three treatments. Controls were 100% distilled water, 10% EM4 with 90% distilled water, and 100% tempeh wastewater, while treatments varied in tempeh wastewater and EM4 concentrations. Chemical analysis revealed variations in total carbon, salinity, phosphorus, and potassium, with undetectable nitrogen content in all formulations. Formulations with 25% tempeh wastewater had lower salinity, phosphorus, and potassium than those with 75% tempeh wastewater. The effects on chili seed germination showed that formulation A3 (25% tempeh wastewater and 10% EM4) resulted in significantly higher germination percentage, mean daily germination, seedling length, and vigor index compared to controls and higher tempeh wastewater formulations. Overall, 25% tempeh wastewater formulations demonstrated superior germination and growth, suggesting tempeh liquid fertilizer as an eco-friendly and effective option for chili cultivation. Further research is needed to optimize its composition and application in agriculture.

INTRODUCTION

In recent years, Malaysia has embraced the versatility of tempeh, a traditional Indonesian soy-based delicacy. Originally popular in vegetarian and vegan diets, tempeh has found its wat into diverse culinary landscapes, especially as meat alternative in Malaysian cuisine [1]. Crafted through the fermentation of soybeans with *Rhizopus oligosporus* mold, tempeh provides a firm, nutty texture, and has gained popularity beyond its Indonesian roots [2]. It is now widely available in various forms, including fresh, frozen, or dried, particularly regions with significant Malay populations [2].

Tempeh production generates a by-product known as liquid tempeh wastewater, rich in soluble compounds, nutrients, and organic matter. This liquid, often discarded, has potential applications as an eco-friendly organic fertilizer due to its nutrient content and microbial activity [1, 3]. Despite its conventional classification as waste, liquid tempeh wastewater can be repurposed, offering an environmentally sustainable alternative to synthetic fertilizer [3].

Previous studies have demonstrated the efficacy of utilizing organic waste as a liquid fertilizer, highlighting its potential to enhance plant growth and soil health. For instance, the application of food waste fermentation liquids as external carbon sources has been shown to significantly enhance biological nitrogen and phosphorus removal in wastewater treatment processes [4]. Similarly, the use of thermal hydrolyzed food waste liquor has been explored as a liquid organic fertilizer, offering a viable alternative to synthetic fertilizers [5]. Furthermore, fermented livestock liquid has been successfully applied as a liquid fertilizer in crop production, underscoring the broader potential of organic waste conversion in agriculture [6].

Effective Microorganisms 4 [EM4] emerge is a microbial inoculant comprising a diverse consortium of beneficial microorganisms, including yeast, phototrophic bacteria, and lactic acid bacteria [7]. Its applications in environmental and agricultural contexts have garnered attention and amplified microbial diversity and activity when introduced into soil or organic substrates [7, 8]. Renowned for its ability to suppress harmful bacteria, mitigate organic waste odours, and accelerate organic material decomposition, EM4 stands as a pivotal agent in various agricultural endeavours, promoting eco-friendly and sustainable waste management and agricultural practices [7, 9].

Local chilli production in Malaysia declined significantly over the years, resulting in an ability to meet market demands. In 2020, Malaysia produced 28,264 tonnes of red chili, a decrease of 54.75% compared to 2016, leading to increased importation from neighboring countries like China, India, and Thailand, which rose from 49,069 tonnes in 2016 to 66,295 tonnes in 2020 [10]. Globally, chili production holds paramount economic and nutritional niche, integral to various cuisines worldwide and serving as a primary income source for many farmers [11]. Adopting efficient farming techniques, including soil health preservation, pest control, and water resource optimization, becomes essential for maximizing chili output [12]. Within this framework, organic liquid fertilizers emerge as critical allies, bolstering chili crop yield and quality to meet the demands of global markets [13].

However, the evidence underscores the variability in organic matter composition within industrial effluents, with improper management potentially leading to ecosystemic harm [14]. The unchecked release of organic chemicals into aquatic environments poses direct and indirect threats to human health, underlining the necessity for responsible waste management practices [14, 15]. Liquid waste from the tempeh industry often meets a similar fate, being disposed of due to a perceived lack of commercial value [16].

Moreover, tempeh liquid waste harbours essential nutrients like nitrogen [N], phosphorus [P], and potassium [K], pivotal for plant growth and soil microbial proliferation. At appropriate concentrations, industrial liquid tempeh waste holds promise as a safe and eco-friendly organic liquid fertilizer [17]. Yet, widespread reliance on synthetic fertilizers, despite their deleterious environmental consequences such as soil degradation and diminished fertility, persists due to limited public awareness regarding the potential of organic waste conversion [18].

Despite the burgeoning interest in liquid fertilizer production from tempeh wastewater, research on optimizing the fermentation stage remains scarce. Liquid fertilizers derived from industrial wastewater, including tempeh, are gaining traction for their eco-friendliness, cost-effectiveness, and nutrient-rich composition [17, 18]. Hence, this study endeavours to refine liquid fertilizer derived from tempeh wastewater fermentation by incorporating EM4 and evaluate its impact on chili seed germination, offering insights into sustainable agricultural practices in Malaysia.

MATERIALS AND METHODS

Tempeh liquid fertilizer was prepared using wastewater collected from a small-medium enterprise (SME) engaged in tempeh production in Kuantan, Pahang, Malaysia. The samples were obtained during the third phase of tempeh production, precisely after the fermentation and pressing processes. Effective microorganisms 4 (EM4) were utilized as an inoculant, sourced from a local supplier. Chili seeds were purchased from an online store located in Petaling Jaya, Selangor**.**

Preparation of Organic Liquid Fertilizer

The method for preparing tempeh liquid fertilizer was adapted from Hartini et al. [3] Tempeh wastewater was subjected to filtration. Subsequently, 300 mL of liquid waste was boiled for 15 to 20 minutes. The heated liquid waste was then divided into three glass beakers. EM4 microbial inoculant was added to two beakers at concentrations of 5% and 10% (v/v) each. The remaining beaker served as the control without EM4. Each beaker was sealed with aluminium foil and kept at room temperature for a week. Successful production of the liquid fertilizer was indicated by a sweet smell upon opening. The resultant liquid fertilizers were then diluted with distilled water to obtain different concentrations as outlined in **Table 1**.

Table 1. Formulations of tempeh wastewater (25% and 75%) and EM4 (5% and 10%).

Formulation	Tempeh Wastewater (%)	EM4 (%)	Distilled Water (%)
C1*	0	0	100
C2*	0	10	90
C3*	100	0	0
A1		0	75
A2	25	5	70
A3		10	65
B1		0	25
B2	75	5	20
B3		10	15

*C1: control 1, C2: control 2, C3: control 3.

Analysis of tempeh liquid fertilizer

The analysis of tempeh liquid fertilizer was carried out. Parameters including total of carbon (TOC), salinity, nitrogen, phosphorus and potassium were analyzed using in-house method based on APHA 5310, conductivity meter, CHNS analyzer, and APHA 3120 for phosphorus and potassium analysis, respectively.

Analysis of chilli seeds germination

Chili seeds were germinated under controlled conditions for up to 14 days. Germination parameters including final germination percentage (FGP), mean germination time (MGT), mean daily germination (MDG), seedling length, and vigor index I and vigor index II. All analyses were conducted in triplicates.

Final Germination Percentage (FGP)

The FGP estimates the proportion of seeds that have successfully germinated into seedlings, providing crucial information on the viability of the seed population [19]. A higher FGP value indicates a greater level of germination within the seed population. FGP was calculated using the following formula:

$$\text{FGP (\%)} = \frac{\text{number of normally sprouted seeds on the } 14^{th} \text{ day}}{\text{number of seeds sown}} \times 100 \quad \text{(Eqn. 1)}$$

Mean Germination Time (MGT)

MGT was calculated based on the day when the majority of seeds germinated, indicating the average time required for seeds to develop into seedlings [20]. MGT was calculated using the following formula:

$$\text{MGT (day)} = \frac{\sum_{i=1}^{l} n_i t_i}{\sum_{i=1}^{l} n_i} \quad \text{(Eqn. 2)}$$

Where n_i = number of normally sprouted seeds on the ith day; $n_i t_i$ = number of normally sprouted seeds during the ith period of time.

Mean Daily Germination (MDG)

MDG was calculated as the ratio of final cumulative germination (%) to the total number of intervals for final germination [21]. MDG was calculated using the following formula:

$$\text{MDG} = \frac{\text{final cumulative germination (\%)}}{\text{total number of intervals for final germination}} \quad \text{(Eqn. 3)}$$

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Seedling Length

After 14 days of seedlings, seedlings were carefully removed from the petri dish, and both root and shoot lengths were measured using a standard scale.

Vigor Index (I) and Vigor Index (II)

Vigor Index (I) and Vigor Index (II) were calculated using the following formula:

$$\text{Vigor index (I)} = \frac{\text{FGP x Seedling length (cm)}}{100} \quad \text{(Eqn. 4)}$$

$$\text{Vigor index (II)} = \frac{\text{FGP x Seedling dry weight (g)}}{100} \quad \text{(Eqn. 5)}$$

Statistical Analysis

Statistical analyses were performed using SPSS Statistics 27 software. The effect of tempeh liquid fertilizer formulation was assessed using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Values of $p<0.05$ were considered statistically significant.

RESULTS

Chemical properties of tempeh liquid fertilizer

The total of carbon (TOC), salinity, nitrogen, phosphorus, potassium, and pH of the formulated tempeh liquid fertilizer are presented in **Table 2**. Notably, formulation A1 exhibited the highest TOC concentration among all formulations. Additionally, formulations B1, B2, and B3 showed higher levels of salinity, phosphorus, and potassium, respectively. Nitrogen content was undetectable in all formulations. The pH values for all formulations ranged from 6.7 to 7.32. Overall, the salinity, phosphorus, and potassium content in formulations containing 25% tempeh wastewater (A1, A2, and A3) ranged from 1.4 psu to 1.61 psu, 0.65 mg/L to 2.72 mg/L, and 215.3 ppm to 253.5 ppm, respectively. In contrast, formulations containing 75% tempeh wastewater (B1, B2, and B3) exhibited higher levels of salinity, phosphorus, and potassium ranging from 4.24 psu to 4.59 psu, 3.2 mg/L to 4.9 mg/L, and 627.1 ppm to 664.4 ppm, respectively (**Table 2**).

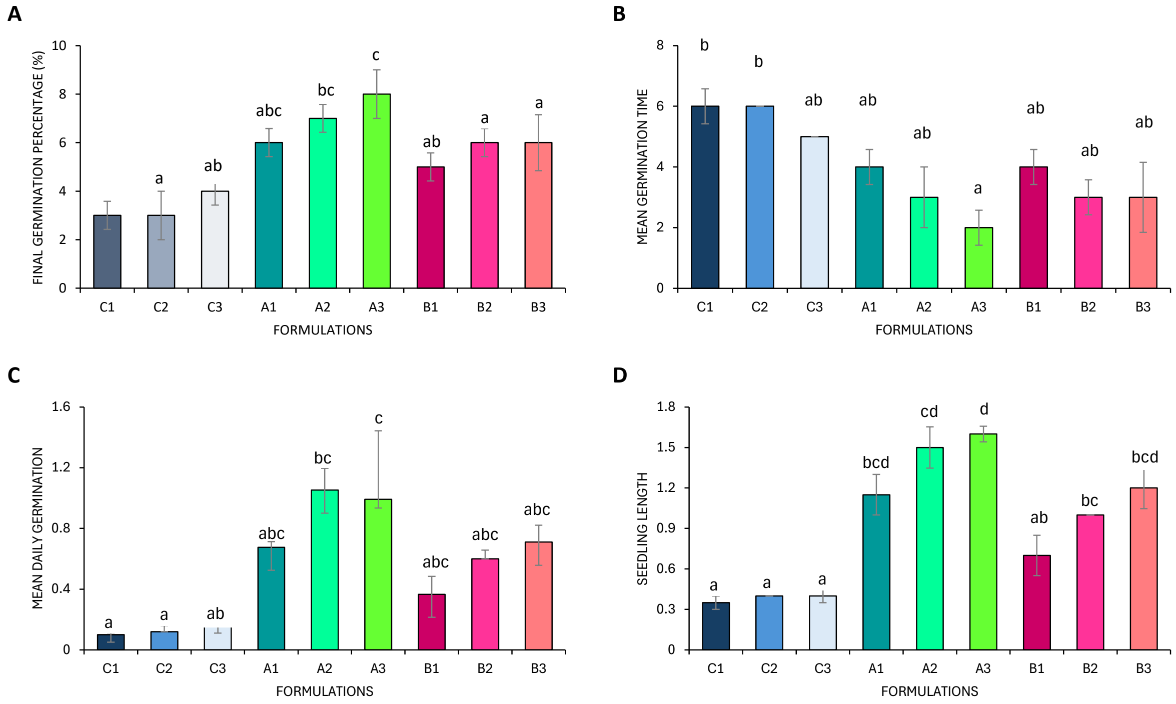

Fig. 1. Effects of different formulations of tempeh liquid fertilizer and EM4 in chilli seeds germination and seedling growth.

Table 2. Chemical properties of tempeh liquid fertilizer formulations.

Formulation	TOC (mg/L)	Salinity (psu)	Nitrogen (mg/L)	Phosphorus (mg/L)	Potassium (ppm)	pH
A1	2,030	1.4		0.65	253.5	6.7
A2	305	1.61		2.72	215.3	6.57
A3	210	1.56	*Not*	1.3	237.1	6.61
B1	566	4.33	*detected*	3.2	664.4	7.32
B2	565	4.24		4.78	627.1	6.97
B3	399	4.59		4.9	649	6.88

*TOC; total of carbon. Formulation A1, A1, and A3 contained 25% tempeh wastewater. Formulation B1, B2, and B3 contained 75% tempeh wastewater.

Effects of different formulations of tempeh liquid fertilizer on chilli seeds germination and seedling growth

The effects of tempeh liquid fertilizer on chili seed germination and seedling growth are illustrated in **Fig. 1**. The final germination percentage (FGP) shown in **Fig. 1(a)** was significantly higher in formulation A3 (25% tempeh wastewater and 10% EM4) compared to all control groups and formulation B1, B2, and B3. Conversely, the mean germination time (MGT) demonstrated in **Fig. 1(b)** was significantly lower in formulation A3 compared to all control groups (C1 and C2). Additionally, mean daily germination (MDG) and seedling length (SL) shown in **Fig. 1(c)** and **Fig. 1(d)** were significantly higher in formulation A3 compared to all control groups.

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Vigor index I demonstrated that formulations A2 and A3 were significantly higher compared to control groups (C1 and C2). Similarly, vigor index II was significantly higher in formulation A3 compared to the control group (C1) (**Fig. 2 (a)** and **(b)**). Overall, formulations with 25% tempeh wastewater demonstrated higher FGP, MDG, SL, vigor index I, and vigor index II, and lower MGT compared to formulations containing 75% tempeh wastewater.

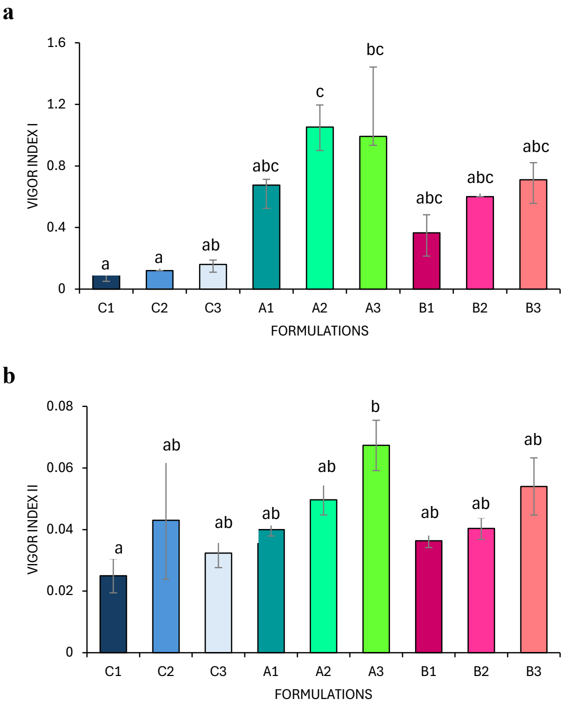

Fig. 2. Effects of different formulations of tempeh liquid fertilizer and EM4 on (a) vigor index I and (b) vigor index II.

DISCUSSION

The utilization of tempeh liquid waste as an organic liquid fertilizer, augmented with Effective Microorganisms 4 [EM4], represents a promising approach towards sustainable agriculture. Organic liquid fertilizers, such as those derived from tempeh wastewater, offer significant potential in enhancing crop yield by providing essential nutrients in an easily absorbable form [22]. The inclusion of EM4 in the fertilizer formulation accelerates the fermentation process, leading to improved nutrient availability and microbial activity, which are critical for plant growth [23].

The chemical analysis of the tempeh liquid fertilizer formulations revealed variation in nutrient content, particularly in total organic carbon [TOC], salinity, phosphorus, and potassium levels. These variations emphasize the importance of optimizing nutrient balance through careful formulation design, as these parameters play pivotal roles in determining the fertilizer's efficacy for plant growth and development [13, 22]. High saline content, particularly from NaCl, can inhibit the absorption of macronutrients and micronutrients, such as nitrogen, potassium, calcium, and iron, by plant roots [24]. Formulations containing 25% tempeh wastewater exhibited lower salinity levels but higher concentrations of phosphorus and potassium compared to those with 75% tempeh wastewater. Lower salinity levels are beneficial, as excessive salinity can impede nutrient absorption. This highlights the need to manage salinity in organic fertilizers to create a more favorable environment for seed germination and plant growth.

The neutral pH range observed in all formulations (6.7 to 7.32) is conducive to plant growth [3,25], suggesting that the tempeh liquid fertilizer could be compatible with various plant species. Although variations in carbon content were observed with different EM4 concentrations, it is important to consider their implications for nutrient availability and soil health [3,8]. The higher TOC levels observed in formulation A1 may enhance microbial activity, supporting the decomposition of organic matter and the release of nutrients such as nitrogen, phosphorus, and potassium, which are essential for seed germination [26]. However, the undetectable nitrogen content across all formulations suggests that further optimization is required to improve nitrogen availability in the fertilizer, potentially through the integration of nitrogen-rich organic materials or additional microbial inoculants [4].

Regarding chili seed germination and seedling growth, the efficacy of EM4 augmentation in tempeh liquid fertilizer formulations was evident. Formulation A3, comprising 25% tempeh wastewater and 10% EM4, demonstrated superior FGP and MDG rates, indicating accelerated seedling emergence compared to control groups and higher wastewater concentration formulations. These results align with previous studies showing the positive effects of nutrient-rich organic fertilizers on seedling vigor and early growth dynamics [27, 28]. The combination of nutrient-rich wastewater and beneficial microorganisms introduced by EM4 likely contributed to the enhanced seedling vigor observed in this study [3, 8, 29]. These findings emphasize the role of EM4 in promoting seedling vigor and early growth. The nutrient-rich composition of the fertilizer, particularly its phosphorus and potassium content, likely played a key role in enhancing seedling vigor, as these nutrients are crucial for promoting root development and regulating water uptake in plants [5].

The incorporation of EM4 into the tempeh liquid fertilizer provided essential nutrients and potentially introduced beneficial microorganisms. These microorganisms may have contributed to establishing a favorable rhizospheric environment for seed germination and seedling growth [8]. While the exact microbial interaction were not measured, EM4 likely stimulated microbial activity, promoting nutrient cycling and soil health [30]. Additionally, EM4 might have alleviated salinity stress associated with liquid waste from fermentation, thereby enhancing seedling vigor and resilience. The potential mechanisms highlight the multiple benefits of incorporating EM4 into tempeh liquid fertilizer formulations, not only for nutrient provision but also for soil health and stress tolerance [8, 16].

MGT provides valuable insights into the kinetics of seed germination and seedling establishment. The observed shorter mean germination time [MGT] in treatments enriched with wastewater and EM4 suggests faster seedling emergence, which is critical for early seedling vigor and successful crop establishment [31]. Treatments containing these components exhibited significantly shorter MGTs compared to control groups, indicating accelerated germination and vigor (**Fig. 1 [b], 1 [c]**, and **Fig. 2**). These findings emphasize the role of fertilizer composition, particularly the combination of tempeh wastewater and microbial inoculants like EM4, in modulating seed germination kinetics and early seedling development [32].

The MDG rate and seedling length provide critical insights into dynamics of seedling growth and early vigor. The differences observed between treatment groups emphasize the influence of fertilizer composition on seedling development [27]. Variations in seedling growth may have been influenced by

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challenges encountered in the laboratory setup, such as nutrient availability and environmental conditions. Future studies should explore optimized conditions for seedling growth to maximize the effectiveness of tempeh liquid fertilizer. Vigor indices offer comprehensive metrics for assessing seed vigor and early seedling growth. Formulations incorporating 25% tempeh wastewater and EM4 showed significant increases in seedling length and vigor indices, indicating the potential of nutrient-rich organic fertilizers to enhance seedling performance. These enhancements are likely due to improved nutrient availability and microbial inoculant activity, which contribute to overall crop productivity. The observed differences across formulations underscore the role of organic fertilizers in promoting early seedling establishment and growth potential [33].

While the laboratory setup provided valuable data, variations in seedling growth may have been influenced by external factors such as nutrient availability and environmental conditions. Challenges such as inconsistent nutrient absorption and varying microbial activity in controlled environments can affect results. Future research should focus on optimizing growth conditions and fertilizer composition to maximize the effectiveness of tempeh liquid fertilizer. Vigor indices, which serve as comprehensive metrics for assessing seed vigor and early seedling growth, consistently demonstrated superior results in treatments containing both tempeh wastewater and EM4, further confirming the beneficial effects of these formulations [29].

Moreover, these findings highlight the importance of continued refinement of organic fertilizers to ensure their environmental and agricultural efficacy. Future research should explore novel approaches to enhance nutrient availability and microbial activity within the fertilizer matrix. The integration of biological and chemical methods to remove potential pollutants and pathogens from tempeh wastewater before its use as a fertilizer may also improve its overall effectiveness. Long-term field trials under diverse agroecological conditions are necessary to assess the real-world performance of tempeh liquid fertilizer across different cropping systems.

In addition to its agronomic benefits, repurposing tempeh liquid waste into organic fertilizers contributes to broader sustainability goals by promoting circular economy principles and reducing waste generation. This approach valorizes tempeh production by-products and mitigates environmental pollution associated with liquid waste disposal [34]. Furthermore, adopting organic liquid fertilizers reduces the dependency on synthetic inputs, thereby lowering agriculture’s environmental footprint while promoting soil health and biodiversity [35].

CONCLUSION

This study explored the potential of utilizing tempeh wastewater for producing eco-friendly and affordable organic liquid fertilizer. The results demonstrate that tempeh wastewater, particularly when combined with microbial inoculants like EM4, holds promise as a sustainable fertilizer option. Among the formulations tested, those with 25% tempeh wastewater showed the most favorable outcomes, with significantly higher final germination percentage (FGP), mean daily germination (MDG), seedling length, vigor indices I and II, and lower mean germination time (MGT) compared to both the control and higher concentration formulations. These findings emphasize the importance of microbial inoculants and nutrient composition in promoting robust seedling establishment and early crop productivity.

However, chemical analysis revealed that the formulations did not fully meet the desired standards for total carbon, salinity, nitrogen, and potassium (3, 16). Only phosphorus and pH levels met the necessary criteria for optimal plant growth, pointing to the need for further refinement in the formulation process. To maximize the effectiveness of tempeh liquid fertilizer, proper treatment methods for wastewater, such as biological and chemical processes to remove pollutants and pathogens, should be implemented.

Future research should focus on conducting long-term field trials, comparing tempeh-based fertilizers with commercial alternatives, and exploring alternative germination environments. Collaboration with experts in soil science and agronomy will also be essential to optimize nutrient analysis and refine fertilizer formulations. Ultimately, repurposing tempeh wastewater as a fertilizer supports sustainability efforts by reducing waste, minimizing environmental impact, and promoting a circular economy within agricultural systems.

AUTHORS CONTRIBUTIONS

Khairiah Mohd Mokhtar: contributed to analysis of data and drafting the manuscript; Wan Norfazilah Wan Ismail: planned and conducted the study and drafting the manuscript; Nurul Huda Abu Bakar: Provided samples and drafting the manuscript; Nor Adila Mhd Omar: planned the study design, supervised data analysis and interpretation, and manuscript preparation.

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DATA AVAILABILITY STATEMENT

Data available on request.

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