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Microbial consortium formulation in liquid organic fertilizer for managing bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*), rice blast (*Pyricularia oryzae*), and enhancing rice productivity

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Abstract. *Nurcahyanti SD, Masnilah R, Budiman SA, Tanzil AI, Nurdika AAH. 2024. Microbial consortium formulation in liquid organic fertilizer for managing bacterial leaf blight (*Xanthomonas oryzae *pv.* oryzae*), rice blast (*Pyricularia oryzae*), and enhancing rice productivity. Biodiversitas 25: 2208-2220.* The productivity of rice is significantly influenced by soil fertility and plant diseases as limiting factors. The overreliance on synthetic fertilizers and pesticides in rice cultivation has led to declining land quality. We propose the utilization of Liquid Organic Fertilizers (LOF) enriched with beneficial microbes as a sustainable solution to maintain soil fertility and protect plants from pathogens. Our research specifically focuses on the formulation of LOF enriched with the microbial consortium to enhance rice productivity and suppress bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*), and rice blast (*Pyricularia oryzae*) diseases. The three LOF treatments were formulated from vegetable and fruit waste (FV), Rice Straw (RS), and Cattle Manure (CM) with a microbial consortium consisting of *Pseudomonas putida* Pf10, *Bacillus subtilis* JB12, and *Trichoderma* sp. The results showed that the LOF from the three materials is suitable for the growth of the microbial consortium with a population of 10^4-10^9 CFU/mL, has macro-micronutrient content, pH at 6.5-6.8, and C-organic to support plant growth. The applications of the three types of LOF were able to suppress the severity of bacterial leaf blight by up to 21% and rice blast by 4% at eight weeks after inoculation. All LOF treatments are capable of enhancing the growth and production of rice. There were no significant differences in LOF materials for disease suppression, growth, and rice production.

Keywords: Liquid organic fertilizer, microbial consortium, rice, *Pyricularia oryzae*, *Xanthomonas oryzae* pv. *oryzae*

Abbreviations: LOF: Liquid Organic Fertilizer, Xoo: *Xanthomonas oryzae* pv. *oryzae*

INTRODUCTION

Rice productivity, as Indonesia's main staple food source, is significantly influenced by soil fertility and plant pathogen infection. Bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*), with potential yield losses ranging from 20 to 100% (Yang et al. 2020), and rice blast (*Pyricularia oryzae*), capable of reducing yields by 10 to 30% (Mandal et al. 2023), are two of the major diseases affecting rice. Rice cultivation involves fertilizers and pesticides to ensure high productivity and protection against pathogens. Currently, farmers heavily rely on chemical fertilizers and pesticides in rice cultivation. However, excessive use of synthetic chemical substances negatively impacts soil and environmental quality. Therefore, minimizing the use of synthetic chemical inputs by transitioning to environmentally friendly alternatives is a priority to address this issue (Martínez-Alcántara et al. 2016).

Moreover, using organic fertilizers and beneficial microbial inputs can serve as an alternative for improving soil quality and providing essential nutrients for plants (Ginting 2019; Doni et al. 2022). For instance, organic fertilizers can be applied as Liquid Organic Fertilizer (LOF). The raw materials for LOF include agricultural, market garbage, and livestock wastes. LOF contains macro elements N, P, K, and Mg, and microelements Zn, Mn, Cu, Mo, B, Mo, and Fe (Raden et al. 2017). Additionally, LOF also contains amino acids, organic acids, vitamins, and plant growth hormones that contribute to improving soil quality. LOF with soluble nutrients also tends to be more easily absorbed by plants and more evenly distributed in soil than solid-formulation organic fertilizers (Solihin et al. 2019; Bahua and Gubali 2020). Solid organic fertilizers exhibited a slow-release characteristic, requiring a longer time for the mineralization. Consequently, the nutrients were not immediately available for plants (Fahrurrozi et al. 2019).

Applying LOF as a source of both macro and micronutrients plays a crucial role in promoting rice growth (Effendi et al. 2023), making the plants more resistant to pathogen infections. However, it remains essential to suppress pathogen populations directly and induce plant defense mechanisms to ensure a disease-free crop. This can be achieved by harnessing beneficial microorganisms that act as biocontrol agents against pathogens in rice. Therefore, combining several microbes that can synergize will result in a microbial consortium. This approach enhance the potential of microbes and their formulations in pathogen biocontrol (Denaya et al. 2021; Maciag et al. 2023). Previous research has explored the potential use of various microbial species and their combinations in the microbial consortium to suppress the severity of rice blast and bacterial leaf blight diseases while enhancing rice productivity (Kurniawati et al. 2021; Widjayanti et al. 2023).

Developing a microbial consortium involves strategically selecting microorganisms with complementary attributes, ensuring a comprehensive and effective defense against various pathogens. Bacteria from the genera *Pseudomonas*, *Bacillus*, and the fungus *Trichoderma* are among the beneficial microbes that have been studied for their ability to suppress bacterial leaf blight development (Yang et al. 2021; Prabawati et al. 2019) and rice blast diseases (Chou et al. 2020; Marwan et al. 2021). The biocontrol mechanisms involve the production of antimicrobial compounds by *Pseudomonas* spp. (Dimkić et al. 2022), Induced Systemic Resistance (ISR) by *Bacillus* sp. (Tsotetsi et al. 2022), or spatial-nutrient competition and the production of cell wall-degrading enzymes against pathogenic hyphae by *Trichoderma* sp. (Yao et al. 2023; Guzmán-Guzmán et al. 2023). Adding these three beneficial microbes genera to the formulation can enhance the quality and functionality of LOF, especially for plant pathogen biocontrol.

Liquid organic fertilizer, with its nutrient content, has the potential to serve as a growth medium for the microbial consortium. This allows LOF and the microbial consortium integration, making the application more efficient. In addition, the interaction between the microbial consortium and the LOF may lead to synergistic effects, further promoting plant growth and resilience to pathogens. This research aims to assess the effectiveness of the microbial consortium formulation consisting of *Pseudomonas putida*, *Bacillus subtilis*, and *Trichoderma* sp. in LOF with different raw materials in suppressing the severity of bacterial leaf blight, rice blast, as well as enhancing the growth and production of rice plants. Furthermore, the study will explore the intricate mechanisms underlying the interaction between the microbial consortium and the LOF, shedding light on the potential for sustainable and eco-friendly agricultural practices.

MATERIALS AND METHODS

Time, place, and experimental design

The research was conducted at the Laboratory of Plant Protection Technology, Soil Chemistry, and Greenhouse of the Faculty of Agriculture, Universitas Jember, from June to December 2023. The study employed a Completely Randomized Design (CRD) with four treatments of different organic waste materials combined with the bacterial consortium for Liquid Organic Fertilizer (LOF), each replicated five times. The treatments in this research are listed in Table 1.

Isolation of *Pyricularia oryzae*

The isolation of *P. oryzae* was performed using the method described by Kadeawi et al. (2021). Necks of rice panicles with blast symptoms (Tables 2-3) from the field were cut to a length of 7 cm and incubated on a petri dish lined with wet tissue. Incubation was carried out for two days at room temperature under bright light. Conidia of *P. oryzae* were observed under a light microscope and collected for pure culture on PDA (Potato Dextrose Agar) medium.

Subculturing of *Xanthomonas oryzae* pv. *oryzae*

Xanthomonas oryzae pv. *oryzae* (Xoo) isolates utilized in this study were obtained from the Plant Pest Organism Collection of the Faculty of Agriculture, Universitas Jember. Subculturing was done by retrieving one loop of bacterial suspension stored in 25% glycerol medium, transferring it into 5 mL of sterile water, and vortexing until mixed. The suspension was then streaked onto Yeast Peptone Glucose Agar (YPGA) medium and incubated at room temperature for 48 hours (Nurcahyanti et al. 2021). The single colony of Xoo was then streaked in YPGA medium to obtain a pure culture.

Mass production of *Pseudomonas putida* Pf10, *Bacillus subtilis* JB12, and *Trichoderma* sp

Pseudomonas putida Pf10, *Bacillus subtilis* JB12, and *Trichoderma* sp. were individually cultivated on YPGA, King's B, and Potato Dextrose Agar (PDA) media (Figure 1). *B. subtilis* JB12 and *P. putida* Pf10 were incubated for 48 hours on their respective medium, while *Trichoderma* sp. was incubated for 7 days on the PDA medium. Mass production of *P. putida* Pf10 and *B. subtilis* JB12 was done by inoculating 3 loops of each bacterial colony into 2 different 100 mL of sterile liquid Yeast Peptone Glucose (YPG) medium in a 250 mL Erlenmeyer flask. Concurrently, *Trichoderma* sp. was grown in a Potato Dextrose Broth (PDB) medium. The liquid medium containing each distinct microbial isolate was then agitated at a speed of 150 rpm using a rotary shaker for 48 hours for both bacteria and 7 days for *Trichoderma* sp. (Kumar and Ashraf 2019; Nurdika and Nurcahyanti 2019; Nurcahyanti et al. 2021).

Table 1. List of treatments in this research

Code	Treatment
Control	Plant without treatment
FV	LOF from fruit and vegetable waste + Microbial consortium
RS	LOF from rice straw + Microbial consortium
CM	LOF from cattle manure + Microbial consortium

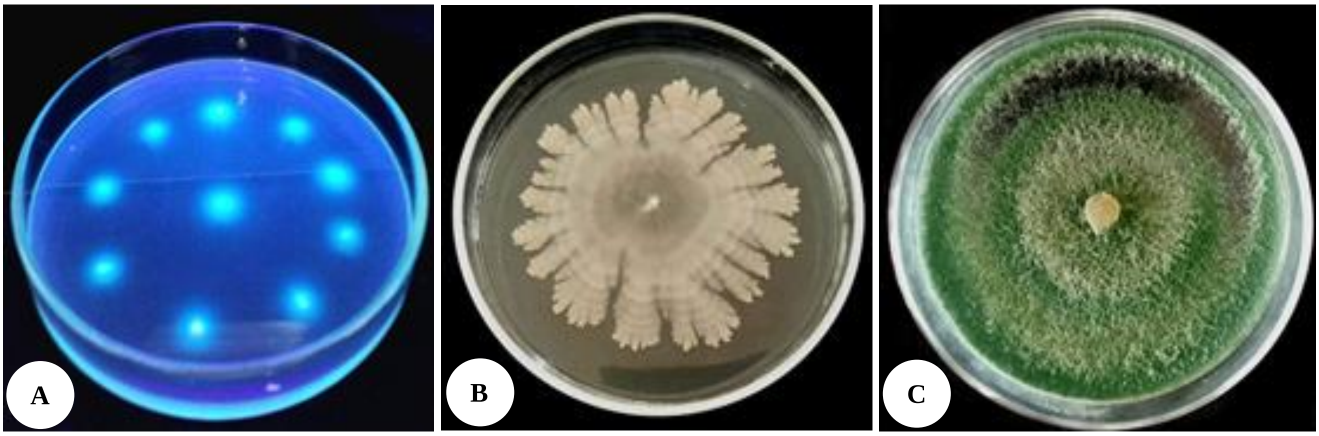

Figure 1. Three microbial consortium isolates were incorporated into the LOF formulation: A. *Pseudomonas putida* Pf10, B. *Bacillus subtilis* JB12, and C. *Trichoderma* sp.

Production of Liquid Organic Fertilizer (LOF) and addition of microbial consortium

Moreover, 5 kilograms of organic waste materials (vegetable and fruit waste, rice straw, and cattle manure) were combined with 15 liters of water, additional nutrient sources, and EM4 (Effective Microorganisms) as a bioactivator. The mixture was stirred thoroughly and incubated in a sealed barrel for two weeks with daily agitation. After incubation, the mixture underwent filtration. The filtrate obtained served as liquid organic fertilizer (Yusnaini et al. 2022).

The microbial consortium formulation was prepared by combining LOF, additional materials for carrier, and nutrient source for the microbes. Subsequently, it was inoculated with beneficial microbes (*P, putida* Pf10 and *B. subtilis* JB12 at a density of 10^9 CFU/mL and *Trichoderma* sp. at 10^7 spores/mL. Each microbe was introduced in a volume of 10 mL into the 90 mL LOF, resulting in a total volume of 120 mL.

Analysis of liquid organic fertilizer formulation

Characteristics of LOF, analysis of nutrient and microorganisms content

The characteristics of the LOF were observed in terms of color, odor, and pH once the liquid organic fertilizer was completed. The pH measurements were conducted using a Martini Mi 151 pH meter instrument. Essential nutrient content analysis of the liquid fertilizer was conducted to determine the content of macroelements Nitrogen (N), Phosphorus (P), Potassium (K), organic matter, and microelements Copper (Cu), Zinc (Zn), and Iron (Fe) according to the standard method by Balai Penelitian Tanah (2005). Total-N was measured using the Kjeldahl method. P_2O_5 and C-organic were measured using the spectronic method. K, Fe, Cu, and Zn were measured using the AAS method. Microbial content was assessed using the total plate count method to calculate the abundance of fungi and bacteria in LOF before inoculation of the microbial consortium.

Growth of microbial consortium in LOF

Observation of microbial consortium growth in LOF was done using the total plate count method. *B. subtilis* JB12, *P. putida* Pf10 with a density of 10^8 CFU/mL, and *Trichoderma* sp. at 10^6 spores/mL were each inoculated in a volume of 10 mL into 90 mL of LOF that had been sterilized using an autoclave at 121°C and 1 atm pressure. The mixtures were then incubated at room temperature for 48 hours. The mixtures were diluted to 10^{-4}, 10^{-5}, and 10^{-6}. A total of 100 µL from each dilution was taken and cultured on YPGA (for *B.subtilis* JB12), Kings'B (for *P. putida* Pf10), incubated for 48 hours, and on PDA (for *Trichoderma* sp), then incubated for 7 days. As a control, the microbial consortium isolates were inoculated to sterilized water, incubated for 48 hours, and cultured using the same method.

Effect on rice seedling growth

Rice seeds were soaked in sterile water for 24 hours for the imbibition process. Seed treatment was then performed by immersing the rice seeds in each LOF according to the treatment for 20 minutes at a dosage of 20 mL/L. Subsequently, 100 seeds were planted in a medium of sterilized soil and compost (3:1). Observations were made on germination rate, plant height, and root length after one week of germination.

Testing on formulation effectiveness in managing bacterial leaf blight, rice blast, and enhancing rice productivity

Rice planting

The research was conducted in a greenhouse to assess the effectiveness of the LOF formulation in controlling bacterial leaf blight and rice blast, as well as enhancing rice productivity. Rice seeds (IR 64 Variety) were soaked in water for 24 hours and subsequently immersed in the LOF formulation for 20 minutes at a 20 mL/L dosage. The seeds were then planted in a soil and compost mixture (3:1) medium and nurtured for 21 days. Afterward, seedlings were uprooted and their roots were immersed in the LOF formulation at a dosage of 20 mL/L for 20 minutes before being planted in pots containing the same planting medium. In each pot, there were 2 clusters of rice plants. Each cluster consisted of 5 seedlings previously sown and selected based on their growth and free from any signs of rice disease. Fertilization was carried out using Urea with a dosage of 250 Kg/ha, SP36 at 100 kg/ha, and KCl at 50 kg/ha. Additional LOF application was performed through spraying one week after planting and repeated during the rice's flowering and grain formation stages with a dosage of 20 mL/L.

Pathogen inoculation

This study comprises two experiments involving the inoculation of *Xanthomonas oryzae* pv. *oryzae* (Xoo) and *Pyricularia oryzae*. In each experiment, rice plants were inoculated using a 10 mL suspension of Xoo (at 10^8 CFU/mL) or *P. oryzae* spores (at 10^6 spores/mL) per plant 28 days after transplanting. Inoculation was performed by spraying the pathogen suspension onto the rice plants until the entire leaf surface was covered. Inoculation was carried out in the greenhouse in the afternoon to avoid direct sunlight exposure and high temperatures. Maintenance was performed by ensuring soil moisture, controlling pests, and managing weeds.

Observation

Effectiveness of disease control

Parameters for assessing the effectiveness of the LOF formulation in controlling bacterial leaf blight and rice blast diseases involved calculating the incubation period, disease incidence, severity, and the Area Under Disease Progress Curve (AUDPC). The incubation period was calculated from pathogen inoculation to the appearance of the first symptoms. Disease incidence was calculated based on the percentage of infected plants compared to the total number of plants observed. Disease severity for bacterial leaf blight and rice blast was calculated based on the following formula:

$$\text{Disease Severity} = \frac{\Sigma(\text{ni x vi})}{\text{N x V}} \; x \; 100\%$$

Where: ni: Number of plants observed with the score I; vi: Disease severity score I; V: Highest disease severity score; N: Total of observed plant

The area under disease progress curve (AUDPC) value was calculated based on the following formula:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2}\right) (t_{i+1} - t_i)$$

Where: n: Total number of observations; Y_{1+1}: Disease severity at the i^{th} observation + 1; Y_1: Disease severity at the i^{th} observation; t_{1+1}: time at the i^{th} observation + 1; t_1: time at the i^{th} observation

Plant growth and production

Observations on plant growth and production include measurements of height, number of tillers, percentage of productive tillers, number of panicles, number of grains, percentage of filled grains, weight of 1000 seeds, and weight of seeds per hill. Rice harvesting was carried out at 115 days after planting.

Table 2. Severity score of BLB disease in rice (IRRI 2002):

Score	Symptoms
0	There are no symptoms.
1	There is a symptom of 1-2 mm long spots around the inoculation point.
2	Symptoms form a circular shape like an eclipse about 2-3 cm long.
3	Symptoms begin to elongate to less than ½ of the leaf length.
4	Symptoms widen and begin to coalesce, the top of the leaf begins to experience tissue death, extending approximately ¼ from the lower part of the leaf surface which is the point of inoculation.
5	Blight symptoms coalesce, the tops of the leaves become dry, and the symptoms extend to ½ of the leaf length.
6	Symptoms extend to ¼ from the underside of the leaf.
7	Symptoms extend too close to the underside of the leaf and almost destroy the entire leaf.
8	Blight symptoms destroy the entire leaf blade and extend to about ½ of the leaf midrib.
9	All leaves and midribs are infected.

Table 3. Severity score of rice blast disease (IRRI 2002; Namrata et al. 2019):

Score	Symptoms
0	There are no symptoms.
1	Small brown spots appear, each the size of a pinhead.
2	Small, round necrotic spots, ash-gray in color, slightly elongated, measuring 1-2 mm in length, with brown edges; numerous spots are typically found on the lower surface of the leaves.
3	Spots resembling the condition in score 2, but with several larger spots appearing on the upper surface of the leaves.
4	Diamond-shaped blast lesions appear, measuring 3 mm or more, with the affected leaf area being less than 2%.
5	Diamond-shaped blast lesions, measuring 3 mm or more, with the affected leaf area ranging from 2 to 10%.
6	Diamond-shaped blast lesions, measuring 3 mm or more, with the affected leaf area ranging from 11 to 25%.
7	Diamond-shaped blast lesions, measuring 3 mm or more, with the affected leaf area ranging from 26 to 50%.
8	Diamond-shaped blast lesions, measuring 3 mm or more, with the affected leaf area ranging from 51 to 75%. Some leaves are starting to die.
9	All leaves are dead, with the affected leaf area exceeding 75%.

Population of pathogens transmitted to rice grains

Observations on Xoo and *P. oryzae* transmitted to rice grains were conducted by weighing 1 gram of harvested rice grains, subsequently crushed with a mortar, and placed in 9 mL of sterile water in a test tube. The suspension was serial diluted, and 100 µL of each dilution was cultured on YPGA medium for Xoo and PDA for *P. oryzae*. The media were then incubated for 48 hours for Xoo and 7 days for *P. oryzae*, followed by population counting. Observations were conducted on rice seeds harvested 115 days after planting.

Data analysis

Disease control, plant growth, and production data were analyzed using Analysis of Variance (ANOVA). If the data showed a significant difference, post hoc analysis using the Duncan Multiple Range Test (DMRT) was performed with a confidence level of 5%. The analysis was conducted using IBM SPSS Statistics Software version 22.

RESULTS AND DISCUSSION

Characteristics, nutrient, and microorganism content of liquid organic fertilizer

The LOF underwent complete decomposition after two weeks of incubation, resulting in a filtrate that exhibited changes in color and odor (Table 4, Figure 2.A). LOF derived from vegetable-fruit, and rice straw displayed a light brown filtrate with a pungent and slightly pungent odor. Conversely, LOF derived from cattle manure exhibited a black filtrate with a strongly pungent odor. Overall, the pH levels of all three types of LOF were considered neutral, ranging from 6.5 to 6.8.

The nutrient content in each treatment of LOF showed variations and high content of different elements (Table 5). Generally, vegetable-fruit waste and cattle manure-based LOF contain higher nutrient content than the LOF from rice straw. LOF derived from vegetable and fruit waste exhibited the highest total N (0.05%), C-organic (0.56%), Cu (0.47 ppm), and Zn (0.67 ppm). LOF derived from cattle manure excelled in P2O5, K2O, and Fe content, with 0.0056%, 0.0053%, and 39.95 ppm, respectively.

Microbial content observations in the LOF showed fungi and bacteria with similar characteristics but distinct population composition growth in all treatments (Table 6). LOF derived from vegetable and fruit waste tends to have the highest bacterial population (1.32 x 10^8 CFU/mL) but with the lowest fungal population (3.51 x 10^3 CFU/mL). LOF derived from rice straw and cattle manure tends to have a more balanced population bacteria and fungi than vegetable-fruit-waste-based LOF. Morphologically, only two types of bacteria colonies are observed in all treatments (Figures 2.B and 2.C). The fungi (Figures 2.D and 2.E) that thrive exhibit morphological differences. LOF from fruit-vegetables waste and cattle manure both having small colony sizes with white mycelium, in contrast to the rice straw LOF fungi which tend to have larger colonies.

Table 4. Characteristics of liquid organic fertilizer

LOF material	Characteristics		
	Color	**Odor**	**pH**
Fruit and vegetable	Light brown	Pungent (++)	6.5
Rice straw	Light brown	Slightly Pungent (+)	6.8
Cattle manure	black	Strongly Pungent (++++)	6.8

Table 5. Nutrient content of liquid organic fertilizer

LOF material	Macro element (%)			Micro element (ppm)			C-organic
	N-total	**P_2O_5**	**K2O**	**Fe**	**Cu**	**Zn**	
Fruit and vegetable	0.05	0.0029	0.0051	22.22	0.47	0.67	0.56
Rice straw	0.04	0.0033	0.0051	16.14	0.33	0.43	0.20
Cattle manure	0.04	0.0056	0.0053	39.95	0.43	0.57	0.36

Table 6. Microorganism content of liquid organic fertilizers

LOF material	Fungi population (CFU/mL)	Fungi colony description	Bacterial population (CFU/mL)	Bacterial colony description
Fruit and vegetable	3.51 x 10^3	1 type: small, white mycelium	1.32 x 10^8	Two types: 1. white, round, mucoid, convex 2. white, irregular, dry, flat
Rice straw	4.53 x 10^6	1 type: large, white mycelium	2.65 x10^7	Two types: 1. white, round, mucoid, convex 2. white, irregular, dry, flat
Cattle manure	2.45 x 10^6	1 type: small, white mycelium	2.35 x10^6	Two types: 1. white, round, mucoid, convex 2. white, irregular, dry, flat

Rice seedling growth

The seed germination rate of rice in all treatments reached 100%, with the highest root length observed in the cattle manure-based LOF treatment at 12.58 cm, followed by the vegetable-fruit waste LOF treatment at 11.81 cm, and rice straw at 11.84 cm. The plant height in all three treatments did not significantly differ, with vegetable-fruit LOF at 25.51 cm, rice straw at 25.55 cm, and cattle manure at 25.62 cm (Table 7, Figure 3). Overall, the growth of rice seedlings in all POC treatments was better compared to the control. LOF did not hinder germination or seedling growth. In the control treatment, the growth started with the development of the plumule, while in all LOF treatments, the initial development started in the radicle. Subsequently, the growth in POC treatments was faster compared to the control.

Growth of microbial consortium in LOF

P. putida Pf10, *B. subtilis* JB12, and *Trichoderma* sp. can thrive well in all three LOF treatments (Table 8). The pH, which tends to be neutral, the relatively adequate nutrient content, and a C/N ratio of less than 12 make these LOFs suitable for the growth of all three species. *B. subtilis* JB12 exhibited a high population across all LOF treatments, ranging from 10^8 to 10^9. *P. putida* Pf10 had a lower population range than to *B. subtilis* JB12, ranging from 10^7 to 10^8. Meanwhile, *Trichoderma* sp. showed the lowest overall microbial population growth, ranging from 10^6 to 10^7.

The effect of LOF formulation with microbial consortium on the development of bacterial leaf blight, plant growth, and rice production

Development of bacterial leaf blight disease on rice

Symptoms of bacterial leaf blight disease can occur at all rice growth stages. These symptoms include streaks on the leaves, which later turn yellowish-white and eventually dry, leading to leaf death (Figure 4.A). The bacteria infect through stomata, hydathodes, and wounds on the leaves. Subsequently, the bacteria proliferate in the intercellular spaces and enter the xylem tissue.

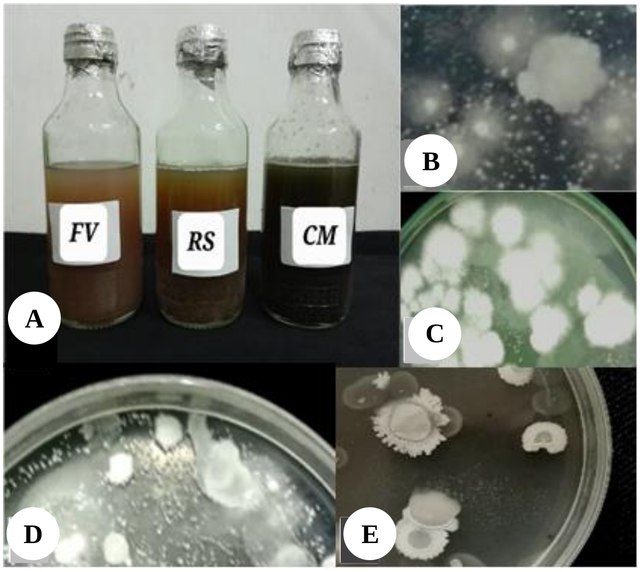

Figure 2. A. Characteristics of liquid organic fertilizer, differences in LOF color. Fungi isolated from LOF: B. Small colony with white mycelium characteristics. C. Large colony with white mycelium. Bacteria isolated from LOF. D. Bacteria with white, round, mucoid, and convex characteristics. E. bacteria with white, irregular, dry, and flat characteristics

Table 7. Rice seedling growth at 7 days after sowing

Treatment	Rice seedling growth		
	Germination rate (%)	**Root length (cm)**	**Plant height (cm)**
Control	100	11.70b	21.80b
Fruit and vegetable	100	11.81ab	25.51a
Rice straw	100	11.84ab	25.55a
Cattle manure	100	12.58a	25.62a

Note: Values with different letters in the same column are significantly different at p<0.05 based on Duncan's Multiple Range Test

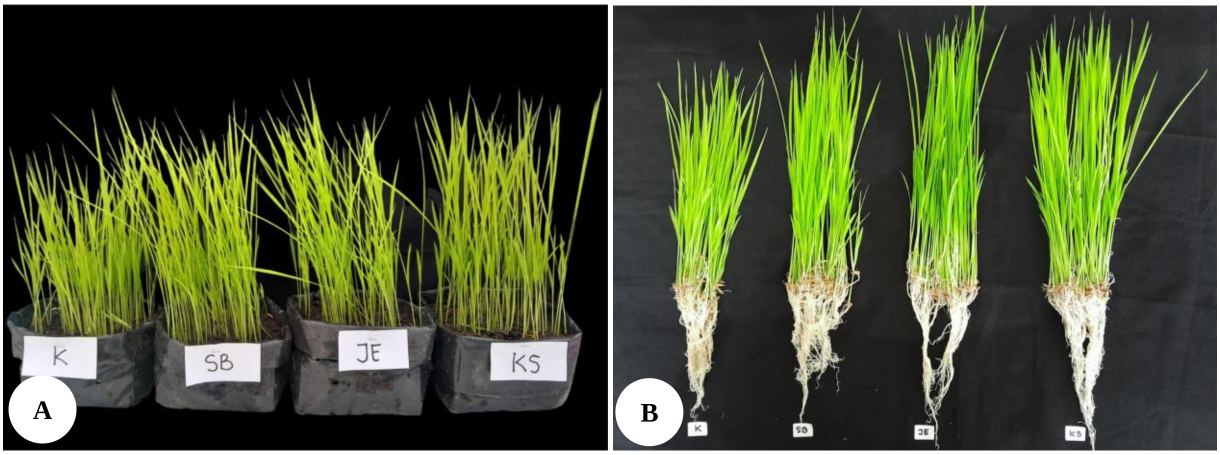

Figure 3. Growth of rice seedlings from left to right in each picture: Control, LOF from fruit and vegetable, LOF from rice straw, and LOF from cattle manure. A. Rice seedling height comparison; B. Root length comparison

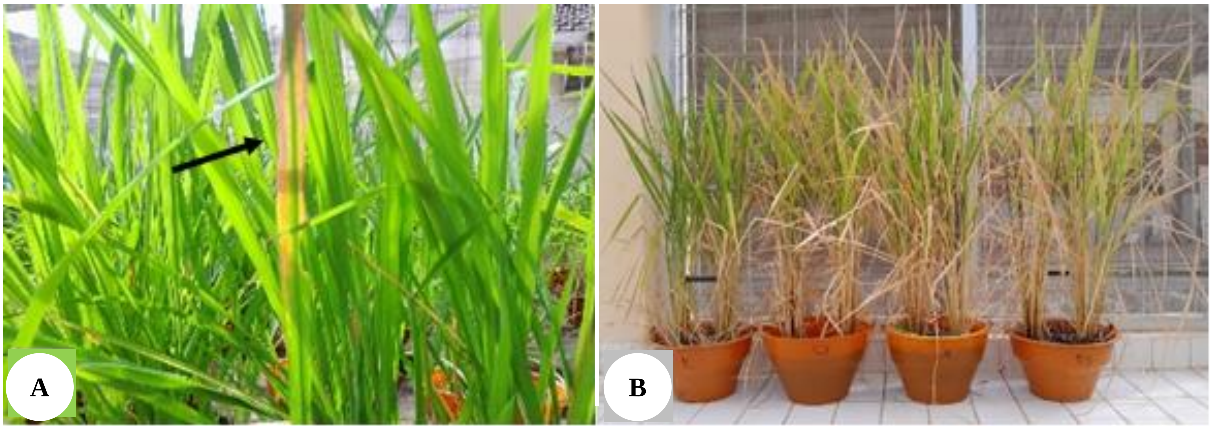

Figure 4. Symptoms of bacterial leaf blight on rice: A. Necrotic symptoms starting from the edges, characteristic of bacterial leaf blight. B. Comparison of bacterial leaf blight symptoms in the three treatments. From left to right: control, LOF derived from fruit-vegetable waste, LOF from rice straw, and LOF from cattle manure

Applying LOF with a microbial consortium does not extend the incubation period of bacterial leaf blight. Symptoms of bacterial leaf blight appear 3-5 days after inoculation in all treatments. The incidence of bacterial leaf blight disease is also not different in all treatments, reaching 100%, indicating that all plants are infected with Xoo. However, applying LOF with a microbial consortium can reduce the severity of the disease (±21%) compared to the control (24%). There is no significant difference between the three LOF treatments in suppressing the severity of bacterial leaf blight.

In contrast to disease severity, the population of Xoo in harvested seeds is lowest in the control treatment, at 1.71 x 10^2 CFU/gram, while in the three LOF treatments around 10^3 CFU/mL. The control treatment's harvested rice seeds tend to be dominated by empty and green grains. In contrast, the three LOF treatments exhibit fully ripe, plump, and yellow grains. The lower population of Xoo observed in the control treatment is suspected to be due to the abundance of empty grains, resulting in a lower transmission of the pathogen to the seeds compared to the LOF treatment, where the grains are filled and susceptible to Xoo infection.

The development of bacterial leaf blight disease severity in all treatments was slow until the fourth week. This condition is suspected to be influenced by the planting during the dry season. The hot weather caused the bacteria to take considerable time to develop and infect rice, although all treatments showed symptoms within five days after inoculation. Bacterial leaf blight disease progressed more rapidly from the 4th to the 8th week after inoculation (Figure 5). The value of the area under the disease progress curve (AUDPC) is directly proportional to the severity of the disease, with the highest observed in the control (249.3) (Table 9), followed by other treatments at lower levels. This indicates that overall, the control experienced the highest total disease severity over the 8-week observation period compared to all LOF treatments.

Growth and rice production in bacterial leaf blight-infected plants

The application of liquid organic fertilizer with the addition of a microbial consortium significantly increased the height of rice plants compared to the control (Figure 6). However, no significant difference was observed in plant height among the various types of LOF.

Table 8. Growth of microbial consortium in liquid organic fertilizer at 72 hours

Treatment	The population of microbial consortium (CFU/mL)		
	P. putida Pf10	***B. subtilis JB12***	***Trichoderma* sp.**
Control	3.21 x 10^4	2.34 x 10^4	2.21 x 10^4
Fruit and vegetable	5.22 x10^8	6.54 x 10^9	2.25 x 10^6
Rice straw	2.35 x 10^7	3.23 x 10^8	2.32 x 10^7
Cattle manure	2.17 x10^8	3.43 x 10^9	6.75 x 10^7

Table 9. Bacterial leaf blight disease development after 8 weeks of inoculation

Treatment	Incubation period (days)	Disease incidence (%)	Disease severity (%)	AUDPC	Xoo population in seeds (CFU/gram)
Control	3-5	100	24.64a	249.3	1.71 x10^2
FV	3-5	100	21.57b	214.7	2.52 x 10^3
RS	3-5	100	21.35b	212.4	2.43 x 10^3
CM	3-5	100	21.61b	222.9	1.06 x 10^3

Note: Values with different letters in the same column are significantly different at $p < 0.05$ based on Duncan's Multiple Range Test. FV: Fruit-Vegetable waste, RS: Rice Straw, CM: Cattle Manure

Table 10. Growth and production of bacterial leaf blight-infected rice at 8 weeks after inoculation

Treatment	Growth and production (8 weeks after inoculation)						
	Height (cm)	Total tillers	Productive tillers (%)	Total grains.panicle	Filled grains/panicle (%)	1000-grain weight (g)	Yield per plant (g)
Control	60.36c	10.88d	60.04b	79.99d	57.23b	15.79b	8.87c
FV	78.12a	13.76b	76.23a	105.06a	86.72a	24.99a	30.21a
RS	78.23a	13.77a	76.67a	103.07b	86.87a	24.95a	30.70a
CM	76.20b	12.45c	73.51a	94.86c	86.48a	25.37a	26.34b

Note: Values with different letters in the same column are significantly different at $p < 0.05$ based on Duncan's Multiple Range Test. FV: Fruit-Vegetable waste, RS: Rice Straw, CM: Cattle Manure

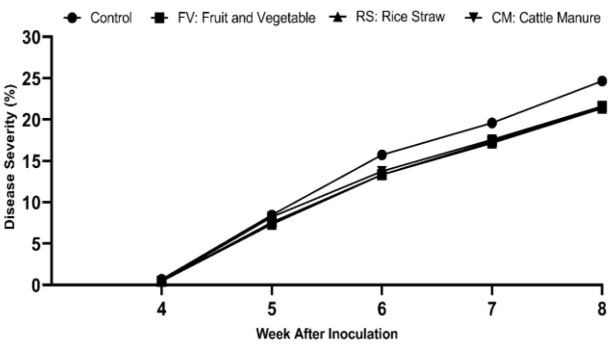

Figure 5. Development of bacterial leaf blight disease severity in rice from 4 to 8 weeks after inoculation

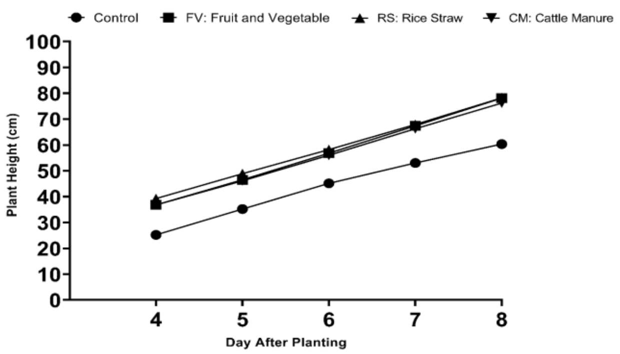

Figure 6. The height of rice infected with bacterial leaf blight up to 8 weeks after *Xanthomonas oryzae* pv. *oryzae* inoculation

Observations on all agronomic parameters (Table 10) indicate that LOF and the microbial consortium can enhance the growth and production of rice plants compared to the control. Variations in growth and production appear to be influenced by the type of applied LOF. Overall, LOF derived from vegetable-fruit waste and rice straw has a more pronounced effect on enhancing growth and production than LOF from cattle manure. However, parameters such as the percentage of productive tillers, percentage of filled grains per panicle, and 1000-grain weight do not exhibit significant differences.

The LOF derived from vegetable-fruit waste and rice straw showed no significant differences in all growth and production parameters except for the total number of grains per panicle, which was higher in the LOF from vegetable-fruit waste. Based on yield per hill parameters, the treatment with LOF derived from vegetable-fruit waste and rice straw proved the most significant, with yields per plant of 30.21 grams and 30.70 grams, respectively, compared to the control with only 8.87 grams.

The effect of liquid organic fertilizer with microbial consortium on the development of rice blast disease, plant growth, and rice production

Development of rice blast disease

Unlike bacterial leaf blight, rice blast disease caused by *P. oryzae* induces distinct symptoms during infections in the vegetative and generative stages. Infections during the vegetative stage result in blast symptoms on leaves and nodes (Figure 7.A). Infections during the generative phase cause failure in grain-filling, exhibiting symptoms such as neck rot, and brown spots on panicles and grains. Lesions on leaves and nodes appear as diamond-shaped necrotic lesions, ranging from gray to white with brown margins.

Blast disease exhibits an incubation period ranging from 6 to 10 days with 100% disease incidence in all treatments. The severity of blast disease is remarkably low, only reaching 6.00% in the control and suppressed by the application of LOF formulations to 4.46% in the vegetable-fruit treatment, 4.44% in the rice straw treatment, and 4.89% in the cattle manure treatment (Table 11). The raw materials of LOF do not influence the severity of the disease.

The low severity of blast disease is suspected to prevent *P. oryzae* from being transmitted to rice grains. The severity of rice blast disease develops slowly until the 5th week (Figure 8). Subsequent increases occur from the 6th to the 8th week, but overall, the severity remains low. This condition is suspected to be influenced by the hot weather, hindering the growth and infection of the *P. oryzae* fungus. Based on the AUDPC values, the control exhibited the highest total severity (38.08), followed by the other significantly lower LOF treatments.

Growth and rice production in rice blast-infected plants

Liquid organic fertilizer treatments with microbial consortium enhance the height of rice plants compared to the control (Figure 9). The increase in plant height starts from the 4th week after planting, indicating linear development until the 8th week in all treatments. The final observations in the 8th week reveal the highest result in the treatment with LOF derived from rice straw (78.58 cm), which is not significantly different from vegetable-fruit (76.76 cm) and cattle manure (75.83 cm) treatments but significantly differs from the control (61.75 cm).

The application LOF with microbial consortium generally enhances the growth and production of rice compared to the control, based on parameters such as the number of tillers, percentage of productive tillers, number of grains/panicle, percentage of filled grains/panicle, 1000-grain weight, and yield/plant (Table 12). There is no significant difference in all agronomic parameters among the LOF treatments. This indicates that applying LOF and microbial consortium plays a role in stimulating plant growth and production, and the differences in the raw material do not significantly affect its effectiveness.

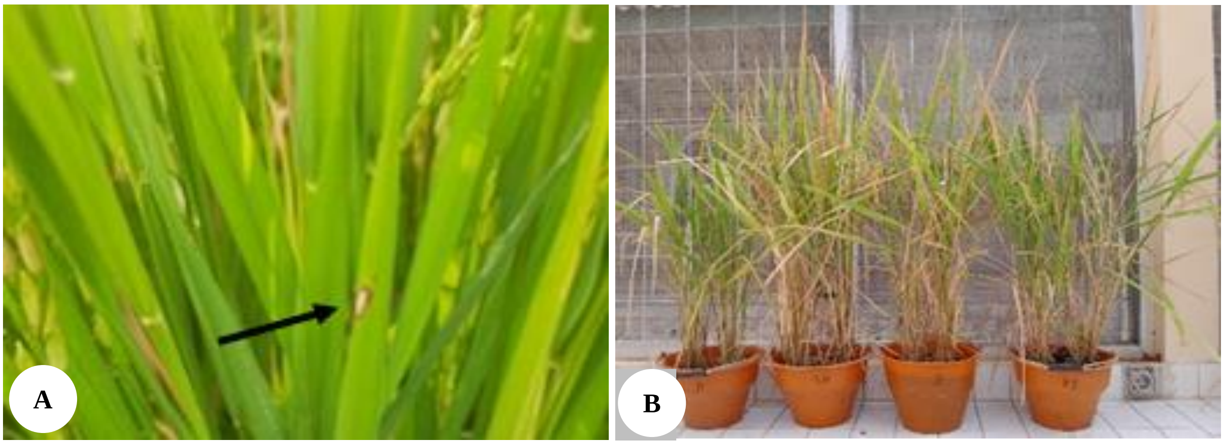

Figure 7. Symptoms of Rice Blast: A. Diamond-shaped necrotic lesions on rice leaves are the characteristic of blast disease. B. Comparison of plant with blast disease symptoms in the three treatments. From left to right: control, LOF derived from fruit-vegetable waste, LOF from rice straw, and LOF from cattle manure

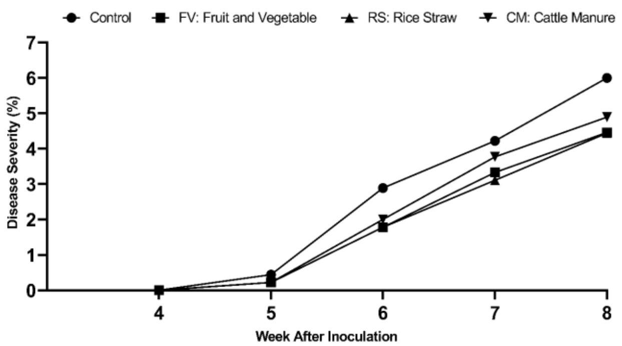

Figure 8. Development of rice blast disease severity from 4 to 8 weeks after inoculation

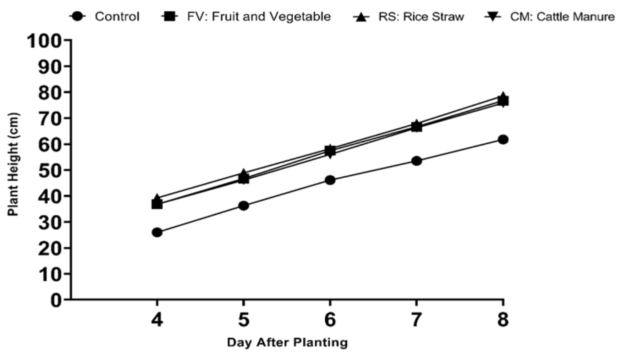

Figure 9. Height of rice infected with rice blast disease up to 8 weeks after *Pyricularia oryzae* inoculation

Table 11. Rice blast disease development after 8 weeks of inoculation

Treatment	Incubation period (days)	Disease incidence (%)	Disease severity (%)	AUDPC	*P.* oryzae population in seeds (CFU/gram)
Control	6 - 10	100	6.00a	38.08	0
FV	6 - 10	100	4.46b	25.64	0
RS	6 - 10	100	4.44b	24.86	0
CM	6 - 10	100	4.89b	28.73	0

Note: Values with different letters in the same column are significantly different at $p < 0.05$ based on Duncan's Multiple Range Test. FV: Fruit-Vegetable waste, RS: Rice Straw, CM: Cattle Manure

Table 12. Growth and production of rice blast-infected rice at 8 weeks after inoculation

Treatment	Growth and production (8 weeks after inoculation)						
	Height (cm)	Total tillers	Productive tillers (%)	Total grains.panicle	Filled grains/panicle (%)	1000-grain weight (g)	Yield per plant (g)
Control	61.75c	9.7b	61.48b	73.58b	54.64b	18.31b	8.83b
FV	76.76b	14.69a	76.02a	115.01a	86.98a	24.29a	30.26a
RS	78.58a	14.02a	75.45a	112.33a	86.77a	24.33a	28.76a
CM	75.83b	14.08a	75.14a	111.68a	87.02a	24.22a	28.22a

Note: Values with different letters in the same column are significantly different at $p < 0.05$ based on Duncan's Multiple Range Test. FV: Fruit-Vegetable waste, RS: Rice Straw, CM: Cattle Manure

Discussion

In general, LOF enriched with a microbial consortium with three different materials has effectively reduced the severity of bacterial leaf blight and rice blast, while improving rice growth and yield. The three LOFs differ in their characteristics, nutrients, and microorganisms content. However, no significant differences observed regarding disease suppression, function as plant growth promoters, and yield enhancement. While LOF or microbial consortia have been widely studied separately (Sah et al. 2018; Ginting 2019; Effendi et al. 2023; Sahlan et al. 2023), their combined use has not been explored much. Nonetheless, it holds great potential as an efficient approach to addressing rice productivity and disease issues.

The materials utilized for Liquid Organic Fertilizer (LOF) influence the variations in its characteristics. The pH in LOF was influenced by its material and the activity of microorganisms converting sugars into lactic or acetic acid (Phibunwatthanawong and Riddech 2019). The pH values of LOF within the range of 6.5 - 6.8 in this research meet the minimum criteria for organic fertilizers and soil conditioners set by the Ministry of Agriculture of the Republic of Indonesia No. 261/KPTS/SR.310/M4/2019. The pH values are also crucial as they impact plant growth and soil quality (Tsaniya et al. 2021).

These differences in LOF nutrient content are presumed to be influenced by the type of materials and their ease of breakdown by microorganisms. Raden et al. (2017) stated that using EM4 as a bioactivator with various beneficial microorganisms tends to support the decomposition process of the material for LOF, resulting in a high content of total-N, C-organic, Fe, Cu, and Zn. LOF from the decomposition of plant waste, such as vegetables and fruits, tends to have high macro and micronutrient content (Ji et al. 2017; Lesik et al. 2019). However, previous research indicates that animal waste can also become a source of these elements (Yusnaini et al. 2022). Based on the quality standards of LOF according to The Ministry of Agriculture Indonesia, regulation No. 261/KPTS/SR.310/M4/2019, both macro and micronutrient contents of LOF in this research are below the standard and do not meet the minimum criteria suitable for plants. Nevertheless, the microbial consortium has the potential to utilize these nutrients for growth, as their nutritional requirement are relatively lower than plants

The microorganisms isolated from the LOF originate from the materials and are also presumed from EM4 as a bioactivator. Microorganisms in the EM4 solution helped decompose organic materials from the utilized waste into simple organic compounds. These microorganisms utilize macromolecules as a source of energy for their growth. Effective Microorganism (EM) technology involves beneficial bacterial species, including lactic acid bacteria (*Lactobacillus* sp, *Streptococcus lactis*), photosynthetic bacteria, yeast, actinomycetes, and fermenting fungi (Domenico et al. 2019; Pszczółkowski et al. 2023). These beneficial microorganisms are advantageous for plants and improve soil condition, especially when combined with the added microbial consortium.

The suppression in bacterial leaf blight and rice blast disease development is presumed to occur due to the microbial consortium in LOF acting as a biocontrol agent against Xoo and *P. oryzae*. The added microbial consortium is crucial in suppressing disease development through antibiosis, growth competition, parasitism, and inducing plant defense mechanisms. Previous research reported the role of *Bacillus* sp and *Pseudomonas* sp as biocontrol agents for Xoo (Parida et al. 2016; Sharma et al. 2022; Resti et al. 2020). *P. aeruginosa* BRp3, as an example, is capable of inducing defense-related enzymes in rice such as peroxidase, catalase, polyphenol oxidase, and Phenylalanine Ammonia Lyase (PAL) during Xoo infection (Yasmin et al. 2017). *Pseudomonas* sp., as a biological control agent, is also capable of producing antimicrobial compounds such as phenazines, pyrrolnitrins, pyoluteorins, phloroglucinols, cyclic lipopeptides, hydrogen cyanide (HCN), siderophore and also several volatile organic compounds (Dimkić et al. 2022).

Phyllosphere bacteria are potential biological control agents against Xoo due to their similar in growth conditions. For instance, *B. cereus* E165, isolated from rice leaves, exhibits antagonistic activity against Xoo (Widjayanti et al. 2023). In this study, *B. subtilis* JB12 was isolated from the soybean phyllosphere. This bacterium has been previously reported to suppress the growth of pathogenic bacteria from the *Xanthomonas* genus by producing antimicrobial compounds (Nurcahyanti et al. 2021). It also influences the activity of phenol and peroxidase, playing a role in plant defense mechanisms (Nurcahyanti et al. 2023).

Bacillus sp. can inhibit the spore germination of *P. oryzae* through the activity of antimicrobial compounds, effectively controlling blast disease (Prabawati et al. 2019). *Trichoderma* sp. has previously been reported as a biocontrol agent for *P. oryzae*, effectively reducing the severity of blast disease, both on leaves and neck blast, and increasing rice productivity (Kumar and Ashraf 2019;

Chou et al. 2020). *Trichoderma* sp. produces hydrolytic enzymes such as chitinase and cellulase, which can degrade the cell walls of *P. oryzae* (Putri et al. 2022). Additionally, *Trichoderma* sp. can contribute to plant resistance induction by triggering enzymes associated with resistance mechanisms linked to the SA and JA pathways (Islam et al. 2023). Lower disease development correlates positively with the growth and production of rice. Therefore, the role of the microbial consortium in suppressing the severity of both pathogens is crucial for rice plant productivity.

The faster growth of rice seedlings, increase in plant height, number of tillers, and plant yield in the LOF treatment is presumed to be due to additional nutrients from LOF and the role of microbial consortium as a plant growth promoter. The availability of additional macro-nutrients N, P, and K from LOF supports rice growth, increases the number of grains, and enhances their quality. The better nutrient absorption levels of N, P, and K in LOF treatments are directly proportional to the improvement in rice growth and productivity (Bahua and Gubali 2020). LOF also serves as a Fe, Cu, and Zn micronutrients source. The application of macronutrients supplemented with the availability of micronutrients has been reported to contribute to plant height, productive tillers, and increased grain yield in rice (Siddika et al. 2016). The foliar application of LOF through spraying enhances the efficiency of micro-nutrient provision compared to direct soil application (Saquee et al. 2023). Both macro and micronutrients present in LOF also play a role in the growth of the microbial consortium.

The liquid organic fertilizer enriched with a microbial consortium significantly increased plant height and tiller number compared to the control, indicating the role of this treatment in plant growth and productivity. Generally, several previous studies demonstrate a positive correlation between applying organic fertilizers on rice growth and production (Ginting 2019; Effendi et al. 2023; Ilahude et al. 2023). However, some studies show no significant difference in rice growth following the application of LOF, despite its higher nutrient content than LOF in this study (Solihin et al. 2019). It is suspected that the microbial consortium also acts as a plant growth-promoting agent and modifies the nutrient composition in LOF, making it more readily available to the plants. Ríos-Ruiz et al. 2020 mentioned that tapplying bacterial consortium can increase rice production and improve nitrogen fertilizer use efficiency.

The microbial consortium, primarily composed of the three genera or species used in this study, is also reported to act as a growth promoter for rice (Ngalimat et al. 2021). Microbial consortia involving bacteria from the *Bacillus* genus have previously been reported to function as growth promoters for rice, evidenced by an increase in the number of leaves and plant height. However, it did not affect root length (Resti et al. 2020). The root lengths influenced by treatments in this study are suspected to be due to the higher microbial diversity within the consortium and the additional nutrients from LOF. The study reports the role of *P. aeruginosa* BRp3 in producing IAA and phosphate solubilization (Yasmin et al. 2017). *B. pumilus* strain TUAT-1, when combined with applying N fertilizer, can enhance rice height, root growth, number of tillers, and rice yield (Win et al. 2018). The role of Plant Growth Promoting Bacteria (PGPB) in producing growth-promoting metabolites and improving nutrient uptake efficiency enables the microbial consortium to support plant growth even when the macro-micro nutrient content in LOF is limited.

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