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Assessment of local wisdom biofertilizer formulas on enhancing microbial diversity and photosynthate allocation in acid-stressed maize

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[2]Doctoral Program of Agricultural Science, Faculty of Agriculture, Universitas Padjadjaran. Jl. Raya Bandung Sumedang KM 21, Jatinangor, Sumedang 45363, West Java, Indonesia
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Abstract. *Irwandhi, Kamaluddin NN, Khumairah FH, Prihatiningsih N, Simarmata T. 2025. Assessment of local wisdom biofertilizer formulas on enhancing microbial diversity and photosynthate allocation in acid-stressed maize. Asian J Agric 9: 112-121.* The use of biofertilizers derived from local wisdom practices presents a promising sustainable agricultural strategy for enhancing crop resilience in acidic soils. This study aimed to assess the effectiveness of the Local Wisdom Biofertilizer (LWB) formula by examining the chemical and biological characteristics of various LWB formulations and their impact on growth-promoting microorganisms (GPMs) diversity (bacteria, fungi, and actinomycetes) and maize growth characters. A Randomized Complete Block Design (RCBD) was employed, testing six LWB formulations (F1 to F6) at four dosage levels (0, 2, 4, and 6%) to assess their effects on key growth traits of maize grown in suboptimal soils. Results revealed significant variation in organic carbon, nitrogen, phosphorus, and potassium across formulations, with F3 demonstrating the highest organic carbon (14.80%) and potassium (0.08%). In comparison, F2 exhibited the highest total nitrogen (4.07%), and F5 had the highest phosphorus content (0.03%). Formulation F6 demonstrated the highest concentrations of nitrogen-fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB) among the treatments. The treatments F3K3, F4K3, F5K1, F5K3, F6K1, and F6K2 effectively enhanced maize plant height, with F3K3 and F5K3 having a particularly significant effect on chlorophyll content. Additionally, F1K1 led to the largest bacterial population, F5K1 supported the greatest fungal population, and F6K2 exhibited the highest actinomycetes population. Correlation analysis indicated a negligible relationship between chlorophyll content and other agronomic traits, with values ranging from -0.02 to 0.01. Principal Component Analysis further analyzed the influence of LWB on various plant traits. These findings underscore the potential of integrating local wisdom biofertilizers into sustainable soil management practices, especially in regions grappling with soil acidity. Future research should delve into the microbial and biochemical mechanisms underlying these benefits to optimize biofertilizer formulations for broader agroecological applications.

Keywords: Acid soil, eco-friendly fertilizer, GPMs, local microorganism, maize

INTRODUCTION

Maize (*Zea mays*) holds significant economic value due to its wide range of uses, including food for humans and livestock and as a raw material for various industries (de Matos Nascimento et al. 2020). In 2023, maize production in Indonesia decreased by 12.5% compared to 2022, mainly due to a 10.03% reduction in the area harvested that year (BPS 2023). This condition is exacerbated by Indonesia's corn production system, which still needs to improve production techniques and farming status (Fitriatin et al. 2017). It is also due to the change of corn land into land for other food commodities and the conversion of agricultural land to non-agricultural land (Khumairah et al. 2019).

The enhancement of maize productivity in Indonesia can be achieved by expanding cultivated land (extensification). One potential area for development is suboptimal land subjected to acidic stress. More than 30% of the global land area comprises acidic soils, with 50% of the potentially arable land classified as acidic (Basak and Biswas 2016). Most of Indonesia's land is in the Inceptisols order, and 52.0 million ha of Inceptisols have the potential to be developed (Fitriatin et al. 2021). In tropical areas with high rainfall, Inceptisols are susceptible to loss of nutrients and organic matter, resulting in degraded soil (Hindersah et al. 2022).

Cultivation of maize in Inceptisols faces several challenges due to the inherently low soil fertility and organic matter content and the acidic soil reaction of these soils (Sofyan and Sara 2019). Low availability of nutrients in the growth phase will inhibit several plant metabolic processes, thereby inhibiting flower formation and reducing crop yields (Wei et al. 2017). This decrease in crop yields occurs on acidic soils due to the limited availability of nutrients such as phosphate (P), molybdenum (Mo), and magnesium (Mg) in the soil, as well as the toxicity of aluminium (Al) and manganese (Mn) to plants (Shi et al. 2017).

Enhancing nutrient availability in acidic soils can be achieved through fertilization efforts (Simanjuntak and Setiawan 2021). Fertilizers are applied to increase the nutrients required for optimal plant growth (Hamid and Tanweer 2021). In recent years, the reliance on inorganic fertilizers

has risen sharply (Muktamar et al. 2016). Excessive use of inorganic fertilizers causes a decrease in soil fertility (Gawęda et al. 2020). High doses of inorganic fertilizer in the long term can increase soil compaction, reduce organic matter, and disrupt the balance of nutrients, thereby reducing soil fertility. Inorganic fertilizers are expensive and have limited availability (Yang et al. 2022). However, there is hope in the form of local wisdom biofertilizer, a promising alternative technology that could potentially replace or reduce the dependence on inorganic fertilizers.

Local Wisdom Biofertilizer (LWB) is a fermented liquid from natural materials rich in growth-promoting microorganisms (GPMs). These microorganisms can break down organic matter, serving as biofertilizers, decomposers, and organic pesticides (Roeswitawati and Ningsih 2018). LWB is a biofertilizer that can improve plant leaf health and promote growth. Materials that can be used include cow urine, vegetable waste, fruit waste, banana tubers, leaves, and banana stems (Retnowati and Katili 2021). Other materials, such as seaweed waste, molasses, and earthworm castings, can also be used (Arfarita et al. 2022). The organic materials in LWB contribute to soil health restoration, increased fertilizer efficiency, and improved crop productivity (Simarmata et al. 2016). LWB fertilizers contain a variety of plant growth-promoting rhizobacteria (PGPR), such as *Azospirillum* sp., *Azotobacter* sp., and *Bacillus* sp. (Retnowati and Katili 2021). Each of these PGPRs can mobilize, facilitate, and enhance nutrient availability by transforming nutrients from forms that are not readily accessible into those that are through biological processes (Simarmata 2013). Applying PGPR as biofertilizers in maize cultivation can increase yields while reducing inorganic fertilizer use by up to 50% (Fitriatin et al. 2014, 2015). Given this potential, the current research is aimed at developing an LWB formula by examining the characteristics of the LWB formula, the diversity of GPMs in each formulation, and the effect of LWB fertilizers in enhancing the growth and development of maize on suboptimal soils, as well as to identify the best formula.

MATERIALS AND METHODS

Location

The formulation and analysis of the Local Wisdom Biofertilizer (LWB) were conducted at the Soil Biology, Soil Fertility, and Plant Nutrition Laboratory, Faculty of Agriculture, Universitas Padjadjaran, Sumedang, West Java, Indonesia from March to May 2024. Subsequently, the bioassay of LWB was carried out from June to August 2024 in a greenhouse located at the Ciparanje Experimental Farm, Faculty of Agriculture, Universitas Padjadjaran (6°54'58.46"S 107°46'18.1"E) (Figure 1). The experimental site is located in a tropical region about 752 meters above sea level. The research location has an annual temperature of around 19-30°C, with a relative humidity level of 39-84%. The soil used is included in the Inceptisols soil classification obtained from Jatinangor. This soil has the characteristics of a slightly acidic pH of 5.83 with low available phosphorus (1.23 ppm) and an organic carbon content of 1.66% (Fitriatin et al. 2024).

Selection and formulation of LWB

The LWB formula was developed based on an existing biofertilizer formulation known as Jakaba (*Jamur Keberuntungan Abadi*), which was discovered by farmers in Kedung Dowo Village, Arjasa Sub-district, Situbondo District, East Java, Indonesia (7°45'30"S 114°09'10"E), as illustrated in Figure 1. This formulation was further refined into six LWB formulas. Each material used in the LWB formulations, as detailed in Table 1, was placed into a 60 L fermentor equipped with an air outlet. Initially, 25 L of water was added, and the materials were homogenized using a turbo mixer. Additional water was added until the final volume reached 30 L. Each formula was fermented for 60 days and monitored by observing aroma and microbial activity. After the fermentation process is complete, the solution is filtered using an 80-100 mesh sieve and transferred to a jerry can for storage until there is a decrease in microbial activity.

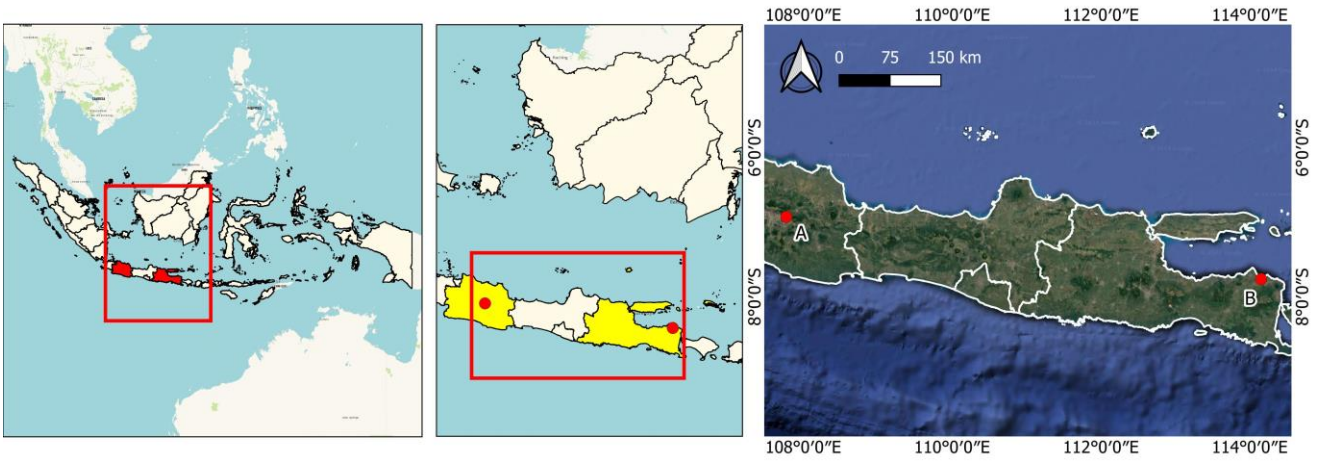

Figure 1. A. Locations of the development and bioassay of the local wisdom biofertilizer in Ciparanje Experimental Farm, Sumedang, West Java, Indonesia; B. The discovery of the Jakaba formula in Kedung Dowo Village, Situbondo District, East Java, Indonesia

Table 1. Composition of materials in each local wisdom biofertilizer formulation

Formulation	Composition
F1	Seaweed (2.5 kg), *Azolla* (2.5 kg), molasses (300 mL), rice husk charcoal (1.25 kg), and yeast (30 g)
F2	Snail shell extract (2.5 kg), crab shell extract (2.5 kg), shrimp shell extract (2.5 kg), eggshell extract (2.5 kg), and rabbit urine 600 mL
F3	Seaweed (1.25 kg), *Azolla* (1.25 kg), molasses (300 mL), rice husk charcoal (625 g), yeast (30 g), snail shell extract (1.25 kg), crab shell extract (1.25 kg), shrimp shell extract (1.25 kg), eggshell extract (1.25 kg), and rabbit urine 600 mL
F4	Rice bran (2 kg), MSG (20 g), shrimp paste (10 g), brown sugar (200 g), lime powder (3 tbsp), bamboo root soaking solution (2 L), and bean sprouts (200 g)
F5	Seaweed (1.25 kg), *Azolla* (1.25 kg), molasses (300 mL), rice husk charcoal (625 g), yeast (30 g), rice bran (1 kg), MSG (10 g), shrimp paste (5 g), brown sugar (100 g), lime powder (1.5 tbsp), bamboo root soaking solution (1 L), and bean sprouts (100 g)
F6	snail shell extract (1.25 kg), crab shell extract (1.25 kg), shrimp shell extract (1.25 kg), eggshell extract (1.25 kg), rabbit urine 600 mL, rice bran (1 kg), MSG (10 g), shrimp paste (5 g), brown sugar (100 g), lime powder (1.5 tbsp), bamboo root soaking solution (1 L), and bean sprouts (100 g)

Note: F1: Plant-based LWB, F2: Animal-based LWB, F3: Plant and animal-based LWB, F4: Jakaba, F5: Nabati Jakaba-based LWB, F6: Animal Jakaba-based LWB

Chemical analysis of LWB

Nitrogen total (%)

The nitrogen content analysis of the fertilizer was conducted using the Kjeldahl method, which involves several stages, including sample digestion, distillation, and titration (Gani et al. 2023). A completely homogenized sample of LWB, weighing precisely 250.0 mg, was cautiously transferred into a Kjeldahl flask. Subsequently, 3 mL of H_2SO_4 and 0.25-0.50 g of selenium were added to the mixture, shaken until completely homogenized. The mixture was then allowed to stand for 2 to 3 hours. The digestion was performed incrementally at temperatures between 150 and 350°C until a clear solution was obtained. After the solution was cooled, a small volume of distilled water was added to inhibit crystallization.

The distillate solution was poured into a boiling flask to a total volume of 250 mL, and deionized water was added until the flask reached the halfway. The distillate reservoir was set up in a 100 mL Erlenmeyer flask, which contained 10 mL of 1% boric acid and three drops of Conway indicator. The distillation procedure involved the addition of 20 mL of 40% sodium hydroxide (NaOH). The distillation was regarded as complete once the solution volume in the Erlenmeyer flask reached 75. The resulting solution was then titrated with 0.05 N H_2SO_4 until a transition from green to pink occurred. The volume of H_2SO_4 used was measured, and the procedure was repeated to obtain the blank sample. The nitrogen content was calculated using the following formula:

$$\text{Nitrogen (\%)} = (Vc - Vb) \times N \times 14 \times fk$$

Where:
Vc: volume H_2SO_4 titration (mL)
Vb: volume H_2SO_4 blank (mL)
N: normality of raw solution H_2SO_4
14: atomic weight of nitrogen
100: % conversion
fk: moisture content correction factor

Organic carbon (%)

According to Gani et al. (2023), the analysis of Organic-C content in LWB can be conducted in several stages. Initially, a sample of LWB weighing 50 to 100 mg is transferred into a 100 mL volumetric flask. Then, 5 mL of a 2N $K_2Cr_2O_7$ solution is introduced, and the mixture is shaken well. Subsequently, 7 mL of H_2SO_4 is added to the solution and left to stand for 30 minutes. To prepare a 250 ppm C standard solution, 5 mL of a 5000 ppm C standard solution is pipetted into a 100 mL volumetric flask. Next, 5 mL of H_2SO_4 and 2N $K_2Cr_2O_7$ solution are added, following the same method used for the 250 ppm C standard solution. Each solution is then diluted to the 100 mL mark and mixed thoroughly. The absorbance of each solution is measured with a spectrophotometer at a wavelength of 651 nm. A standard curve is constructed to derive the linear equation, which allows for the determination of the x-value of the solution. The organic-C content is calculated using the following formula.

$$\text{Organic-C (\%)} = \text{ppm curve} \times \text{mL} \times \text{fk}$$

Where:

ppm curve: the sample rate was derived from the regression curve showing the relationship between the standard series rate and the reading, adjusted by subtracting the blank value.

fk: moisture correction factor = 100 / (100 - % moisture content)

Phosphate and potassium analysis (%)

Phosphorus content was measured using spectrophotometry, while potassium was determined using flame photometry. A 500 mg sample of LWB from each formula was weighed and placed into a digestion flask. Then, 0.5 mL of $HClO_4$ and 5 mL of HNO_3 were thoroughly mixed into the solution. The mixture was heated in a reactor, starting at 100°C. After the yellow vapors had dissipated, the temperature was raised to 200°C. The digestion process was considered complete when white vapors were observed, and approximately 0.5 mL of liquid remained in the flask. The solution was then cooled and

diluted with deionized water to a final volume of 50 mL. After mixing, the mixture was filtered using W-41 filter paper (Extract A). Next, 1 mL of Extract A was transferred into a 20 mL test tube, and then 9 mL of deionized water was added and mixed until the solution became homogeneous, resulting in a 10-fold dilution (Extract B).

The absorbance of potassium, and the absorption values of extract B and the potassium standard solution, were documented. Each potassium standard solution received 1 mL of extract B, and the mixtures were then placed in individual 20 mL test tubes. Next, 9 mL of phosphate coloring reagent and standard solution were added to each sample. The mixtures were thoroughly mixed using a vortex mixer and allowed to sit for 15 to 25 minutes. The absorbance of each sample and potassium standard solution was measured and recorded at a wavelength of 693 nm. Standard curves for both potassium and phosphate samples were created to formulate linear equations, enabling the calculation of concentrations (ppm).

Biological analysis of LWB

The isolation of nitrogen-fixing bacteria was carried out using selective media, namely Jensen's medium (sucrose; 20 g L^{-1}, $CaCO_3$: 2 g L^{-1}, $MgSO_4$: 0.5 g L^{-1}, Na_2MoO_4: 0.0005 g L^{-1}, NaCl: 0.5 g L^{-1}, K_2HPO4: 1 g L^{-1}, $FeSO_4$: 0.1 g L^{-1}, Agar: 15 g L^{-1}) and Pikovskaya agar medium (dextrose: 10 g L^{-1}, $Ca_3(PO_4)_2$: 5 g L^{-1}, $(NH_4)2SO_4$: 0.5 g L^{-1}, $MgSO_4.7H_2O$: 0.1 g L^{-1}, yeast extract: 0.5 g L^{-1}, $FeSO_4.7H_2O$: 0.0001 g L^{-1}, KCl: 0.2 g L^{-1}, $MnSO_4.7H_2O$: 0.0001 g L^{-1}, Agar: 15 g L^{-1}, pH 7). The cultures were then incubated at room temperature for 48 hours.

Biological assay of LWB on maize

This study utilized a Randomized Complete Block Design (RCBD) with treatments comprising six LWB formulations (F1, F2, F3, F4, F5, and F6) and three LWB dosage levels (2%, 4%, and 6%) as well as a control group (without LWB application). The experiment involved a total of 19 treatments, each replicated twice. Maize seeds were sown in polybags filled with 1 kg of soil, and 10 mL of LWB was applied according to each treatment specification. Plant maintenance included daily watering based on field capacity and was continued until 28 days after planting (DAP). Plant height, measured from the base to the plant tip, was the growth parameter. Chlorophyll content was assessed using an Opti-Sciences Chlorophyll Meter CCM 200 Plus. At 28 DAP, the maize plants were harvested, and root length was measured from the base to the longest root. Shoots and roots were separated using scissors, and fresh weights were recorded with a digital balance. Samples from each treatment group were transferred to paper envelopes and dried in an oven until they reached a stable weight. The final dry weights of both the shoots and roots were documented. The total biomass for each treatment was calculated by summing the dry weights of shoots and roots (Fitriatin et al. 2020). The rhizosphere microbial population was analyzed using the Serial Dilution Plate Method, as described by Ben-David and Davidson (2014). Soil samples were cultivated on various media: Potato Dextrose Agar for fungi, Nutrient Agar for bacteria, and Starch Casein Agar for actinomycetes.

Data analysis

The impacts of various treatments on the measured variables were assessed using ANOVA with SPSS (Statistical Package for the Social Sciences) software. Significant differences were identified through Duncan's test, with significance established at $P<0.05$. Correlation analysis was conducted using Pearson correlation testing, and Principal Component Analysis (PCA) was also performed. Data visualization of the research findings was accomplished using Prism 9 software.

RESULTS AND DISCUSSION

Chemical properties of LWB

The results of the chemical analysis for each LWB formula, as illustrated in Figure 2, indicate that each formula exhibits distinct levels of organic carbon, nitrogen, phosphorus, and potassium. The organic carbon content ranges from 0.40 to 14.80%, with formula F3 exhibiting the highest concentration at 14.80%. Nitrogen levels vary between 0.94 and 4.07%, with formula F2 containing the greatest amount at 4.07%. Phosphorus concentrations range from 0.01 to 0.03%, with formula F5 demonstrating the highest phosphorus content at 0.03%. Potassium levels vary from 0.01 to 0.08%, with formula F3 again showing the highest potassium content at 0.08%. These variations in nutrient content can be attributed to the different materials utilized in each formula. The nutrient composition in each formula plays a crucial role in supporting plant growth and development. Organic fertilizers containing organic matter, nitrogen, phosphorus, potassium, and other essential elements can serve as a valuable source of plant nutrition (Ma et al. 2022).

Biological properties of LWB

Biological analysis of LWB shows that each formula contains growth plant microorganisms (GPM) with different populations (Figure 3). Formulation F6 has the highest population of nitrogen-fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB) compared to other formulas. This microbial population plays an essential role in supporting plant growth and development. NFB enhances nitrogen availability to plants, while the high population of PSB increases phosphate availability. The diversity of GPM populations in LWB also acts as a decomposer agent, aiding in the breakdown of organic matter in the biofertilizer (Batara et al. 2016). Furthermore, the LWB formula can also contain growth hormones such as Indole-3-acetic acid (IAA), cytokinin (zeatin), cytokinin (kinetin), and gibberellin, which can be used to support plant growth (Sodiq et al. 2019). Research by Sodiq et al. (2021) showed that LWB contains GPMs such as *Bacillus cereus* and *Lysinibacillus* sp., which can be used as a biofertilizer inoculant consortium.

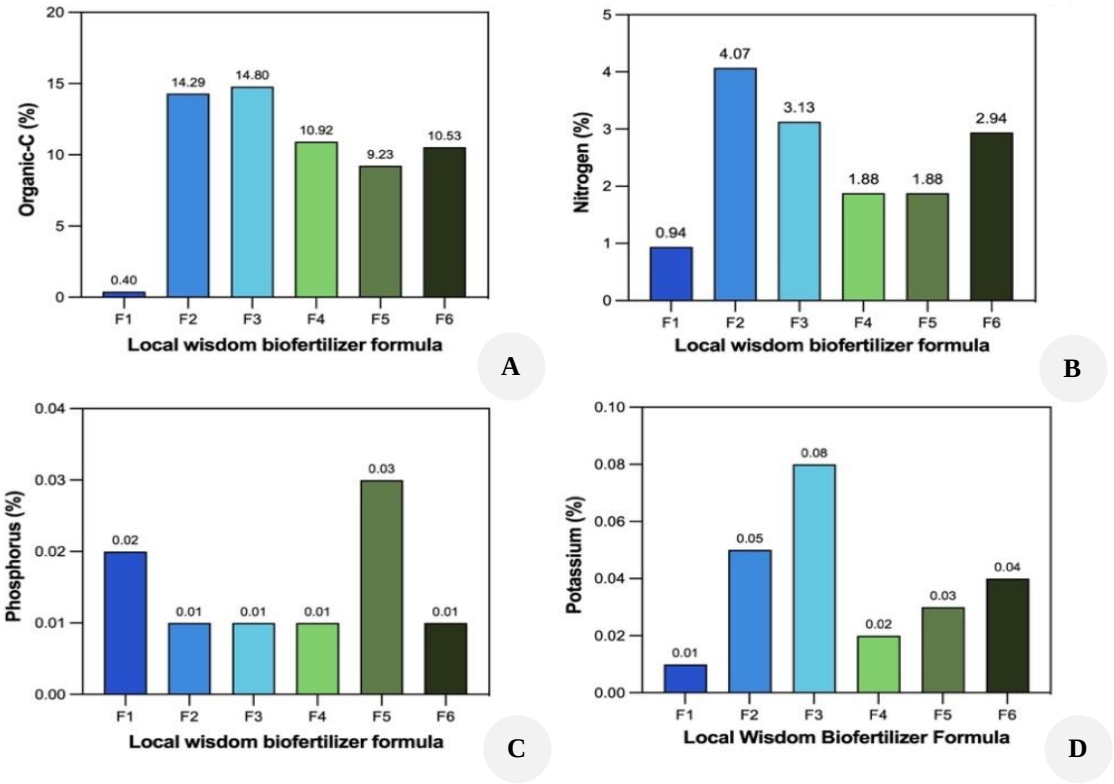

Figure 2. Macronutrient content of local wisdom biofertilizer. A. Organic-C; B. Nitrogen; C. Phosphorus; D. Potassium. F1: Plant-based LWB, F2: Animal-based LWB, F3: Plant and animal-based LWB, F4: Jakaba, F5: Nabati Jakaba-based LWB, F6: Animal Jakaba-based LWB

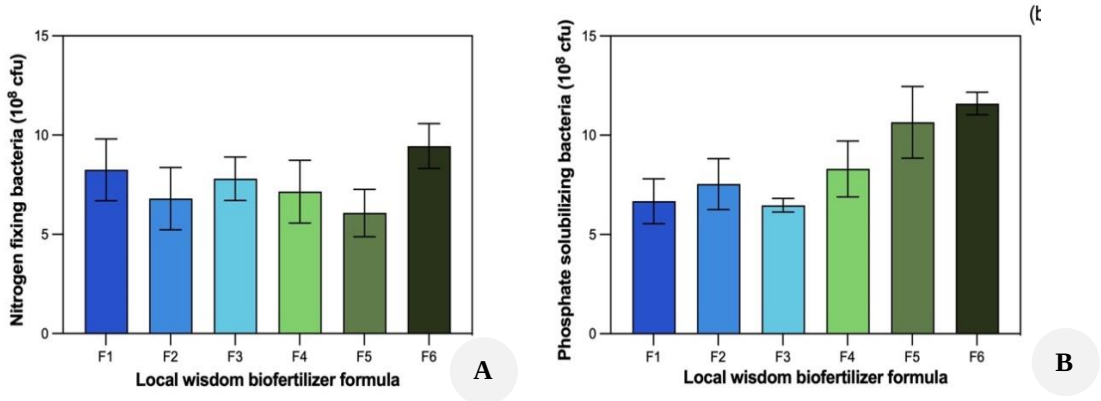

Figure 3. Diversity of growth-promoting microorganism populations in the LWB formula. F1: Plant-based LWB, F2: Animal-based LWB, F3: Plant and animal-based LWB, F4: Jakaba, F5: Nabati Jakaba-based LWB, F6: Animal Jakaba-based LWB

The influence of formulation and dosage of LWB on the growth traits of maize

The application of local biofertilizer formulas and dosages influenced the morphological characteristics of maize (Table 2). The treatments F3K3, F4K3, F5K1, F5K3, F6K1, and F6K2 effectively increased maize plant height. F3K3 and F5K3 treatments significantly affected the chlorophyll content in corn plants. This condition may be related to an increase in the microbial population in the corn rhizosphere. Adding organic fertilizer increases nutrient absorption by roots through the proliferation of beneficial bacterial groups. This finding is consistent with Lidbury et al. (2019), who demonstrated that the application of organic fertilizers significantly increased bacterial community biomarkers, particularly Bacteroidetes. Bacteroidetes are abundant members of the plant microbiome that suppress pathogens and play a critical role in rhizosphere phosphorus mobilization, a nutrient that often limits plant growth.

Table 2. Morphological traits of maize by different formulas and dosages of local wisdom biological fertilizers

Treatment	PH	Cl	WWS	DWS	WWR	DWR	BM
F0K0	37.5a	6.0a	2.0	0.27	2.27	1.24	1.52
F1K1	37.5a	6.9a	2.1	0.22	3.34	1.73	1.94
F1K2	43.5a	6.7a	2.6	0.31	5.51	3.01	3.32
F1K3	44.5a	6.0a	2.5	0.36	3.16	1.74	2.09
F2K1	41.5a	7.2a	2.7	0.35	5.26	2.96	3.31
F2K2	45.5a	6.0a	2.7	0.23	4.53	2.62	2.84
F2K3	47.5a	6.5a	3.5	0.51	6.48	3.78	4.29
F3K1	43.0a	6.8a	2.2	0.20	3.70	2.16	2.36
F3K2	47.5a	6.6a	2.5	0.36	4.49	2.59	2.95
F3K3	49.5b	9.4b	3.1	0.37	3.05	1.74	2.11
F4K1	43.5a	7.85a	2.4	0.34	3.69	2.00	2.34
F4K2	45.0a	7.7a	2.5	0.31	2.90	1.62	1.92
F4K3	50.0b	6.8a	3.2	0.40	3.06	2.14	2.53
F5K1	48.5b	5.6a	3.2	0.34	4.50	2.64	2.98
F5K2	43.0a	7.4a	2.4	0.25	2.49	1.30	1.55
F5K3	48.5b	8.2b	2.4	0.27	4.66	2.78	3.05
F6K1	49.5b	5.9a	3.0	0.46	5.29	3.02	3.47
F6K2	48.5b	7.3a	3.2	0.48	8.34	5.00	5.48
F6K3	44.0a	7.1a	2.1	0.22	3.09	1.86	2.07

Note: Numbers followed by the different letters in a column are significantly different based on the Duncan test at p≤0.05. PH: Plant height, Cl: Chlorophyll, WWS: Wet weight of the shoots, DWS: Dry weight of the shoots, WWR: Wet weight of the root, DWR: Dry weight of the root, BM: Biomass

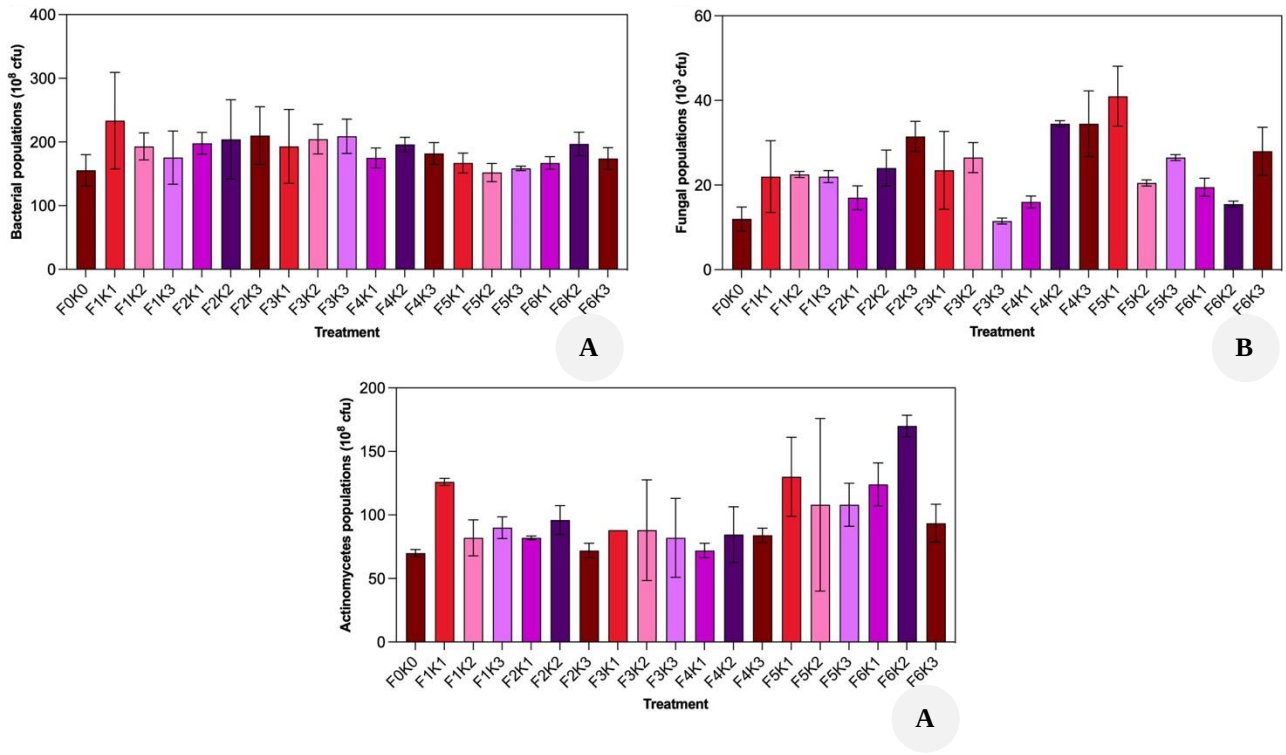

Figure 4. Microbial population the rhizosphere of maize after application of LWB with a combination of formula (F) and dosage levels (K). F1: Plant-based LWB, F2: Animal-based LWB, F3: Plant and animal-based LWB, F4: Jakaba, F5: Jakaba vegetable-based LWB, F6: Animal Jakaba-based LWB. K0: without LWB, K1: LWB dose 2%, K2: LWB dose 4%, K3: LWB dose 6%

Microbial population of local wisdom biofertilizer formulation

The use of LWB on maize plants significantly impacted the microbial populations in the rhizosphere. The F1K1 treatment produced the highest bacterial population, while the F5K1 formulation produced the largest fungal population. Meanwhile, F6K2 produced the highest actinomycete population (Figure 4). This is in line with Fitriatin et al. (2017), which shows that the addition of biofertilizer with P fertilizer can increase the bacterial

population in the soil. The use of organic fertilizers can diminish the growth of pathogenic bacteria like Acinetobacter while promoting greater diversity within rhizosphere bacterial communities (Ma et al. 2022). Microbial communities in the rhizosphere are essential in maintaining soil fertility and supporting plant growth. Microbes in the rhizosphere play a role in the decomposition of organic matter, pathogen management, and nutrient cycling. The microbial content in biofertilizers added to the soil also helps provide essential nutrients for plant growth (Melini et al. 2023). Biofertilizers consist of various microorganisms, including bacteria, that can dissolve phosphate to be available to plants. The presence of phosphate-solubilizing bacteria in the soil allows them to adapt to their environment and compete with native microbes found in the soil (Janati et al. 2023).

The increase in microbial population due to the addition of LWB can occur due to the influence of organic material content in each formula. Organic materials can increase microbial activity, thereby encouraging rhizosphere microbial diversity. Increasing organic matter in the soil also improves soil structure, nutrient retention, and water retention capacity (Kamaa et al. 2011). Apart from that, it also influences the structure of bacterial and fungal communities in the rhizosphere. Changes in microbial community structure can enhance the symbiotic relationship between plants and microbes. This increased interaction can support plant growth and resistance to environmental stress. In addition, rhizosphere microbial diversity also increases resistance to pathogens and reduces the potential for crop failure due to unfavorable soil conditions (Ma et al. 2022).

Table 3. Morphological traits variance revealed by principal components

	PC1
Eigenvalue	4.158
Variability (%)	59.40%
Cumulative %	59.40%

Table 4. Principal component analysis of morphological traits in maize

	PC1
PH	0.199
Cl	0.004
WWS	0.857
DWS	0.805
WWR	0.946
DWR	0.951
BM	0.968

Note: PH: Plant height, Cl: Chlorophyll, WWC: Wet weight of the shoot, DWS: Dry weight of the shoots, WWR: Wet weight of the root, DWR: Dry weight of the root, BM: Biomass

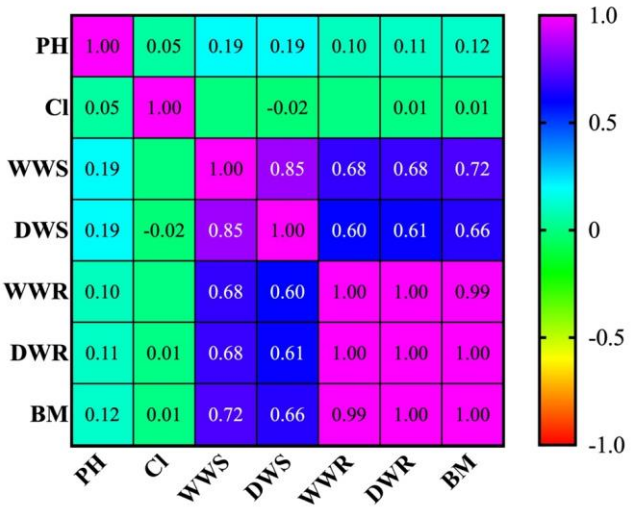

Figure 5. Correlation matrix between morphological characteristics of corn plants and the effect of biofertilizer application. Color reflects the value of the correlation coefficient (r) on a scale from -1 to 1. The traits analyzed include PH: Plant height, Cl: Chlorophyll content, WWS: Wet weight of the shoots, DWS: Dry weight of the shoots, WWR: Wet weight of the roots, DWR: Dry weight of the roots, and BM: Overall biomass. High positive correlation values are shown in dark blue to purple, while values close to zero are shown in bright green to light blue

Pearson's Correlation Analysis of growth traits in maize

Correlation analysis was conducted to assess the relationships among various morphological traits of maize, including plant height, chlorophyll content, wet and dry weights of the shoots, wet and dry weights of the roots, and overall biomass. The pairwise Pearson's correlation analysis in Figure 5 shows that each plant character has a different degree of association. Chlorophyll (Cl) is a pigment in leaves essential to photosynthesis. In the photosynthesis process, Cl plays a role in capturing and converting sunlight into energy in the form of glucose. However, the results of correlation analysis show that chlorophyll has a very weak or almost negligible correlation with other characters, with correlation values ranging from -0.02 to 0.01. This weak correlation indicates that although Cl is essential in photosynthesis, Cl does not directly influence all plant growth characteristics. Characteristics such as root development, disease resistance, or water use efficiency are likely influenced by other factors, such as environmental conditions or microbial interactions (Li et al. 2018). The strength of the correlation can be interpreted using the absolute value of r, very strong (0.90-1.00), strong (0.70-0.89), moderate (0.40-0.69), weak (0.10-0.39), and negligible (0.00-0.10) (Pengphorm et al. 2024). The near-zero correlation values indicate that changes in chlorophyll content do not necessarily correspond to changes in other traits. This condition is likely caused by the adaptation of corn plants to environmental stress, which causes fluctuations in chlorophyll concentration so that it does not have a significant effect on other characteristics (Talebzadeh and Valeo 2022). This condition can occur due to the response

expressed by genes in plant leaves influenced by the LWB formula application. Genes expressed differently in chlorophyll and chloroplast biogenesis are the biomolecular reasons for the formation of green or yellow colors in leaves (Mei et al. 2022). However, to find out the deeper mechanism of this finding, further research is needed on the mechanism of LWB fertilizer in influencing chlorophyll content so that it does not damage other characteristics. The traits, including plant height, wet and dry weights of the shoots, wet and dry weights of the roots, and total biomass, show positive correlations, ranging from moderate to strong positive relationships. This positive association suggests that increases in the others often accompany an increase in one trait. High total biomass in corn is associated with greater wet weight and dry weight of roots, as well as with higher wet weight and dry weight of shoots (Walne et al. 2021).

Morphological traits diversity based on PCA

The use of biofertilizers on maize plants can affect a range of plant characteristics, and the variability of these traits can be thoroughly assessed using Principal Component Analysis (PCA) (Zulchi et al. 2024). PCA helps to describe the variation in variables (plant traits), reveal trait diversity, and explain potential data variance (Acquaah 2012). When applied to maize traits, PCA identified a significant first principal component with an eigenvalue of 1, contributing to 59.40% of the observed variation, as shown in Table 3. This first principal component (PC1) contributes primarily to variations in biomass. Broad trait diversity is generally assessed using a significant dissimilarity coefficient or an Euclidean distance exceeding 1 (Karuniawan et al. 2021). Principal Component 1 (PC1), which primarily represents plant height (PH) with a loading of 0.199 (see Table 4), also captures the effects of other traits. With an eigenvalue of 4.158, PC1 explains 59.40% of the overall trait diversity in maize, serving as a crucial component in conveying information regarding trait diversity and the relationships among superior traits in the biplot diagram.

The biplot shown in Figure 6 accounts for 59.40% of the total variance in the characters, as represented by PC1. In the PC1 biplot, variables with shorter vectors from the origin, such as Cl (chlorophyll) and PH (plant height), demonstrate minimal variation. In contrast, variables with longer vectors, including WWS (wet weight of the shoots), WWR (wet weight of the root), DWC (dry weight of the shoots), DWR (dry weight of the root), and BM (biomass), demonstrate a higher degree of variability. These findings align with previous research, which indicates that vector lengths in PCA biplots reflect the magnitude of variability in specific traits and their influence on the overall variation of the dataset (Jolliffe and Cadima 2016).

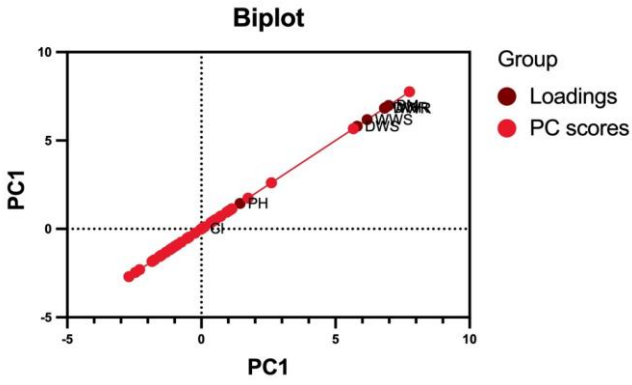

Figure 6. Principal Component Analysis (PCA) biplot showing the distribution of principal component scores (PC scores) and loadings on morphological traits of maize plants. Variables such as PH: Plant height, Cl: Chlorophyll, WWS: Wet weight of the shoots, DWS: Dry weight of the shoots, WWR: Wet weight of the root, DWR: Dry weight of the root, BM: Biomass appear to contribute differently to the principal component. The relationship between variables is described by the direction and length of the loading vector, where variables with the same direction of length vectors have a high positive correlation

In Figure 6, the variation in maize plant traits is indicated by vector lengths, suggesting that the diversity in plant response is likely influenced by individual traits and the availability of nutrients due to biofertilizer application. Previous studies have shown that biofertilizers enhance nutrient uptake efficiency, leading to significant variation in plant growth traits (Bhardwaj et al. 2014). The pattern of variation identified in this study underscores the importance of nutrient-driven physiological responses in maize plants, particularly in traits such as biomass and root shoot weight. These results further support the role of biofertilizers as sustainable agricultural inputs, driving variability in growth traits while enhancing overall crop resilience and productivity (Prasad et al. 2019).

In conclusion, formulation F3 exhibited the highest levels of organic carbon (14.80%) and potassium (0.08%), while F2 recorded the highest total nitrogen content (4.07%), and F5 had the greatest phosphorus content (0.03%). Formulation F6 demonstrated the highest concentrations of nitrogen-fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB) among the treatments. The treatments F3K3, F4K3, F5K1, F5K3, F6K1, and F6K2 effectively enhanced maize plant height, with F3K3 and F5K3 having a particularly significant effect on chlorophyll content. In addition, F1K1 led to the largest bacterial population, F5K1 supported the largest fungal population, and F6K2 demonstrated the highest actinomycetes population. Correlation analysis shows a minimal relationship between Cl content and other characters, with correlation values ranging from -0.02 to 0.01.

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