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RICE-HUSK BIOCHAR AND TRICHODERMA FORTIFICATION MODULATE THE CHEMICAL AND MICROBIOLOGICAL PROFILES OF ORGANIC-WASTE LIQUID FERTILIZER

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Abstract

Liquid organic fertilizer (LOF) derived from mixed organic residues may promote nutrient circularity; however, its chemical quality and microbial viability depend on formulation and fermentation conditions. This study assessed the effects of rice-husk biochar and Trichoderma spp. fortification on LOF produced from banana peel, pineapple peel, rabbit urine, and molasses in a passively aerated stacked-bucket system. A randomized complete block design included four treatments with four replicates: unfortified LOF (P1), Trichoderma alone (P2), 1% w/v biochar (P3), and biochar plus Trichoderma (P4). Chemical variables were analyzed by ANOVA followed by Tukey's HSD test at 5%, whereas microbial and metal data were interpreted descriptively. Fortification did not affect pH (7.20–7.22). P3 had the highest organic C (0.58%) and C/N ratio (5.92), whereas P4 had the highest total N (0.12%), P (0.22%), and K (0.62%). P2 had the highest total fungal count (7.2×10^5 CFU mL^{-1}) and recoverable Trichoderma population (6.5×10^5 CFU mL^{-1}); corresponding values in P4 were 3.8×10^5 and 3.5×10^5 CFU mL^{-1}. Co-fortification improved macronutrient profiles but did not maximize Trichoderma recovery from the liquid fraction. Further optimization and plant-based efficacy testing are required.

Keywords: liquid organic fertilizer; organic waste; passive-aerobic fermentation; rice-husk biochar; Trichoderma

INTRODUCTION

Recycling organic waste into liquid fertilizer is a circular-economy strategy that can reduce disposal burdens while returning carbon and nutrients to agricultural production systems. Liquid organic fertilizers can be produced from crop residues, animal wastes, digestates, and agro-industrial by-products; however, their N, P, K, organic C, pH, and microbial composition vary widely with feedstock and processing conditions. This variability constrains dose consistency, storage stability, microbiological safety, and the reproducibility of plant responses (Chand et al., 2026; Kumareswaran et al., 2024).

The mixture of banana peel, pineapple peel, rabbit urine, and molasses represents plant- and animal-derived organic residues with contrasting characteristics. Fruit peels supply sugars, minerals, and lignocellulosic fractions; rabbit urine provides soluble N; and molasses supplies readily metabolizable carbon. Although this combination may support fermentation, high dilution and separation of the liquid and solid fractions may reduce the recovery of carbon, nutrients, and microorganisms in the final product. Recent evidence also indicates that plant- and animal-derived LOFs differ in their effects on soil fertility, nutrient uptake, and shallot yield, underscoring the importance of feedstock-specific formulation (Ansyori et al., 2026). LOF quality should therefore be verified using measured chemical and microbiological attributes rather than color, odor, or pH alone (Abidin et al., 2024; Wu et al., 2023).

Research on biochar and microbial inoculants has primarily focused on solid-state composting, whereas evidence for diluted LOF produced by passive-aerobic fermentation with leachate recirculation remains limited. Few studies have simultaneously evaluated pH, C/N ratio, organic C, N, P, K, total fungal count, Trichoderma population, Pb, Zn, and partial compliance with technical requirements. The novelty of this study lies in the direct comparison of rice-husk biochar, Trichoderma spp., and their co-fortification in a stacked-bucket system to identify trade-offs between nutrient enrichment and inoculant recovery in the liquid fraction. This study aimed to evaluate the chemical

and microbiological responses of organic-waste LOF to these three fortification strategies. We hypothesized that the formulation producing the most favorable macronutrient profile would not necessarily support the highest Trichoderma population.

LITERATURE REVIEW

Biochar has a porous structure, high surface area, surface charge, functional groups, and an ash fraction that can influence pH, ammonium adsorption, phosphorus transformation, carbon retention, humification, and microbial community composition. However, biochar responses are not universal because they depend on feedstock, pyrolysis temperature, application rate, particle size, substrate properties, and oxygen availability (Li et al., 2023; Wang et al., 2023). Meta-analyses and mechanistic studies indicate that biochar can reduce carbon and N losses, but under some conditions it may also accelerate organic matter transformation by altering fungal communities and enzymatic activity (Ye et al., 2023; Yin et al., 2023; Zhang et al., 2024).

Co-application of biochar and microbial inoculants can improve composting by enhancing lignocellulose degradation, N transformation, humification, and P mobilization. These effects have been reported for municipal solid waste, straw, and mixed green waste; however, most studies used solid matrices and higher biochar application rates than those used in LOF systems (Li et al., 2025; Luo et al., 2026; Tahsini et al., 2025; Zhao et al., 2025). A directly relevant tiered-bucket study showed that fermentation system and bioactivator type produced distinct effects on LOF-compost maturity and macronutrient content, with Trichoderma favoring stabilization and EM4 tending to retain higher nutrient concentrations (Ansyori et al., 2025). Findings from solid-state composting therefore cannot be directly extrapolated to liquid fermentation systems involving leachate recirculation and phase separation.

Trichoderma spp. are saprotrophic fungi that produce hydrolytic enzymes and bioactive metabolites and may therefore support the bioconversion of lignocellulosic residues. Their performance depends on strain identity, substrate availability, pH, aeration, microbial competition, and propagule viability (Alias et al., 2022; Lima et al., 2024). Biochar can provide microorganisms with microhabitats, attachment surfaces, and physical protection, but its performance as a carrier is governed by porosity, soluble carbon, P, pH, feedstock, and pyrolysis temperature (Bolan et al., 2023; Debode et al., 2024). This framework suggests that co-fortification may improve the nutrient profile of LOF without necessarily increasing the number of viable Trichoderma propagules recovered from the liquid fraction.

METHOD

Study Period and Location

The study was conducted from January to April 2026. LOF fermentation was carried out at the Agricultural Technology Laboratory, Universitas Satu Nusa Lampung. Chemical analyses were conducted at the Laboratory of the Lampung Center for the Application of Agricultural Modernization and the Integrated Laboratory, Universitas Lampung, whereas microbiological analyses were performed at the Plant Protection Laboratory, Politeknik Negeri Lampung.

Materials and Formulations

Each 15-L fermentation unit contained 2 kg of ripe banana peel, 2 kg of pineapple peel, 2 L of fresh rabbit urine, 500 mL of molasses, and water to a final volume of 15 L. The fruit peels were cut into approximately 1–2 cm pieces before mixing. Rice-husk biochar was added at 1% (w/v), equivalent to 150 g per 15 L. The Trichoderma spp. inoculum was applied at 0.5 g L^{-1} using a suspension with a minimum density of 10^6 spores mL^{-1}.

Passive-Aerobic Fermentation System

Fermentation was conducted in two stacked 20-L buckets. The upper bucket served as the solid-substrate reactor and was perforated at the bottom to allow leachate to drain into the lower bucket. The lower bucket collected the leachate and was fitted with a sampling valve. Passive aeration was provided by two perforated PVC pipes installed vertically in the upper bucket and by ventilation holes in the lid and upper wall. The collected leachate was recirculated to the upper bucket every three days. After 30 days of fermentation, the leachate was held for 14 days and then filtered to obtain the LOF. Changes in color, odor, pH, temperature, and sediment formation were monitored as process indicators but were not used as the sole criteria of maturity.

Experimental Design

The experiment followed a randomized complete block design with four treatments and four replicates, giving 16 experimental units. Each treatment represented one fortification formulation, as presented in Table 1.

Table 1. LOF fortification treatments

Treatment	Formulation
P1	Unfortified LOF
P2	LOF + Trichoderma spp.
P3	LOF + rice-husk biochar
P4	LOF + rice-husk biochar + Trichoderma spp.

Chemical and Microbiological Analyses

pH was measured with a digital pH meter calibrated using pH 4 and 7 buffer solutions. Organic C was determined by the Walkley–Black method, total N by the Kjeldahl method, total P by UV–Vis spectrophotometry after wet digestion, and total K by flame photometry. The C/N ratio was calculated by dividing organic C by total N. Total fungi were enumerated by plate counting on Potato Dextrose Agar and expressed as CFU mL^{-1}. The Trichoderma population was enumerated on a selective medium and confirmed from colony morphology. Pb and Zn were determined using an in-house method with an Agilent 5900 ICP-OES instrument.

Statistical Analysis and Quality Assessment

Chemical data in Table 2 were analyzed using SPSS version 26. Normality, homogeneity of variance, and additivity were assessed using the Shapiro–Wilk, Bartlett, and Tukey tests, respectively. Data meeting these assumptions were subjected to analysis of variance at $\alpha = 0.05$. When treatment effects were significant, means were separated using Tukey's honestly significant difference (HSD) test at the 5% level. Means followed by different letters within a column were considered significantly different. Because the source data for Tables 3 and 4 did not include dispersion measures or post hoc groupings, the microbiological, Pb, and Zn results were interpreted descriptively and were not used to infer statistically significant differences.

Regulatory quality was assessed partially against Decree of the Minister of Agriculture No. 261/KPTS/SR.310/M/4/2019. The estimated macronutrient sum was calculated as total N + (total P × 2.2914) + (total K × 1.2046), thereby converting P to P_2O_5 and K to K_2O. Comparisons of Pb and Zn were considered indicative because laboratory results were reported in mg L^{-1}, whereas the technical requirements are expressed in ppm or on another concentration basis (Ministry of Agriculture of the Republic of Indonesia, 2019).

RESULTS AND DISCUSSION

pH, C/N Ratio, and Nutrient Contents

Table 2. Effects of fortification on pH, C/N ratio, and nutrient contents of LOF

Treatment	pH	C/N	Organic C (%)	Total N (%)	Total P (%)	Total K (%)
P1	7.20 ± 0.03 a	5.33 ± 0.85 ab	0.49 ± 0.03 bc	0.09 ± 0.01 b	0.16 ± 0.01 d	0.41 ± 0.03 c
P2	7.20 ± 0.02 a	4.28 ± 0.54 b	0.45 ± 0.02 c	0.11 ± 0.02 ab	0.20 ± 0.01 b	0.48 ± 0.02 b
P3	7.22 ± 0.04 a	5.92 ± 0.84 a	0.58 ± 0.02 a	0.10 ± 0.01 ab	0.19 ± 0.01 c	0.47 ± 0.03 b
P4	7.20 ± 0.03 a	4.35 ± 0.32 b	0.51 ± 0.03 b	0.12 ± 0.01 a	0.22 ± 0.01 a	0.62 ± 0.02 a

Values are means ± standard deviations of four replicates. Within a column, means followed by different letters differ significantly according to Tukey's HSD test at the 5% level.

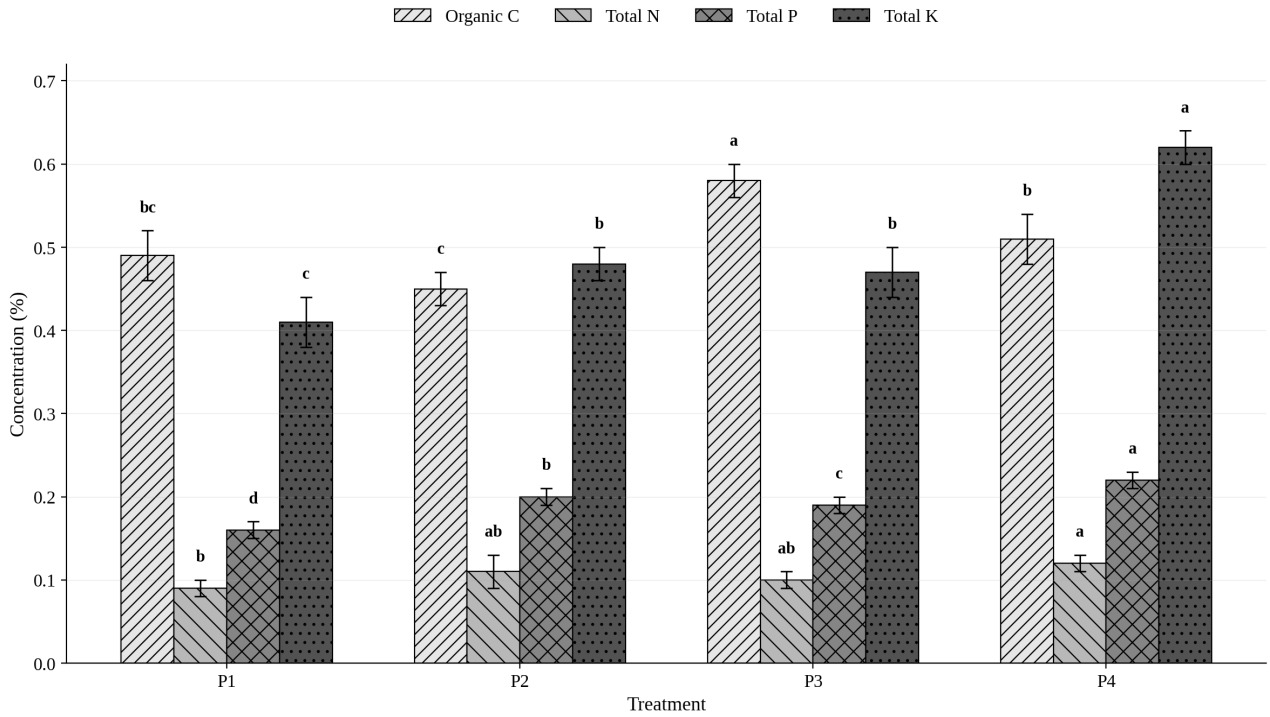

Figure 1. Organic C and macronutrient profiles of LOF. Bars represent means ± standard deviations; different letters within the same variable indicate significant differences according to Tukey's HSD test at the 5% level.

Fortification did not affect LOF pH. All treatments belonged to the same statistical group and varied within a narrow range of 7.20–7.22. This result indicates that the buffering capacity of the system was more influential than the alkalinity of biochar or inoculant metabolism. Biochar can increase or stabilize pH through its ash fraction and surface functional groups, but the response depends on pyrolysis temperature, application rate, initial substrate pH, organic acid production, and aeration (Li et al., 2023; Wang et al., 2023). In practical terms, all formulations were near neutral, and the 0.02-unit difference has negligible agronomic significance.

P3 had the highest C/N ratio (5.92) and differed significantly from P2 (4.28) and P4 (4.35), whereas P1 (5.33) was intermediate. The higher ratio in P3 primarily reflected increased organic C without a proportional increase in N. Conversely, P2 and P4 had lower ratios because their N concentrations were relatively higher. The C/N ratio of the liquid fraction cannot be used alone as a maturity indicator because dilution, particle sedimentation, and the partitioning of C and N between liquid and solid fractions can alter this value. Biochar and inoculants may also modify mineralization, humification, and N retention through different mechanisms (Li et al., 2025; Ye et al., 2023).

P3 had the highest organic C concentration (0.58%), 18.4% higher than P1. P4 contained 0.51% and differed from P3, whereas P2 had the lowest value (0.45%). Biochar alone increased carbon recovery in the liquid fraction, but this effect was attenuated when Trichoderma was added. This pattern may reflect accelerated carbon mineralization by fungi or retention of biomass and carbon particles in the solid fraction. Biochar may enhance humification and carbon retention, yet it can also accelerate organic matter transformation by altering fungal communities and cellulase activity (Yin et al., 2023; Zhang et al., 2024). Because dissolved organic carbon, particulate carbon, CO_2 evolution, and biochar distribution within the reactor were not measured, the dominant mechanism remains uncertain.

P4 had the highest total N concentration (0.12%), representing a 33.3% increase over P1 (0.09%) and a significant difference from the control. P2 (0.11%) and P3 (0.10%) were intermediate. The biochar–Trichoderma combination was more effective than the control in retaining or releasing total N; however, this result does not establish a synergistic interaction because the treatment structure was not analyzed factorially. Biochar can adsorb NH_4^+ and reduce N loss, whereas inoculants can accelerate organic matter decomposition; the final outcome is governed by the balance among mineralization, microbial immobilization, volatilization, and phase partitioning (Li et al., 2025; Wang et al., 2023). Measurements of NH_4^+, NO_3^-, organic N, and NH_3 and N_2O emissions are needed to resolve the underlying mechanisms.

Total P showed clear separation among treatments. P4 had the highest concentration (0.22%), 37.5% greater than P1, followed by P2 (0.20%), P3 (0.19%), and P1 (0.16%); all means belonged to different significance groups. Biochar may contribute P through its ash fraction and alter adsorption–desorption equilibria, whereas microbial activity may release organic P or immobilize it in biomass. Biochar has been reported to mediate P-fraction transformation through changes in microbial communities, but an increase in total P does not necessarily indicate greater plant-available P (Zhao et al., 2025). Therefore, P fractionation and measurement of soluble orthophosphate are required before increased P availability to plants can be inferred.

Total K exhibited the largest response. P4 reached 0.62%, 51.2% higher than the control, and differed significantly from all other treatments. P2 and P3 were in the same statistical group at 0.48% and 0.47%, respectively, whereas P1 had the lowest value (0.41%). Potassium is readily released from plant tissues because it is not structurally incorporated into organic molecules; banana peel is also a K-rich material. The biochar–Trichoderma combination may have enhanced K release from plant tissues while also contributing K from the mineral fraction of biochar. However, the relative contribution of these mechanisms cannot be resolved without characterizing biochar ash, soluble K, and volume balance. Overall, Figure 1 shows that P4 was superior for N, P, and K, whereas P3 was superior for organic C.

Total Fungal Count and Trichoderma Population

Table 3. Total fungal and Trichoderma populations in LOF

Treatment	Total fungal count (CFU mL^{-1})	Trichoderma population (CFU mL^{-1})
P1	2.5×10^4	Not detected
P2	7.2×10^5	6.5×10^5
P3	3.5×10^4	Not detected
P4	3.8×10^5	3.5×10^5

"Not detected" indicates that Trichoderma colonies were not observed using the method applied.

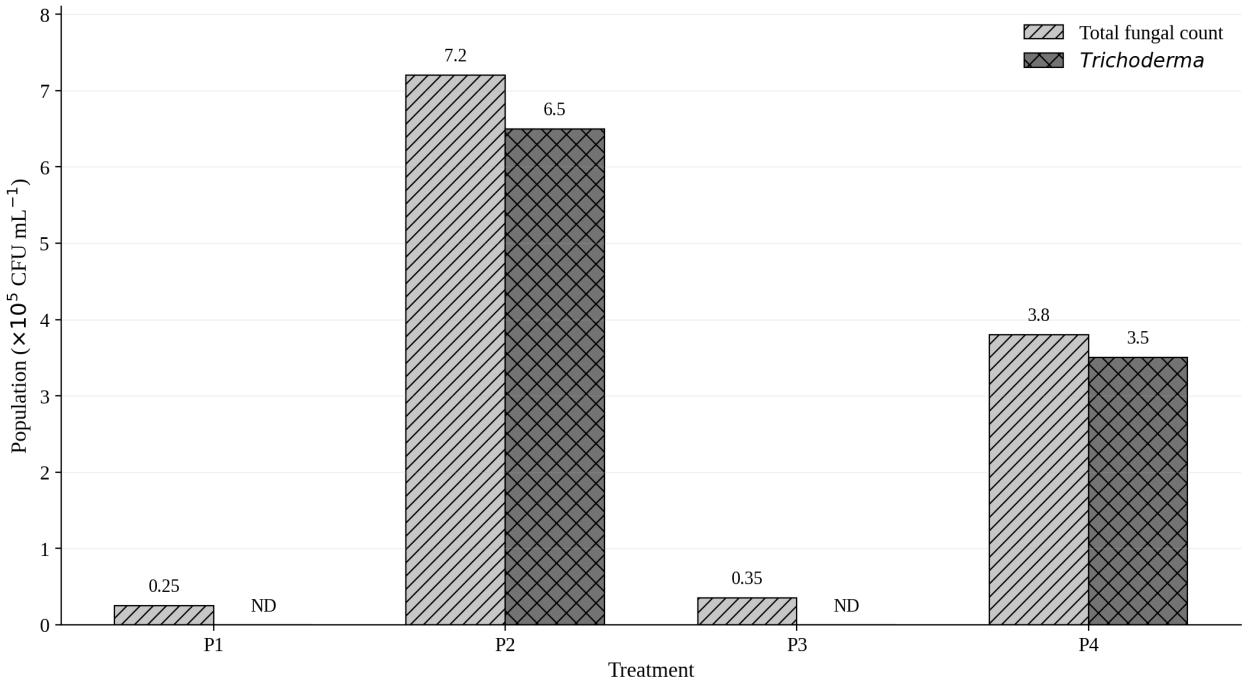

Figure 2. Total fungal and Trichoderma populations across the four formulations (×10^5 CFU mL^{-1}). The graph is descriptive; ND = not detected.

Descriptively, P2 had the highest total fungal count, 7.2×10^5 CFU mL^{-1}, or 28.8 times that of P1. P4 ranked second (3.8×10^5 CFU mL^{-1}), followed by P3 (3.5×10^4 CFU mL^{-1}) and P1 (2.5×10^4 CFU mL^{-1}). Numerically, biochar alone increased the total fungal count by approximately 40% relative to the control, whereas the biochar–Trichoderma combination produced a count 15.2 times that of the control. Nevertheless, the total fungal count in P4 remained 47.2% lower than in P2. This pattern indicates that Trichoderma inoculation alone produced the greatest

fungal recovery from the liquid fraction, whereas adding 1% biochar did not increase the total fungal count relative to P2. Trichoderma was detected only in formulations that received the inoculum. P2 reached 6.5×10^5 CFU mL^{-1}, whereas P4 yielded 3.5×10^5 CFU mL^{-1}, 46.2% lower than P2. This finding does not support the claim that rice-husk biochar, at the rate and under the system tested, acted as a carrier that enhanced Trichoderma recovery from the liquid fraction. Biochar may have retained propagules on its surface or within the solid fraction, thereby reducing their recovery in the leachate, or its chemical properties may not have been suitable for the strain used. Biochar carrier performance is strongly influenced by feedstock, pyrolysis temperature, pH, porosity, soluble carbon, and P (Bolan et al., 2023; Debode et al., 2024). Propagule distribution in the solid and liquid fractions should therefore be quantified separately before carrier performance is concluded.

High colony counts alone do not demonstrate agronomic functionality, pathogen antagonism, or product stability. Enzyme activity, strain identity, P solubilization, production of plant-growth-promoting metabolites, storage viability, pathogenicity, and plant efficacy were not evaluated. Trichoderma has broad bioconversion potential, but its performance is highly dependent on the strain, substrate, and fermentation conditions (Alias et al., 2022; Lima et al., 2024). Based on the available data, P2 is more promising as a source of viable inoculum in the liquid fraction, whereas P4 is more promising for macronutrient enrichment.

Pb, Zn, and Partial Compliance with Technical Requirements

Table 4. Pb and Zn concentrations in LOF

Treatment	Pb (mg L^{-1})	Zn (mg L^{-1})
P1	0.1981	0.0803
P2	0.1037	0.1554
P3	0.0401	0.9208
P4	0.0795	0.3044

Comparisons among treatments are descriptive.

Pb concentrations ranged from 0.0401 to 0.1981 mg L^{-1}. P3 had the lowest value, whereas P1 had the highest. Numerically, all values were well below the maximum Pb limit of 5 ppm specified for LOF, although equivalence between mg L^{-1} and ppm depends on sample density and the reporting basis. The lower Pb values in biochar-amended treatments are consistent with the sorption capacity of biochar; however, without dispersion measures and analysis of dissolved and particulate fractions, these differences cannot be considered statistically significant or explained mechanistically.

Zn concentrations ranged from 0.0803 to 0.9208 mg L^{-1} and were descriptively highest in P3. Zinc is an essential micronutrient rather than solely a contaminant. All values were far below the prescribed micronutrient range of 25–500 ppm. Thus, Zn did not indicate excessive contamination, but its concentration also did not meet the range required for micronutrient fortification. Nutrient and potentially toxic element dynamics during organic-waste processing are influenced by feedstock, pH, mineralization, concentration through mass loss, and binding to organic and mineral fractions (Gao et al., 2023).

Table 5. Partial assessment of LOF quality against minimum technical requirements

Parameter	Observed range	Technical requirement	Partial interpretation
pH	7.20–7.22	4–9	Within the prescribed range
Organic C	0.45–0.58%	Minimum 10% (w/v)	Does not meet requirement
$N + P_2O_5 + K_2O$	Estimated 0.95–1.37%	2–6% (w/v)	Does not meet requirement*
Pb	0.0401–0.1981 mg L^{-1}	Maximum 5 ppm	Numerically below the limit*
Zn	0.0803–0.9208 mg L^{-1}	25–500 ppm	Numerically below the prescribed range*
Total fungal count	2.5×10^4–7.2×10^5 CFU mL^{-1}	≥1×10^5 CFU mL^{-1} for liquid fungal biofertilizers	P2 and P4 meet; P1 and P3 do not meet

Source of requirements: Ministry of Agriculture of the Republic of Indonesia (2019). The estimated $N + P_2O_5 + K_2O$ was calculated as total N + (total P × 2.2914) + (total K × 1.2046). An asterisk denotes an indicative comparison because the units and reporting basis of laboratory results must be aligned with the regulatory basis. The assessment

did not include all mandatory parameters, including organic N, other micronutrients, Escherichia coli, Salmonella spp., pathogenicity, stability, and functional efficacy.

Integrated Interpretation, Study Limitations, and Formulation Implications

The results reveal distinct formulation targets. P3 was most effective in increasing organic C and maintaining the C/N ratio, P4 was most effective in increasing N, P, and K, and P2 had the highest descriptive total fungal count (7.2×10^5 CFU mL^{-1}) and Trichoderma population (6.5×10^5 CFU mL^{-1}). No single formulation was therefore superior across all attributes. The principal finding is a divergence between chemical quality and inoculant recovery: biochar–Trichoderma co-fortification improved the macronutrient profile, but total fungi and Trichoderma recovered from the liquid fraction were lower than with the inoculant alone.

The absence of simultaneous superiority may be explained by the highly diluted system, the low biochar rate of 1%, leachate recirculation, and separation of liquid and solid fractions. This context dependence is consistent with previous tiered-bucket results in which fermentation atmosphere and bioactivator treatment produced different quality outcomes rather than a single universally superior formulation (Ansyori et al., 2025). Co-application of biochar and inoculants in solid-state composting can enhance lignocellulose degradation and nutrient retention (Luo et al., 2026; Tahsini et al., 2025), but in the present system biochar may have altered the partitioning of particles, microbial cells, and nutrients between phases. Characterization of biochar pH, electrical conductivity, cation-exchange capacity, surface area, ash content, total C, P, K, and particle size is required to link material properties to LOF responses.

Study limitations include the absence of measurements of soluble nutrient fractions, pH and temperature dynamics, gaseous emissions, propagule partitioning between biochar and leachate, Trichoderma strain identity, storage stability, microbiological safety, and plant responses. The agronomic relevance of these formulations should ultimately be confirmed through plant-based testing; in Ultisol-grown shallot, plant- and animal-derived LOFs applied with reduced NPK improved soil fertility, nutrient uptake, and yield, although field validation was still recommended (Ansyori et al., 2026). From a product-development perspective, P4 may serve as a starting formulation for N-P-K enrichment, whereas P2 is more suitable for a liquid formulation with a higher Trichoderma density. Nevertheless, all formulations contained organic C and estimated $N + P_2O_5 + K_2O$ below the minimum technical requirements.

CONCLUSION

Rice-husk biochar and Trichoderma spp. fortification did not affect LOF pH but altered the C/N ratio and the concentrations of C, N, P, and K. P3 had the highest organic C and C/N ratio, whereas P4 had the highest total N, P, and K. Descriptively, P2 had the highest total fungal count (7.2×10^5 CFU mL^{-1}) and Trichoderma population (6.5×10^5 CFU mL^{-1}). Total fungi and Trichoderma in P4 were 47.2% and 46.2% lower than in P2, respectively. Thus, co-fortification improved the macronutrient profile but did not maximize inoculant recovery from the liquid fraction. Based on the minimum count for liquid fungal biofertilizers, P2 and P4 met the requirement, whereas P1 and P3 did not. Pb was numerically below the maximum limit, whereas Zn remained below the prescribed micronutrient range. All formulations met the pH requirement, but organic C and estimated $N + P_2O_5 + K_2O$ did not meet the minimum technical requirements for LOF.

Further optimization should address the solid-to-water ratio, biochar dose and properties, inoculation timing, and separation of the solid and liquid fractions. Analyses should include dissolved and particulate organic C, NH_4^+, NO_3^-, P fractions, soluble K, biochar physicochemical properties, strain identification, shelf life, microbiological safety, and plant-based efficacy tests. P4 is the most promising formulation for developing an N–P–K-enriched LOF, whereas P2 is the most promising for a Trichoderma-based liquid formulation.

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