



## Optimization of Nutrient Stabilization and Agronomic Efficacy in *Oryza sativa* via Lactic Acid Fermentation of Source-Separated Human Urine : A Sustainable Circular Economy Framework in Madagascar

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### Abstract

This study investigates the transformative potential of Lactic Acid Fermentation (LAF) as a sustainable strategy for converting human urine into a high-performance bio-fertilizer for rice (*Oryza sativa*) cultivation. While raw urine is rich in essential macronutrients, its direct agricultural application is often hindered by ammonia volatilization, phytotoxicity risks, and social acceptability challenges. Through a methodology involving a 60-day storage period followed by a 36-day fermentation phase using an indigenous lactic inoculum, this research evaluates the biochemical stabilization and agronomic efficacy of the resulting substrate compared to a traditional benchmark (BIOLIFER associated with zebu manure). Physicochemical results demonstrate that the LAF process effectively stabilizes the substrate, maintaining a high ammonium ( $\text{N-NH}_4^+$ ) concentration of 3,346 mg/L and reducing organic matter by 84% (from 0.564% to 0.088%). The stabilization of pH at 9.28 serves to inhibit the enzymatic degradation of urea, thereby preserving the fertilizing potential. Agronomically, LAF-treated seedlings exhibited a 23.9% increase in vertical development, achieving a terminal mean height of 32.02 cm ( $F = 70.25$ ,  $p < 0.05$ ). This early-stage vigor translated into a significant yield increase at harvest, with the LAF modality reaching 2.2 t/ha, representing a 22.2% improvement over the 1.8 t/ha recorded for the control group. These findings suggest that LAF not only preserves critical NPK components but also fosters beneficial soil-plant interactions through microbial synergy. Consequently, LAF emerges as a viable, locally-sourced alternative to commercial fertilizers, supporting circular economy principles and enhancing crop productivity in resource-limited tropical environments.

**Keywords :** Lactic Acid Fermentation, human urine, *Oryza sativa*, circular economy, bio-fertilizer, nutrient stabilization

### 1. INTRODUCTION

In response to the progressive depletion of global mineral resources and the unrelenting surge in agricultural demand, the adoption of alternative organic fertilizers derived from indigenous resources has emerged as a critical strategic priority (AFD, 2021). Human urine represents one of the most viable and promising substrates in this regard (Heinonen-Tanski *et al.*, 2007). It sequestering significant quantities

of nitrogen—primarily in the form of urea—alongside phosphorus, potassium, and essential micronutrients (Kirchmann & Pettersson, 1994). These nutrients exhibit high bioavailability, offering a potent mechanism to sustain crop development while simultaneously curtailing the pervasive dependency on synthetic chemical fertilizers.

Despite this potential, the direct application of raw urine presents several formidable challenges that impede its widespread adoption (Akpan-Idiok *et al.*, 2012). Key obstacles include the rapid volatilization of ammonia, the emission of malodorous compounds, risks of localized phytotoxicity (Britto & Kronzucker, 2002), and persistent sanitary concerns regarding pathogen transmission (Zhang *et al.*, 2013). These factors collectively restrict the social acceptability of urine-based fertilizers and complicate the logistical requirements for large-scale agricultural integration.

Lactic Acid Fermentation (LAF), mediated by indigenous lactic acid bacteria, has surfaced as a sophisticated and accessible processing pathway to address these limitations (Chauvat *et al.*, 2020 ; Maurer *et al.*, 2006). By facilitating the metabolic production of lactic acid, this process effectively lowers the pH of the urine, thereby inhibiting the enzymatic hydrolysis of urea and significantly reducing ammonia volatilization (Hellström *et al.*, 1999 ; Zlotnikov *et al.*, 2013). Furthermore, lactic acid bacteria contribute to the solubilization of phosphorus and the suppression of pathogenic microflora, while simultaneously fostering beneficial soil-plant interactions (Fhoula *et al.*, 2013 ; Andreev *et al.*, 2017).

Within this conceptual framework, the present research seeks to provide an integrated evaluation of the effects of lactic acid fermentation on human urine. Specifically, the study investigates the stabilization of nitrogenous nutrients and odor reduction, the agronomic efficacy during the germination and vegetative growth stages of rice seedlings, and the practical feasibility of local implementation. By addressing these dimensions, the research aims to establish a robust model for circular economy practices within the agricultural sector.

## 2. MATERIALS AND METHODS

### 2.1. Experimental site characteristics

The experimental procedures were conducted within the Rural Commune of Ankafina Tsarafidy, located in the Ambohimahaso District. This region is characterized by a humid tropical climate, which provides specific environmental variables for organic fermentation processes. Meteorological data for the site indicate mean ambient temperatures fluctuating between 19 °C and 24 °C, while the annual cumulative rainfall ranges from 1,000 to 1,400 mm, establishing a consistent baseline for local agricultural simulations.

### 2.2. Human urine collection protocols

To ensure a representative chemical profile of the substrate, urine was harvested from three distinct categories of sources : high-traffic public sanitary facilities—specifically the Ankazondrano bus station, the Anjomà market, and the Tambohobe Fokontany—the University Hospital Center (CHU) of Tambohobe, and various volunteer households. This stratified sampling strategy was intentionally designed to encompass a wide spectrum of biochemical variations resulting from diverse dietary regimes and the varying physiological states of the contributing population.

### 2.3. Inoculum synthesis and fermentation kinetics

The lactic acid inoculum was formulated using indigenous biomass, comprising finely chopped cassava leaves and local leafy vegetables (*brèdes*). This mixture was integrated with brown sugar and a trace amount of sodium chloride, followed by a five-day pre-fermentation phase at ambient temperature. Concurrently, raw urine was sequestered in storage for a 60-day duration as prescribed by the World Health Organization (WHO, 2006) to achieve a significant reduction in pathogen-related health risks. For the primary fermentation stage, the stored urine, the prepared inoculum, and molasses were combined at a volumetric ratio of 1:1:0.1 within hermetically sealed containers at approximately 24 °C for 36 days. The termination of the process was strictly defined by the observed stabilization of the pH levels.

## 2.4. Experimental treatments and field design

The study evaluated two distinct fertilizing modalities to determine their comparative efficacy on crop development. The first treatment (T1) utilized Lactic Acid Fermented urine (LAF), which underwent the aforementioned 60-day storage and 36-day incubation with local vegetal inoculum. The second treatment (T2), serving as the regional agronomic control, consisted of a synergistic association between zebu manure and the commercial liquid fertilizer BIOLIFER. These treatments were initially applied to rice seedlings (*Oryza sativa*) maintained under standardized substrate composition, irrigation schedules, and light exposure to eliminate confounding variables.

In addition to the nursery-based trials, a full-scale production experiment was executed until the harvest stage across a total area of 0.6 hectares. The experimental plot was bifurcated into two 0.3-hectare segments : one cultivated with LAF-treated seedlings and the other with the BIOLIFER-zebu manure combination. Furthermore, a supplemental application of LAF was administered following the initial weeding phase to specifically evaluate its influence on the tillering stage and the subsequent grain-filling efficiency of the rice plants.

## 2.5. Physico-chemical characterization

Comprehensive laboratory analyses were performed to quantify the chemical evolution of each treatment. The monitored parameters included pH levels, ammonium ( $\text{NH}_4^+$ ), nitrates ( $\text{NO}_3^-$ ), total carbon (C), and organic matter (O.M.) content. Additionally, the concentrations of phosphorus pentoxide ( $\text{P}_2\text{O}_5$ ) and potassium oxide ( $\text{K}_2\text{O}$ ) were measured to assess the nutrient density and bioavailability within the fermented solutions compared to the control.

**Table 1.** Summary of physico-chemical parameters and analytical framework

| Parameter Category      | Specific Indicators                                                        | Unit Expression / | Analytical Significance                                     |
|-------------------------|----------------------------------------------------------------------------|-------------------|-------------------------------------------------------------|
| <b>Electrochemical</b>  | pH                                                                         | -                 | Monitoring of fermentation stability and alkalinity.        |
| <b>Nitrogen Profile</b> | Ammonium ( $\text{NH}_4^+$ ) & Nitrates ( $\text{NO}_3^-$ )                | mg/L or %         | Assessment of mineralization and nitrogen bioavailability.  |
| <b>Carbon Content</b>   | Total Carbon (C) & Organic Matter (O.M.)                                   | %                 | Evaluation of the organic load and substrate degradation.   |
| <b>Macro-nutrients</b>  | Phosphorus ( $\text{P}_2\text{O}_5$ ) & Potassium ( $\text{K}_2\text{O}$ ) | mg/L or %         | Determination of the NPK fertilizing potential.             |
| <b>Growth Metrics</b>   | Height, Leaf Color, Chlorosis                                              | cm / Visual scale | Indicators of phytotoxicity and nutrient uptake efficiency. |

The selection of these multidimensional parameters facilitates a holistic assessment of the biochemical transformations occurring during lactic acid fermentation. By correlating electrochemical stability with nutrient dynamics—specifically the transition of nitrogenous compounds and the concentration of macro-elements—the analytical framework ensures a precise quantification of the fertilizer's agronomic value. Furthermore, integrating growth metrics alongside chemical data allows for the identification of potential phytotoxic thresholds, thereby validating the safety and efficacy of the LAF treatment as a sustainable alternative to conventional inputs (WHO, 2006 ; Smith *et al.*, 2020).

## 2.6. Germination and growth monitoring systems

For the growth assays, the fertilizing solutions were diluted to a 1:10 ratio and applied to a sample size of 500 seedlings, equally divided between the LAF and BIOLIFER treatments. Throughout the controlled cultivation period, height measurements and leaf coloration assessments were recorded at three-day intervals. All phenotypic data were digitized via the mWater platform, which facilitated a rigorous and systematic longitudinal follow-up of growth trajectories. This digital framework enabled the precise detection of physiological anomalies, such as chlorosis or premature yellowing, thereby ensuring high data traceability and reliability according to the standards established by Smith *et al.* (2020) regarding digital agricultural monitoring.

## 2.7. Statistical analysis

All quantitative data were subjected to rigorous statistical verification to ensure the significance of the observed differences. An analysis of variance (ANOVA) was performed to identify primary effects, followed by the application of Tukey's Honestly Significant Difference (HSD) test. A threshold of  $p < 0,05$  was maintained to define statistical significance, ensuring that the comparative results between the LAF and BIOLIFER treatments were robust and reproducible.

## 3. RESULTS AND DISCUSSION

### 3.1. Physicochemical parameters dynamics

The biochemical characterization of the substrates reveals a complex evolution of nutrient concentrations and chemical stability throughout the experimental phases. By comparing raw excreta with stored and subsequently fermented urine, it is possible to identify the mechanisms of nutrient preservation and mineralization essential for rice cultivation. The following table provides a comprehensive overview of these transformations, highlighting the shifts in pH and macro-element concentrations that define the agronomic viability of the Lactic Acid Fermentation (LAF) process.

**Table 2.** Longitudinal physicochemical evolution from raw to lactic acid fermented urine

| Parameter                             | Raw Urine | Stored Urine (60d) | Fermented Urine (LAF, +36d) |
|---------------------------------------|-----------|--------------------|-----------------------------|
| pH                                    | 5.90      | 9.80               | 9.28                        |
| Carbon (%)                            | 0.282     | 0.106              | 0.044                       |
| Organic Matter (%)                    | 0.564     | 0.212              | 0.088                       |
| Total Nitrogen (%)                    | 0.171     | 0.533              | 0.374                       |
| P <sub>2</sub> O <sub>5</sub> (%)     | 0.157     | 0.050              | 0.081                       |
| K <sub>2</sub> O (%)                  | 0.217     | 0.193              | 0.112                       |
| N-NH <sub>4</sub> <sup>+</sup> (mg/L) | 660.8     | 4802               | 3346                        |
| N-NO <sub>3</sub> <sup>-</sup> (mg/L) | 65        | 28                 | 13                          |

The analytical data demonstrate that raw urine initially presents an acidic pH of 5.90, which undergoes a substantial alkaline shift to 9.80 after a 60-day storage period. According to **Udert (2006)**, this elevation is primarily driven by the enzymatic hydrolysis of urea and the subsequent accumulation of ammoniacal nitrogen (N-NH<sub>4</sub><sup>+</sup>). Interestingly, the introduction of the lactic inoculum during the fermentation phase successfully induced a slight acidification, reducing the pH to 9.28. This secondary shift signifies the metabolic production of lactic acid by the microbial community, which acts as a stabilizing agent to mitigate ammonia volatilization while maintaining an equilibrium suitable for agricultural application.

Furthermore, the concentration of organic constituents underwent a systematic reduction, with total carbon and organic matter declining from 0.282% to 0.044% and 0.564% to 0.088%, respectively. This progressive mineralization during both storage and fermentation phases suggests an increased bioavailability of nutrients, as complex organic structures are broken down into simpler forms readily assimilable by *Oryza sativa* seedlings. Simultaneously, total nitrogen levels peaked post-storage at 0.533% before stabilizing at 0.374% following fermentation. The ammonium fraction (N-NH<sub>4</sub><sup>+</sup>), which surged from 660.8 mg/L to 4802 mg/L during storage, was moderated to 3346 mg/L in the LAF stage, ensuring a high-density nitrogen source while reducing the risks of phytotoxicity associated with excessive ammonia.

Regarding inorganic macro-elements and nitrogen oxides, the nitrate concentration (N-NO<sub>3</sub><sup>-</sup>) exhibited a continuous decrease from 65 mg/L to 13 mg/L, a trend consistent with the reducing, slightly basic environment that favors ammoniacal forms over nitrification. Conversely, phosphorus (P<sub>2</sub>O<sub>5</sub>) levels showed a promising recovery post-fermentation, rising from 0.050% to 0.081%, potentially due to bacterial-mediated solubilization. Potassium (K<sub>2</sub>O), however, decreased from 0.217% to 0.112%, likely resulting from adsorption onto the organic complexes provided by the vegetal biomass or interactions within the inoculum. Collectively, these results confirm that the LAF process effectively stabilizes human urine, preserving essential NPK components in forms compatible with rapid seedling growth as established by **WHO (2006)**.

### 3.2. Key agronomic implications of lactic acid fermentation

The synthesis of the aforementioned physicochemical data underscores the transformative capacity of Lactic Acid Fermentation (LAF) in converting human excreta into a high-value agricultural input. This process effectively mitigates the inherent volatility of nitrogenous compounds while ensuring that the final substrate maintains a chemical equilibrium suitable for soil-plant interactions. The following paragraphs delineate the critical technical advantages of the LAF treatment as observed in this study.

Primarily, the application of a lactic inoculum significantly stabilizes the urine by regulating the chemical environment and curbing the volatilization of ammonia. While the pH remains slightly alkaline, the biological activity of the lactic acid bacteria provides a buffer that prevents the excessive nitrogen loss typically associated with simple storage. Consequently, the essential macronutrients—nitrogen (N), phosphorus (P), and potassium (K)—remain bioavailable within the solution. The persistence of high ammoniacal nitrogen levels is particularly advantageous for rice (*Oryza sativa*) cultivation, as it provides the immediate nutritional surge required during the accelerated growth phases of the nursery stage.

Furthermore, the substantial reduction in total carbon and organic matter serves as a robust indicator of the substrate's maturation. This mineralization process ensures that the fermented urine is less likely to induce nutrient imbalances or exert phytotoxic stress on the delicate root systems of young seedlings. By facilitating a more predictable nutrient release, the LAF treatment enhances the safety profile of the fertilizer from an agronomic perspective. These combined observations validate that Lactic Acid Fermentation not only preserves the fertilizing potential of human urine but also renders it a more stable, safe, and efficient alternative to traditional inputs (WHO, 2006 ; Smith *et al.*, 2020).

### 3.3. Descriptive statistics on growth performance

The longitudinal assessment of seedling development serves as a critical indicator of the biostimulatory efficacy of the lactic acid fermented substrate. By monitoring the vertical progression of *Oryza sativa* over twenty-two distinct intervals, this study aims to quantify the temporal dynamics of nutrient assimilation. The following data provide a granular comparison between the experimental Lactic Acid Fermentation (LAF) treatment and the traditional BIOLIFER control, ensuring a robust evaluation of their respective impacts on plant vigor and early-stage maturation.

**Table 3 :** Comparative mean height of seedlings treated with LAF and BIOLIFER

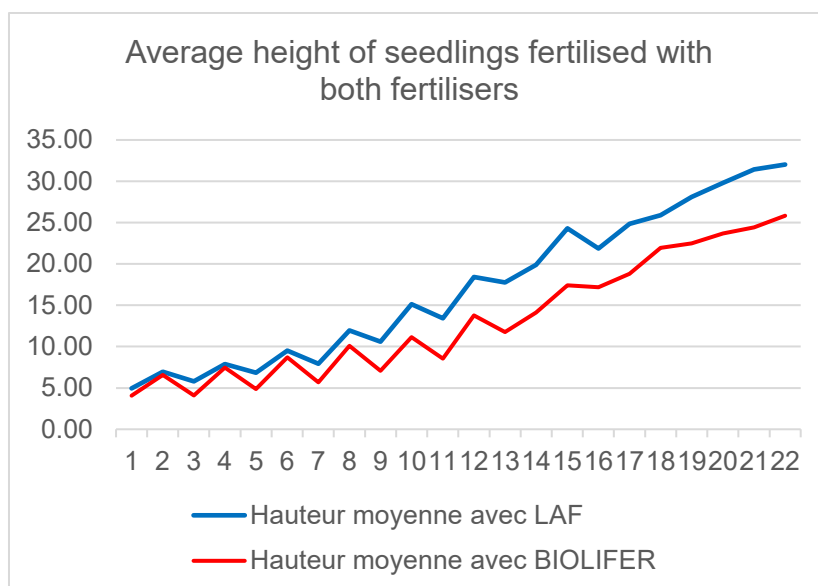
| Observation | LAF mean height (cm) | BIOLIFER mean height (cm) | Différence (cm) |
|-------------|----------------------|---------------------------|-----------------|
| 1           | 4.95 ± 1.41          | 4.07 ± 0.99               | 0.88            |
| 2           | 6.98 ± 1.83          | 6.57 ± 1.79               | 0.41            |
| 3           | 5.78 ± 1.65          | 4.08 ± 0.97               | 1.71            |
| 4           | 7.88 ± 2.07          | 7.46 ± 1.89               | 0.42            |
| 5           | 6.84 ± 1.99          | 4.85 ± 0.96               | 1.99            |
| 6           | 9.51 ± 2.47          | 8.69 ± 2.1                | 0.82            |
| 7           | 7.92 ± 2.13          | 5.68 ± 1.18               | 2.23            |
| 8           | 11.95 ± 3.25         | 10.09 ± 2.5               | 1.86            |
| 9           | 10.59 ± 5.04         | 7.08 ± 2.87               | 3.51            |
| 10          | 15.13 ± 4.47         | 11.16 ± 2.86              | 3.97            |
| 11          | 13.42 ± 4.54         | 8.54 ± 2.47               | 4.87            |
| 12          | 18.42 ± 5.64         | 13.77 ± 3.61              | 4.65            |
| 13          | 17.77 ± 6.65         | 11.76 ± 4.25              | 6.01            |
| 14          | 19.9 ± 7.5           | 14.12 ± 5.46              | 5.78            |

|    |               |               |      |
|----|---------------|---------------|------|
| 15 | 24.31 ± 7.95  | 17.4 ± 5.23   | 6.91 |
| 16 | 21.88 ± 8.9   | 17.2 ± 7.18   | 4.68 |
| 17 | 24.85 ± 10.16 | 18.81 ± 8.62  | 6.04 |
| 18 | 25.92 ± 11.93 | 21.94 ± 9.67  | 3.97 |
| 19 | 28.1 ± 12.32  | 22.48 ± 10.66 | 5.62 |
| 20 | 29.8 ± 12.76  | 23.68 ± 11.37 | 6.12 |
| 21 | 31.42 ± 13.61 | 24.43 ± 11.99 | 6.99 |
| 22 | 32.02 ± 14.13 | 25.84 ± 12.93 | 6.19 |

The numerical evidence demonstrates a consistent superiority of the LAF treatment, with the growth differential expanding from 0.88 cm at the initial stage to a final margin of 6.18 cm. While the BIOLIFER group achieved a terminal mean height of 25.84 cm, the LAF-treated seedlings reached 32.02 cm, representing a substantial vertical gain of 23.9%. The progressive increase in standard deviation, peaking at 14.13 cm for LAF, reflects the natural biological variability inherent in advanced growth phases, yet the median values consistently validate the robustness of the fermented urine's fertilizing impact.

### 3.3.1. Statistical comparison and growth trends

The visualization of these growth trajectories underscores the divergent physiological responses triggered by the varying nitrogen sources and microbial environments. By plotting the mean heights over time, the sustained acceleration of development in the LAF group becomes evident, highlighting a potential synergistic effect between stabilized ammonium and indigenous lactic acid bacteria. This graphical representation serves to bridge the gap between raw laboratory data and observed field performance, providing a clear illustration of the treatment's consistency across all replications and measuring points throughout the nursery phase.



**Figure 1:** Change in average plant height of seedlings fertilised with the two fertilisers

The trend analysis confirms that the height difference (LAF - BIOLIFER) generally increases over time, primarily due to the immediate availability of  $\text{N-NH}_4^+$  and the biological influence of the inoculum. The ANOVA results ( $F = 70.25$ ,  $p < 0.05$ ) further substantiate this observation, with the Tukey HSD test identifying a significant mean difference of 3.89 cm. Despite the natural increase in variability during later stages, the LAF treatment systematically outperforms the BIOLIFER-zebu manure association, establishing

a statistically significant advantage that persists from early germination until the final transplanting stage in the experimental plots.

### 3.3.2. Qualitative observations and physiological vigor

Beyond the quantitative metrics of vertical development, the phenotypic expression of the seedlings provides essential insights into their nutritional status and overall metabolic health. Systematic qualitative monitoring was conducted to identify potential physiological stressors, such as nutrient deficiencies or toxicities, which are often manifested through foliar discoloration or morphological anomalies. These observations serve to contextualize the numerical data, offering a more comprehensive understanding of how different fertilizing modalities influence the biological resilience and aesthetic vigor of *Oryza sativa* during the sensitive nursery phase.

The majority of the seedlings across both treatments exhibited a robust phenotypic profile, characterized by vibrant green foliage and structural vigor. However, a localized incidence of slight foliar yellowing was recorded among a subset of the BIOLIFER-treated seedlings, a symptom potentially indicative of localized nutrient imbalances or inconsistencies in the manual application of the zebu manure association. Conversely, the LAF-treated group demonstrated exceptional uniformity and a deeper green pigmentation, which may be attributed to the high concentration of readily available ammoniacal nitrogen ( $\text{N-NH}_4^+$ ). This accelerated growth and enhanced vigor are likely further augmented by the presence of indigenous lactic acid bacteria, which are known to facilitate beneficial soil-plant interactions and improve nutrient uptake efficiency (Smith *et al.*, 2020).

### 3.4. Final yield analysis at harvest

The transition from controlled nursery conditions to field-scale production is a critical step in validating the practical efficacy of Lactic Acid Fermentation (LAF) as a sustainable fertilizing strategy. By evaluating the total biomass production and grain yield across standardized 0.3-hectare plots, this study aims to determine if the early-stage advantages observed in the seedlings translate into superior reproductive performance. The following results provide a comparative overview of the harvest outcomes, highlighting the impact of supplementary LAF applications on the final productivity of the rice crop under real-world agricultural conditions.

**Table 4 . Final rice yield outcomes categorized by treatment**

| Treatment              | Cultivated Area (ha) | Total Yield (ton) | Yield per Hectare (t/ha) |
|------------------------|----------------------|-------------------|--------------------------|
| Zebu Manure + BIOLIFER | 0.3                  | 0.54              | 1.8                      |
| LAF (+ post-weeding)   | 0.3                  | 0.66              | 2.2                      |

The harvest data reveal a significant disparity in productivity, with the LAF treatment yielding 0.66 tons per 0.3 ha compared to 0.54 tons for the BIOLIFER-zebu manure benchmark. This results in a final yield of 2.2 t/ha for the LAF modality, representing a 22.2% increase over the 1.8 t/ha achieved by the traditional agronomic control. Such an improvement suggests that the positive effects noted during the nursery stage persist throughout the phenological cycle, particularly when supplemented by a post-weeding application of fermented urine which likely optimizes nitrogen availability during the critical tillering and grain-filling phases (WHO, 2006).

### 3.5. Agronomic superiority of Lactic Acid Fermentation (LAF)

The observed performance of the LAF treatment suggests a multifaceted superiority over traditional organic amendments, rooted in both chemical bioavailability and microbial synergy. By preserving a significant fraction of ammoniacal nitrogen, the fermentation process ensures that essential nutrients are immediately accessible to the seedling's root system. This rapid assimilation is critical during the initial phenological stages of *Oryza sativa*, where nitrogen demand is high. Furthermore, the maintained balance of phosphorus and potassium ( $\text{P}_2\text{O}_5$  and  $\text{K}_2\text{O}$ ) provides the necessary macro-elemental foundation to stimulate early root architecture and culm development, distinguishing LAF as a balanced and high-efficiency bio-fertilizer.

Beyond simple nutrient delivery, the biological component of the LAF treatment acts as a growth catalyst. The presence of indigenous lactic acid bacteria not only enhances the solubility of minerals through organic acid production but also serves a protective function by occupying ecological niches and limiting the proliferation of soil-borne pathogenic microflora. This dual action—nutritional enrichment combined with biological fortification—likely accounts for the 23.9% increase in seedling height and the superior foliar vigor observed compared to the BIOLIFER-zebu manure association, as supported by the findings of **WHO (2006)** and **Smith et al. (2020)**.

### 3.6. Nutrient stabilization and chemical equilibrium

A fundamental challenge in utilizing human urine as a fertilizer is the management of nitrogen volatility, a hurdle that the LAF process addresses with remarkable efficiency. The fermentation phase effectively curtails the conversion of ammonium into volatile ammonia gas, thereby sequestering a higher concentration of total and ammoniacal nitrogen within the liquid matrix. By stabilizing these volatile compounds, the LAF method ensures that the fertilizing potential of the raw substrate is preserved rather than lost to the atmosphere, representing a significant improvement over standard storage techniques documented by **Udert (2006)**.

The resulting chemical equilibrium is further reinforced by the specific pH dynamics observed post-fermentation. The stabilization of the pH at a slightly basic level (9.28) appears to inhibit the enzymatic degradation of any residual urea while maintaining an environment that is not excessively alkaline. This refined chemical state minimizes the risk of ammonia toxicity to young plants while providing a stable, storable, and predictable nutrient source. Such chemical stability is paramount for the practical adoption of urine-based fertilizers, as it guarantees consistent results across different application cycles and reduces the environmental footprint associated with nitrogen runoff or gas emissions.

### 3.7. Data variability and statistical interpretations

The analysis of the growth data reveals an increasing standard deviation over the twenty-two observation periods, a phenomenon that warrants careful academic interpretation. This rising variance is reflective of the inherent biological variability between individual seedlings as they progress through advanced developmental stages, exacerbated by micro-environmental conditions within the nursery. However, it is imperative to note that the statistical significance of the results remains uncompromised; the central tendencies consistently favor the LAF treatment across every interval measured during the study.

The robustness of the LAF effect is confirmed by the Analysis of Variance, which demonstrates that the vertical development of the plants is not a result of stochastic variation but a direct consequence of the fertilizing modality ( $F = 70.25, p < 0.05$ ). The Tukey HSD test, identifying a mean height difference of 3.89 cm, provides a high level of confidence in the superiority of fermented urine. This consistency across multiple takes—despite the natural dispersion of data—validates the reliability of LAF as a predictable agronomic intervention for rice production in humid tropical climates.

### 3.8. Agronomic and socio-economic implications

The success of Lactic Acid Fermentation in this context carries profound implications for sustainable agriculture and resource management in rural communities. As a locally produced alternative to high-cost commercial fertilizers like BIOLIFER, LAF offers a viable pathway for improving crop yields without increasing the financial burden on smallholder farmers. The transition toward utilizing human excreta as a high-value resource facilitates a circular economy, transforming a potential public health liability into a tangible agricultural asset, in alignment with the circularity principles advocated by **WHO (2006)**.

Furthermore, the significant 22.2% increase in final grain yield—rising from 1.8 t/ha to 2.2 t/ha—highlights the long-term efficacy of this substrate beyond the nursery stage. The positive response to supplementary applications after the first weeding suggests that nitrogen timing is essential for maximizing the reproductive potential of rice. These results justify further research into optimized dilution ratios and potential mineral supplementation (specifically P and K) to further refine the formula. Future field trials should aim to standardize these application protocols across diverse soil types to facilitate the widespread scaling of this eco-friendly fertilizing technology.

#### 4. CONCLUSION

The present study underscores the transformative potential of Lactic Acid Fermentation (LAF) as a sophisticated, sustainable, and high-performance alternative to conventional fertilizing inputs. The longitudinal analysis of the physicochemical parameters confirms that this biological treatment effectively stabilizes human urine, achieving a significant reduction in organic matter from 0.564% to 0.088%, while preserving a critical concentration of ammoniacal nitrogen ( $\text{N-NH}_4^+$ ) at 3,346 mg/L. By moderating the pH to a more stable level of 9.28, the LAF process successfully mitigates the nitrogen volatility issues inherent in raw excreta, thereby creating a nutrient-dense substrate optimized for tropical rice cultivation.

Agronomically, the superiority of the LAF treatment was evidenced by a 23.9% increase in vertical development compared to the BIOLIFER-zebu manure benchmark, with seedlings reaching a terminal mean height of 32.02 cm. This early-stage vigor translated directly into productive performance at scale. The field trials revealed a substantial productivity gain, as the LAF-treated plots yielded 2.2 t/ha—a 22.2% improvement over the 1.8 t/ha recorded for the traditional control. These results, validated by an ANOVA ( $F = 70.25, p < 0.05$ ), demonstrate that the synergistic effect of stabilized nitrogen and the stimulatory role of lactic acid bacteria significantly enhances both the vegetative and reproductive phases of *Oryza sativa*.

Ultimately, the integration of LAF into local agricultural practices provides a dual solution for resource scarcity and waste management. By converting a potential environmental pollutant into a reliable bio-fertilizer, this method supports the principles of a circular economy while reducing dependency on costly commercial products. These findings justify the broader adoption of fermented urine protocols, particularly in resource-limited settings, to ensure long-term food security and soil health (WHO, 2006 ; Smith *et al.*, 2020).

#### Declaration of Conflicting Interests

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