

Effects of organic and mineral fertigation on the yield and nutritional quality of lettuce (*Lactuca sativa* L.)

Efeitos da fertirrigação orgânica e mineral no rendimento e na qualidade nutricional da alface (*Lactuca sativa* L.)

Efectos de la fertirrigación orgánica y mineral sobre el rendimiento y la calidad nutricional de la lechuga (*Lactuca sativa* L.)

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## **ABSTRACT**

The current high demand for regenerative production systems has stimulated the development and use of biofertilizers as an alternative to mineral fertilizers. This study evaluated the agronomic performance and nutritional quality of lettuce (Lactuca sativa L.) subjected to different types of fertigation in a very clayey dystrophic Red Latosol soil and in a greenhouse. The experiment was conducted in a randomized block design with four treatments/fertigation: mineral (T1 - AM), Hortbio® (T2 - HB), commercial biofertilizer (T3 - BC), and a control with irrigation using only water (T4 - CO). Four replicates were used. Morpho-agronomic and nutritional variables were evaluated. The results indicated that mineral fertigation promoted greater biomass accumulation, while biofertilizers showed intermediate and very similar performance among themselves. As for the nutritional quality of lettuce plants, biofertilizers showed the best results, with mineral fertilization showing the worst results. This was probably due to the formation of precipitates and other insoluble forms of micronutrients by the high activity of phosphate, carbonate, and hydroxyl ions in the soil solution caused using highly soluble fertilizers in fertilizers in fertigation with mineral fertilizers. This study is aligned with emerging themes such as regenerative agriculture, climate justice and just transition, sustainable development goals, and the Brazilian environmental legal framework.

**Keywords:** Biofertilizers. Regenerative Agriculture. Climate Change Adaptation. Climate Change Mitigation. Plant Growth-Promoting Microorganisms.

## **RESUMO**

A grande demanda atual por sistemas de produção regenerativos tem estimulado o desenvolvimento e uso de biofertilizantes como alternativa à adubação mineral. O presente trabalho avaliou o desempenho agronômico e a qualidade nutricional de alface (Lactuca sativa L.) submetida a diferentes tipos de fertirrigação em um Latossolo Vermelho distrófico muito argiloso e em casa de vegetação. O experimento foi conduzido em delineamento de blocos ao acaso, com quatro tratamentos/fertirrigação: mineral (T1 - AM), Hortbio® (T2 - HB), biofertilizante comercial (T3 - BC) e um controle com irrigação apenas com água (T4 -CO). Foram utilizadas quatro repetições. Variáveis morfo-agronômicas e nutricionais foram avaliadas. Os resultados indicaram que a fertirrigação mineral promoveu maior acúmulo de biomassa, enquanto os biofertilizantes apresentaram desempenho intermediário e muito parecido entre eles. Já quanto à qualidade nutricional das plantas de alface, os biofertilizantes apresentaram os melhores resultados, com a adubação mineral apresentando os piores resultados. Isso se deu, provavelmente, pela formação de precipitados e outras formas insolúveis de micronutrientes pela alta atividade de íons fosfato, carbonato e hidroxila na



solução do solo causada pelo uso de fertilizantes muito solúveis na na fertirrigação com adubos minerais. O presente estudo se alinha com temas emergentes como a agricultura regenerativa, a justiça climática e transição justa, os objetivos de desenvolvimento sustentável e com o arcabouço legal ambiental brasileiro.

Palavras-chave: Biofertilizantes. Agricultura Regenerativa. Adaptação às Mudanças Climáticas. Mitigação das Mudanças Climáticas. Microrganismos Promotores do Crescimento Vegetal.

## **RESUMEN**

La elevada demanda actual de sistemas de producción regenerativos ha estimulado el desarrollo y el uso de biofertilizantes como alternativa a los fertilizantes minerales. Este estudio evaluó el rendimiento agronómico y la calidad nutricional de la lechuga (Lactuca sativa L.) sometida a diferentes tipos de fertirrigación en un suelo latosol rojo distrófico muy arcilloso y en un invernadero. El experimento se llevó a cabo en un diseño de bloques aleatorios con cuatro tratamientos/fertirrigación: mineral (T1 - AM), Hortbio® (T2 - HB), biofertilizante comercial (T3 -BC) y un control con riego solo con agua (T4 - CO). Se utilizaron cuatro réplicas. Se evaluaron variables morfoagronómicas y nutricionales. Los resultados indicaron que la fertirrigación mineral promovió una mayor acumulación de biomasa, mientras que los biofertilizantes mostraron un rendimiento intermedio y muy similar entre sí. En cuanto a la calidad nutricional de las plantas de lechuga, los biofertilizantes mostraron los mejores resultados, mientras que la fertilización mineral mostró los peores resultados. Esto se debió probablemente a la formación de precipitados y otras formas insolubles de micronutrientes por la alta actividad de los iones fosfato, carbonato e hidroxilo en la solución del suelo, causada por el uso de fertilizantes altamente solubles en la fertirrigación con fertilizantes minerales. Este estudio está en consonancia con temas emergentes como la agricultura regenerativa, la justicia climática y la transición justa, los objetivos de desarrollo sostenible y el marco jurídico medioambiental brasileño.

Palabras clave: Biofertilizantes. Agricultura Regenerativa. Adaptación al Cambio Climático. Mitigación del Cambio Climático. Microorganismos Promotores del Crecimiento Vegetal.

## 1 INTRODUCTION

Sustainable agriculture is gaining more space in everyday production systems (Brunelle *et al.*, 2024). This is happening not only because of the need to mitigate the environmental impacts of this economic activity, but also because of the need to adopt resilient systems adapted to global climate change (GCC). The Intergovernmental Panel on Climate Change (IPCC, 2021) emphasizes the use



of nature-based solutions as an adaptation strategy, further strengthening this strategy. Solutions that meet the IPCC's co-benefit benchmark, i.e., that can promote greenhouse gas emission mitigation, carbon sequestration, and adaptation to GCC, should be prioritized (IPCC, 2023).

Among the recommended strategies, the use of bio-inputs is one of them (Kurniawati et al., 2023). These products have the potential to increase the tolerance of agricultural systems to climate change, reducing the impacts of abiotic stresses on the productivity and quality of products derived from this economic activity. Biofertilizers, for example, in addition to their primary function of providing nutrients to crops, contain a series of Plant Growth Promoting Microorganisms (PGPM) such as Arbuscular Mycorrhizal Fungi (AMF) (Jumrami et al., 2022) and Plant Growth-Promoting Rhizobacteria (PGPR) (Péres-Bernal et al, 2025), which are capable of releasing hormone-like chemical compounds used by cultivated plants to perform different physiological functions, such as auxins (IAA) (Santos et al., 2020), which participate in various processes such as root emission and morphology, apical dominance, plant growth, geotropism, phototropism, and fruit development (Alatzas, 2013). The mechanisms of adaptation to abiotic stresses can be described as improved nutrient absorption, optimization of photosynthetic processes, promotion of superior oxidizing activity, and improved reproductive capacity (Jumrami et al., 2022). These microorganisms have the potential to play a critical role in increasing tolerance to stresses such as drought, salinity, nutritional deficiency, and heat, establishing symbiosis with more than 80% of terrestrial plants (Tang et al., 2022). Other compounds such as abscisic acid, gibberellins, jasmonates, and strigolactones can promote such adaptation to abiotic stresses by adjusting root growth and stomatal closure.

Lettuce (*Lactuca sativa* L.) is one of the most widely consumed leafy vegetables in Brazil and worldwide (Neves *et al.*, 2017). In the Federal District (DF), for example, it is a socio-economic activity of paramount importance, with its cultivation linked to both corporate and family farming. It is the vegetable with the largest planted area in the DF, occupying 1,414.23 ha. Approximately 23,000 tons are produced annually by 862 rural producers (EMATER DF, 2024). It generates about five jobs per hectare, making it an important source of income for family



farming (Resende *et al.*, 2007). Production costs have increased significantly recently, partly due to GCC, reaching up to 56%, harming producers with low investment potential and access to credit (EMATER DF, 2024), and are therefore important for achieving the IPCC frameworks for climate justice and just transition.

Hortbio® is a biofertilizer widely used in organic agriculture that has an open formula and non-commercial character, registered by Embrapa Hortalicas. Bomfim et al. (2024), when microbiologically characterizing this biofertilizer, found high biodiversity, about 217 isolates, including 120 bacteria, including actinomycetes, 61 yeasts, and 36 filamentous fungi. Groups such as Bacillus, Klebsiella, Kurthia, Enterococcus, Staphylococcus, and Pseudomonas are present. The greatest biodiversity was found 10 days after inoculation with efficient microorganisms (EM), a period coinciding with the highest production of IAA, which is therefore the recommended period for use. Despite their nutritional potential and adaptation to abiotic stresses, their raw materials vary greatly depending on the batch, requiring constant care and characterization, as well as continuous development in search of greater product homogeneity (Cajamarca et al., 2019a). Hortbio® has already been evaluated for the cultivation of lettuce in greenhouses and pots by Cajamarca et al. (2019b) and Bomfim et al. (2023), proving to be efficient but variable depending on the type of lettuce and the cultivar used. Lima et al. (2024) showed the potential of using this biofertilizer to mitigate the negative effects of the average temperature projected for the end of the century in Brazil.

For all the above reasons, and to increase the maturity level of the use of Hortbio® biofertilizer for lettuce cultivation, the aim of this study was to evaluate the productivity and nutritional quality of this vegetable when grown in soil with different inputs applied by fertigation in a greenhouse.



## **2 MATERIAL AND METHODS**

#### 2.1 LOCAL OF STUDY

The experiment was performed at Embrapa Hortaliças, located in the rural area of Brasília, Federal District, Brazil. The geographical coordinates are 15°56'S, 48°08'W and the altitude is 997.6 m. A greenhouse with plastic covering was used, with the following dimensions: 7 m width x 50 m length. The plastic cover was low-density polyethylene with a thickness of 150 microns and antiaphid screen. The crop was grown in soil classified as dystrophic Red Latosol with a very clayey texture. The local climate is Aw (tropical savanna with rainfall concentrated in summer) in the Köppen-Geiger classification. The plant beds were covered with black plastic mulching and fertigation was applied by drip irrigation. The fertigation hoses were located under the mulching to prevent any type of microbiological contamination of the lettuce crop. The black mulch also served to reduce microbiological contamination by warming the plant beds.

## 2.2 EXPERIMENTAL DESIGN

The experimental design used was randomized blocks (RB) with four treatments and four replicates. The treatments used were fertigation with: T1 – mineral fertigation (AM); T2 - Hortbio® fertigation (HB); T3 - commercial biofertilizer fertigation (BC); and T4 - irrigation with water without added fertilizers (CO – control). Each experimental plot contained 12 plants. The production cycle used was 45 days. All treatments were standardized to supply 160 kg/ha of nitrogen. For mineral fertilization, the recommendations for the use of soil correctives and fertilizers in Minas Gerais: 5th Approach (Ribeiro *et al.*, 1999) were used. It was carried out two productive cycles.

Previously, the soil acidity was corrected and base saturation adjusted to 70% using dolomitic limestone. Mineral treatment was composed of a planting fertilization carried out by adding 60 kg/ha of P<sub>2</sub>O<sub>5</sub>. Cover fertigation was carried out using 160 kg/ha of N and 80 kg/ha of K<sub>2</sub>O through fertigation distributed



throughout the crop cycle with three weekly applications. The beginning of all fertigation processes for all treatments occurred 10 days after the transplanting of seedlings. Hortbio®, in turn, was applied based on the nitrogen supply used for mineral fertilization (160 kg/ha), thus resulting in a different nutrient balance in relation to the other elements in comparison with mineral fertigation due to its chemical composition. The same occurred with the use of the commercial biofertilizer. Table 1 shows the chemical and physicochemical composition of Hortbio® determined by Cajamarca *et al.* (2019a).

Table 1. Chemical and physicochemical characteristics of Hortbio® biofertilizer determined in different studies.

Study	рН	С	N	Ρ	K	S	Ca	Mg	Fe
					g/L				mg/L
Ca- jamarca et al.	6.0	200.3#	4.5	0.81	2.93	0.46	3.91	1.00	121.15
(2019a)									

Legend:

pH - hydrogen ion potential C - Carbon; N - Nitrogen; C/N - Carbon-Nitrogen rate; P - Phosphorus; K - Potassium; S -

ogen; C/N – Carbon-Nitrogen rate; P – Phosphorus; K – Potassium; S – Sulfur; Ca – Calcium; Mg – Magnesium; Fe – Iron

Source: The authors modified from Cajamarca et al. (2019).

The following morpho-agronomic variables were determined: fresh weight, dry weight, head diameter, and number of leaves. The concentrations of macro and micronutrients in lettuce plants were also evaluated, and the levels of calcium, phosphorus, potassium, magnesium, sulfur, iron, manganese, copper, zinc, and boron were determined. Plant nutrition was determined jointly from plants harvested in both production cycles.

## 2.3 STATISTICAL ANALYSIS

The data initially underwent screening and outliers were excluded, being replaced by the average of the remaining repetitions. They were also subjected to analysis of variance (ANOVA). The averages were then grouped using Duncan's test. SISVAR 5.6 statistical software was used for these analyses.

To integrate the morpho-agronomic and nutritional variables, a quantitative classification of the best treatments based on the synthesis of multiple variables



was determined using the average rank. This was calculated using only the variables that showed a statistically significant difference to avoid bias and preserve statistical sensitivity. To calculate the average rank, each variable used has its result converted into the follow position between the treatments used (one is attributed for the better treatment, 2 for the second one, and so on). Those variables that belonged to two groups of average classification, for example, ab, received intermediate values, which in this case corresponded to 1.5.

The individual ranks (by variables) were then integrated to compose those referring to morpho agronomic and nutritional variables. For this purpose, the arithmetic mean of the individual ranks was used. Finally, the morpho agronomic and nutritional ranks were also integrated by arithmetic means, obtaining the best treatments. It should be noted that the lower the final value corresponds to the better the treatment performance.

## 2.4 ARTIFICIAL INTELLIGENCE USES

Generative artificial intelligence (AI) as ChatGPT 5, Microsoft Copilot, Data Analyst, and DeepL were used to support data analysis, pre-writing, and translation from Portuguese to English. It was also used to produce figures and graphs. These tools were used only as support, always monitored and corrected by humans.

## **3 RESULTS AND DISCUSSION**

Table 2 shows the productivity data for the two production cycles, while Table 3 shows the treatments ranking obtained from morpho-agronomic characteristics used to calculate the average rank, for each productive cycle. Table 4, in turn, shows nutritional quality of the lettuce produced and Table 5 the reclassification of these variables for average rank calculation.

Among the productivity-related variables evaluated, only dry mass and number of leaves did not show significant ANOVA at a 5% probability in the first cycle. All other variables in this cycle, as well as all variables in the second cycle,



showed statistically significant differences. The attributes Ca, Mg, S, Fe and Mn, related to the nutritional quality of the lettuce produced, did not show significant ANOVA.

T1 showed better results for all morpho-agronomic variables. However, there was a greater difference in these attributes in the first cycle than in the second cycle, which is to be expected, since the use of biofertilizers results in slower nutrient release and continuous improvement in soil quality, leading to better results in the medium and long term. Biofertilizers can improve microbial biodiversity and nutrient cycles, which positively affects agricultural growth and productivity over time. They also have a lower nutrient release rate, allowing for sustained availability and enrichment of soil quality (Liu *et al.*, 2022).

In the first cycle, the fresh mass observed in T1, for example, showed a productivity about 53% higher than the average of the other three treatments, which had their averages classified in the same group by Duncan's test. In the second cycle, for this same variable, production was about 39% higher. In the first cycle, mineral fertilization still showed better results in head diameter formation, while the other three treatments showed lower and statistically equal results. In the second cycle, T1 continued to show the best results for all variables but was statistically equal to T3 for head diameter and statistically equal to T2 and T3 for number of leaves. T2 also did not differ statistically from T3 for head diameter. For fresh mass, T2 and T3 showed the second highest values statistically speaking, while T4 showed the lowest value among all treatments. Finally, for dry mass, in the second cycle, T1 showed the highest value, T2 and T3 showed intermediate values, and T2 also did not differ from T4.

Long-term application of biofertilizers has the potential to improve soil quality and nutrient dynamics, resulting in better crop growth in the medium and long term. This effect is particularly noticeable in successive agricultural crops (Kumawat *et al.*, 2023). In addition, consistent biofertilizer inoculations can increase agricultural productivity in monocultures and intercropping systems, with greater gains as gradual nutrient release occurs (Pérez-Bernal *et al.*, 2025).

Biofertilizers can also synchronize nutrient release with plant demand (Pezzaroli et al., 2020), especially in long-cycle plants. It is possible that the



kinetics of nutrient release using biofertilizers to produce fast-cycle crops, such as lettuce, may not be able to meet the needs in the initial cycles, but as soil quality and health improve, the results may stabilize. The sustained and continuous release of nutrients by biofertilizers, becoming a continuous source for plant nutrition and promoting soil quality improvement, can contribute to better agronomic performance in the medium and long term (Maaz *et al.*, 2025).

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Table 2. Morphological, agronomic, and productive characteristics of lettuce treated with different forms of fertigation.

Treatment	Fresh	Dry mass	Head	Number of	Fresh	Dry mass	Head	Number of		
	mass	(g)	diameter	leaves	mass	(g)	diameter	leaves		
	(g)		(cm)		(g)		(cm)			
		1º ciclo				2º ciclo				
T1 - AM	673,4 a	20,0 ns	18,3 a	27,6 <sup>ns</sup>	307,8 a	15,3 a	16,1 a	23,0 a		
T2 - HB	346,2 b	15,9 ns	13,7 b	23,1 <sup>ns</sup>	165,3 b	9,00 bc	13,7 b	22,6 a		
T3 - BC	306,4 b	14,0 ns	12,6 b	20,8 ns	209,0b	11,5 b	14,5 ab	22,0 a		
T4 - CO	297,2 b	12,0 ns	11,9 b	21,3 <sup>ns</sup>	87,7 c	6,8 c	12,4 b	15,8 b		

Averages followed by the same letter are equal according to Duncan's test at a 5% probability. Legend: T1 – mineral fertigation (AM); T2 - Hortbio® fertigation (HB); T3 - commercial biofertilizer fertigation (BC); and T4 - irrigation with water without added fertilizers (CO – control). nsNot significant at a 5% probability.

Source: The authors

Table 3. Reclassification for calculating the average rank of morphological, agronomic, and productive characteristics of lettuce treated with different forms of fertigation.

Treatment	Fresh	Dry mass	Head	Number of	Fresh	Dry mass	Head	Number of	
	mass	(g)	diameter	leaves	mass	(g)	diameter	leaves	
	(g)		(cm)		(g)		(cm)		
	1º ciclo				2º ciclo				
T1 - AM	1	NC	1	NC	1	1	1	1	
T2 - HB	2	NC	2	NC	2	2.5	2	1	
T3 - BC	2	NC	2	NC	2	2	1.5	1	
T4 - CO	2	NC	2	NC	3	3	2	2	

Legend: NC – Not considered to the average rank calculation. T1 – mineral fertigation (AM); T2 - Hortbio® fertigation (HB); T3 - commercial biofertilizer fertigation (BC); and T4 - irrigation with water without added fertilizers (CO – control).

Source: The authors

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Table 4. Nutritional quality of lettuce produced with different forms of fertigated.

Treatment	Ca	Р	K	Mg	S	Fe	Mn	Cu	Zn	В
	mg.kg <sup>-1</sup>									
T1 - AM	9713,1 ns	3789,2 a	88182,4 ab	2582,0 ns	2387,0 ns	296,4 ns	74,4 <sup>ns</sup>	5,6 b	44,3 b	5,6 b
T2 - HB	9530,6 ns	3476,8 ab	94256,2 a	2930,0 <sup>ns</sup>	3359,3 ns	339,7 ns	72,3 ns	9,8 a	51,8 ab	11,4 a
T3 - BC	8250,9 ns	3114,4 b	77784,4 ab	2617,0 ns	3159,4 ns	396,7 ns	73,1 <sup>ns</sup>	8,1 ab	61,4 a	11,4 a
T4 - CO	9275,6 ns	3056,9 b	76209,8 b	2722,0 ns	2739,5 ns	368,7 ns	85,1 <sup>ns</sup>	8,6 ab	67,2 a	12,0 a

Averages followed by the same letter are equal according to Duncan's test at a 5% probability. Legend: T1 – mineral fertigation (AM); T2 - Hortbio® fertigation (HB); T3 - commercial biofertilizer fertigation (BC); and T4 - irrigation with water without added fertilizers (CO – control). nsNot significant at a 5% probability.

Source: The authors

Table 5. Reclassification for calculating the average rank of nutritional quality characteristics of lettuce treated with different forms of fertigated.

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Treatment	Ca	Р	K	Mg	S	Fe	Mn	Cu	Zn	В
	mg.kg <sup>-1</sup>									
T1 - AM	NC	1	1.5	NC	NC	NC	NC	2	2	2
T2 - HB	NC	1.5	1	NC	NC	NC	NC	1	1.5	1
T3 - BC	NC	2	1.5	NC	NC	NC	NC	1.5	1	1
T4 - CO	NC	2	2	NC	NC	NC	NC	1.5	1	1

Legend: NC – Not considered to the average rank calculation. T1 – mineral fertigation (AM); T2 - Hortbio® fertigation (HB); T3 - commercial biofertilizer fertigation (BC); and T4 - irrigation with water without added fertilizers (CO – control).

Source: The authors



Regarding the nutritional quality of lettuce fertirrigated with different types of fertilizers, ANOVA proved to be insignificant for the following attributes: Ca, Mg, S, Fe, and Mn. For the element P, T1 and T2 presented the best results. T2, in turn, also did not differ statistically from treatments T3 and T4, and therefore, it is a treatment whose result can be considered intermediate. For K, the best result was observed for T2, which did not differ statistically from T1 and T3. The latter, in turn, also did not differ statistically from T4. The Cu concentration was higher for T2, not differing statistically from T3 and T4. The worst result was observed for T1, which did not differ statistically from T3 and T4. Zn, in turn, showed higher concentrations in T4 and T3, not differing statistically from T2. T2 belongs to both the group that presented the best result and the average treatment of the group that presented the worst result (T1). B, in turn, was more concentrated in plants fertigated with HB and BC, as well as those irrigated only with water. These values differed statistically from the treatment with mineral fertilization.

In terms of nutrition, the good results found for the treatment that used only water for irrigation are noteworthy. This may be due to a series of processes, such as lower leaf growth, which, when associated with the absorption of micronutrients from the soil, which had been used for vegetable crops for over 20 years, resulted in a higher amount of micronutrients in milligrams per kilogram of plant produced. Another potential explanation is the non-formation of precipitates, which may have occurred in mineral fertilization, with the formation of phosphates, hydroxides, and carbonates of Mn, Zn, Cu, and B. In the case of B, it is possible that borates were also formed. Additionally, in the mineral fertilization treatment, no micronutrients were used. Finally, competition for adsorption sites in soil clays, especially with macronutrients, may also have contributed to this. These hypotheses are reinforced by the higher P values observed for mineral fertilization, an element added in fertilization.

The availability and fractions of P in soils influence the relative uptake of micronutrients (Dutta *et al.*, 2024). Long-term agricultural systems are still capable of promoting nutrient recycling, improving the available stock of micronutrients (Bhatt *et al.*, 2023). It shows that high concentrations of P via



mineral fertilizer can interfere with the absorption of essential micronutrients through precipitation and competitive synergies with secondary elements and the microbiological complexity of the soil. Sheperd & Oliverio (2024) show that the presence of high ionic phosphorus activity in soils can promote precipitation and competition with essential micronutrients, reducing their absorption. The precipitation of heavy metals by phosphates, hydroxides, and carbonates under high doses of P was also discussed by Ahmed *et al.* (2024). Competition between macro and micronutrients for clay adsorption sites affecting the availability of the latter is also reported by Zhao *et al.* (2023). However, He *et al.* (2024) demonstrate that the presence of macronutrients can reduce the retention of critical micronutrients by soil clays, highlighting the importance of competition in the availability of the elements that make up the latter group.

Biofertilizers containing humic acid, associated with PGPR, promote greater nutritional efficiency through the formulation of nanocapsules that promote the controlled release of micronutrients (Hamed *et al.*, 2024). Biofertilizers are sources of micronutrients with mechanisms such as complexation by humic substances that promote the gradual release of ions, a positive effect on rhizosphere microbiota, promoting solubilization and absorption (Bakki *et al.*, 2024; Ghazanfar *et al.*, 2024; Maaz *et al.*, 2025; Pérez-Bernal *et al.*, 2025).

## Calculation of the Average Rank for variables linked to morphoagronomic/productive characteristics

How to calculate: Sum all valid ranks (ignore "NC") and divide by the number of characteristics considered (number of valid values).

## AM:

Reclassification values: 1 (FM1), 1 (HD1), 1 (FM2), 1 (DM2), 1 (HD2), 1 (NL2)

Total: 6 variables with statistically significant difference

Average Rank for AM: (1 + 1 + 1 + 1 + 1 + 1) / 6 = 1.00

HB:

Reclassification values: 2 (FM1), 2 (HD1), 2 (FM2), 2.5 (DM2), 2 (HD2), 1 (NL2)



Total: 6 variables with statistically significant difference

Average Rank for HB: (2 + 2 + 2 + 2.5 + 2 + 1) / 6 = 1.92

BC:

Reclassification values:2 (FM1), 2 (HD1), 2 (FM2), 2 (DM2), 1.5 (HD2), 1

(NL2)

Total: 6 variables with statistically significant difference

Average Rank for BC: (2 + 2 + 2 + 2 + 1.5 + 1) / 6 = 1.75

CO:

Reclassification values: 2 (FM1), 2 (HD1), 3 (FM2), 3 (DM2), 2 (HD2), 2 (NL2)

Total: 6 variables with statistically significant difference

Average Rank for CO: (2 + 2 + 3 + 3 + 2 + 2) / 6 = 2.33

Where: FM1 – Fresh Mass in the first productive cycle; HD1 – Head Diameter in the first productive cycle; FM2 - Fresh Mass in the second productive cycle. DM2 – Dry Mass in the second productive cycle; HD2 - Head Diameter in the second productive cycle; NL2 – Number of Leaves in the second productive cycle.

Table 6 summarizes the final classification of the Average Rank for morpho-agronomic and/or productivity variables while Table 7 presents a summary of the Average Rank calculated for variables related to lettuce plant nutrition. Additionally, Table 8 shows the Integrated Average Rank (average of the ranks calculated for the morpho-agronomic/productivity and nutritional quality variables of the plants).

Table 6. Summary of the final classification of the Average Rank for morphoagronomic/productivity variables

Treatment	Averange Rank					
AM	1.00					
BC	1.75					
НВ	1.92					
CO	2.33					

Legend: T1 – mineral fertigation (AM); T2 - Hortbio® fertigation (HB); T3 - commercial biofertilizer fertigation (BC); and T4 - irrigation with water without added fertilizers (CO – control).

Source: The authors



# Calculation of the Average Rank for variables linked to nutritional quality of lettuce plants

## AM:

Reclassification values: 1 (P), 1.5 (K), 2 (Cu), 2 (Zn), 2 (B)

Total: 5 variables with statistically significant difference

Average Rank for AM: (1 + 1.5 + 2 + 2 + 2) / 5 = 1.7

HB:

Reclassification values: 1.5 (P), 1 (K), 1 (Cu), 1.5 (Zn), 1 (B)

Total: 5 variables with statistically significant difference

Average Rank for HB: (1.5 + 1 + 1 + 1.5 + 1) / 5 = 1.2

BC:

Reclassification values:2 (P), 1.5 (K), 1.5 (Cu), 1 (Zn), 1 (B)

Total: 5 variables with statistically significant difference

Average Rank for BC: (2 + 1.5 + 1.5 + 1 + 1) / 5 = 1.4

CO:

Reclassification values:2 (P), 2 (K), 1.5 (Cu), 1 (Zn), 1 (B)

Total: 5 variables with statistically significant difference

Average Rank for CO: (2 + 2 + 1.5 + 1 + 1) / 5 = 1.5

Table 7. Summary of the final classification of the Average Rank for plant nutrition characteristics

Treatment	Averange Rank
HB	1.20
BC	1.40
CO	1.50
AM	1.70

Legend: T1 – mineral fertigation (AM); T2 - Hortbio® fertigation (HB); T3 - commercial biofertilizer fertigation (BC); and T4 - irrigation with water without added fertilizers (CO – control).

Source: The authors



## **Calculation of the integrated Average Rank**

Table 8. Integrated average rank for the morphoagronomic/productivity and nutritional quality variables of lettuce plants

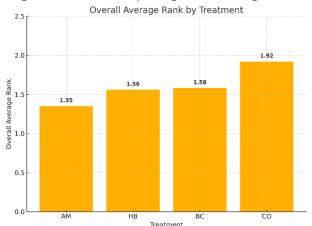
Treatment	Rank Productivity	Rank Nutritional Quality	Integrated Average Rank
AM	1.00	1.70	(1.00 + 1.70)/2 = 1.35
HB	1.92	1.20	(1.92 + 1.20)/2 = 1.56
ВС	1.75	1.40	(1.75 + 1.40)/2 = 1.58
СО	2.33	1.50	(2.33 + 1.50)/2 = 1.92

Legend: T1 – mineral fertigation (AM); T2 - Hortbio® fertigation (HB); T3 - commercial biofertilizer fertigation (BC); and T4 - irrigation with water without added fertilizers (CO – control).

Source: The authors

Figure 1, in turn, shows, through a column chart, the integrated Average Rank for each treatment used (mineral fertilizer, biofertilizers, and control).

Figure 1. Integrated Average Rank for each treatment used to produce lettuce grown in soil and greenhouse with drip fertigation and irrigation.



Legend: T1 – mineral fertigation (AM); T2 - Hortbio® fertigation (HB); T3 - commercial biofertilizer fertigation (BC); and T4 - irrigation with water without added fertilizers (CO – control).

Source: The authors

The Average Rank calculation clearly shows the better performance of mineral fertilization when analyzing morphoagronomic and productivity attributes. The commercial biofertilizer, in turn, obtained the second-best result, but was much less efficient than the first. The same was true for Hortbio® (third best) and the control without fertilization (last position). However, in terms of nutritional aspects, the picture is quite different, with Hortbio® showing the best position, followed by commercial biofertilizer, control without fertilization, and mineral



fertilization. When the two average ranks are combined, mineral fertigation shows the best performance (1.35), closely followed by Horbio (1.56) and commercial biofertilizer (1.58). The control treatment had the worst result, with a value far from those observed for the other treatments (1.92). Strategies for quantitative ranking of multiple treatments in agricultural sciences have become an increasingly used alternative, reducing the subjectivity of decisions and conclusions of the work carried out, as shown by those conducted by Brown *et al.* (2022) and Tohid & Olafson (2025).

## **4 CONCLUSIONS**

- 1. Mineral fertigation provided better agronomic performance in terms of production, as shown by Duncan's test and by the Mean Rank for this variable;
- 2. Fertigation with Hortbio provided better nutrition for lettuce plants, as also shown by Duncan's test and the Mean Rank;
- 3. The integrated Mean Rank showed that mineral fertilization was the best treatment, closely followed by biofertilizers and, finally and far behind the first two, by control without fertilization;
- 4. The better nutritional effect of biofertilizers in terms of micronutrient absorption is probably linked to the formation of organic complexes capable of making these elements available for absorption by lettuce plants;
- The worse nutritional effect of mineral fertigation is probably due to the formation of unavailable forms of micronutrients with phosphate, carbonate, and hydroxyl ions;
- 6. Biofertilizers gradually release their nutrients, which, for a fast-cycle crop such as lettuce, can translate into productivity through improved soil health in the medium and long term;
- 7. This work is aligned with emerging agendas such as Sustainable Development Goals (SDGs) number 1 (poverty eradication), 2 (zero hunger and sustainable agriculture), 3 (health and well-being), 10 (reduction of inequalities), 11 (sustainable cities and communities), 12 (responsible consumption and production), and 13 (climate action);



- 8. It is also aligned with various public policies such as the National Bioinputs Program, the National Urban and Peri-Urban Agriculture Program, the National Agroecology and Organic Agriculture Plan, the National Environmental Policy, the National Water Resources Policy, the National Climate Change Plan, the National Adaptation Plan, and the National Solid Waste Plan, among others. Ultimately, this study is related to Article 225 of the Brazilian Federal Constitution;
- As it is a crop widely used in family farming, this article also aligns with the concepts of climate justice and just transition, which are benchmarks of the IPCC.

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