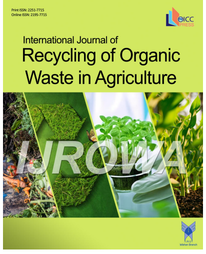

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Effect of mineral liquid organic fertilizer on soil and morphological/physiological parameters in *ICA Cerinza* bean cultivation until flowering stage

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Abstract:

Purpose: The production of beans and food in general at the global level has been threatened by the high costs of industrially synthesized fertilizers. Therefore, the goal of this work was to evaluate the effect of a mineral organic liquid fertilizer (MOLF) on the chemical properties of the soil and the morphological and physiological parameters in *ICA Cerinza* bean cultivation up to the flowering stage.
Method: The crop was established in open fields in bags and randomly distributed. Aerobic fermented MOLF and commercial liquid fertilizer were evaluated up to the flowering stage. The behavior of micro and macronutrients in the soil was determined using Mehlich-3 and Bray II extractants. In addition, physiological parameters were evaluated in the plants.
Results: The incorporation of MOLF in *ICA Cerinza* bean cultivation increased the content of available P, with results very similar to those of commercial fertilizer. Higher values were observed in chlorophyll content compared to commercial fertilizer. Fluorescence data showed heterogeneous behavior in treatments and which was associated with nutrient content in the soil. Oxalic, citric, formic, acetic, lactic, and succinic acids were identified, with their concentration being higher in the early stages of growth and their production was closely related to the P content and the addition of MOLF.
Conclusion: This study demonstrates the potential of using MOLF in bean crops, improving the availability of nutrients in the soil and promoting the secretion of organic substances that can contribute to nutrient mobilization, contributing to fundamental stages such as flowering.

Keywords: Organic acids; Solid urban organic waste; Nutrient availability; Liquid waste

1. Introduction

Legumes are crops of great global importance due to their impact on human health (Barman et al., 2018). *Phaseolus vulgaris*, commonly known as the common bean, is considered a food that can serve as an alternative to both plant and animal-based sources due to its high content of protein and micronutrients such as iron (Fe) and zinc (Zn) (Ganesan and Xu, 2017). Additionally, it contains a wide variety of phytochemical compounds with biological activity that may influence glycemic control and cardiovascular risk factors in type 2 diabetes mellitus (Jenkins et al., 2012), decreasing inflammatory response and improving insulin sensitivity (Sánchez-Tapia et al., 2020). It helps prevent insulin resistance and metabolic endotoxemia by modulating the gut microbiota (McNabney and Henagan, 2017) and reduces the pro-inflammatory state and enhances metabolic characteristics in individuals with overweight and obesity (Hermsdorff et al., 2011), leading many communities worldwide to maintain its production. In Colombia, bean cultivation represents one of the most important dietary components due to its high protein content and provides economic support to small and medium scale producers. The significance of bean cultivation in the national territory has resulted in the designated hectares not decreasing considerably; for example, in 2020 there was a decrease of about 9% in the area planted compared to 2019, which led to an 11.8% reduction in production (agricultura, 2021); however, the trend in planted areas showed an increase for 2021, where it is noted that between January and April 2020, 35,200 ha were set aside, and for the same period in 2021, this increased to 40,911 ha for bean planting (agricultura, 2021), as a consequence of the action plans implemented by national, departmental, and local government entities.

On the other hand, the production of beans and food production in general, worldwide, has been threatened by the high costs of industrially synthesized fertilizers. In the case of Colombia, increases of up to 43% in acquisition costs have discouraged producers due to low profitability. Consequently, this has led to the implementation of solid or liquid organic fertilizers from animal sources, plant residues (Chatzistathis et al., 2021), or urban organic solid waste (UOSW) to enhance crop production. It has been shown that their incorporation contributes to the recovery and improvement of the physical, chemical, and microbiological properties of the soil in the medium and long term (Triharyanto et al., 2022; Iqbal et al., 2019; Itelima et al., 2018). Different studies have demonstrated that this practice can reduce the use of highly soluble fertilizers (Bohórquez-Sandoval et al., 2024), improve production yields, and generate economic gains (Chew et al., 2019; Malick et al., 2019). However, the low concentrations of micro and macronutrients have limited its implementation in the agricultural sector; this issue has been addressed by incorporating minerals in the form of apatites, dolomites, and sulfates, as reported by García et al. (2021). Additionally, the transformation of these leachates into fertilizers enables the return of organic nutrients that can be utilized by soil microorganisms and plants, thus preventing potential damage to water effluents and soils (Lucero-Sorbazo et al., 2022).

The benefits of organic fertilizers in crops are associated with the contribution of components such as dissolved organic matter, nutrients, microorganisms, and organic substances (Cuy et al., 2024) as well as the various actions they can perform in biogeochemical cycles, such as fixing or mineralizing N, solubilizing organic or mineral P. Furthermore, changes in the physical, chemical, and microbiological properties of the soil can affect the phenological stages of the crop, such as germination, flowering, and harvest (Alzamel et al., 2022). Therefore, promoting microbial diversity helps prevent further soil degradation (Bebber and Richards, 2022), given that the world's agricultural soils are deteriorating largely due to agricultural activities (Wuepper et al., 2020). Thus, the objective of this study was to evaluate the effect of a liquid mineral organic fertilizer (LMOF) obtained through the fermentation of leachate from UOSW in the municipality of Paipa-Boyacá and its influence on the chemical properties of the soil and the morphological and physiological parameters of the Cerinza ICA bean crop up to the flowering stage.

2. Materials and methods

Leachate collection

Leachate was collected from the composting plant of the Red Vital public utilities company, located in the municipality of Paipa, Boyacá,Colombia. It was transported in plastic containers to the Soil Laboratory of the Fundación Universitaria Juan de Castellanos, located at the San Francisco de Asís Veterinary Clinic in Soracá. The chemical characteristics are summarized in Appendix 1. The content of heavy metals (Ni, Cr, and Pb) in the leachate can be referenced in previously published works (García et al., 2021).

Production of fermented organic fertilizer

The leachate was enriched with rock phosphate (RP), potassium (K) in the form of sulfate, nitrogen as urea, and minerals such as Mg, Zn, Mn, and Fe as sulfates. In summary, the formulation included 5% RP, 2.5% K, 5% molasses, 5% magnesium sulfate and urea, and 0.5% sulfates of Mn, Zn, and Fe. Once the mixture was prepared, the fermentation period (aerobic) for the LMOF was 15 days, and it was manually stirred once daily. The chemical characteristics are summarized in Appendix 2.

Initial soil collection

Soil collection was carried out at the San Isidro Labrador experimental farm at the Juan de Castellanos University Foundation, on a plot previously cultivated with potatoes and fertilized with NPK. The chemical characteristics of the soil are presented in Appendix 3 and in the Results and Discussion section.

Study area

The research was conducted at the San Isidro Labrador Experimental Farm of the Faculty of Agricultural and Environmental Sciences of the Fundación Universitaria Juan de Castellanos, located in Soracá, Boyacá, Colombia (at an altitude of 2,799 msnm, latitude $5^\circ 30' 02''$ N, and longitude $73^\circ 20' 00''$ W) during the months of September to December 2022.

Bean crop planting

Certified *ICA Cerinza* bean seeds (*Phaseolus vulgaris* L.) were commercially acquired. A completely randomized block design was used to arrange four treatments with three replicates; each repetition consisted of 14 experimental units. The trial was established in the field in two kg plastic bags, as shown in Appendix 4. In summary, the treatments were no fertilizer addition (T0), 5% molasses-fermented leachate (M-FL) (T1), P and K-rich MOLF at 5% (T2), and commercial liquid fertilizer (CLF) (T3). After the treatments were established, two *ICA Cerinza* bean seeds were manually planted in each of the bags at a depth of two cm. Fertilization was carried out at three physiological stages corresponding to 1) leaf development, 2) floral organ appearance, and 3) flowering. The treatments are summarized in Appendix 5.

Pest control

Progressive applications of granulated agricultural molluscicide with metaldehyde as the active ingredient were carried out for slug control following the product instructions.

Analytical methods

Morphological behavior

Fresh and dry matter content was determined at 56 and 88 days after sowing (das) by randomly selecting three plants from each treatment. The fresh matter for the stem and root was calculated by weighing each part using an analytical balance. The plants were placed in paper bags and taken to a forced-air oven at a temperature of 80 °C until reaching a constant weight to determine the dry matter. The dimensions of the stem (from the base to the apex of the main stem) and root were measured using a tape measure.

Physiological behavior

Stomatal conductance (gs) and chlorophyll index (CI) were measured between 4:00 p.m and 6:00 p.m, using three plants from each treatment. Measurements were carried out on the upper third of the bean plant leaves using a Delta–T Devices Type AP4 diffusion porometer and a SPAD-502 meter. The evaluations were conducted during the stages of lateral shoot formation (LSF), floral organ appearance (FOA), and flowering (FL). Fluorescence determination was performed using an OR-PAM fluorometer between 5:00 pm and 6:00 pm and was evaluated during FOA and FL stages.

Fertilizer characterization

The pH and EC were measured with a pH meter from Hanna and a conductivity meter. Organic nitrogen content was determined using the Kjeldahl method, hereinafter referred to as Total Kjeldahl Nitrogen (TKN). Total Organic Carbon (TOC) was analyzed using a Multi N/C 2100 analyzer (Analytik Jena) located in the Catalysis group at the Universidad Pedagògica y Tecnològica de Colombia. TP and SP were determined following the methodology described in the AOAC (Association of Official Agricultural Chemists). All parameters were determined only for leachates. Minerals (Na, Fe, Zn, Mg, Mn, and Ca) was analyzed using a PerkinElmer model 400 atomic absorption spectrophotometer after performing closed acid wet digestion in a FOSS Digestor^{TM} 2508, using sulfuric and nitric acid. Digestion was completed until the sample became colorless.

Nutrient behavior in soil

Soil samples (both initial and from bean cultivation) were collected at a depth of 0 – 30 cm. These samples were dried in an oven at 90 °C for 16 hours, crushed using a mortar, sieved (2 mm mesh size), packed in polyethylene bags, and stored for subsequent analysis. Available phosphorus was extracted using the Bray II method (P BII) (Bray and Kurtz, 1945) and quantified using the vanadomolybdophosphoric acid colorimetric method with a UV-Visible spectrophotometer at a wavelength of 420 nm. pH was determined using a 0.01 M $CaCl_2$ solution in a 1:5 sample-to-solution ratio, and the electrical conductivity (EC) was measured using distilled water in a 1:5 sample-to-water ratio (Minasny et al., 2011). The concentrations of available minerals (K, Na, Fe, Zn, Mg, Mn, and Ca) were assessed using the Mehlich-3 (M-3) extraction solution (Mehlich, 1984) and further measured with a PerkinElmer model 400 atomic absorption spectrometer. Organic acids were extracted according to the methodology proposed by Zhu et al. (2021) and Ali et al. (2011), and subsequently analyzed using high performance liquid chromatography (HPLC) on a Shimadzu LC-2030 C3D instrument at the Animal Nutrition Laboratory of the Fundación Universitaria Juan de Castellanos-Soracá. The analysis conditions were as follows: mobile phase 15 mM phosphate buffer adjusted with H_3PO_4 to pH 2.0 and acetonitrile in a 94:6 ratio, flow rate of 0.2 mL/min, temperature of 30 °C, two serially connected VDSpher PUR C18-E columns, 3 μm, 250 × 4.6 mm, injection volume 20 μL, and a wavelength of 210 nm.

Data analysis

The assumptions of normality (Shapiro-Wilks test) and homoscedasticity (Levene's test) were validated. Repeated-measures ANOVA along with Bonferroni's test ($P < 0.05$) was used to determine significant differences between treatments. The Pearson correlation coefficient was used for the analysis of the relationships among chemical variables, organic acids, and physiological variables. Additionally, the principal component analysis (PCA) was performed to explore the data structure. All statistical analyses were conducted using R software version 4.3.2, employing the “Corrplot" and “factoextra" packages.

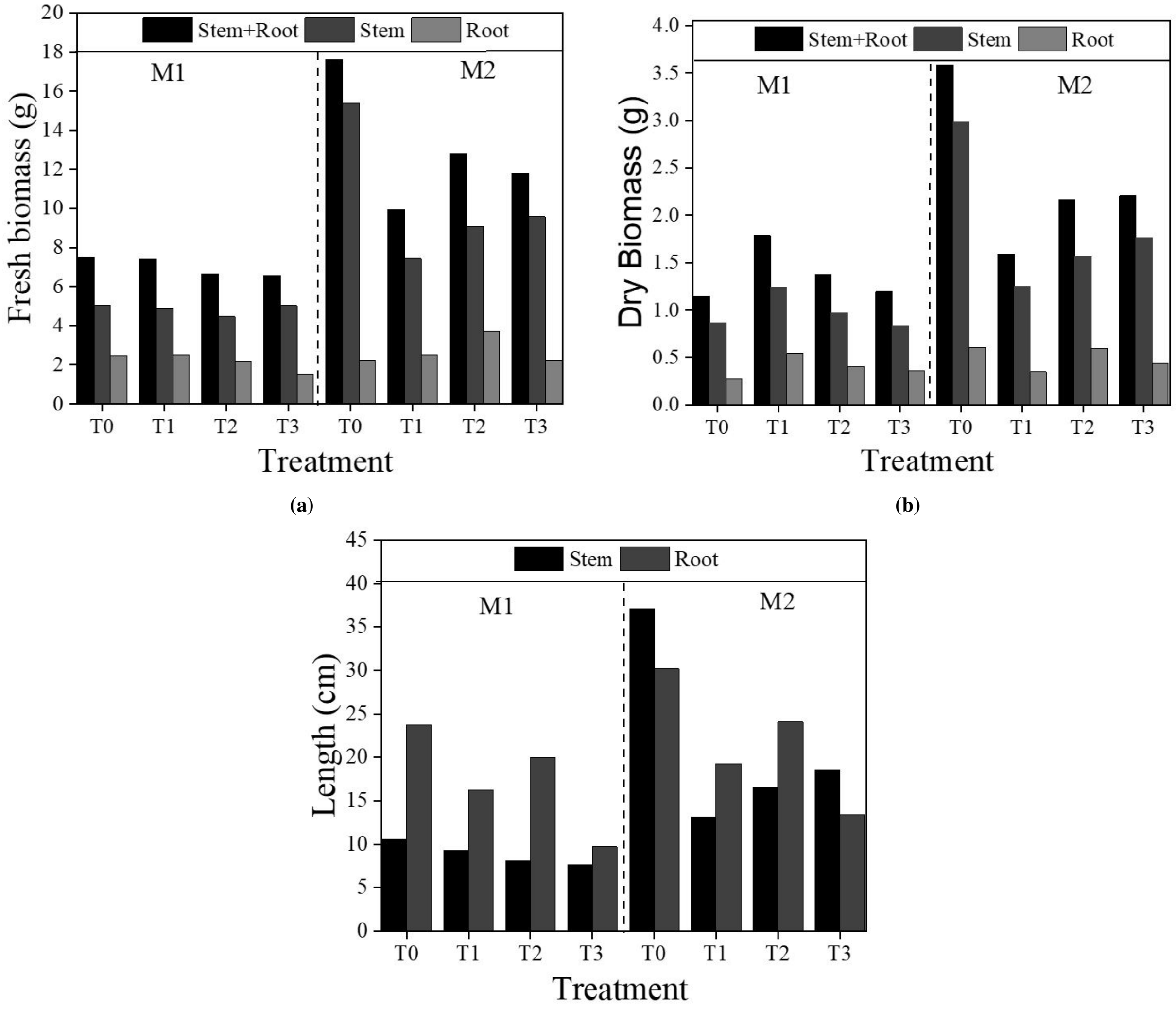
3. Results and discussion

Morphological parameters

Biomass and length of bean plants

The Fig. 1 displays the behavior of biomass (fresh and dry) and length (root and stem) of bean crops at two sampling times, designated month 1 (M1) and month 2 (M2). The data indicate that plant biomass during the first month was not influenced by the addition of different types of fertilizer and showed similar behavior to T0. However, in the second month, both fresh and dry biomass of root + stem and stem alone were affected by the treatments and followed this order: T0 > T2 > T3 > T1. In contrast, root growth displayed an exhibited pattern in the following descending order: T2 > T1 > T0 > T3. Additionally, there was a decrease in stem size among treatments in the first month in the following descending order: T0 > T1 > T2 > T3. However, in the second month, the order was T0 > T3 > T2 > T1. Lastly, the root length in both months exhibited the same behavior, showing the following descending order: T0 > T2 > T1 > T3, with greater values observed in treatments where no minerals were added.

The incorporation of organic and inorganic fertilizers into soil to enhance the growth and yield of crops is a well-established practice in the agricultural sector. Research has demonstrated positive effects on total biomass, fresh biomass, and root-stem length. For example, Zandvakili



(a) **(b)**

(c)

Figure 1. Physiological Parameters Content in Bean Cultivation in month one and two. Fresh Biomass (A), Dry Biomass (B), Stem and Root Length (C).

et al. (2019) assessed physiological and productive parameters in lettuce crops, where organic fertilizer increased biomass by 72% compared to the control treatment. Similarly, Roy and Kashem (2014) evaluated three organic amendments—rice straw and *Ageratum conyzoides* residues (crushed mix, compost, and vermicompost)-in *Phaseolus vulgaris* cultivation. The data showed increases in root length of 23.4%, 20.5%, and 17.7% for the crushed mix, compost, and vermicompost, respectively, compared to the control. Furthermore, Ji et al. (2017) evaluated various organic liquid fertilizers derived from shrimp extracts (L1), plant decomposition (L2), vermicompost (L3), seaweed extracts (L4), fish extracts (L5), chemical fertilizer (CF), and a control (CT) at the time of transplant and 30 days post-transplant of chrysanthemum. The data revealed that after 60 days of treatment, positive effects on root size and dry weight were observed in all treatments, with L1 showing the most significant effects. Specifically, L1 resulted in increases of approximately 62% in root dry weight and 60% in total root length compared to CF.

In this study, the organic fertilizer resulted in increases in root dry weight of 27.11% and root length of 44.5% compared to CLF; however, stem weight and length were approximately 12% higher in CLF than in MOLF. This behavior could be related to the availability of Nitrogen (NH$_4^+$ and NO$_3^-$) and the presence of microorganisms such as Rhizobium, which act symbiotically with the plant facilitate N uptake (Masson-Boivin and Sachs, 2018). The absorption of available species like N enhances the development and vegetative growth of crops (Cossani and Sadras, 2019). On the other hand, it may also be associated with changes in soil fertility, where minerals may be more readily available for uptake by the plant, as described by Azam (1990); this could influence the photosynthetic process and affect plant biomass (Jannoura et al., 2014). The greatest root length was observed in T0 likely due to low levels of macro- and micronutrients, as mentioned by Gruber et al. (2013). Finally, the behavior of T0 in the measured variables could be associated with the initial mineral content present in the soil and utilized by the plant; moreover, the role of root exudates in enhancing the availability of elements such as P cannot be ruled out (Tawaraya et al., 2013). The standard errors for the physiological parameters are in the supporting information (sections 8.7 and 8.8).

Physiological parameters

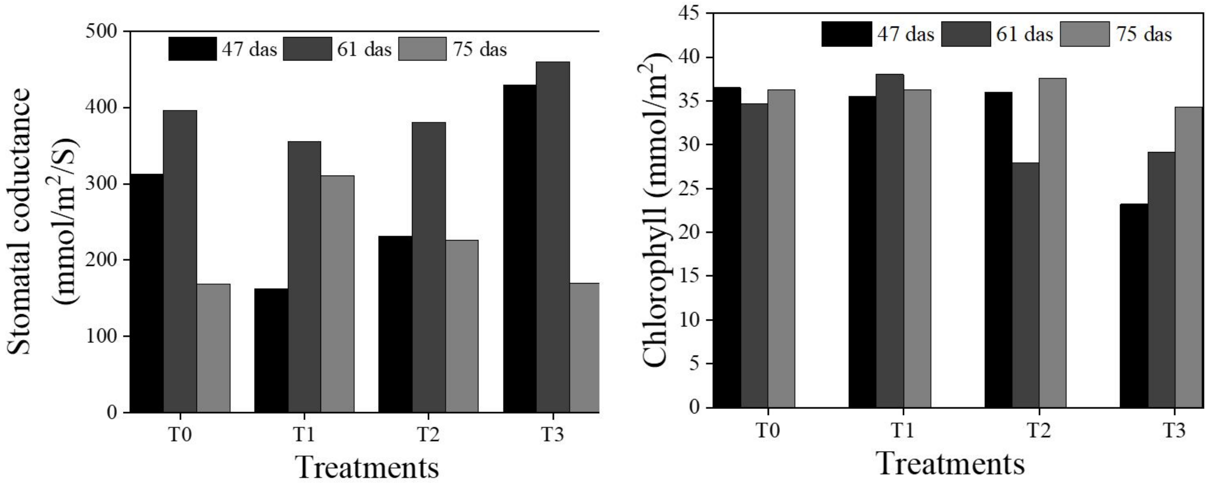
Stomatal Conductance (gs) and Chlorophyll Index (CI)

The values obtained for gs and CI are shown in Fig. 2. The gs readings at the FOA stage showed the highest values across all treatments in the following descending order: T3 > T0 > T2 > T1, followed by the LSF stage (except for T1) and finally, the lowest values corresponding to the FL stage in the order of T1 > T2 > T3 > T0. On the other hand, the CI values for T0, T1, and T2 showed no statistically significant differences at the FL and LSF stages. Similar values were observed for T2 and T3 at the FOA stage, which were lower than those of T0 and T1. Concurrently, T3 showed a gradual increase, but at all stages, the CI was lower than the values of T0 and T1. The behavior of the gs and CI data could be linked to the presence of micro and macronutrients; for example, the higher performance of T3 in the FOA stage and LSF stage could be associated with the incorporation of NPK, which may enhance stomatal conductance by promoting the photosynthetic process (Rawat et al., 2022; Zangani et al., 2021). Moreover, the behavior of gs in T3 might be related to osmotic stress events or nutrient imbalances (Li et al., 2022), as a result of the high nutrient availability present in the incorporated liquid fertilizer, particularly N content, which can affect the production and concentration of hormones, like abscisic acid (ABA), which plays a key role in the regulation of stomatal closure. Additionally, some studies suggest that abscisic acid can regulate genes that respond to N (Garciarrubio et al., 1997; Smoczynska et al., 2022; Wang et al., 2023), which may have contributed to the closure of stomata and reduced stomatal conductance.

Additionally, the data obtained for T1 during the FL stage can be associated with the incorporation of organic species (organic acids) and essential nutrients (K and P) present in M-FL and the soil; these have been shown influence on the regulation of stomatal opening and closing, ensuring adequate gas exchange and metabolic regulation in plants (Hasanuzzaman et al., 2018; Lin et al., 2020). Thus, adequate levels of available nutrients can improve the photosynthetic rate, which in turn generates greater stomatal conductance. Authors such as Zangani et al. (2021) reported similar results with the addition of three different concentrations of N and P in a *Brassica napus L.* crop, where the application of 100 kg/ha of N significantly increased leaf stomatal conductance during the late flowering stage, and the application of 150 kg/ha of P significantly increased leaf stomatal conductance during the early flowering stage compared to the control. On the other hand, studies have reported that the CI can be used as an indirect indicator of foliar N concentration, since nitrogen levels significantly increase the chlorophyll content in leaves (Zangani et al., 2021). Finally, the constant fluctuations observed in the order of T1 > T2 > T3 in the CI might also be associated with mollusk infestation (slugs), which could have affected chlorophyll synthesis and led to a decrease in CI. The damage caused by this type of pest possibly led to the degradation of chlorophyll or hindered its production (Al-Zahrani et al., 2020; Moser et al., 2020).

Chlorophyll fluorescence parameters (CF)

Table 1 presents data related to CF in bean cultivation during the FOA stages and concluding at FL. The data indicate that: 1) the addition of fertilizers (organic and inorganic) has a positive effect on F_0 at the FOA stage; however, at the end of FL, F_0 values were lower for T1, T2, and T3 compared to T0; 2) F_m data show similar values for T1 and T2 but are lower compared to T0 at FOA, and heterogeneous results were observed for all treatments at the end of FL, with the highest for T0; 3) the highest values of Y.II were observed at the FOA stage for T0 and T3, followed by T2 and T1, however, at the end of FL, the values of Y.II for T0 and T2 were similar, and T3 presented the lowest value; 4) the highest ETR value was observed in T3 during FOA and in T0 at the end of FL; the lowest values were recorded in T2 and T3, respectively; 5) qP values showed a greater impact in T3 at FOA, but at the end of FL, treatments T0 and T1 were the highest, compared to T0 and T3; 6) for qL, T3 and T0 showed the highest values at the FOA stage, and at the



(a) (b)

Figure 2. Behavior of: (A) Stomatal Conductance (gs) and (B) Chlorophyll Index (CI). Formation of lateral shoots 47 das, appearance of the floral organ 61 das, flowering 75 das.

Table 1. Results of physiological variables of Fluorescence in different stages of *ICA Cerinza* bean cultivation.

Treatments	F_0 (a.u)	F_m (a.u)	Y.II (a.u)	ETR ($mmol/m^2s^2$)	qP (a.u)	qL (a.u)	qN (a.u)	NPQ (a.u)
				Sample 1 (M1)				
T0	140.0 ± 14.45^a	232.3 ± 28.76^a	0.40 ± 0.02^a	20.9 ± 1.15^a	0.67 ± 0.02^a	0.45 ± 0.05^a	0.18 ± 0.13 b	0.17 ± 0.13 b
T1	169.0 ± 16.97^a	220.5 ± 24.75^a	0.23 ± 0.01^b	12.3 ± 0.42^{ab}	0.44 ± 0.01^b	0.28 ± 0.02^b	0.57 ± 0.05^a	0.83 ± 0.14^a
T2	145.5 ± 10.50^a	219.0 ± 7.00^a	0.33 ± 0.07^{ab}	17.7 ± 3.65^b	0.60 ± 0.10^a	0.42 ± 0.08^a	0.39 ± 0.17^{ab}	0.49 ± 0.29^{ab}
T3	155.7 ± 13.61^a	261.3 ± 26.08^a	0.40 ± 0.02^a	21.3 ± 1.27^a	0.70 ± 0.02^a	0.50 ± 0.02^a	0.10 ± 0.02^b	0.09 ± 0.09^b
				Sample 2 (M2)				
T0	152.3 ± 36.00^a	271.0 ± 68.74^a	0.43 ± 0.03^a	23.1 ± 1.50^a	0.71 ± 0.07^a	0.49 ± 0.13^a	-0.25 ± 0.37^a	-0.09 ± 0.15^a
T1	137.5 ± 7.78^a	220.0 ± 1.41^a	0.38 ± 0.03^{ab}	20.0 ± 1.77^{ab}	0.67 ± 0.05^a	0.50 ± 0.05^a	-0.12 ± 0.16^a	-0.05 ± 0.09^a
T2	116.0 ± 18.08^a	196.0 ± 43.92^a	0.40 ± 0.04^a	21.3 ± 2.34^a	0.57 ± 0.29^a	0.55 ± 0.08^a	-0.02 ± 0.05^a	-0.01 ± 0.04^a
T3	141.7 ± 15.01^a	201.7 ± 35.02^a	0.29 ± 0.05^b	15.5 ± 2.40^b	0.66 ± 0.07^a	0.52 ± 0.07^a	-0.03 ± 0.10^a	-0.01 ± 0.06^a

Sample Collection 1: Appearance of Floral Organ (69 das) and Sample Collection 2: End of Flowering Stage (89 das). Initial Fluorescence (F_0), Maximum Chlorophyll Fluorescence in the State of Dark Adaptation (F_m), Electron Transport Rate (ETR), Coefficient of Past Photochemical Quenching based on the Puddle Model (qP), Effective Quantum Efficiency (Y.II), Coefficient of Photochemical Quenching based on the Lake Model (qL), Non-Photochemical Quenching of Variable Chlorophyll Fluorescence (qN), and Non-Photochemical Quenching (NPQ). Treatments followed by the same letter within the same column are not statistically different from each other ($p < 0.05$).

FL stage, the values for all treatments ranged from 0.49 to 0.55, with the highest value recorded for T2; 7) finally, qN and NPQ data show positive values at the FOA stage, with the greatest effect in T1 compared to other treatments, and at the end of FL, the greatest effects were observed in T0 presenting a negative value.

The results of CF serve has a tool for understanding of photosynthetic metabolism and identifying plant performance under different stress conditions (abiotic and biotic), such as water deficiency, high/low temperatures, nutrient scarcity, heavy metal toxicity, air pollution, and the presence of pests (Brestic and Zivcak, 2013; Murata et al., 2007). The results obtained for F_0 can be associated with possible biotic stress that the plants experienced due to herbivory by mollusks of the genus *Deroceras*; as demonstrated by Pellissier (2013), who found that herbivory negatively affected the photosynthesis of Abies alba and Rubus fruticosus, influencing the F_0 values.

On the other hand, the F_m data showed the following descending order: T3 < T0 < T1 < T2; with the highest value observed for the commercial liquid fertilizer. This result can be attributed to the presence of highly soluble minerals in the CLF, which contribute to improved photosynthetic efficiency by facilitating light capture and electron transfer (Chatzistathis et al., 2021; Gong et al., 2021). Notable among these are N, Mg, P, and K; which have been shown to contribute to the synthesis of chlorophyll and other pigments necessary for photosynthesis (Jaquetti et al., 2014; Kalaji et al., 2018). Authors such as Gong et al. (2021) found that the incorporation of N as a fertilizer improved the photosynthetic efficiency of proso millet (Panicum miliaceum) leaves; this facilitated stomatal opening and increased in the maximum quantum yield of photosystem II (PSII) photochemistry and the photochemical quenching coefficient. Additionally, N, Mg, P, and K have a positive influence on light capture and facilitate electron transfer in the photosynthetic process, which is reflected in higher F_m values; behavior that has been associated with their role as cofactors of photosynthetic enzymes (Hänsch and Mendel, 2009; Kalaji et al., 2018). The positive response in T2 suggests that the addition of minerals, along with other organic components of the leachate, could improve the plants' ability to perform photosynthesis.

Effective quantum yield (Y.II)

On the other hand, Y.II is considered an important parameter because it allows for the estimation of the efficiency in converting light energy into chemical energy. According to Kitajima and Butler (1975), normal values range from 0.75 to 0.85; this ratio is proportional to the quantum yield. The values presented in this study range from 0.23 to 0.40, significantly below the ranges established by Kitajima and Butler (1975) and Smolikova et al. (2023) who found values of 0.65 when measuring green leaves in pea crops under natural light conditions. According to Öquist et al. (1992), decreases in Y.II values may be related to two processes: the first is influenced by a decrease in the rate constant of PSII photochemistry, and the second by increases in the rate constant of non-radiative dissipation of excitation energy, both due to impairments in the PSII reaction centers. Additionally, authors such as Zalamena et al. (2015) indicated that metals like Zn can negatively affect Y.II behavior since they interfere with the activity of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) or disrupt light absorption by photosynthetic pigments. In this study, it is evident that a Zn content of 0.72 ppm in T1 reduced the value of Y.II by 42.5% compared with T0 in month 1; however, for Zn concentrations of 1.64 ppm in T3 and 0.43 ppm in T0, the highest values of Y.II were recorded. In month 2, the available Zn content was similar across all treatments, and the Y.II data for T0, T1, and T2 showed no statistically significant differences. The data from this study do not align with that reported by Zalamena et al. (2015), indicating that other variables may significantly impact Y.II. Moreover, Suh et al. (2002) observed negative effects on Y.II values with the addition of excess Fe in hydroponically grown Pisum sativum L. In this study, the highest Y.II values were observed in M2 compared to M1 and coincided with the lowest contents of available Fe. The impairments in Y.II have been related to the fact that high Fe contents in tissues can cause damage to the photosynthetic apparatus as a result of the formation of reactive oxygen species (ROS) (Xing et al., 2010; Yruela, 2009). Finally, we consider that one of the circumstances that most affected the behavior of Y.II was the presence of mollusks (slugs), which resulted in 1) physical damage to the leaves, 2) the presence of mollusks typically results in high saliva secretions on the leaf surface, promoting cellular damage due to the presence of enzymes and other substances (Rashad et al., 2023), and 3) potential indirect damage due to secondary water stress caused by decreased water and nutrient uptake due to mechanical damage to the bean plant.

Electron transport rate (ETR) and photochemical quenching (qP)

ETR and qP have been associated as indicators of 1) photosynthetic process efficiency and 2) the ability of plants to regulate the use of absorbed light energy in chloroplasts (Murchie and Lawson, 2013). ETR and qP data exhibit higher values in M2 compared to M1, except for T3. This behavior could be linked to nutrient availability. For instance, Ali et al. (2021) assessed the impact of different biochar doses (20, 40, and 60 tons/ha) and two nitrogen levels (270 and 360 kg N/ha) on rice cultivation (*Oryza sativa* L.). The authors found that qP and ETR values increased as the doses of biochar and nitrogen are raised, compared to treatments where only N was added. These results were associated with the effects of biochar on microbial consortia that secrete enzymes responsible for the N cycle in the soil, such as glutamine synthetase (GS), nitrate reductase (NR), and glutamine 2-oxoglutarate aminotransferase (GOGAT), facilitating higher N absorption rates in plants. On the other hand, ETR and qP could be influenced by either a deficiency or excess of minerals such as Fe (Shi et al., 2022), P (Suárez et al., 2023), Mg (Jaghdani et al., 2021), K (Xu et al., 2020), and Zn (Huang et al., 2023); which showed heterogeneous behavior across the treatments (see Table 2), in addition to damage caused by slugs that can alter ETR.

Fraction of open PSII centers (qL)

qL provides insight into the proportion of open or active reaction centers of PSII at any given time (Lee et al., 2013). High qL values indicate greater efficiency in the photosynthetic process and an enhanced ability to absorb light energy to initiate the photosynthetic electron transport chain. The data showed higher qL values in M2 compared to M1 across all treatments, suggesting that M1 experienced higher stress conditions, which predominantly affected the qL values. Authors such as Ruban et al. (2012), Demmig-Adams (1990), Ruban and Horton (1995), Ruban et al. (1993), and Baker and Horton (1987) have noted that the nature of qL is highly heterogeneous, and the values can be associated with factors such as: 1) pigment content, 2) trapped protons, 3) aggregated light-harvesting complex II (LHCII), and 4) photo damage to RCII itself. All these factors are related to the effective nutrient uptake by the plant, which plays specific roles in pigment synthesis, protein complex stability, and the utilization of solar energy for the photosynthetic process. Finally, we believe that the low qL values in M1 were related to greater damage at this stage caused by slugs, as mentioned in the section on Effective Quantum Yield (Y.II).

Non-photochemical quenching (NPQ) and quantum yield of non-photochemical quenching (qN)

NPQ and qN have been associated with protective mechanisms against light or environmental stress. NPQ provides information on the dissipation of excess light energy as heat, a process that helps prevent: 1) overexcitation of the photosystems and 2) the generation of reactive oxygen species

Table 2. Behavior of micro and macronutrients in soil in bean cultivation.

Treatments	Fe	Zn	Mn	Mg	Ca	Na	K
Units	Ppm						
Initial data soil	67.03	0.95	9.33	22.80	182.00	48.30	117.00
M1							
T0	87.28 ± 17.50	0.43 ± 0.02	9.61 ± 1.59	31.45 ± 7.66	202.33 ± 14.62	30.34 ± 3.86	101.69 ± 21.3
T1	76.37 ± 9.76	0.72 ± 0.16	11.88 ± 0.42	25.99± 5.37	356.63 ± 11.04	32.71 ± 5.12	93.87 ± 6.15
T2	114.67 ± 9.10	0.25 ± 0.09	11.20± 1.75	27.98 ± 3.54	203.95 ± 7.94	16.12 ± 1.81	159.67 ± 3.53
T3	68.85 ± 1.92	1.64 ± 0.17	13.42 ± 2.36	27.68 ± 4.96	368.85 ± 14.85	20.70 ± 2.68	182.57 ± 7.70
M2							
T0	68.05 ± 3.34	1.15 ± 0.23	3.89 ± 0.46	32.93 ± 4.40	193.12 ± 4.03	50.59± 5.20	99.11 ± 5.63
T1	66.32 ± 0.91	1.09± 0.15	7.56 ± 0.49	35.45 ± 4.40	189.75 ± 18.33	47.80 ± 5.21	90.59 ± 2.31
T2	55.01± 4.30	1.66 ± 0.19	4.85 ± 0.37	32.58 ± 2.36	170.59 ± 3.82	45.33 ± 4.27	90.96 ± 2.77
T3	58.66 ± 2.55	1.11 ± 0.73	7.40 ±0.42	30.96 ± 3.15	162.30 ±8.01	56.42 ± 4.27	108.02 ± 2.98

Means ± standard deviation

(ROS). On the other hand, qN is related to the efficiency of the NPQ process. The data showed higher NPQ values with organic fertilizer treatments (T1 and T2), which could be related to several factors, such as increased nutrient absorption, changes in the microbial fauna, presence of organic compounds like acids found in the fertilizers; produced by the degradation of organic matter and secreted by the plant. Thus, the results of this research show that the addition of liquid organic fertilizers can, in addition to contributing to nutrient availability, influence energy dissipation mechanisms under environmental stress, such as the presence of slugs, with treatments T1 and T2 being the most affected by this pest. On the other hand, negative NPQ values could be associated with low light intensity conditions, which resulted in low NPQ activity. Finally, there is a positive correlation between NPQ and qN.

Soil quality parameters

pH behavior

The pH values for the first and second samplings are shown in Fig. 3. The data indicate an increase in pH of 21.4% and 23.4% at 56 and 88 days, respectively, compared to the initial value (4.74). These results were similar to those obtained by Phibunwatthanawong and Riddech (2019), Malick et al. (2019) and Lee (2010), who reported slightly acidic pH values in soil after the application of organic fertilizer, derived from the use of organic waste. These effects may be related to the presence of sugars in the substrate, which are converted into organic acids by microbial activity (Phibunwatthanawong and Riddech, 2019). Additionally, organic fertilizers may influence the soil's buffering capacity, thereby reducing pH fluctuations. In the second sampling (88 das), the reported values for T1 are slightly more acidic (around 5.5) than the initial values. This could be associated with the incorporation and secretion of organic acids that contribute to the decrease in soil pH, leading to the release of minerals not previously available in the soil (Adeleke et al., 2017). Moreover, Sugiyama and Yazaki (2012) suggest that root exudates from some leguminous plants (citric and malic acid) also serve to acidify the surrounding soil to acquire phosphate, a result that can explain the acidic behavior in all treatments. On the other hand, the moderately acidic pH of T2 in M1 may be associated with the salts added in the MOLF.

EC behavior

The EC data are presented in Fig. 4. The initial soil electrical conductivity (0.32 mS/cm) recorded a significant increase with the addition of fertilizer for all treatments. Authors such as Roy et al. (2010); Chang et al. (2010) report a gradual increase in EC in soils fertilized with organic manures, as observed in this study for T2, where the increase can be attributed to the rise in soluble salt content imparted by the organic components provided by the fertilizers. This is due to the solubilization of soluble ions such as chloride, sulfate, sodium, and other inorganic ions present in the fertilizers, and organic species formed through the mineralization of organic matter, which increases soil EC (Chang et al., 2010; Chan et al., 2016; Ramesha et al., 2021). However, an increase was also observed in T3 due to the presence of ionic salts, such as nitrates, phosphates, and potassium ions that can dissociate in the soil and contribute to the EC values. Finally, for T0, this increase can be attributed to the presence of nitrifying nodules, which enhance the availability of nitrogen in the soil by fixing atmospheric nitrogen and converting it into organic nitrogen that plants can use. Thus, the release of nitrogen into the soil can increase ion concentration and EC (Masson-Boivin and Sachs, 2018; Niedziński et al., 2011).

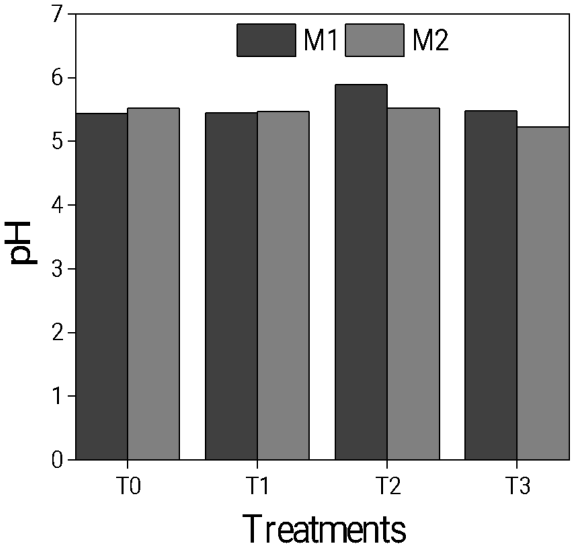


Figure 3. Soil pH behavior with the addition of liquid organic mineral fertilizer during the cultivation of *ICA Cerinza* bean.

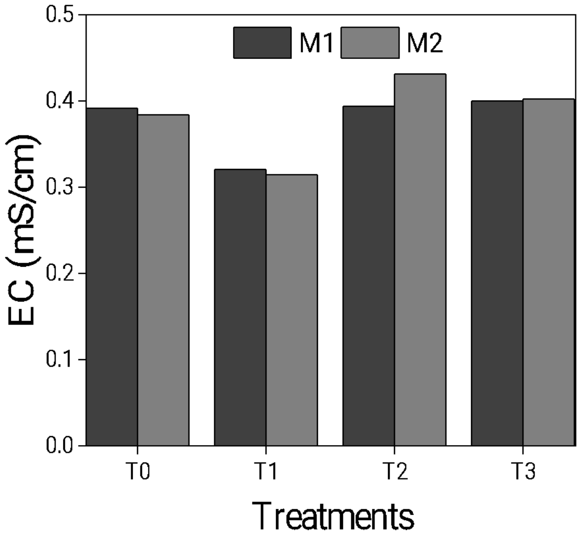


Figure 4. Soil EC behavior with the addition of liquid organic mineral fertilizer during the cultivation of *ICA Cerinza* bean.

Bray II phosphorus behavior

The results shown in Fig. 5 illustrate the effect of fertilizer addition on the phosphorus content compared to the control treatment T0. The application of M-FL (T2) recorded a phosphorus content very close to that obtained with CLF (T3), with values of 54.9 ppm and 53.5 ppm (M1); and

48.4 ppm and 53.7 ppm (M2) respectively. Research by Ullah et al. (2022) obtained similar results, where they demonstrated that soil phosphorus increased by 35% with the application of a commercial liquid NPK-rich fertilizer and by 21.8% with the application of barnyard manure. In contrast, Ayeni et al. (2012) and Hammad et al. (2020) report an opposite effect on phosphorus availability with the addition of organic fertilizers and commercial fertilizers, showing a higher phosphorus content in organic fertilizers. This is because this type of fertilizer contains a wider range of phosphorus that is readily available to plants compared to the nutrients available in the soil. Furthermore, they demonstrated that organic fertilizers can be favorably compared with commercial fertilizers in terms of nutrient release and crop yield. The low phosphorus concentrations in T0 and T2 are the result of not having a direct source of P (it is worth noting that MOLF does not report phosphorus-solubilizing microorganisms like M-FL, unpublished data). However, studies by Sulieman and Tran (2015), Valentine et al. (2017), and Vardien et al. (2016) have shown that legumes can develop adaptation strategies to P deficiency, such as processes involving acquisition, assimilation, exportation, recycling, and a lower rate of phosphorus absorption. They also maintain phosphorus homeostasis in nodules during shortages, and Vardien et al. (2016) point out that low P levels are accompanied by a decrease in the net rate of P absorption, as well as in the uptake of P by these nodules, which could explain the slight increase in P in M2 for T0. The slight decrease in P content in treatments T1, T2, and T3 in the second month can be associated with the plant's utilization of this element, as the plant requires it during the flowering stage to promote flower and seed formation (He et al., 2019).

Micro and macronutrient behavior

The behavior of minerals in the soil is described in Table 2. The data show the following changes from M1 to M2 for treatments T0:T0, T1:T1, T2:T2, and T3:T3. For Fe, a decreases of 22.02%, 13.16%, 52.03%, and 14.80% was observed; for Zn, there were increases of 164.41%, 51.55%, 567.13%, and 32.28%; for Mn, the content decreases by 59.52%, 36.37%, 56.66%, and 44.86%; for Mg, there was an increases of 4.70%, 36.38%, 16.41%, and 11.86%; for Ca, there was a decreases of 4.55%, 46.79%, 16.37%, and 56.00%; for Na, there was an increases of 66.74%, 46.15%, 182.14%, and 172.52%; and finally, K decreases by 2.54%, 3.49%, 43.03%, and 40.84%, respectively. Additionally, when comparing the data for M1, decreases in element concentration were evident from T0 to T1 in Fe, Mg, and K; T0 to T2 in Zn, Mg, and Na; T0 to T3 in Fe, Mg, and Na. Increases in M1 were observed from T0 to T1 in Zn, Mn, Ca, and Na; T0 to T2 in Fe, Mn, Na, and K; and from T0 to T3 in Zn, Mn, Na, and K. Finally, when comparing data for M2, decreases in element concentrations were noted from T0 to T1 in Fe, Zn, Mg, and Ca; T0 to T2 in Fe, Ca, Na, and K; T0 to T3 in Fe, Zn, Mg, and Ca, while increases in M2 were observed from T0 to T1 in Mn, Na, and K; T0 to T2 in Zn, Na, and K; and from T0 to T3 in Fe, Mg, and Ca, respectively.

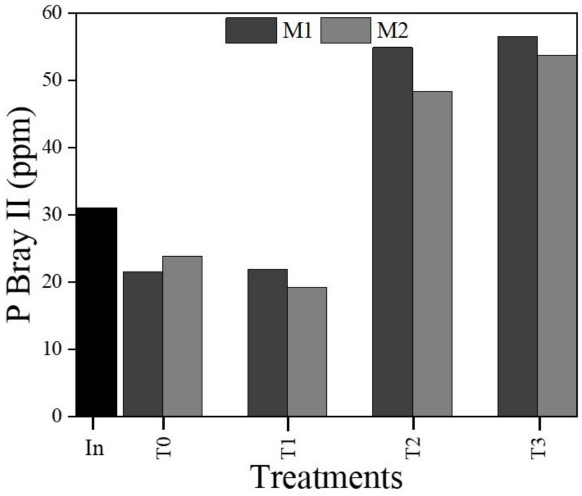


Figure 5. Behavior of soil phosphorus availability with the addition of liquid organic mineral fertilizer in the cultivation of *ICA Cerinza* bean.

The data indicate that the content of K and Ca was significantly influenced in T2 and T3 compared to T0. This is due to the addition of organic and inorganic fertilizers containing soluble salts, that increase the concentration of available cations of both Ca and K for uptake by the plant (Chen et al., 2007). For other cations, studies by Antil and Singh (2007) reported that the application of organic fertilizers based on poultry manure and sludge increased the content of available P, K, Zn, Fe, Mn, and Cu in the soil. The declines in mineral content from M1to M2 may be associated with the absorption of minerals by the plant, thereby reducing the concentration in the soil (Jacoby et al., 2017). Additionally, increases in other cations, such as Mg and Na were observed, aligning with findings by Ahanger et al. (2021), who determined that the use of nano compost based on fresh cow manure significantly improved the concentrations of N, P, K, and Na by 60.5%, 36.1%, 64.3%, and 6.6% at 100 mg/kg in soil. Finally, the behavior may be linked to effects on the microbiological activity in the soil from symbiotic bacteria, which can contribute to nutrient mobilization (Chen et al., 2007; Jacoby et al., 2017). The difference in nutrient availability could also be associated with the presence of acidic organic species like the organic acids identified in this study, as mentioned in the section on organic acid behavior.

Organic acids (OA) behavior

HPLC analysis reported the presence of six OA: oxalic acid (OxA), citric acid (CA), formic acid (FA), acetic acid (AA), lactic acid (LA), and succinic acid (SA). However, the initial soil sample, succinic acid was not detected. Additionally, the concentrations of the acids were below the quantification limit, which is why quantitative data for the acids in the initial soil are not reported. The data displayed the following trends from M1 to M2 when comparing T0:T0, T1:T1, T2:T2, and T3:T3: for OxA, increases of 116.8%, 5.5%, 9.5%, and 21.3% were observed; for CA, content decreased by 100.0%, 57.0%, 51.0%, and 85.6%; for FA, there was a decrease of 88.4%, 14.4%, 71.8%, and 45.9%; for AA, the content decreased by 81.7%, 96.6%, 47.5%, and 99.4%;

and for LA and SA, a 100.0% decrease was observed in all treatments (Fig. 6). In summary, the acid content was heterogeneous for each treatment. Studies on bean cultivation, such as that reported by Shen et al. (2002), identified the presence of AA, CA, and tartaric acid (TA), which were attributed to root exudation in different common bean genotypes, using various dosages of P and in the absence of P. The results showed that the concentration of OA was dependent on the P content in the soil; lower P content led to higher contents of AA, CA, and TA. The authors mentioned that this compartment could be associated with an increase in acid transport from shoots to roots; possibly through phloem, vascular tissue, and specific transport substances. Low levels of P can affect enzymatic activities, such as the activity of phosphoenolpyruvate carboxylase (Ohwaki and Hirata, 1992; Hoffland et al., 1989), and influence OA content. It has been observed that P deficiencies activate adaptation mechanisms (generation of OA) to enhance the absorption of P fixed in the soil. This behavior was noted in T0 and T1, where the lowest available P values were recorded; however, T3 presented a different behavior, with the highest contents of CA and SA, aligning with the higher available P values. Moreover, various studies show that P and K solubilizing microorganisms, as part of their mechanisms to enhance the availability of these nutrients, secrete a wide variety of OA as clarified by (Meena et al., 2014; Alori et al., 2017) in their review articles; where their proportion is associated with the type of organisms present and the carbon sources incorporated into the soil. For instance, Patel et al. (2008) observed that Citrobacter sp. favored the production of acetic acid in the presence of sucrose and fructose, and gluconic acid in the presence of glucose and maltose, respectively. In this regard, we consider that the behavior in the production of OA in M1 and M2 is linked to the incorporation of the carbon source, which could alter chemical and biological processes including microbial synthesis, redox reactions, degradation of organic matter, and root secretion of chemical species by the plant (Helten et al., 2022; Macias-Benitez et al., 2020; Wei et al., 2018).

Statistical analysis

The correlation coefficients for the samples collected at 55 days are shown in Appendix 6, displaying the following characteristics: strong positive correlations between Zn content with Ca and AC; pH with Fe; strong negative correlations: Fe concentration with Ca and Zn; moderate correlations: Mn content with Zn and Ca/EC with various nutrients; and finally, low or null correlations between P BII concentration and Na. Furthermore, Fig. 7a, presents the biplot representing the first two principal components of the data taken at 55 days. The first component accounts for 30.3% of the variance and the second for 25.9% (together explaining 56.2% of the cumulative variance). The most contributing variables in Dimension 1 were Zn, Ca, Mn and AC. This dimension might reflect the variability in soil composition related to the presence of elements like Zn and Ca, as well as the influence of Na and soil pH, moreover, T3 showed a higher association with these variables. In Dimension 2, the most contributing variables were Na, Fe, K, P BII, pH. Dimension 2 may be related to the spatial distribution of elements like Na and Fe, and the influence of K and P on these variations. The presence of Na and Fe could be inversely proportional in certain soil areas. Fig. 7b displays the biplot of principal components of the second sampling (88 das). Dimension 1 accounts for 32.33% of the total variance and Dimension 2 for 23.39%. Together,

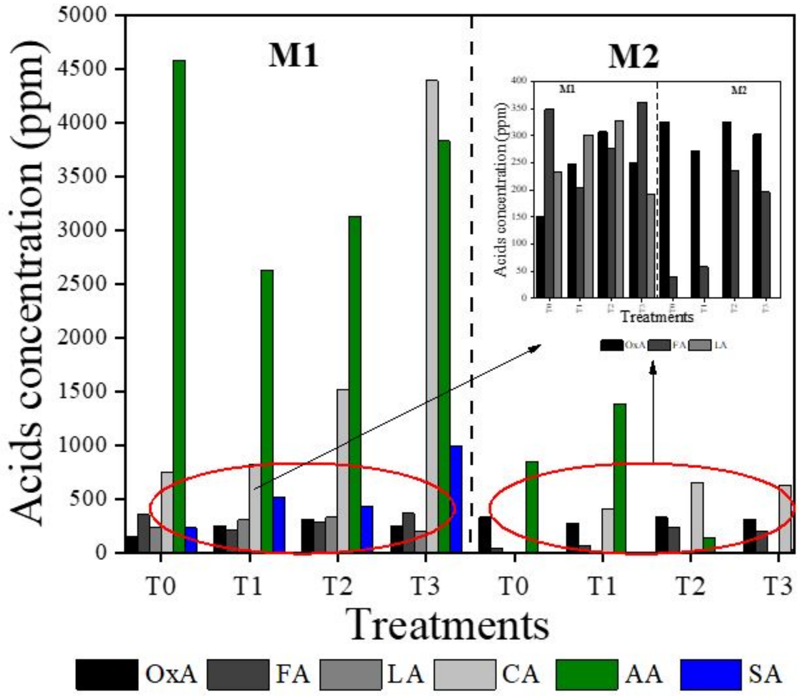


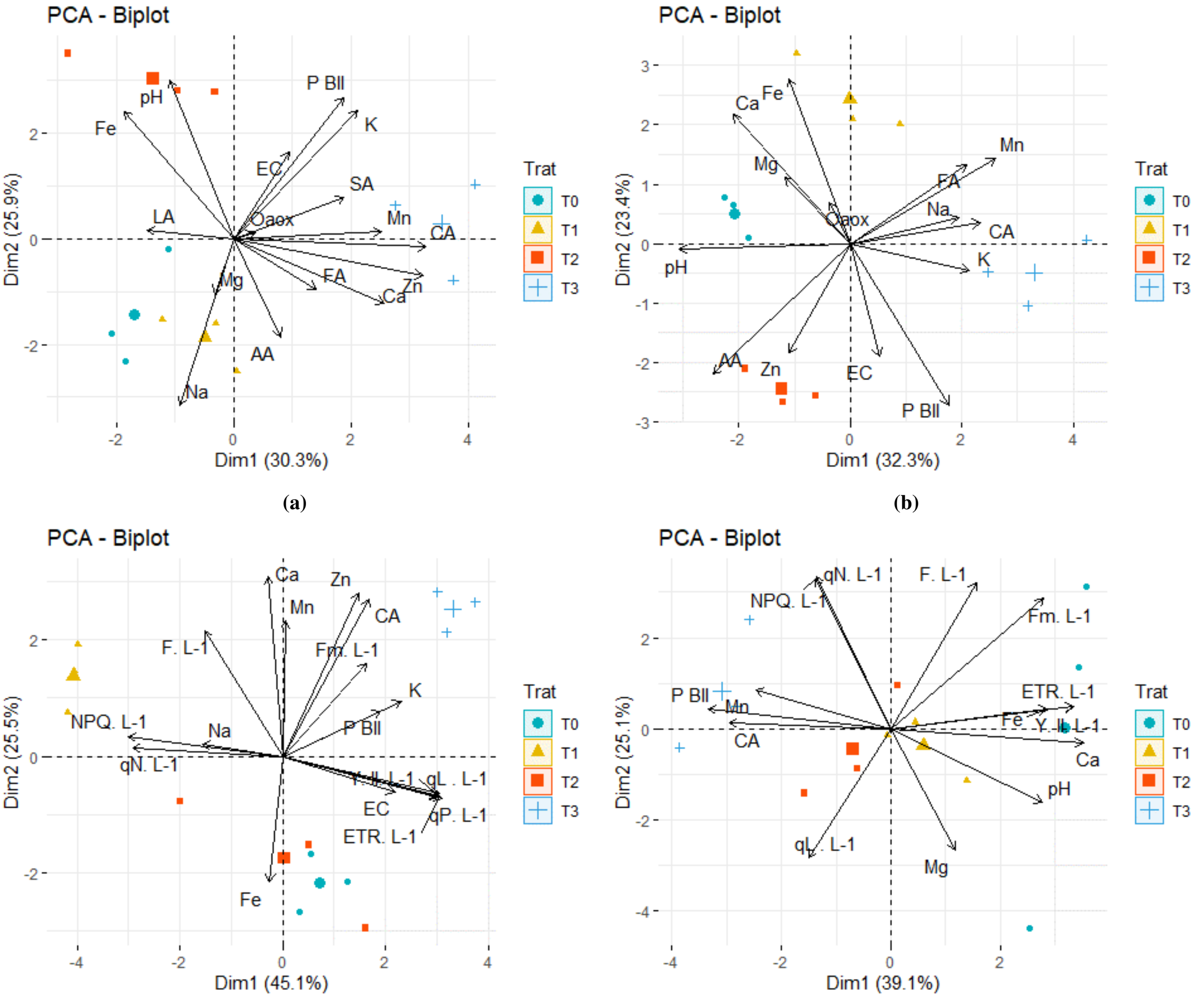
Figure 6. Behavior of soil organic acids with the addition of liquid organic mineral fertilizer in the cultivation of *ICA Cerinza* bean. Oxalic Acid (OxA), Formic Acid (FA), Lactic Acid (LA), Citric Acid (CA), Acetic Acid (AA), Succinic Acid (SA).

the first two dimensions explain 55.72% of the variability. Additionally, the correlation coefficients for the samples collected at 88 days are shown in Appendix 7, featuring the following characteristics: strong positive relationship between Mn and AC; negative relationship between pH and AA, negative relationship between Zn and EC, and positive relationship between Ca and Fe.

Fig. 7c, presents the biplot with the first two principal components for chemical and physiological variables taken at 55 days and 61 days, respectively. This graph shows that the first two components explain 70.6% of the total variability. The variables “ETR. L-1", “Y. II. L-1", “qP. L-1", “qN. L-1", and “qL. L-1" stand out as the main contributors to Dimension 1, which appears to relate to photosynthetic processes. On the other hand, the variables Ca, Fe, Mn, Zn, and K are the most influential in Dimension 2, which appears to be associated with the concentration of certain nutrients, suggesting possible response patterns or interactions in the soil. Fig. 7d, displays the biplot with the first two principal components for chemical and physiological variables taken at 89 days and 88 days, respectively; the variability explained by the first two components is 64.2%. It is observed that variables “Y. II. L-1", “ETR. L-1", Fe, and Ca exhibit a positive correlation in Dimension 1, while showing a negative correlation with variables P BII, Mn, and AC. These latter variables, in turn, show an association with T3. Finally, in Appendix 8, the analysis of comparisons between different treatments (T0, T1, T2, T3) for each variable measured at two different times (55 and 88 das) is shown, where statistical tests were used to evaluate whether there are significant differences between the groups.

Final observation

RedVital, through the transformation of UOSW has successfully reduced waste disposal in landfills by 50%, achieving the recovery of biodegradable material via the fermentation of leachate to produce pathogen-free, heavy metal-free MOLF. Additionally, research has identified the presence of organic molecules within the fertilizer, including oxalic, butyric, malic, and acetic acids, among others, which can positively influence the solubilization of phosphorus (P) present in phosphate rock, a key source of P for MOLF production (Cuy et al., 2024). Moreover, MOLF contains microbial populations that contribute to soil microbial enrichment



(a) (b)

(c) (d)

Figure 7. Principal components analysis of the soil samples at 55 das (a), 88 das (b), soil and physiological variables at 55 days and 61 days (c) and soil and physiological variables at 89 days and time 88 (d).

(García et al., 2021). One of the main advantages of using organic fertilizers, in general, is their positive impact on soil properties, particularly the increase in effective cation exchange capacity (CEC), chelation processes with ions (Al^{3+} and Fe^{3+}), aggregate formation, and water retention (Bohórquez-Sandoval et al., 2024). These characteristics can positively influence soil health and support crop development. In turn, these findings highlight the potential of MOLF as a sustainable alternative to conventional fertilization, contributing to improved environmental sustainability.

4. Conclusion

The incorporation of MOLF in the *ICA Cerinza* bean crop increased the available P content with concentrations similar to those in the inorganic fertilizer treatment, due to the presence of organic acids that persist over time. On the other hand, it was evident that the behavior of the Chlorophyll Index (CI) in T0, T1, and T2 was more consistent than in T3, and was within the ranges of 35 to 50 SPAD for the measured phenological stages, while T3 was below the CI range, which could be linked to the presence of micro micronutrients and microorganisms. Additionally, fluorescence data showed heterogeneous behavior in the cross treatments, and this was associated with nutrient content in the soil, changes in the microorganism fauna, and the presence of slugs. Therefore, liquid organic fertilizers can contribute to nutrient availability and influence energy dissipation mechanisms under environmental stress. Furthermore, six organic acids (oxalic, citric, formic, acetic, lactic, and succinic) were identified, with higher concentrations observed in the early growth stages, and their production was closely related to P content and the addition of MOLF. Finally, this study demonstrates the potential of using MOLF in crops of national interest, improving soil nutrient availability and promoting the secretion of organic substances that can aid in nutrient mobilization.

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Authors contributions

Vargas-Cuy AM: Experimental development, research, data analysis, writing, original draft. García-Molano JF: Research, data analysis, project lead, writing, original draft. Moreno-Amaya LA: Statistical analysis, writing of the statistical section of the draft. Páez-Guevara LA: Research, data analysis, writing, original draft, project lead.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix 1. Chemical characteristics of UOSW composting leachate.

Parameter	Unit	Value
pH	–	4.40
EC	mS/cm	17.82
TOC	g/L	35.13
Fe	Ppm	2.03
Mn	Ppm	13.53
Zn	Ppm	9.10
K	Ppm	52.62
Na	Ppm	100.12
Ca	Ppm	990.56
Mg	Ppm	76.15
TKN	g/L	0.33
TP	g/L	0.49

Appendix 2. Chemical characteristics of MOLF derived from UOSW composting leachate.

Parameter	Unit	Value
pH	–	3.43
EC	mS/cm	67.36
TOC	g/L	39.09
Fe	Ppm	67.36
Mn	Ppm	20.55
Zn	Ppm	70.59
K	ppm	1133.48
Na	ppm	56.92
Ca	ppm	2728.56
Mg	ppm	196.18
TKN	g/L	5.53
TP	g/L	0.77
SP	g/L	0.54

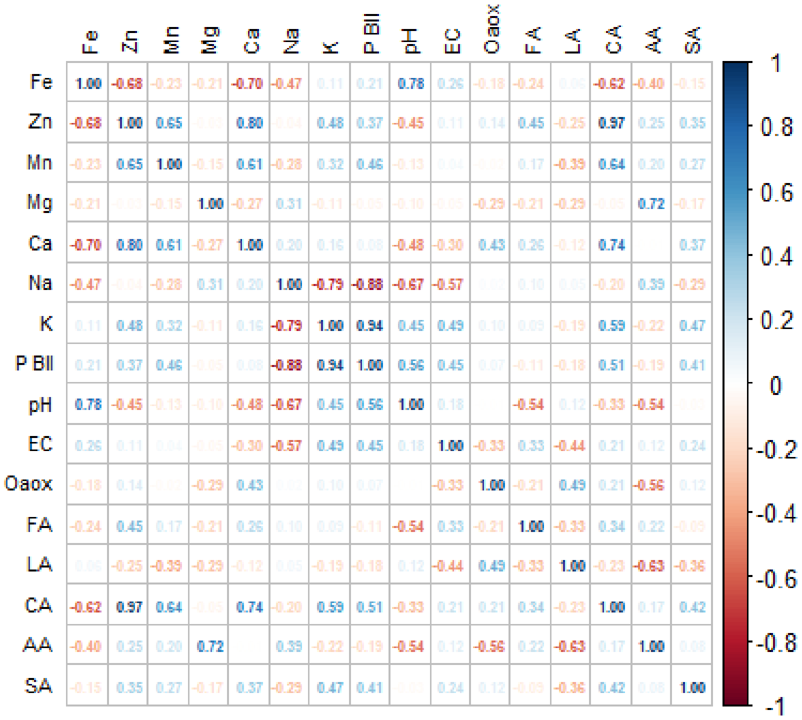
Appendix 3. Chemical characteristics of the soil used in the cultivation of *ICA Cerinza* beans.

Parameter	Unit	Value
pH	–	4.74
EC	mS/cm	0.32
OM	%	
Fe	ppm	67.03
Mn	ppm	9.33
Zn	ppm	0.95
K	ppm	117.00
Na	ppm	48.30
Ca	ppm	182.00
Mg	ppm	22.80
P BII	ppm	31.02

Appendix 4. Bean cultivation in the San Francisco de Asís Experimental Farm of the FUJDC-Soracá-Boyacá.

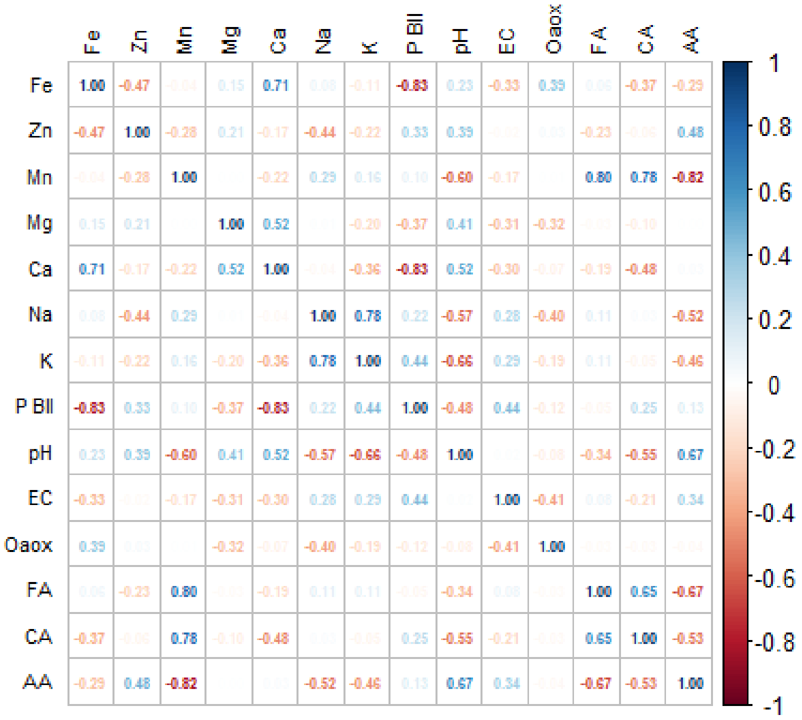
Appendix 5. Summary of the treatments used for the development of the *ICA Cerinza* bean crop.

Physiological state	Treatment	Proportions	Total plant dose
Leaf development	T0	-	-
	T1	95 mL of water 5 mL of M-FL	100 mL
	T2	95 mL of water 5 mL de MOLF	
	T3	95 mL de water 5 mL de CLF	
Floral organ appearance	T0	-	-
	T1	142.5 mL of water 7.5 mL M-FL	150 mL
	T2	142.5 mL of water 7.5 mL MOLF	
	T3	142.5 mL of water 7.5 ml of CLF	
Flowering	T0	-	-
	T1	190 mL of water 10 mL de M-FL	200 mL
	T2	190 mL of water 10 mL of MOLF	
	T3	190 mL of water 10 mL of CLF	



	Fe	Zn	Mn	Mg	Ca	Na	K	P BII	pH	EC	Oaox	FA	LA	CA	AA	SA
Fe	1.00	-0.68	-0.23	-0.21	-0.70	-0.47	0.11	0.21	0.78	0.26	-0.18	-0.24	0.06	-0.62	-0.40	-0.15
Zn	-0.68	1.00	0.65	-0.03	0.80	-0.04	0.48	0.37	-0.45	0.11	0.14	0.45	-0.25	0.97	0.25	0.35
Mn	-0.23	0.65	1.00	-0.15	0.61	-0.28	0.32	0.46	-0.13	0.04	-0.02	0.17	-0.39	0.64	0.20	0.27
Mg	-0.21	-0.03	-0.15	1.00	-0.27	0.31	-0.11	-0.05	-0.10	-0.05	-0.29	-0.21	-0.29	-0.05	0.72	-0.17
Ca	-0.70	0.80	0.61	-0.27	1.00	0.20	0.16	0.08	-0.48	-0.30	0.43	0.26	-0.12	0.74		0.37
Na	-0.47	-0.04	-0.28	0.31	0.20	1.00	-0.79	-0.88	-0.67	-0.57	0.02	0.10	0.05	-0.20	0.39	-0.29
K	0.11	0.48	0.32	-0.11	0.16	-0.79	1.00	0.94	0.45	0.49	0.10	0.09	-0.19	0.59	-0.22	0.47
P BII	0.21	0.37	0.46	-0.05	0.08	-0.88	0.94	1.00	0.56	0.45	0.07	-0.11	-0.18	0.51	-0.19	0.41
pH	0.78	-0.45	-0.13	-0.10	-0.48	-0.67	0.45	0.56	1.00	0.18		-0.54	0.12	-0.33	-0.54	-0.03
EC	0.26	0.11	0.04	-0.05	-0.30	-0.57	0.49	0.45	0.18	1.00	-0.33	0.33	-0.44	0.21	0.12	0.24
Oaox	-0.18	0.14	-0.02	-0.29	0.43	0.02	0.10	0.07		-0.33	1.00	-0.21	0.49	0.21	-0.56	0.12
FA	-0.24	0.45	0.17	-0.21	0.26	0.10	0.09	-0.11	-0.54	0.33	-0.21	1.00	-0.33	0.34	0.22	-0.09
LA	0.06	-0.25	-0.39	-0.29	-0.12	0.05	-0.19	-0.18	0.12	-0.44	0.49	-0.33	1.00	-0.23	-0.63	-0.36
CA	-0.62	0.97	0.64	-0.05	0.74	-0.20	0.59	0.51	-0.33	0.21	0.21	0.34	-0.23	1.00	0.17	0.42
AA	-0.40	0.25	0.20	0.72		0.39	-0.22	-0.19	-0.54	0.12	-0.56	0.22	-0.63	0.17	1.00	0.08
SA	-0.15	0.35	0.27	-0.17	0.37	-0.29	0.47	0.41	-0.03	0.24	0.12	-0.09	-0.36	0.42	0.08	1.00

Appendix 6. Pearson correlation matrix of chemical variables and organic acids at 55 das.



	Fe	Zn	Mn	Mg	Ca	Na	K	P BII	pH	EC	Oaox	FA	CA	AA
Fe	1.00	-0.47	-0.04	0.15	0.71	0.08	-0.11	-0.83	0.23	-0.33	0.39	0.06	-0.37	-0.29
Zn	-0.47	1.00	-0.28	0.21	-0.17	-0.44	-0.22	0.33	0.39	-0.02	0.03	-0.23	-0.06	0.48
Mn	-0.04	-0.28	1.00		-0.22	0.29	0.16	0.10	-0.60	-0.17		0.80	0.78	-0.82
Mg	0.15	0.21		1.00	0.52	0.01	-0.20	-0.37	0.41	-0.31	-0.32	-0.03	-0.10	
Ca	0.71	-0.17	-0.22	0.52	1.00	-0.04	-0.36	-0.83	0.52	-0.30	-0.07	-0.19	-0.48	0.03
Na	0.08	-0.44	0.29	0.01	-0.04	1.00	0.78	0.22	-0.57	0.28	-0.40	0.11	0.03	-0.52
K	-0.11	-0.22	0.16	-0.20	-0.36	0.78	1.00	0.44	-0.66	0.29	-0.19	0.11	-0.05	-0.46
P BII	-0.83	0.33	0.10	-0.37	-0.83	0.22	0.44	1.00	-0.48	0.44	-0.12	-0.05	0.25	0.13
pH	0.23	0.39	-0.60	0.41	0.52	-0.57	-0.66	-0.48	1.00	0.02	-0.08	-0.34	-0.55	0.67
EC	-0.33	-0.02	-0.17	-0.31	-0.30	0.28	0.29	0.44	0.02	1.00	-0.41	0.08	-0.21	0.34
Oaox	0.39	0.03		-0.32	-0.07	-0.40	-0.19	-0.12	-0.08	-0.41	1.00	-0.03	-0.03	-0.04
FA	0.06	-0.23	0.80	-0.03	-0.19	0.11	0.11	-0.05	-0.34	0.08	-0.03	1.00	0.65	-0.67
CA	-0.37	-0.06	0.78	-0.10	-0.48	0.03	-0.05	0.25	-0.55	-0.21	-0.03	0.65	1.00	-0.53
AA	-0.29	0.48	-0.82		0.03	-0.52	-0.46	0.13	0.67	0.34	-0.04	-0.67	-0.53	1.00

Appendix 7. Pearson correlation matrix of chemical variables and organic acids at 88 das.

Appendix 8

Appendix 8: Comparative results between treatments

Table 8.1 shows the results of comparisons between different treatments (T0, T1, T2, T3) for each variable measured at two different time points (55 and 88 days after the beginning of the experiment). Statistical tests were used to evaluate if significant differences exist between groups. The p-values indicate the probability of obtaining results as extreme as those observed, assuming no real differences; lower p-values indicate greater evidence of significant differences. Asterisks indicate significance (*). The common convention is used that a p value < 0.05 is considered significant, $p < 0.01$ highly significant and $p < 0.001$ highly significant. Finally, variables that did not show statistically significant differences between treatments were not incorporated in the results. This suggests that, for those specific variables, the experimental conditions and treatments applied did not generate statistically significant changes at the two time points considered.

Table 8.2 shows the data from the analysis of the effect of time in each treatment, considering a period of 55 to 88 days after planting. The values indicated with asterisks represent statistically significant differences. The results suggest that there are significant variations in some variables as a function of time, depending on the treatment applied.

Table 8.3 shows the results of the multiple comparisons of the treatments for the chlorophyll variable at the four time points and shows statistically significant changes at each of the four time points considered, and the magnitude of these variations is influenced by the treatment applied.

On the other hand, a temporal analysis was carried out at the different sampling times (Appendix Table 8.4). These results indicate that there are significant differences in the variable analyzed between the different times intervals considered. For example, noticeable changes are observed between the periods of 47 to 61 days after planting in treatments 2 and 3, 47 to 75 days in treatment 3 and 61 to 75 days in treatments T0, T2 and T3, suggesting a significant influence of time on the variable evaluated.

Table 8.5 shows the significant differences in conductance between treatments at different periods. At 47 days, T0-T1 and T0-T2 are significant, while T1-T3 and T2-T3 are highly significant. At 75 days, T0-T1, T0-T2, T1-T3, and T2-T3 are significant. At 89 days, T0-T1, T0-T3 and T1-T2 are significant. These results highlight the temporal influence and differences between treatments on conductance.

The temporal analysis for conductance is shown in Table 8.6. These results reveal significant differences in the variable analyzed over the different time intervals considered. During the period from 47 to 61 days after planting, treatments T2 and T3 exhibit values below 0.05, which conclusively indicates a significant difference in that time interval. On the other hand, in the period from 61 to 89 days after sowing (dds), no significant differences were observed in any of the four treatments.

Appendix 8.1. Results of Multiple Comparison Tests for evaluation of significant differences between treatments for the chemical variables at 55 and 88 das.

	Samples 55 das					
Parameter	T0-T1	T0-T2	T0-T3	T1-T2	T1-T3	T2-T3
Fe	0.56	0.216	0.23	0.00009***	0.265	0.01*
Zn	0.126	0.077	0.005**	0.04*	0.029	0.002***
Mn	0.172	0.463	0.021*	0.594	0.431	0.385
Ca	0.006	0.815	0.01*	0.005***	0.392	0.005
Na	0.354	0.032	0.103	0.05	0.064	0.196
K	0.606	0.033*	0.04*	0.006**	0.006**	0.188
PBII	0.757	0.004**	0.004**	0.007**	0.001**	0.723
pH	0.803	0.049	0.566	0.046*	0.641	0.01
AC	0.245	0.881	0.012*	0.155	0.002**	0.002**
	Samples 88 das					
Fe	0.487	0.092	0.035*	0.036*	0.06	0.441
Zn	0.808	0.038*	0.957	0.105	0.950	0.378
Mn	0.001**	0.166	0.016*	0.022*	0.723	0.001***
Ca	0.724	0.035	0.047	0.26	0.213	0.115
Na	0.671	0.011*	0.308	0.682	0.205	0.100
K	0.151	0.082	0.029*	0.810	0.016*	0.005**
PBII	0.026	0.0003***	0.004**	0.002**	0.005**	0.123
pH	0.383	0.728	0.017	0.390	0.110	0.017
CA	0.003**	0.012*	0.074	0.006**	0.918	0.149

(*) $P < 0.05$, (**) $P < 0.01$, (***) < 0.001

Appendix 8.2. Results of Multiple Comparison Tests to evaluate significant differences at times 55 and 88 das in chemical variables.

Parameter	T0	T1	T2	T3
Fe	0.229	0.242	0.016*	0.011*
Zn	0.040*	0.169	0.009**	0.251
Ca	0.408	0.010**	0.039*	0.003**
Na	0.015*	0.122	0.014*	0.012*
K	0.821	0.375	0.002**	0.015*
pH	0.232	0.828	0.037*	0.038*
AA	0.008**	0.040*	0.223	0.039*
CA	0.079	0.059	0.254	0.007**

(*) $P < 0.05$, (**) $P < 0.01$, (***) < 0.001

Appendix 8.3. Results of Multiple Comparison Tests for evaluation of significant differences between treatments at different times for the Chlorophyll variable.

	T0-T1	T0-T2	T0-T3	T1-T2	T1-T3	T2-T3
47 das	0.18900	0.74300	0.00060**	0.75800	0.00100**	0.00000***
61 das	0.12100	0.00002***	0.01600	0.00070**	0.01900*	0.05560
75 das	0.15000	0.06000	0.18000	0.00200**	0.00003***	0.00001***
89 das	0.12000	0.08600	0.00500*	0.77000	0.00336**	0.11300

(*) $P < 0.05$, (**) $P < 0.01$, (***) < 0.001

Appendix 8.4. Results of Multiple Comparison Tests to evaluate significant differences at times 55 and 88 das for the Chlorophyll variable.

Days	T0	T1	T2	T3
47-61	0.486	1.000	0.009**	0.007**
47-75	1.000	1.000	0.245	0.004***
47-89	0.202	1.000	1	1.000
61-75	0.024*	1.000	0.003**	0.018*
61-89	1.000	1.000	1.000	1.000
75-89	0.648	1.000	1.000	0.009**

Appendix 8.5. Results of Multiple Comparison Tests to evaluate significant differences between treatments at different times for the conductance variable.

Days	T0-T1	T0-T2	T0-T3	T1-T2	T1-T3	T2-T3
47 das	0.019*	0.05*	0.123	0.1	0.005*	0.015
61 das	0.246	0.423	0.074	0.397	0.001**	0.060
75 das	0.040*	0.010*	0.956	0.153	0.030*	0.020*
89 das	0.023*	0.579	0.015*	0.008*	0.267	0.199

(*) $P < 0.05$, (**) $P < 0.01$, (***) < 0.001

Appendix 8.6. Results of Multiple Comparison Tests to evaluate significant differences at times 55 and 88 das for the variable conductance.

Days	T0	T1	T2	T3
47-61	0.486	1.000	0.009**	0.007**
47-75	1.000	1.000	0.245	0.004***
47-89	0.202	1.000	1	1.000
61-75	0.024*	1.000	0.003**	0.018*
61-89	1.000	1.000	1.000	1.000
75-89	0.648	1.000	1.000	0.009**

(*) $P < 0.05$, (**) $P < 0.01$, (***) < 0.001

Appendix 8.7. Standard Error of Physiological Parameters in Bean Cultivation at 55 Days.

Treatment	Fresh biomass stem root	Fresh biomass stem	Fresh biomass root	Dry biomass stem root	Dry biomass stem	Dry biomass root	Stem length	Root length
T0	0.771	0.363	0.417	0.013	0.024	0.011	0.520	2.670
T1	0.429	0.330	0.193	0.120	0.135	0.032	0.478	1.027
T2	0.393	0.098	0.360	0.066	0.068	0.032	0.120	1.401
T3	0.401	0.273	0.163	0.126	0.116	0.050	0.087	0.462

Appendix 8.8. Standard Error of Physiological Parameters in Bean Cultivation at 88 Days.

Treatment	Fresh biomass stem root	Fresh biomass stem	Fresh biomass root	Dry biomass stem root	Dry biomass stem	Dry biomass root	Stem length	Root length
T0	0.925	0.911	0.015	0.272	0.221	0.051	5.622	1.732
T1	0.252	0.391	0.159	0.057	0.083	0.029	0.726	1.471
T2	0.677	0.259	0.461	0.091	0.048	0.048	0.404	3.484
T3	0.842	0.953	0.269	0.347	0.353	0.086	2.566	0.913