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# Application of *Trichoderma viride* immobilized on rice husk under reduced synthetic fertilization in yellow chili pepper (*Capsicum baccatum* L.) var. pendulum

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**Introduction:** The intensive use of synthetic fertilizers in horticultural crops generates economic and environmental impacts, particularly in already degraded soils. Sustainable strategies are therefore required to reduce mineral fertilizer inputs without compromising crop productivity. This study evaluated the effect of an organic amendment containing *Trichoderma viride* immobilized on rice husk as a strategy to reduce mineral fertilization in yellow chili pepper (*Capsicum baccatum* L.) var. pendulum.

**Methods:** Four treatments were evaluated in a completely randomized block design with three replications: T1 (complete fertilization with 200:66:42 kg ha$^{-1}$ N:P:K), T2 (135:48:21 kg ha$^{-1}$ N:P:K without organic amendment), T3 (135:48:21 kg ha$^{-1}$ N:P:K plus organic amendment at 0.5 kg plant$^{-1}$), and T4 (organic amendment only). Crop variables (height, chlorophyll index, root depth, fruit number, yield, and dry matter) were evaluated.

**Results:** Full fertilization (T1) increased plant height and showed a slight tendency toward higher chlorophyll index and fruit number values. However, yields under reduced fertilization (T2 and T3) were statistically similar to T1, indicating that mineral fertilization could be reduced by 30% without significant yield penalties under the evaluated conditions. The addition of the organic amendment in T3 did not significantly increase yield compared with T2, but promoted greater dry matter allocation to leaves, stems, and roots, suggesting a potential positive effect on vegetative development.

**Discussion:** The results indicate that reducing mineral fertilization in yellow chili pepper is feasible without compromising productivity. The incorporation of *T. viride* immobilized on rice husk may represent a complementary strategy for agroecological transition while promoting the recycling of agro-industrial residues. Nevertheless, because the findings derive from a single growing season, multi-year studies are required to validate these results and to assess long-term effects on soil properties, microbiota dynamics, commercial scalability, and economic viability.

KEYWORDS
**agro-industrial waste, microbial immobilization, organic amendment, reduced fertilization, *Trichoderma viride***

## 1 Introduction

The yellow chili pepper (*Capsicum baccatum* L.) var. pendulum is a crop native to the Andean regions of South America, specifically Bolivia, Peru, and Ecuador, where favorable climatic and soil conditions support its growth (Bosland and Votava, 2012). The plant has an erect growth habit and can reach an average height of 122cm (Rojas et al., 2016). The fruits reach a length of 10 to 15 centimeters (although some accessions can achieve greater length) and display a deep orange coloration upon ripening; these thin-walled peppers possess a fruity taste with notes reminiscent of berries and an intense, sharp heat (Ugas and Mendoza, 2012; DeWitt and Bosland, 2009). In Peru, the yellow chili pepper, also known as pickled chili pepper or mirasol chili pepper after dehydration, is one of the most important species of the *Capsicum* genus due to its high commercial value and cultural significance in the Andean regions (Bosland and Votava, 2012; Jäger et al., 2013). Its fruits are essential to Peruvian gastronomy and represent a significant source of income for small farmers, the primary producers of this crop (Jäger et al., 2013).

According to the Ministry of Agrarian Development and Irrigation (MIDAGRI) (2024), the cultivated area of yellow chili pepper in Peru between August 2023 and July 2024 reached 5,739 hectares, with a total production of 60,111 tons. Of this total, the region of Arequipa contributed 254 hectares of cultivated area and a production of 1,044 tons. This production is supported by both, domestic and international demand, driven by the boom in Peruvian gastronomy, where chili pepper is a key ingredient (Aguirre-Sosa et al., 2023). However, the production of this crop, like all others, faces challenges due to inadequate management practices and the threat of climate change (Mazuela, 2013). Among these challenges, the excessive use of synthetic fertilizers stands out as a vector of diffuse pollution of water bodies, atmospheric pollution from nitrous oxide emissions, soil degradation due to the deterioration of its structure, and the loss of organic matter and microbial diversity (Savci, 2012; Tripathi et al., 2020; González-Marquetti et al., 2019). This problem arises from the definition of nutrient management plans based on empirical strategies, or the lack of documented strategies that integrate practices to optimize the use of N and P, thereby reducing their environmental impact (Wu and Ge, 2019; Weil and Brady, 2017). In this context, there is a need to implement sustainable strategies that maintain or improve crop yields while minimizing the environmental and economic impact.

The use of beneficial microorganisms, such as those of the *Trichoderma* genus, has gained relevance as a management alternative capable of improving crop growth and yield by increasing nutrient availability in the soil, acting as biological control (Zin and Badaluddin, 2020; Ji et al., 2020), and stimulating the production of plant hormones, such as organic acids, auxins, and gibberellins (López-Valenzuela et al., 2022). Additionally, *Trichoderma* strengthens the adaptive capacity of crops by contributing to their tolerance to abiotic stresses, such as drought, salinity, and extreme temperatures (Kashyap et al., 2017). In particular, *Trichoderma viride* can produce secondary metabolites, such as chitinases, release hydrolytic enzymes, and compete effectively with pathogenic organisms for resources, allowing it to induce defense responses in the host plant and contribute to disease suppression (Błaszczyk et al., 2014; Abu-Tahon and Isaac, 2020; Naglot et al., 2015). Thus, the biocontrol effect of this species promotes plant growth (Thilagavathi et al., 2007). Additionally, *T. viride* solubilizes inorganic phosphorus and mineralizes organic phosphorus, increasing its availability in the soil and its subsequent uptake by seedlings. Thus, it has been documented to regulate root morphology, increase dry weight, improve chlorophyll fluorescence, and increase the content of photosynthetic pigments, thereby promoting plant growth (Song et al., 2023) when added.

On the other hand, the use of organic amendments is a widely used practice due to its potential contribution to crop productivity and soil health. This requires a thorough understanding of the amendment and the recipient agroecosystem to minimize risks and enhance benefits (Urra et al., 2019). Thus, the combined use of solid organic amendments and beneficial microorganisms has been documented to increase the positive effects on yield in various agricultural systems. For example, the simultaneous application of compost with beneficial microorganisms (Arbuscular Mycorrhizal Fungi and Plant Growth Promoting Rhizobacteria) has shown significant improvements in soil fertility and the yield of various crops (Meddich et al., 2020). Similarly, the integration of poultry manure with phosphate-solubilizing bacteria has been shown to increase the availability of essential nutrients and optimize plant growth, particularly in calcareous soils (Adnan et al., 2019). Specifically for *Trichoderma* spp., its application combined with compost has promoted phosphorus absorption and increased the concentration of photosynthesis-related metabolites, resulting in a significant increase in dry biomass (Vinci et al., 2018) and the promotion of early growth in various crops (Yazdani et al., 2011).

Rice husks have proven to be a viable alternative for incorporation into the soil due to their ability to increase organic carbon (Linam et al., 2023), improve soil physical properties, and increase crop growth and yield (Ebaid and El-Refaee, 2007; Mishra et al., 2017; Bassuony, 2015). Furthermore, their rich composition of cellulose (38%), lignin (27%), and proteins (4%) provides an ideal support for beneficial microorganisms, improving soil-plant interactions (Arévalo et al., 2017). However, despite growing evidence on the benefits of synergistic interactions between organic amendments and microorganisms, there is a notable lack of research specifically evaluating the effect of the combining rice husks and *T. viride* as a productivity improvement strategy. This could be particularly relevant in coastal valleys where rice is widely cultivated, such as in the Arequipa region (MIDAGRI, 2024), generating large volumes of husks as a residue from grain processing (between 20% and 33% of its total weight (Koopmans and Koppejan, 1997)). Based on the documented benefits of *T. viride* as a plant growth promoter and rice husks as organic amendments, we hypothesize that *T. viride* immobilized on rice husks will enable a reduction in mineral fertilization while maintaining comparable yields in yellow pepper cultivation. In this context, the present study aims to evaluate the effect of *T. viride* immobilized on rice husks as a strategy to reduce mineral fertilization in the cultivation of yellow pepper *(Capsicum baccatum* L.) var. pendulum.

# 2 Materials and methods

## 2.1 Study area

The research was conducted at the Agrarian Experimental Station (EEA) in Arequipa, Perú, located within National Institute of Agrarian Innovation (INIA, in the Santa Rita de Siguas district, Arequipa province, Arequipa region. The experimental plot was located at 16°28'24,1632"S, 72°6' 44,21"W, 1285 m.a.s.l (Supplementary Figure 1). The study area has an average annual precipitation of 0.11 mm, with minimum temperatures reaching 9.3°C in July and maximum temperatures ranging between 25 and 26°C from September to April. These averages were calculated based on historical data (1949-2013) from the Majes meteorological station (16°20'8.4"S, 72°9'8.92"W), managed by the National Meteorology and Hydrology Service of Peru (SENAMHI, 2024). Additionally, during study months (April 2024 - February 2025), meteorological parameters were collected from the Santa Rita meteorological station, also managed by SENAMHI Supplementary Figure 2) located adjacent to the experimental plot.

## 2.2 Experimental design

The study was established under a Completely Randomized Block Design (CRBD) with four treatments and three blocks (n=3). The treatments were related to the application of amendments based on rice husk inoculated with *Trichoderma viride* (Table 1). Each treatment was randomly assigned within each block as shown in Supplementary Figure 1B. Each experimental unit consisted of 70 plants, with a total of 840 plants throughout the experiment. The area of each experimental unit was 42 $m^2$ (6 m × 7 m).

## 2.3 Physical and chemical characteristics of soils

Before installing the experiment, a composite sample was extracted from the plough layer of the soil (0.30 m deep). The sample was submitted to physicochemical characterization at the Soil, Water and Foliar Laboratory of INIA Arequipa (LABSAF-INIA Arequipa). The results showed, 21.9 mg $kg^{-1}$ of available phosphorus (Olsen and Sommers, 1982), and 497 mg $kg^{-1}$ of available potassium (Semarnat, 2002), a bulk density (BD) of 1.3 g $cm^{-3}$ (Blake and Hartge, 1986), a pH of 7.9 (McLean, 1982); an electrical conductivity (EC1:5) of 246.2 mS $m^{-1}$; and an organic matter content (OM) of 1.06% (Walkley and Black, 1934). The textural class corresponds to a sandy loam with a composition of 73% sand, 5% clay, and 23% silt (Bouyoucos, 1962).

TABLE 1 Experimental treatments.

| Treatment | Treatment description |
|---|---|
| T1 | 200:66:42 kg $ha^{-1}$ for N:P:K |
| T2 | 135:48:21 kg $ha^{-1}$ for N:P:K |
| T3 | 135:48:21 kg $ha^{-1}$ for N:P:K + *T. viride* immobilized on rice husk (0.5 *kg* $plant^{-1}$) |
| T4 | *T. viride* immobilized on rice husk (0.5 kg $plant^{-1}$) |

## 2.4 Experimental plot management

The experimental plot was established in April 2024. The land was previously subsoiled, plowed, and leveled using a John Deere 6110D agricultural tractor. A tracked furrower attached to the tractor was used to construct the beds, which were 0.30 m high and spaced 1.20 m apart.

Subsequently, a surface drip irrigation system was installed, initially with one strip per furrow (16 mm inner diameter, in-line drippers every 20 cm, and a flow rate of 1.2 liters per hour at a nominal operating pressure of 60 kPa). At 120 days after transplant (DAT), a second strip was added per bed to optimize irrigation distribution. A fertigation arch with a Venturi injector was installed at the entrance to the plot. This system enabled irrigation during the campaign, with a total volume of 7550 $m^3$ $ha^{-1}$, and an irrigation frequency of every two days: one hour in the early stages of the crop and another, one and a half hours from the beginning of flowering. The irrigation depth (ID) was calculated according to the soil water balance formula proposed by Allen et al. (1998).

$$ID = ET_c - Pe.$$

$$ET_c = ET_0 \times Kc.$$

Where $ET_c$ is the crop evapotranspiration (mm), Pe is the effective precipitation (mm), $ET_0$ is the reference crop evapotranspiration (mm), and Kc is the crop coefficient. Pe was calculated according to the USDA methodology (Smith, 1992). The $ET_0$ value was calculated with the Penman-Monteith equation modified by FAO (Allen et al., 1998) from climate data recorded by the SENAMHI Pampa de Majes meteorological station. Kc varied according to the development of the chili crop (Allen et al., 2006).

Transplanting of the yellow chili pepper seedlings of the 'Pacae' cultivar took place on April 25, 2024 (seedlings were purchased from a local nursery). At that time, the seedlings had an average height of 18 cm and were fully developed, with six leaves. Planting was carried out with a 0.6 m spacing between plants and 1.2 m between beds, which allowed reaching a planting density of approximately 13,888 plants $ha^{-1}$. Tutoring was implemented in all treatments at 95 DAT. Harvesting was carried out at four successive times (every 21 days), beginning at 248 DAT and concluding at 306 DAT.

Phytosanitary control was carried out through programmed applications based on previous evaluations. The insecticides Imidacloprid (400 ml $ha^{-1}$ in 400 L water) and Lambda-cyhalothrin (400 ml $ha^{-1}$ in 400 L water) were used alternately for the control of *Thrips* and *Bemisia tabaci*; the insecticide Alpha-cypermethrin (600 ml $ha^{-1}$ in 400 L water) was only used twice (50 and 80 DAT) for the control of *Agrotis ipsilon* Rott larvae. The first two insecticides were applied starting at 50 DAT, alternating them every 15 days or according to the phytosanitary evaluations results. *Phytophthora* spp. presence was recorded during crop development. For its control, foliar applications of Phosetyl-Al (ALIETTE®) were performed at a rate of 2 kg $ha^{-1}$ at two time points, at 203 and 224 DAT. This application was targeted to plants that showed signs of the disease. We used this product because it does not interfere with the growth of *Trichoderma viride* (Dhanya et al., 2016).

## 2.5 Fertilization

The fertilization dose implemented in the study was based on that proposed by Tirado (2014), who recommends applying 200:66:208 kg ha$^{-1}$ for N:P:K in the yellow chili crop. However, the high content of available potassium in the soil (497 mg kg$^{-1}$) recorded in the physicochemical characterization, justified the application of a reduced dose at a maintenance level equivalent to 20% of the initial recommendation (42 kg ha$^{-1}$ K). Consequently, treatment T1 received an adjusted nutritional formulation of 200:66:42 kg ha$^{-1}$ for N: P:K, respectively. On the other hand, treatments T2 and T3 were established with a significantly lower dose (135:48:21 kg ha$^{-1}$ for N, P and K), corresponding to a reduction of 32.5% N, 26.7% P, and 50% K compared to T1. Treatment T4 corresponded to the non-fertilization control, i.e., no nutrients were applied. The fertilizers used were: ammonium nitrate, monoammonium phosphate and potassium nitrate, applied by fertigation.

The fertilizer dose was distributed throughout the crop (Tirado, 2014). During the vegetative stage, 24% of nitrogen (N), 61% of phosphorus (P) and 13% of potassium (K) were applied. At the flowering stage, 27% N, 12% P, and 16% K were supplied. During fruiting, 26% N, 14% P, and 36% K were added. Finally, at the ripening stage, 23% N, 12% P, and 35% K were incorporated.

## 2.6 Microbial inoculation

A total of 6.9 tons of rice husks per hectare were used as the base substrate. This one was washed five times with sanitized irrigation water in a concrete pond containing 10% commercial hypochlorite. The beneficial fungus *T. viride* was used in the form of a commercial product called TRICHOMAX, which was provided by Soluciones Agrosostenibles S.A.C. (Solagro), based in Trujillo, Peru. This product corresponds to a natural isolate of *T. viride* with an approximate concentration of $1 \times 10^{12}$ conidia·kg$^{-1}$ and is registered with SENASA under the number PBUA 393.

Was inoculated as follows: 60 liters of water and 47 kg of the commercial product (*T. viride*) were used to wash the spores. The remaining suspension was filtered to remove insoluble particles. The filtered suspension was then mixed with 40 kg of molasses. The final solution was diluted in 750 liters of water and added to 6.9 t ha$^{-1}$ of rice husk throughout a 20-liter backpack sprayer. The resulting moist amendment obtained was applied to the soil at a dose of 0.5 kg per plant, at a distance of 0.2 m from the plant neck and at a depth of 0.2 m, specifically in treatments T3 and T4, 74 days after transplanting (DAT), due to logistical constraints in the delivery of the inoculum by the supplier. The original experimental protocol specified its application at the time of transplant establishment. However, application was delayed until 74 DAT. This deviation from the original protocol constitutes a limitation of the study, as it may have influenced fungal colonization and, consequently, treatment efficacy.

## 2.7 Determination of biometric variables and yield components

Using a 3.5 m tape measure, plant height was measured from the neck to the stem apex in ten representative plants per experimental unit. Measurements were taken at the time of *T. viride* incorporation, immobilized on rice husks, into the soil for the corresponding treatments (74 DAT), then at 109 DAT, 151 DAT, 186 DAT, 221 DAT, and 248 DAT (start of the first harvest). After that, crop growth was not registered. For the same evaluation periods, the chlorophyll index (SPAD) was recorded using the portable CONICA MINOLTA SPAD-502 Plus Olympus device. For this purpose, three plants per treatment were randomly selected, and three readings were taken for each, choosing three mature leaves from the upper part of the plant (Islam et al., 2014). Measurements were taken weekly, in the morning between 8:00 and 9:00 am (Yadava, 1986), avoiding direct sunlight to prevent reading errors and photoinhibition (Han et al., 2022). Additionally, the number of days to flowering was recorded when more than half of the population in each experimental unit reached 50% full flowering (Conte et al., 2022). To determine the yield components, three representative plants were chosen per experimental unit. The number of fruits per plant was counted and classified into categories following local criteria (Table 2). In addition, the dry matter of the harvested plants was evaluated by separating the fruits, the aerial parts, and the root parts. The biomass was measured after drying each part in an oven at 70°C for 72 hours (Gandica and Peña, 2015; Casierra and García, 2005; Jinagool and Arom, 2023). From these measurements, the following biomass allocation indicators were calculated: RWR (Root Weight Ratio), which represents the proportion of root dry matter relative to total biomass; SWR (Shoot Weight Ratio), corresponding to the proportion of aboveground dry matter (leaves, stems, and fruits) in relation to total biomass; and SRR (Shoot-to-Root Ratio), which expresses the relationship between aboveground and belowground biomass (Siaga et al., 2024).

Upon completion of the final harvest, root depth (cm) was determined in the sampled plants. Three plants per experimental unit were selected, and a soil pit measuring 40 x 40 x 40 cm was excavated at a distance of 20 cm from each plant collar, using a spade and pickaxe to expose the soil profile without compromising root system integrity. The vertical distance from ground level to the deepest point reached by the roots was measured using a measuring tape, and recorded as the maximum rooting depth for each evaluated plant.

## 2.8 Statistical analysis

Repeated measures variables (plant height and chlorophyll index) were analyzed using generalized linear mixed models (GLMM) in R version 4.4.3, considering plot as random effect. Model selection was performed based on AICc, applying Gamma distribution with logarithmic link. Model assumptions were validated through simulated

TABLE 2 Yellow chili pepper classification into categories according to traditional local criteria.

| Category | Characteristics |
|---|---|
| First | Undamaged, with stem, uniform color, and length >16 cm. |
| Second | With some deformities, with stem, uniform color, and 10–16 cm long. |
| Third | With some deformities, with stem, color 50% discolored, and length <10 cm. |
| Fourth | Fruits that are still soft or overripe; it shows damage (mechanical, insect, or disease-related). It generally does not reach the market. |

residuals. Fixed effects significance was determined by likelihood ratio tests. Yield, dry matter, and root depth variables were analyzed through ANOVA, followed by Tukey's test when significant differences were detected. Normality and homoscedasticity were verified with Shapiro-Wilk and Bartlett tests, respectively. Discrete variables (number of fruits per plant and days to flowering) were analyzed using Kruskal-Wallis test with Dunn's *post hoc* analysis. All tests used $\alpha = 0.05$. ANOVA was performed using INFOSTAT software version 2020 (Di Rienzo et al., 2020), while other analyses were conducted in R version 4.4.3 (R Core Team, 2025).

# 3 Results

## 3.1 Height of the plant and root depth

All factors significantly affected plant height (Supplementary Table 1). Treatment effects were highly significant ($\chi^2 = 129.1$, $p < 0.001$), as were temporal effects (Days: $\chi^2 = 1781.4$, $p < 0.001$; $D^2$: $\chi^2 = 566.8$, $p < 0.001$). The model explained 97% of height variation. Plant height followed a sigmoidal pattern across treatments (Figure 1), with three distinct phases: slow initial growth to flowering (143 DAT), rapid growth during flowering (143–168 DAT), and plateau to harvest (248 DAT). Treatment T1 achieved the highest final height (128.7 cm), followed by T2, T3, and T4. Growth differences became pronounced after flowering onset, with T1 maintaining superior performance throughout the reproductive phase. The average root depth recorded in the different treatments ranged between 31.8 cm (T1) and 29.37 cm (T4), with no significant differences between all treatments (Supplementary Table 2).

## 3.2 Chlorophyll index (SPAD) by development periods

The generalized linear mixed model revealed significant effects of treatment ($\chi^2 = 55.8$, df = 3, $p < 0.01$), time ($\chi^2 = 23.4$, df = 1, $p < 0.01$), and their interaction ($\chi^2 = 54.4$, df = 3, $p < 0.01$) on the chlorophyll index (Supplementary Table 3). The marginal and conditional pseudo-$R^2$ values (0.64 and 0.65, respectively) indicate a good model fit.

Throughout the growing season, T1 tended to exhibit higher chlorophyll index values; however, no significant differences were detected among T1, T2, and T3, as indicated by the overlap of confidence intervals (Figure 2). In contrast, T4 consistently showed significantly lower SPAD values compared with all fertilized treatments during most of the experimental period. All treatments displayed a characteristic decline in chlorophyll index after the flowering stage (150 DAT), with the most pronounced decrease observed in T4, which lacked mineral fertilization. Treatments T1, T2, and T3 followed similar senescence patterns.

## 3.3 Fresh yield

Significant differences were observed in the number of fruits per plant among treatments ($p < 0.05$) (Figure 3). Treatment T1 recorded the highest value, significantly outperforming T4. T2 and T3 did not differ statistically from the other treatments but showed a tendency to present higher values compared to T4. In relative terms, fruit number in T4 was 47% lower than in T1, whereas T2 and T3 showed reductions of 24% and 33%, respectively, compared to the maximum value obtained in T1.

The analysis of yellow chili yields according to commercial categorization (Table 3) shows variations among the different treatments evaluated. In category C1 (first category), treatments T2 and T3 showed the highest values, although without significant differences respect to T1 and T4. For category C2 (the second category), T1 showed the highest yield, which was significantly higher than T4. In contrast, T2 and T3 showed intermediate values, without statistical differences compared to the other treatments. In category C3 (third category), T1 registered the highest value, differing significantly from T3 and T4. Regarding the C4 category (discard category), no significant differences were observed between treatments, although there was a tendency to present higher values

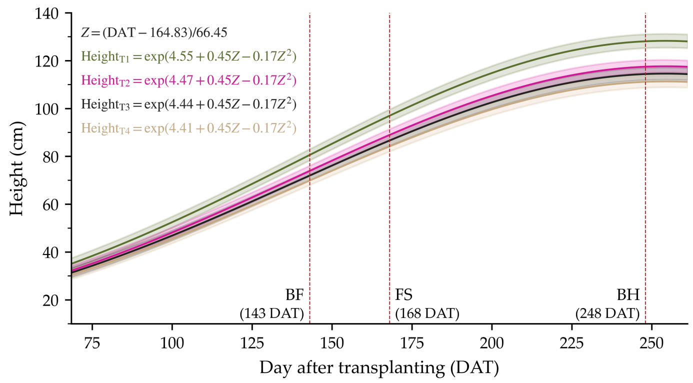


FIGURE 1
Plant height across the evaluated days under constant treatment effects. Z: Standardized values of days after transplanting. The mean number of days is 164.83 with a standard deviation of 66.45. The solid curves represent the plant height values obtained from third-order polynomial equations. The shaded bands around each curve represent the 95% confidence intervals for each treatment. T1 = 200:66:42 kg $ha^{-1}$ for N:P:K (full fertilization), T2 = 135:48:21 kg $ha^{-1}$ for N:P:K, T3 = 135:48:21 kg $ha^{-1}$ for N:P:K + *T. viride* immobilized on rice husk (0.5 kg $plant^{-1}$), T4 = *T. viride* immobilized on rice husk only (0.5 kg $plant^{-1}$). The vertical dashed lines indicate the beginning of flowering (BF), fruiting stage (FS), and the beginning of harvest (BH).

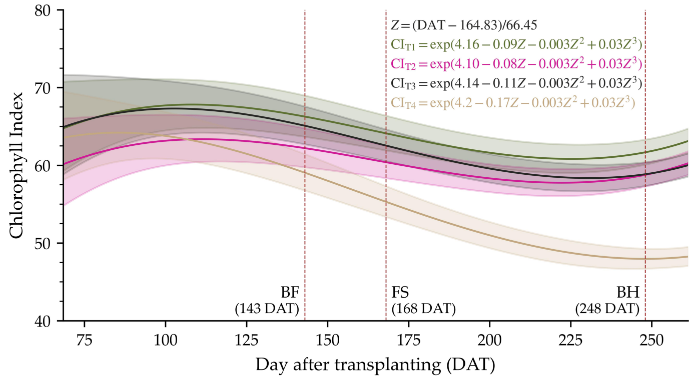


FIGURE 2
Plant chlorophyll index (CI) values across the evaluated days under constant treatment effects. Z: Standardized values of days after transplanting (DAT). The mean number of days was 164.83 with a standard deviation of 66.45. The solid curves represent the fitted chlorophyll index values obtained from third-order polynomial equations. The shaded bands around each curve represent the 95% confidence intervals for each treatment. T1 = 200:66:42 kg $ha^{-1}$ for N:P:K (full fertilization), T2 = 135:48:21 kg $ha^{-1}$ for N:P:K, T3 = 135:48:21 kg $ha^{-1}$ for N:P:K + *T. viride* immobilized on rice husk (0.5 kg $plant^{-1}$), T4 = *T. viride* immobilized on rice husk only (0.5 kg $plant^{-1}$). The vertical dashed lines indicate the beginning of flowering (BF), fruiting stage (FS), and the beginning of harvest (BH).

in T1 and lower values in T4. As for total yield (Y), T1 reached the highest value, although it was statistically similar to the other treatments. Numerically, this represented up to 79% more yield compared to T4. Finally, the commercial yield (CY) defined as the sum of categories C1, C2, and C3 was statistically similar among the four treatments; however, T1 and T2 tended to reach the highest values and T4 showed the lowest value.

## 3.4 Dry matter percent and distribution at harvest

Regarding the percentage of dry matter in fruits, it was observed that treatment T4 presented the highest value, being significantly higher ($p < 0.05$) than treatments T1 and T2, and statistically similar to T3. Regarding root dry matter, treatment T3 exhibited the highest percentage, differing significantly from T1 and T4, but without statistically significant differences from T2. In terms of dry matter accumulation in stems and leaves, treatment T3 reached the highest value, being significantly higher than T4, but without statistical differences with T1 and T2 (Figure 4).

Total dry matter analyses revealed that treatment T1 exhibited the highest biomass accumulation, being statistically similar to treatments T2 and T3, but significantly superior to T4. The latter recorded the lowest biomass accumulation, representing approximately 59% of the biomass obtained in T1. Regarding biomass allocation indices (RWR, SWR, and SRR), no significant differences were observed between treatments (Table 4).

# 4 Discussion

## 4.1 Vegetative development and physiological behavior

As it was expected, the higher the doses of mineral fertilization, the higher the red-hot chili pepper (*Capsicum annuum* L.) yields were reported (Ahmed and Abdelkader, 2020). However, the height differences between non (T4) and medium rates (T3) of mineral fertilization were not significant for treatments with *T. viride* immobilized on rice husk. This suggest that the benefits of this microorganism may be manifested in other agronomic parameters, such as disease resistance or nutrient use efficiency, rather than height (Romero et al., 2022). Excessive vegetative growth does not necessarily translate into higher productivity or agronomic efficiency (Thomas et al., 1978).

The chlorophyll index showed significant differences among treatments at reproductive stages. This trend could be explained by the higher availability of nitrogen in treatments with mineral fertilization, an essential element for chlorophyll synthesis (Costa et al., 2018). However, the statistical similarity of chlorophyll levels between T3 and T1 in early stages, and, T2 and T3 in later stages, indicating that rice husk inoculated with *T. viride* could improve nitrogen use efficiency. Aligned with that, it was reported that *T. viride* enhances the efficiency of nitrogen fertilizing (i.e. improves N uptake, promotes plant growth, and reduce N losses by leaching or volatilization) reducing the environmental impact (Paul et al., 2024; Xu et al., 2017; Wang et al., 2018).

Flowering is mainly determined by external environmental factors (e.g. photoperiod and temperature/greening) in combination with endogenous signals (e.g. genotype and plant age) (Li et al., 2016; Iannucci et al., 2008). Therefore, neither chemical fertilization rates nor the addition of organic amendments affected flowering initiation.

Furthermore, root depth similarities among treatments, was attributed to the presence of a plow pans at approximately 30 cm depth throughout the experimental field. This compacted soil layer, related to the prolonged use of shallow rotary tillage, may have restricted the growth of deeper roots (Sun et al., 2023; Whalley et al., 2000). We were not considered a limiting factor for crop establishment, because approximately 72% of the chili pepper root system within the first 25 cm of the soil profile (Hulugalle and Willatt, 1987).

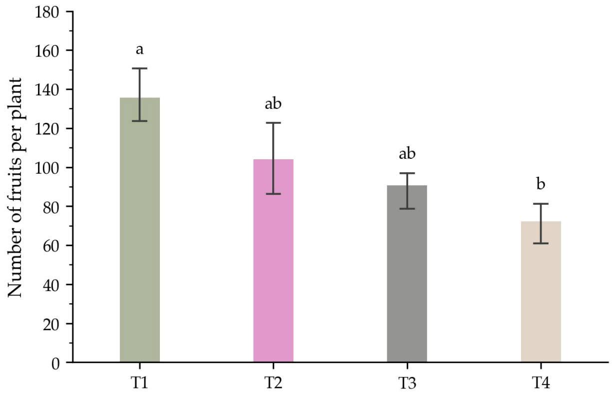


FIGURE 3
Number of fruits per plant in response to different treatments: T1 = 200:66:42 kg ha$^{-1}$ for N:P:K (full fertilization), T2 = 135:48:21 kg ha$^{-1}$ for N:P:K, T3 = 135:48:21 kg ha$^{-1}$ for N:P:K + *T. viride* immobilized on rice husk (0.5 kg plant$^{-1}$), T4 = *T. viride* immobilized on rice husk only (0.5 kg plant$^{-1}$). Different letters represent statistical significance among treatments at $p < 0.05$ for Dunn Test.

## 4.2 Yield components and commercial categorization

Despite inconclusive results regarding differences in total yield, T1 exhibited trends toward higher yield and greater fruit number relative to the other treatments, related to the higher nutrient inputs provided under full fertilization (Tirado, 2014; Ruiz, 2015; Irigoyen Diaz and Laos Ossa, 2008). However, T1 tends to have higher values in the non-commercial category (C4) and higher yields in C2 (≈36.8% of its total yield), while the other treatments tend to distribute their yields between categories C2 and C1 (≈30.9%–37.1%). This suggests that a higher fruit yield does not always translate into better commercial quality. Specifically, T3 numerically showed a greater proportion of fruits in category C1 (≈36.9%) without an increase in fruit number, suggesting a tendency toward improved commercial fruit distribution, accompanied by greater allocation to root and aerial biomass (Figure 4). Align with that, some research pointed out the positive effect of *Trichoderma* over the growth of *Capsicum annuum* L. in reduced mineral fertilization rates, the enhancement of the reproductive organs quality, nutrient uptake, yield parameters, and soil phosphorus availability in the post-harvest period (Duan et al., 2023; Senadheera et al., 2024; Song et al., 2024).

Therefore, the application of *T. viride* in pepper cultivation may contribute to physiological improvement; however, its direct effect on yield could not be clearly distinguished under the conditions of this study meter. Increases were observed in both aerial and root structures, indicating greater vegetative vigor of the crop, which may improve crop vigor, although this did not translate into statistically significant yield increases in the present study (Vazallo et al., 2013; Singh and Sharma, 2019; Pineda-Cotrina et al., 2022). These results were achieved despite the organic amendment being applied at 74 days after transplanting, suggesting that earlier application at transplanting may improve fungal establishment and should be evaluated in future studies.

Although T4 showed substantially lower numerical yield values, differences in total yield were not statistically significant. This trend may be associated with limited nutrient availability, particularly nitrogen, phosphorus, and potassium, due to the absence of mineral fertilization (Kirkby, 2012; Weil and Brady, 2017). Moreover, the delayed application of *T. viride* at 74 DAT may have further restricted effective root colonization and the establishment of beneficial plant–microbe interactions (Bandara and Kang, 2024). Under these unfertilized conditions, the incorporation of rice husk inoculated with *Trichoderma* likely stimulated microbial activity, leading to a temporary immobilization of mineral N and reducing its availability for plant uptake during early growth stages. This effect is consistent with previous studies indicating that carbon inputs enhance microbial N immobilization by increasing microbial demand for inorganic N, particularly when organic substrates supply bioavailable carbon (Cao et al., 2021). Although the rice husk presented a moderate C:N ratio (29) (Mushtaq et al., 2019), this parameter alone is not sufficient to predict N immobilization, as the chemical quality and decomposability of organic carbon are stronger regulators of microbial N dynamics (Chen et al., 2024). In this sense, *Trichoderma* inoculation may have accelerated the decomposition of lignocellulosic fractions, promoting microbial proliferation and intensifying transient competition with plants for available nitrogen.

Our results partially support the hypothesis. Yield was maintained under 30% reduced mineral fertilization; however, comparable productivity was also observed in the reduced-fertilization treatment without *T. viride* (T2), indicating that the specific contribution of *T. viride* to yield maintenance could not be clearly isolated. Nevertheless, the greater root and shoot biomass observed

TABLE 3 Yields of yellow chili according to commercial categorization.

| Treatments | C1 (t ha$^{-1}$) | C2 (t ha$^{-1}$) | C3 (t ha$^{-1}$) | C4 (t ha$^{-1}$) | Y (t ha$^{-1}$) | CY (t ha$^{-1}$) |
|---|---|---|---|---|---|---|
| | n.s. | * | * | n.s. | n.s. | n.s. |
| T1 | 12.2 ± 0.5 | 20.9 ± 2.5 $^{a}$ | 12.3 ± 0.8 $^{a}$ | 11.1 ± 2.5 | 56.6 ± 4.3 | 45.4 ± 3.5 |
| T2 | 16.9 ± 4.5 | 17.8 ± 1.5$^{ab}$ | 10.8 ± 0.6 $^{ab}$ | 6.0 ± 1.4 | 51.4 ± 7.2 | 45.5 ± 5.9 |
| T3 | 16.3 ± 3.0 | 13.7 ± 0.7$^{ab}$ | 7.5 ± 1.3 $^{b}$ | 7.0 ± 2.6 | 44.5 ± 0.7 | 37.6 ± 2.0 |
| T4 | 9.9 ± 0.7 | 11.6 ± 0.9$^{b}$ | 6.7 ± 1.0 $^{b}$ | 3.3 ± 1.3 | 31.6 ± 3.2 | 28.3 ± 2.1 |

C1, first category; C2, second category; C3, third category; C4, fourth category or discard; Y, total yield; CY, commercial yield (C1 + C2 + C3). * meas significance (p-value < 0.05) in ANOVA; n.s. means non-significant. Different letters in each testing parameter represent statistical significance among groups at $p < 0.05$, as determined by the Tukey Test.

TABLE 4 Total dry matter (g) and biomass distribution indicators (RWR, SWR, and SRR) in yellow chili pepper plants.

| Treatments | Total dry matter (g) | RWR | SWR | SRR |
|---|---|---|---|---|
| | * | n.s. | n.s. | n.s. |
| T1 | 1065.7 ± 86.7 [a] | 0.1 ± 0.0 | 0.9 ± 0.0 | 12.2 ± 0.1 |
| T2 | 972.9 ± 109.3 [ab] | 0.1 ± 0.0 | 0.9 ± 0.0 | 8.9 ± 1.7 |
| T3 | 947.7 ± 45.5 [ab] | 0.1 ± 0.0 | 0.9 ± 0.0 | 7.8 ± 1.0 |
| T4 | 630.2 ± 51.4 [b] | 0.1 ± 0.0 | 0.9 ± 0.0 | 7.5 0.7 |

Data are presented as mean ± standard error. * means significance (p-value < 0.05) in ANOVA; n.s. means non-significant. Different letters represent statistical significance at p < 0.05 for Tukey Test.

in T3 suggests that *T. viride* promoted vegetative development, which may confer agronomic benefits not fully captured by yield measurements alone.

## 4.3 Dry matter storage and partitioning of assimilates

The dry matter distribution results revealed a significant pattern. At the fruit level, the T4 treatment exhibited the highest dry matter content, which was significantly higher than that of T1 and T2, and did not differ from T3. This result may reflect an adaptive strategy under nutrient limitation, where plants prioritize the allocation of photo-assimilates to reproductive organs. The involvement of *T. viride*, known to enhance photosynthetic efficiency, carbon metabolism, and biomass partitioning, could also contribute to this pattern (Harman et al., 2004; Shoresh et al., 2010; Herrera-Parra et al., 2017). At the root level, T3 (combination of reduced fertilization with *T. viride*) could have favored a more robust root development, which can improve water and nutrient uptake. *Trichoderma* species are known to colonize roots, stimulate growth, and modulate phytohormones such as auxins and abscisic acid, thereby enhancing root development in horticultural crops (Duan et al., 2023; Contreras-Cornejo et al., 2022; Fazeli-Nasab et al., 2022; Moran-Diez et al., 2021). At the level of stems and leaves, the results suggest that T3 may promote biomass allocation not only in roots but also in aerial tissues, likely by enhancing nutrient uptake and photosynthetic efficiency (Bedine et al., 2022; Subramaniam et al., 2022; Vij et al., 2022).

In terms of total dry matter, the greater biomass accumulation in treatment T1 is likely attributed to the high nutrient supply, particularly nitrogen, which is known to enhance total dry matter production (Xiang et al., 2018; Wang et al., 2021; Vadillo et al., 2024). However, this condition also produced low biomass allocation to roots, reflected in a low RWR value and high SRR, which may indicate reduced investment in belowground structures under conditions of high nutrient availability (Poorter et al., 2012).

Conversely, in treatments T3 and T4, where *T. viride* was immobilized in rice husk, a redistribution of biomass toward the root system was observed. This is consistent with reports by Harman et al. (2004), who demonstrated that species of the genus *Trichoderma* promote root development.

In T3, although the total dry matter did not significantly exceed that of T2, suggesting that the beneficial effect of the fungus may be more related to nutrient use efficiency rather than direct biomass increase. Furthermore, the lower SRR in T3 and T4 reflects an adaptive plant strategy of allocating a greater proportion of resources to the root system, which has been described as common response under conditions of lower nutrient availability or beneficial associations (He et al., 2020).In this context, the implementation of the use of *T. viride* immobilized on rice husks as an organic amendment represents an innovative agricultural technique that could strengthen the competitive advantage of Peruvian organic food exporters, aligning with global preferences for sustainable products (de Almeida, 2025). This innovation assumes greater relevance, considering that Peruvian gastronomy has experienced significant expansion worldwide, capturing the attention of diverse regions and major cities with its flavors, textures, colors and culinary excellence, thereby demand for authentic Peruvian ingredients, such as yellow chili (Aguirre-Sosa et al., 2023). This international gastronomic growth offers strategic opportunity to apply sustainable production techniques that meet both international quality standards and the sustainability expectations of contemporary consumers.

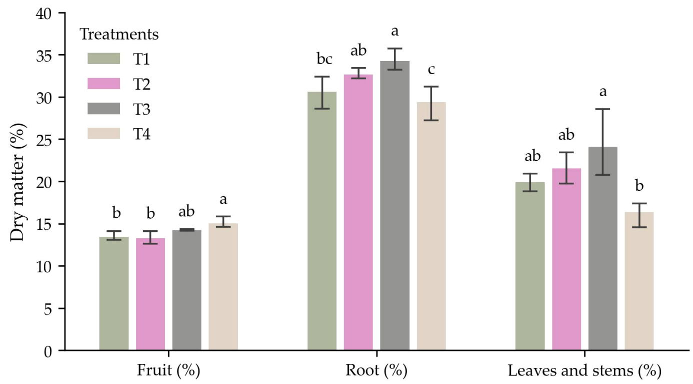


FIGURE 4
Dry matter percentages over time in response to different treatments: T1 = 200:66:42 kg ha$^{-1}$ for N:P:K (full fertilization), T2 = 135:48:21 kg ha$^{-1}$ for N:$P_2O_5$:$K_2O$, T3 = 135:48:21 kg ha$^{-1}$ for N:P:K + *T. viride* immobilized on rice husk (0.5 kg plant$^{-1}$), T4 = *T. viride* immobilized on rice husk only (0.5 kg plant$^{-1}$). Different letters above bars indicate significant differences between treatments for each plant part, as determined by Tukey's multiple comparison test of means (p < 0.05).

### 4.4 Study limitations and future research directions

This study presents several limitations. The delayed application of T. viride at 74 DAT may have restricted optimal root colonization and underestimated the bioformulation's full agronomic potential. The absence of a fully unfertilized control without *Trichoderma*, given that T4 included the organic amendment, limits the interpretation of baseline soil dynamics and the independent contribution of each factor. The single-season design, conducted under specific edaphoclimatic conditions of the arid agroecosystem of Santa Rita de Siguas, restricts the generalizability of results across broader environmental contexts. Although the compaction layer at approximately 30 cm and the recalcitrant nature of rice husk (C:N 29:1) were considered in the discussion, their interactions with treatment effects could not be fully characterized. Furthermore, the lack of rhizosphere microbiological analyses, economic assessments, and fruit nutritional quality evaluations limits comprehensive interpretation of the findings.

Future research should prioritize multi-year field experiments across diverse agroecological conditions to validate these results and assess their temporal stability. Experimental designs should incorporate a fully unfertilized control without biological inoculants and a treatment combining full fertilization with *T. viride*. Optimizing inoculation timing, particularly at transplanting, and inoculum density will be key to improving colonization efficiency. Investigations into the molecular mechanisms of *Trichoderma*-plant-soil interactions, combined with rhizosphere microbiological analyses, would deepen understanding of the observed growth-promoting effects. Finally, economic feasibility studies, fruit nutritional quality assessments, and commercial scalability evaluations remain priorities for the practical implementation of these strategies in Peruvian horticultural systems.

## 5 Conclusions

This study suggests that a 30% reduction mineral fertilization in *Capsicum baccatum* L. var. pendulum can be achieved without significant yield penalties under the conditions evaluated. The results revealed a defined physiological trade-off: intensive conventional fertilization (T1) increased plant height and showed a slight tendency to enhance chlorophyll index and fruit number per plant; however, yields obtained under reduced fertilization (T2, T3) were statistically similar to those of T1, indicating the possibility of maintaining comparable productivity levels with lower fertilizer inputs. The incorporation of *T. viride* immobilized in rice husk (T3) did not generate additional yield gains compared with T2, although it was associated with greater root and shoot biomass allocation, suggesting a positive effect on vegetative development rather than on yield enhancement among. These findings contribute to the growing body of research on biologically based nutrient management strategies integrating beneficial microorganisms and organic residues within reduced-input farming systems. They support continued exploration of integrated agricultural systems combining beneficial microorganisms with organic amendments as complementary strategies for reduced-input farming. Such approaches may contribute to agroecological transition pathways in economically important Peruvian horticultural crops while also creating opportunities for rice processing waste valorization. However, further validation under diverse agronomic conditions is required to confirm the consistency and practical relevance of this bioformulation for sustainable pepper production.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

RP-C: Formal analysis, Investigation, Writing – original draft. SL-E: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. AQ: Investigation, Methodology, Writing – original draft. RF-M: Supervision, Writing – review & editing. RS-A: Supervision, Writing – review & editing.

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## Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2026.1711962/full#supplementary-material

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