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RESEARCH ARTICLE
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Assessment of Biofertilizers and Humic Acids on the Growth of Coffee Varieties in Nursery: Experimental Study in Chanchamayo, Peru

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ABSTRACT

The rising cost of chemical fertilizers has increasingly threatened the economic sustainability of coffee cultivation in Peru, leading to decreased yields across plantations. This study evaluates the effects of biofertilizers on the growth and biomass of three coffee varieties. Using a full factorial design (A × B), factor A consisted of three coffee varieties; (Castillo, Catuaí, and Obata) while factor B included five fertilization treatments; soil fertilization with humic acids (FEAH), soil fertilization with Trichoderma (FET), soil fertilization with mycorrhizae (FEM), soil fertilization alone (FE), and a control without fertilization (SF), resulting in 15 unique treatments. Biometric measurements were included like plant height, leaf count, stem diameter, total fresh biomass, and total dry biomass. The Castillo variety demonstrated the most promising results, with an average plant height of 15.8cm, stem diameter of 4.30mm, 12.14 leaves, fresh biomass of 10.82g per plant, and total dry biomass of 2.26g per plant. Comparative analysis of treatments revealed that FEAH and FEM provided the most substantial improvements in growth metrics and biomass, outperforming FET, FE, and the control. This study not only addresses the sustainability challenges facing coffee cultivation in Peru but also offers critical insights into eco-friendly fertilization practices that promote economic and environmental resilience in coffee farming.

Keywords: Humic acids, Biofertilizers, Fertilization, Organic coffee

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INTRODUCTION

Coffee production is a vital economic activity in many countries, one strategy for its management and conservation is to delimit areas suitable for coffee production, where climatic and soil conditions favor the cultivation of various varieties of this plant (López-Carmona et al., 2021). However, the challenges associated with soil fertility and agricultural sustainability have driven the search for alternatives to conventional chemical fertilizers. In this context, biofertilizers and humic acids have emerged as promising solutions to improve the growth and productivity of coffee plants (Chávez-Díaz et al., 2022). Coffee cultivation in Peru is primary source of income for approximately 225 000 families and generating over 2 million jobs. The country has established itself as the largest exporter of organic coffee worldwide (MIDAGRI, 2021). However, the sector faces critical challenges, including high fertilizer costs and inadequate management practices during the nursery phase. These issues have led to a reduction in plantation lifespans, shorter renewal periods, low productivity, and rising production costs. Continued soil degradation and limited access to modern agricultural technologies have prompted many growers to abandon their crops. To ensure the production of high-quality coffee plants, it is essential to focus on efficient nutrition and adequate development tailored to specific varieties, which are crucial for success in the field. Ensuring plant health and establishing a vigorous root system that optimizes water absorption and nutrient efficiency is necessary (ANACAFÉ, 2022).

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Recent literature emphasizes innovative technological alternatives aimed at enhancing coffee production quality, particularly through the use of biofertilizers and humic acids. Humic acids, components of soil organic matter, have been shown to positively influence plant physiology and soil fertility even at low application rates. It enhances nutrient absorption, leading to improved growth rates, yield quality, and increased tolerance to abiotic stresses (Magaña Arteaga & González Fuentes, 2015; Silvera-Pablo et al., 2024). Various studies reported that these substances have beneficial effects both indirectly on soil physical properties and directly on physiological and biochemical processes in plants, stimulating growth and improving yield levels (Cesco et al., 2002; Pedranzani et al., 2015). For instance, Ochoa & Licona (2017) studied the Lempira coffee variety in a nursery setting with humic acids and synthetic fertilizers. Their findings indicated no significant differences in root dry weight between treatments after 60 days, underscoring the potential of humic acid combined with nitrogen fertilizer. The biofertilizers, composed of beneficial microorganisms, not only provide essential nutrients to the soil, but also promote the health of the soil microbiome, resulting in more robust and resilient plant growth (Beltran-Pineda & Bernal-Figueroa, 2022). On the other hand, humic acids, derived from the decomposition of organic matter, improve soil structure, increase water retention, and facilitate the availability of nutrients for plants (Cruz-Cárdenas et al., 2021). The combination of these two organic amendments could offer synergies that significantly enhance the development of coffee varieties in nurseries.

Another promising approach involves the use of arbuscular mycorrhizae, which form symbiotic relationships with over 80% of plant species. In coffee cultivation across various countries, mycorrhizae are utilized to reduce chemical fertilizer dependence (Berruti et al., 2016). These fungi enhance nutrient absorption (particularly phosphorus) and improve overall plant health without completely replacing chemical fertilizers. Research indicates that mycorrhizae can reduce fertilizer use by 50-80% over time (Perez et al., 2011; Jaramillo, 2011). In Peru, commercial products often contain species such as *Glomus intraradices* and *Glomus mosseae*, although their widespread use remains limited. Rivillas Osorio (2003) demonstrated positive effects on seedling growth when coffee varieties were inoculated with various mycorrhizal species during nursery phases. Furthermore, Cano (2011) mentions that arbuscular mycorrhizae provide benefits to coffee cultivation.

Additionally, *Trichoderma*, a beneficial anaerobic fungus known for its saprophytic or parasitic behavior, has gained traction in sustainable agriculture. It acts as a biological control agent against root diseases while promoting plant growth by enhancing nutrient uptake and modifying the rhizosphere (Castro & Rivillas, 2012). Bacusoy & Fienco (2023) reported successful applications of Trichoderma in rice crops aimed at eco-sustainable production. Guicalpi (2009) evaluated its effects on the Caturra variety in nurseries, achieving notable growth metrics with specific application rates. Navarrete et al. (2022) reported an increase in biomass and disease resistance in coffee plants treated with specific biofertilizers. Likewise, research carried out by López-Carmona et al. (2021) showed improvements in soil quality and nutrient use efficiency through the application of humic acids.

Despite these advancements in biofertilizers and humic acids for coffee cultivation in nurseries, there remains a gap in understanding their effects on other varieties such as Castillo, Catuaí, and Obata. Therefore, the objective of this study is to evaluate the impact of biofertilizers and humic acids on the growth of these three coffee varieties under nursery conditions in Pichanaqui, Chanchamayo.

MATERIALS & METHODS

Experiment Site

The study was carried out in the province of Chanchamayo, in the district of Pichanaqui, department of Junín, Peru (Fig. 1). At an altitude of 563 masl (Coordinates: 11°03′16″S 75°19′45″W). This location has an average temperature of 27°C and an annual rainfall of 3300mm (Senahmi, 2021).

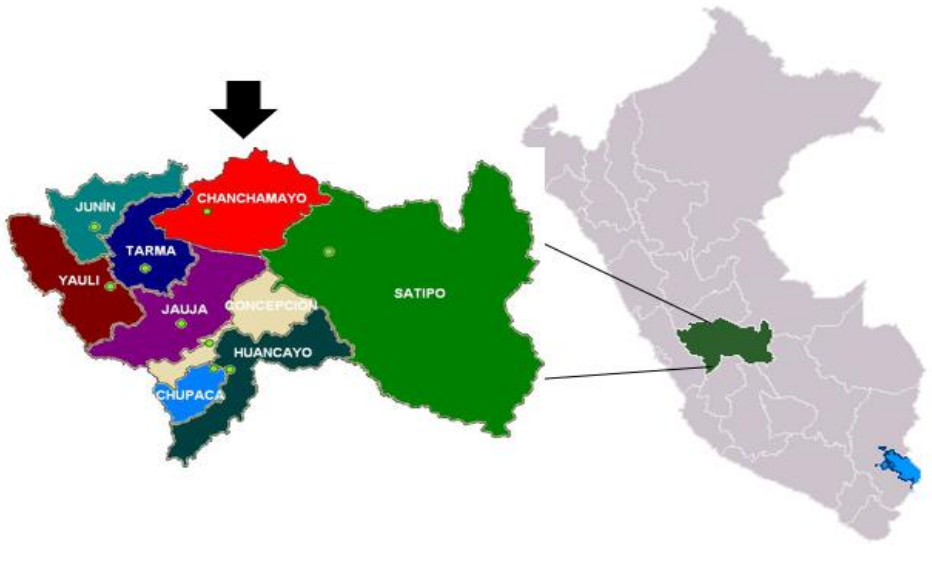

Fig. 1: Location of the study area in Chanchamayo, Peru.

Biological Material and Characteristics of the Treatments

Three coffee varieties—Castillo, Catuai, and Obata—were used from nurseries affiliated with the ACPC Coffee Agrarian Cooperative in Pichanaqui, Chanchamayo, Peru. Each treatment consisted of 10 plants of the same variety, arranged in a linear fashion and inoculated with microorganisms (*Trichoderma* and *Mycorrhizae*) along with humic acids. A control group, which received no fertilizer, was fertilized with minerals (Silvera-Pablo et al., 2024).

Installation, Handling, Inoculation and Fertilization of Treatments

One seedling was placed per bag and transplanted on September 5, 2021. Microorganisms were applied in each treatment at 30 and 60 days. Fertilization was done by irrigation. Humic acids were applied twice: when the first and third pair of true leaves appeared. Pruning was done 120 days after transplant. During the nursery phase, irrigation, fertilization, and weed control were carefully managed. Match stage seedlings were used in 5 x 9" nursery bags with a 3:1 mix of agricultural soil and sand, enriched with organic guano. Substrate analysis showed optimal conditions for nursery growth. The dose applied according to the treatment was: mycorrhizal complex: 2g/bag (first 2cm), Trichoderma: 30g/row (0.3 g/seedling), humic acid: 5mL/L (15% concentration), and mineral fertilization: 51ppm N, 7.9ppm P_2O_5, and 93ppm K_2O, administered twice (10mL/plant when the first pair of leaves appear and 20mL/plant when the third pair of leaves appear) (Cruz-Cárdenas et al., 2021).

Biometric and Biomass Accumulation Parameters

Physical parameters were determined 120 days after transplantation. Plant height was quantified (using a ruler graduated in cm to measure from the base of the stem to the terminal bud of the coffee plant), stem diameter (using a caliper in mm, measuring the stem diameter at 2cm from the base), number of leaf pairs (counting leaf pairs per plant), fresh weight (g) of the aerial part, roots and the total per plant (the fresh weight of the aerial and root parts were weighed separately and added together to obtain the total weight). In addition, the dry weight (g) of the aerial part, roots and the total per plant was determined (samples were previously dried in an oven at 60°C for 72 hours, and then the weights were recorded in a similar way to the fresh weight) (Navarrete et al., 2022).

Experimental Design

A full factorial design A × B with three levels of variety and five levels of fertilizers was used (Table 1). The treatments applied to each variety (Castillo, Catuaí, and Obata) included humic acids, a Mycorrhizal Consortium (MVA), and Trichoderma, in addition to treatment with mineral fertilization and another without fertilization. It is important to mention that all treatments with biofertilizers and humic acids applied the fertilization dose determined for the mineral fertilization treatment (soluble fertilizers). An analysis of variance of the factorial design and a comparison of means (Tukey test) were performed in case of finding significance at 95% (P=0.05), using the agricolae statistical package of the R Project 4.2.1 an RStudio program (Montgomery, 2013; Mendiburu, 2022).

Table 1: Full factorial design A × B (5x3) for the treatments and coffee varieties

Factor	Level 1	Level 2	Level 3	Level 4	Level 5
Technology	Humic acids	*Mycorrhizal*	*Trichoderma*	Conventional	Without fertilization
Variety	Castillo	Catuaí	Obata		
Treatments			Codification		
	Technology				Variety
T1	Humic acids				Castillo
T2	Humic acids				Catuaí
T3	Humic acids				Obata
T4	*Mycorrhizal*				Castillo
T5	*Mycorrhizal*				Catuaí
T6	*Mycorrhizal*				Obata
T7	*Trichoderma*				Castillo
T8	*Trichoderma*				Catuaí
T9	*Trichoderma*				Obata
T10	Conventional				Castillo
T11	Conventional				Catuaí
T12	Conventional				Obata
T13	Without fertilization				Castillo
T14	Without fertilization				Catuaí
T15	Without fertilization				Obata

RESULTS & DISCUSSION

Biometric Growth Parameters: Plant Height, Number of Leaves, Stem Diameter

Fig. 2 shows the coffee varieties that were used across different applied technologies. The application of humic acids has shown significant differences between the

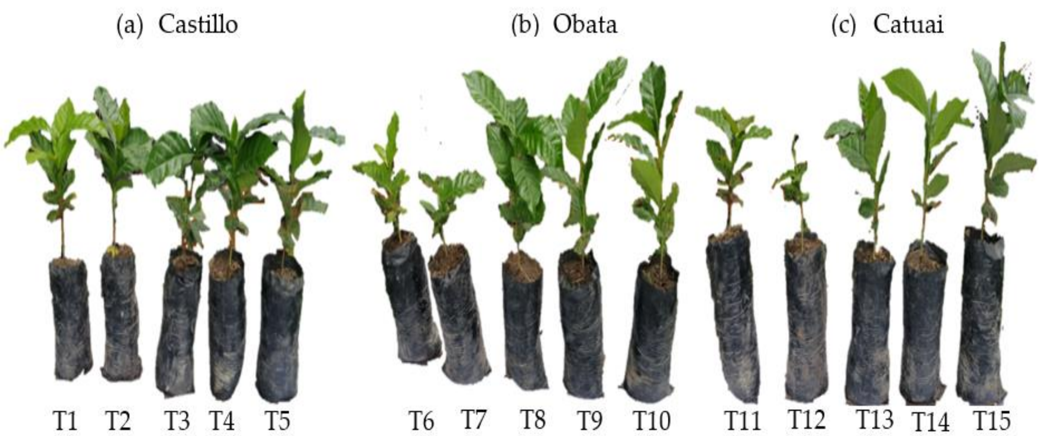

Fig. 2: Treatment-Induced Variations in Coffee Varieties: Analysis of (a) Castillo, (b) Obata, and (c) Catuai

Table 2: Evaluation of physical parameters: ANOVA results and factorial model coefficients.

Factor	Plant height (cm)	Number of leaves	Stem diameter (mm)
		ANOVA	
Technology	<0.05	<0.05	<0.05
Variety	<0.05	<0.05	<0.05
Technology*Variety	0.004	0.007	<0.05
		Coeficients of models	
		Technology	
Humic acids	1.837[a]	0.993[a]	0.563[a]
Trichoderma	1.037[a]	0.327[ab]	0.320[a]
Mycorrhiza	1.453[a]	0.593[a]	0.226[a]
Conventional	-2.163[b]	-0.673[bc]	-0.440[b]
Without fertilization	-2.163[b]	-1.240[c]	-0.670[b]
		Variety	
Castillo	2.437[a]	1.600[a]	0.364[a]
Catuai	-1.043[b]	-0.740[b]	-0.207[b]
Obata	-1.033[b]	-0.860[b]	-0.157[b]

Different letters in the same column indicate significant differences (P≤0.05).

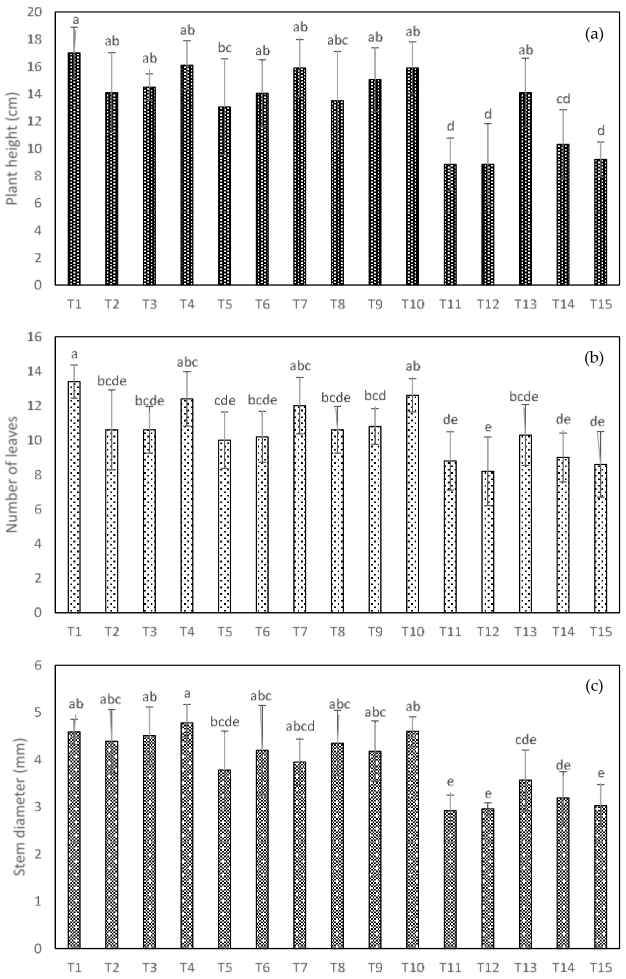

Fig. 3: Influence of Treatments on Key Growth Variables in Coffee Trees: (a) Plant height, (b) Number of leaves and (c) Stem diameter (Different letters indicate significant differences at P≤0.05).

treatments and technologies used, as shown in Fig. 3. Table 2 presents significant influences of the interaction between technology and coffee varieties. The coefficients of the factorial model indicate that the use of humic acids, together with Trichoderma and Mycorrhiza, improves the response variables, while conventional methods and processes without fertilization tend to decrease them. In terms of coffee varieties, the Castillo variety exhibits superior growth characteristics, including greater plant height, leaf count, and stem diameter, compared to the Catuai and Obata varieties, which show reduced values. Specifically, the Castillo coffee variety achieved an average height of 17.00cm, a leaf count of 13.4, and a stem diameter of 4.59mm, outperforming all other treatments and varieties evaluated. These findings align with Puspita Sari & Abdoellah (2017), who reported that the application of humic acids to the substrate (15 g/kg) significantly promoted the growth of coffee seedlings. This positive effect of humic substances is also corroborated in other crops; for example, Arancon et al. (2003) observed similar benefits in cucumber growth, while El-Helaly (2018) noted improvements in carrot development. Additionally, Narro (2007) highlighted that humic substances directly influence coffee crop growth, particularly in terms of plant height.

Several studies have demonstrated the positive effects of humic acids on plant growth, primarily by enhancing cell membrane permeability (Nardi et al., 2002; Arteaga et al., 2006). These substances also function as growth regulators, notably stimulating root development (Chen et al., 2004). Research further highlights that the Castillo coffee variety offers significant advantages in agronomic management, resilience to environmental conditions and adaptability to production systems, which may influence its performance (Arcila et al., 2007; Orozco et al., 2011). Additionally, Castillo (2005) emphasizes that humic acids facilitate nutrient transfer from roots to shoots, promote nutrient accumulation in leaves, and activate enzymes that enhance early plant development, leaf expansion, and root growth. The application of mycorrhizae and humic acids improved corn development and production, especially at high doses. Liquid humic acid showed better results than granulated humic acid, although there was no impact on the chronological age of the crop (Navarrete et al., 2022).

In coffee plants, mycorrhizal fungi exhibited effects statistically similar to those of Trichoderma and humic acids (Table 2). Coffee production in Costa Rica focuses on Caturra and Catuaí hybrids, which have replaced traditional varieties such as Villalobos and Typica since the mid-20th century (Smith, 2018). Other varieties are also grown, such as Bourbon, Geisha and Obatá, the latter belonging to the Sarchimor group, with Robusta genetics (World Coffee Research, 2020; Quesada-Román et al., 2022), with the Castillo variety achieving notable performance. This variety reached a height of 15.9cm, produced 12 leaves, and developed a stem diameter of 3.95mm, outperforming the other varieties evaluated. These findings align with those of Hernández-Acosta et al. (2020), who reported that inoculating coffee plants with the mycorrhizal consortiumcmgrp (*Glomus claroides, Rhizophagus diaphanus,* and *Paraglomus albidum*) significantly increased plant height, by 17.74% in the Garnica variety and 16.50% in Caturra, compared to controls. Similarly, Ibarra-Puón et al. (2014) observed improved plant height in Robusta coffee 140 days after inoculation with the same consortium. Del Aguila et al. (2018) evaluated nine arbuscular mycorrhizal consortia in Arabica coffee seedlings of the Catuaí variety and identified three consortia that increased plant height by 10.65% compared to controls. Furthermore, in chemically degraded soils—characterized by salinity, low phosphorus availability, and water deficits—the symbiosis between coffee plants and mycorrhizae plays a critical role (Tristão et al., 2006). On the other hand, soil type and quality and humidity could have influenced the diversity of endophytes and coffee yield (Hosseyni Moghaddam et al., 2021).

Biomass Accumulation Parameters: Fresh Weight, Dry Weight and Dry Matter Percentage

The application of humic acids resulted in significant differences across treatments and technologies (Fig. 3). The Castillo variety exhibited superior performance, reaching an average height of 17.00cm, 13.4 leaves, and a stem diameter of 4.59mm, outperforming all other evaluated varieties and treatments. These results are consistent with those of Puspita Sari and Abdoellah (2017), who reported that applying humic acids (15 g/kg) to the substrate positively influenced coffee growth. Soils suitable for coffee cultivation should be deep (at least 1 m), with loamy textures and a thick layer of leaf litter (López-Carmona et al., 2021). Castillo-González et al. (2024) found that fungal communities on coffee leaves in Costa Rica exhibited greater endophyte diversity in mature leaves under conventional management. While the coffee variety showed an unclear influence and solar exposure had an insignificant effect, agroforestry and organic farming practices stood out for reducing pathogens and promoting mutualistic fungi. The development of biofertilizers supports environmental sustainability and agricultural productivity by leveraging microorganisms that enhance soil fertility and crop yield through processes such as nitrogen fixation and phosphate solubilization (Beltran-Pineda & Bernal-Figueroa, 2022).

Table 3 highlights the positive effects of humic acids on coffee growth, particularly in enhancing fresh matter production. These compounds exert a phytohormonal effect, promoting growth in young coffee plants during the nursery stage (Jana et al., 2010). Similarly, Kulikova et al. (2003) found that humic substances facilitate nutrient absorption, especially of those nutrients in deficiency, leading to increased biomass. These findings align with those reported for other crops, such as tomatoes (Zaller, 2007) and peppers (Berova & Karanatsidis, 2009), where significant increases in biomass were also observed.

They met significant positive effects of mycorrhizae, similar to those of humic acids, on fresh aerial biomass, root weight and total biomass (Fig. 4). These microorganisms enhance biometric traits and biomass production in coffee plants. Hernández-Acosta et al. (2020) reported similar results, showing that mycorrhizal inoculation in coffee plants increased dry matter and plant height compared to non-inoculated controls.

The results demonstrate positive effects on the growth and biomass accumulation of coffee seedlings during the nursery stage (Fig. 4), with the Castillo variety exhibiting superior performance. This variety achieved a height of 16.1cm, 12 leaves per plant, and a stem diameter of 4.78mm, outperforming all other treatments, except for those involving humic acids. These results may be attributed to the bioprotective role of Trichoderma, which enhances root health and influences stem thickness (Fig. 4). Soil microbial activity and its associated benefits are strongly influenced by unsustainable intensive agricultural practices and climatic conditions, which alter soil characteristics at the physical, chemical, and biological levels, including temperature, humidity, salinity, aeration, redox state, nutrient bioavailability and pH (Ibarra-Villarreal et al., 2021; Cruz-Cárdenas et al., 2021).

Guilcapi (2009) reported that Trichoderma spp. positively affect seedling health by combating a wide range of phytopathogenic fungi in both soil and air, thereby improving plant height and biomass. Additionally, Trichoderma has been shown to effectively control root diseases caused by Fusarium, Rhizoctonia, and Pythium, as well as pathogens that form sclerotia, such as Sclerotinia and Sclerotium. Similar benefits have been observed in other crops, such as rice (Bacusoy & Fienco, 2023).

Rhizobium species not only fix atmospheric nitrogen but also promote plant growth, yield, and the number of nodules per root, while mobilizing phosphorus (Saharan and Nehra, 2011). Recent research on this bacterial genus as a plant growth promoter has focused on its effects on: i) the structure of root-associated microbial communities (Jha et al., 2020); ii) the development of biofertilizers for various legume crops (Passricha et al., 2020); iii) the introduction of Rhizobium cells into seeds using vacuum technology to prevent inoculum loss (Lekatompessy et al., 2020); and iv) the evaluation of the effect of co-inoculation of Rhizobium and endomycorrhizal spores (Kiuk et al., 2019; Chávez-Díaz et al., 2022).

Table 3: Statistical Analysis: ANOVA and Factorial Model Coefficients for Biomass Parameters.

Factor	Root fresh weight (g)	Air part fresh weight (g)	Total weight (g)	Root dry weight (g)	Dry weight aerial part (g)	Total dry weight (g)	% Dry material
			ANOVA				
Technology	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Variety	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Technology * Variety	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.0084
			Coeficients of model				
			Technology				
Humic acids	1.087[a]	1.053[ab]	2.147[a]	0.154[a]	0.332[a]	0.487[a]	0.005[a]
Trichoderma	-0.180[bc]	0.020[bc]	-0.153[bc]	-0.019[b]	0.024[bc]	0.0046[bc]	0.007[ab]
Mycorrhiza	0.287[b]	1.220[a]	1.513[ab]	0.018[b]	0.276[ab]	0.295[ab]	0.000[ab]
Conventional	-0.547[c]	-0.713[cd]	-1.320[cd]	-0.089[b]	-0.236[cd]	-0.325[cd]	-0.010[c]
Without fertilization	-0.647[c]	-1.580[d]	-2.187[d]	-0.064[b]	-0.397[d]	-0.461[d]	-0.003[bc]
			Variety				
Castillo	0.833[a]	1.640[a]	2.460[a]	0.128[a]	0.420[a]	0.549[a]	0.005[a]
Catuai	-0.427[b]	-0.840[b]	-1.260[b]	-0.067[b]	-0.182[b]	-0.249[b]	0.001[a]
Obata	-0.407[b]	-0.800[b]	-1.200[b]	-0.061[b]	-0.238[b]	-0.299[b]	-0.006[b]

Different letters in the same column indicate significant differences at $P \leq 0.05$.

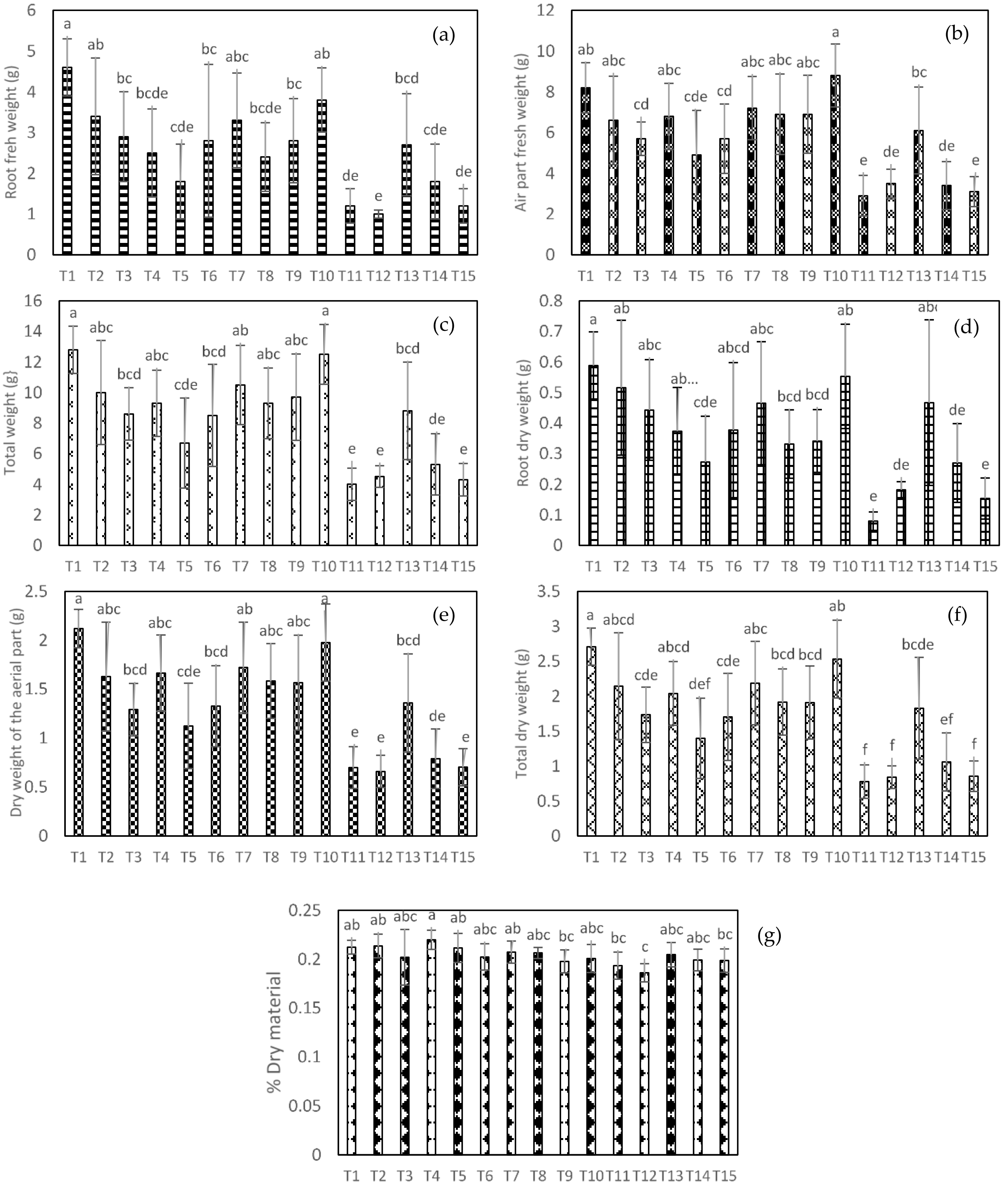

Fig. 4: Comprehensive analysis of treatment effects on coffee tree growth: (a) Root fresh weight, (b) air part fresh weight, (c) Total weight, (d) Root dry weight, (e) Dry weight of the aerial part, (f) Total dry weight and (g) % dry material. Different letters indicate significant differences at P≤0.05.

Among plant growth-promoting fungi, strains from the Glomus genus have been extensively studied. These fungi mitigate the effects of water stress on plants (Mota et al., 2020) and increase plant growth when co-inoculated with growth-promoting bacteria (Nadeem et al., 2014). Additionally, various Trichoderma strains have been investigated for their: i) antagonistic and mycoparasitic potential against phytopathogens; ii) ability to enhance plant growth under abiotic stress conditions (Hermosa et al., 2012); iii) capacity to increase pigment content in plants (Metwally and Al-Amri, 2020); and iv) Potential to improve soil microbiota and enzymatic activity (Zhang et al., 2020).

Conclusion

The results of this study underscore the substantial benefits of incorporating humic acids during the nursery phase, particularly in enhancing the growth of coffee seedlings across the three evaluated varieties. Notably, the Castillo variety exhibited significant improvements in biometric parameters: plant height, leaf number, and stem diameter, along with marked increases in both fresh and dry biomass, distinguishing it from the other varieties. Trichoderma demonstrated a specific role as a bioprotective agent, contributing to enhanced crop resilience. These findings emphasize the importance of adopting tailored approaches that consider the unique characteristics of each variety while addressing the transition to the final planting field.

To effectively apply these results in practice, it is crucial to recognize the potential of humic acids in fostering vigorous growth during the critical nursery phase. Furthermore, the use of mycorrhizal fungi as universal enhancers of root quality offers significant promise, while the bioprotective effects of Trichoderma warrant further exploration. The successful implementation of these strategies requires a thorough understanding of genetic variations and the adoption of precise management practices, ensuring optimal outcomes as these findings are integrated into coffee production systems.

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