Research article

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Revista Facultad Nacional de Agronomía

UNIVERSIDAD NACIONAL DE COLOMBIA

Trichoderma harzianum application improves turmeric (*Curcuma longa* L.) growth, yield and rhizome quality

Aplicación de *Trichoderma harzianum* mejora el crecimiento, rendimiento y calidad del rizoma de cúrcuma (*Curcuma longa* L.)

https://doi.org/10.15446/rfnam.v79.118981

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ABSTRACT

Turmeric (*Curcuma longa* L.) is a high-value medicinal crop of growing economic and pharmaceutical relevance. However, optimizing its yield and quality remains a major challenge in tropical agricultural systems. This study evaluated the effects of *Trichoderma harzianum* on agronomic performance, yield, and rhizome quality of turmeric. A field trial was conducted in Iquitos, Peru, using a randomized complete block design with four treatments (0, 2, 4, and 6 g of *T. harzianum* conidia per plant). Applications were performed at transplanting (0 days) and at 60, 120, and 180 days after planting. Agronomic traits such as plant height, number of leaves and shoots, and fresh and dry rhizome yield and rhizome quality (protein and curcuminoid content) were assessed. Rhizome production varied significantly among treatments ($P \leq 0.01$). Observed yields increased from 6.52 kg per plot in the control to 8.92 kg at 4 g for fresh yield, and from 0.87 to 1.18 kg per plot for dry yield. Tukey's test confirmed that all inoculated treatments outperformed the control ($P<0.05$), with no differences among inoculated groups. Regression modeling predicted an optimal fresh yield of 9.14 kg per plot (10.83 t ha^{-1}) at 5.27 g per plant and a maximum curcuminoid content of 7.13% at 2.30 g per 100 g of dry rhizome. Yields declined at higher doses. These findings support *T. harzianum* as a promising biostimulant to improve turmeric productivity under tropical field conditions, requiring validation in broader production environments.

Keywords:
Agronomic performance
Biostimulant
Rhizome yield
Secondary metabolites
Turmeric

CITATION: Lavado-SolSol A, Villacrés-Vallejo J, Ramírez-Chung J, Marín-Sisley P, Barreto-Salcedo C and González-Aspajo G (2026) *Trichoderma harzianum* application improves turmeric (*Curcuma longa* L.) growth, yield and rhizome quality. Revista Facultad Nacional de Agronomía Medellín, 79: e118981. doi: https://doi.org/10.15446/rfnam.v79.118981

RESUMEN

La cúrcuma (*Curcuma longa* L.) es un cultivo medicinal de alto valor con creciente relevancia económica y farmacéutica. Sin embargo, optimizar su rendimiento y calidad continúa siendo un reto en los sistemas agrícolas tropicales. El presente estudio evaluó el efecto de *Trichoderma harzianum* sobre el desarrollo agronómico, el rendimiento y la calidad del rizoma de cúrcuma. El ensayo de campo se realizó en Iquitos, Loreto (Perú), durante la campaña agrícola 2023. Se empleó un diseño de bloques completos al azar con cuatro tratamientos (0, 2, 4 y 6 g de conidios de *T. harzianum* por planta) y cinco repeticiones. Las aplicaciones se efectuaron al trasplante (0 días) y a los 60, 120 y 180 días después de la siembra. Se evaluaron parámetros de crecimiento (altura de planta, número de hojas y brotes), rendimiento de rizoma fresco y seco, y calidad del rizoma (proteínas y curcuminoides). La producción de rizoma varió significativamente entre tratamientos ($P<0,01$). El rendimiento fresco observado aumentó de 6,52 kg por parcela en el control a 8,92 kg en 4 g por planta; el rendimiento seco pasó de 0,87 a 1,18 kg por parcela. La prueba de Tukey confirmó que todos los tratamientos inoculados superaron al control ($P<0,05$), sin diferencias entre los grupos tratados. El modelo de regresión predijo un rendimiento fresco óptimo de 9,14 kg por parcela (10,83 t ha^{-1}) a 5,27 g por planta y un máximo de curcuminoides de 7,13% a 2,30 g por 100 g de rizoma seco. Dosis superiores redujeron el rendimiento. Estos hallazgos respaldan el uso de *T. harzianum* como biestimulante prometedor para mejorar la productividad de cúrcuma en condiciones tropicales, requiriendo validación en diferentes entornos productivos.

Palabras clave:
Desarrollo agronómico
Bioestimulantes
Rendimiento de rizoma
Metabolitos secundarios
Cúrcuma

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Received: May 26, 2025; Accepted: September 22, 2025

Global turmeric (*Curcuma longa*) production continues to be dominated by India, which accounts for approximately 80% of world output. The country has strengthened its export leadership, shipping 176,325 tons valued at USD 341 million in FY 2024–25, representing 66% of global turmeric exports, according to the Ministry of Commerce & Industry of India (Koushal et al. 2024; Press Information Bureau, Government of India 2025).

The international turmeric market shows dynamic growth patterns, particularly evident in Peru's emergence as a significant regional exporter. Peru exported 3,951 tons of turmeric in 2024, a 27.2% increase compared with 2023, and continued this trajectory with 2,346.6 tons exported in the first five months of 2025, a 58.2% increase over the same period in 2024. Peru's export strategy focuses mainly on European markets, with the Netherlands receiving 47.6% and Spain 12.7% of Peruvian turmeric shipments in 2024, distributed through leading exporters in various forms, including fresh turmeric, powder, and dehydrated flakes (PromPerú 2025).

The growing global demand for food, intensified by the dual pressures of population growth and climate change, requires innovative agricultural strategies that enhance crop productivity and quality while minimizing environmental impacts (Anderson et al. 2020). Among the proposed alternatives, the use of biofertilizers has emerged as a promising complement to synthetic agrochemicals, which are associated with negative effects on human health, soil fertility, and ecosystem stability (Daniel et al. 2022; Saputro and Kurniawati 2024).

Among these biofertilizers, *Trichoderma harzianum* Rifai, a well-characterized species within the *Trichoderma* genus, has gained significant attention due to its multifaceted roles in promoting plant growth, enhancing nutrient uptake, and providing biocontrol against phytopathogens (Nosheen et al. 2021; Alzate et al. 2024). Despite extensive studies on its benefits, knowledge gaps remain regarding its specific effects on the yield and rhizome quality of *C. longa*, particularly under tropical field conditions such as those found in Peru.

Curcuma longa, a member of the Zingiberaceae family, is widely recognized for its culinary and medicinal applications, largely attributed to its curcuminoid content (Puglia et al. 2021). Its growing economic value is reflected in its extensive use in the food, cosmetic, and pharmaceutical industries. Peru has recently emerged as a notable exporter (Costa et al. 2023). However, its cultivation faces challenges from both biotic and abiotic stressors that limit yield and quality (Bela 2023). The integration of *T. harzianum* as a biofertilizer presents a potential solution for improving the agronomic performance of turmeric; nevertheless, empirical studies evaluating its efficacy in this context are lacking (Nosheen et al. 2021).

Recent studies have shown that *T. harzianum* enhances both root and shoot development, overall plant vigor, and stress resilience (Ahmad et al. 2022; Milton et al. 2020). Its action mechanisms include the secretion of growth-promoting compounds, nutrient competition, and the induction of systemic resistance (Kumar et al. 2024, 2022). Additionally, its ability to suppress soil-borne pathogens makes it particularly suitable for turmeric cultivation, reducing disease incidence and improving crop health (Singh et al. 2023), which is vulnerable to fungal infections (Patel et al. 2023). However, region-specific interactions remain underexplored, especially in Peruvian agroecosystems.

This study aimed to evaluate the effects of *T. harzianum* on the yield and rhizome quality of *C. longa* under tropical field conditions, focusing on growth performance and secondary metabolite accumulation in response to different conidial doses. The outcomes of this research are expected to provide empirical evidence on the efficacy of *T. harzianum* as a biofertilizer for turmeric cultivation in tropical agroecosystems, contributing to the development of sustainable agricultural practices tailored to the needs of Peruvian turmeric producers.

MATERIAL AND METHODS

Study site and experimental design

The field experiment was conducted from January to August 2023 at the Cultivation and Conservation Field for Amazonian Medicinal Plants, located at the Facultad de Agronomía, Universidad Nacional de la Amazonía Peruana (UNAP), along Zungarococha Highway, San Juan Bautista District, Loreto, Peru (03°46'13.2"S, 73°22'10.4"W) at 126 meters above sea level (masl).

Climatic conditions were obtained from the Servicio Nacional de Meteorología e Hidrología del Perú (SENAMHI), with average temperatures ranging from 26.8 to 29.1 °C, total rainfall of 1,437 mm (January–May) and 387.6 mm (June–August), and relative humidity of 83–91%. The experimental design, including treatments, timeline, and evaluated parameters, is illustrated in Figure 1.

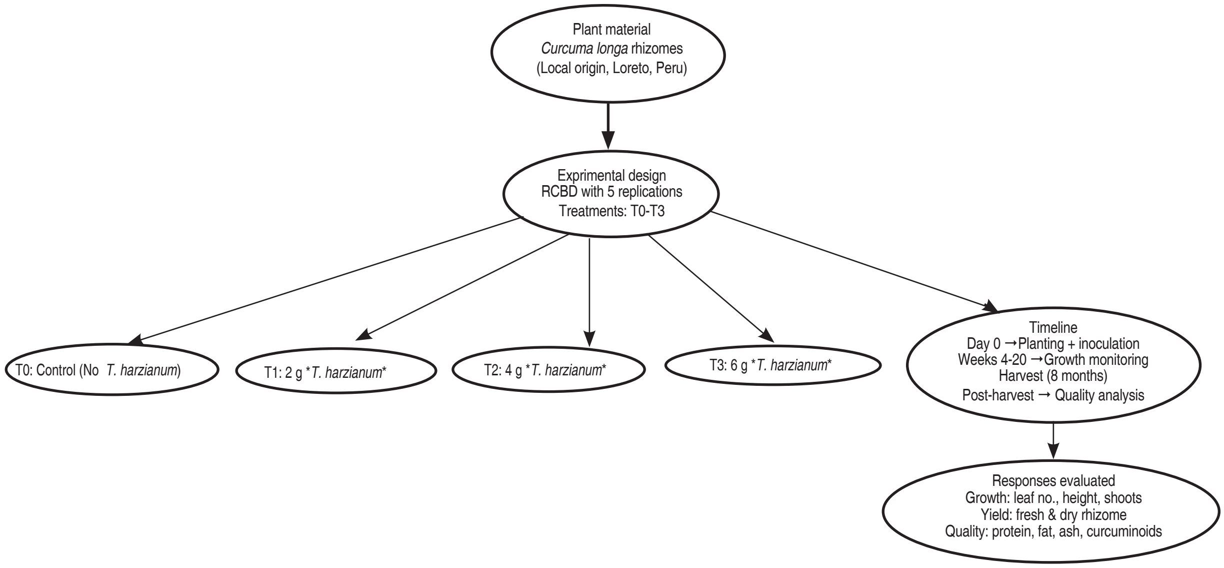

Figure 1. Experimental design of turmeric (*Curcuma longa* L.) field trial showing treatments (T_0–T_3), application timeline, and evaluated parameters.

The soil was classified as sandy loam (70% sand, 17% silt, and 13% clay), with a pH of 6.55, high phosphorus (76.9 ppm), low potassium (78 ppm), and medium–low organic matter content (2.34%). Soil properties were determined following standard analytical methods.

A randomized complete block design was employed, comprising four treatments and five replications. Each experimental unit (5 m^2 per plot) included two rows with 18 plants each. Treatments consisted of increasing doses of *T. harzianum* conidia: T_0 (control): 0 g; T_1: 2 g (6.4×10^9 conidia); T_2: 4 g (12.8×10^9 conidia); and T_3: 6 g (19.2×10^9 conidia). Applications were performed at transplanting and repeated every 60 days, for a total of four applications throughout the crop cycle.

Trichoderma harzianum preparation

Spores of *T. harzianum* were provided by the Servicio Nacional de Sanidad Agraria del Perú (SENASA), Lima. Replication was conducted at the Microbiology Laboratory of the Instituto de Medicina Tradicional (IMET), Iquitos. Initial spore viability was assessed using the plate count method, showing $3.2\text{x}10^9$ viable conidia per gram. For mass propagation, the protocol was modified from Naeimi et al (2020), where 50 g of *T. harzianum* conidia were inoculated into 500 g of sterilized rice (autoclaved at 121 °C for 20 minutes) supplemented with 60 mL of sterile distilled water in sealed Ziploc bags. The moisture content was adjusted to approximately 40% to optimize fungal growth. The bags were manually agitated daily for 30 seconds to ensure uniform distribution and prevent substrate compaction, then incubated under controlled conditions with a 12-hour light/dark photoperiod at 25±2 °C for 5 days in a laminar flow cabinet. After incubation, the colonized substrate showed characteristic green coloration, indicating successful *Trichoderma* sporulation. The inoculated substrate was subsequently dried under sterile conditions in a laminar flow cabinet to reduce moisture content to <10% and stored at 16 °C in airtight glass containers with silica gel desiccant until use. Prior to field application, spore concentration was determined by serial dilution and plating on potato dextrose agar, confirming viable spore counts of $3.2\text{x}10^9$ conidia per gram of dried substrate.

Curcuma longa crop management

The samples were taxonomically identified by preparing flowering voucher samples, which were deposited at the *Herbarium Amazonense* (AMAZ), Iquitos-Perú. under accession code AMAZ 42787. Rhizomes containing 3–5 viable buds were planted in 20 cm-deep holes, spaced 0.60 m between rows and 0.50 m between plants. Agronomic practices included hilling every 30 days, manual weeding every 15 days, limited irrigation during peak heat, and fertilization three months after planting. Harvesting was conducted eight months after transplanting.

Data collection

A zigzag sampling pattern was used to select 10 representative plants per plot, excluding border plants. Selection criteria included plant height $\geq$30 cm, higher leaf number, and shoot vigor. Measured variables included the number of leaves, plant height (collar to apex), number of shoots, and rhizome fresh and dry weights using a Dival Model DS digital balance. All measurements were performed at the end of the experiment, 240 days after planting.

Rhizome quality analysis

Rhizome quality analyses were performed on a dry weight basis at the Laboratorio de Compuestos Bioactivos - Instituto Tecnológico de la Producción (ITP), Lima. Protein content was determined using the Kjeldahl method; ash content by incineration in a Barnstead Thermolyne 48000 muffle furnace; fat content using Soxhlet extraction (Buchi E-800). Curcuminoid content was determined by UV-Visible spectrophotometry following the validated method of Hazra et al. (2015), in wich a 500 μg mL^{-1} curcumin standard was prepared in methanol and diluted to 5–20 μg mL^{-1} for calibration curve construction, with absorbance measurements read at 421 nm using a Thermo Scientific Genesys 180 spectrophotometer and results expressed as curcumin equivalents per gram of dry sample.

Statistical analysis

Data were analyzed using parametric methods within a randomized complete block design consisting of four treatments and five replications. Analysis of variance (ANOVA) was performed to assess treatment effects, followed by Tukey's HSD test for mean separation (α=0.05). The treatment sum of squares was partitioned into linear, quadratic, and cubic polynomial components using orthogonal contrasts for deeper interpretation of dose-response relationships. Regression analysis was also performed to model variable responses and identify the optimal conidial dose. All statistical analyses were conducted using SISVAR and RStudio (v4.4.1).

RESULTS AND DISCUSSION

Agronomic characteristics of *Curcuma longa*

The agronomic variables of *C. longa* were significantly affected by the application of *T. harzianum* (Table 1). Leaf number and plant height showed highly significant differences ($P\leq0.01$), while shoot number varied significantly at $P\leq0.05$. Quadratic models were fitted for leaf number and plant height, and a cubic model for shoot number (Figure 2).

Table 1. Analysis of variance of the regression of the agronomic characteristics, yield and quality of *C. longa* by *T. harzianum* action.

	Agronomic Characteristics						Yield			
	Leaves (N°)		Height (cm)		Shoots (N°)		RF (kg per plot)		RS (kg per plot)	
VF	Fc	Pr>Fc	Fc	Pr>Fc	Fc	Pr>Fc	Fc	Pr>Fc	Fc	Pr>Fc
Dose	13.45	0.000**	11.12	0.000*	3.97	0.035*	21.74	0.000**	18.48	0.000**
Linear	25.07	0.000**	14.37	0.003*	6.31	0.03*	43.57	0.000**	43.57	0.000**
Quadratic	11.62	0.005*	14.95	0.002*	0.43	0.53	8.06	0.020*	8.06	0.015*
Cubic	-	-	-	-	5.19	0.042*	-			-

FR: Fresh rhizome. DR: Dry rhizome. Fc: F calculated; Pr>Fc: probability value. *Significance at $P\leq0.05$; ** $P\leq0.01$.

According to treatment means (Table 2), plants inoculated with 4 g developed more vigorous vegetative growth than the control, with averages of 47.25 leaves, 61.50 cm in height, and 1.33 shoots per plant. Regression analysis estimated the maximum responses at intermediate doses: 49.11 leaves at 4.30 g (Figure 2A), 63.28 cm in height at 3.88 g (Figure 2B), and 1.38 shoots per

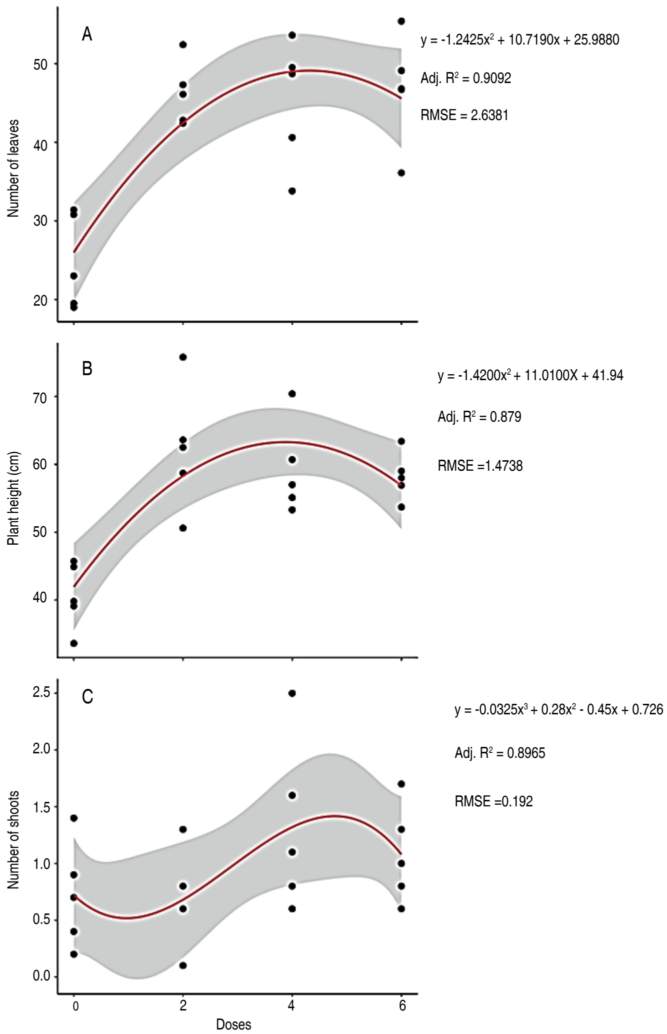

Figure 2. Quadratic regression models showing the effects of *T. harzianum* doses on **A**. leaf number, **B**. plant height, and **C.** shoot number.

Table 2. Effects of *Trichoderma harzianum* doses on agronomic traits and rhizome yield in *Curcuma longa*, with mean separation by Tukey's test.

Treatment	Leaves (N°)	Height (cm)	Shoots (N°)	RF (kg per plot)	RS (kg per plot)
T_0 (**0 g**)	24.7±2.7[b]	40.6±2.2[b]	0.65±0.1[a]	6.52±0.6[a]	0.87±0.07[b]
T_1 (**2 g**)	46.2±1.8[a]	62.2±4.1[a]	1.25±0.1[a]	8.42±0.5[b]	1.14±0.07[a]
T_2 (**4 g**)	45.2±3.6[a]	59.3±3.0[a]	1.45±0.1[a]	8.44±0.5[b]	1.15±0.07[a]
T_3 (**6 g**)	46.8±3.1[a]	58.2±1.6[a]	0.98±0.1[a]	9.02±0.4[b]	1.22±0.05[a]
F-value	13.94	11.56	1.59	4.421	5.33
P-value	<0.0001	<0.0002	0.23	<0.019	<0.009

Means followed by different letters in the same column differ significantly by Tukey's test (α=0.05). RF: Fresh rhizome; RS: Dry rhizome.

plant at 4.30 g (Figure 2C). At higher doses, the models predicted reductions in all three traits. Visual differences among treatments at harvest are shown in Figure 3.

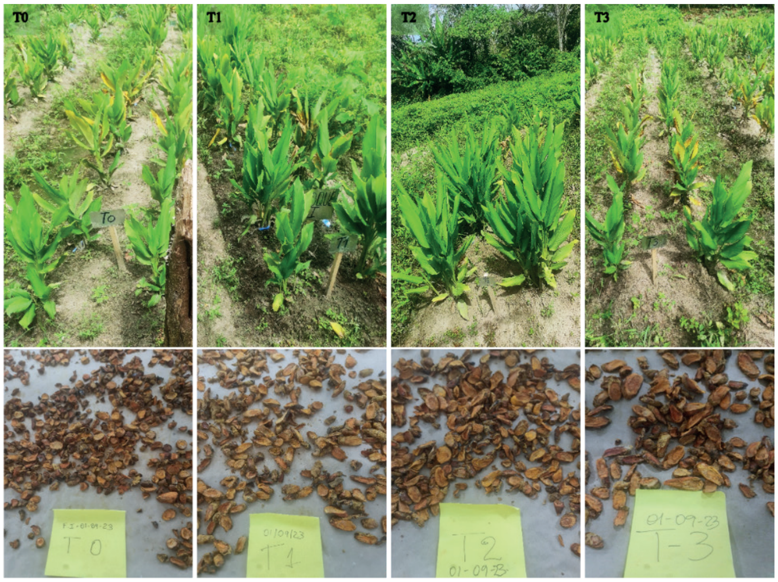

Figure 3. Growth performance of turmeric (*Curcuma longa* L.) under *Trichoderma harzianum* application: control (T_0) and three inoculation doses (T_1–T_3).

The enhanced vegetative development observed with *T. harzianum* application aligns with findings reported by Verma et al. (2019), who documented significant improvements in turmeric growth parameters under organic practices involving *T. harzianum* seed treatment at 5 g kg^{-1} seed rhizome plus soil application. The beneficial effects can be attributed to the biocontrol and growth-promoting capabilities of *Trichoderma* species documented by Tripura et al. (2018), who reported that the combined application of the recommended dose of fertilizer with *T. harzianum* resulted in higher growth parameters, including plant height, pseudostem diameter, leaf area, and leaf-area index in turmeric.

The quadratic response patterns observed for leaf number and plant height suggest optimal bioagent concentrations exist, beyond which diminishing returns occur. This dose-dependent response has been documented in integrated management systems by several researchers. Altaf et al. (2022) found that *T. harzianum* effectiveness in sustainable management approaches varied with application methods, while Nanda et al. (2024) demonstrated that moderate concentrations of biocontrol agents, including *T. harzianum* provided optimal results in sesame cultivation compared to higher doses. The cubic model for shoot number indicates more complex interactions between *T. harzianum* and plant developmental processes, possibly related to hormonal balance modifications.

Yield of *Curcuma longa* rhizomes

Rhizome production also varied significantly among treatments ($P \leq 0.01$; Table 1). Fresh and dry yields fitted quadratic regression models (Figure 4).

The observed data (Table 2) indicated increases from 6.52 kg per plot in the control to 8.92 kg at 4 g for fresh yield, and from 0.87 to 1.18 kg per plot for dry yield. The models estimated maxima slightly above these values, with 9.14 kg per plot (10.83 t ha^{-1}) at 5.27 g for fresh yield (Figure 4A) and 1.23 kg per plot (1.44 t ha^{-1}) at ~5 g for dry yield (Figure 4B). At doses greater than 5 g, yields decreased. Tukey's test (Table 2) confirmed significant differences between the control and all inoculated treatments, but no differences among the treated groups.

The yield improvements obtained with *T. harzianum* application demonstrate substantial economic benefits for turmeric cultivation. The 36.8% increase in fresh yield observed at optimal doses is notable because Tripura et al. (2018) reported the highest benefit-cost ratio of 4.87 with RDF + VAM + *T. harzianum* treatment combinations. Studies evaluating biocontrol efficacy show that *T. harzianum* can significantly improve plant performance through multiple mechanisms, including disease suppression and enhanced nutrient uptake (Jakatimath et al. 2017).

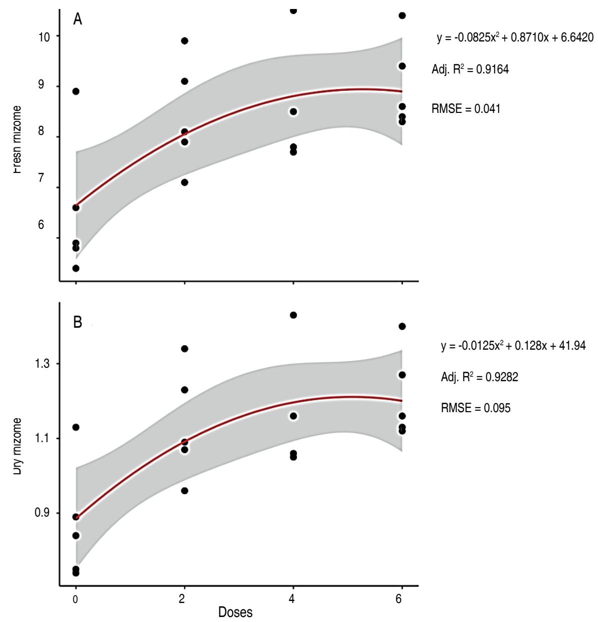

Figure 4. Quadratic regression models of rhizome yield response to fertilizer doses. **A**. Fresh and **B**. Dry.

The quadratic response pattern for both fresh and dry yields indicate an optimal inoculum density of around 5 g per application, beyond which competitive effects may limit benefits. Wagh et al. (2017) demonstrated that bioagents, including *T. harzianum* exhibited optimal mycelial growth inhibition at specific concentrations, with effectiveness declining at higher doses. Similar patterns have been observed by Gaur et al. (2011), who found that *T. harzianum* paint formulations showed optimal effectiveness at intermediate concentrations for disease management in citrus. The strong correlation between fresh and dry yield responses suggests that *T. harzianum* influences both water accumulation and dry matter partitioning to rhizomes.

Rhizome quality evaluation

Chemical composition of the rhizomes was also influenced by *T. harzianum* (Table 1). Protein, fat, and ash contents followed cubic models, while curcuminoids adjusted to a quadratic response (Figure 5).

Mean comparisons (Table 2) showed that protein increased from 7.20% in the control to 8.45% at 4 g, while curcuminoids rose from 6.59% in the control to 6.98% at 2 g. The regression models estimated higher values than those observed in the treatments, with 8.72% protein at 5.31 g (Figure 5A) and 7.13% curcuminoids at 2.30 g (Figure 5D). Fat and ash contents fluctuated across doses without a defined optimum (Figures 5B–C).

The enhancement of protein content (17.4% increase) and curcuminoids (5.9% increase) represents improvements in rhizome quality. Research on *C. longa* chemical composition has shown substantial variations depending on cultivation practices and environmental conditions. Setzer et al. (2021) documented major variations in essential oil components among five different *C. longa* varieties cultivated in North Alabama, with ar-turmerone ranging from 6.8-32.5%, α-turmerone from 13.6-31.5%, and β-turmerone from 4.8-18.4%, indicating that cultivation practices strongly influence bioactive compound profiles.

The mechanisms underlying quality improvements may involve enhanced secondary metabolite biosynthesis pathways. Gururani et al. (2022) demonstrated that cultivation practices significantly affect phytochemical composition and biological activities in *C. longa* accessions from different altitudes, with variations in major compounds including turmerone, germacrone, eucalyptol, caryophyllene, and α-curcumene. Environmental and biological factors can modulate the production of these bioactive compounds through enhanced antioxidant activities and improved nutrient metabolism.

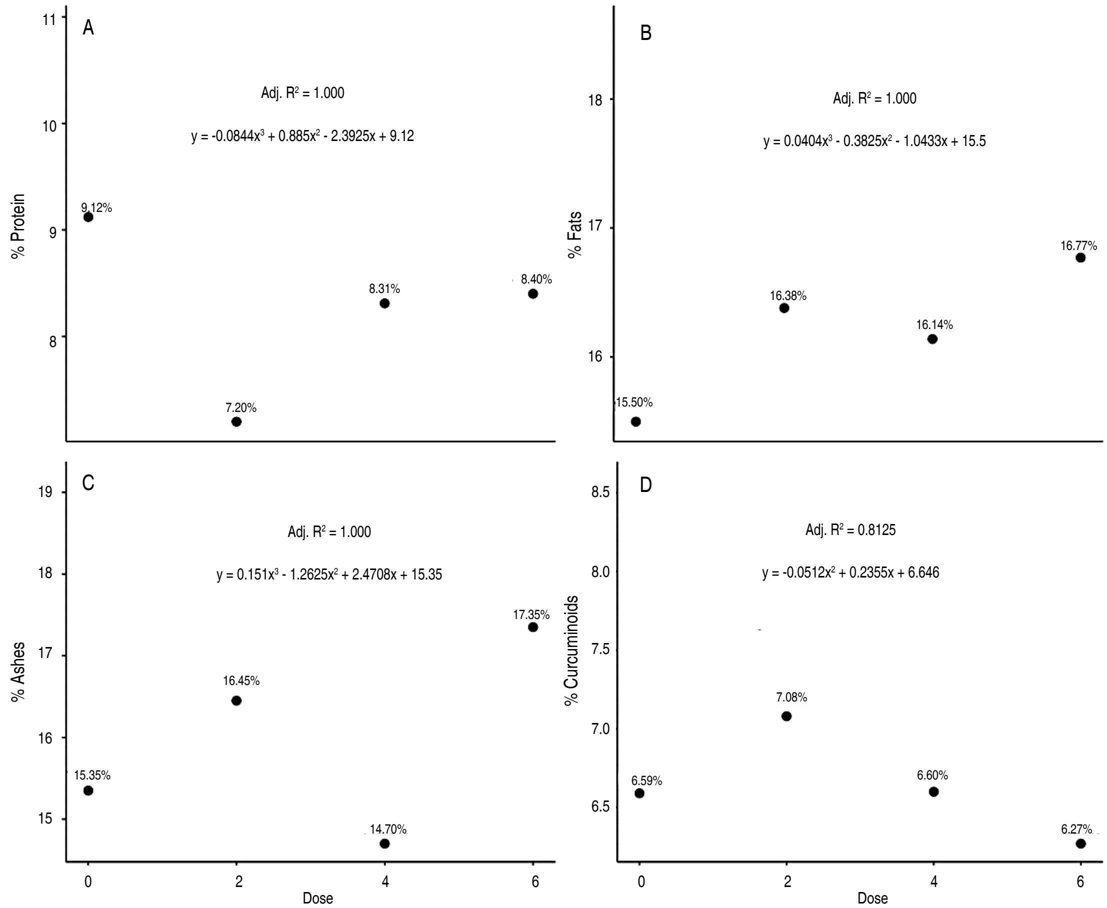

Figure 5. Polynomial regression models showing the relationship between *T. harzianum* doses and rhizome quality attributes: **A**. Protein content, **B**. Fat content, **C**. Ash content, and **D.** Curcuminoid content.

The quadratic response pattern for curcuminoids, with an optimum at 2.30 g, suggests that lower bioagent concentrations may be more effective for enhancing secondary metabolite production compared with vegetative growth parameters. Marchant et al. (2022) reported that cultivation conditions significantly influence phytochemical profiles in *C. longa*, with specific treatments increasing polyphenols, flavonoids, and curcumin synthesis while boosting radical scavenging activity. The differential optima observed (2.30 g for curcuminoids vs. 5.31 g for protein) indicate that *T. harzianum* applications can be tailored according to production goals.

The observed improvements in curcuminoid content are particularly significant given their commercial and therapeutic value. Fernández-Marín et al. (2021) demonstrated that cultivation methods influence the final properties of *C. longa* oil, including total phenolic content and antioxidant properties, with optimized conditions yielding 10.32% extraction efficiency and improved bioactive. Similarly, Baka (2023) reported that *C. longa* rhizome extract showed enhanced phenolic content and bioactive properties when plants were subjected to specific cultivation treatments, with curcumin content reaching 3,220.8 μg g^{-1} dry weight. The integrated approach of combining biological treatments with optimized cultivation practices offers potential for sustainable enhancement of both yield and quality parameters in medicinal plant production systems.

CONCLUSION

The application of *T. harzianum* significantly enhanced the growth, yield, and rhizome quality of *C. longa* under tropical field conditions. Regression modeling revealed dose-dependent responses that maximize both vegetative development and secondary metabolite accumulation, confirming the dual functionality of *T. harzianum* as both biofertilizer and biostimulant. These findings contribute to the growing body of knowledge on microbial-based bioenhancers in tropical agricultural systems, providing empirical evidence for their integration as sustainable alternatives to conventional synthetic inputs.

This research advances our understanding of plant-microbe interactions in tropical agroecosystems, particularly regarding the optimization of biostimulant applications for high-value medicinal crops. The results support the broader adoption of biological approaches in sustainable agriculture, addressing the increasing global demand for environmentally responsible crop production practices.

ACKNOWLEDGMENTS

The authors express gratitude to the Instituto Tecnológico de la Producción (ITP) for technical support and Dr. Maritza Barriga Sánchez for conducting the proximate analysis of *C. longa* rhizomes.

CONFLICT OF INTERESTS

The authors declare no known financial or personal conflicts of interest that could have influenced the work reported in this manuscript.

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