AGRONOMY (AGRONOMIA)

Agrária —Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.21, n.1, e4342, 2026
Recife, PE, UFRPE. www.agraria.pro.br
DOI: 10.5039/agraria.v21i1e4342 - Protocol 4342
Subm. 05/08/2025 • App. 21/12/2025• Pub. 23/01/2026

Application of *Trichoderma* spp. at different times on the growth and nutrition of grafted dwarf cashew seedlings

João Marcos Rodrigues dos Santos[1*], Carlos Alberto Kenji Taniguchi[2], Christiana de Fátima Bruce da Silva[2], Adriana Guirado Artur[1]

1 Universidade Federal do Ceará, Fortaleza, CE, Brasil. E-mail: joaorodriguesestagio.embrapa@gmail.com; driguirado@yahoo.com.br
2 Embrapa Agroindustria Tropical, Fortaleza, CE, Brazil. E-mail: carlos.taniguchi@embrapa.br; christiana.bruce@embrapa.br;

ABSTRACT: *Trichoderma* species are widely recognized for promoting plant growth and improving plant nutritional status. This study aimed to evaluate the effects of *Trichoderma longibrachiatum* (LPPC299) and *Trichoderma asperellum*, applied at different times (at sowing, after grafting, and combined application), on the growth and nutrition of grafted dwarf cashew seedlings 'BRS 226'. The experiment was conducted in a completely randomized design with seven treatments and ten replicates. At 120 days after germination, growth-related mechanisms, biometric parameters, dry matter production, and plant nutritional status were evaluated. The LPPC299 isolate applied after grafting promoted a significant increase in stem diameter and leaf nitrogen content, while combined applications increased stem diameter. *T. asperellum* mainly enhanced leaf area and leaf dry matter. Total biomass production and most nutrient contents did not differ significantly among treatments. The isolates did not produce indole-3-acetic acid; however, they were able to solubilize calcium phosphate and produce siderophores in vitro, independently of application type and timing. It is concluded that the effects of *Trichoderma* are isolate-dependent, with LPPC299 being the most promising isolate for improving the performance of grafted dwarf cashew seedlings.

Keywords: *Anacardium occidentale* L.; fruit cultivation; nutrient uptake; plant growth-promoting fungi; sustainable agriculture

Aplicação de *Trichoderma* spp. em diferentes épocas sobre o crescimento e a nutrição de mudas enxertadas de cajueiro-anão

RESUMO: Espécies de *Trichoderma* são amplamente reconhecidas por promover o crescimento vegetal e melhorar o estado nutricional das plantas. Este estudo teve como objetivo avaliar o efeito de *Trichoderma longibrachiatum* (LPPC299) e *Trichoderma asperellum* aplicados em diferentes épocas (semeadura, após enxertia e aplicação combinada) sobre o crescimento e a nutrição de mudas enxertadas de cajueiro-anão 'BRS 226'. O experimento foi conduzido em delineamento inteiramente casualizado, com sete tratamentos e dez repetições. Aos 120 dias após a germinação, foram avaliados mecanismos relacionados ao crescimento, parâmetros biométricos, produção de matéria seca e estado nutricional das plantas. O isolado LPPC299 aplicado após a enxertia promoveu aumento significativo no diâmetro do caule e no teor de nitrogênio foliar, enquanto aplicações combinadas aumentaram o diâmetro do caule. *T. asperellum* destacou-se principalmente no incremento da área foliar e da matéria seca das folhas. A produção total de biomassa e a maioria dos nutrientes não apresentaram diferenças significativas entre os tratamentos. Os isolados não produziram AIA, mas apresentaram capacidade de solubilizar fosfato de cálcio e produzir sideróforos in vitro, de forma independente do tipo e do momento de aplicação. Conclui-se que os efeitos de *Trichoderma* são dependentes do isolado, sendo o LPPC299 o mais promissor para o incremento do desempenho de mudas enxertadas de cajueiro-anão.

Palavras-chave: *Anacardium occidentale* L.; fruticultura; absorção de nutrientes; fungos promotores de crescimento de plantas; agricultura sustentável

* João Marcos Rodrigues dos Santos. E-mail: joaorodriguesestagio.embrapa@gmail.com (Corresponding author)
Associate Editor: Mário de Andrade Lira Junior.

This is an open-access article distributed under the Creative Commons Attribution 4.0 International License.

INTRODUCTION

Among tropical fruit trees, cashew (*Anacardium occidentale* L.) holds significant socioeconomic potential in both national and international markets (Anusuya et al., 2020; Lima et al., 2020). However, cashew nut production declined from approximately 147 thousand tons in 2022 to 126 thousand tons in 2023, representing a 13.9% decrease. The reduction in average yield was even more pronounced, dropping from 347 kg ha^{-1} in 2022 to 293 kg ha^{-1} in 2023 a decline of 15.6% (IBGE, 2023). This decline can be largely attributed to the heterogeneity of orchards, which often results from the lack of high-quality seedlings used in the establishment or replanting of cashew orchards (Santos et al., 2020).

The use of clonal grafted seedlings is essential for the establishment of productive orchards. Seedling quality is strongly correlated with plant survival in the field and plays a pivotal role in ensuring uniform, productive, and sustainable orchard systems (Moreira et al., 2023). From this perspective, achieving economically sustainable orchards requires the adoption of strategies that confer multiple agronomic benefits, ranging from enhanced plant growth and nutrition to minimal impact on the soil–plant system.

In this context, fungi of the genus *Trichoderma* have been widely recognized for their ability to confer numerous benefits to host plants (Moreno-Lora et al., 2022; Guzmán-Guzmán et al., 2025). These fungi colonize the root system by forming structures resembling appressoria, penetrating the root epidermis and establishing themselves between the epidermis and cortex. This interaction directly stimulates plant growth (both shoots and roots), improves nutrient availability and uptake, enhances the plant's defense responses against pathogens and pests, and increases resistance to both biotic and abiotic stresses (Tyśkiewicz et al., 2022; Woo et al., 2023; Asghar et al., 2024).

To elicit these benefits, *Trichoderma* spp. produce phytohormones such as auxins, cytokinins, and gibberellins, as well as various enzymes. They also solubilize essential elements such as phosphorus and micronutrients, and produce siderophores, among other mechanisms critical for plant development and growth (Contreras-Cornejo et al., 2024; Raju & Punamalai, 2025).

These mechanisms have been consistently reported in cashew cultivation systems. Santos et al. (2021) demonstrated that inoculation with *T. longibrachiatum* and *T. asperellum* promoted significant improvements in the growth of dwarf cashew rootstocks, particularly in plant height, stem diameter, leaf area, and total biomass, under nursery conditions. Although nutrient accumulation in leaves was not significantly altered, the authors highlighted that growth promotion was associated with phosphate solubilization and siderophore production, reinforcing the role of *Trichoderma* as a multifunctional plant growth–promoting microorganism. These findings support the relevance of *Trichoderma* in cashew seedling production systems and underscore its potential to enhance early vigor and substrate efficiency, providing a strong experimental basis for further investigation in grafted cashew seedlings.

This study aims to address critical knowledge gaps concerning the use of *Trichoderma* spp. as bioinputs in the production of grafted dwarf cashew seedlings. Although *Trichoderma* is well established as a plant growth-promoting microorganism, its effects on the nutrition and development of grafted perennial fruit crops, particularly cashew remain underexplored. There is limited information on how different *Trichoderma* isolates affect nutrient uptake, early seedling vigor, and the performance of specific scion–rootstock combinations under nursery conditions.

Given this context, we hypothesize that the inoculation of *Trichoderma* isolates into the planting substrate may represent a bioeconomic and sustainable strategy capable of enhancing growth and improving the nutritional status of grafted dwarf cashew seedlings. Therefore, the objective of this study was to evaluate the ability of different *Trichoderma* isolates to promote growth and nutrient uptake in grafted dwarf cashew seedlings.

MATERIALS AND METHODS

Experimental location and design

The experiment was carried out in the Pacajus Experimental Field, belonging to Embrapa Tropical Agroindustry (Figure 1). The region's climate is classified as tropical with dry winter (Aw), according to Köppen's climate classification (Köppen, 1918).

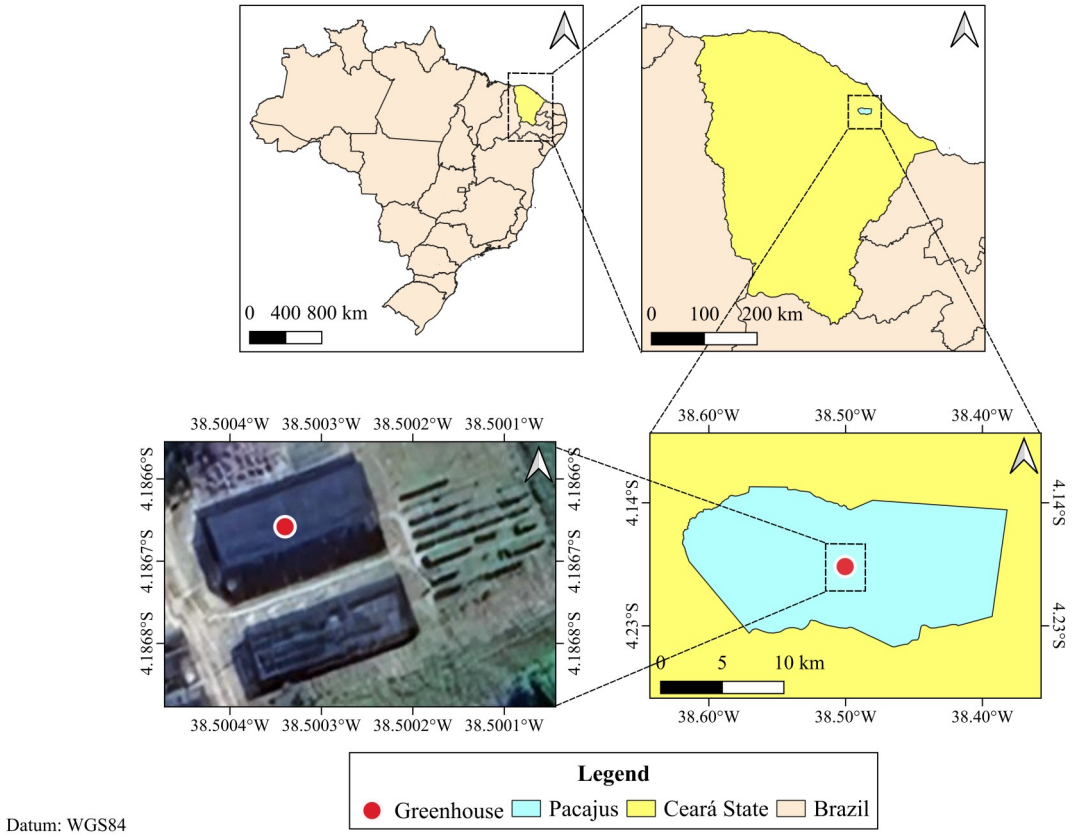

Figure 1 - Map of the geographical location of the experimental site.

The experiment was conducted under a 50% shade net in the rootstock production phase and under full sun in the grafted seedling phase. The selection of *Trichoderma* isolates for this study was based on their demonstrated biotechnological potential in previous *in vitro* and *in vivo* screenings with other plant species. The design was completely randomized, with seven treatments: T1: control without isolate; T2: treatment with *T. longibrachiatum* isolate at sowing (LPPC299 S); T3: treatment with commercial isolate *T. asperellum* at sowing (Commercial S); T4: treatment with *T. longibrachiatum* isolate after grafting (LPPC299 G); T5: treatment with *T. asperellum* isolate after grafting (Commercial G); T6: treatment with *T. longibrachiatum* isolate at sowing and after grafting (LPPC299 SG); and T7: treatment with *T. asperellum* isolate at sowing and after grafting (Commercial SG), with ten replicates. Each experimental unit consisted of one tube containing one dwarf cashew seedling.

Experiment setup and grafting process

The experiment was set up using 288 cm^3 tubes, previously disinfected with 3% sodium hypochlorite. The tubes were filled with sterile commercial substrate (120 °C for 1 hour, repeated after 24 hours), and then a seed nut of the genotype 'CCP 06' was planted in the center of each tube, 2 cm deep in the substrate, in a vertical position with the tip down.

Substrate inoculation was performed using two types of inoculums: *Trichoderma asperellum* (Commercial) and the isolate The *Trichoderma longibrachiatum* strain used in this study was previously identified at the molecular level by Sanó et al. (2022) through sequencing of the ITS/5.8S rRNA region. Genomic DNA was extracted from fungal mycelium, followed by PCR amplification using ITS4 and ITS5 primers, Sanger sequencing, and sequence assembly using the Phred/Phrap/Consed pipeline. The resulting sequences were deposited in GenBank (accession numbers OL652611 and OL652612) and confirmed as *T. longibrachiatum* based on BLASTN similarity searches. This prior molecular characterization ensures the taxonomic reliability of the strain employed in the present study.

The LPPC299 isolate was reactivated in Petri dishes containing PDA (potato, dextrose and agar) culture medium and incubated for seven days with a 12-hour photoperiod at 28 °C. The inoculum was produced from these colonies, using three discs (5 mm each) of PDA medium containing mycelium and conidia of the isolate. These discs were aseptically allocated to Erlenmeyer® flasks containing 50 g of parboiled rice + 30 mL of deionized water (both sterilized in an autoclave at 121 °C for 15 minutes). The inoculum was kept in a BOD incubator at 28 °C and under a 12-hour photoperiod for 15 days. After this period, all material was transferred aseptically to sterile kraft paper envelopes, dried in a BOD oven at temperature of 32 °C for five days, and then crushed

using a blender. Conidia concentration was adjusted to 3.2 x 10^9 conidia mL^{-1} with a Neubauer chamber. For the commercial biological input, the concentration used was 5.4 x 10^9 conidia mL^{-1}.

Trichoderma inocula were applied in the form of a suspension, with the selected dose (based on the recommendation of the commercial input) diluted in distilled and sterilized water, and 10 mL of the suspension/seedling was applied on top of the nut seeds for the treatments applied at sowing (rootstock) and deposited on the substrate surface, near the seedling stem for those applied after grafting.

Sixty days after germination, the grafting process was carried out by side grafting, using scions of the 'BRS 226' genotype. Irrigation was carried out 24 hours after inoculation of the isolates.

Seedling growth and nutritional status evaluations

Evaluations related to seedling growth were performed at 120 days after germination. The seedlings were evaluated for: a) height: from the collar to the apical bud (determined with a tape measure - cm); b) stem diameter: measured with a digital caliper 5 cm above the substrate; c) number of leaves: determined by counting all fully expanded leaves; and d) leaf area: measured using the leaf area integrator LI-3100C, LI-COR® - cm^2.

The seedlings were separated into leaves, stems, and roots, washed with running water, 3% hydrochloric acid (HCl) (v:v), and deionized water, placed in a paper bag, and kept in a forced air circulation oven at 65 °C until reaching constant mass. After drying, leaf dry matter, stem dry matter, total shoot dry matter, and root dry matter were determined. Then, the samples were ground in a Wiley mill, passed through 1-mm-mesh sieves, and stored in containers.

To evaluate the nutritional status of cashew seedlings, leaf tissue samples were collected and subjected to standardized chemical digestion procedures. The concentrations of phosphorus, potassium, calcium, magnesium, sulfur, copper, iron, manganese, and zinc were determined following nitric-perchloric acid digestion. This method ensures the complete oxidation of organic matter, allowing for accurate quantification of macro- and micronutrients using inductively coupled plasma optical emission spectrometry. For total nitrogen analysis, sulfuric acid digestion was employed, followed by distillation and titration using the Kjeldahl method. These analytical procedures were conducted in accordance with established protocols for plant tissue analysis to ensure precision and reliability of the nutrient content data. For the boron content, the samples were incinerated in a muffle furnace, followed by quantification by spectrophotometry using the azomethine-H method, according to the methodology described by Miyazawa et al. (2009).

Subsequently, nutrient accumulation in the leaves was determined based on the results of dry matter and nutrient content in the leaves.

Re-isolation of fungal isolates from plant tissue

To check for the presence of the isolates in plant tissue, re-isolation was carried out through indirect plating of roots (Alfenas et al., 2016). The fragments were deposited in Petri dishes containing PDA culture medium, supplemented with rose bengal (0.05 g L^{-1}) and incubated at 28 °C, with a 12-h photoperiod for 7 days. To obtain the pure isolates, after 7 days, a disc (3 mm) containing mycelium and conidia was subcultured to another Petri dish, containing PDA medium and incubated under the same conditions as described above. Slides were prepared to visualize the characteristic fungal structures of *Trichoderma* under a light microscope (40 x) (Abreu & Pfenning, 2019). These isolates were the same as those used in all trials of the present study.

Detection of plant growth promotion-related mechanisms in fungal isolates

a) *In vitro* production and quantification of indole-3-acetic acid

The capacity of the isolates to qualitatively and quantitatively produce indole-3-acetic acid (IAA) was measured by transferring three discs of PDA culture medium (5 mm each), containing mycelia and conidia to 250 mL Erlenmeyer® flasks, containing 50 mL of PD (potato dextrose) broth (sterilized at 121 °C), in the absence and presence of L-tryptophan. 5 mL of L-tryptophan solution (100 µg mL^{-1}) was used for each 50 mL of liquid medium.

All the material contained in the Erlenmeyer® flasks was subjected to orbital shaking at 150 rpm and temperature of 28 °C for 7 days, after which the colonized medium was centrifuged at 5,000 rpm for 15 minutes, and the supernatant was reserved. Indole-3-acetic acid was quantified by following the method described by Gordon & Weber (1951), using 2 mL of Salkowski's reagent + 4 mL of supernatant, and keeping the mixture in the dark at 28 °C for 25 minutes to obtain the pink color. After the reaction time, the phytohormone was quantified in a spectrophotometer at 530 nm wavelength, and the concentrations in µg mL^{-1} were calculated from the standard curve with known concentrations of the synthetic form of the phytohormone (0 to 100 µg mL^{-1}). The readings were then used to calculate the concentration of IAA in the samples.

b) *In vitro* solubilization and quantification of calcium phosphate

We selected calcium phosphate as the target substrate because it is widely used as a standard model for screening phosphate-solubilizing microorganisms, allowing reliable comparison with previous studies involving Trichoderma spp.

In addition, Ca–P forms are commonly found in tropical and semi-arid soils, where cashew is typically cultivated. Although Al–P and Fe–P complexes are also relevant in acidic soils, their solubilization mechanisms are often more complex and substrate-specific.

The capacity of *Trichoderma* isolates to solubilize phosphates was evaluated by adding 100 mL of modified NBRIP culture medium (Oliveira et al., 2012) in a 250 mL Erlenmeyer® flask, followed by sterilization in an autoclave for 15 minutes at 121 °C. After cooling, three discs (5 mm each) containing PDA + mycelium culture medium and conidia were added to the medium. The material was exposed to orbital shaking at 150 rpm and temperature of 28 °C for 7 days, centrifuged at 5,000 rpm for 15 minutes, and the supernatant was reserved.

Phosphorus (P) content was quantified by using 0.5 mL of supernatant, 5.0 mL of distilled water, and 2.5 mL of vanadate-molybdate solution (Malavolta et al., 1989). After 20 minutes of reaction under light, soluble phosphorus was quantified in a spectrophotometer at 420 nm wavelength. Solubilized P content was determined using the colorimetric method described by Murphy & Riley (1962), subtracting the P contained in the treatments from the content in the control sample (NBRIP medium + phosphate and without inoculum). Phosphorus contents in the samples were calculated by constructing a standard curve of KH_2PO_4 with known contents (8 to 160 µg L^{-1}).

c) *In vitro* biosynthesis and quantification of siderophores

The potential of *Trichoderma* isolates in the biosynthesis of siderophores was determined by the universal method of Schwyn & Neilands (1987). This method recommends the addition of 100 mL of PD (potato dextrose) culture medium to a 250 mL Erlenmeyer® flask, with subsequent sterilization in an autoclave at 121 °C for 15 minutes. After cooling, three discs (5 mm each) of PDA medium containing mycelia and conidia were added to the liquid medium. All Erlenmeyer® flaks were placed on a benchtop incubator with 150 rpm orbital shaking at 28 °C for 7 days.

After the shaking period, centrifugation was performed at 5000 rpm, and the supernatant was reserved. In glass tubes, 2 mL of the supernatant was mixed with 2 mL of the CAS (chrome azurol S) solution. The mixture was kept for 1 hour at room temperature and protected from light. Then, reading was performed in a spectrophotometer, at 630 nm of absorbance. Calculations were performed using the equation defined by Payne (1988).

Statistical analysis

The normality of residuals was assessed using the Shapiro -Wilk test, while the homogeneity of variances was evaluated using the Bartlett test. The data obtained were subjected to analysis of variance by the F test and, when significant, the means of the growth and nutrient variables were compared using the Scott-Knott test at a 5% probability level. The student's t-test compared means of phosphate solubilization and siderophore biosynthesis analyses. The data were analyzed using the AgroEstat software.

RESULTS AND DISCUSSION

Seedling growth and nutritional status evaluations

In the production of grafted 'BRS 226' dwarf cashew seedlings, significant differences were observed among treatments for stem diameter (Table 1). Seedlings inoculated with *Trichoderma longibrachiatum* after grafting (LPPC299 G), as well as those inoculated at sowing and re-inoculated after grafting with either *T. longibrachiatum* (LPPC299 SG) or *T. asperellum* (Commercial SG), exhibited significantly larger stem diameters compared to the control. Given the similar effects of these treatments on this variable, the single application of *T. longibrachiatum* after grafting (LPPC299 G) appears to be the most advantageous for nursery adoption, as it reduces bioinput and labor costs (Table 1).

These findings are consistent with those reported by Santos et al. (2021), who observed increases in leaf area and stem diameter following inoculation of *T. longibrachiatum* in dwarf cashew rootstocks, relative to the control. Stem diameter is a critical parameter, as it is directly associated with seedling growth and survival under field conditions. Several studies suggest that stem diameter is the most reliable indicator of seedling quality at planting, given its strong correlation with vigor. Seedlings with thicker stems tend to produce a greater number of buds, promoting the emergence of leaf primordia and subsequent shoot formation after transplanting. Moreover, these seedlings are better able to withstand sudden environmental fluctuations, such as strong winds (Moreira et al., 2023).

The differential responses observed between *T. asperellum* and isolate LPPC299 suggest distinct colonization and persistence strategies in cashew seedlings. For *T. asperellum*, the significant effect only under combined application (at sowing and after grafting) indicates that repeated inoculation may be necessary to maintain an effective population during critical developmental stages, particularly considering the physiological and microbiological disturbance caused by grafting. In contrast, LPPC299 showed consistent effects when applied either at sowing or after grafting, suggesting greater adaptability and stability in the rhizosphere and plant tissues. These results highlight that the effectiveness of *Trichoderma* strains is strongly strain-dependent and influenced by application timing and frequency.

Regarding leaf area, the highest values were recorded in seedlings treated with *T. asperellum*, both at sowing (Commercial S) and after grafting (Commercial G), as well as with *T. longibrachiatum* applied at sowing (LPPC299 S), when compared to the control treatment (Table 1).

Table 1 - Growth of grafteed seedlings of dwarf cashew 'BRS 226', as a function of the application of *Trichoderma* isolates

Treatments	Height	Diameter	Leaf area	Number of leaves
	cm	mm	cm^2	-
Control	21.61 a	6.55 b	115.19 b	7.25 a
LPPC299 S	21.78 a	6.89 b	136.49 a	6.75 a
Commercial S	22.45 a	6.94 b	128.83 a	6.50 a
LPPC299 G	20.73 a	7.48 a	95.74 b	6.13 a
Commercial G	22.42 a	7.09 b	145.49 a	7.62 a
LPPC299 SG	22.25 a	7.31 a	112.15 b	6.12 a
Commercial SG	21.88 a	7.65 a	108.99 b	6.00 a
Standard deviation	1.1	0.65	29.73	1.51
Coefficient variation (%)	5.04	9.11	24.69	22.87

Means followed by the same letter in the columns do not differ by Scott-Knott test at 5% probability level.

The treatments significantly affected only the leaf dry matter production of grafted dwarf cashew seedlings. Increases of 25.0%, 18.2%, and 17.0% in leaf dry matter were observed following the application of *T. asperellum* after grafting (Commercial G), at sowing (Commercial S), and *T. longibrachiatum* at sowing (LPPC299 S), respectively, compared to the control (Table 2). These results are closely associated with the leaf area data presented in Table 1, indicating that seedlings with larger leaf areas tended to accumulate greater leaf dry matter.

Table 2 - Dry matter production of grafted seedlings of dwarf cashew 'BRS 226', as a function of the application of *Trichoderma* isolates

Treatments	Dry matter				
	Leaves	Stem	Roots	Shoot	Total
	..... g $plant^{-1}$				
Control	0.88 b	2.28 a	1.76 a	3.16 a	4.92 a
LPPC299 S	1.03 a	2.36 a	1.78 a	3.39 a	5.18 a
Commercial S	1.04 a	2.33 a	1.69 a	3.37 a	5.07 a
LPPC299 G	0.71 b	2.43 a	1.74 a	3.14 a	4.89 a
Commercial G	1.10 a	2.53 a	1.66 a	3.64 a	5.31 a
LPPC299 SG	0.77 b	2.38 a	1.75 a	3.15 a	4.91 a
Commercial SG	0.82 b	2.35 a	1.81 a	3.17 a	4.98 a
Standard deviation	0.21	0.40	0.50	0.45	0.84
Coefficient variation (%)	24.13	17.15	28.80	13.87	16.71

Means followed by the same letter in the columns do not differ by Scott-Knott test at 5% probability level.

Yu et al. (2023) inoculated *Trichoderma longibrachiatum* in *Pinus massoniana* seedlings and observed that the fungus significantly increased shoot dry matter compared to the non-inoculated control. With respect to the nutritional status of grafted dwarf cashew seedlings, the treatments significantly affected only leaf nitrogen (N) content (Table 3). Inoculation with *T. longibrachiatum* after grafting (LPPC299 G) led to a 16% increase in leaf N content relative to non-inoculated seedlings. This result is consistent with the findings of Yu et al. (2023), who reported enhanced N accumulation in *Pinus massoniana* seedlings following inoculation with *T. longibrachiatum*.

Fungi of the genus *Trichoderma* are known to enhance soil fertility through rapid colonization of the rhizosphere and plant root systems, as well as through the release of urease, an enzyme that plays a direct role in nitrogen mineralization (Asghar & Kataoka, 2021; Aruna et al., 2025).

Table 3 - Macronutrient contents in leaves of grafted seedlings of dwarf cashew 'BRS 226', as a function of the application of *Trichoderma* isolates.

Treatments	N	P	K	Ca	Mg	S
			..... g kg^{-1}			
Control	12.63 b	1.08 a	9.89 a	4.85 a	3.03 a	0.86 a
LPPC299 S	12.91 b	1.03 a	9.50 a	4.15 a	2.69 a	0.90 a
Commercial S	12.28 b	1.09 a	9.82 a	4.54 a	2.68 a	0.83 a
LPPC299 G	14.68 a	1.13 a	11.13 a	4.87 a	3.09 a	1.00 a
Commercial G	11.79 b	1.09 a	10.47 a	4.17 a	2.43 a	0.82 a
LPPC299 SG	13.28 b	1.29 a	11.12 a	4.92 a	2.90 a	0.92 a
Commercial SG	11.71 b	1.05 a	10.32 a	4.36 a	2.83 a	0.90 a
Standard deviation	1.11	0.12	0.91	0.50	0.26	0.09
Coefficient of variation (%)	8.72	11.63	8.82	11.05	9.53	10.33

Means followed by the same letter in the columns do not differ by Scott-Knott test at 5% probability level.

Table 4 - Micronutrients contents in leaves of grafted seedlings of dwarf cashew 'BRS 226', as a function of the application of *Trichoderma* isolates

Treatments	Cu	Fe	Zn	Mn	B
			..... mg kg^{-1}		
Control	4.75 a	42.50 a	14.50 a	127.50 a	20.90 a
LPPC299 S	4.25 a	37.25 a	11.75 a	120.75 a	16.75 a
Commercial S	5.00 a	33.50 a	12.25 a	133.75 a	18.93 a
LPPC299 G	5.25 a	51.25 a	12.50 a	128.00 a	19.01 a
Commercial G	4.75 a	43.25 a	12.25 a	108.50 a	19.52 a
LPPC299 SG	5.50 a	42.50 a	13.75 a	124.50 a	15.73 a
Commercial SG	4.50 a	36.25 a	12.50 a	117.75 a	20.03 a
Standard deviation	0.65	19.42	2.16	23.44	2.75
Coefficient variation (%)	13.47	47.45	16.89	19.06	14.71

Means followed by the same letter in the columns do not differ by Scott-Knott test at 5% probability level.

Although the application of the *T. longibrachiatum* strain after grafting (LPPC299 G) resulted in higher leaf nitrogen content, this increase did not translate into greater leaf area or leaf dry matter production (Tables 1 and 2); on the contrary, reductions in these variables were observed. This can be attributed to the nutrient concentration effect, as no significant differences were found among treatments regarding the total accumulation of nitrogen or other nutrients in the leaves of dwarf cashew seedlings. In this context, variations in biomass production may have influenced nutrient concentrations: lower biomass may have led to nutrient concentration in the tissues, whereas greater biomass could have resulted in nutrient dilution, without necessarily affecting total nutrient uptake by the plant (Tables 5 and 6).

These findings suggest that *Trichoderma* isolates may exhibit species-specific responses depending on the host plant. Consequently, the microorganism-plant interaction is dynamic and regulated by biochemical signals, including root exudates released by the plant, which are recognized by the fungus and/or vice versa. Such signaling is essential for establishing a functional interaction that enables the plant to fully benefit from the presence of the microorganism (Ling et al., 2022).

Table 5 - Accumulation of macronutrients in leaves of grafted seedlings of dwarf cashew 'BRS 226', as a function of the application of *Trichoderma* isolates

Treatments	N	P	K	Ca	Mg	S
	..... mg plant^{-1}					
Control	11.23 a	0.98 a	8.85 a	4.35 a	2.69 a	0.77 a
LPPC299 S	13.24 a	1.06 a	9.84 a	4.29 a	2.78 a	0.93 a
Commercial S	12.70 a	1.13 a	10.29 a	4.67 a	2.78 a	0.85 a
LPPC299 G	9.93 a	0.76 a	7.72 a	3.26 a	2.09 a	0.66 a
Commercial G	13.02 a	1.22 a	11.62 a	4.61 a	2.69 a	0.91 a
LPPC299 SG	10.23 a	0.97 a	8.50 a	3.85 a	2.26 a	0.72 a
Commercial SG	9.63 a	0.87 a	8.44 a	3.56 a	2.31 a	0.73 a
Standard deviation	2.34	0.22	2.27	0.92	0.55	0.14
Coefficient of variation (%)	20.53	22.76	24.43	22.56	22.00	18.72

Means followed by the same letter in the columns do not differ by Scott-Knott test at 5% probability level.

Table 6 - Accumulation of micronutrients in leaves of grafted seedlings of dwarf cashew 'BRS 226', as a function of the application of *Trichoderma* isolates

Treatments	Cu	Fe	Zn	Mn	B
	..... µg plant^{-1}				
Control	4.22 a	37.80 a	12.80 a	110.82 a	18.25 a
LPPC299 S	4.40 a	37.37 a	12.07 a	125.02 a	17.22 a
Commercial S	5.22 a	32.75 a	12.62 a	133.50 a	19.72 a
LPPC299 G	3.60 a	34.52 a	8.32 a	86.95 a	13.10 a
Commercial G	5.35 a	47.32 a	13.67 a	120.27 a	21.47 a
LPPC299 SG	4.22 a	31.20 a	10.57 a	96.55 a	12.62 a
Commercial SG	3.70 a	29.85 a	10.17 a	95.90 a	16.47 a
Standard deviation	1.16	14.36	2.78	24.89	4.05
Coefficient of variation (%)	26.50	40.08	24.30	22.66	23.88

Means followed by the same letter in the columns do not differ by Scott-Knott test at 5% probability level.

The *Trichoderma* isolates evaluated in this study were not able to biosynthesize indole-3-acetic acid (IAA), either in the absence or presence of L-tryptophan. Consequently, the number of leaves in the grafted dwarf cashew seedlings was not influenced by the application or reapplication of the isolates, possibly due to the inability of these microorganisms to synthesize auxin.

For *Trichoderma* isolates to produce auxins, plants must first synthesize signaling compounds that are recognized by the microorganisms. In response, the fungi emit signals that enable the microbial colonization process (Ling et al., 2022). Plant roots also release sugars that serve as a food source for microorganisms, facilitating efficient colonization (Howard et al., 2020; Vries et al., 2020). If these compounds are not properly recognized, the interaction may be compromised, preventing both the fungus and the plant from deriving mutual benefits. This likely explains the absence of auxin production by the isolates under study.

Despite their inability to produce IAA, the isolates demonstrated the capacity to solubilize calcium phosphate in vitro (Figure 2) and to produce siderophores (Figure 2). However, no significant differences were observed among the treatments. The timing of inoculation, whether at sowing, after grafting, or at both stages, did not affect the ability of the isolates to solubilize calcium phosphate or to produce siderophores. These findings indicate that such traits are intrinsic to the isolates.

Phosphate solubilization is a well-documented trait among *Trichoderma* species and is primarily attributed to the secretion of organic acids, such as gluconic and citric acids,

which lower the pH of the surrounding environment and facilitate the release of phosphorus from insoluble complexes (Bedine et al., 2022). This metabolic capacity is often constitutive, meaning it can occur independently of the presence of a host plant or its root exudates (Bononi et al., 2020). The present findings are consistent with this understanding, as inoculation timing had no significant impact on phosphate solubilization activity.

Similarly, siderophore production, characterized by the secretion of low-molecular-weight compounds with high affinity for ferric ions (Fe^{3+}) is another well characterized mechanism exhibited by *Trichoderma* isolates (Cui et al., 2025). These molecules improve iron acquisition under iron-limited conditions, thereby benefiting both the fungus and the host plant. This is particularly relevant in tropical soils, where iron fixation is common. The literature suggests that siderophore production is often a constitutive response, especially under iron-deficient conditions such as those simulated in the present study (Xie et al., 2024).

Thus, the results indicate that phosphate solubilization and siderophore production by the *Trichoderma* isolates occurred independently of inoculation type and timing, reinforcing the notion that these are inherent metabolic traits. While these mechanisms are recognized as important for plant growth promotion, their effectiveness in planta may also depend on additional factors, such as successful root colonization, compatibility with plant root exudates, and interactions with the native soil microbiota (Esparza-Reynoso et al., 2021).

In this context, it is important to emphasize that the in vitro evaluation of siderophore production and phosphate solubilization reflects the intrinsic functional potential of each isolate rather than the influence of the inoculation strategy. Therefore, differences observed among treatments in nursery conditions should not be interpreted as direct modulation of these metabolic traits by the application method, but rather as a consequence of strain-specific performance and their interaction with plant and environmental factors. This distinction is essential to avoid overinterpretation of application effects on mechanisms that are fundamentally isolate-dependent.

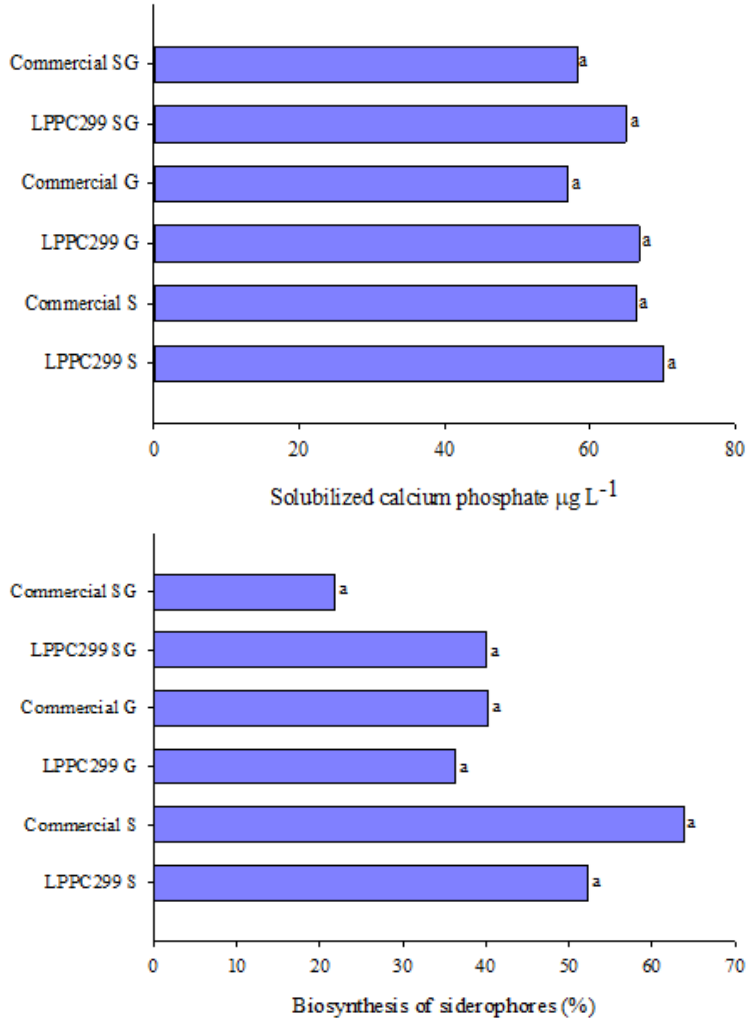

Figure 2 - Contents of solubilized calcium phosphate - µg L^{-1} and biosynthesis of siderophores - % by the *Trichoderma* isolates under study according to the treatments.

Means followed by the same letter do not differ according to the t test at 5% probability; Soluble phosphorus (test F.: 1.36^{ns}; Coefficient variation (%): 11.91; Standard deviation: 1.44) and siderophores (test F.: 1.32^{ns}; Coefficient variation: 51.11; Standard deviation: 7.05).

CONCLUSIONS

This study demonstrates that the effects of *Trichoderma* isolates on cashew seedling growth and nutrition are strongly strain-dependent and influenced by the interaction between inoculation timing and plant developmental stage.

Among the evaluated isolates, only LPPC299 promoted a significant increase in leaf nitrogen content, whereas the other isolate did not induce a similar response. This contrasting behavior highlights that plant nutritional responses cannot be generalized at the species level and are governed by isolate-specific metabolic and colonization traits. Furthermore, the absence of consistent nitrogen responses under combined or alternative application timings indicates that inoculation strategy alone does not guarantee enhanced nutrient accumulation.

These findings reinforce the need for caution when extrapolating results across isolates and application methods.

Although the present results provide relevant evidence of functional variability among *Trichoderma* strains, additional experiments are recommended to confirm the observed nitrogen responses and to clarify the physiological and microbiological mechanisms underlying these differences.

Overall, our results emphasize that the successful use of *Trichoderma* in cashew seedling production requires careful isolate selection, rather than reliance on application timing alone.

ACKNOWLEDGEMENTS

The authors would like to thank the Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) for granting a scholarship to the first author under the process BMD-0008-01029.01.08/18 and BMD-0008-01029.01.08/23. The authors thank also the Embrapa Agroindústria Tropical (SEG: 03.13.12.007.00.00), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for funding this research (C.F.B.S. thanks proc. 444339/2014-0, 405401/2021-3, and 304901/2022-9).

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contributions: Conceptualization: JMRS, CAKT, CFBS, AGA; Data Curation: JMRS, CAKT, CFBS, AGA; Formal Analysis: JMRS, CAKT, CFBS, AGA; Funding Acquisition: JMRS, CAKT, CFBS, AGA; Resources: JMRS, CAKT, CFBS, AGA; Visualization: JMRS, CAKT, CFBS, AGA; Writing – original draft: JMRS, CAKT, CFBS, AGA; Writing – review & editing: JMRS, CAKT, CFBS, AGA.

Conflicts of interest: The authors declare that there is no conflict of interest, whether professional or financial, that could have influenced the article.

Funding: The Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico - FUNCAP (BMD-0008-01029.01.08/18 and 0008-01029.01.08/23), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES (Finance Code 001), the Embrapa Agroindústria Tropical (SEG: 03.13.12.007.00.00) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (444339/2014-0, 405401/2021-3, and 304901/2022-9).

LITERATURE CITED

Abreu, L.M.; Pfenning, L.H.; O gênero *Trichoderma.* In: Meyer, MC.; Mazaro, S.M.; Silva, J.C. (Eds.). *Trichoderma*: uso na agricultura. 1.ed. Brasília: Embrapa, 2019. p. 163-179. http://www.alice.cnptia.embrapa.br/alice/handle/doc/1117296. 28 Jul. 2025.

Alfenas, A.C.; Ferreira, F. A.; Mafia, R. G.; Gonçalves, R. Isolamento de fungos fitopatogênicos. In: Alfenas, A.C.; Mafia, R.G. (Eds.). Métodos em fitopatologia. 2.ed. Viçosa: editora UFV, 2016. p. 55-91.

Anusuya, A.; Balasubramaniam, P.; Krishnaveni, T.R.S. Socio-economic characteristics of the cashew growers of Tamil Nadu – An analysis. International Journal of Current Microbiology and Applied Sciences, v. 9, n. 7, p.1790-1801, 2020. https://doi.org/10.20546/ijcmas.2020.907.205.

Aruna, K.; Sucharita, K.; Suresh, P.; Priyadarshini, S.; Vineela, D.R.S.; Kumar, K.V.K. Exploitation of *Trichoderma* spp. for soil and crop health in sustainable agriculture. International Journal of Current Microbiology and Applied Sciences, v. 14, n. 1, p.60-71, 2025. https://doi.org/10.20546/ijcmas.2025.1401.006.

Asghar, W.; Craven, K.D.; Kataoka, R.; Mahmood, A.; Asghar, N.; Raza, T.; Iftikhar, F. The application of *Trichoderma* spp., an old but new useful fungus, in sustainable soil health intensification: A comprehensive strategy for addressing challenges. Plant Stress, v. 12, e100455, 2024. https://doi.org/10.1016/j.stress.2024.100455.

Asghar, W.; Kataoka, R. Effect of co-application of *Trichoderma* spp. with organic composts on plant growth enhancement, soil enzymes and fungal community in soil. Archives of Microbiology, v, 203, p.4281-4291, 2021. https://doi.org/10.1007/s00203-021-02413-4.

Bedine, M.A.B.; Iacomi, B.; Tchameni, S.N.; Sameza, M.L.; Fekam, F.B. Harnessing the phosphate-solubilizing ability of *Trichoderma* strains to improve plant growth, phosphorus uptake and photosynthetic pigment contents in common bean (*Phaseolus vulgaris*). Biocatalysis and Agricultural Biotechnology, v. 45, e102510, 2022. https://doi.org/10.1016/j.bcab.2022.102510.

Bononi, L.; Chiaramonte, J.B.; Pansa, C.C.; Moitinho, M.A.; Melo, I. S. Phosphorus-solubilizing *Trichoderma* spp. from Amazon soils improve soybean plant growth. Scientific Reports, v. 10, e2858, 2020. https://doi.org/10.1038/s41598-020-59793-8.

Contreras-Cornejo, H.A.; Schmoll, M.; Esquivel-Ayala, B.A.;

González-Esquivel, C.E.; Rocha-Ramírez, V.; Larsen, J. Mechanisms for plant growth promotion activated by Trichoderma in natural and managed terrestrial ecosystems. Microbiological Research, v. 281, e127621, 2024. https://doi.org/10.1016/j.micres.2024.127621.

Cui, H.; Cheng, Q.; Jing, T.; Chen, Y.; Li, X.; Zhang, M.; Qi, D.; Feng, J.; Vafadar, F.; Wei, Y.; Li, K.; Zhao, Y.; Zhou, D.; Xie, J. *Trichoderma virens* XZ11-1 producing siderophores inhibits the infection of Fusarium oxysporum and promotes plant growth in banana plants. Microbial Cell Factories, v. 24, e22, 2025. https://doi.org/10.1186/s12934-024-02626-4.

Esparza-Reynoso, S.; Ruíz-Herrera, L.F.; Pelagio-Flores, R.; Macías-Rodríguez, L.I.; Martínez-Trujillo, M.; López-Coria, S.S-N.; Herrera-Estrella, A.; López-Bucio, J.; *Trichoderma atroviride* emitted volatiles improve growth of *Arabidopsis* seedlings through modulation of sucrose transport and metabolism. Plant Cell Environmental, v. 44, n. 6, p.1961–1976, 2021. https://doi.org/10.1111/pce.14014.

Gordon, S.A.; Weber, R.P. Colorimetric estimation of indole acetic acid. Plant Physiology, v. 26, n. 1-2, p.192-195, 1951. https://doi.org/10.1016/0003-2697(76)90514-5.

Guzmán-Guzmán, P.; Etesami, H.; Santoyo, G. Trichoderma: a multifunctional agent in plant health and microbiome interactions. BMC Microbiology, v. 25, e434, 2025. https://doi.org/10.1186/s12866-025-04158-2.

Howard, M.M.; Munoz, C.A; Kao-Kniffin, J.; Kesseler, A. Soil microbiomes from fallow fields have species-specific effects on crop growth and pest resistance. Frontiers in Plant Science, v. 11, e1171, 2020. https://doi.org/10.3389/fpls.2020.01171.

Instituto Brasileiro de Geografia e Estatística - IBGE. Levantamento sistemático da produção agrícola: Tabela 2 - Área, produção e rendimento médio - Confronto das safras de 2022 e estimativas para 2023 - Brasil. julho, 2023. https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/9201-levantamento-sistematico-da-producao-agricola.html. 29 Aug. 2023.

Köppen, W. Klassifikation der klimate nach Temperatur, Niederschlag und Jahreslauf. Petermanns Geographische Mitteilungen, v. 64, p.193–248, 1918.

Lima, G.S.; Silva, J.B.; Soares, L.A.A.; Gheyi, H.R.; Nobre, R.G.; Souza, L.P. Physiological indices and phytomass partition in precocious dwarf cashew clones irrigated with saline waters. Comunicata Scientiae, v. 11, e3196, 2020. https://doi.org/10.14295/cs.v11i0.3196.

Ling, N.; Wang, T.; Kuzyakov, Y. Rhizosphere bacteriome structure and functions. Nature Communications, v. 13, e836, 2022. https://doi.org/10.1038/s41467-022-28448-9.

Malavolta, E.; Vitti, G.C.; Oliveira, S.A. Metodologia para análise de elementos em material vegetal. In: Malavolta, E.; Vitti, G.C.; Oliveira, S.A. (Eds.). Avaliação do estado nutricional das plantas: princípios e aplicações. 2.ed. Piracicaba: Associação Brasileira para Pesquisa da Potassa e do Fosfato, 1989. p. 135-189.

Miyazawa, M.; Pavan, M. A.; Muraoka, T.; Carmo, C. A. F. S.; Melo, W. J. Análise química de tecido vegetal. In: Silva, F. C (Ed.). Manual de análises químicas de solos, plantas e fertilizantes. 2. ed. Brasília: Embrapa Informação Tecnológica; Embrapa Solos, 2009. p. 191-234. http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/330496. 22 Jun. 2025.

Moreira, G.G.; Hakamada, R.; Silva, R.M.L.; Lemos, C.C.Z.; Florentino, A.L.; Gonçalves, J.L.M. Seedling morphological characteristics on survival, uniformity, and growth during a full short rotation in Eucalyptus grandis x e. Urophylla plantation. Forests, v. 14, n. 9, e1756, 2023. https://doi.org/10.3390/f14091756.

Moreno-Lora, A.; Sousa-Ortega.; Recena, R.; Perea-Torres, F.; Delgado, A. Microbial inoculants improve nutrients uptake and yield of durum wheat in calcareous soils under drought stress in the Mediterranean region. Archives of Agronomy and Soil Science, v. 69, n. 11, p.2233–2247, 2022. https://doi.org/10.1080/03650340.2022.2146099.

Murphy, J.; Riley, J.P.A. Modified single solution method for determination of phosphate in natural waters. Analytical Chemistry Acta, v. 27, p.31-36, 1962. https://doi.org/10.1016/S0003-2670(00)88444-5.

Oliveira, A.G.; Chagas, A.F.; Santos, G.R.; Miller, L.O.; Chagas, L.F.B. Potencial de solubilização de fosfato e produção de AIA por *Trichoderma* spp. Revista Verde de Agroecologia e Desenvolvimento Sustentável, v. 7, n. 3, p.149-155, 2012. http://dx.doi.org/10.18378/rvads.v7i3.1338.

Payne, S.M. Iron and virulence in the family Enterobacteriaceae. Critical Reviews in Microbiology, v. 16, n. 2, p.81-111, 1988. https://doi.org/10.3109/10408418809104468.

Raju, Y.; Punamalai, G. A review on the overview of *Trichoderma* - a versatile biocontrol agent and plant growth promotor. Frontiers in Environmental Microbiology, v. 11, n. 2, p.19-25, 2025. https://doi.org/10.11648/j.fem.20251102.11.

Sanó, L.; Oliveira, L.L.B.; Leão, M.D.M.; Santos, J.E.A.; Medeiros, S.C.; Schneider, F.; Sousa, A.B.O.; Taniguchi, C.A.K.; Muniz, C.R.; Grangeiro, T.B.; Silva, C.F.B. *Trichoderma longibrachiatum* as a biostimulant of micropropagated banana seedlings under acclimatization. Plant Physiology and Biochemistry, v. 190, p.184-192, 2022. https://doi.org/10.1016/j.plaphy.2022.09.008.

Santos, J.M.R.; Taniguchi, C.A.K.; Silva, C.F.B.; Natale, W.; Artur, A.G. *Trichoderma* in the promotion of growth and nutrition of dwarf cashew rootstock. Revista Ciência Agronômica, v. 52, n. 4, e20207697, 2021. https://doi.org/10.5935/1806-6690.20210053.

Santos, R.M.; Natale, W.; Taniguchi, C.A.K.; Corrêa, M.C.M.; Serrano, L.A.L.; Artur, G.A. Association of controlled-

release and foliar fertilizers in the production of grafted dwarf cashew seedlings. Journal of Plant Nutrition, v. 43, n. 7, p.1048-1056, 2020. https://doi.org/10.1080/01904167.2020.1711934.

Schwyn, B.; Neilands, J.B. Universal chemical assay for the detection and determination of siderophore. Analytical Biochemistry, v. 160, n. 1, p.47-56, 1987. https://doi.org/10.1016/0003-2697(87)90612-9.

Tyskiewicz, R.; Nowak, A.; Ozimek, E.; Jaroszuk-Sciset. *Trichoderma*: the current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. International Journal of Molecular Sciences, v. 23, n. 4, e2329, 2022. https://doi.org/10.3390/ijms23042329.

Vries, F.T.; Griffiths, R.I.; Knight, C.G.; Nicolitch, O.; Williams, A. Harnessing rhizosphere microbiomes for drought-resilient crop production. Science, v. 368, n. 6488, p.270-274, 2020. https://doi.org/10.1126/science.aaz5192.

Woo, S.L.; Hermosa, R.; Lorito, M.; Monte, E. *Trichoderma*: a multipurpose, plant-beneficial microorganism for eco-sustainable agriculture. Nature Reviews Microbiology, v. 21, p.312-326, 2023. https://doi.org/10.1038/s41579-022-00819-5.

Xie, B.; Wei, X.; Wan, C.; Zhao, W.; Song, R.; Xin, S.; Song, K. Exploring the biological pathways of siderophores and their multidisciplinary applications: a comprehensive review. Molecules, v. 29, n. 10, e2318, 2024. https://doi.org/10.3390/molecules29102318.

Yu, C.; Jiang, X.; Xu, H.; Ding, G. *Trichoderma longibrachiatum* inoculation improves drought resistance and growth of *Pinus massoniana* seedlings through regulating physiological responses and soil microbial community. Journal of Fungi, v. 9, n. 7, e624, 2023. https://doi.org/10.3390/jof9070694.