

Application of nitrogen combined with *Trichoderma harzianum* in sugar apple and custard apple seedlings

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ABSTRACT

This study aimed to evaluate the growth-promoting potential of *Trichoderma harzianum* in sugar apple (*Annona squamosa* L.) and custard apple (*Annona reticulata* L.) seedlings. The seedlings were subjected to different nitrogen rates combined with *Trichoderma* application strategies. The design used was a randomized complete block, with a 3 × 5 factorial arrangement of *Trichoderma* application strategy and nitrogen rates, and three replications. The *Trichoderma* application strategy included: S₁ – control (no application); S₂ – IBLF 006 WP applied at planting; and S₃ – IBLF 006 WP applied at planting and again 30 days after seedling emergence. Evaluated variables included relative chlorophyll index (RCI), chlorophyll a (Chl a), chlorophyll b (Chl b), plant height (PH), number of leaves (NL), shoot fresh mass (SFM), and shoot dry mass (SDM). In sugar apple, *Trichoderma* increased shoot dry mass by 29.4% (S₂) and 16.8% (S₃) in the absence of nitrogen of fertilization and promoted greater plant height at low rates (0–25 kg ha⁻¹). In custard apple, S₃ resulted in higher fresh and dry shoot biomass, particularly at low (0–25 kg ha⁻¹ N) and high (75–100 kg ha⁻¹ N) nitrogen rates. The application of strain IBLF 006 (*Trichoderma harzianum*) is not recommended for the production of sugar apple (*Annona squamosa* L.) and custard apple (*Annona reticulata* L.) seedlings, either alone or in combination with different nitrogen rates, as the observed benefits were limited and inconsistent. Therefore, under the experimental conditions, a nitrogen rate of 100 kg ha⁻¹ provided the best overall results and is recommended for nursery seedling production.

Keywords: *Annona squamosa* L.; *Annona reticulata* L.; *Trichoderma* spp.; Bioinputs.

Aplicação de nitrogênio combinado com *Trichoderma harzianum* em mudas de pinhas e condessa

RESUMO

O objetivo deste trabalho foi avaliar o possível potencial promotor de *Trichoderma harzianum* em mudas de *Annona squamosa* L. e *Annona reticulata* L. sob diferentes doses de nitrogênio. O delineamento utilizado foi o de blocos casualizados, em esquema fatorial, 3 x 5 (tratamentos e doses), com três repetições, sendo os tratamentos relacionados ao *Trichoderma* spp. foram S₁ – controle, sem aplicação da cepa, S₂ – IBLF 006 WP (*Trichoderma harzianum*) aplicado no plantio e S₃ – IBLF 006 WP aplicado no plantio e 30 dias após a emergência. As variáveis avaliadas foram índice relativo de clorofila (IRC), clorofila a (CLa) e clorofila b (CLb), altura de planta (ALTP), número de folhas (NFOL), massa fresca da parte aérea (MFPA) e massa seca da parte aérea (MSPA). Na pinha, o *Trichoderma* aumentou a massa seca da parte aérea em 29,4% (S₂) e 16,8% (S₃) na ausência de adubação nitrogenada, além de promover maior altura de plantas em baixas doses de nitrogênio (0–25 kg ha⁻¹). Na condessa, S₃ resultou em maiores valores de massa fresca e massa seca da parte aérea, especialmente em doses baixas (0–25 kg ha⁻¹ N) e altas de nitrogênio (75–100 kg ha⁻¹ N). A aplicação da cepa IBLF 006 (*Trichoderma harzianum*) não é recomendada para a produção de mudas de pinha (*Annona squamosa* L.) e condessa (*Annona reticulata* L.), seja de

forma isolada ou em combinação com diferentes doses de nitrogênio, visto que os benefícios observados foram limitados e inconsistentes. Portanto, nas condições experimentais, a dose de nitrogênio de 100 kg ha⁻¹ proporcionou os melhores resultados e é recomendada para a produção de mudas em viveiro.

Palavras-chave: *Annona squamosa* L.; *Annona reticulata* L.; *Trichoderma* spp.; Biopromotor.

1. Introduction

The Food and Agriculture Organization of the United Nations (FAO) reports that “global trade in tropical fruits has expanded to unprecedented levels in recent years,” with production primarily concentrated in Asian and Latin American countries and largely intended for domestic consumption (FAO, 2020). The diverse health benefits and unique flavors of exotic fruits have contributed to increased demand in major markets. The Centre for the Promotion of Imports from Developing Countries notes that the value of European imports of exotic fruits rose by 40% over the past five years, with the Netherlands serving as the principal importer (CBI, 2024).

Although Brazilian flora contains a vast diversity of edible fruits, only a small proportion of this potential is currently recognized and utilized, despite generating BRL 91 billion in production and a 21% increase from 2023 to 2024 (IBGE, 2024; Teixeira et al., 2019).

The *Annonaceae* family, the largest within the order *Magnoliales*, comprises a wide variety of tropical and subtropical fruit trees and shrubs, many of which are native species that have been cultivated for centuries (Couvreur et al., 2019). Within this family, only the genera *Annona* and *Asimina* include species that produce edible fruits. The four most widely cultivated and commercially significant *Annona* fruits are *Annona muricata* (sour sop), *Annona cherimola* (atemoya), *Annona squamosa* (sugar apple), and *Annona reticulata* (custard apple), in descending order of importance (Brannan et al., 2025; Faria et al., 2025).

Sugar apple is commercially important due to its medicinal and nutritional properties, which include vitamins A, B, C, E, and vitamin K1 (phylloquinone), antioxidants, polyunsaturated fatty acids, essential minerals, and a distinctive aroma and flavor (Liu et al., 2013). In contrast, custard apple remains under-studied, with limited information available on its cultivation. This species is currently considered semi-domesticated and is primarily found in backyard orchards or natural environments in tropical and warm regions (Fuentes et al., 2022). Its average productivity of 5.35 t ha⁻¹ is low, largely due to the cultivation systems employed, but could be enhanced through the adoption of improved technologies such as optimized nutrient management (Lemos, 2014).

Effective fertilization management is essential for crops that are highly dependent on soil quality, such as *Annonaceae* species, which, although generally

resilient, extract substantial quantities of nutrients from the soil. Both nutrient deficiency and excess can adversely affect fruit quality (Sá et al., 2021). For seedlings, appropriate fertilization creates optimal growth conditions and reduces vulnerability to biotic and abiotic stresses. In particular, nitrogen (N) enhanced plant height, stem diameter, leaf production, biomass accumulation, and Dickson Quality Index, with the greatest responses observed at N rates ranging from 100 to 200 mg dm⁻³ in *Annona crassiflora*, while also increasing N concentration and accumulation in plant tissues, thereby promoting seedling establishment and supporting faster and more stable early growth (Costa et al., 2019).

Utilizing microorganisms to enhance the rhizosphere environment is a promising strategy, as these organisms play a critical role in maintaining long-term soil fertility. Beneficial bacteria and fungi can suppress plant pathogens and are widely implemented in agricultural and sustainable production systems (Zydlik et al., 2021). The fungus *Trichoderma* spp. is prevalent in decomposing organic matter, and its root colonization efficiency increases under stress conditions (Poveda, 2020). However, most research on *Trichoderma* spp. has focused on economically significant crops, with limited studies on fruit species and even fewer examining interactions with nitrogen fertilization.

Information on the interaction between *Trichoderma* spp. and nitrogen fertilization in fruit crops remains limited. However, several studies have reported positive effects of these fungi on seedling growth and nutrient acquisition. In *Citrus aurantifolia*, *Trichoderma harzianum* promoted plant growth and physiological performance under reduced fertilization rates (Abdelmoaty et al., 2022). Similarly, positive responses have been reported in papaya (*Carica papaya* L.) and cashew (*Anacardium occidentale* L.) seedlings (Araújo et al., 2019; Santos et al., 2021), including improvements in vigor and improved nutrient utilization.

The objective of this study was to evaluate the effects of different *Trichoderma harzianum* application strategies and nitrogen rates on the growth of *Annona squamosa* L. and *Annona reticulata* L. seedlings.

2. Material and Methods

The experiment was conducted at the State University of Goiás (UEG), Ipameri Campus, in a

greenhouse measuring $30 \times 7 \times 3.5$ meters. The structure was constructed of metal and fully covered with 25% shade cloth. During the experimental period, mean air temperature ranged from approximately 23.5 to 33 °C, relative humidity averaged 72-85%, and incident solar radiation inside the greenhouse was estimated at 4.1-4.9 kWh m⁻² day⁻¹. The regional climate is classified as Aw (tropical savanna) according to the Köppen-Geiger classification system, with wet summers, dry winters, and an average annual temperature of 20 °C (Alvares et al., 2013).

A randomized complete block design was employed in a 3×5 factorial arrangement (*Trichoderma harzianum* application strategy $\times$ doses), with three replications, resulting in a total of 45 experimental units. The application strategies were: S₁, control without strain application; S₂, IBLF 006 WP (*Trichoderma harzianum* – Ecotrich®) applied at planting; and S₃, IBLF 006 WP applied at planting and again 30 days after emergence, at a dose of 0.5 kg p.c. ha⁻¹ diluted in 150 L ha⁻¹ (1×10^{10} CFU g⁻¹). A volume of 4 mL of the suspension was sprayed per pot, approximately $1.33 \times$

10^8 CFU pot⁻¹ per application, using a manual sprayer with a 550 mL capacity. Nitrogen rates were 0, 25, 50, 75, and 100 kg ha⁻¹ (urea). Additionally, 60 kg ha⁻¹ of P₂O₅ (single superphosphate) and 60 kg ha⁻¹ of K₂O (potassium chloride) were applied, following Araújo et al. (2007).

Each experimental unit consisted of an 8 L pot filled with dystrophic Red-Yellow Latosol of clayey texture (Santos et al., 2018), the mean value of the three plants within each experimental unit was used for all evaluations. The soil was sieved, amended with 3.5 g of limestone per kilogram based on soil analysis, and incubated for 40 days.

Soil moisture was monitored throughout the experimental period by the gravimetric method, based on periodic weighing of the pots. Irrigation was performed every two days, and at each irrigation event, water was added to restore the soil to approximately 80% of its water-holding capacity. The volume applied varied according to water loss from the pots, averaging approximately 420 mL pot⁻¹ per irrigation.

Table 1. Principal chemical characteristics of the soil (0–20 cm depth) prior to fertilizer or lime application. Ipameri, GO, Brazil, 2025.

Characteristics	pH	OM	P _(resin)	H+Al	K	Ca	Mg	SB	CEC	V%
	CaCl ₂	g dm ⁻³	mg dm ⁻³	mmol dm ⁻³						
Soil	5.1	24.6	9.1	30.5	4.2	18.5	7.6	30.3	60.8	49.8

pH – active acidity; OM – organic matter; P – available phosphorus; H+Al – potential acidity; K – available potassium; Ca – exchangeable calcium; Mg – exchangeable magnesium; CEC – effective cation exchange capacity; V% – base saturation.

After fertilization with the designated doses, six sugar apple (*Annona squamosa* L.) seeds were sown per pot. After 20 days, thinning was conducted to retain three plants per pot. Evaluations occurred 90 days after sowing. The variables assessed included relative chlorophyll index (RCI), chlorophyll a (Chl a), and chlorophyll b (Chl b), all measured using a CFL1030 chlorophyll meter (SN0359). RCI values were reported in SPAD units, while chlorophyll a and b were expressed in dimensionless units, based on measurements from two fully expanded leaves in the median portion of each plant. Plant height (PH) was calculated as the mean distance from the soil surface to the apical meristem and expressed in centimeters. The number of leaves (NL) was recorded as the total per pot, excluding leaves <1 mm. Shoot fresh mass (SFM) and shoot dry mass (SDM) were determined by placing samples in kraft paper bags, drying them in a forced-air oven at 65 °C for 72 hours, and weighing them in grams for seedlings of both *Annona squamosa* L. and *Annona reticulata* L.

Data were first tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Bartlett’s test. Analysis of variance and polynomial regression analyses were then performed

using SISVAR software (Ferreira, 2011). The linear and quadratic models were tested, and model selection was based on the significance of regression coefficients by the t test ($p \leq 0.05$), coefficient of determination (R^2).

3. Results and Discussion

Significant interactions between *Trichoderma harzianum* application strategy and nitrogen rates were observed for most evaluated variables ($p \leq 0.05$). However, chlorophyll a in sugar apple exhibited significance ($p \leq 0.01$) only for the treatment source of variation, and both chlorophyll a and b in custard apple were also exceptions (Table 2). The coefficients of variation ranged from 6.7% for the relative chlorophyll index to 15.6% for dry shoot mass, and from 3.4% to 6.9% across the *Annona* species, indicating good experimental precision. These results demonstrate divergent responses among treatments and suggest a high probability of identifying the optimal nitrogen dose and treatment for both crops.

The control treatment S₁ (without fungal application) demonstrated superior performance in the relative chlorophyll index (34.4 kg ha^{-1} – $29.40 \text{ g chlorophyll}^{-1}$, expressed as SPAD units) in sugar apple compared to treatment S₂ (single application), which

exhibited improved performance with increasing doses. In contrast, treatment S₃ (two applications) produced a response similar to the control and resulted in a reduction in performance at nitrogen rates exceeding 50 kg N ha⁻¹ (Figure 1). The high hygroscopicity of urea and its rapid hydrolysis in the soil may temporarily alter salt concentration and nutrient dynamics in the rhizosphere, potentially affecting the metabolic activity and colonization efficiency of *Trichoderma harzianum*, consequently, the benefits of inoculation may be diminished under conditions of high nitrogen availability.

In custard apple, the application strategy S₃ was more effective in increasing photosynthetic pigment concentrations at nitrogen rates near 75 kg ha⁻¹, with stabilization observed beyond this threshold (approximately $\pm$ 30 SPAD units), suggesting a growth-promoting effect. Chlorophyll a is the most abundant pigment in plants, present in the reaction centres and light-harvesting complexes of nearly all oxygenic photosynthetic organisms, including higher plants,

algae, and cyanobacteria. In contrast, chlorophyll b is the second most abundant chlorophyll and functions exclusively as an accessory pigment in the light-harvesting system (Bjorn et al., 2009; Chen, 2014).

According to Paraginski et al. (2025), peach rootstocks [*Prunus persica* (L.) Batsch] did not show a positive response to inoculation with *Trichoderma asperellum* when compared with the treatment receiving only controlled-release fertilizer. The authors observed estimated reductions in chlorophyll b (-27.1%) and total chlorophyll (a+b) (-9.64%) contents, whereas chlorophyll a showed only a slight estimated increase (+0.09%). In addition, the inoculation promoted estimated simultaneous reductions in plant height (-1.78%), stem diameter (-2.43%), leaf number (-7.45%), and leaf area (-22.44%). These results indicated that the response to the fungus depends on the nutritional status of the plant and may not provide additional benefits when nutrient supply is already adequate, suggesting a potential impairment in the quality of the produced seedlings.

Table 2. Summary of mean squares for the variables relative chlorophyll index (RCI), plant height (PH), number of leaves (NL), and shoot fresh mass (SFM), shoot dry mass (SDM), as affected by three *Trichoderma* application strategies and five nitrogen rates in *Annona squamosa* L. and *Annona reticulata* L. Ipameri, Goiás State, Brazil, 2025.

Sugar Apple (<i>Annona squamosa</i> L.)	S.V.	DF	RCI	Chl a	Chl b	PH	NL	SFM	SDM
	Strategy	2	0.95 ^{n.s.}	32.30*	1.97 ^{n.s.}	18.74*	10.05 ^{n.s.}	2.91 ^{n.s.}	0.52 ^{n.s.}
	Dose	4	16.09**	1.59 ^{n.s.}	0.44 ^{n.s.}	20.48**	191.36**	47.23**	4.62**
	S x D	8	17.5**	17.63 ^{n.s.}	2.03*	11.21**	149.93**	13.34**	1.80**
	Block	2	2.90**	21.37 ^{n.s.}	1.12 ^{n.s.}	0.52 ^{n.s.}	44.00 ^{n.s.}	14.50*	1.36*
	Error	28	3.24	9.02	0.85	3.01	40.40	3.70	0.42
	CV (%)		6.73	13.44	19.00	7.14	13.65	15.12	15.66
Custard Apple (<i>Annona reticulata</i> L.)	F.V.	DF	RCI	Chl a	Chl b	PH	NL	SFM	SDM
	Strategy	2	4.35 ^{n.s.}	14.08 ^{n.s.}	1.18 ^{n.s.}	72.38**	374.40**	16.19**	1.52**
	Dose	4	34.32**	16.40 ^{n.s.}	1.72 ^{n.s.}	13.18**	116.30**	24.13**	2.17**
	S x D	8	28.38**	13.13 ^{n.s.}	1.06 ^{n.s.}	8.84**	247.21**	4.33**	0.50**
	Block	2	6.05 ^{n.s.}	4.15 ^{n.s.}	0.29 ^{n.s.}	0.20	1.84 ^{n.s.}	0.10	0.01 ^{n.s.}
	Error	28	1.95	7.79	0.74	1.82	11.80	0.17	0.02
	CV (%)		5.48	13.48	19.76	6.03	6.91	3.61	3.36

n.s., * and ** - not significant, significant at 5% and 1% probability levels by the F test, respectively; CV (%) = coefficient of variation.

S.V. = Source of variation; DF = Degrees of freedom; RCI = Relative chlorophyll index; Chl a = Chlorophyll a; Chl b = Chlorophyll b; PH = Plant height; NL = Number of leaves; SFM = Shoot fresh mass; SDM = Shoot dry mass; CV = Coefficient of variation.

The application strategy S₂ resulted in higher chlorophyll a concentration, indicating an enhanced capacity for acquiring this compound, which is frequently used as a reference in structural analyses of other chlorophylls and related photopigments. Elevated chlorophyll b values were also observed, with nitrogen rates exerting a more pronounced influence, suggesting improved nutrient assimilation as nitrogen levels increased (Figure 1). The assessment of photosynthetic pigments is fundamental to plant photosynthesis and growth. Spectrophotometry is currently the most widely used method for measuring these pigments due to its

operational simplicity and non-destructive nature. While this technique has been successfully applied in agriculture, it should be supplemented with additional assessment methods for fruit crops (Wei et al., 2024; Huang et al., 2024).

Inoculation with *Trichoderma asperelloides* isolates significantly influences chlorophyll a, b, and total chlorophyll concentrations, as well as the chlorophyll a:b ratio, in maize (Meneses et al., 2022). Furthermore, increased nitrogen availability promotes *Trichoderma* colonisation in the rhizosphere, thereby improving nutrient uptake and chlorophyll synthesis (Singh et al.,

2019). Niaz et al. (2024) identified a strong positive correlation between SPAD values and combined chlorophyll content (a + b), indicating that the non-destructive SPAD method is preferable to the more time-consuming destructive extraction method. This approach enables continuous monitoring and improved management of orchard plants, with reliable results reported for six fruit tree species—mango (*Mangifera indica* L.), banana (*Musa acuminata* Colla), musambi

(*Citrus limetta* Risso), bitter orange (*Citrus aurantium* L.), lemon (*Citrus limon* (L.) Osbeck), and guava (*Psidium guajava* L.)—as well as four annual crops: maize (*Zea mays* L.), rice (*Oryza sativa* L.), cotton (*Gossypium herbaceum* L.), and sugarcane (*Saccharum* spp.). The SPAD technique facilitates rapid seedling assessment, selection of seedlings with higher commercial potential, and early detection of nutrient deficiencies.

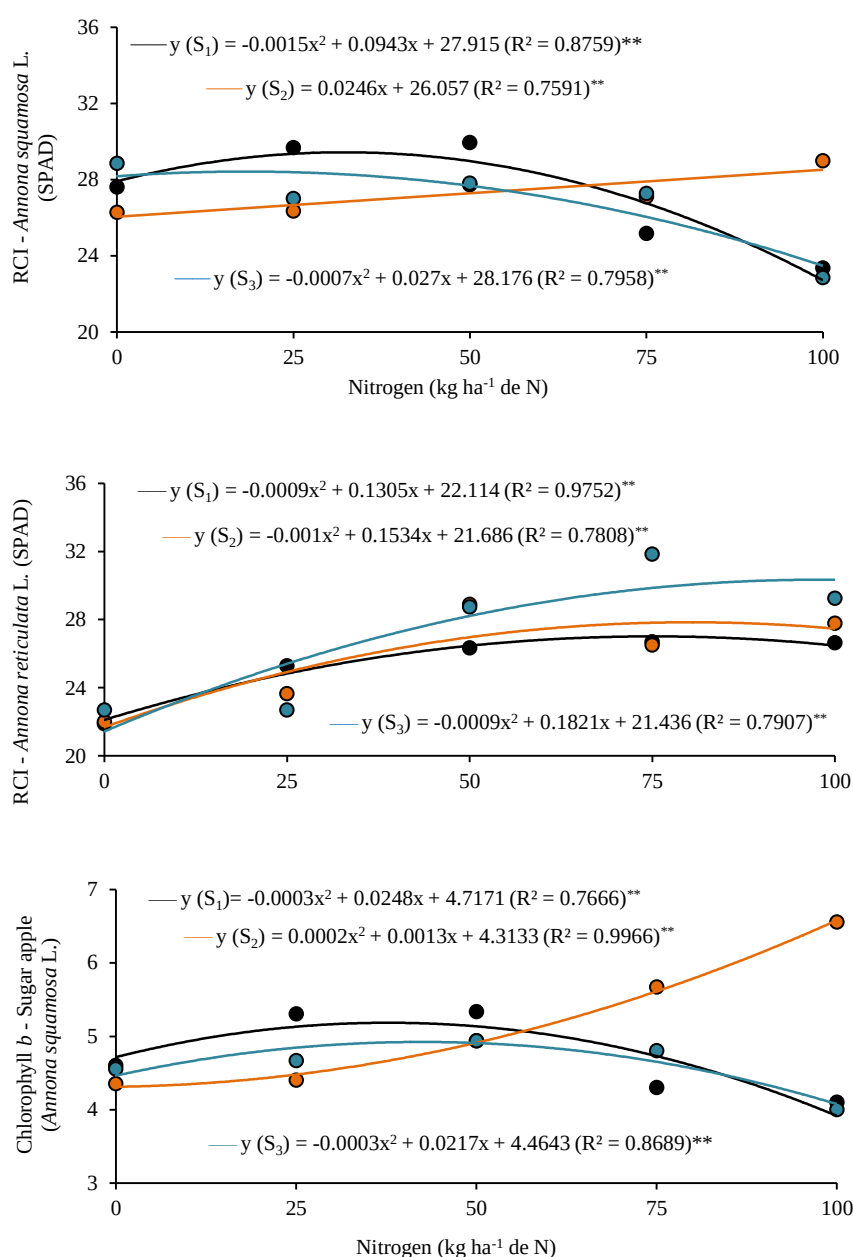


Figure 1. Relative chlorophyll index (RCI), chlorophyll a, and chlorophyll b as a function of different nitrogen rates (0, 25, 50, 75, and 100 kg ha⁻¹ N) and three *Trichoderma harzianum* application strategies [S₁ – control (without application); S₂ – IBLF 006 (application at planting); and S₃ – IBLF 006 (application at planting and 30 days after planting)] in sugar apple (*Annona squamosa* L.) and custard apple (*Annona reticulata* L.).

According to Batista et al. (2003), under nitrogen deficiency in *Annona muricata* (soursop), older leaves mobilize nitrogen to younger leaves and organs, resulting in chlorosis, particularly in older leaves. These symptoms include a gradual loss of green coloration to a

pale green tone, uniformly distributed across the leaf blade, petiole, and veins. Such a nutritional imbalance may subsequently affect plant height and overall performance by reducing photosynthetic rates.

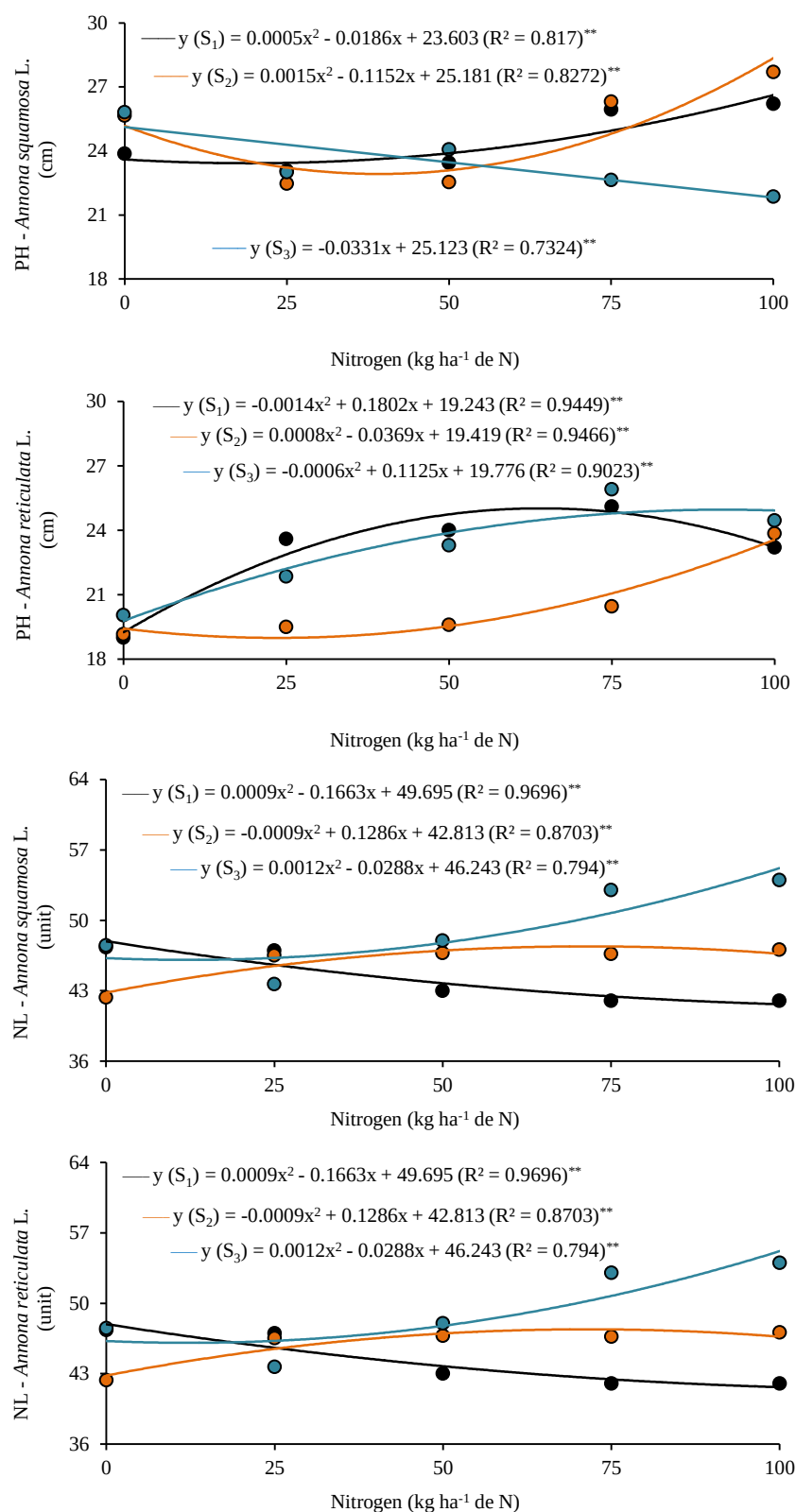


Figure 2. Plant height (PH) and number of leaves (NL) as a function of different nitrogen rates (0, 25, 50, 75, and 100 kg ha⁻¹ N) and three *Trichoderma harzianum* application strategies [S₁ – control (without application); S₂ – IBLF 006 (application at planting); and S₃ – IBLF 006 (application at planting and 30 days after planting)] in sugar apple (*Annona squamosa* L.) and custard apple (*Annona reticulata* L.).

Seedling height is a critical morphophysiological indicator that enables the prediction of establishment success in fruit crops. Taller seedlings are typically associated with more vigorous root systems, greater carbohydrate reserves, enhanced post-transplant

adaptation, improved water-stress tolerance, and increased nutrient-uptake efficiency. In this study, *Trichoderma harzianum* application did not yield additional benefits with increasing nitrogen rates in sugar apple, and a similar trend was observed in custard

apple (Figure 2). However, under low nitrogen rates (0–25 kg ha⁻¹ N), sugar apple seedlings treated with the fungus exhibited higher heights. For instance, at 12 kg ha⁻¹ N, plant height increased by 2.1% and 5.3% compared to the control. The optimal nitrogen dose for sugar apple seedlings was 100 kg ha⁻¹ N, while for custard apple it was approximately 64 kg ha⁻¹ N, resulting in an average plant height of 25 cm (Figure 2). Limited information on Annonaceae cultivation presents a significant challenge, as optimal seedling management practices remain largely undefined. The typically slow initial growth of these species underscores the need to develop and refine cultivation strategies that accelerate growth and increase the probability of reaching maturity. This issue is especially pertinent for conservation, since approximately 40 species in the family are classified as vulnerable or endangered (Brasil, 2022). Due to the similarities among species, the present findings may inform management recommendations for related taxa.

Anozie et al. (2023) reported that while the effects of various organic compounds on *Annona muricata* seedling development differ, organic matter generally enhances seedling growth and development. The study found that soil amended with biochar beneath the surface layer resulted in the highest plant height (26.7 cm), stem diameter (0.49 cm), and leaf number (4.4), compared to amendments with poultry manure, sawdust, or goat manure. These findings suggest that combining organic fertilizers may improve efficiency and produce synergistic effects in cultivation practices.

Leaves fulfill multiple essential functions in plant physiology, serving as primary sites of photosynthesis, reservoirs of mobile nutrients, and environmental sensors that regulate key processes. In this study, fungal inoculation strategy increased leaf number, particularly under S₃ (100 kg ha⁻¹ N) in both species. However, leaf size varied considerably, and a greater number of smaller leaves does not necessarily indicate superior physiological status compared to fewer, larger, and more vigorous leaves (Figure 2). In fruit crops, leaves are especially important during early development, as they are the main sites of photosynthesis and physiological regulation. Photosynthesis is critical for carbohydrate production, which supports vegetative growth and subsequent reproductive development (Zhu et al., 2011).

In sugar apple (*Annona squamosa* L.), the control application strategy (S₁) showed increased efficiency as nitrogen rates increased, with a potential response extending beyond 100 kg ha⁻¹ N, especially for shoot dry mass (Figure 3). In contrast, without nitrogen fertilization, the strategies S₂ and S₃ resulted in increases of 29.4% and 16.8%, respectively. These findings suggest that *Trichoderma* inoculation can

provide benefits even in the absence of mineral fertilization. Urea, a widely used nitrogen fertilizer in agriculture, possesses a high hygroscopic capacity, enabling it to absorb environmental moisture. This property can increase rhizosphere salinity and potentially impair mycelial growth. The impact of urea on root development and its interactions with *Trichoderma* species is complex and influenced by factors such as salt concentration near the root zone, soil conditions, and the presence of other beneficial microorganisms.

In crops such as cacao (*Theobroma cacao*), *Trichoderma* inoculation increased radicle and hypocotyl length and reduced fungal incidence on seeds. In seedlings, plant height and root dry mass also increased, while stem diameter and leaf area remained unaffected (Sousa et al., 2021). These results demonstrate the effectiveness of *Trichoderma* in controlling naturally occurring seedborne fungi.

For custard apple (*Annona reticulata* L.), strategy S₃, with two applications, showed greater stability across increasing nitrogen rates, with more pronounced benefits at both low (0–25 kg ha⁻¹ N) and high (75–100 kg ha⁻¹ N) nitrogen rates for fresh and dry biomass, whereas responses in the other variables were less consistent and, in some cases, showed no benefit at all. Thus, the application of *Trichoderma harzianum* strain IBLF 006 is not recommended for seedling production of *Annona squamosa* L. and *Annona reticulata* L., as the observed improvements were limited and did not consistently surpass the responses obtained with nitrogen fertilization alone under the experimental conditions.

Current agricultural practices are unsustainable over the long term, as excessive use of pesticides and fertilizers has intensified soil desertification and secondary salinization. Salinity stress remains a major threat to global food security (Morton et al., 2019). Liu et al. (2023) evaluated 64 *Trichoderma* isolates for salt tolerance and found that only 19 maintained growth and sporulation capacity under saline conditions, while most isolates were severely affected. Environmental factors, including soil temperature, pH, and salinity, are among the most critical variables influencing the growth and biocontrol efficacy of *Trichoderma* spp. Due to the dynamic nature of microbial interactions, ongoing research is essential to enhance understanding of *Trichoderma* responses to environmental variables and fungicidal compounds, to optimize their application in diverse agricultural systems (Cavalcante et al., 2025).

High nitrogen application rates can substantially interfere with the growth-promoting effects of *Trichoderma* spp., as both nitrogen availability and source, whether organic or inorganic, in the cultivation medium can alter spore production, mycelial growth,

and the efficiency of secreted enzymes. These alterations directly impact the saprophytic and biocontrol capacities of the fungus (Rodríguez-Gómez

and Hobley, 2013), potentially limiting fungal population development under elevated nitrogen inputs.

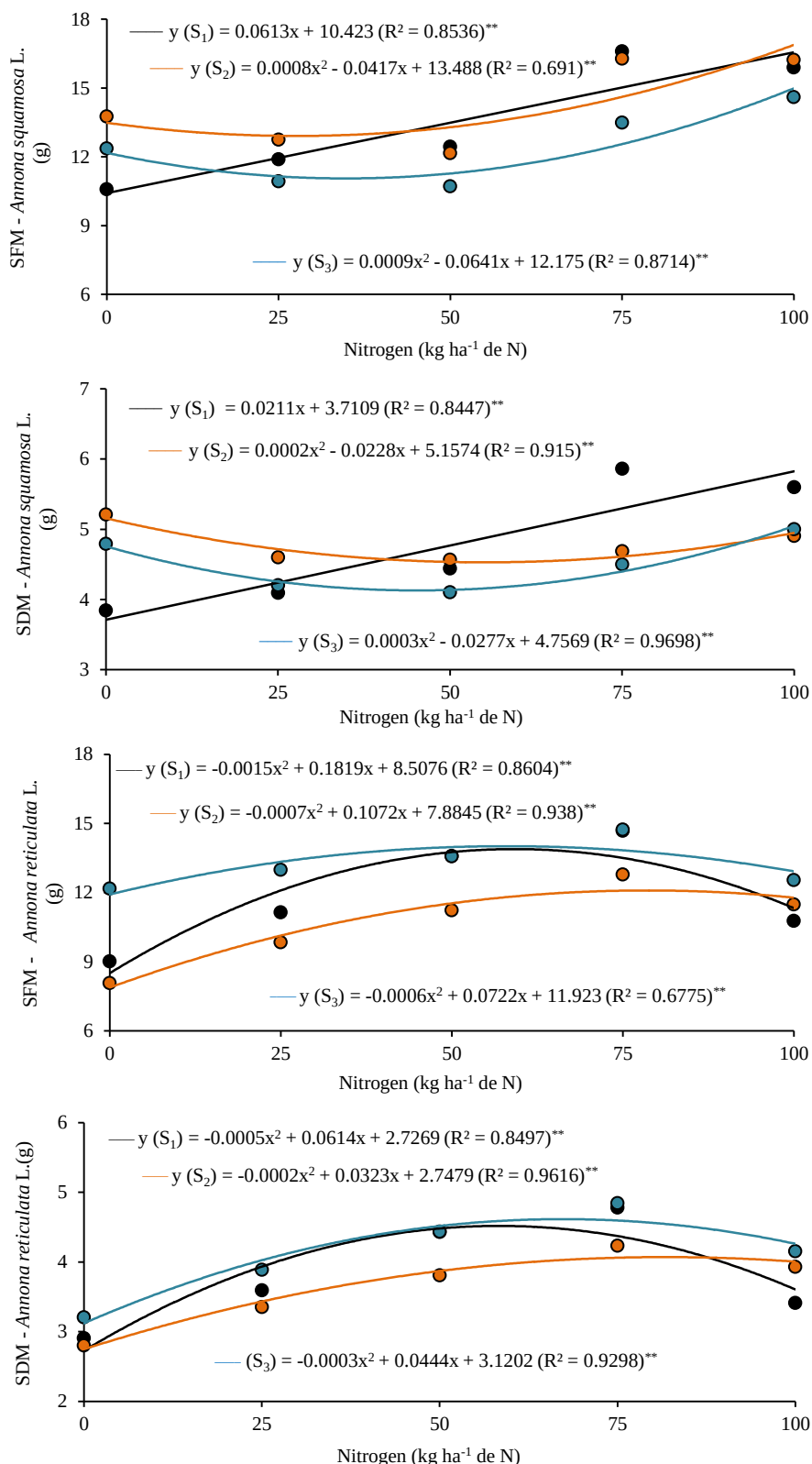


Figure 3. Shoot fresh mass (SFM) and shoot dry mass (SDM) as a function of different nitrogen rates (0, 25, 50, 75, and 100 kg ha⁻¹ N) and three *Trichoderma harzianum* application strategies [S₁ – control (without application); S₂ – IBLF 006 (application at planting); and S₃ – IBLF 006 (application at planting and 30 days after planting)] in sugar apple (*Annona squamosa* L.) and custard apple (*Annona reticulata* L.).

Trichoderma utilizes multiple mechanisms to interact with the environment and other microorganisms, including competition for nutrients,

antibiosis, mycoparasitism, regulation of plant growth, and induction of systemic resistance (Vindas-Reyes et al., 2024). Additionally, *Trichoderma* facilitates plant–

environment and plant–plant interactions through phytohormone production, thereby enhancing nutrient uptake by promoting efficient internal resource management and adaptively regulating the influence of abiotic factors. Collectively, these processes confer a growth-regulatory role to *Trichoderma* within agroecosystems (Hermosa et al., 2013).

4. Conclusions

The application of strain IBLF 006 (*Trichoderma harzianum*) is not recommended for the production of sugar apple (*Annona squamosa* L.) and custard apple (*Annona reticulata* L.) seedlings, either alone or in combination with different nitrogen rates, as the observed benefits were limited and inconsistent. Therefore, under the experimental conditions, a nitrogen rate of 100 kg ha⁻¹ provided the best overall results and is recommended for nursery seedling production.

Authors' Contribution

Conceptualization and methodology: Luciana Maria da Silva, Rikelme Matheus dos Santos Relvas e Fabricio Rodrigues; data collection and curation: Natália Veleda Duailibe e Rikelme Matheus dos Santos Relvas; formal analysis: Natália Veleda Duailibe e Fabricio Rodrigues; data interpretation: Natália Veleda Duailibe, Rikelme Matheus dos Santos Relvas e Fabricio Rodrigues; project administration: Fabricio Rodrigues; supervision: Rithielly Machado Rodrigues de Araújo e Fabricio Rodrigues; preparation of the original draft: Fabricio Rodrigues; writing - revision and editing: Luciana Maria da Silva e Fabricio Rodrigues. All authors read and approved the final version of the manuscript.

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