International Journal of
Plant Biology

MDPI

Article

Rice-Based *Trichoderma* Inocula Enhance Fruit Production of Cutleaf Groundcherry (*Physalis angulata* L.) in the Amazon

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Abstract

In Amazonian Spodosols, soil acidity and nutrient constraints limit the cultivation of cutleaf groundcherry. One sustainable alternative for cultivating this species is the use of *Trichoderma* inoculants, but studies on their use in this crop are scarce. This study evaluated whether rice-based inocula of *Trichoderma harzianum* (TH) CCB-LA101 and *T. viride* (TV) CCB-LA103 improved growth and fruit production of cutleaf groundcherry and whether the responses varied with the application dose. Plants were grown in a randomized complete block design with a 2 × 3 factorial arrangement: two *Trichoderma* species and three inoculum doses applied to the soil (0, 4, and 8 g plant^{-1}; *Trichoderma* concentration of 3.2×10^9 CFU g^{-1}). Species and dose affected fruit width, length, number, and yield, with TH more effective than TV at increasing fruit number and yield. The contrast between inoculated and non-inoculated plants was significant for all traits. The 8 g plant^{-1} dose significantly increased fruit number and yield by 22.8% and 17.7%, respectively, relative to the non-inoculated control, whereas fruit width and fruit length decreased by 8.3% and 7.3%, respectively. Considering the six treatment combinations, the 8 g plant^{-1} TH inoculum (TH8) was the only treatment to significantly increase fruit yield, with net returns for smallholders estimated at USD 1104.46 ha^{-1}. Therefore, under the tested conditions, TH8 could be used to grow cutleaf groundcherry in Amazonian Spodosols.

Keywords: *Trichoderma harzianum*; *Trichoderma viride*; plant growth-promoting fungi; fruit yield; bolsa mullaca; camapu; Spodosol

Academic Editor: Cheng Chang

Received: 5 June 2026
Revised: 13 August 2026
Accepted: 15 August 2026
Published: 18 August 2026

1. Introduction

In the Amazon Basin, crop production on acidic and nutrient-poor soils [1] requires management practices that sustain yields while reducing the dependence on external inputs. Spodosols cover 1.9% of the Amazon's land area [2]. Severe acidity and elevated exchangeable aluminum are their dominant fertility constraints, yet cassava and horticultural crops are regularly grown on them. Thus, improving yields under these conditions without mineral fertilization is a practical rather than a purely theoretical challenge, and microbial inoculants offer one pathway toward this goal [3].

Trichoderma has become one of the most extensively studied beneficial fungi in agriculture, first attracting attention as a biocontrol agent and later as a plant growth promoter. Early research focused on its antagonism against soil-borne pathogens [4], while its capacity as a plant growth-promoting fungus (PGPF) has been recognized [5,6]. *Trichoderma* promotes plant growth through several mechanisms, including nutrient solubilization, phytohormone synthesis, root architecture modification, and induction of systemic responses that broaden tolerance to both biotic and abiotic stresses [7,8]. These effects depend on the fungal species or isolate, host genotype, application method, and environmental conditions of the study. These dependencies justify the field testing of specific host–inoculant combinations [9]. The most commonly used species are *T. harzianum* (TH) and *T. viride* (TV), both of which have been used to increase the yield of tomato and *Capsicum*.

Cutleaf groundcherry, *Physalis angulata* L. ($2n = 4x = 48$), a member of the Solanaceae family, is native to tropical America and has become a widespread weed in tropical, subtropical, and warm-temperate regions [10]. In the Peruvian Amazon, it is called bolsa mullaca, bolsa referring to the papery, balloon-like calyx that envelops each berry and makes the plant immediately recognizable at markets. The plant is a fast-growing annual herb with glabrous, angled stems that typically reach 0.5–1.5 m [10]. Flowers are campanulate, and the calyx inflates around the maturing berry, forming the bag structure for which the plant is named. Berries measure 1–1.5 cm in diameter and weigh up to 2.44 g; the flavor is pleasant, and a natural shelf life of 4–8 weeks makes them suited for fresh-market sale without cold-chain requirements [11]. In Mexico, this species is commercially cultivated using organic fertilizers and pesticides, with fruit yields ranging from 8 to 13 t ha^{-1} and prices ranging from USD 0.30 to 1.00 per kg [11].

This species is an emerging crop with considerable medicinal potential. Traditionally, different parts of the plant are employed in tropical countries to treat 26 diseases, including malaria, asthma, hepatitis, dermatitis, rheumatism, diabetes, tumors, gonorrhea, and liver disorders [12]. Studies have reported anti-inflammatory and anticancer properties of its fruits [13], antileishmanial properties of its roots [14], and antimalarial properties of the whole plant [15].

Although this species is regarded as a weed in other parts of the world, in the Peruvian Amazon it is a crop of high economic value (USD 1.4 kg^{-1}). In this context, the cultivation of cutleaf groundcherry as a fruit crop in Amazonian Spodosols using *Trichoderma* inoculants has not been studied extensively. Increasing its productivity at a low cost without chemical fertilization could support crop diversification and soil conservation and provide additional income sources for smallholders.

One method of inoculating *Trichoderma* in the soil is using carriers such as rice, with the objective of allowing this fungus to survive in adverse open-field conditions. Therefore, this study aimed to compare the effects of rice-based *T. harzianum* and *T. viride* inocula on the agronomic traits and fruit yield of cutleaf groundcherry growing in an open field and to identify the dose that increases fruit yield in Amazonian Spodosol. This study revealed that 8 g plant^{-1} TH inoculum increases fruit yield and net return for smallholders.

2. Materials and Methods

2.1. Site and Soil Analysis

The experiment was conducted at the National University of the Peruvian Amazon (UNAP) in Iquitos, Loreto, Peru (03°46′13.2″ S, 73°22′10.4″ W). The soil at the experimental site was classified as Spodosol. Before the experiment, a composite soil sample was collected from the top 20 cm layer and analyzed at the Laboratorio de Análisis de Suelos,

https://doi.org/10.3390/ijpb17080076

Plantas, Aguas y Fertilizantes, Universidad Nacional Agraria La Molina (UNALM, Lima, Peru). Soil texture was determined using the hydrometer method. Soil pH was measured in a 1:1 (*w*/*v*) soil-to-water suspension, and electrical conductivity (EC) was measured in a 1:1 (*w*/*v*) extract. Organic matter (OM) was determined using the Walkley–Black method [16]. Available phosphorus (P) was extracted using the modified Olsen method [17]. Exchangeable cations Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+} were extracted using 1 M ammonium acetate at pH 7.0 [18], and exchangeable acidity (Al^{3+} + H^{+}) was determined by extraction with 1 N KCl [19]. The soil physicochemical properties are presented in Table 1.

Table 1. Physical and chemical properties of the Spodosol used for cultivation of cutleaf groundcherry. Iquitos, Peru, 2024.

c	Unit	Value
pH (H_2O)	-	5.40
EC	dS m^{-1}	0.98
P	mg kg^{-1}	65.70
Ca^{2+}	$cmol_c$ kg^{-1}	5.19
Mg^{2+}	$cmol_c$ kg^{-1}	0.84
K^{+}	$cmol_c$ kg^{-1}	0.25
Na^{+}	$cmol_c$ kg^{-1}	0.12
Al^{3+} + H^{+}	$cmol_c$ kg^{-1}	0.05
SB	$cmol_c$ kg^{-1}	6.45
CEC	$cmol_c$ kg^{-1}	8.80
OM	g kg^{-1}	28.7
BS	%	73
Sand	%	72
Silt	%	15
Clay	%	13

Legend: EC = electrical conductivity; OM = organic matter; SB = sum of bases; CEC = cation exchange capacity; BS = base saturation.

The values of the climatic variables during the experiment were obtained from the Puerto Almendras/SENAMHI meteorological station (Figure 1). The *Trichoderma* spp. inoculum was prepared in the Microbiology Laboratory of the Institute of Traditional Medicine (IMET-ESSALUD, Iquitos, Peru).

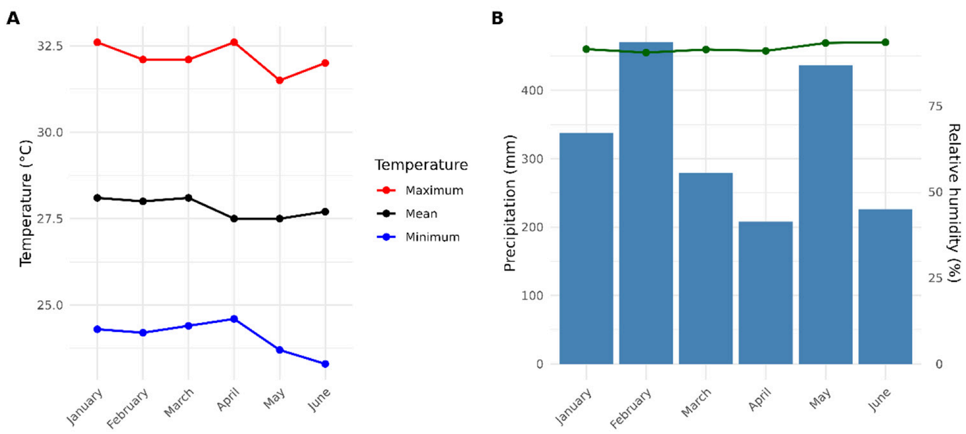

Figure 1. Meteorological conditions during the cutleaf groundcherry experiment. (**A**) Temperature. (**B**) Precipitation and Relative Humidity. Iquitos, Peru, 2024 (Source: SENAMHI).

2.2. Fungal Inoculum and Plant Material

Inocula of *T. harzianum* Rifai CCB-LA101 (5.6×10^9 CFU g^{-1}) and *T. viride* Pers. CCB-LA103 (4.5×10^9 CFU g^{-1}) were provided by the Servicio Nacional de Sanidad Agraria del Perú (SENASA, Lima, Peru). The inocula were multiplied on an autoclaved white rice solid substrate.

Because no breeding program exists for cutleaf groundcherry in Peruvian Amazon, seeds were obtained from the Laboratory of Cultivation and Conservation of Medicinal Plants, Faculty of Agronomy, UNAP. These seeds were collected from a native plant growing in floodplain forests near Iquitos, identified at the Herbarium Amazonense (UNAP), and deposited with the accession number 43165.

2.3. Inoculum Multiplication

A solid-state fermentation method [20] was used, with modifications. Eight polypropylene heat-sealable bags, each containing 500 g of white rice, were autoclaved at 120 °C and 101 kPa for 20 min. Then, under aseptic conditions in a laminar flow chamber, 50 mL of sterile water and 20 g of *Trichoderma* inoculum were added to each bag. The bags were incubated in a laminar flow chamber under a 12:12 h light:dark photoperiod (200 lx) for 8 d, during which abundant sporulation was observed. The fungal cultures were then transferred to 1 L autoclaved hermetic glass jars wrapped in aluminum foil to exclude light. The jars were stored in an incubator at 16 °C for three months, after which the final *Trichoderma* concentration was 3.2×10^9 CFU g^{-1}.

2.4. Experimental Design and Treatments

The experiment was arranged in a randomized complete block design (RCBD) with a 2×3 factorial arrangement of treatments replicated in three blocks. The factors were (1) *Trichoderma* species (*T. harzianum* and *T. viride*) and (2) rice-based inoculum dose (0, 4, and 8 g of total inoculum per plant). The total inoculum dose was divided into two applications: 50% in the seedling phase and 50% in the field. The 0 g dose was used as the control (without *Trichoderma* or the rice substrate). Each experimental plot measured 1×5 m and contained 14 plants at a spacing of 0.70×0.60 m.

2.5. Seedling Production and Inoculation

The seeds were sown in trays filled with topsoil (Figure 2A). Twenty days after sowing, the seedlings were transplanted into 200 mL cups, with half of the assigned inoculum dose (0, 2, or 4 g per cup) placed at the base of each cup (Figure 2B). Cup-grown seedlings were maintained for an additional 30 days before transplantation into field beds on 20 February 2024. The remaining half-dose was incorporated 20 days after the field transplant (Figure 2D), bringing the cumulative totals to 0, 4, and 8 g per plant. No mineral fertilizer or synthetic pesticide was applied in the trial; weed control relied on hand removal throughout.

2.6. Data Collection and Statistical Analysis

Harvesting began on 15 May 2024, and continued for six subsequent harvests at eight-day intervals. At each harvest, plant height, fruit length, and fruit width were measured. At the end of the harvest period, the total number of fruits per plant, weight of 100 fruits, and total fruit yield (t ha^{-1}) were determined. The data were analyzed by two-way analysis of variance (ANOVA). Before ANOVA, data were checked for normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test). When significant effects were found ($p \leq 0.05$), the means were separated using the Scott–Knott test. Statistical analyses were

https://doi.org/10.3390/ijpb17080076

performed using R version 4.5.1 [21]. Treatment means were grouped using the ScottKnott package (v. 4.5.1) [22]. Figures were produced with ggplot2 [23].

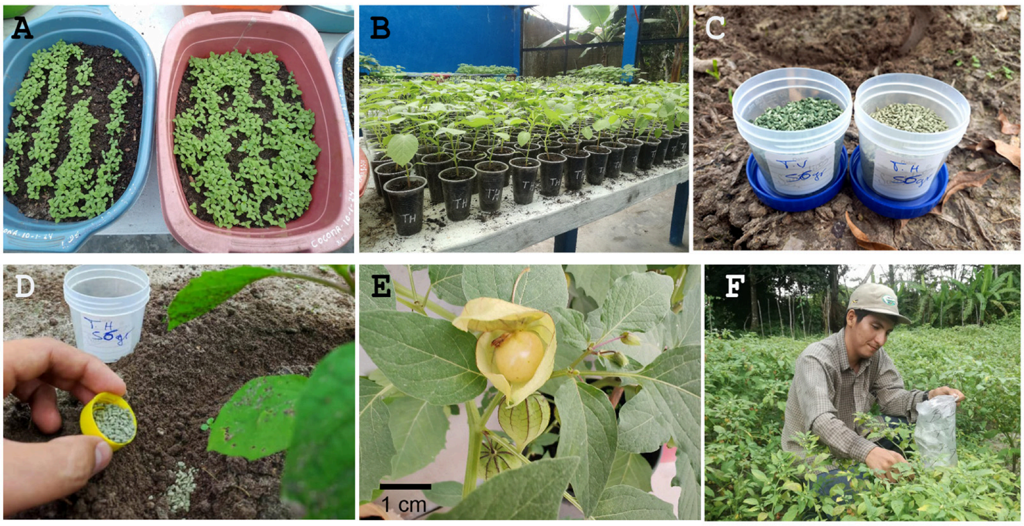

Figure 2. Key steps in conducting the experiment with cutleaf groundcherry inoculated with *Trichoderma* spp. (**A**) Seedlings 10 days after sowing. (**B**) Seedlings 50 days after sowing, ready for field transplantation. (**C**) *Trichoderma viride* Pers. CCB-LA103 (left) and *T. harzianum* Rifai CCB-LA101 (right) inoculum; (**D**) application of *Trichoderma* 20 days after field transplantation; (**E**) mature fruit at the harvest stage; and (**F**) manual harvesting of fruits.

3. Results

3.1. Analysis of Variance

Two-way ANOVA revealed no significant *Trichoderma* species × inoculum dose interaction ($p > 0.05$) for any of the six agronomic traits evaluated (Table 2). Therefore, the main effects of species and inoculum dose were analyzed independently. Species significantly affected fruit width, length, number per plant, and yield, whereas inoculum dose affected all traits except weight of 100 fruits.

Table 2. Two-way analysis of variance for agronomic and yield characteristics of cutleaf groundcherry treated with *Trichoderma harzianum* and *T. viride* inoculum (3.2×10^9 CFU g^{-1}) at three application doses (0.0, 4.0, and 8.0 g plant^{-1}).

		Mean Squared					
Source of Variation	**DF**	**Plant Height (cm)**	**Weight of 100 Fruits (kg)**	**Fruit Width (cm)**	**Fruit Length (cm)**	**Number of Fruits Per Plant**	**Fruit Yield (t ha^{-1})**
Block	2	4.89 ns	0.0001 ns	0.0019 ns	0.0001 ns	2141.2 **	1.66 **
Treatments	5	84.65 ns	0.0004 ns	0.0113 *	0.0074 *	2753.0 **	1.60 **
Species–S	1	41.25 ns	0.0002 ns	0.0259 **	0.0159 *	2713.4 **	1.77 **
Dose–D	2	187.43 *	0.0006 ns	0.0137 *	0.0105 *	5071.4 **	2.65 **
C vs. I	1	368.96 **	0.0013 *	0.0188 *	0.0123 *	8612.9 **	4.03 **
S × D	2	3.56 ns	0.0002 ns	0.0017 ns	0.0000 ns	454.28 ns	0.47 ns
Error	10	29.78	0.0002	0.0023	0.0021	231.32	0.15
Total	17						
CV (%)		8.10	11.97	4.42	4.17	5.36	4.79
Mean		67.38	0.1224	1.10	1.09	283.96	8.19

Legend: *, **, and ns: significant at $p < 0.05$ and $p < 0.01$, and not significant, respectively. C = control (TH0 and TV0), I = inoculated (TH4, TH8, TV4 and TV8).

3.2. Effect of *Trichoderma* Species

The TH produced higher values than the TV for the main yield components (Figure 3). Compared with TV, TH increased fruit width and length by 7.2% and 5.6%, respectively, the number of fruits per plant by 9.0%, and fruit yield by 8.0%. In contrast, *Trichoderma* species did not affect plant height or weight of 100 fruits (Table 2).

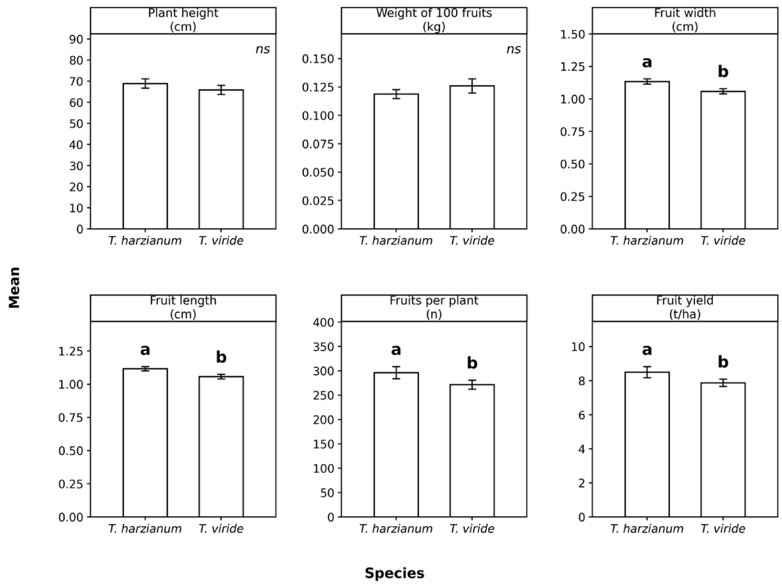

Figure 3. Effect of rice-based inoculum of *Trichoderma harzianum* and *T. viride* (3.2×10^9 CFU g^{-1}) considering the mean of three doses (0.0, 4.0, and 8.0 g $plant^{-1}$) on cutleaf groundcherry. Bars with different letters indicate statistically significant differences among treatments according to Scott–Knott test, $\alpha = 0.05$). Error bars represent the standard error of the mean. The absence of letters indicates no significant differences according to the F test.

3.3. Effect of Inoculum Dose and the Source–Sink Trade-Off

The contrast between the control group (TH0 and TV0) and the inoculated treatments (TH4, TH8, TV4, and TV8) was significant for all six traits evaluated (Table 2), indicating that *Trichoderma* inoculation affects the growth and fruit production of cutleaf groundcherry.

However, when the three doses were considered, a trade-off was observed between the number of fruits and individual fruit size (Figure 4). Compared with the non-inoculated control, the 4 and 8 g $plant^{-1}$ doses significantly increased plant height (by 14.6% and 16.9%), the number of fruits per plant (by 13.9% and 22.8%), and fruit yield (by 9.0% and 17.7%), respectively. Conversely, the 8 g $plant^{-1}$ dose reduced fruit length and width by 7.3% and 8.3%, respectively, whereas the 4 g $plant^{-1}$ dose did not significantly reduce either trait. A plausible interpretation is that *Trichoderma* inoculation at 8 g $plant^{-1}$ increased fruit set, generating more sinks than the assimilate supply of the canopy could sustain at a uniform fruit size, hence the smaller fruits at the highest dose.

3.4. Effects of Six *Trichoderma* Treatment Combinations

ANOVA detected no significant treatment effects on plant height or weight of 100 fruits, but significant effects on fruit width, fruit length, the number of fruits per plant, and fruit yield (Table 2). For the number of fruits per plant and fruit yield, TH inoculum at 8 g $plant^{-1}$ (TH8) showed significantly higher values than all the remaining treatments (Figure 5). This indicates that TH8 was the most effective treatment for increasing fruit yield.

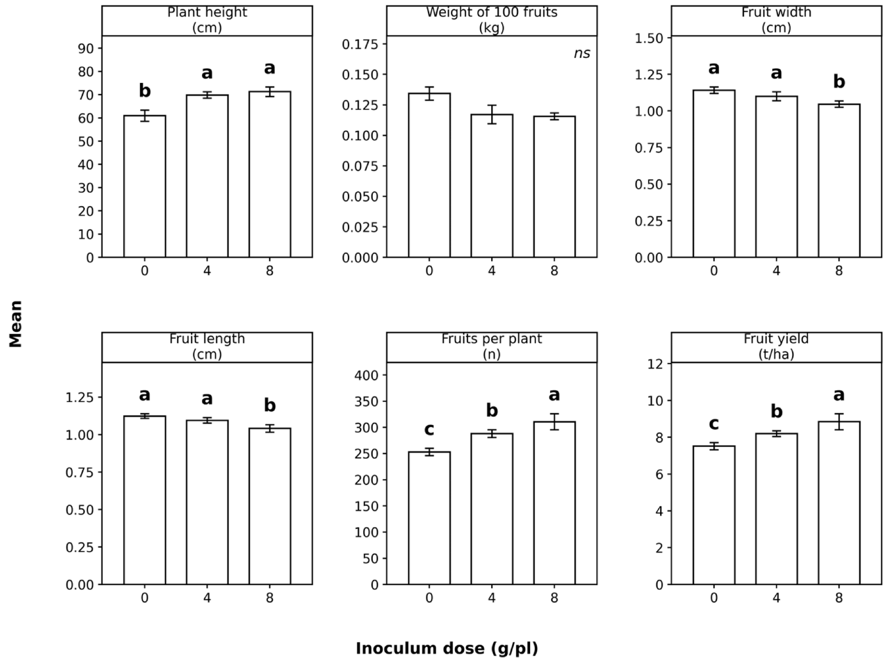

Figure 4. Effect of rice-based *Trichoderma* inoculum dose on plant growth and yield components of cutleaf groundcherry. Bars represent treatment means (n = 3), and error bars represent the standard error of the mean. Different lowercase letters indicate significant differences among inoculum doses according to Scott–Knott test ($p \leq 0.05$). The absence of letters indicates no significant differences according to the F test.

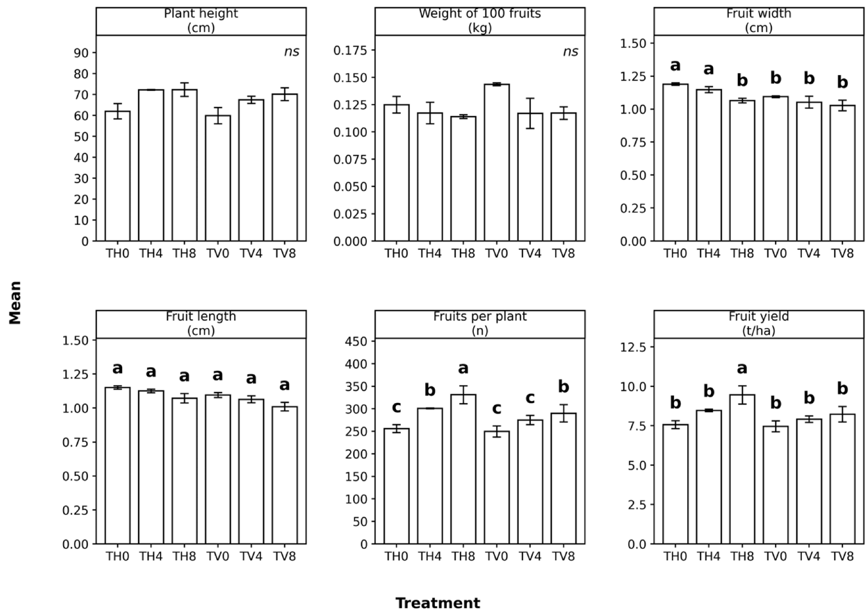

Figure 5. Effects of six *Trichoderma* treatment combinations on plant growth, fruit traits, and yield of cutleaf groundcherry. The treatments consisted of *T. harzianum* at 0, 4, and 8 g plant^{-1} (TH0, TH4, and TH8, respectively) and *T. viride* at 0, 4, and 8 g plant^{-1} (TV0, TV4, and TV8, respectively). Bars represent the mean ± standard error (n = 3). Different letters above the bars indicate significant differences among treatments according to Scott–Knott test at $p < 0.05$. The absence of letters indicates no significant differences according to the F test.

4. Discussion

4.1. Species-Specific Efficacy: T. harzianum as a Superior Growth Promoter

TH CCB-LA101 outperformed TV CCB-LA103 in both the fruit number and total yield of cutleaf groundcherry. This result supports the view that *Trichoderma* effects are species- and isolate-dependent, even within the same host.

The better performance of TH in this study contrasts with reports in tomato, in which TV improved plant growth, yield, and suppression of *Fusarium oxysporum* f. sp. lycopersici more effectively than TH [24]. Nevertheless, TH isolates have demonstrated strong antimicrobial and biocontrol activities in solanaceous crops [25]. These divergent responses across host–inoculant combinations are likely attributable to isolate-specific biochemical profiles, including variations in secondary metabolites, hormone-like signals, and cell wall-degrading enzymes, together with their interactions with the physiological and metabolic status of each host plant [26].

One possible explanation for the superior performance of TH is its efficient root colonization. In tomatoes, Chacón et al. [27] showed that *T. harzianum* CECT 2413 colonizes the root epidermis and cortex, grows mainly through intercellular spaces, and does not invade the vascular tissues. During early colonization, 5–6% of fungal genes were upregulated, including those associated with redox metabolism and nutrient transport. The fungus also exhibited a yeast-like morphology approximately 72 h after colonization [27]. Although the isolate and host plant differed from those in the present study, these observations are consistent with the hypothesis that root colonization efficiency contributes to the agronomic response observed for TH.

Evidence from *T. virens* has shown that apoplastic effector proteins can suppress plant oxidative responses and facilitate inter- and intracellular penetration in maize roots [28]. This indicates that distinct molecular mechanisms underlie the colonization strategies of different *Trichoderma* species, ultimately resulting in divergent effects on host plants.

4.2. Dose Response: Fruit Number–Fruit Size Trade-Off

The final microbial density of the rice-based inoculum (3.2×10^9 CFU g^{-1}) was above the minimum level of 2×10^6 CFU g^{-1} reported for *Trichoderma* formulations and substrates [29]. The main dose-related response was a trade-off between the number and size of fruits. Although the 4 and 8 g $plant^{-1}$ doses increased total yield, the yield gain was mainly associated with a higher number of fruits rather than with larger fruits.

The reduction in fruit mass and dimensions observed at the highest inoculum dose may be associated with changes in source–sink dynamics, nutrient availability, and plant hormone signaling [30]. During fruiting, developing fruits act as strong sinks and compete for photoassimilates produced by photosynthetically active tissues. In addition, *Trichoderma* produces auxin-like compounds and other growth-regulatory metabolites [26,31]. In this context, we hypothesized that a higher inoculum load may have promoted greater fruit initiation, thereby generating more sinks than the canopy could supply at the same rate.

If photoassimilate production and nutrient availability remain relatively constant while the number of sinks increases, each fruit would receive proportionally fewer resources [32]. This hypothesis is consistent with the observed reduction in weight of 100 fruits and fruit dimensions at 8 g $plant^{-1}$. However, this hypothesis could not be tested because we did not evaluate leaf area, photosynthetic rate, nutrient uptake, plant hormone concentrations, and assimilate partitioning.

Stronger competition for carbon and nutrients when fruit load exceeds assimilate supply has also been documented in other crops [33]. However, for cutleaf groundcherry, fruits with a diameter exceeding 1 cm meet the commercial standard in Iquitos markets. Therefore, although the 8 g $plant^{-1}$ dose reduced fruit size, the fruits remained commercially viable.

https://doi.org/10.3390/ijpb17080076

4.3. Determination of the Most Promising Treatment

Considering the six treatments, TH at 8 g plant^{-1} (TH8) produced the highest number of fruits per plant and the highest fruit yield, significantly exceeding all the other treatments. The next most effective treatments for the number of fruits per plant were TH4 and TV8; however, these treatments did not differ significantly from TH0 and TV0 in fruit yield. Consequently, only TH8 could be recommended for use in Spodosols (Podzols, WRB) under the rainy conditions of the Amazon.

4.4. Implications for Sustainable Agriculture in the Amazon

The experimental soil was a sandy loam Spodosol characterized by moderate acidity, low salinity, high available P, and high base saturation (Table 1). The exchangeable acidity was very low, indicating a minimal risk of aluminum toxicity. Nevertheless, the predominance of sand and low clay content indicate limited water and nutrient retention capacity, making organic matter management and split fertilization important under field conditions in this region. From a practical perspective, the yield response to *Trichoderma* inoculation in the absence of chemical inputs is especially relevant for smallholder farmers in the Amazon, for whom mineral fertilizers and registered pesticides are often economically or logistically inaccessible. Because rice-grain inoculum can be produced locally at a low cost, it may lower adoption barriers relative to commercial formulations.

The practical use of *Trichoderma* depends strongly on the delivery method, including seed treatment, soil inoculation, root dipping, foliar spraying, fertigation, and soil drenching [34]. Liquid and solid formulations can be effective following spore extraction and processing [35], but the direct application of colonized rice grains may be advantageous for smallholder systems because it bypasses spore washing, filtration, and formulation processes.

The economic calculations showed that the 4 and 8 g plant^{-1} doses require 95.2 and 190.5 kg ha^{-1} of inoculum, respectively, using a spacing of 0.6 × 0.7 m. Considering that the commercial cost of inoculum in the region is USD 5.88 kg^{-1}, the investment would be USD 559.78 and USD 1120.14 ha^{-1}, respectively, with labor costs for application of USD 117.60 ha^{-1}. At the current farm-gate price in Iquitos (USD 1.40 kg^{-1}) and the yield gain observed with TH at the 4 g (0.562 t ha^{-1}) and 8 g plant^{-1} doses (1.673 t ha^{-1}), the additional gross income to farmers would amount to USD 786.80 and USD 2342.20 ha^{-1}, respectively. After deducting the inoculum and labor costs for application, the estimated net return would be USD 109.42 and USD 1104.46 ha^{-1}, respectively. These returns suggest that rice-based *Trichoderma* inoculum with TH at 8 g plant^{-1} is economically viable for farmers cultivating cutleaf groundcherry in Amazonian Spodosols.

4.5. Limitations and Future Research

Conducting this experiment at a single location in a Spodosol and during the rainy season limits the generalizability of the findings to the Amazonian dry season. The Spodosol in Iquitos is sandy, severely acidic, and low in available phosphorus. However, edaphic conditions across Amazonian Spodosols are not uniform, and the dose–response recorded here may not hold for soils with higher clay fractions, deeper organic horizons, or a different resident microbial community. Testing this dose–response relationship across contrasting soil types, such as Oxisols and seasonally flooded várzea soils, would show whether 8 g plant^{-1} is a site-specific optimum or a rate that holds across Amazonian soil types.

Fruit quality was not assessed in this trial, which represents a limitation of this study. Cutleaf groundcherry is a fresh-market crop; therefore, quantifying sugar content, acidity, flavor, shelf life, and consumer preference could indicate whether *Trichoderma* inoculation affects organoleptic traits. In strawberry, *Trichoderma* did not affect total soluble solids, acidity, pH, firmness, or total polyphenols [36] but affected vitamin C and anthocyanin content [37]. Therefore, incorporating these variables into future trials would substantially strengthen the agronomic and commercial interpretations of the results.

Five aspects of inoculum application require further investigation. (i) The absence of a treatment containing sterilized rice alone, without *Trichoderma*, limits the attribution of the observed effects specifically to the isolates tested. The amount of rice applied may have altered the soil microbiota and fertility, and the effects attributed to *Trichoderma* and inoculum dose may have been partly associated with substrate mineralization. Future studies should include, in addition to the untreated control, treatments containing sterilized rice alone at the corresponding application rates to distinguish the effects of the carrier from those of fungal isolates. (ii) The presence of *Trichoderma* isolates in the roots or rhizosphere was not confirmed because inoculation alone does not ensure the successful association or persistence of the isolate throughout the plant life cycle. Post-inoculation assessments would allow the presence and persistence of isolates to be linked more directly to the responses observed at each dose. (iii) The experiment was conducted in unamended soil, whereas smallholder farmers in the Amazon commonly apply charcoal and organic compost to their home gardens. Whether these amendments would enhance or attenuate the effects of *Trichoderma* observed under unamended soil conditions remains to be determined. (iv) The rice-based *Trichoderma* inoculum was prepared in eight days and stored for three months. Other low-cost, efficient carrier alternatives to rice should be tested, such as sorghum [34], wheat, and corn. (v) Doses higher than 8 g plant^{-1} should be tested to determine the maximum enhancement of fruit yield and economic return.

5. Conclusions

Rice-based *Trichoderma harzianum* CCB-LA101 inoculum was more effective than *T. viride* CCB-LA103 in increasing the fruit number and yield of cutleaf groundcherry under Amazonian Spodosol conditions. The 8 g plant^{-1} inoculum dose produced the greatest yield response for both *Trichoderma* species. The 8 g plant^{-1} *T. harzianum* inoculum (TH8) was the only treatment to significantly increase fruit yield. Thus, under the tested conditions, TH8 could be used to increase fruit yield, with net returns for smallholders estimated at USD 1104.46 ha^{-1}.

Author Contributions: Conceptualization, J.V.-V. and J.R.-C.; methodology, J.V.-V., J.R.-C. and C.A.T.-B.; software, C.A.T.-B.; validation, J.P.J.C.M. and J.R.-C.; formal analysis, C.A.T.-B.; investigation, J.P.J.C.M. and J.R.-C.; resources, J.V.-V.; data curation, J.P.J.C.M. and J.R.-C.; writing—original draft preparation, C.A.T.-B., E.I.A.d.S. and Á.B.B.N.; writing—review and editing, C.A.T.-B., E.I.A.d.S., Á.B.B.N. and J.C.-C.; visualization, J.C.-C.; supervision, J.V.-V.; project administration, J.V.-V.; funding acquisition, J.V.-V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding; however, financial support for this publication was provided by the Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) through the Programa de Apoio à Disseminação do Conhecimento Científico, Tecnológico e Inovador no Âmbito da Pós-Graduação Stricto Sensu—Divulga CT&I (Edital N.º 017/2024) and POSGRAD/FAPEAM.

Data Availability Statement: The original data presented in the study are openly available in https://doi.org/10.5281/zenodo.21980652 (accessed on 10 August 2026) or https://github.com/cesarticonabr/trichoderma (accessed on 10 August 2026).

Acknowledgments: The authors would like to thank UNAP and IMET-ESSALUD for their logistical and institutional support. During the preparation of this manuscript, the authors used Claude Sonnet 4.6 (Anthropic) and Paperpal for English-language editing. The authors have reviewed and edited the output and take full responsibility for the content of this publication.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

TH	*Trichoderma harzianum*
TV	*Trichoderma viride*

References

1. Neto, E.C.D.S.; Pereira, M.G.; Júnior, E.F.F.; da Silva, S.B.; de Carvalho, J.A.; Santos, J.C. Temporal Evaluation of Soil Chemical Attributes after Slash-and-Burn Agriculture in the Western Brazilian Amazon. *Acta Sci. Agron.* **2019**, *41*, 42609. [CrossRef]
2. Quesada, C.A.; Lloyd, J.; Anderson, L.O.; Fyllas, N.M.; Schwarz, M.P.; Czimczik, C.I. Soils of Amazonia with Particular Reference to the RAINFOR Sites. *Biogeosciences* **2011**, *8*, 1415–1440. [CrossRef]
3. Pandey, K.; Saharan, B.S. Soil Microbiomes: A Promising Strategy for Boosting Crop Yield and Advancing Sustainable Agriculture. *Discov. Agric.* **2025**, *3*, 54. [CrossRef]
4. Guzmán-Guzmán, P.; Kumar, A.; de los Santos-Villalobos, S.; Parra-Cota, F.I.; Orozco-Mosqueda, M.D.C.; Fadiji, A.E.; Hyder, S.; Babalola, O.O.; Santoyo, G. *Trichoderma* Species: Our Best Fungal Allies in the Biocontrol of Plant Diseases—A Review. *Plants* **2023**, *12*, 432. [CrossRef] [PubMed]
5. Abdenaceur, R.; Farida, B.; Mourad, D.; Rima, H.; Zahia, O.; Fatma, S.-H. Effective Biofertilizer *Trichoderma* spp. Isolates with Enzymatic Activity and Metabolites Enhancing Plant Growth. *Int. Microbiol.* **2022**, *25*, 817–829. [CrossRef] [PubMed]
6. Bean, K.M.; Kisiala, A.B.; Morrison, E.N.; Emery, R.J.N. *Trichoderma* Synthesizes Cytokinins and Alters Cytokinin Dynamics of Inoculated *Arabidopsis* Seedlings. *J. Plant Growth Regul.* **2022**, *41*, 2678–2694. [CrossRef]
7. Basak, A.; Pallob, A.T.; Jahan, F.; Hasan, M.; Olife, M.R.I.; Saha, A.; Angon, P.B.; Ananna, T.J.; Sakil, M.A. Molecular and Physiological Mechanisms of *Trichoderma*-Induced Plant Defense against Phytopathogens. *Physiol. Mol. Plant Pathol.* **2025**, *141*, 103030. [CrossRef]
8. Galeano, R.M.S.; Ribeiro, J.V.S.; Silva, S.M.; Simas, A.L.D.O.; Guimarães, N.C.D.A.; Masui, D.C.; Corrêa, B.O.; Giannesi, G.C.; de Lima, S.F.; Brasil, M.D.S.; et al. New Strains of *Trichoderma* with Potential for Biocontrol and Plant Growth Promotion Improve Early Soybean Growth and Development. *J. Plant Growth Regul.* **2023**, *43*, 4099–4119. [CrossRef]
9. Bandara, A.Y.; Kang, S. *Trichoderma* Application Methods Differentially Affect the Tomato Growth, Rhizomicrobiome, and Rhizosphere Soil Suppressiveness against *Fusarium oxysporum*. *Front. Microbiol.* **2024**, *15*, 1366690. [CrossRef] [PubMed]
10. Mairura, F. *Physalis angulata* (Cutleaf Groundcherry). *CABI Compend.* **2025**, 1–21. [CrossRef]
11. Vargas-Ponce, O.; Martínez, J.S.; Tavares, M.D.P.Z.; Mares, L.E.V. Traditional Management of a Small-Scale Crop of *Physalis angulata* in Western Mexico. *Genet. Resour. Crop Evol.* **2015**, *63*, 1383–1395. [CrossRef]
12. Rengifo, E.; Vargas-Arana, G. *Physalis angulata* L. (Bolsa Mullaca): Revisión de Usos Tradicionales, Química y Farmacología. *Bol. Latinoam. Caribe Plantas Med. Aromat.* **2013**, *12*, 431–445.
13. Wang, P.; Yang, X.; Hu, Z.; Li, Y.; Yang, J.; Hao, X.; Yuan, C.; Yi, P. UPLC-Q-Orbitrap-MS/MS-Guided Isolation of Bioactive Withanolides from the Fruits of *Physalis angulata*. *J. Agric. Food Chem.* **2023**, *71*, 16581–16592. [CrossRef] [PubMed]
14. da Silva, R.R.P.; da Silva, B.J.M.; Rodrigues, A.P.; Farias, L.H.S.; Silva, M.; Alves, D.T.V.; Bastos, G.D.N.T.; do Nascimento, J.L.M.; Silva, E.O. In Vitro Biological Action of Aqueous Extract from Roots of *Physalis angulata* against Leishmania (Leishmania) Amazonensis. *BMC Complement. Altern. Med.* **2015**, *15*, 249. [CrossRef] [PubMed]
15. Ruiz, L.; Ruiz, L.; Maco, M.; Cobos, M.; Gutierrez-Choquevilca, A.L.; Roumy, V. Plants Used by Native Amazonian Groups from the Nanay River (Peru) for the Treatment of Malaria. *J. Ethnopharmacol.* **2010**, *133*, 917–921. [CrossRef] [PubMed]
16. Walkley, A.; Black, I.A. An Examination of the Degtjareff Method for Determining Soil Organic Matter, and a Proposed Modification of the Chromic Acid Titration Method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
17. Olsen, S.R.; Coles, C.V.; Watanabe, F.S.; Dean, L.A. *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*; U.S. Government Printing Office: Washington, DC, USA, 1954.
18. Page, A.L.; Miller, R.H.; Keeney, D.R. *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*; American Society of Agronomy and Soil Science Society of America: Madison, WI, USA, 1982.
19. Soil and Plant Analysis Council. *Soil Analysis Handbook of Reference Methods*; Soil and Plant Analysis Council Inc.: Boca Raton, FL, USA, 1999.

20. Naeimi, S.; Khosravi, V.; Varga, A.; Vágvölgyi, C.; Kredics, L. Screening of Organic Substrates for Solid-State Fermentation, Viability and Bioefficacy of *Trichoderma harzianum* AS12-2, a Biocontrol Strain Against Rice Sheath Blight Disease. *Agronomy* **2020**, *10*, 1258. [CrossRef]
21. R Core Team. *R: A Language and Environment for Statistical Computing*; R Core Team: Vienna, Austria, 2025.
22. Jelihovschi, E.G.; Faria, J.C.; Allaman, I.B. ScottKnott: A Package for Performing the Scott-Knott Clustering Algorithm in R. *Trends Appl. Comput. Math.* **2014**, *15*, 3–17. [CrossRef]
23. Wickham, H. *Ggplot2: Elegant Graphics for Data Analysis*; Springer: New York, NY, USA, 2016.
24. Jamil, A. Antifungal and Plant Growth Promoting Activity of *Trichoderma* spp. against *Fusarium Oxysporum* f. sp. lycopersici Colonizing Tomato. *J. Plant Prot. Res.* **2021**, *61*, 243–253. [CrossRef]
25. Rahman, M.; Borah, S.M.; Borah, P.K.; Bora, P.; Sarmah, B.K.; Lal, M.K.; Tiwari, R.K.; Kumar, R. Deciphering the Antimicrobial Activity of Multifaceted Rhizospheric Biocontrol Agents of Solanaceous Crops Viz., *Trichoderma harzianum* MC2, and *Trichoderma harzianum* NBG. *Front. Plant Sci.* **2023**, *14*, 1141506. [CrossRef] [PubMed]
26. Contreras-Cornejo, H.Á.; 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. *Microbiol. Res.* **2024**, *281*, 127621. [CrossRef] [PubMed]
27. Chacón, M.R.; Rodríguez-Galán, O.; Benítez, T.; Sousa, S.; Rey, M.; Llobell, A.; Delgado-Jarana, J. Microscopic and Transcriptome Analyses of Early Colonization of Tomato Roots by *Trichoderma harzianum*. *Int. Microbiol.* **2007**, *10*, 19–27. [CrossRef]
28. Nogueira-López, G.; Greenwood, D.; Middleditch, M.; Winefield, C.; Eaton, C.J.; Steyaert, J.M.; Mendoza-Mendoza, A. The Apoplastic Secretome of *Trichoderma virens* During Interaction With Maize Roots Shows an Inhibition of Plant Defence and Scavenging Oxidative Stress Secreted Proteins. *Front. Plant Sci.* **2018**, *9*, 409. [CrossRef] [PubMed]
29. Kumar, S.; Thakur, M.; Rani, A. *Trichoderma*: Mass Production, Formulation, Quality Control, Delivery and Its Scope in Commercialization in India for the Management of Plant Diseases. *Afr. J. Agric. Res.* **2014**, *9*, 3838–3852.
30. Pawar, R.; Rana, V.S. Manipulation of Source-Sink Relationship in Pertinence to Better Fruit Quality and Yield in Fruit Crops: A Review. *Agric. Rev.* **2019**, *40*, 200–207. [CrossRef]
31. Singh, A.; Shukla, N.; Kabadwal, B.C.; Tewari, A.K.; Kumar, J. Review on Plant-*Trichoderma*-Pathogen Interaction. *Int. J. Curr. Microbiol. Appl. Sci.* **2018**, *7*, 2382–2397. [CrossRef]
32. Nakai, H.; Yasutake, D.; Hidaka, K.; Miyoshi, Y.; Eguchi, T.; Yokoyama, G.; Hirota, T. Sink Strength Dynamics Based on Potential Growth and Carbohydrate Accumulation in Strawberry Fruit. *HortScience* **2024**, *59*, 1505–1510. [CrossRef]
33. Alikhani-Koupaei, M.; Khosravinejad, A. Defining the Physiological and Molecular Threshold of Source–Sink Balance Governing Yield and Sugar Metabolism in 'Mazafati' Date Palm (*Phoenix dactylifera* L.). *Biocatal. Agric. Biotechnol.* **2025**, *70*, 103848. [CrossRef]
34. Woo, S.L.; Ruocco, M.; Vinale, F.; Nigro, M.; Marra, R.; Lombardi, N.; Pascale, A.; Lanzuise, S.; Manganiello, G.; Lorito, M. *Trichoderma*-Based Products and Their Widespread Use in Agriculture. *Open Mycol. J.* **2014**, *8*, 71–126. [CrossRef]
35. Swathi, B.; Patibanda, A.K.; Prasadji, J.K.; Krishnayya, P.V.; Ahamed, M.L. Evaluation of Formulations through Testing the Bioefficacy of Selected *Trichoderma* Isolate against *S. rolfsii*. *Int. J. Curr. Microbiol. Appl. Sci.* **2020**, *9*, 301–309. [CrossRef]
36. Gaitán, J.B.M.; Ferrucho, R.L.; Herrera, J.G.Á. Efecto de dos cepas de *Trichoderma* en el control de *Botrytis cinerea* y la calidad del fruto en fresa (*Fragaria* sp.). *Rev. Colomb. Cienc. Hortícolas* **2014**, *8*, 44–56. [CrossRef]
37. Lombardi, N.; Caira, S.; Troise, A.D.; Scaloni, A.; Vitaglione, P.; Vinale, F.; Marra, R.; Salzano, A.M.; Lorito, M.; Woo, S.L. *Trichoderma* Applications on Strawberry Plants Modulate the Physiological Processes Positively Affecting Fruit Production and Quality. *Front. Microbiol.* **2020**, *11*, 1364. [CrossRef] [PubMed]

https://doi.org/10.3390/ijpb17080076