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Trichoderma asperellum-based biostimulant product: Beneficial impact on plant growth and yields

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ABSTRACT

The agricultural sector is a cornerstone of the national economy and faces significant challenges with key crops in Morocco, such as tomato. Establishing an ecological and sustainable cultivation system is therefore essential, including the use of alternatives to synthetic chemical inputs. This study evaluated the effects of *Trichoderma asperellum*, a beneficial fungus with biostimulant and bioprotective properties, on the growth and yield of greenhousegrown tomato (*Solanum lycopersicum*). Four agronomic traits were assessed: fruit number, average fruit weight, yield per harvest, and shoot length. Application of *T. asperellum* enhanced all parameters, with effects depending on the applied concentration, plant developmental stage, and environmental conditions. A moderate volume of 5 L proved most effective during the active growth phase, producing 6.48 kg/m² and up to 57.71 fruits, while also promoting early development and activating plant defense mechanisms. Although overall performance declined by the end of the cycle, the 20 L volume maintained yield, highlighting the potential of *T. asperellum* to improve productivity throughout the growth cycle.

Keywords: Trichoderma asperellum, biostimulant, bioprotection, greenhouse, tomato.

INTRODUCTION

In 2020, global tomato production reached 186.821 million kilograms, cultivated on 5,051,983 hectares, with an average yield of 3.71 kg/m², marking a 3.35% rise compared to the previous year (FAO, 2020). This growth underscores the growing global significance of this crop, particularly for exporting countries such as Morocco (Mzibra *et al.*, 2021). Morocco has notably strengthened its position in the international market by steadily boosting its exports. In 2021, the country was ranked fifth worldwide for tomato exports, and in 2022, it moved up to third place, surpassing Iran and Spain, with export growth exceeding 17% in a single year, the highest increase globally (EastFruit, 2023). Despite this

strong performance, meeting the high demand for tomatoes remains challenging due to abiotic and biotic stresses, including recurrent droughts in Morocco, which have caused heatwaves and water shortages, as well as the emergence of new pests and diseases (Stuch *et al.*, 2020; Bandana *et al.*, 2024). Changing climate conditions are likely contributing to the emergence of diseases such as late blight, leaf curl, and black spot in tomato crops (Bhandari *et al.*, 2021; El Allaoui *et al.*, 2025). Additionally, temperature fluctuations directly impact the development of insects and pests (Peace, 2020; Ouznati *et al.*, 2025).

Previous studies indicate that rising temperatures exacerbate damage caused by fungi, bacteria, and insects (Johkan *et al.*, 2011; Kotba *et al.*, 2020; Albatnan *et al.*, 2025). Consequently,

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farmers increasingly depend on chemical solutions (Eshun *et al.*, 2011; Bandanaa *et al.*, 2024; Boudoudou *et al.*, 2025a, 2025b), which pose significant risks to the health of both producers and consumers, as well as to the environment. Furthermore, resistance to these chemicals reduces the effectiveness of phytosanitary treatments (Elame *et al.*, 2019; El Kaissoumi *et al.*, 2025; Khirallal *et al.*, 2025). Other impacts include the emergence of new pests and increased residue levels in food products (Dhanush *et al.*, 2015).

Extreme climatic conditions can significantly increase production and yield losses in tomato fields, mainly due to the spread of pests and diseases (Bhandari *et al.*, 2021). Research indicates that the productivity of horticultural crops typically declines when temperatures surpass critical physiological thresholds (Bisbis *et al.*, 2018; Bose and Pal, 2023). In Turkey, tomato greenhouses have reported yield reductions ranging from 6% to 53%, with an average of 12.5%, along with higher use of irrigation water, fertilizers, and electricity (Kürklü *et al.*, 2025).

Ensuring consistent yields under difficult temperature conditions without sacrificing quality has become a critical priority for the agricultural industry. Heavy dependence on chemical inputs to control plant diseases and pests is no longer sustainable. Therefore, adopting sustainable farming practices and creating alternatives to pesticides are essential to secure the sector's future and uphold export quality (Gajanana *et al.*, 2006).

Farmers are increasingly adopting organic fertilizers. Finding the right balance between organic and mineral fertilizers is crucial for encouraging healthy plant growth, enhancing quality, and increasing yields. Moreover, the use of microbes that promote plant growth (Sallami *et al.*, 2023a, 2023b; El Allaoui *et al.*, 2023) offers a sustainable and affordable alternative, significantly reducing reliance on chemical inputs (Bender *et al.*, 2016; Toju *et al.*, 2018; Benjelloun *et al.*, 2021; Kulkarni and Joshi, 2024; Xu *et al.*, 2024).

Over the years, agroecology has become a vital approach for rethinking farming and food systems, encouraging more ecological and sustainable practices (Altieri *et al.*, 2020; Idoudi *et al.*, 2024). One example is the use of beneficial microorganisms like Trichoderma, known to boost root growth and rhizosphere activity (Harman *et al.*, 2021; Finkel *et al.*, 2017). Today, many biopesticides and biofertilizers are commercially available, most developed from symbiotic fungi

such as Trichoderma and Gliocladium. Their popularity stems from their abundance, ease of production, low toxicity, and extensive research affirming their effectiveness. These products are used to control pathogens in soil, roots, aerial parts, and even after harvest. Each microbiome is considered a distinct habitat with specific physicochemical conditions and unique microbial communities (Whipps *et al.*, 1988).

Certain species of Trichoderma have been shown to enhance seed germination, seedling emergence, plant growth, and yield (Hmpuni et al., 2006; Chagas et al., 2016; Mouden et al., 2023; El Kaissoumi et al., 2024; Sellal et al., 2024; El Rhoch et al., 2025). They also improve plant defenses, making crops more resistant to pests, diseases, and environmental stresses (Jaroszuk-Ściseł et al., 2019; Cai et al., 2016; El Kaissoumi et al., 2023; Errifi et al., 2024a, 2024b; Adnani et al., 2024a, 2024b). By colonizing roots, this fungus promotes root development, increases seed viability and germination, and encourages flowering, photosynthesis, and fruit quality (Halifu et al., 2019; Qostal et al., 2020a, 2020b; Kribel et al., 2020; Sbbar et al., 2025). Applications of Trichoderma strains have boosted both yield and quality across various crops such as tomato, cucumber, bean, carrot, cotton, and corn (Hoyos-Carvajal et al., 2009; Ouazzani Chahdi et al., 2025a, 2025b). However, research on this topic in Morocco remains limited, highlighting the need for further study. This research aims to increase tomato yields using a Moroccan Trichoderma asperellum-based product with both bioprotective and biostimulant effects in greenhouse cultivation.

MATERIALS AND METHODS

Bioprotective and biostimulant product

The liquid formulation, developed under laboratory conditions, consists of a conidial suspension of *Trichoderma asperellum* KU987252 at a concentration of 2×10° conidia/mL, enriched with specific additives (Ouazzani Chahdi *et al.*, 2019, 2025a) (Figure 1).

Experimental site

The experiment was conducted in a delta-shaped greenhouse (9 m long \times 7 m wide) located

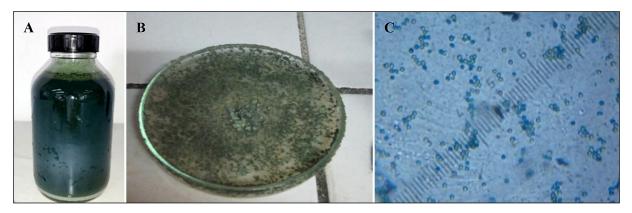


Figure 1. (A) Product formulated from the Trichoderma asperellum strain (KU987252), (B) culture of the strain on PSA medium aged 7 days, (C) Conidia

on the experimental field of the Faculty of Sciences, Ibn Tofaïl University, Kenitra (Morocco). The structure, oriented north—south, was equipped with a single entrance door, side openings for natural ventilation, and a 2 m-wide shading net extending along its entire length. The soil used originated from the Maâmora region, a non-cultivated sandy area. Its main physicochemical characteristics were as follows: pH = 7.53; organic matter = 0.7%; total nitrogen = 0.05%; $P_2O_5 = 0.239\%$; $K_2O = 0.15$ meq/100 g; Mg = 0.20 meq/100 g; and Ca = 7351.5 mg/kg (El Gabardi *et al.*, 2020; 2024).

Inside the greenhouse, five raised beds (8 m long and 40 cm wide) were arranged, each covered with black polyethylene mulch and separated by 40 cm walkways. Irrigation was provided through a T-TAPE drip irrigation system powered by a small motor pump. Environmental parameters (temperature and relative humidity) were recorded daily using a digital thermo-hygrometer (Testo 625). During the experimental period (March–July 2017), temperature ranged

between 25 and 36 °C, and relative humidity varied from 55 to 75%.

Plant material and pre-planting treatment

The plant material consisted of young tomato seedlings (Solanum lycopersicum). On March 24, 2017, the roots of the seedlings, along with their peat substrate, were immersed for 45 minutes in a suspension of Trichoderma asperellum KU987252 prepared at a final concentration of 10⁷ conidia/mL by dilution in distilled water (Figure 2A).

Control seedlings underwent the same procedure but were immersed only in distilled water (Figure 2 B). After treatment, the seedlings were transplanted into the greenhouse (Figure 3) at a density of 25 plants per bed, spaced 30 cm apart within rows and 40 cm from each end of the bed. Irrigation was carried out every three days using well water. Data were collected during three distinct production periods corresponding to the early (May 31–June 20, 2017), mid (June



Figure 2. Soaking the roots of tomato plants: (A) plant soaked in the organic product, (B) control plant soaked in distilled water



Figure 3. Transplanting tomato eedlings in a greenhouse

21–July 3, 2017), and late (July 11–28, 2017) stages of the crop cycle.

Post-planting treatments

After transplantation, the plants received three successive applications of the *Trichoderma* asperellum-based product. The suspensions were prepared in 10 L containers at a concentration of 10⁷ conidia/mL and applied through the drip irrigation system at volumes of 5 L, 10 L, 15 L, and 20 L per bed (Figure 4).

The experimental design followed a randomized complete block layout with five treatments (control, 5 L, 10 L, 15 L, and 20 L of *T. asperellum* suspension). Each treatment was replicated five times, and each replicate consisted of five plants, resulting in a total of 25 plants per treatment.



Figure 4. Treatment of tomato plants with different concentrations of the *T. asperellum*-based product

Applications were performed at 20-day intervals according to the following schedule:

- April 13, 2017 first application;
- May 3, 2017 second application;
- May 23, 2017 third application.

Control beds received the same irrigation volumes using well water only, without conidia.

Growth and yield measurements

Fruit harvests were conducted twice weekly, 15 days after each treatment, on April 27, May 18, and June 8, 2017. Data were categorized into three main production periods corresponding to early (May 31–June 20), mid (June 21–July 3), and late (July 11–28) stages of the crop cycle. For each replicate (five plants), the total number of fruits and total fresh weight were recorded using a precision scale (Figure 5). Yield (kg/m²) was calculated using the formula:

$$Yield = \frac{Total fruit weight (kg)}{Cultivated area (m^2)}$$

At the end of the experiment (July 2017), the shoot length of each plant was measured using a metallic measuring tape.

Statistical analysis

The data were analyzed using a one-way analysis of variance (ANOVA) to assess the effects of different treatment volumes on plant growth and yield parameters. Mean comparisons were conducted using the Least Significant Difference (LSD) test at a 5% probability level (p < 0.05). Each treatment included five replicates, with five plants per replicate.

RESULTS AND DISCUSSION

Greenhouse experiments were conducted to assess the impact of a *Trichoderma asperellum*-based product on tomato (*Solanum lycopersicum*) growth and yield. The experimental setup and tomato growth stages under different *T. asperellum* treatments are illustrated in Figure 6. Evaluations focused on four variables: number of fruits per plant, average fruit weight, total yield per harvest, and shoot length. Treatment with *Trichoderma asperellum* significantly influenced tomato productivity over three evaluation periods, with



Figure 5. Tomato fruits harvested: A) sorted, B) weighed

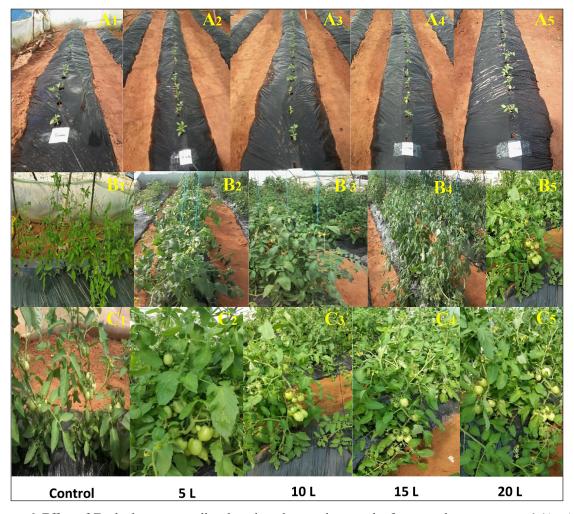


Figure 6. Effect of *Trichoderma asperellum*-based product on the growth of tomato plants: treatment 1 (A_1 – A_5), treatment 2 (B_1 – B_5), and treatment 3 (C_1 – C_5) represent different application volumes at successive growth stages

responses varying according to the applied dose and environmental conditions.

During the first evaluation period (May 31–June 20, 2017), the 5 L and 20 L treatments recorded the highest fruit numbers, with 28.85 and 29 fruits per plant, respectively. However, the

LSD test revealed no statistically significant differences among treatments, suggesting that the effects of *T. asperellum* were not yet fully expressed at this early stage of crop development.

In the second evaluation period (June 21–July 3, 2017), all treated plants produced more fruits

than the control. The 5 L treatment achieved the highest mean number of fruits (57.71), demonstrating the effectiveness of *T. asperellum* in promoting fruiting during the mid-growth stage, when physiological activity is maximal. These findings are consistent with those of El Kaissoumi *et al.* (2024), who observed a sustained increase in tomato fruit number following *T. asperellum* application throughout the growth cycle. Similarly, Mouden *et al.* (2023) reported that both irrigation and foliar spraying with *T. asperellum* enhanced vegetative growth and overall yield.

During the third evaluation period (July 11–28, 2017), a general decline in fruit production was observed across all treatments, particularly with the 5 L volume (16.14 fruits per plant). This decrease may be attributed to unfavorable climatic conditions, such as high temperatures and water deficit, coupled with physiological exhaustion of the plants at the end of the cycle. Such factors may reduce the bioactivity of *T. asperellum* in the soil or limit the plant's responsiveness to its biostimulant effects (Table 1). Tables 2 and 3 present the effects of the different *T. asperellum* application volumes (5, 10, 15, and 20 L) on the average fruit weight (g) and yield (kg/m²) over the three production periods.

During the first evaluation period, *T. asperellum*-treated plants exhibited a marked improvement compared with the control, which averaged 1092.77 g. The 5 L treatment produced the highest average fruit weight (3892.68 g), followed by 10 L (3004.7 g) and 20 L (3156.04 g). The 5 L treatment also achieved the highest yield (6.48 kg/m²), compared with only 1.82 kg/m² in the control. These results highlight the strong positive influence of *T. asperellum* during early vegetative development.

The observed benefits likely stem from the activation of physiological mechanisms supporting early plant growth, enhanced nutrient availability, improved root development, and greater stress tolerance. However, at higher application volumes (10 L and 20 L), the effects tended to decline, suggesting the existence of an optimal threshold. Beyond this point, microbial activity may reach saturation, potentially disturbing soilmicrobe interactions or limiting further benefits.

These observations agree with Illescas *et al.* (2022), who demonstrated that *T. asperellum* regulates the expression of abiotic stress–related genes such as *NAC2* and *DREB2*, thereby improving plant resilience during early growth. The fungus also increases antioxidant enzyme activity,

Table 1. Effect of treatments with the *Trichoderma asperellum*-based product on the average number of tomatoes harvested across three-time intervals

Periods	Product mixture volumes				
	Control	5 liter	10 liter	15 liter	20 liter
05/31/2017 06/20/2017	11.28 _b a	28.85 _{ab} ^a	24.00 _b a	21.85 _b a	29.00 _b a
06/21/2017 07/03/2017	49.57 _a ª	57.71 _a a	52.86 _a ª	51.42 _a ª	54.00 _a ª
07/11/2017 07/28/2017	27.28 _{ab} ^a	16.14 _b ^a	17,14 _b ^a	19.71 _b ª	19.57 _b ª

Note: Two values on the same line, followed by the same letter on superscript, are not significantly different at the 5% level according to the LSD test. Two values on the same column, followed by the same letter on subscript, are not significantly different at the 5% level according to the LSD test.

Table 2. Effect of treatments with a *Trichoderma asperellum*-based product on the average weight of tomatoes harvested during three time intervals (g)

Periods	Product mixture volumes				
	Control	5 liters	10 liters	15 liters	20 liters
05/31/2017 20/06/2017	1092.77 _b ª	3892.68 _{ab} ^a	3004.70 _a ab	2307.74 _b ^a	3156.04 _a ª
21/06/2017 03/07/2017	4539.52 _a ^a	6724.57 _a ^a	5503.11 _a ^a	7464.90 _a a	6682.44 _a ^a
11/07/2017 28/07/2017	2095.00 _{ab} ^a	1176.83 _b ª	1278.77 _b ª	1671.30 _b ª	1568.10 _a ª

Note: Two values on the same line, followed by the same letter on superscript, are not significantly different at the 5% level according to the LSD test. Two values on the same column, followed by the same letter on subscript, are not significantly different at the 5% level according to the LSD test.

notably superoxide dismutase (SOD), while reducing the accumulation of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA), two indicators of oxidative stress (Zhang *et al.*, 2016). Altogether, these mechanisms enhance stress tolerance and promote robust plant development.

The findings of Boorboori and Zhang (2023) further support these results, showing that *T. asperellum* enhances the expression of stress-responsive genes, leading to more vigorous plant growth. Similarly, Scudeletti *et al.* (2021) demonstrated that *Trichoderma* application improves antioxidant enzyme activity and water-use efficiency, two key processes for maintaining optimal growth under controlled cultivation. The responses observed, particularly at moderate doses, may result from a synergistic combination of physiological stimulation and enhanced plant defense, ultimately improving productivity from the earliest growth stages.

During the second period, all treatments, including the control, exhibited a general increase in production. Average fruit weights ranged from 4539.52 g to 7464.9 g, with the highest value recorded for the 15 L treatment, which achieved a maximum yield of 12.44 kg/m². The absence of significant differences among treatments may be

due to favorable climatic conditions that allowed the plants to express their full genetic potential. These results are consistent with Kamanda *et al.* (2022) and Mouria *et al.* (2007), who emphasized the positive impact of *T. asperellum* on the growth of crops such as maize and tomato.

In contrast, during the third period, production decreased across all treatments. Average fruit weights ranged from 1176.83 g to 2095 g, while yields dropped to between 1.96 and 3.49 kg/m². Although the control achieved a slightly higher yield (3.49 kg/m²), the differences among treatments were not statistically significant.

Data presented in Table 4 also show that variations in average fruit weight depended on both the applied volume of *T. asperellum* suspension and the growth period, indicating that the fungus's effect on fruit quality is influenced by dosage and timing of application.

The application of *T. asperellum* significantly promoted the vegetative growth of tomato plants, especially in stem length (Figure 7). The control plants showed the lowest average height (81.33 cm), while the 5 L and 15 L treatments produced the greatest growth, reaching 128 cm and 132.33 cm, respectively. In contrast, the 10 L treatment exhibited limited growth (92 cm), and the 20 L

Table 3. Impact of treatments with the *Trichoderma asperellum*-based product on the average tomato yield (kg/m²) during three time periods

Periods	Product mixture volumes					
	Control	5 liters	10 liters	15 liters	20 liters	
05/31/2017 06/20/2017	1.82 _b a	6.48 _{ab} ^a	5.00 _a ab	3.84 _b ^a	5.26 _a ^a	
06/21/2017 07/03/2017	7.56 _a a	11.20 _a a	9.17 _a ^a	12.44 _a ª	11.13 _a a	
07/11/2017 07/28/2017	3.49 _{ab} ^a	1.96 _b a	2.13 _b ^a	2.78 _b ^a	2.61 _a ^a	

Note: Two values on the same line, followed by the same letter on superscript, are not significantly different at the 5% level according to the LSD test. Two values on the same column, followed by the same letter on subscript, are not significantly different at the 5% level according to the LSD test.

Table 4. The effect of treatments with the *Trichoderma asperellum*-based product on the average weight of tomato fruit (g) during the three time intervals

Periods	Product mixture volumes					
	Control	5 liters	10 liters	15 liters	20 liters	
31/05/2017 20/06/2017	37.00 _a a	34.89 _a a	18.94 _a ^b	39.90 _a ª	39.64 _a ^a	
21/06/2017 03/07/2017	18.58 _a a	13.28 _a a	12.54 _a ^a	23.43 _a a	13.54 _a ^a	
11/07/2017 28/07/2017	21.46 _a a	21.00 _a a	15.67 _a ª	20.12 _a a	26.48 _a ^a	

Note: Two values on the same line, followed by the same letter on superscript, are not significantly different at the 5% level according to the LSD test. Two values on the same column, followed by the same letter on subscript, are not significantly different at the 5% level according to the LSD test.

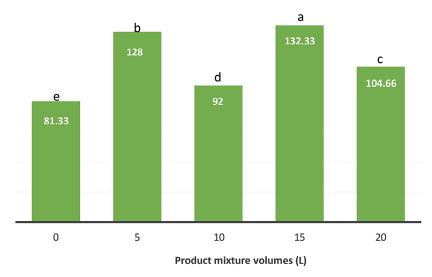


Figure 7. Growth of the aerial parts of tomato plants at the end of the trial after different treatments with the *Trichoderma asperellum*-based product

treatment resulted in intermediate values (104.66 cm). This pattern indicates a nonlinear, dose-dependent response, implying that increasing biofungal concentration does not necessarily result in greater plant growth.

These findings demonstrate that *T. asperellum* exerts its biostimulant effects in a dose-dependent manner, with the most favorable outcomes observed at moderate doses (5 L and 15 L). The enhanced growth may be attributed to improved nutrient uptake, particularly nitrogen and phosphorus and to the production of phytohormones such as auxins and gibberellins by certain *Trichoderma* strains (Harman *et al.*, 2004; Shoresh *et al.*, 2010; Khirallah *et al.*, 2017; Elouark *et al.*, 2025; Kribel *et al.*, 2025).

The reduced growth observed at 10 L and 20 L could be due to rhizosphere saturation, leading to microbial competition, disruption of the plant—microbe balance, or interference with hormonal signaling pathways. These results corroborate those of Illescas *et al.* (2022), who demonstrated that moderate doses of *T. asperellum* enhance the expression of stress-tolerance genes (NAC2 and *DREB2*), increase antioxidant enzyme activity, and improve plant resilience under stress. Conversely, excessively high concentrations may counteract these positive effects, reducing overall treatment efficacy (Ye *et al.*, 2020; Fu *et al.*, 2020; Lanzuise *et al.*, 2022; Sehim *et al.*, 2023; Sudha *et al.*, 2024).

Overall, our findings confirm previous reports indicating that *T. asperellum* functions as a reliable biostimulant under various environmental

conditions, including the presence of pathogens (El Kaissoumi *et al.*, 2023; 2025; Errifi *et al.*, 2024). Ajiboye *et al.* (2022) demonstrated its effectiveness against *Alternaria solani*, while Sehim *et al.* (2023) observed improved vegetative growth, greater leaf production, shoot elongation, and enhanced post-harvest resistance to *Fusarium oxysporum*. Collectively, these studies highlight the dual role of *T. asperellum* as both a biostimulant and a biological control agent.

CONCLUSIONS

The application of *Trichoderma asperellum* offers a promising eco-friendly approach to enhance tomato growth, yield, and resilience in greenhouse settings. Our findings show that moderate doses (5 L and 15 L) optimize production by significantly boosting fruit count, average fruit weight, and above-ground biomass. These beneficial effects probably result from multiple mechanisms, including improved root growth, better nutrient absorption, regulation of abiotic stress—related genes (NAC2, DREB2), and activation of antioxidant enzymes like superoxide dismutase (SOD).

In contrast, higher doses (20 L) did not offer additional benefits and, in some instances, even hindered growth, highlighting a non-linear, dose-dependent response. This emphasizes the importance of carefully adjusting application rates based on environmental conditions and plant developmental stages to maximize the benefits of *T. asperellum*.

Besides promoting growth, *T. asperellum* was also very effective as a biocontrol agent, lowering disease rates. Overall, these results emphasize the dual function of *Trichoderma asperellum* as both a biostimulant and a biological control method, providing a sustainable alternative to chemicals for improving crop health and yields.

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