



Research Article

Efficacy of *Trichoderma* species as Biocontrol Agents for Management of Tomato Fruit Rot

Mohamed F.A. Ahmed^{1*}, Hamam H. Youns², Maha A. El-Abhar², Maryan M. Youssef³ and Ahmed A. Elsis⁴

¹Central Laboratory of Organic Agriculture (CLOA), Agricultural Research Center (ARC), Giza, Egypt; ²Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt; ³Faculty of Agriculture, Cairo University, Egypt; ⁴Faculty of Agriculture, Benha University, Benha, Egypt.

Abstract | Tomato (*Lycopersicon esculentum* Mill.) is a globally remarkable crop, yet its production is severely limited by fungal pathogens that cause fruit rots, particularly those caused by *Alternaria* species. The aims of this study were to evaluate several integrated and eco-friendly strategies for the management of tomato fruit rot and assess their effectiveness in improving yield, fruit quality, and key biochemical attributes. Fungal diseases considerably contribute to substantial losses in tomato fruits throughout handling, storage, and distribution from harvest to the consumer. In this study, several fungi originating from rotten tomato fruits in Menoufia Governorate, Egypt, were isolated and identified as *A. solani* and *A. alternata*, where *A. solani* was more prevalent and virulent, causing severe early blight symptoms, particularly in the Super strain B hybrid (recording 96.30% incidence and 34.36% severity). Several biocontrol agents, including *Trichoderma album*, *T. atroviride*, *T. harzianum*, *T. viride*, and the commercial product Blight Stop (*T. harzianum*) exhibited a strong *in vitro* antagonistic activity against *A. solani*, with *T. album* achieving the highest inhibition (90.28%). *In vivo*, all bio-agents and Blight Stop reduced disease incidence and severity of tomato fruit rot, with *T. album* achieving the greatest control efficiency, recording 73.56% and 74.76% during 2023/24–2024/25 growth seasons, respectively, followed by *T. atroviride* and Blight Stop. Additionally, these treatments enhanced multiple yield components, including fruit quality (total soluble solids “TSS”, vitamin C, protein content, carbohydrates), and several other biochemical traits such as flavonoids and phenolics content, and defense-related enzymes (*i.e.*, peroxidase (PO), polyphenol oxidase (PPO), chitinase, and β -1,3-glucanase), with *T. album* consistently recorded the highest values. These findings highlight the effectiveness of *Trichoderma* spp. as biocontrol agents and promising alternatives to chemical pesticides for suppressing post-harvest tomato diseases and improving plant performance, thereby underscoring their potential as sustainable and ecofriendly tools for disease management.

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***Correspondence** | Mohamed F.A. Ahmed, Central Laboratory of Organic Agriculture (CLOA), Agricultural Research Center (ARC), Giza, Egypt; **Email:** mohamed_faah@yahoo.com

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) is a globally important crop and a key component of Egyptian agriculture, with over 153,000 hectares cultivated and about 6.7 million tons produced annually (Liu *et al.*, 2022; FAO, 2024). However, its production is heavily constrained by fungal diseases such as fruit rots, early blight, and wilts, which account for over 50% of yield losses (Zhu *et al.*, 2023; Akila *et al.*, 2024; Ramezani *et al.*, 2024). Due to the environmental and health risks of the chemical fungicides, use of biological control agents has emerged as a sustainable alternative for disease management (Ahmed, 2013; Venkatesan and Muniyan, 2024). *Trichoderma* spp. (e.g., *T. album*, *T. atroviride*, *T. harzianum*, *T. viride*, and *Blight stop*) effectively controlled tomato fruit rot caused by *Alternaria* spp. through mycoparasitism, antibiosis, competition, and induced systemic resistance, with *T. harzianum* showing the highest efficacy (Harman *et al.*, 2004; Harman, 2006; Ahmed *et al.*, 2017; Akila *et al.*, 2024). Additionally, these treatments enhanced yield and fruit quality (*i.e.*, total soluble solids TSS, vitamin C, protein content, and carbohydrates content) by improving plant growth and metabolic activity (Shoresh *et al.*, 2010; Kumar *et al.*, 2017; Tsolakidou *et al.*, 2024). They also stimulated several biochemical defenses, including increased phenolics, flavonoids, and activity of defense enzymes such as peroxidase (PO), polyphenol oxidase (PPO), chitinase, and β -1,3-glucanase, with *T. harzianum* consistently induced the strongest responses (Pieterse *et al.*, 2014; Harman, 2024). Additionally, *T. viride* acts as a biofungicide against multiple soil- and fruit-borne pathogens (Hajji *et al.*, 2025). *Trichoderma* spp. promote plant growth and immune response by producing secondary metabolites and defense-related enzymes, including chitinase, PO, and PPO, which increase resistance to microbial infections (Yao *et al.*, 2023; Akila *et al.*, 2024; Karačić *et al.*, 2024). The objectives of this study were to assess several integrated and ecofriendly strategies for managing tomato fruit rot, and enhancing yield, fruit quality, and key biochemical attributes.

Materials and Methods

Tomato cultivars

Five tomato hybrid varieties, mainly Super Strain B, Super Strain B Regular, Eliza, Castle Rock, and Super Altery were obtained from the Vegetable Research

Department, Horticulture Research Institute, Agricultural Research Centre (ARC), Giza, Egypt to evaluate their susceptibility to tomato fruit rot post-harvest disease.

Isolation and identification of the tomato early blight pathogen

During autumn 2023, 100 infected tomato leaves showing early blight symptoms were collected from fields of Menoufia Governorate, Egypt, representing different tomato cultivars, including Super Strain B, Super Strain B Regular, Eliza, and Castle Rock. Infected tissues were cut into small pieces (≈ 2 mm) using a disinfected scissor, surface sterilized with 0.5% NaOCl for 2–3 min, rinsed three times with sterile distilled water, and air dried before being aseptically placed onto the surface of Potato Dextrose Agar (PDA) plates (Naik *et al.*, 2010). Ten plates were used, each containing five pieces of infected leaf tissue. The inoculated plates were incubated at 25 ± 2 °C under a 12-h light/12-h dark photoperiod for 7 d. After incubation, the developed fungal hyphae were picked, purified using single spore colony technique, sub-cultured on PDA slants, and stored at 5–10°C for further study. The isolated fungi were identified based on their morphological characteristics according to several standard keys described by Singh (1982); Barnett and Hunter (1987); Simmons (2007).

Pathogenicity tests

Preparation of *Alternaria* sp. inoculum

Alternaria sp. was cultured on PDA plates at 27°C under a 12 h light/dark cycle for 15 d (DeMers, 2022). Conidia were collected by adding 5 ml sterile distilled water and scraping the culture with a sterile spatula. The inoculum was adjusted to 5×10^6 spores/ml using a haemocytometer (Schmeyer *et al.*, 2024).

Greenhouse experiment

The study was conducted in 2024 at ARC greenhouse facilities, Egypt. Five tomato hybrids were tested for their susceptibility to *A. solani*. Seedlings of tomato were transplanted into steam-sterilized soil in pots each containing 2.7 kg of light clay soil, with three seedlings per pot. Each replicate consisted of 30 seedlings distributed among 10 pots. Plants were spray inoculated using a hand-held atomizer with 30 ml of the prepared spore suspension, whereas control seedlings were sprayed with 30 ml of sterile distilled water. Three replicates were maintained for each tomato hybrid.

Disease assessment

After inoculation, the seedlings were covered with plastic bags for 48 h to maintain high humidity and grown under normal conditions in a completely randomized design. Disease severity was evaluated after two weeks using a 0–9 scale based on the infected leaf area (Latha *et al.*, 2009). The standard disease severity (%) was evaluated according to the following formula:

$$\text{Disease severity (\%)} = \frac{\sum (n \times r)}{NR} \times 100$$

Where: n = Number of infected leaves on the plant; N= Total number of leaves examined; r = Numerical rate of infected leaves, and R= Highest numerical rate.

Biocontrol agents

Four biocontrol agents (BCAs), *Trichoderma album*, *T. atroviride*, *T. harzianum*, and *T. viride* were provided by the Biological Control Production Unit, Central Laboratory of Organic Agriculture (CLOA), Agricultural Research Centre (ARC), Giza, Egypt.

Laboratory assays

In vitro antagonistic activity

The antagonistic agents *i.e.*, *Trichoderma album*, *T. atroviride*, *T. harzianum*, *T. viride*, and Blight stop (*T. harzianum*) were individually added at the rate of 10 ml to 125 ml to conical flasks each containing 90 ml PDA before solidification, resulting in a final concentration of 10% (v/v). A disc of *A. solani* was cut individually from each actively growing culture using a sterile cork borer and aseptically placed at the center of each seeded plate. Control plates were inoculated with *A. solani* only. Each treatment was replicated three times and the plates were incubated at 25±2 °C (El-Tanany *et al.*, 2018). Incubation continued until full growth of the fungal pathogen in the control plates. The *in vitro* antagonistic efficacy was evaluated by calculating the percentage reduction in radial mycelial growth compared to the control, following the standard method reported by Ahmed (2013).

$$\% \text{ Reduction in linear growth} = \frac{G1 - G2}{G1} \times 100$$

Where; G1: Growth of the pathogenic fungus in the control plates, G2: Growth of the pathogen in the treatment plates.

Field experiments

Inocula preparation of the fungal biocontrol agents

The BCAs were cultured on Gliotoxin fermentation (GF) broth medium and incubated for 10 d at 25 ± 2 °C under dark conditions to promote propagule production (Ahmed, 2005, 2013). After incubation, each broth culture was homogenized using an electric blender for 2 min, to obtain a uniform suspension, and the propagule concentration was adjusted to approximately 30 × 10⁶ propagule/ml. Inoculum of each bio-agent was applied at the rate of 1 L per 50 L of water.

Biofungicide

Blight Stop, a biocidal formulation containing a wild isolate of *T. harzianum* (30 × 10⁶ cfu/ml) was used for comparison with the tested fungal BCAs. It was applied at a rate of 1 L per 50 L of water and provided by the Biological Control Production Unit, Central Laboratory of Organic Agriculture (CLOA), ARC.

Field study setup

Field trials were conducted in Sadat City, Menoufia Governorate, Egypt, over two seasons (2023/2024 and 2024/2025) to evaluate an ecofriendly method used for managing tomato fruit rot. Tomato fruit-rot pathogen was naturally present in the used soil. Experiments used three replicated plots in a completely randomized block design, each plot measuring 112.5 m² with three ridges (25 × 1.5 m) separated by 50 cm (Ahmed *et al.*, 2022). Tomato seeds (Super Strain B hybrid) were sown in a nursery foam tray on 1st September and grown seedlings with 3–4 true leaves were transplanted 45 d later on 15th October (Arafa *et al.*, 2022). Each ridge received 50 transplants of the Super Strain B hybrid, identified as highly susceptible to fruit rot based on prior pathogenicity tests. During the 2023/2024 and 2024/2025 growing seasons, composts were applied to the field soil at 1.2 kg/m² and irrigated daily. After 15 d, Super Strain B tomato transplants were planted (Ahmed *et al.*, 2022).

Treatment of seedlings

Sixty-day-old seedlings were subjected to preharvest individual treatment using diluted (1:50) suspensions of *T. album*, *T. atroviride*, *T. harzianum*, and *T. viride* (30 × 10⁶ cfu/ml). The commercial biofungicide Blight Stop (*Trichoderma* spp.) was applied individually and supplemented with 5% Arabic gum and 5% potassium soap to enhance adhesion, where the mixture was allowed to stand for 10 min. prior to application

(Ahmed *et al.*, 2022). Treatments were applied as foliar sprays twice at 21-d intervals, starting on 1st of November during the 2023/2024 and 2024/2025 growing seasons, while untreated plants served as the control (El-Tanany *et al.*, 2018). Each treatment had three replicated plots, each consisting of three ridges, with a regular drip irrigation and standard organic practices. Field conditions were approximately 25 ± 5°C with 60 ± 10% relative humidity.

Disease assessment and different crop parameters

Data collection

At the end of each growing season, three plants were randomly sampled from each plot for laboratory analysis. Recorded parameters included fruit rot disease incidence, plant survival after 90 d of transplanting, and disease severity assessed 28 d after treatment (Ahmed *et al.*, 2023).

Disease severity (DS %) was measured using the scale of Poly and Srikanta (2022) and calculated as follows:

$$\text{Disease severity (\%)} = \frac{\sum nxr}{5N} \times 100$$

Where n is the number of plants in each rating, r is the maximum rating (5), and N is the total number of plants.

The percentages of disease incidence and efficacy of each bio-agent were calculated according to Ahmed *et al.* (2023); Shi *et al.* (2023) using the following equations:

$$\text{Disease incidence (DI \%)} = \frac{\text{No. of rotted fruits}}{\text{Total No. of examined fruits}} \times 100$$

Also, % efficacy for each bio-agent was calculated as follows:

$$\text{Efficacy of bioagent treatment(\%)} = \frac{\text{Mean of control disease parameters} - \text{Bio-agent treatment}}{\text{Mean of control disease parameters}} \times 100$$

Different agricultural measures and analyses

Yield measurements

Yield components were assessed by recording the number of fruits per plant, average fruit weight (g/fruit), and total fruit yield (g/plant) from three randomly selected replicate per treatment (Ahmed *et al.*, 2022).

Total proteins content

Total protein content was determined in tomato leaf

tissues collected from three randomly selected plants per treatment, with each plant representing one biological replicate. Protein content was measured using the Bradford (1976) method.

Vitamin C content

Vitamin C content was determined in tomato fruits collected from three randomly selected plants per treatment, with each plant representing one biological replicate. Ascorbic acid content was determined by its ability to decolorize 2,6-dichlorophenol-indophenol dye, with absorbance measured at 518 nm (AOAC, 2005; Ofor *et al.*, 2015).

Total carbohydrates content

Total carbohydrate content was determined in tomato fruits collected from three randomly selected plants per treatment, with each plant representing one biological replicate. Carbohydrates were quantified using the phenol-sulfuric acid method after extraction with HClO₄. Absorbance was measured at 490 nm and expressed as µg glucose/g fresh weight (Dubios *et al.*, 1956; Crompton and Birt, 1967).

Total soluble solids (TSS)

Total soluble solids (TSS) were determined in tomato fruit juice using a digital refractometer and expressed as °Brix, with three biological replicate per treatment. (Tigchelaar, 1986).

Total phenolics and flavonoids

Total phenolic and flavonoid contents were determined in tomato leaf tissues collected from three randomly selected plants per treatment, with each plant representing one biological replicate. Total phenolics were estimated using the Folin–Ciocalteu method (Singleton *et al.*, 1999), while total flavonoids were determined using the aluminium chloride colorimetric method (Rice-Evans *et al.*, 1996).

Enzyme activity

Enzyme activities were determined in tomato leaf tissues obtained from three randomly selected plants per treatment (n= 3). Crude enzyme extracts were prepared from fresh leaf tissues according to several established protocols. Briefly, 1 g of fresh leaf tissue was homogenized in chilled potassium phosphate buffer (0.1 M, pH 7.0) using a pre-cooled mortar and pestle. The homogenate was centrifuged at 10,000–12,000 × g for 15–20 min. at 4 °C. The resulting supernatant was collected and used as the crude

enzyme extract for subsequent biochemical assays. Polyphenol oxidase (PPO), peroxidase (PO), chitinase, and β -1,3-glucanase activities were determined using standard spectrophotometric methods as described by Ishaaya (1971); Hammerschmidt *et al.* (1982); Boller and Mauch (1988); Ni *et al.* (2001); Sun *et al.* (2006), respectively. Enzyme activities were expressed as activity units per gram of fresh tissue per unit time ($\Delta A \text{ min}^{-1} \text{ g FW}^{-1}$), according to the respective assay procedures.

Statistical analyses

The collected data were subjected to analysis of variance (ANOVA). The treatment means were compared using the least significant difference (LSD) test at the 5% probability level ($p \leq 0.05$), according to Snedecor and Cochran (1989).

Results

Isolation, purification, and identification of fungi from rotted tomato fruits

A total 85 fungal isolates recovered from rotten tomato fruits in Menoufia Governorate, Egypt, were purified and identified as *A. solani* and *A. alternata* based on their cultural, morphological and microscopic characteristics (Table 1). The findings revealed that Sadat City had the highest incidence of *Alternaria* spp., with recorded frequencies of 36.36 for *A. solani* and 40.00 for *A. alternata*, followed by Kafr Dawoud (33.33 and 36.00, respectively). In general, *A. solani* was more prevalent (33 isolate) than *A. alternata* (25 isolate). Consequently, Sadat City was selected as the main study site for evaluating the different eco-friendly biocontrol strategies against tomato fruit rot.

Table 1: Frequency (%) of *Alternaria* spp. isolated from rotten fruits of tomato collected during 2023/24 growing season.

Different locations	Frequency of <i>Alternaria</i> spp.			
	<i>A. solani</i>		<i>A. alternata</i>	
	No.	(%)	No.	(%)
Ashmoun district	3	9.09	2	8.00
Bagour district	7	21.21	5	20.00
Birkat El Sabaa district	4	12.12	3	12.00
Kafr Dawood	11	33.33	9	36.00
Sadat city	12	36.36	10	40.00
Total	33	-	25	-

Pathogenicity assessment and susceptibility evaluation of tomato cultivars to early blight disease

Data presented in Table 2 indicate that all *Alternaria* spp. caused typical early blight symptoms with variable disease incidence and severity among the tested tomato cultivars. *A. solani* was the most virulent pathogen, especially the Super strain B hybrid, which exhibited the highest disease incidence (96.3%) and severity (34.36%). In contrast, the other cultivars showed a comparatively lower susceptibility, with Super Alteray recording the lowest incidence (64.2%) and severity (15.35%) when treated with *A. solani*. Overall, due to the high virulence of *A. solani* and the high susceptibility of the Super strain B hybrid, both were selected as the model pathosystem for subsequent experiments aimed to evaluating the effective biocontrol strategies.

Table 2: Pathogenicity assessment and susceptibility evaluation of tomato cultivars to early blight disease under greenhouse conditions.

Different tomato cultivars (cv.)	Sadat city			
	<i>A. solani</i>		<i>A. alternata</i>	
	Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)
Super stain B hybrid	96.3	34.36	86.0	43.0
Super strain Bnormal	76.1	20.50	63.3	14.1
Super alteray	64.2	15.35	49.4	9.3
Castel rock	81.0	27.30	69.5	16.2
Elisa	68.4	21.40	53.2	11.4
L.S.D. _{0.05}	Cultivars (cv.)=1.29		Isolates (I)= 1.31	cv.×I= 2.62

The mean values were calculated from three randomly selected replicate for each treatment.

In vitro inhibitory potential of the selected antagonists on *Alternaria solani*

Data presented in Table 3 indicate the *in vitro* assay used to assess the antagonistic potential of several *Trichoderma* spp. bio-agents against the mycelial growth of *A. solani*, revealed that all tested antagonists substantially suppressed the fungal development with varying efficiencies. Among them, *T. album* exhibited the strongest inhibitory effect, achieving 90.8% growth reduction, followed closely by *T. atroviride* (88.72%). The commercial bio-fungicide Blight Stop (*T. harzianum*) displayed a moderate effectiveness (78.32%), while *T. harzianum* demonstrated a comparatively lower inhibition (72.18%). *T. hamatum* was the least effective antagonist, though it still

provided a considerable reduction in fungal growth (69.16%), indicating that all bio-agents possessed varying degrees of biocontrol potential against the fungal pathogen.

Table 3: Inhibitory effects of *Trichoderma* spp. bio-agents on the inhibition (%) of *Alternaria. solani* linear growth after 5 d of incubation at 25±2°C.

Antagonists	<i>A. solani</i>	
	Linear growth (mm)	Reduction (%)
<i>T. album</i>	8.7	90.28
<i>T. atroviride</i>	10.1	88.72
<i>T. hamatum</i>	27.6	69.16
<i>T. harzianum</i>	24.9	72.18
Blight stop (<i>T. harzianum</i>)	19.4	78.32
Control untreated	89.5	0.00
L.S.D at 1% for: Pathogenic fungus (P)	= 0.52	A×P=1.20
Antagonists (A)	= 0.68	

The mean values were calculated from three randomly selected replicate for each treatment.

Field experiments

Effect of bio-agent's treatment on the management of tomato fruit rot under field conditions

Results presented in Table 4 reveal that all tested biological agents *T. album*, *T. atroviride*, *T. harzianum*, *T. viride*, and the commercial bio-product Blight Stop effectively reduced tomato fruit rot compared to the untreated control under field conditions during the 2023/24 and 2024/25 growing seasons. The degree of disease suppression differed among the applied treatments but remained consistent across both seasons. *T. album* showed the highest efficacy,

achieving a disease control (%) of 73.56% and 74.76%, respectively. This was followed by *T. atroviride*, which also demonstrated a strong performance with 70.60% and 71.76% control. The bio-preparation Blight Stop provided a moderate disease reduction, recording 64.76% and 66.76% across the two seasons. In contrast, *T. hamatum* exhibited the lowest effectiveness, achieving 58.12% and 59.07% disease control only. These findings highlight the superior performance of *T. album* and *T. atroviride* as promising biocontrol agents for managing tomato fruit rot under field conditions.

Influence of biological treatments on tomato yield parameters under field trials during 2023/24–2024/25 growing seasons

The data presented in Table 5 clearly demonstrate that application of the tested antagonistic fungi and Blight Stop at a dilution of 1:50, considerably improved tomato yield parameters, with more pronounced effects observed during the 2023–2024 growing season than during the 2024/2025 season. Among the evaluated treatments, *T. album* exhibited the highest efficacy, markedly improving yield components, with 47.3 fruits/ plant, an average fruit weight of 104.4 g, and a total fruit yield of 4938.12 g/ plant. In contrast, *T. hamatum* showed the lowest improvement in yield, recording 37.1 fruits/ plant, an average fruit weight of 90.3 g, and a total fruit yield of 3729.39 g/ plant. Meanwhile, *T. atroviride*, Blight Stop, and *T. harzianum* displayed intermediate effects on yield attributes, producing 44.8, 41.3, and 39.3 fruits/ plant, with average fruit weights of 102.4, 101.0, and 95.3 g, and total fruit yields of 4587.52, 3747.10, and 3745.29 g/ plant, respectively, during the 2023/2024 growing season.

Table 4: Effect of treating tomato plants with different bio-agents under field conditions during the growing seasons (2023/24–2024/25).

Different treatments	2023/24 growing season				2024/25 growing season			
	Disease incidence %	Disease severity %	Mean	Efficacy* %	Disease incidence %	Disease severity %	Mean	Efficacy* %
<i>T. album</i>	15.4	4.3	9.85	73.56	14.2	4.3	9.25	74.76
<i>T. atroviride</i>	16.7	5.2	10.95	70.60	15.5	5.2	10.35	71.76
<i>T. hamatum</i>	24.1	7.1	15.6	58.12	22.9	7.1	15	59.07
<i>T. harzianum</i>	22.5	7.7	15.1	59.46	21.3	7.7	14.5	60.44
Blight stop (<i>T. harzianum</i>)	18.2	6.5	12.35	66.85	17.0	6.5	11.75	67.94
Control	59.1	15.4	37.25	0.00	57.9	15.4	36.65	0.00
LSD at 5%	2.83	0.88			2.81	0.88		

Where; *Efficacy of biological treatments (%) = [(Mean of Control disease parameters – Treatment)/ Mean of Control disease parameters)] ×100. The mean values were calculated from three randomly selected replicate for each treatment.

Table 5: Effectiveness of different bio-agent's treatment applications on tomato yield parameters under field conditions during 2023/24 and 2024/25.

Different treatments	2023/24 growing season			2024/25 growing season		
	No. of fruit/ plant	Average fruit weight/ (g/fruit)	Fruit yield (g/plant)	No. of fruit/ plant	Average fruit weight/ (g/fruit)	Fruit yield (g/plant)
<i>T. album</i>	47.3	104.4	4938.12	45.3	102.4	4638.72
<i>T. atroviride</i>	44.8	102.4	4587.52	42.8	100.4	4297.12
<i>T. hamatum</i>	37.1	90.3	3729.39	37.3	88.3	3293.59
<i>T. harzianum</i>	39.3	95.3	3745.29	39.3	93.3	3666.69
Blight stop (<i>T. harzianum</i>)	41.3	101.0	3747.10	41.4	99.0	4098.60
Control	13.4	45.6	1979.04	10.7	43.6	466.52
LSD at 5%	0.88	2.12	10.22	0.77	2.10	10.12

The mean values were calculated from three randomly selected replicate for each treatment.

During the 2024/2025 growing season, *T. album* demonstrated the highest efficacy in enhancing tomato yield components, producing the greatest number of fruits/ plant (45.3), highest average fruit weight (102.4 g), and consequently the highest total fruit yield (4638.72 g/ plant). In contrast, *T. hamatum* exhibited the lowest efficacy, recording 37.3 fruits/ plant, an average fruit weight of 88.3 g, and a total fruit yield of 3293.59 g/ plant. Intermediate effects were observed with *T. atroviride*, Blight Stop, and *T. harzianum*, which recorded 42.8, 41.4, and 39.3 fruits/ plant, respectively. Their corresponding average fruit weights were 100.4, 99.0, and 93.3 g, while total fruit yields reached 4297.12, 4098.60, and 3666.69 g/ plant, respectively. Overall, these findings indicate that *T. album* was the most effective treatment for improving the major yield attributes of tomato in the 2024/2025 growing season.

Evaluation of different biological treatments on tomato fruit quality under field conditions throughout the two growing seasons (2023/24–2024/25)

The analyses presented in Table 6 clearly indicate that all applied treatments remarkably enhanced tomato fruit quality parameters, compared to the untreated control across both 2023/2024 and 2024/2025 growing seasons. The assessed quality attributes were Total soluble solids (TSS), vitamin C content, protein content, and total carbohydrates, which are critical indicators of both the nutritional value and market acceptability of the tomato fruits. Among the tested treatments, *T. album* demonstrated the highest efficacy, consistently recording superior values for all measured parameters in both seasons. Specifically, TSS reached 6.7% and 4.7%, vitamin C content increased to 47.2 and 44.95 mg/100 g fresh

weight (FW), protein content reached 4.35 and 4.20 g/100 g FW, and total carbohydrates were 35.82 and 33.82 mg/100 g FW during the first and second seasons, respectively. The second most effective treatment was *T. atroviride*, which also produced notable improvements in fruit quality. It recorded TSS values of 6.4% and 4.4%, vitamin C contents of 44.7 and 42.60 mg/100 g FW, protein levels of 3.75 and 3.60 g/100 g FW, and total carbohydrates of 34.50 and 32.50 mg/100 g FW in the two respective seasons. These obtained results suggested a strong and consistent role of certain *Trichoderma* species in enhancing the biochemical composition of the tomato fruits. Blight Stop (*T. harzianum*) ranked the third among the evaluated treatments in terms of biocontrol efficacy and also produced notable improvements in fruit quality. It recorded TSS values of 6.3% and 4.3%, vitamin C contents of 44.10 and 41.90 mg/100 g FW, protein levels of 3.40 and 3.25 g/100 g FW, and total carbohydrate contents of 33.67 and 31.67 mg/100 g FW during the 2023–2024 and 2024–2025 seasons, respectively. In contrast, *T. hamatum* exhibited the lowest effectiveness among the biological treatments. Overall, the current findings emphasize the superiority of *T. album*, followed by *T. atroviride*, in improving tomato fruit quality under field conditions, highlighting their potential as effective ecofriendly biostimulants in sustainable tomato production systems.

Influence of biological treatments on the activity of tomato leaf flavonoids and total phenols under field conditions during two growing seasons (2023/24–2024/25)

The data presented in Table 7 clearly display that all applied treatments considerably enhanced the accumulation of flavonoids and total phenolic

Table 6: Evaluation of the effect of different bio-agent's treatments on tomato fruit quality under field conditions throughout two growing seasons (2023/24–2024/25).

Different treatments	2023/24 growing season				2024/25 growing season			
	TSS*	Vitamin C (mg/100g FW)	Protein (g/100g FW)	Carbohydrate (mg/g FW)	TSS*	Vitamin C (mg/100g FW)	Protein (g/100g FW)	Carbohydrate (mg/g FW)
<i>T. album</i>	6.7	47.2	4.35	35.82	4.7	44.95	4.20	33.82
<i>T. atroviride</i>	6.4	44.7	3.75	34.50	4.4	42.60	3.60	32.50
<i>T. hamatum</i>	6.0	39.8	2.08	27.97	4.0	37.90	1.93	25.97
<i>T. harzianum</i>	6.2	40.6	2.65	28.85	4.2	38.53	2.50	26.85
Blight stop (<i>Trichoderma</i> spp.)	6.3	44.1	3.40	33.67	4.3	41.90	3.25	31.67
Control	3.7	20.0	0.85	14.35	1.7	18.00	0.70	12.35
LSD at 5%	0.22	2.14	0.12	1.32	0.18	2.12	0.11	1.29

Where; *TSS: Total soluble solids. The mean values were calculated from three randomly selected replicate for each treatment.

Table 7: Influence of bio-agent's treatments on the activity of tomato leaf flavonoids and total phenols under field conditions during the two growing seasons (2023/24–2024/25).

Different treatments	2023/24 growing season		2024/25 growing season	
	Flavonoids	Total phenols	Flavonoids	Total phenols
<i>T. album</i>	55.0	200.2	53.0	198.2
<i>T. atroviride</i>	50.9	196.0	48.9	194.0
<i>T. hamatum</i>	42.9	171.5	40.9	169.6
<i>T. harzianum</i>	44.4	181.4	42.4	179.4
Blight stop (<i>T. harzianum</i>)	44.9	186.3	42.9	184.3
Control	30.0	126.0	28.0	124.0
LSD at 5%	1.33	2.22	1.32	2.11

The mean values were calculated from three randomly selected replicate for each treatment.

compounds in tomato leaves, compared to the untreated control during both the 2023/2024 and 2024/2025 growing seasons. These biochemical parameters are key indicators of induced plant defense and antioxidant capacity. Among the tested treatments, *T. album* exhibited the highest stimulatory effect, recording flavonoid and total phenol contents of 55.0% and 200.20% in the first season, and 53.0% and 198.20% in the second season, respectively. This superior performance highlights its strong role in activating plant secondary metabolism and enhancing resistance mechanisms. Treatment with *T. atroviride* ranked the second, which showed a noticeable increase in flavonoid and total phenol contents, recording 50.9% and 196.0% in the first season, and 48.9% and 194.00% in the second season,

respectively. Blight stop (*T. harzianum*) as a biocide came at the third effective treatment compared to the bio-agents, recording flavonoid and total phenol contents of 44.9% and 186.3% in the first season, and 42.9% and 184.30% in the second season, respectively. In contrast, the remaining treatments produced moderate improvements. *T. hamatum* showed the least influence on flavonoid content (42.9% and 40.9%) and phenolic accumulation (171.5% and 169.6%) during the two growing seasons 2023/24 and 2024/25, respectively. Overall, the obtained results confirmed the effectiveness of *Trichoderma* spp., particularly *T. album*, in promoting the biochemical defense responses in the treated tomato plants.

Impact of different treatments on tomato plant enzyme responses in the field throughout two growing seasons (2023/24–2024/25)

The results presented in Table 8 clearly indicate that all evaluated treatments remarkably enhanced the activities of key defense-related enzymes, mainly PO, PPO, chitinase, and β -1,3-glucanase in tomato leaves, compared to the untreated control during both 2023/2024 and 2024/2025 growing seasons. These enzymes play crucial roles in plant defense mechanisms against pathogens infection. Among the tested treatments, *T. album* displayed the highest activities of all the evaluated defense-related enzymes during both growing seasons, recording PO, PPO, chitinase, and β -1,3-glucanase activities of 219.00 ΔA min./ g FW, 13.25 ΔA min./ g FW, 28.50 μmol N-acetylglucosamine min./ g FW, and 5.95 μmol glucose equivalents min./ g FW, respectively, in the first season. The corresponding activities in the second season were 217.00 ΔA min./ g FW, 11.95 ΔA min./

g FW, 27.20 μmol N-acetylglucosamine min./ g FW, and 4.92 μmol glucose equivalents min./ g FW, respectively. This superior performance reflected its strong ability to induce systemic resistance and enhance plant biochemical defenses. Treatment with *T. atroviride* ranked the second, exhibiting comparatively high activities of the evaluated defense-related enzymes. In the 2023/2024 season, PO, PPO, chitinase, and β -1,3-glucanase activities reached 210.00 ΔA min./ g FW, 13.06 ΔA min./ g FW, 25.90 μmol N-acetylglucosamine min./ g FW, and 5.45 μmol glucose equivalents min./ g FW, respectively. The corresponding values in 2024/2025 were 208.00 ΔA min./ g FW, 11.76 ΔA min./ g FW, 24.60 μmol N-acetylglucosamine min./ g FW, and 4.42 μmol glucose equivalents min./ g FW, respectively.

Blight Stop ranked the third among the evaluated treatments, expressing comparatively high activities of the assessed defense-related enzymes. During the 2023/2024 season, PO, PPO, chitinase, and β -1,3-glucanase activities reached 208.00 ΔA min./ g FW, 13.05 ΔA min./ g FW, 24.85 μmol N-acetylglucosamine min./ g FW, and 5.22 μmol glucose equivalents min./ g FW, respectively. In the 2024/2025 season, the corresponding activities were 206.00 ΔA min./ g FW, 11.75 ΔA min./ g FW, 23.55 μmol N-acetylglucosamine min./ g FW, and 4.19 μmol glucose equivalents min./ g FW, respectively. In contrast, *T. hamatum* exhibited the lowest activities of the evaluated defense-related enzymes among the tested treatments. During the 2023/2024 season, PO, PPO, chitinase, and β -1,3-glucanase activities reached 190.00 ΔA min./ g FW, 12.80 ΔA min./ g FW, 20.00 μmol N-acetylglucosamine min./ g FW, and 4.20 μmol glucose equivalents min./ g FW, respectively. The corresponding activities during the

2024/2025 season were 188.00 ΔA min./ g FW, 11.50 ΔA min./ g FW, 18.70 μmol N-acetylglucosamine min./ g FW, and 3.17 μmol glucose equivalents min./ g FW, respectively. The remaining treatments induced moderate increases in enzyme activities relative to the untreated control. Overall, these findings demonstrate the capacity of *Trichoderma* spp. to enhance defense-related enzymatic responses in tomato plants under field conditions, with *T. album* exhibiting the most pronounced stimulatory effect.

Discussion

In recent years, it has become evident that extensive use of the chemical pesticides in agriculture poses serious risks to both the environment and human health due to their high toxicity. These chemicals disrupt the natural biological balance, leading to the emergence of new pest species, cause a decline in populations of beneficial natural enemies, and the accumulation of harmful residues within the human food chain (Ahmed et al., 2022; Daraban et al., 2023).

Fungal pathogen's isolation from rotten tomato fruits in Menoufia Governorate led to the recovery of *A. solani* and *A. alternata* as the predominant pathogens, with *A. solani* occurring more frequently due to its stronger virulence and adaptation to tomato tissues (Rotem, 1994; Agrios, 2005). Both species are widely distributed and are the major contributors to fruit rot and postharvest losses, particularly under favourable environmental conditions such as high humidity and moderate temperatures (Thomma, 2003; Chaerani and Voorrips, 2006; Thomidis et al., 2023). Previous studies confirmed the dominance of *Alternaria* spp. in infected tomato fruits, often representing a large

Table 8: Effect of treating tomato with bio-agents on the activity of peroxidase (PO), polyphenol oxidase (PPO), chitinase and β -1,3 glucanase enzymes under field conditions during 2023/24 and 2024/25 growing seasons.

Different treatments	2023/24 growing season				2024/25 growing season			
	PO	PPO	Chitinase	β -1,3 glucanase	PO	PPO	Chitinase	β -1,3 glucanase
<i>T. album</i>	219	13.25	28.50	5.95	217	11.95	27.20	4.92
<i>T. atroviride</i>	210	13.06	25.90	5.45	208	11.76	24.60	4.42
<i>T. hamatum</i>	190	12.80	20.00	4.20	188	11.50	18.70	3.17
<i>T. harzianum</i>	203	12.90	22.50	4.83	201	11.60	21.20	3.80
Blight stop (<i>T. harzianum</i>)	208	13.05	24.85	5.22	206	11.75	23.55	4.19
Control	158	5.95	7.50	0.50	156	4.65	6.20	0.40
LSD at 5%	2.62	0.14	0.24	0.11	2.44	0.12	0.22	0.10

Where: PO: Peroxidase, PPO: Polyphenol oxidase. The mean values were calculated from three randomly selected replicate for each treatment.

proportion of the fungal population (Gherbawy *et al.*, 2018; Khaskheli *et al.*, 2021). Their prevalence is attributed to their ecological adaptability, rapid growth, and ability to produce enzymes and mycotoxins that enhance tissue colonization and deterioration (Bessadat *et al.*, 2017; Habib *et al.*, 2021), highlighting the need for effective management strategies (Nazarbekova *et al.*, 2025).

Pathogenicity tests of the isolated *Alternaria* spp. confirmed their strong virulence on tomato, typically producing early blight symptoms such as concentric leaf spots, stem lesions, and fruit rot, in consistence with the previous studies reported by Rotem (1994); Agrios (2005). These results fulfilled Koch's postulates, verifying that these obtained isolates were the causal agents of the disease, in agreement with Chaerani and Voorrips (2006). Tomato cultivars showed a variable susceptibility, reflecting the substantial role of host genetics in disease development, as also noted by Roy *et al.* (2019). The resistant cultivars limit the microbial infection through several structural and biochemical defenses, while environmental factors particularly high humidity, moderate temperatures (20–30 °C), and prolonged leaf wetness may enhance the disease severity (Razdi *et al.*, 2021). Additionally, differences in virulence among the current isolates were observed, with *A. solani* generally more aggressive than *A. alternata*, probably due to its production of cell wall-degrading enzymes and toxins that facilitate host tissue colonization (Singh *et al.*, 2019; Iqbal *et al.*, 2023).

The current discussion confirmed that all tested bio-agents effectively suppressed *A. solani*, with *Trichoderma* spp. *i.e.* *T. album*, *T. atroviride*, *T. harzianum*, and *T. viride* demonstrated a superior biocontrol performance through multiple suggested mechanisms, including mycoparasitism, antibiosis, competition, induction of plant defense responses, and the secretion of lytic enzymes (*i.e.*, chitinases, glucanases, proteases) and antifungal metabolites (Harman *et al.*, 2004). The observed high efficacy of *T. album* and *T. atroviride* was consistent with previous studies, which displayed that *Trichoderma* spp. produced antifungal metabolites and cell wall-degrading enzymes, which inhibited pathogen growth and enhanced host resistance (Vinale *et al.*, 2008). Additionally, the relatively lower efficacy observed for *T. harzianum* and *T. hamatum* supported the concept that biocontrol efficiency is a strain-dependent and

is influenced by genetic and environmental factors affecting enzyme production and adaptability (Benítez *et al.*, 2004). Commercial formulations such as Blight Stop displayed a moderate effectiveness, supporting their role as ecofriendly alternatives despite their slightly lower activity (Ahmed *et al.*, 2022; Zhang *et al.*, 2022). Overall, these obtained results aligned with several previous studies emphasizing the importance of *Trichoderma* spp. as highly effective biocontrol agents in sustainable plant disease management (Harman, 2006; Singh *et al.*, 2019; Karthika *et al.*, 2020).

The present field results clearly demonstrated that all tested *Trichoderma* spp. based treatment's considerably reduced tomato fruit rot compared to the untreated control, confirming their effectiveness as biological control agents under natural conditions. The superior performance of *T. album*, which achieved the highest disease reduction across both tested seasons, may be attributed to its strong rhizosphere competence, rapid colonization, and ability to produce a wide range of antifungal metabolites (Harman *et al.*, 2004). These include secondary compounds and hydrolytic enzymes that directly inhibit pathogen growth and enhance plant defense responses. Similar findings were reported by Harman (2006), who described *Trichoderma* spp. as highly effective biocontrol agents due to their combined mechanisms of mycoparasitism, antibiosis, and induction of systemic resistance. Furthermore, Vinale *et al.* (2008) emphasized that certain *Trichoderma* strains can stimulate plant metabolism and improve resistance against fungal pathogens under both greenhouse and field conditions.

The current obtained results indicated that all tested *Trichoderma*'s-based treatments remarkably improved tomato yield parameters under field conditions, with *T. album* expressing the highest effectiveness by enhancing fruit number, weight, and total yield. This superior performance was most likely due to its strong ability to promote plant growth, improve nutrient uptake, and suppress pathogens through multiple mechanisms (Harman *et al.*, 2004; Harman, 2006). These findings agree with several previous studies reporting that *Trichoderma* spp. enhance crop productivity by stimulating plant growth and inducing resistance (Hermosa *et al.*, 2012). Other treatments, including *T. atroviride*, *T. harzianum*, and the commercial product Blight Stop, expressed

moderate and variable effects. This variation may be attributed to species- and strain-specific differences in their ability to colonize plant tissues, produce bioactive metabolites, and activate plant defense mechanisms, resulting in a differential induction of the defense-related enzymes. [Benítez et al. \(2004\)](#); [Vinale et al. \(2008\)](#) reported that the biocontrol efficacy of the *Trichoderma* spp. depends strongly on the type strain along with its interaction with the host plant and the pathogen. Overall, these findings support the use of biocontrol agents, particularly *T. harzianum*, as sustainable alternatives to chemical inputs for enhancing tomato productivity ([Harman et al., 2021](#); [Ahmed et al., 2021](#); [Ahmed et al., 2022](#)).

All the tested *Trichoderma*-based treatments, including the individual biocontrol agents and the commercial *Trichoderma*-based Blight Stop product, markedly improved tomato fruit quality compared to the untreated control, as evidenced by enhanced physicochemical and nutritional attributes such as TSS, vitamin C, protein content, and carbohydrate content across both seasons. *T. album* proved to be the most effective treatment, consistently producing the highest values for all the tested quality parameters, followed by *T. atroviride*. This superior performance was likely due to the ability of *T. album* to enhance nutrient uptake, stimulate plant metabolism, and promote the biosynthesis of secondary metabolites, which improve fruit nutritional quality and reduce pathogen pressure, allowing greater allocation of resources to fruit development ([Hermosa et al., 2012](#); [Reddy et al., 2016](#)). These findings are consistent with previous studies demonstrating that *Trichoderma* spp. act as plant growth promoters and biostimulants, improving both yield and fruit quality through physiological and biochemical modulation ([Harman et al., 2004](#); [Harman, 2006](#)). Additionally, [Vinale et al. \(2008\)](#) reported that *Trichoderma* spp. can enhance plant metabolic processes and increase the accumulation of beneficial compounds in crops. The relatively lower performance of *T. hamatum* further confirmed that the efficiency of *Trichoderma* strains varies depending on their biological activity and environmental adaptability ([Benítez et al., 2004](#)). Among treatments, *T. harzianum* was the most effective due to its ability to produce phytohormones and bioactive metabolites, improve nutrient availability, and stimulate antioxidant accumulation ([Harman et al., 2004](#); [Vinale et al., 2008](#)).

Blight Stop markedly improved tomato fruit quality compared to the untreated control, as reflected by increases in TSS, vitamin C, protein, and carbohydrate contents across both growing seasons. These improvements may be attributed to the ability of *Trichoderma*-based bioproducts to stimulate plant growth, nutrient acquisition, and secondary metabolism, thereby enhancing the accumulation of nutritionally important fruit constituents ([Vukelić et al., 2021](#)). Similar improvements in tomato fruit quality following *Trichoderma* application have been reported, including enhanced TSS and ascorbic acid, supporting the potential of *Trichoderma*-based products as sustainable tools for producing higher-quality tomato fruits ([García-Latorre et al., 2025](#)). Overall, these findings support the roles of the tested bio-agents and Blight stop in improving tomato fruit quality in sustainable production systems.

All *Trichoderma* spp. and Blight stop-based treatments considerably increased flavonoid and total phenolic contents in tomato leaves compared to the untreated control, indicating an enhanced plant defense and antioxidant capacity across both seasons. *T. album* showed the strongest stimulatory effect, followed by *T. atroviride*, while *T. hamatum* was the least effective. The elevated accumulation of these secondary metabolites suggested the activation of plant defense pathways and improved resistance to stress. These findings are consistent with previous studies demonstrating that *Trichoderma* spp. can induce systemic resistance and stimulate the production of phenolics and flavonoids as part of plant defense mechanisms ([Harman et al., 2004](#); [Shoresh et al., 2010](#)). This enhancement in flavonoid and total phenolic contents was linked to the activation of plant defense mechanisms, as these secondary metabolites function as antioxidants and antimicrobial compounds that improve stress tolerance ([Lattanzio et al., 2006](#); [Hermosa et al., 2012](#)). Among treatments, *T. harzianum* displayed the highest effect due to its ability to induce systemic resistance and stimulate the phenylpropanoid pathway *via* activation of defense enzymes such as phenylalanine ammonia-lyase (PAL) ([Harman, 2006](#); [Vinale et al., 2008](#); [Ahmed et al., 2017](#); [El-Khalifa et al., 2022](#)).

All the presently evaluated *Trichoderma* spp. and Blight stop-based treatments substantially enhanced the activities of key defense-related enzymes, mainly PO, PPO, chitinase, and β -1,3-glucanase in tomato leaves, compared to the untreated control across both

growing seasons. *T. album* demonstrated the highest enzyme induction, followed by *T. atroviride*, while *T. hamatum* showed the lowest activity. The increased levels of these enzymes indicated the activation of several plant defense mechanisms, including strengthened cell walls, degradation of pathogen cell components, and enhanced resistance to infection. These obtained results are consistent with previous findings reporting that *Trichoderma* spp. induce systemic resistance by stimulating defense-related enzymes and biochemical pathways (Shoresh *et al.*, 2010; Harman *et al.*, 2021; Harman, 2024).

Conclusions and Recommendations

This study demonstrated the potential of integrated, eco-friendly biocontrol strategies for managing tomato fruit rot while improving yield, fruit quality, and plant defense responses. Among the isolated pathogens, *Alternaria solani* was more prevalent and virulent than *A. alternata*, causing a severe disease in the tomato Super Strain B hybrid, recording 96.30% incidence and 34.36% severity. All evaluated *Trichoderma* spp. and the commercial *Trichoderma*-based product Blight Stop exhibited a substantial antagonistic activity against *A. solani*, both *in vitro* and *in vivo*. *T. album* showed the greatest biocontrol efficacy, achieving 90.28% mycelial growth inhibition *in vitro* and 73.56–74.76% disease-control efficiency across the 2023/2024 and 2024/2025 growing seasons, respectively. Moreover, *T. album* consistently produced the greatest improvements in yield components, TSS, vitamin C, protein content, carbohydrates, flavonoids, total phenolics, and defense-related enzymes, including PO, PPO, chitinase, and β -1,3-glucanase. Overall, these findings confirm that *Trichoderma* spp., particularly *T. album*, represent promising sustainable and environmentally compatible alternatives to the conventional fungicides used for suppressing tomato fruit rot pathogen, and simultaneously enhance crop productivity, fruit nutritional quality, and innate defense capacity under field conditions. Based on the present findings, *T. album* is recommended as a promising eco-friendly biocontrol agent for the integrated management of tomato fruit rot caused by *Alternaria solani*. Its superior disease-suppressive activity, along with its positive effects on yield, fruit quality, and plant defense responses, recommends its potential as a sustainable alternative to the conventional fungicides.

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Novelty Statement

This study presents an integrated approach combining biological control agents and the bioproduct Blight Stop as ecofriendly alternatives to chemical pesticides used for the management of tomato diseases and improves tomato fruit quality and safety for human use.

Authors' Contributions

MFAA: Conceptualization, methodology, validation, investigation, resources, writing original draft, writing review and editing, visualization, project administration, and supervision.

HHY: Conceptualization, methodology, validation, investigation, resources, writing review and editing, visualization, and funding acquisition.

MAE-A: Methodology, formal analysis, investigation, data curation, writing original draft, writing review and editing, and visualization.

MMY: Software, data curation, and writing review and editing.

AAE: Writing original draft, statistical analysis, and writing review and editing.

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