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Using *Trichoderma harzianum* to stimulate defensive enzymes in tomato plants infected with *Alternaria solani*, which causes early blight disease, in vivo and in vitro

Sujood Mukhles Alhadethy[1], **Rajaa Fadhil Hamdi**[2*]

[1]Hadetha Secondary School for Girls, Department of Hadetha Education, Haditha, Anbar, Iraq
[2]Department of Biology, College of Science, University of Anbar, Anbar, Iraq

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Corresponding author
Rajaa Fadhil Hamdi
sc.moh_n2002@uoanbar.edu.iq

ABSTRACT

A pot experiment for the 2024–2025 agricultural season was conducted using a completely randomized design to evaluate the effectiveness of *Trichoderma harzianum* in the biological control of *Alternaria solani*, the cause of early blight on tomato plants, in vivo and in vitro. The bioactivity of the 15-day culture filtrate extracted from *T. harzianum* was assessed against pathogenic *A. solani* fungi. The dual culture assay (DCA) was calculated to evaluate the efficacy of *T. harzianum* on the pathogenic fungus in vitro. Chitinase and β-glucanase enzymes in *T. harzianum* were measured to determine their role in fungal cell degradation. To understand the role of *T. harzianum* filtrate in stimulating defensive enzymes in plants (in vivo), the activities of phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), peroxidase (POD), and catalase (CAT) were measured in tomato plants after treatment with *T. harzianum* filtrate.

The results showed that, in the dual culture assay, the largest mean diameter of *Trichoderma* was (80.00 ± 0.71) in 7 days (168 hr), while *A. solani* showed the largest diameter (25.00±0.71) in 4 days and then decreased to the lowest diameter (10.20±0.66) in 7 days. The age of *Trichoderma* filtrate and the incubation period had significant differences ($P \leq 0.05$) in *A. solani* colonies. The 15-day extracts of *T. harzianum* had significant cytotoxic effects toward A. solani. The results of chitinase and β-glucanase enzymes extracted from the culture medium of *T. harzianum* showed 2.87 and 1.68 Units/mL, respectively, compared with the control (non-*Trichoderma* cultured medium), which gave 0.021 and 0.013 Units/mL, respectively. The results also showed that the defensive enzymes in tomato plants (PAL, PPO, POD, and CAT) showed higher levels in plants treated with *Trichoderma* filtrate than in untreated plants, further supporting the role of *Trichoderma* filtrate in stimulating plant resistance. In vitro findings using several methodologies indicate that *T. harzianum* was the most effective in suppressing *A. solani* growth. It may serve as a possible biocontrol agent in the future.

Keywords: *Alternaria solani, CAT, Chitinase, POD, Trichoderma harzianum*

1 INTRODUCTION

Early blight is a common fungal disease that affects many agricultural crops, especially tomatoes and potatoes, and is caused by the genus Alternaria, such as Alternaria *solani* [1]. This disease causes significant losses in agricultural production by destroying leaves and reducing photosynthetic efficiency, thereby affecting crop growth and productivity [2]. Regular chemical fungicides provide limited protection against this disease; as a result, farmers are increasingly turning to integrated pest management strategies that combine cultural prac-

tices, resistant crop varieties, and biological controls to mitigate the impact of early blight. By employing these methods, they aim to foster healthier crops while minimizing reliance on chemical treatments [3]. To identify an environmentally safe, cost-effective method to combat this disease, attention is now focused on the use of biocontrol agents in agriculture. *Trichoderma* species, especially T. harzianum, have been widely tested and used as biocontrol agents [3]. Instead of directly controlling pathogens, bioagents use other mechanisms to enhance host resistance to disease, helping to achieve sustainable agricultural practices; consequently, awareness and research in the agricultural sector have increased recently [4]. Because the continuous use of chemical pesticides poses ecological risks, can promote pathogen adaptation, and contributes to environmental pollution, the shift toward sustainable agriculture and reduced chemical pesticide use has increased interest in biological control as an effective and environmentally friendly strategy for combating plant pathogens [5, 6]. Among the most promising bioactive agents in this field, *Trichoderma* spp. are among the most important fungal agents used to control plant pathogens through the production of antifungal compounds and the stimulation of plant immunity [7]. Studies indicate that *Trichoderma* can inhibit the growth of *A. solani* through cell wall-degrading enzymes, such as chitinases and glucanases, and can also induce systemic resistance (ISR) in infected plants. It is also easy to propagate and apply, making it an ideal choice in integrated plant disease management (IPM) programs.

This research aims to assess the effectiveness of *T. harzianum* in the biological control of early blight disease caused by *A. solani*, and to evaluate its effects on plant growth and its role in stimulating plant defense enzymes against the pathogen. This study contributes to enhancing the understanding of the role of *Trichoderma* in biological control, which may help develop sustainable strategies to protect crops and improve agricultural production.

2 MATERIAL AND METHODS

2.1 Fungal isolates

2.1.1 *Trichoderma harzianum*

The identified strain was obtained from the Mycology and Plant Pathology Research Laboratory, Department of Biology, College of Science, University of Anbar.

2.1.2 *Alternaria solani*

The identified strain was obtained from the College of Agriculture, Department of Plant Protection, University of Anbar, and is registered in GenBank under accession number pp858982.

2.2 Potato dextrose broth (PDB) preparation

The medium was prepared by adding 200 g of potato to 500 mL of distilled water, then boiling in a conical flask for 20–30 minutes. The mixture was filtered through a piece of gauze, then 20 g of dextrose was added, and the volume was brought to 1 L. The medium was distributed into conical flasks, sealed with cotton plugs, and sterilized using an autoclave at 121 °C and 15 pounds/inch for 25 minutes.

2.3 Preparation *T. harzianum* filtrate

Potato dextrose broth (PDB) was prepared for the *T. harzianum* filtrate. The medium was inoculated with a piece of active mycelium of *T. harzianum* (7 days old), then incubated in a shaking incubator at 25–28 °C and 120–150 rpm for 15 days to promote secondary metabolite production. After incubation, the culture was filtered through Whatman filter paper and centrifuged at 5000 rpm for 10 min to separate the mycelium from the filtrate.

2.4 Extraction of secondary metabolites from *T. harzianum* liquid filtrate.

The culture filtrates of *T. harzianum* collected at 15 days were extracted with ethyl acetate three times at a final ratio of 1:1. The combined organic fractions were desiccated using $MgSO_4$ and subsequently evaporated under reduced pressure at 35 °C. The red-brown residues were dissolved in methanol and stored at -21 °C until further analysis [8].

1. The filtrate was extracted with ethyl acetate (3 times) at a 1:1 ratio.
2. The combined organic fractions were dried (MgSO4).
3. Evaporation was performed under reduced pressure at 35 °C.
4. The red-to-brown residues recovered were dissolved in methanol.
5. Samples were stored at -20 °C until the analysis was done.

2.5 Effect of *T. harzianum* filtrate on the growth of the pathogenic fungus *A. solani* in vitro.

Five discs (0.5 cm diameter) were taken from the PDA on which the *Trichoderma* fungus grew and placed in each flask of PDB prepared as described above, then incubated for 15 days at 27 °C. After the incubation period, the fungal cultures were filtered using a sterile Buchner funnel under sterile conditions. The filtrate was then passed, under sterile conditions, through a Millipore filter containing filter paper with 22-micrometer holes, with each flask processed separately. Potato dextrose agar (PDA) was prepared and dispensed into 500 mL flasks. After sterilization was complete, *Trichoderma* filtrate was added at a ratio of 2 mL: 18 mL to PDA. The media containing the filtrate were poured into sterile dishes. After solidification, the plates were inoculated with 0.5 cm discs of the pathogenic fungus *A. solani* and incubated at 27 °C. When fungal growth in the control treatment (PDA without *Trichoderma* filtrate) reached the edge of the plate, growth diameter was measured by calculating the average of two perpendicular diameters from the back of the colony passing through the center of the colony [9].

2.6 Dual culture assay

The dual-culture assay was used to evaluate the inhibitory activity of *Trichoderma* spp. against the pathogenic fungus *A. solani*. The competitive capability for direct interaction among *Trichoderma* strains was assessed using dual-culture assay techniques [10]. In this assay, potato dextrose agar (PDA) medium was prepared and poured into sterile Petri dishes. The center of the first half of the dish was inoculated with a 1 cm disk taken from the edge of a five-day-old *T. harzianum* colony growing on PDA medium, while the edge of the other half of the dish was inoculated with a similar disk of *A. solani* growing on PDA medium (five days old). The dishes were incubated at 25 ± 2 °C, and colony diameters were measured after a 7-day incubation period. The degree of opposition was estimated according to a five-degree scale; the degree of antagonism was calculated after the growth of the first fungal colony in the control treatment reached the edge of the dish, according to the scale indicated in [11], consisting of five degrees:

1. The biocontrol fungus covers the entire surface of the dish, preventing the pathogenic fungus from growing.
2. The biocontrol fungus covers two-thirds of the plate's surface, while the pathogenic fungus covers the remaining third of the plate.
3. The biocontrol fungus covers half of the plate's surface, and the pathogenic fungus covers the other half of the plate.
4. The biocontrol fungus covers one-third of the plate's area, while the pathogenic fungus covers two-thirds of the plate.
5. The pathogenic fungus completely covers the dish.

2.7 Estimation of chitinase and beta-glucanase enzymes

The di-nitro salicylic acid (DNS) solution, chitin solution, beta-glucan solution, and phosphate buffer solution (pH 6) were prepared as described in [12, 13].

2.8 Czapek dox sucrose-free liquid medium

Sucrose-free liquid medium (CD) was prepared by dissolving 0.02 g of ferrous sulfate ($FeSO_4$), 0.5 g of potassium chloride (KCl), 0.5 g of magnesium sulfate ($MgSO_4 \cdot H_2O$), 1 g of potassium monohydrogen phosphate (K_2HPO_4), and 2 g of sodium nitrate ($NaNO_3$) in 1000 mL of distilled water [14].

2.9 *Trichoderma* cultivation in cd liquid medium enriched with chitin and glucan

The CD liquid medium, free of sucrose and enriched with 1% chitin, was prepared to estimate the chitinase enzyme. The medium was prepared according to [15]. Medium free of the fungus was used as a control treatment. The same method was used to prepare the glucanase enzyme stimulation medium by replacing chitin with glucan.

2.9.1 Preparation of crude enzyme filtrate

After the incubation period, the enzyme production medium was filtered through a Whatman No. 1 filter paper in a Buchner funnel to collect the filtrate, which was then centrifuged at 5000 rpm for 10 minutes. The supernatant, representing the crude enzyme, was collected and stored at 4 °C until use.

2.9.2 Estimation of chitinase activity

Chitinase enzyme activity was measured as described by [16].

2.9.3 Estimation of glucanase activity

This enzyme was estimated as above, except that the enzyme was incubated with the glucanase solution rather than the chitin solution, and the standard curve was prepared using glucose.

2.10 Pot experiments (in vivo)

A pot experiment was conducted using a completely randomized design to grow tomato plants in soil mixed with peat moss (ratio of 1:2) after autoclave sterilization. Treatments were applied to the plants by soaking and spraying with *T. harzianum* filtrate to determine its effect on plant defensive enzymes and to prevent infection by the fungus *A. solani*.

2.11 Tomato plant treatments (experiment design)

The pot experiment was conducted to evaluate the effect of *T. harzianum* filtrate (age 15 days) on defensive enzymes in tomato plants, with three replicates for each treatment, as follows:
Treatment 1 = control (tomato plant without any treatment (zero))
Treatment 2 = control (tomato plant treated with *T. harzianum* filtrate)
Treatment 3 = tomato plant infected with *A. solani*
Treatment 4 = tomato plant treated with *T. harzianum* filtrate, then infected with *A. solani* (preventive)
Treatment 5 = tomato plant infected with *A. solani* then treated with *T. harzianum* filtrate (therapeutic)

2.12 Preparation of the enzymatic plant extract

One gram of tomato root was taken for each treatment, washed well, and cut into small pieces. The root was crushed in a ceramic mortar placed in an ice bath, and 10 mL of phosphate buffer (pH 6) was added. The homogenate was filtered with filter paper, placed in tubes, and centrifuged in a refrigerated centrifuge at 4 °C and 5,000 rpm for 20 minutes to separate the filtrate from the precipitate. The plant filtrate, representing the enzymatic extract, was then collected.

2.13 Estimation of the activity of phenylalanine ammonia lyase, polyphenol oxidase, peroxidase, and catalase enzymes

The activity of phenylalanine ammonia-lyase (PAL) was determined by the rate of conversion of L-phenylalanine to cinnamic acid, as described by [17]. Enzymatic activity of polyphenol oxidase (PPO) was determined using the method described by [18]. Peroxidase (PO) activity was determined according to [19], and catalase (CAT) activity was measured using the method reported by [20].

2.14 Statistical analysis

The Statistical Package for the Social Sciences (SPSS) (2019) was used to detect the effect of differences in incubation period on study parameters. Least significant difference (LSD) (0.05) was used to compare means in this study.

3 RESULTS AND DISCUSSION

3.1 Dual culture technique

The results in Table 1 and Figure 1 indicated a significant difference ($P \leq 0.01$) in the colony diameters of *T. harzianum* and *A. solani* with increasing incubation time. The largest mean diameter of *Trichoderma* was 80.00 ±0.71 at 7 days (168 hr), while *A. solani* showed a diameter of 10.20 ±0.66 on the same day. The biocontrol fungus covered two-thirds of the Petri dish surface, while the pathogenic fungus covered the remaining third, with antagonism degrees 1 (Figure 2C) and 2 (Figure 2A, B) according to the scale of [21].

Table 1 Diameter of *Trichoderma* and *A. solani* colonies (mm) in the Dual Culture Technique (DCT)

Incubation Period (Hour)	Means ± SE	
	Diameter of *Trichoderma* (mm)	Dimeter of *A. solani* (mm)
72 (3 day)	45.00 ± 0.71e	20.00 ± 0.71 b
96 (4 day)	55.00 ± 0.71 d	25.00 ± 0.71 a
120 (5 day)	60.00 ± 1.00c	25.20 ± 0.66 a
144 (6 day)	72.20 ± 1.77 b	20.00 ± 0.71 b
168 (7 day)	80.00 ± 0.71 a	10.20 ± 0.66c
L.S.D.	3.134 **	2.035 **
P-value	0.0001	0.0001
** (P ≤ 0.01).		

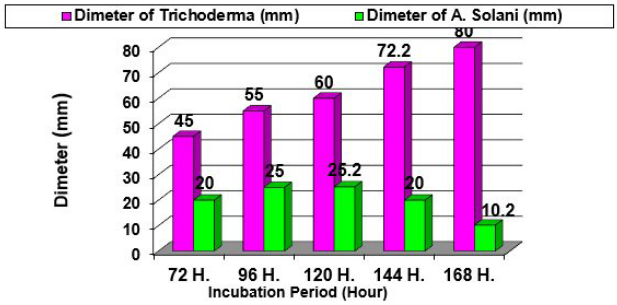

Fig. 1 Diameter of *Trichoderma* and A.*solani* Colony (mm) in Dual Culture Technique (DCT)

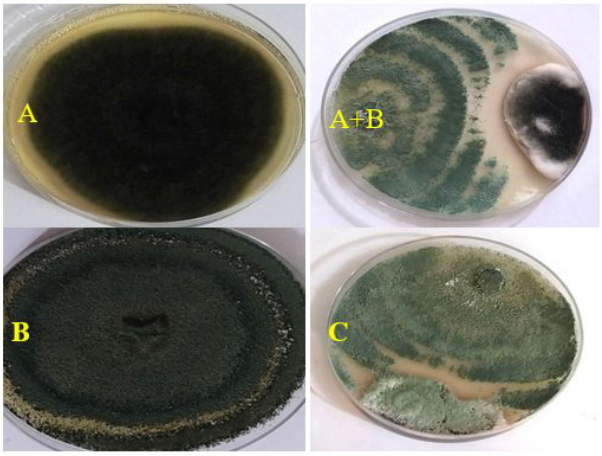

Fig. 2 Antagonistic activity of *Trichoderma* against *A. solani* in the Dual Culture Assay in vitro. (A) *A. solani*, (B) Trichoderma, (A+B) *A. solani* with Trichoderma, (C) *Trichoderma* covers all *A. solani* colonies

3.2 Effect of *trichoderma* filtrate on the growth of the pathogenic fungi *A. solani* in vitro

The age of *Trichoderma* filtrate and the incubation period had significant differences (P≤0.05) on *A. solani* colony diameter. When the filtrate age was 3 days, the largest diameter of *A. solani* was 52.00 ±1.87 mm during an incubation period of 168 h, whereas the diameter of *A. solani* decreased to 40.00 ±2.28 mm with a filtrate age of 15 days and an incubation period of 168 h, compared with the control, which gave a diameter that reached 70.00 mm (Table 2; Figures 3 and 4).

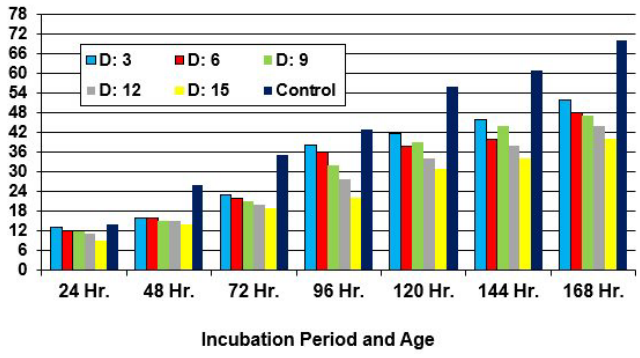

Fig. 3 Effect of Incubation Period and Age of *Trichoderma* Filtrate in *A. solani*, diameter in mm

Fig. 4 Efficacy of *Trichoderma* filtrate age on *A. solani* diameter (mm)

3.3 Chitinase and β-glucanase enzymes in trichoderma

The chitinase enzyme activity in the Trichoderma-cultured medium was significantly higher (2.87 Units/mL) than in the control (non-Trichoderma-cultured medium) (0.021 Units/mL). In addition, β-glucanase enzyme activity extracted from the culture media showed significant differences, with higher activity in Trichoderma-cultured medium (1.68 Unit/mL) compared with the control (non-*Trichoderma* cultured medium) (0.013 Unit/mL) (Table 3).

3.4 Activity of defense enzymes in the tomato plant

Table 4 shows the activity of four enzymes that are important in plant defense responses under different treatments, where enzymatic activity was measured in units (units/min/g fresh weight). These enzymes include phenylalanine ammonia lyase (PAL), an essential enzyme in the phenolic pathway that catalyzes the production of phenolic compounds involved in resistance against pathogens. Its activity was very low in treatment 1 (0.33 unit/min/g) but increased significantly in the remaining treatments, reaching the highest value in treatment 5 (4.32 unit/min/g). Polyphenol oxidase (PPO) contributes to the production of oxidized phenolic compounds that enhance plant resistance. Treatment 1 recorded the lowest PPO activity (0.13 unit/min/g), while treatment 5 showed the highest activity (0.88 unit/min/g). Peroxidase (POD) stimulates the formation of lignin and other defense molecules in cell walls, thereby enhancing resistance to pathogens. Treatment 1 recorded the lowest POD activity (1.87 units/min/g), whereas treatment 5 showed the highest activity (3.11 units/min/g). Catalase (CAT) is essential for eliminating hydrogen peroxide (H_2O_2), a product of oxidative stress. The lowest CAT value was in treatment 1 (0.45 unit/min/g), and the highest value was in

Table 2 Effect of *Trichoderma* Filtrate Age on *A. solani*

Diameter of *A. solani* (mm)							
Age of Filtrate m (day)	Incubation Period (Hour)						
	24	48	72	96	120	144	168
3	13.00 ±0.31 a	16.00 ±0.83 b	22.80 ±0.86 b	38.00 ±0.54 b	41.80 ±0.58 b	46.00 ±1.14 b	52.00 ±1.87 b
6	12.00 ±0.71ab	16.00 ±0.83 b	22.00 ±0.83 b	36.00 ±1.81 b	37.80 ±0.66c	40.00 ±2.28 cd	48.00 ±2.05bc
9	12.00 ±0.54ab	15.00 ±0.71 b	21.00 ±1.14bc	32.00 ±1.14c	39.00 ±1.58bc	44.00 ±1.41bc	47.00 ±3.39 bc
12	11.00 ±1.00ab	15.00 ±0.71 b	20.00 ±1.58bc	27.60 ±2.13 d	34.00 ±1.58 d	38.00 ±1.94de	44.00 ±1.41 cd
15	9.00 ±2.12 b	14.00 ±0.71 b	19.00 ±0.71c	22.00 ±0.71e	31.00 ±0.94 d	34.00 ±2.07e	40.00 ±2.28 d
Control	14.00 ±0.00a	26.00 ±0.00a	35.00 ±0.00a	43.00 ±0.00a	56.00 ±0.00a	61.00 ±0.00a	70.00 ±0.00a
LSD value	3.014 *	2.029 *	2.085 *	3.761 *	3.079 *	4.854 *	6.122 *
Means having with the different letters in same column differed significantly. *($P \leq 0.05$).							

treatment 5 (3.08 unit/min/g). Values at the significance level (LSD 0.05) indicate significant differences among treatments.

Table 3 Chitinase and β-Glucanase enzymes in different transaction conditions

Transactions	Chitinase (Unit/ml)	β-Glucanase (Unit/ml)
Trichoderma culture medium	2.87	1.68
Control (without-Trichoderma cultured medium)	0.021	0.013

Table 4 Enzyme activity in tomato plants treated with different treatments

Treatments	Phenylalanine ammonia lyse Unit min- $\mathbf{1g^{-1}f.w}$	Polyphenol oxidase Unit min-1g $^{-1}$**f.w**	Peroxidase Unit min-1g $^{-1}$ f.w	Catalase Unit min-1g $^{-1}$**f.w**
1	0.33	0.13	1.87	0.45
2	4.23	0.79	3.02	2.99
3	3.8	0.46	2.59	2.56
4	4.11	0.67	2.9	2.87
5	4.32	0.88	3.11	3.08
$LSD_{0.05}$	0.21	0.14	0.26	0.11

4 DISCUSSION

The present investigation revealed (Table 1, Fig. 2) that *T. harzianum* exhibited antagonism toward the *A. solani*pathogen responsible for Alternaria disease. The dual culture assay showed that *Trichoderma* isolates exhibited in vitro mycoparasitic activity against *A. solani*. Within 7 days post-inoculation, the *Trichoderma* isolates successfully overgrew and sporulated on the *A. solani* pathogen colony, with concurrent growth of these antagonistic isolates in close proximity to the pathogen. The antagonistic *Trichoderma* isolates examined in this work may serve as a source of innovative biological fungicides, particularly against Alternaria pathogens such as *A. solani*, while mitigating the adverse effects associated with chemical fungicides [22]. This may be attributed to Trichoderma's ability to compete with pathogenic fungi for nutrients in the environment, as well as to its production of chemical compounds, such as enzymes and hormones, that inhibit the pathogen. It may also alter the environment around the pathogenic fungus, leading to reduced or inhibited growth [19].

Our results in Table 2 and Figure 4 showed that *Trichoderma* filtrate inhibits and reduces *A. solani* growth in Petri dishes, likely because it contains many chemical compounds that play important roles against pathogens. Our study agreed with [23, 24], which demonstrated that certain biological control agents, specifically *Trichoderma* spp., can achieve conidial production inhibition of up to 90% against *A. solani* under laboratory conditions. Study [25] noted that the mycelial growth of *A. solani* (exceeding 90%) was significantly reduced in the presence of *Trichoderma* species (T. harzianum, T. hamatium, and T. viride) at elevated concentrations. Moreover, [26] highlighted the toxicity of *Trichoderma* spp. toward *A. solani*. The anticipated mechanisms proposed to explain antagonism include competition and antibiosis (progression, penetration, sporulation, and colonization) [27].

The effect of *Trichoderma* filtrate on *A. solani* may be related to the destruction of the fungal cell wall, leading to fungal death and inhibition of spore production due to the production of chitinase and beta-glucanase enzymes, which were detected in our study [28]. *Trichoderma* filtrate contains many enzymes, including chitinase and glucanase (Table 3), which degrade pathogenic cell walls. This result agrees with numerous studies indicating that *Trichoderma* predominantly manages root, shoot, and postharvest infections through antagonistic properties activated by several biocontrol pathways. Studies [29, 30] contend that *Trichoderma* facilitates indirect biocontrol

of fungal soil-borne diseases by competing for nutrients and space, altering environmental circumstances, enhancing plant development, strengthening plant defense mechanisms, and inducing antibiosis, or through direct biocontrol via mycoparasitism. *Trichoderma* commences the production of hydrolytic or lytic enzymes during mycoparasitic interactions, such as glucanase, chitinase, and protease, which degrade the chitin polymer in the cell wall of the fungal pathogen [31]. This agrees with our study.

Moreover, *Trichoderma* has the capacity to produce antibiotics or low-molecular-weight diffusible chemicals, including tricholin, harzianic acid, peptaibols, viridins, 6-pentyl-pyrone, and heptelidic acid [14]. The interplay of indirect and direct biocontrol processes is influenced by *Trichoderma* spp., crop plants, and environmental conditions, including nutrient availability, pH, temperature, and iron content [12]. Consequently, *Trichoderma* spp. may serve as effective biofungicides and alternative agents against soil-borne fungal infections [32].

According to this study's findings, tomato plants treated with native *Trichoderma* bioagents and then challenged with *A. solani* showed increased induction of defense-related enzymes, including PO, PPO, and PAL. These results concur with those of several researchers. Cucumber peroxidase activity was enhanced by a bioformulation of T. virens sprayed on leaves and blossoms [33]. The elevated control rate observed for the treatments can be ascribed, among other factors, to their capacity to induce the accumulation of reactive oxygen species, as demonstrated by the activation of enzymes associated with oxidative stress (catalases, peroxidase, and lipoxygenase). The buildup of reactive oxygen species can engage in defense processes, exert toxic effects on pathogens, contribute to hypersensitivity reactions, and serve as secondary signaling factors in the signal transduction cascade that activates defense genes and promotes cellular protection. Additionally, it may act as a response mechanism (peroxidase activation) in plants against *A. solani* infection [34, 35]. Analyses of catalase, peroxidase, and lipoxygenase enzyme activities revealed that the fungus filtrate contains molecules with elicitor potential, which may originate from proteins, toxins, oligosaccharides, or other components produced by microorganisms that activate the primary defense mechanisms in tomato plants [36].

The secondary metabolites produced by *Trichoderma* spp. include peptides, peptaiboles, polyketides, pironas, and volatile and non-volatile molecules [21, 37]. These compounds have a variety of functional uses, including the production of enzymes, biofungicides, plant growth promoters, proteins, pigments, and antibiotics [27]. These molecules have a variety of effects on plants, including increasing resistance, removing toxins, and deactivating enzymes produced by phytopathogens during infection. They also solubilize nutrients that are not bioavailable to plants in their elemental form [38]. *T. harzianum* has been identified as a biopesticide for many insect pests, whereas hexadecenoic acid and 7,10-octadecadienoic acid have been classified as pesticides, nematicides, and insecticides [39].

Trichoderma species are heterotrophic saprobionts that compete with other microbes, inhibiting their growth. Among their modes of action are the synthesis of metabolites and antibiotics, as well as hyperparasitism, which encourages the development of systemic resistance in plants [8]. The findings support the capacity of *T. harzianum* to trigger defensive mechanisms in infected tomato plants. Genes involved in the salicylic acid (SA) and ethylene and jasmonate (ET/JA)-mediated signaling pathways were up-regulated, indicating activation of hormone-mediated signaling; however, this may occur via promoting the expression of many genes involved in defensive responses (such as protease inhibitors, resistance proteins like CC-NBS-LRR), and hormone interaction. Harzianic acid therapy also enhanced tomato's response to infection [10].

The genus *Trichoderma* may adapt to and flourish in a variety of environmental settings. This genus has shown promise in the long-term control of fungal-induced crop diseases. *Trichoderma* species are prevalent in the rhizosphere of plants and are among the most commonly isolated soil-dwelling fungi. As parasites and antagonists of numerous phytopathogenic fungi, these opportunistic, avirulent plant symbionts shield plants from illness [11]. Organisms, including Trichoderma, Aspergillus, Penicillium, Alternaria, and Fusarium, are widely employed in biotechnological applications and organic chemistry. Their use as a paradigm for plastic biodegradation and chemical analysis has been recorded in extensive research [40].

Depending on the substrate and surrounding conditions, fungi can produce secondary metabolites. Because it facilitates the formation of metabolites, an appropriate medium for the production of bioactive secondary metabolites is crucial [41]. It is established that secondary metabolites containing aldehyde groups, particularly unsaturated aldehydes, exhibit bioactivity and have been

demonstrated to suppress seed germination, pollen germination, pathogenic fungi, and bacteria [42]. Moreover, hydrazide derivatives in *Trichoderma* contain several bioactive groups and exhibit diverse biological activities. Hydrazide has been shown to exhibit antibacterial, anticancer, antitubercular, anti-inflammatory, and antifungal properties [43].

Strategies may be created to utilize these fungi for the extraction of bioactive substances. Moreover, employing endophytes as prospective factories for the synthesis of secondary metabolites could transform agricultural, medicinal, and biotechnological research in the imminent future [13].

5 CONCLUSION

The findings indicate that these *Trichoderma* strains possess antimicrobial properties and represent potential natural sources of biologically active chemicals. In vitro findings derived from several methodologies indicate that *T. harzianum* exhibited the most effective inhibition of *A. solani* growth. The results showed that *Trichoderma* stimulated the plant defense response, leading to increased activity of enzymes involved in the production of defensive compounds and in resistance to oxidative stress. These interactions likely involved a prebiotic or a stimulatory agent that activated the plant immune system. *Trichoderma* may serve as a prospective biocontrol agent. Because *Trichoderma* demonstrated high efficacy in combating the disease and reducing its severity, and in improving plant growth by stimulating defense enzymes against the pathogen, we conclude that *Trichoderma* could be an environmentally friendly and sustainable alternative for controlling early blight in tomato plants.

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Conflict of interest

The authors declare no conflict of interest.

Consent to publish

N/A

Ethical approval

N/A

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