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Research Article

The effect of *Funeliformis mosseae* and *Trichoderma harzianum* on early blight disease in tomato

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Abstract
Early blight, caused by *Alternaria alternata*, is a major tomato disease that leads to significant economic losses. This study aimed to evaluate the effectiveness of two fungi, *Funeliformis mosseae* and *Trichoderma harzianum*, as potential biological control agents against the disease. Initially, the inhibitory effect of *T. harzianum* on the pathogen's colony growth was assessed under laboratory conditions using the dual-culture method, where *T. harzianum* reduced pathogen colony growth by 70%. Subsequently, the efficacy of *F. mosseae* and *T. harzianum*, applied individually and in combination, was tested against *A. alternata* in greenhouse conditions using a completely randomized design with three replications per treatment. Various parameters were measured, including chlorophyll a, b, and total content; total phenol; activities of catalase and peroxidase enzymes; and plant growth traits such as stem number, stem height, fresh and dry weight of aerial parts, root length, and fresh and dry weight of roots. Statistical analysis revealed that the application of *T. harzianum* and *F. mosseae* enhanced antioxidant enzyme activity (catalase and peroxidase) and phenolic compound levels, leading to improved growth and increased dry matter content in tomatoes under pathogen infection conditions.
Keywords: Peroxidase, Phenolic compounds, Dual-culture, Catalase

مقاله پژوهشی

تأثیر *Funeliformis mosseae* و *Trichoderma harzianum* بر بیماری سوختگی زودهنگام گوجه‌فرنگی

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چکیده
سوختگی زودهنگام، ناشی از *Alternaria alternata* یک بیماری مهم گوجه‌فرنگی است، که خسارت اقتصادی قابل توجهی به محصول وارد می‌کند. هدف از این پژوهش بررسی کارایی دو قارچ *Funeliformis mosseae* و

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Trichoderma harzianum برای یافتن روش مناسب مبارزه زیستی بیماری بود. بدین منظور ابتدا با روش کشت متقابل تاثیر بازدارندگی از رشد پرگنه بیمارگر توسط *T. harzianum* در شرایط آزمایشگاه بررسی شد، که *T. harzianum* باعث کاهش ۷۰ درصدی رشد پرگنه بیمارگر شد. سپس مایه‌زنی گوجه‌فرنگی با *F. mosseae* و *T. harzianum* ، به تنهایی و باهم به صورت ترکیبی علیه *A. alternata* در شرایط گلخانه در قالب طرح کاملاً تصادفی، در سه تکرار برای هر تیمار بررسی شدند. شاخص‌هایی نظیر میزان کلروفیل a، b و کل، فنل کل، آنزیم‌های کاتالاز و پراکسیداز و صفات رشدی گیاه شامل تعداد ساقه، ارتفاع ساقه، وزن تر و خشک اندام‌های هوایی، طول ریشه و وزن تر و خشک ریشه اندازه‌گیری شدند. تجزیه و تحلیل آماری داده‌های این آزمایش نشان داد که، کاربرد *T. harzianum* و *F. mosseae* با افزایش فعالیت آنزیم‌های آنتی‌اکسیدانت مانند کاتالاز و پراکسیداز و ترکیب‌های فنلی، باعث بهبود رشد و افزایش ماده خشک گوجه‌فرنگی در شرایط آلودگی به بیمارگر می‌شوند.

واژگان کلیدی: پراکسیداز، ترکیب‌های فنلی، کشت متقابل، کاتالاز

Introduction مقدمه

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop grown globally for its edible fruits. Early blight caused by *Alternaria* species is one of the major tomato diseases worldwide, causing losses of up to 86% of yield (Bavand et al. 2023). However, fungal pathogens, including *A. alternata*, can cause significant damage to tomato plants, reducing both the quantity and quality of the crop. Several *Alternaria* species, including *Alternaria solani* (Ellis and F. Martin) L.R. Jones, and *A. alternata* (Fr.) Keissl are known to cause disease (Adhikari et al. 2017). Limited soil and water resources have constrained the cultivation of tomatoes, making pest and disease control crucial for maintaining high production levels, in Iraq. *A. alternata*, the causal pathogen of tomato black spot, is a major threat to tomato crops worldwide, causing significant losses in yield (Coelho 2022). Although fungicides can be used to control this disease, their long-term effects on human health and the ecosystem are a concern (Mahmood et al. 2016). Biocontrol agents, offer a sustainable and effective alternative for controlling early blight disease in tomato plants (Lahlali et al. 2022).

The aim of this study was to evaluate the efficacy of biocontrol agents; *Funeliformis mosseae* and *Trichoderma harzianum*, individually and in combination, to combat *A. alternata* infection in tomato plants. Overall, our study highlights the potential of biocontrol agents as effective alternatives to chemical pesticides for managing early blight disease in tomato plants. By developing a suitable biocontrol protocol using *T. harzianum* and *F. mosseae*, we can reduce the use of harmful fungicides while maintaining high production levels.

Materials and Methods مواد و روش‌ها

Isolation, Purification, & Identification of fungal isolates

Thirty five fungal isolates as the causal agent of early blight of tomato were obtained from infected plants in Basra province of Iraq. The hyphal tip method were performed to purify the desired isolates. The purified isolates were then subjected to various tests to identify the causative agent of the observed disease symptoms in the tomato plants (Bavand et al. 2023).

From Basra province, 50 soil samples representing diverse soil types including; peat soil, grassland soil, and windy sandy soil in various depths (0, 10, 20, 30, 50, and 100 cm) were collected. Each sample, approximately 300 g, was secured in sterile plastic bags, promptly transported to the laboratory in a refrigerated container, and preserved at 4ºC. For the isolation of the *T. harzianum* species, a dilution technique was employed, as detailed by Elad and Chet (1981). Post the incubation, fungal mycelia were separated from the liquid medium via centrifugation at 10,000 rpm and 4°C, followed by two successive washes using distilled water. Based on distinctive morphological traits, the fungi were identified, purified, and archived in micro-tubes for future reference (Mirzaeipour et al. 2023). Separately, the *F. mosseae* isolate was provided by the Department of Plant Pathology, College of Agriculture, Basra University, Iraq.
Morphological identification of pathogen and biocontrol fungal isolates was carried out following established protocols. The identification of *T. harzianum* species was based on the guidelines provided by Gams and Bissett (2002), whereas the identification of *Alternaria* species followed the guidelines outlined by Woudenberg et al. (2015).

Molecular identification of Alternaria and Trichoderma species

DNA extraction from fungal mycelium was performed using the method described by Doyle and Doyle (1987). For ITS rDNA amplification, a 25 µl PCR reaction mixture was prepared, comprising 2.5 µl of 10X reaction buffer, 5 µl of dNTP mix, 0.3 µl of Taq polymerase, 100 ng of template DNA, 2.5 µl of $MgCl_2$, and 1 µl each of the forward primer 2AAF (5'-TGTCAGTAACTACAGATG-3') and reverse primer 3AAR (5'-ATGGATGCTAGACCTTTGCTGAT-3'). The thermal cycling process began with an initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute. The amplified PCR products were analyzed via gel electrophoresis on a 1% agarose gel run at 95 V for 1.5 hours in 1X TBE buffer. To visualize the DNA, the gel was stained with 12 µl of SYBR-safe (10,000X concentration). The bands were documented using the BioDoc Gel Documentation System UVP camera (California, USA). For sequencing the ITS1 and ITS2 regions, the PCR products were sent to Macrogen Co., South Korea. Phylogenetic analysis was conducted using MEGA11 software (Tamura et al. 2021).

Assay of the effect of T. harzianum on colony growth of A. alternata in vitro

To ascertain the antagonistic potential of *T. harzianum* against *A. alternata*, a dual-culture method was employed. Discs of 0.5 cm diameter, taken from a four-day-old PDA culture of both *A. alternata* and *T. harzianum*, were inoculated onto a fresh PDA plate, positioned approximately 1 cm from the plate's edge. The plates were subsequently incubated at 28°C for a duration of five days. This antagonistic evaluation was replicated twice, with each instance conducted in triplicate. To quantify the inhibitory effect, radial growth measurements of the examined fungal isolates were taken. The inhibition percentage was determined using the formula: Inhibition percentage = C-T/C×100, where C and T: the diameter of the fungal growth in the control and treatment, respectively.

Assay of the effect of F. mosseae and T. harzianum on early blight disease in tomato under greenhouse conditions

To assess the antagonistic potential of *F. mosseae* and *T. harzianum* against *A. alternata* in greenhouse conditions and its consequent effect on tomato plant growth variables relative to control, the following procedure was adopted: Pots with a 5 kg capacity were filled using a 1:2 ratio mixture of soil to peat moss. *T. harzianum* was propagated on sterile wheat grains, which,

after three days, were combined with soil pre-inoculated with *F. mosseae*. Subsequently, 50 g of both *F. mosseae*-inoculated and sterile millet wheat seeds were added to the pots, which were then sown with tomato seeds and irrigated as required. Once the tomato seedlings developed 5-6 leaves, they were treated with a foliar spray of the pathogen at a concentration of 10^6 spores/ml. After 50 days, parameters including; catalase activity (U mF.protein^{-1}), peroxidase activity (U mF.protein^{-1}), total phenol (µg /g), chlorophyll a (mg F/fw), chlorophyll b (mg F/fw), total chlorophyll (mg F/fw), shoot dry weight (gr), root dry weight (gr), root length (cm), number of stems, number of flowers, number of fruits, fruit weight (gr) and number of leaf spots were evaluated. The experiment was designed in a Complete Random Design (CRD) format. The seven treatments were undertaken including; *A. alternata*, *T. harzianum*, *F. mosseae, F. mosseae +A. alternata, T. harzianum + A. alternata, T. harzianum + F. mosseae + A. alternata* and control (healthy plant).

Enzymatic activity of tomato inoculated with F. mosseae, T. harzianum and A. alternata

Leaf samples were homogenized in liquid nitrogen, mixed with potassium phosphate buffer, and centrifuged at 14,000 rpm at 4°C to obtain supernatant rich in enzymes like catalase and peroxidase. For catalase activity, a mix of sodium phosphate buffer, hydrogen peroxide, and the enzyme extract was prepared and the absorbance was measured at 240 nm using a spectrophotometer. For peroxidase, a blend of potassium phosphate buffer, guaiacol, hydrogen peroxide, and enzyme extract was used, and absorbance was gauged at 290 nm.

Assay of total phenol

First, 500 mg of plant tissue were placed in 3 ml of 80% homogenized methanol in a bain-marie at 70 °C for 3 h. Then, centrifuged twice at 9000 rpm for 15 min. To 50 µl of methanolic extract, 500 µl of Folin Ciocaltio solution was added. After 5 min, 500 µl of 7% sodium carbonate solution were added. The total phenol in UV spectrophotometer was estimated at 765 nm.

Assay of chlorophyll

To measure the amount of chlorophyll, 0.2 g of the fresh leaves of the tested plants were ground in a Chinese mortar containing 15 ml of 80% acetone, after filtering with filter paper, their absorbance in UV spectrophotometer was estimated at 640, 660, and 470 nm.

Statistical analysis

Data were analyzed in a completely randomized design comprising seven treatments with three replicates each, using the Least Significant Difference (L.S.D) method. SAS software was employed to discern significant differences in enzyme activity across treatments (P = 0.05).

Results یافته‌ها

Based on morphological and molecular studies, *A. alternata* (NCBI Gen Bank No. OM976647) and *T. harzianum* (NCBI Gen Bank No. OM976643) were closely related to other *A. alternata*, and *T. harzianum* isolates (Figures 1,2).

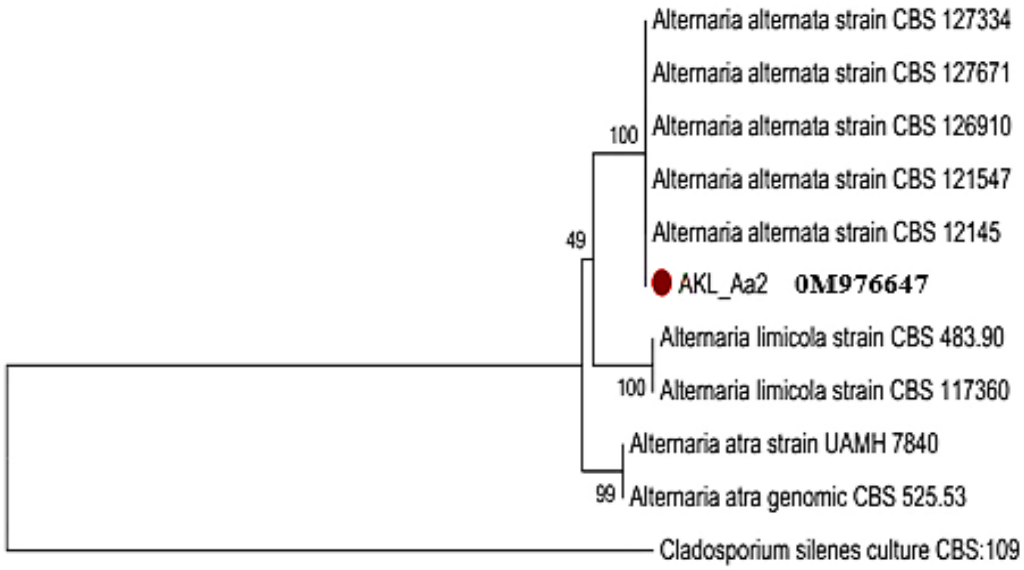

Figure 1. This phylogenetic tree illustrates the genetic relationship of *Alternaria alternata* isolate, based on ITS sequences, with other *A. alternata* isolates, and confirming the identification of the pathogen responsible of early blight disease in tomato.

شکل۱. این درخت تبارزایی رابطه ژنتیکی نزدیک، بر اساس توالی‌های ITS ، جدایه *Alternaria alternata* را با سایر جدایه‌های این قارچ نشان می‌دهد و شناسایی عامل بیماری سوختگی زودهنگام گوجه‌فرنگی را تایید می‌کند.

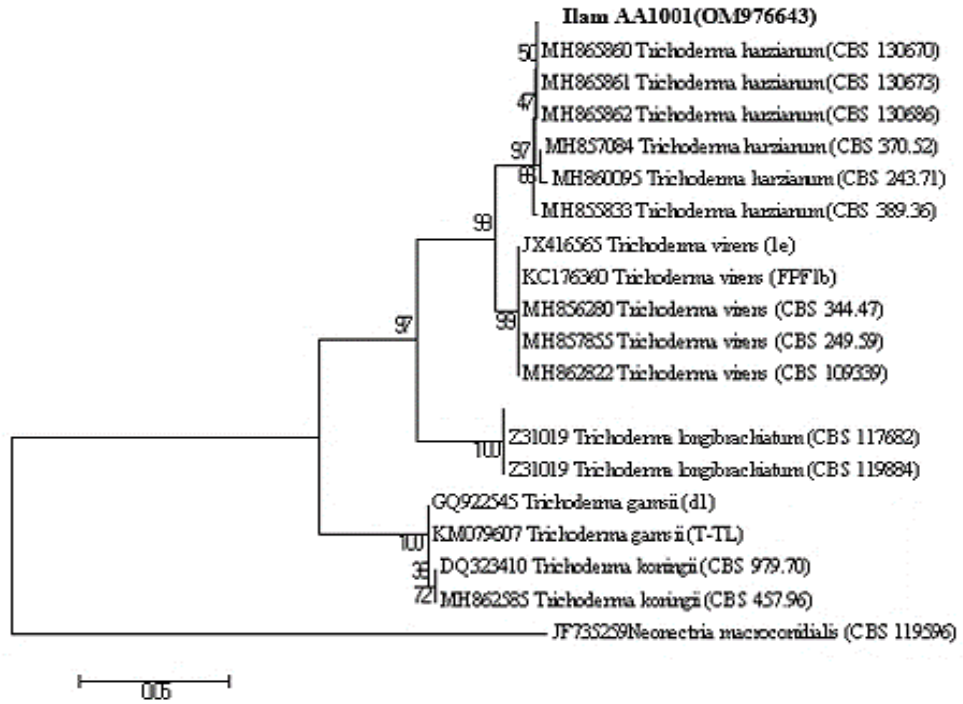

Figure 2. This phylogenetic tree presents the genetic relationship of the *Trichoderma harzianum* isolate, with other *T. harzianum* isolates.

شکل ۲. این درخت تبارزایی، شباهت ژنتیکی نزدیک جدایه *Trichoderma harzianum* را با سایر جدایه‌های این قارچ نشان می‌دهد.

The effect of T. harzianum isolate on A. alternata in vitro

The growth inhibition percentage of *A. alternata* by *T. harzianum* in dual-culture reached over 70% (Fig. 3). *T. harzianum* has been previously identified as a potent biocontrol agent with strong antagonistic properties against various pathogens (Harman et al. 2004). Its ability to reduce the growth of *A. alternata* is consistent with findings of Chaverri and Samuels (2003), where the pathogen's growth was effectively inhibited in vitro.

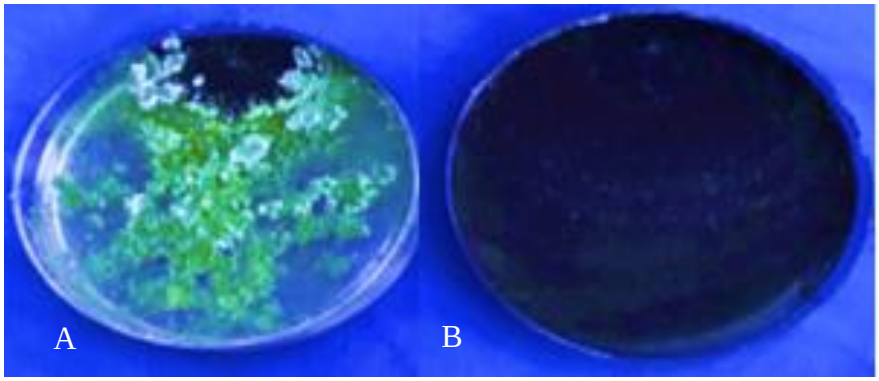

Figure 3. A. The antagonistic interaction between *Trichoderma harzianum* and *Alternaria alternata* in vitro, showing a reduction in the pathogen colony growth. B. The control, where *Alternaria alternata* grows uninhibited.

شـــکل ۳. A. بـرهمکنش بـین *Trichoderma harzianum* و *Alternaria alternata* را در شـرایط آزمایشـگاهی نشـان می‌دهد که کاهش رشد پرگنه بیمارگر مشاهده می‌شود. B . شاهد، جایی که *Alternaria alternata* بدون مهار رشد می‌کند.

The effect of F. mosseae and T. harzianum on early blight disease in tomato under greenhouse conditions

Enzymatic Activity

Alternaria increased the activity of catalase in tomato. However, the presence of *T. harzianum*, when combined with *Alternaria*, further elevated the catalase activity (0.620 U mF.protein^{-1}). Mycorrhizal fungus increased peroxidase activity (0.411 U mF.protein^{-1}) in tomato under the influence of *Alternaria alternata* (Table 1). Enhanced enzymatic activity in plants can be a direct response to stress or infection, acting as a defense mechanism (Mandal et al. 2009). The increased activity of both catalase and peroxidase indicates a heightened defense response in tomatoes treated with biocontrol agents against *Alternaria alternata* infection.

Phenolic Content

Tomato plants exhibited the highest phenol content when treated with a combination of mycorrhizal and *T. harzianum* fungi under *Alternaria alternata* infection. The highest (2.423 micrograms/g) and the lowest (0.933 micrograms/g) amount of phenol in tomato plants were obtained with the combined application of mycorrhizal and *T. harzianum* fungi under the conditions of the pathogen infection and the control treatment, respectively (Table 1, Fig.4). Phenolic compounds play a crucial role in plant defense mechanisms against pathogenic infections (Saini et al., 2024). The observed increase in phenolic content and enhanced growth

Table 1. The effect of *Funeliformis mosseae*, *T. harzianum harzianum* and *Alternaria alternata* pathogenic fungi on tomato plant traits.

جدول ۱. اثر قارچ‌های *Funeliformis mosseae*، *T. harzianum harzianum* و بیمارگر *Alternaria alternata* بر صفات بوته گوجه‌فرنگی.

Traits	Catalase activity	Peroxidase activity	Total Phenol	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Shoot dry weight	Root dry weight (gr)	Root length (cm)	No. of stems	No. of flowers	No. of fruits	Fruit weight (gr)	No. of leaf spots
Control	0.356b	0.223cd	0.933c	0.585c	0.330c	0.915c	2.504cd	0.955ab	39.00ab	10.67bc	5.33bc	1.00c	4.75b	0.33b
A.alternata	0.579a	0.174cd	1.452bc	0.355c	0.175d	0.530d	2.047d	0.665b	28.33b	8.67d	4.67cd	1.33bc	2.78b	7.00a
T.harzianum	0.284b	0.138d	1.167bc	0.606bc	0.359bc	0.965bc	4.071a	1.022a	43.67a	12.67a	6.67ab	2.33a	4.31b	0.67b
F.mosseae	0.380b	0.332ab	1.463b	0.835ab	0.489ab	1.324ab	3.542ab	0.843a	40.33ab	12.00ab	7.33a	1.00c	11.94a	0.67b
F. mosseae+ A.alternata	0.394b	0.411a	1.107b	0.613bc	0.354bc	0.967bc	3.361ab	0.921ab	46.33a	10.00cd	7.00a	1.67b	9.83a	1.00b
T. harzianum + A.alternata	0.620a	0.323ab	1.392b	0.547bc	0.215cd	0.762cd	2.970bc	0.852ab	38.67ab	9.67cd	5.00cd	1.00c	9.43a	1.33b
T. harzianum + F. mosseae + A.alternata	0.314b	0.300bc	2.423a	1.054a	0.552a	1.606a	2.293bc	1.134a	46.33a	10.00cd	3.67d	1.00c	5.16b	1.00b

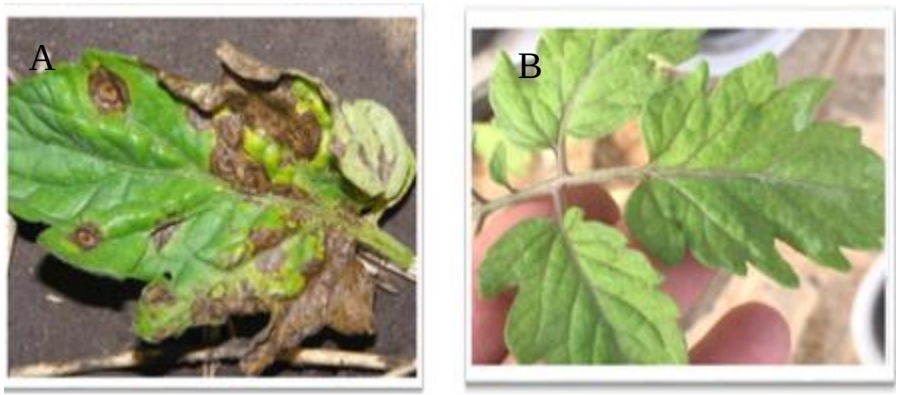

Figure 4. A. The control, where *Alternaria alternata* inoculated alone. B. The antagonistic interaction between *Trichoderma harzianum* + *Funeliformis mosseae* and *Alternaria alternata* in greenhouse, showing a reduction in the disease symptoms.

شکل ۴. A. شاهد را نشان می‌دهد، جایی که *Alternaria alternata* به تنهایی تلقیح شده است. B. برهمکنش *Trichoderma harzianum* + *Funeliformis mosseae* و *Alternaria alternata*را در گلخانه نشان می‌دهد، که نشان دهنده کاهش شدت علایم بیماری است.

parameters might be a synergistic effect of the bio-agents in mitigating the stress caused by *Alternaria alternata.*

Chlorophyll Content

The highest amount of chlorophyll a (1.054 mg/g fresh weight of leaves) was observed with the combined application of *F. mosseae*, *T. harzianum* under *Alternaria alternata* contamination conditions, which was not significantly different from the application of mycorrhizal fungi separately. *Alternaria* fungus decreased the amount of chlorophyll a in tomato leaves, although it was not statistically significantly different from the control treatment (Table 1).

The highest amount of chlorophyll b (0.552 mg/g fresh weight of leaf) was observed with the combined application of *F. mosseae* and *T. harzianum* under *Alternaria alternata* contamination conditions, which was not significantly different from the application of mycorrhizal fungi individually. *Alternaria* fungus had the lowest (0.175 mg/g fresh weight of leaf) amount of chlorophyll b in tomato leaves. *T. harzianum* fungus had no significant effect on the amount of chlorophyll b in the condition of contamination with *Alternaria alternata* and it was in the same statistical group with *Alternaria alternata* (Table 1).

The highest amount of total chlorophyll (1.606 mg/g fresh weight of leaf) with the combined application of *F. mosseae. T. harzianum,* was observed under the condition of contamination with *Alternaria* fungus, which was not significantly different from the application of mycorrhizal fungus separately. *Alternaria* fungus accounted for the lowest amount of total chlorophyll in tomato leaves (0.530 mg/g fresh weight of leaf) (Figure 6). *T. harzianum* fungus had no significant effect on the amount of total chlorophyll in the condition of contamination with *Alternaria* fungus and it is in the same statistical group with *A. alternata* fungus (Table 1).

Tomato plants treated with both *F. mosseae* and *T. harzianum* under *Alternaria alternata* infection showed the highest chlorophyll content, with no significant difference when using mycorrhizal fungi separately. *A. alternata* reduced chlorophyll levels, but *T. harzianum* showed no significant effects on its own. Chlorophyll content is an indicator of plant health and productivity. The positive effect of *F. mosseae* and *T. harzianum* could be attributed to their symbiotic relationship with plant roots, enhancing nutrient uptake and thus plant health (Smith and Read, 2008).

Plant Growth and Fruit Production

Tomato stem length was not affected by antagonistic and pathogenic fungi.

These bio-agents also positively impacted plant growth parameters. The highest shoot dry weight (4.071 g per plant) was obtained by using of *T. harzianum harzianum* and *Alternaria alternata* had the lowest amount of shoot dry weight (2.047 g per plant).

The highest root dry weight (1.134 g per plant) was observed with the use of *T. harzianum* + *Funeliformis* + *Alternaria alternata* and *Alternaria alternata* had the lowest amount of plant fresh weight (0.665 g per plant).

Alternaria alternata reduced the number of secondary stems in tomato. *T. harzianum* fungus caused an increase in the number of stems in tomato and it was not significantly different from mycorrhizal fungi and they accounted for the highest number of secondary stems in tomato plants. The application of mycorrhizal fungus increased the number of flowers in tomato plants under both conditions of absence and contamination with *A. alternata* fungus. But *T. harzianum* fungus only increased the number of flowers in tomato under conditions of non-contamination with *A. alternata*.

The highest number of fruits per plant was observed with the application of *T. harzianum* under the condition of non-contamination with *A. alternata*, and in the next stage, the simultaneous application of mycorrhizal fungus and *A. alternata* resulted in the highest number of fruits per tomato plant.

Mycorrhizal fungi increased the weight of tomato fruit under the conditions of absence and pathogenicity of *A. alternata*. *T. harzianum* fungus caused an increase in the weight of tomato fruit under the condition of contamination with *A. alternata* fungus. Biocontrol agents like mycorrhizal fungi have been known to enhance plant productivity by improving nutrient uptake and providing a better defense against pathogens (Gianinazzi et al. 2010). *T. harzianum's* positive impact on fruit weight even under *A. alternata* infection underscores its potential as a beneficial bio-agent in sustainable agriculture (Harman et al. 2004).

Correlation between Characteristics

The chlorophyll content of tomato leaves showed a positive and significant correlation with peroxidase enzyme activity and phenol, and a negative and significant correlation with the number of infected spots on the leaf. There was a positive and significant correlation between catalase enzyme activity and the number of infected spots. A positive and significant correlation was observed between fruit weight, shoot dry weight, number of flowers and peroxidase enzyme activity. The number of infected leaf spots had a negative and significant correlation with the dry weight of roots and aerial parts, stem length, number of stems and root length. Root length showed a positive and significant correlation with root dry weight. A positive and significant correlation was also observed between the number of stems, the dry weight of aerial parts, the number of flowers and the number of fruits.

Discussion بحث

In the *T. harzianum* confrontation test, it was observed that this fungus limits the growth of *A. alternata* and is able to advance on the mycelium that causes the disease. Considering that *T. harzianum* and *Funeliformis* species prevented the growth of the pathogenic fungus, it can be concluded that the food competition and also the produced metabolite can be effective in controlling the disease agent. Regarding the type of substances in the metabolites of *T. harzianum*, a lot of research has been done by other researchers and it has been determined that the enzymes cellulase, chitinase, beta 1-3 glucanase, endoglucanase, exoglucanase and also antibiotics such as Trichotoxin, Paracelsin, Alamethicin, Trichodermin, Gliotoxin and Viridin is produced by various *T. harzianum* strains that play an effective role in biological control (Sperandio and Filho 2021). In the current research, it was found that the use of *T. harzianum* and *Funeliformis* isolates during seed planting increased the growth indicators and also reduced the symptoms of wave spot disease. This success of *T. harzianum* or *Funeliformis* species can be the result of the rapid establishment of these isolates on the root and the soil around it, which did not allow the establishment of the pathogenic fungus and thus reduced the disease. The use of two isolates in combination caused a significant increase in growth factors of tomato.

According to the above description, the type of plant and its root secretions, the variety and amount of microorganisms that stimulate plant growth and their ability to colonize around the root, and the physical and chemical conditions of the soil, and especially the type of soil microbial flora, finally, they create a complex interaction in the soil, so that any change in this interaction can change the conditions of plant growth (Colla et al. 2015). Therefore, it is important to find the right time and amount of adding the antagonist to the soil and seeds to

control the disease, and in order to achieve the proper control of the disease, it is necessary to gain sufficient knowledge of the cause of the disease, the occurrence of the disease, the appropriate substrate, and the appropriate composition and formulation of the antagonist. Therefore, more studies are needed in this field. In general, it can be said that because a biocontrol agent has no harm to humans, animals, and even the host plant, it is more economical than chemical poisons, and it does not have a risk in terms of increasing pathogen resistance and residual toxins in food and surrounding waters, in the increasing development of sustainable agriculture, one of the important indicators of which is the health and safety of products, it is necessary to pay more attention to scientific research in the field of biological control, especially to investigate the effects of antagonistic species from different aspects and to integrate it as an essential part of the management plan.

In conclusion, this research provides compelling evidence for the efficacy of *F. mosseae* and *T. harzianum* as sustainable and organic approaches to manage early blight disease and rot in tomato plants. The combined application of these bio-agents emerges as a particularly promising strategy, with implications for enhanced crop productivity and disease resistance. Future investigations into the molecular mechanisms governing these interactions could further advance the understanding and application of biocontrol agents in agriculture.

Conclusion نتیجه‌گیری

Conclusively, the use of *T. harzianum* and *F. mosseae* has showcased significant potential in not just disease control but also in improving plant growth parameters. The combined application seems to be particularly effective, hinting at a symbiotic enhancement in their action against pathogens. Future studies could further delve into the molecular mechanisms underpinning these interactions for more targeted agricultural interventions. Our findings highlight a pioneering approach in integrating mycorrhizal and antagonistic fungi for effective tomato disease management, potentially revolutionizing biocontrol strategies.

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