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RESEARCH

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Biological control of arbuscular mycorrhizal fungi and *Trichoderma harzianum* against *Fusarium oxysporum* and *Verticillium dahliae* induced wilt in tomato plants

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Abstract

Background Arbuscular mycorrhizal fungi (AMF) and *Trichoderma harzianum* are effective bioagents against *Fusarium oxysporum* and *Verticillium dahliae* in tomato plants. The objective of the research was to evaluate the in vivo antagonistic activity of AMF and *T. harzianum* against Verticillium and Fusarium wilt by enhancing the growth and resistance of tomato plants. A completely randomized experimental design was used, consisting of twelve treatments with nine replicates for each treatment. The treatments included combinations of AMF and *T. harzianum* inoculation, infection or non-infection by *F. oxysporum* and *V. dahliae,* while also considering individual and combined treatments. Mycorrhization rates, growth parameters, disease severity, disease progression, and the impact on disease mitigation were evaluated.

Results The study revealed the superiority of AMF over *T. harzianum*, resulting in a significant enhancement in the overall extent of mycorrhizal colonization in tomato plants co-inoculated with *T. harzianum*. Moreover, AMF treatments and the AMF + *T. harzianum* consortium contributed to the improvement in growth among all plants infected with *V. dahliae* and *F. oxysporum*. Both AMF and *T. harzianum* significantly reduced the progression of Fusarium wilt, resulting in reductions of 45.14 and 44.91%, respectively, than the untreated plants infected with *F. oxysporum* (initial disease severity of 75.54%). *T. harzianum* demonstrated greater efficacy in reducing *V. dahliae* infection, with a reduction of 34.45% compared to 28.26% for AMF, starting from an initial disease severity of 69.85%. Thus, *T. harzianum* demonstrated greater effectiveness in controlling disease, particularly Verticillium wilt.

Conclusion The target application of disease control methods in tomato plants revealed the effectiveness of both AMF and *T. harzianum* in mitigating Fusarium wilt. Furthermore, *T. harzianum* demonstrated a higher level of effectiveness against Verticillium wilt. These findings emphasize the potential of AMF and *T. harzianum* as sustainable alternatives in agriculture, providing a viable option to decrease dependence on fungicides.

Keywords Biocontrol, *Trichoderma harzianum*, Arbuscular mycorrhizal fungi, *Verticillium dahliae*, *Fusarium oxysporum*, *Lycopersicon esculentum*

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Background

Crop pests, such as diseases, insects, and weeds, result in substantial annual crop yield losses worldwide, even with the extensive application of chemical pesticides. Nonetheless, the reliance on pesticides poses risks to both human health and the environment, while also fostering the emergence of pesticide-resistant pests (Tudi et al. 2021).

To address these challenges, alternative biological control approaches have emerged, including the utilization of symbiotic endophytic fungi such as arbuscular mycorrhizal fungi (AMF) and certain species of the *Trichoderma* genus. These approaches have demonstrated their potential in controlling a wide range of plant pathogens, allowing for more environmentally friendly agricultural practices.

AMF, which are classified within the phylum Glomeromycota, play a vital role in plant development by not only stimulating growth but also enhancing the plants resistance against a range of pests (Boutaj et al. 2022). Moreover, commercial products based on *Trichoderma harzianum* such as *T. Trianum* (Kthiri et al. 2020) or researched strains like *T. virens* (Jogaiah et al. 2018) and *T. pubescens* (Behiry et al. 2023) have shown promising results as biocontrol agents (BCA).

Tomato (*Lycopersicon esculentum* Mill.) production holds significant importance in Algeria's agricultural economy. Nonetheless, this crop faces considerable susceptibility to a variety of pathogens. On the other hand, climate change and human activities are causing ecosystem alterations, resulting in reduced biodiversity and creating favorable conditions for the proliferation of pathogens (Cavicchioli et al. 2019). Diseases such as Verticillium wilt, caused by the soil-borne fungal pathogen *V. dahliae,* is particularly challenging to control. However, promising results have been observed in combating this disease with antagonists from the *Trichoderma* genus (Kong et al. 2022) and AMF (Boutaj et al. 2022).

Similarly, the biological control of Fusarium wilt caused by *Fusarium oxysporum* is facilitated by some *Trichoderma* species (Erazo et al. 2021) and AMF (Devi et al. 2022). AMF act as beneficial biocontrol agents, enhancing plant immunity against pathogens, while contributing to plant nutrition and modulating the microbial balance in the mycorrhizosphere (Vishwakarma et al. 2022). Furthermore, certain species of *Trichoderma* exert control over pathogens by directly impacting them. They stimulate plant innate immunity through the use of natural and synthetic elicitors (Takehara et al. 2023*). Trichoderma* species also employ mechanisms such mycoparasitism (Panchalingam et al. 2022). To enhance the effectiveness of biocontrol agents, researchers have been investigating the use of BCA consortia to achieve synergistic effects. These consortia often possess multiple biocontrol mechanisms, resulting in enhanced biocontrol outcomes. Furthermore, the coexistence of symbiosis and root pathogenesis within the same plant is a common occurrence in nature. Interestingly, studies have shown that certain strains of *Trichoderma* have the ability to influence the activity of AMF (Martinez et al. 2004). However, the current understanding of the in vivo interaction between these two processes and their impact on plant development remains limited.

The objective of this study is evaluating the potential of AMF and *T. harzianum* both individually and in combination, as bio-protectors of tomato plants in vivo, under greenhouse conditions. The focus will be on controlling two common pathogens, *Verticillium dahliae* and *Fusarium oxysporum*.

Methods

Tomato material and growth conditions

The experiment was conducted in the greenhouse of Badji Mokhtar University, locality of Sidi Ammar, Annaba province, Algeria (36° 81′ 58′′ N; 7° 71′ 36′′ E). The Rio Grande tomato variety was sterilized with 2% sodium hypochlorite and then sown in autoclaved peat for 1 h at 120 °C, two consecutive times. The seedlings were then placed in individual pots and kept in the greenhouse under daylight conditions at a temperature of 20–25 °C and a relative humidity of 60–70%. The tomato plants were watered twice a week. One-month-old plants were used for the experiment.

Fungal material

Four fungal strains were utilized in the study. Two antagonists, including arbuscular mycorrhizal fungi (AMF) and *T. harzianum,* as well as the two pathogens *V. dahliae* and *F. oxysporum.*

Arbuscular mycorrhizal fungi

The AMF used were provided in the form of a commercial inoculum, Symbivit (INOCULUM plus, France). Symbivit is a granular formulation containing propagules of six AMF species (*Claroideoglomus claroideum*, *Claroideoglomus etunicatum*, *Funneliformis geosporum*, *Funneliformis mosseae*, *Glomus micro-aggregatum* and *Rhizophagus intraradices*) that have been sporulated and stored on an inert support (clay) in the presence of technological additives. The AM fungal inoculum contains 20 infective propagules g^{-1} (SYMBIVIT Database, July 2023).

Trichoderma harzianum

The *T. harzianum* strain was provided by Valorization and Conservation of Biological Resources (VALCOR) laboratory, M'Hamed Bougara University of Boumerdes. The *T. harzianum* isolate was sequenced and identified as *Trichoderma harzianum,* listed in the GenBank database on NCBI under the code OL587563 (Reghmit et al. 2022). It was characterized by its high in vitro capacity to control *V. dahliae*. Multiplication of *T. harzianum* was performed on potato dextrose medium (PDB) supplemented with 50 mg/l streptomycin. *T. harzianum* spores were added to the culture medium and incubated at 150 rpm and 25 ± 1°C for 7 days. The density of the suspensions was adjusted to 106 CFU/ml.

Fusarium oxysporum and *Verticillium dahliae*

Strains of the pathogens *V. dahliae* and *F. oxysporum* were provided by the VALCOR laboratory. The pathogenicity test for these two species was previously conducted by the VALCOR laboratory, with *V. dahliae* (R1) tested by Tihar-Benzina et al. (2016) and *F. oxysporum* tested by Bennacer et al. (2022). Multiplications were carried out separately on potato dextrose medium (PDB) supplemented with 50 mg/l streptomycin, following the same procedure as for *T. harzianum*, with incubation at 150 rpm and 25 ± 1 °C for a period of 14 days.

In vivo antagonistic assay

The experiment was conducted using a completely randomized block design. Tomato seeds were germinated on January 18, 2020, and the young seedlings were transplanted in two-liter polyethylene bags on February 18, 2020. Each plant was inoculated with 200 infectious fungal propagules of AM, which were deposited near the roots. The application of *T. harzianum* and both pathogens (*V. dahliae* and *F. oxysporum*) was carried out at 13 and 14 weeks from the start of the experiment, respectively. Inoculation of these plants was performed by infecting each tomato plant with 10 ml/plant of a suspension containing *V. dahliae* and *F. oxysporum* spores (106 spore ml^{-1}) through single applications. A total of twelve treatments were conducted, each repeated nine times. All the possible treatments, including individual treatment (AMF + *V. dahliae,* AMF + *F. oxysporum, T. harzianum* + *V. dahliae, T. harzianum* + *F. oxysporum, and* AMF + *T. harzianum)* as well combination treatments (AMF + *T. harzianum* + *V. dahliae* and AMF + *T. harzianum* + *F. oxysporum*), were applied using the two bioinoculants (AMF and *T. harzianum*). Additionally, one control treatment (without pathogens), positive treatments with pathogens inoculated (*V. dahliae and F. oxysporum),* and antagonist treatments (AMF, *T. harzianum*) were conducted.

Plant parameters

After 18 weeks of transplanting, on July 20, 2020, nine tomato plants were considered for measurement and evaluation of the following parameters: mycorrhizal colonization intensity of tomato roots, growth parameters (above-ground and root fresh biomass) and diseases incidence on tomato plants inoculated with *V. dahliae* and *F. oxysporum*.

The intensity of mycorrhizal colonization in the root system (M% and A%) was determined using staining technique described by Phillips and Hayman (1970). Root samples from five plants per treatment were used, and observations were made on 30 root fragments of 1 cm. The intensity of colonization of the root cortex was estimated in relation to the entire root system and expressed as a percentage. Arbuscular colonization was also evaluated and expressed as a percentage. The MYCOCALC program and the method described by Trouvelot et al. (1986) were used for annotation and evaluation.

- Growth biomass increase (GB) was calculated for above-ground and root fresh biomass by comparing the mean biomass of plants inoculated with antagonistic agents against control and positive treatments. The calculations were performed for individual treatments (AMF + *V. dahliae,* AMF + *F. oxysporum, T. harzianum* + *V. dahliae, T. harzianum* + *F. oxysporum, and* AMF + *T. harzianum)* as well as the combination treatments (AMF + *T. harzianum* + *V. dahliae* and AMF + *T. harzianum* + *F. oxysporum*). GB was expressed as a percentage and calculated using the formula:

$$\text{GB}(\%) = \frac{(\text{Biomass gain of treated plants} - \text{Biomass gain in control or positive treatments})}{\text{Biomass gain in control or positive treatments}} \times 100$$

- Length of the branches showing symptoms of diseases (DS), such as yellowing, browning or wilting of the leaves, or the presence of necrosis, was measured at the end of the experiment. Disease incidence (%) was calculated for each plant by the following formula:

$$\text{DS}(\%) = \frac{\text{Lengths of the diseased stems}}{\text{Lengths of the total stems}} \times 100$$

Statistical analysis

Intensity and arbuscular colonization of the root system by AMF were assessed according to two factors: infection with *V. dahliae* or *F. oxysporum* pathogens in presence or absence of T. *harzianum*. The data obtained were analyzed using a two-way ANOVA with a general linear model. Post-hoc pairwise comparisons were conducted using Tukey's honestly significant difference (HSD) test in the R software, version 4.0.2, with the R commander package version 2.8-0 (Downie 2016). The growth parameters were subjected to a three-way ANOVA (AMF * *T. harzianum* * *V. dahliae*) and (AMF * *T. harzianum* * *F. oxysporum*). Mean comparisons between different treatments were performed at a significance level of 5% using Tukey's HSD test with R 4.0.2 and Rcmdrpackage, version 2.8-0.

Results

AMF root cortex colonization of tomato plants

Estimation of tomato root colonization by AMF revealed varying levels among the different applied treatments, including AMF, AMF + *V. dahliae*, AMF + *F. oxysporum*, AMF + *T. harzianum*, AMF + *T. harzianum* + *V. dahliae* and AMF + *T. harzianum* + *F. oxysporum.* The intensity of root cortex colonization was high in all treatments, ranging from 52.09% in the AMF treatment to 67.13% in the AMF + *V. dahliae* treatment (Fig. 1a). A significant effect was observed between the AMF treatment and all other treatments, except for the AMF + *T. harzianum* + *F. oxysporum* treatment. Arbuscules were more abundant in plants inoculated with *T. harzianum* and infected by *V. dahliae* or by *F. oxysporum* (Fig. 1b). The lowest values were recorded in plants co-inoculated with *T. harzianum* and infected with *F. oxysporum* (3.2%).

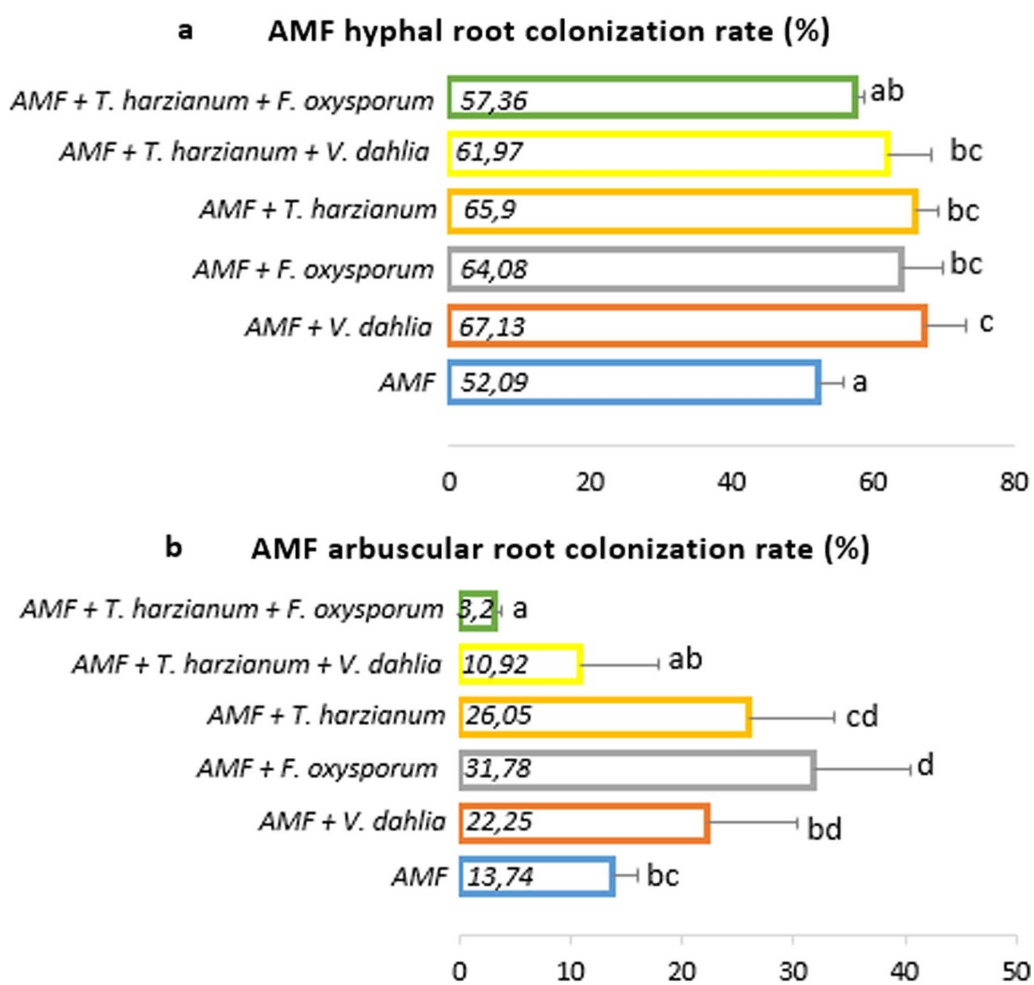

Fig. 1 Arbuscular mycorrhizal fungi (AMF) colonization of tomato plants, mean values with different letters are significantly different ($P > 0.05$), **a** Arbuscular mycorrhizal fungi (AMF) hyphal root colonization rate, **b** AMF arbuscular root colonization rate

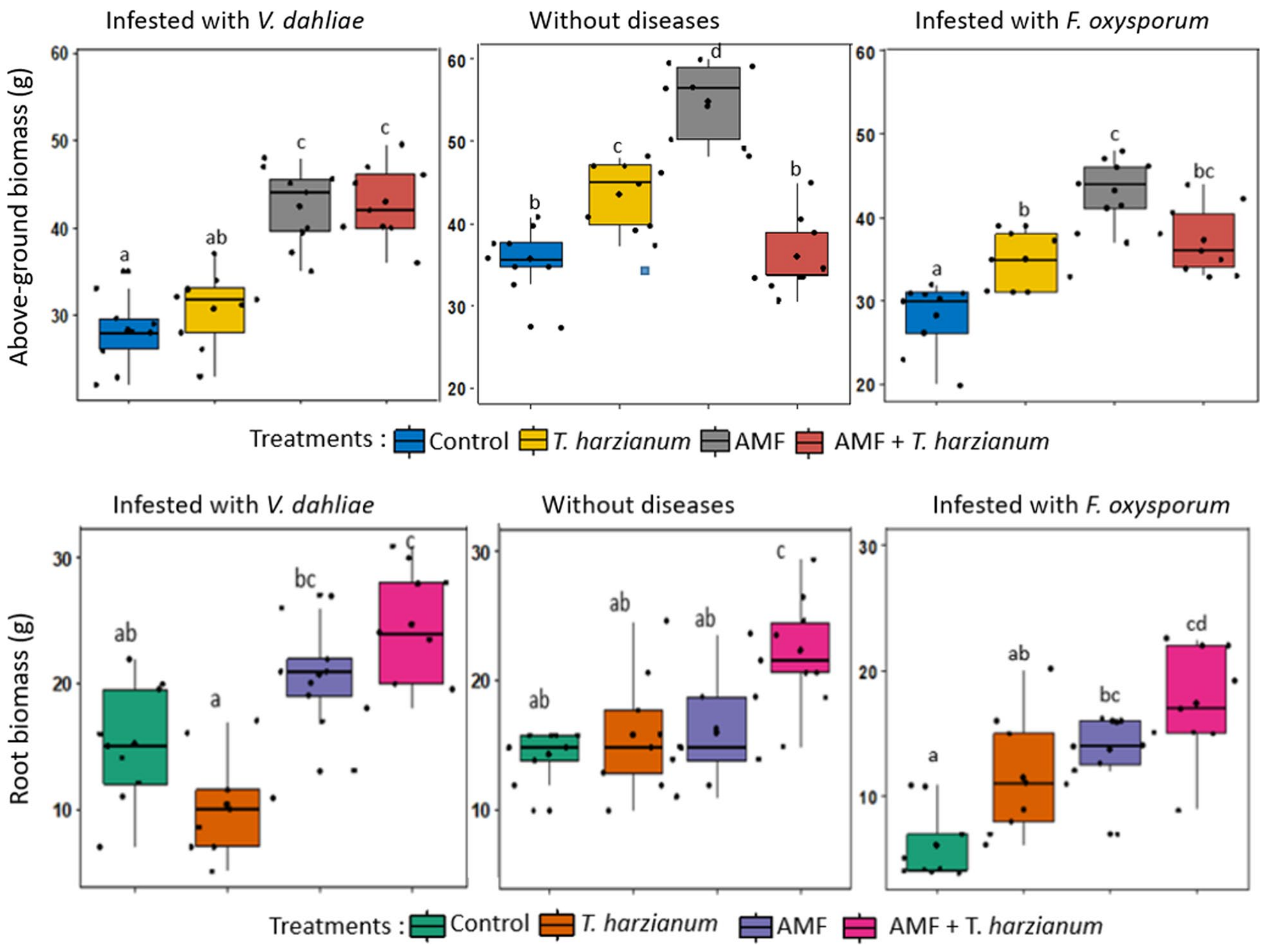

Fig. 2 Effect of arbuscular mycorrhizal fungi (AMF) and *Trichoderma harzianum* on tomato growth in the presence of fungal disease (Verticillium and Fusarium wilt), mean values with different letters are significantly different ($P > 0.05$)

Biocontrol activity of *F. oxysporum* and *V. dahliae* wilt by biostimulation of tomato growth

Above-ground biomass growth

In this study, various treatments were applied to tomato plants to evaluate their effects on above-ground and root biomass. The analysis of mean biomass growth in tomato plants inoculated with AMF, *T. harzianum*, and AMF + *T. harzianum*, both in the presence and absence of *V. dahliae* or *F. oxysporum* pathogens, showed fluctuating biomass values (Fig. 2). When comparing the above-ground biomass measurements of treated groups to the untreated control group, inoculation with AMF resulted in a significant increase of 52.68%. Similarly, the treatment with *T. harzianum* improved above-ground biomass, with a gain of 21.25%. However, the combined inoculation of AMF and *T. harzianum* did not show a significant difference compared to the control.

In the case of plants infected with *V. dahliae*, notable growth stimulation was observed in both the AMF and AMF + *T. harzianum* treated groups. These treatments resulted in significant increases in above-ground biomass, with gains of 50.31 and 52.09%, respectively, when compared to the untreated plants and the positive control group infected with *V. dahliae*. However, *T. harzianum* did not have a significant effect on above-ground biomass growth. When considering plants infected with *F. oxysporum*, the above-ground biomass showed substantial improvements, with gains of 52.94, 23.6, and 32.06% for the AMF, *T. harzianum*, and AMF + *T. harzianum* treatments, respectively, as compared to the *F. oxysporum*-infected plants (Table 1).

Root biomass growth

In terms of root biomass, the AMF + *T. harzianum* treatment demonstrated the highest effectiveness than the untreated control group. This treatment significantly stimulated root biomass growth, resulting in an increase of 56.62%. When comparing root biomass measurements of plants infected with *V. dahliae*, the AMF + *T. harzianum* treatment was the most efficient,

Table 1 Effect of arbuscular mycorrhizal fungi (AMF) and *Trichoderma harzianum* on above-ground and root biomass growth of tomato plants infected or not with *Verticillium dahliae* and *Fusarium oxysporum*

Treatments	Above-ground biomass (g)	Root biomass (g)	Gains of above-ground biomass (%)	Gains of root biomass (%)
Growth increase compared to control				
Control	35.00 ± 3.93 (b)	14.44 ± 2.12 (ab)		
AMF	53.44 ± 4.45 (d)	16.16 ± 4.01 (ab)	52.68	11.91
T. harzianum	42.44 ± 3.93 (c)	16.00 ± 4.69 (ab)	21.25	10.80
AMF + *T. harzianum*	35.16 ± 4.43 (b)	22.66 ± 4.44 (c)	0.45	56.62
Growth increase compared to plant infected with *V. dahliae*				
V. dahliae	28.16 ± 4.21 (a)	15.16 ± 4.8 (ab)		
AMF + *V. dahliae*	42.33 ± 4.60 (c)	20.66 ± 4.27 (bc)	50.31	36.27
T. harzianum + *V. dahliae*	30.64 ± 4.29 (ab)	10.32 ± 4.06 (a)	8.80	− 31.92
AMF + *T. harzianum* + *V. dahliae*	42.83 ± 4.30 (c)	24.66 ± 4.81 (c)	52.09	62.66
Growth increase compared to plant infected with *F. oxysporum*				
F. oxysporum	28.22 ± 4.23 (a)	6.00 ± 3.00 (a)		
AMF + *F. oxysporum*	43.16 ± 3.98 (c)	13.72 ± 2.96 (bc)	52.94	128.66
T. harzianum + *F. oxysporum*	34.88 ± 3.48 (b)	11.44 ± 4.66 (ab)	23.6	90.66
AMF + *T.harzianum* + *F.oxysporum*	37.27 ± 4.07 (bc)	17.38 ± 4.45 (cd)	32.06	189.66

Values with different letter(s) within a column are significantly different at $p < 0.05$ by Turkey's multiple range test. Values are the means (± standard error) of nine replicates

resulting in root biomass augmentation of 62.66%. Similarly, the AMF treatment increased root biomass, by 36.27%. However, *T. harzianum* treatment recorded the lowest value. For the plants infected with *F. oxysporum*, the best treatments were AMF, AMF + *T. harzianum*, (13.72 g and 17.38 g, respectively), corresponding to increases of 128.66 and 189.66%. Plants infected with *F. oxysporum* showed the lowest values (Fig. 2).

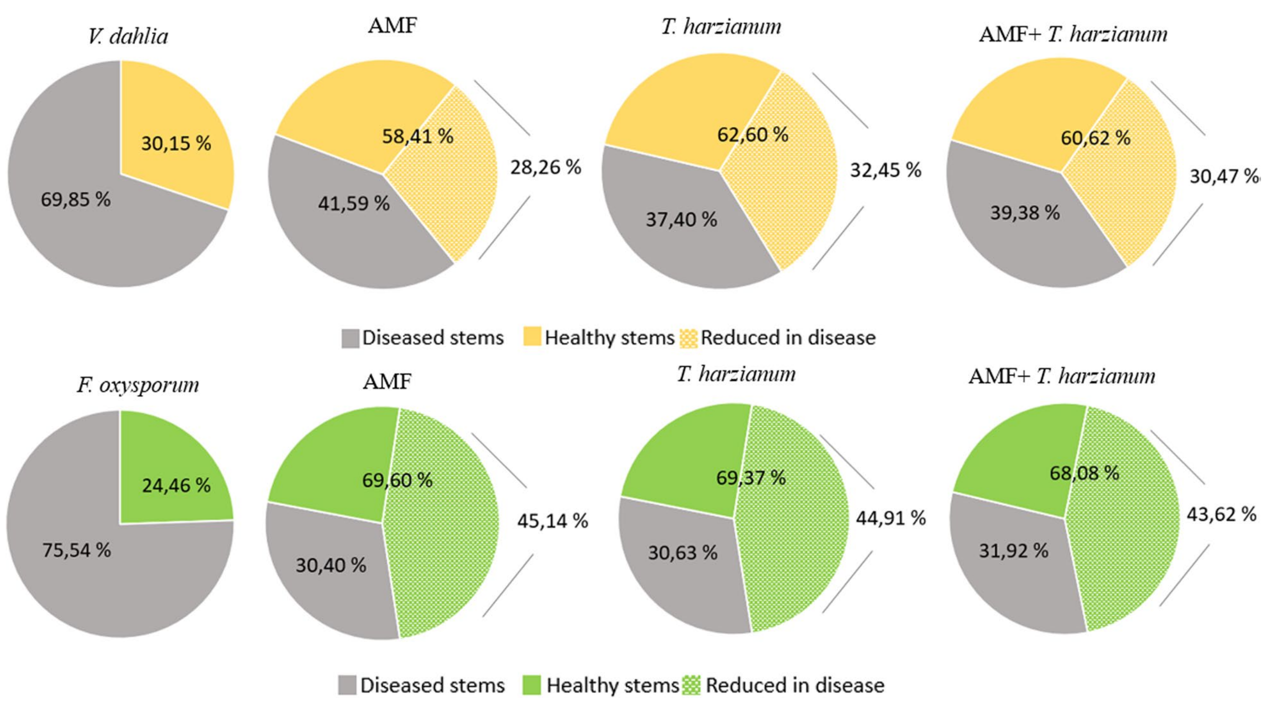

Fig. 3 Incidence rate of Verticillium and Fusarium wilt and antagonistic effect of arbuscular mycorrhizal fungi (AMF) and *Trichoderma harzianum*

Incidence of disease in tomato plants inoculated with *V. dahliae* and *F. oxysporum*

Tomato plants infected with *V. dahliae* and *F. oxysporum* without any treatment, showed high disease incidence rates (Fig. 3). However, a mitigation in disease incidence was observed for all the applied treatments, including AMF, *T. harzianum,* and their synergistic effect (AMF + *T. harzianum).* For tomato plants infected with *V. dahliae,* the mean disease incidence rate was 69.85%, with only 30.15% of healthy stems. Notably, maximum protection was observed in tomato plants treated with *T. harzianum*, which showed a 32.45% decrease in disease incidence. AMF and AMF + *T. harzianum* treated plants exhibited a disease mitigation, respectively by 28.26 and 30.47%. The combination of AMF and *T. harzianum* did not show additional effects compared to individual treatments. Furthermore, tomato plants inoculated with *F. oxysporum* presented the highest mean disease incidence rate, surpassing that of *V. dahliae*, with 75.54% of affected stems and only 24.46% remaining healthy. However, disease mitigation was observed for AMF, *T. harzianum,* and AMF + *T. harzianum*, resulting in decreases of 45.14, 44.91 and 43.62% in disease incidence rates, respectively.

Discussion

In this study, co-inoculation of AMF and *T. harzianum* in greenhouse conditions has been shown to be effective in controlling diseases caused by *V. dahliae and F. oxysporum*. However, it is important to note that the effectiveness of these symbiotic association in enhancing resistance or tolerance may vary between AMF symbionts and *T. harzianum*. They probably operate through multiple and various mechanism. AMF ensures protection through competition control. The presence of *T. harzianum* and both pathogenic fungi led to a significant increase in overall mycorrhizal colonization in the plants. However, plants inoculated solely with AMF displayed the lowest colonization rates. Furthermore, the presence of *T. harzianum* and the pathogens resulted in a higher intensity of arbuscules. The activation of specific plant defense mechanisms in response to AMF colonization is one of the ways mycorrhizal fungi provide protection to plants. A study showed that by regulating the competitive balance, AMF exclude harmful strains or species that can be detrimental to the plant (Harman et al. 2021). However, it's worth noting that AMF and soil-borne plant diseases compete for root tissues, which may lead to direct competition for space. Martínez-Medina et al. (2009) reported that multi-taxa AMF inoculation significantly increase root colonization, and the presence of *T. harzianum* also enhanced AMF colonization. Furthermore, inoculation with *T. harzianum* showed better results than the colonization by wild populations of microbes alone (Hafiz et al. 2022).

Several studies have demonstrated that increased mineral uptake in AMF colonized plants is associated with greater disease resistance (Li et al. 2013). When nutrient concentrations were high, an accompanying increase in plant biomass was observed (Bhantana et al. 2021). The productivity of plants depends on their overall health and interactions with rhizospheric microorganisms, whether symbiotic or pathogenic. Under the present experimental conditions, in the absence of disease, tomato plants inoculated with AMF and *T. harzianum* showed improved growth than uninoculated plants, particularly in terms of above-ground biomass. AMF treatment was the most effective. However, co-inoculation (AMF + *T. harzianum*) did not have a significant effect on above-ground biomass compared to the control, which is consistent with the findings of Molina-Lores et al. (2022). The authors reported the superior performance of AMF (*Glomus cubense*) in enhancing plant nutrition than *T. harzianum* and its combined effect (*Glomus cubense* + *T. harzianum*), attributing it to structural improvements in the plant, including an increase in stem diameter (Molina-Lores et al. 2017). AMF treatment induced better growth of above-ground and root biomass, even in plants infected with *V. dahliae* or *F. oxysporum* leading to improved resistance against these pathogens.

In the case of Fusarium wilt, all treatments (AMF, *T. harzianum* and AMF + *T. harzianum*) effectively improved above-ground biomass. For plants affected by Verticillium wilt, the most effective treatment was AMF, either alone or in combination with *T. harzianum*, resulting in enhanced above-ground biomass compared to *T. harzianum* treatment. Regarding root systems, *T. harzianum* did not show any increase in biomass in plants infected with these two diseases. Root biomass was significantly boosted by the AMF + *T. harzianum.* Mycorrhizae compensated for the loss of biomass and root function caused by pathogen infection by enhancing nutrient and water uptake, indirectly reducing pathogen damage and improving disease resistance in host plants (Ma et al. 2021). Previous studies by Lee and Whang (2016) and Benzina-Tihar et al. (2020) linked poor root system growth in tomato plants inoculated with certain *Trichoderma* species to the production of indole acetic acid (IAA), which promotes stem and coleoptile growth and inhibits root growth. Kakabouki et al (2021) reported that greater root colonization by arbuscular mycorrhizal fungi following *T. harzianum* inoculation, suggesting that *T. harzianum* contributes to the growth-enhancing activity of AMF. Linderman (1994) suggested that microbial antagonists of pathogenic fungi do not interfere with

AMF, and may even promote the development of mycosymbionts and facilitate AMF formation.

Furthermore, few studies have demonstrated the sensitivity of a pathogen strain to biological control agents, depending on their modes of action. Buck and Jeffers (2004) reported variability in the susceptibility of pathogenic strains to biocontrol agents that act through nutrient competition. *V. dahliae*, for instance, was found less susceptible to *T. harzianum* than AMF that act through nutrient competition.

In this study, the incidence of wilt caused by *F. oxysporum* was higher than to *V. dahliae*. The application of AMF and *T. harzianum*, either alone or in combination, effectively reduced the severity of Verticillium and Fusarium wilt. *T. harzianum* showed greater effectiveness in reducing Verticillium wilt compared to AMF treatments, while both AMF and *T. harzianum* demonstrated similar effectiveness against Fusarium wilt. The application of AMF + *T. harzianum* consortium did not show any additional effect compared to individual treatments. Molina-Lores et al. (2022) stated that the combined presence of *T. harzianum*, known for its biopesticide effect, and *Glomus cubense*, known for its bio-fertilizing effect, yields stronger outcomes compared to their individual applications. Linderman (1994) highlighted the potential of harnessing the prophylactic abilities of AM fungi in conjunction with other rhizosphere microorganisms that exhibit antagonistic properties against root pathogens used in biological control practices. This involves utilizing *Trichoderma* species in combination with mycorrhizae, resulting in a positive and synergistic impact on the overall health of specific plants. Guzmán-Guzmán et al. (2023) have also emphasized that *Trichoderma* remains the preferred choice in the development of biological control programs.*Trichoderma* acts directly on the pathogen population in the rhizosphere by releasing metabolites that impact pathogen growth, including extracellular enzymes capable of deteriorating their cell wall (Rao et al. 2016). Furthermore, *Trichoderma* showcases its protective capabilities through various mechanisms, such as the synthesis of silver nanoparticles and the development of innovative formulations like nano-emulsions, as highlighted by Konappa et al. (2021). Moreover, inoculating plants with *T. harzianum* elicits a range of defense responses at the histopathological, biochemical, and gene expression levels, effectively providing protection against diseases, as outlined by De Britto and Jogaiah (2022).

Conclusions

The findings further enhance our understanding of the potential of arbuscular mycorrhizal fungi and *T. harzianum* as biocontrol agents for the management of wilt diseases in tomato plants. Their utilization revealed distinct defense mechanisms. AMF demonstrated a protective effect by increasing the mycorrhizal surfaces, thereby improving plant nutrition and promoting robust growth and ultimately strengthening their resistance against pathogens. On the other hand, *T. harzianum* acted directly on the pathogens. While all treatments, including the application of AMF, *T. harzianum*, and their combined synergistic treatment exhibited a slowdown in disease progression for both wilt diseases, the distinct synergistic effect was not prominently observed. This study highlighted the specificity of biocontrol agents in their interaction with pathogens. Both AMF and *T. harzianum* exhibited effectiveness in combating Fusarium wilt, which demonstrated a higher incidence rate than to Verticillium wilt. Additionally, *T. harzianum* demonstrated superior control in the case of Verticillium wilt. The results taken together emphasize the potential of AMF and *T. harzianum* as sustainable alternatives in agriculture, offering a viable solution to reduce reliance on fungicides and promote environmentally and health-conscious practices.

Abbreviations

AMF	Arbuscular mycorrhizal fungi
BCA	Biocontrol agents
DS	Symptoms of diseases
PDB	Potato dextrose medium
F. oxysporum	*Fusarium oxysporum*
GB	Growth biomass increase
T. harzianum	*Trichoderma harzianum*
VALCOR laboratory	Valorization and conservation of biological resources laboratory
V. dahliae	*Verticillium dahliae*

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Author contributions
MHA contributed in all experiments, data analysis and interpretation and writing the manuscript; MC performed investigation; BF contributed to identification and donor of the fungal material isolates, methodology, and revision of the manuscript; HN contributed to data analysis and graphical display; RN prepared fungi material and performed identification of *T. harzianum* isolate; ZH wrote the first draft manuscript; and KH performed writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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The datasets used and analyzed during the current study are indicated in the manuscript and are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication

All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare that they have no competing interest.

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