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Determination of the efficacy of *Trichoderma koningii* and *Rhizophagus irregularis* against Fusarium wilt disease in tomato

Trichoderma koningii ve *Rhizophagus irregularis*'in domateste Fusarium solgunluk hastalığına karşı etkinliğinin belirlenmesi

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ABSTRACT

This study investigated the potential of *Trichoderma koningii* and *Rhizophagus irregularis* as environmentally friendly as an alternative to chemical control for Fusarium wilt disease in tomato under greenhouse conditions. The research focused on how these *T. koningii* and *R. irregularis* interacted and their impact on plant growth and disease resistance. *T. koningii* alone significantly reduced disease severity (DS = 0.83; DSI = 29.33%) compared to the control group infected with *Fusarium oxysporum* f. sp. *lycopersici* (FOL). However, the most effective protection came from combining both *T. koningii* and *R. irregularis* (DS = 0.33; DSI = 14.33%), achieving a level comparable to healthy controls. This combined treatment not only displayed superior disease resistance but also showed the highest chlorophyll content (Chl a = 5.62 mg g^{-1} Fresh Weight; Chl b = 3.11 mg g^{-1} Fresh Weight; Chl T = 8.74 mg g^{-1} Fresh Weight), indicating a stronger ability to counteract the chlorophyll degradation caused by FOL infection. Furthermore, tomato plants co-inoculated with *T. koningii* and *R. irregularis* exhibited the most robust antioxidant response, evident in significantly higher activities of antioxidant enzymes (superoxide dismutase = 46.17 units g^{-1} ml^{-1} min^{-1}, peroxidase activity = 5.66 units g^{-1} ml^{-1} min^{-1}, and catalase activity = 104.42 units g^{-1} ml^{-1} min^{-1}) and total phenolic content (3.14 mg g^{-1}). These findings suggest that the combined application of *T. koningii* and *R. irregularis* has the potential to be a more effective and environmentally friendly strategy for managing Fusarium wilt disease and promoting overall plant health in tomato compared to using either *T. koningii* or *R. irregularis* alone.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), a Solanaceae cultivated in North Africa for over 4000 years (Robinson and Decker-Walters 1997), is a major global crop (reached nearly 45.8 million metric tonnes in 2024). In Tunisia, it is a major agricultural product, with annual production estimated at 980000 tonnes (FAO 2024). Cultivation thrives across

diverse Tunisian regions with varying soil and climate conditions (Elbekkay et al. 2021, Rhouma et al. 2021). Inter-annual tomato yields exhibited significant variation attributable to biotic and abiotic stressors. Notably, the fungus *Fusarium oxysporum* f.sp. *lycopersici* (FOL), responsible for Fusarium wilt disease, negatively impacted tomato production (Keinath et al. 2020, Ozan and Aşkın 2006, Ozan and Maden 2004). This pathogen likely reduces the total number of fruits, total fruit weight, and individual fruit size (Keinath et al. 2019). Annual yield losses caused by FOL can be substantial, varying depending on factors such as the severity of the infection, the cultivar's susceptibility, and environmental conditions. In severe cases, yield losses can exceed 50% (Keinath et al. 2019, Keinath et al. 2020).

Driven by population growth, agricultural intensification practices incorporating fungicides have become widespread to maintain crop yields from limited arable land (Mousa et al. 2021). While fungicides effectively control diseases, their overuse disrupts soil ecosystems, promotes fungicide resistance in plant pathogens, and leaves behind harmful residues (Rhouma et al. 2023). Biological control agents (BCAs) present a promising alternative for disease management (Hajji-Hedfi et al. 2023). Unlike fungicides, BCAs represent a sustainable approach that promotes a healthy soil environment and avoids toxic residues (Matrood and Rhouma 2022).

Among BCAs, arbuscular mycorrhizal fungi (AMF) and *Trichoderma* spp. are particularly promising (Matrood and Rhouma 2022). *Trichoderma* spp., avirulent saprophytes, act against various fungal plant pathogens through a multi-pronged approach including antibiotic and enzyme production, directly parasitizing fungi, and inducing plant resistance and competition in the root zone (Guzmán-Guzmán et al. 2023). Additionally, *Trichoderma* spp. promote plant growth and physiological activity (Kubiak et al. 2023). AMF represent a crucial category of BCAs renowned for their capacity to establish mutually beneficial symbiotic associations with plants (Paliwoda and Mikiciuk 2020). These symbiotic interactions have been extensively documented to exert a positive influence on crop performance, enhancing overall plant vigor and productivity (Chen et al. 2013). Within the diverse spectrum of AMF, the *Glomaceae* family encompasses a significant number of fungal species (Dodd et al. 2000). Notably, considerable research efforts have been directed towards investigating the specific impact of *R. irregularis* (formerly known as *Glomus intraradices*) on the growth and development of diverse crops, with a particular emphasis on its role in mitigating the detrimental effects of biotic and abiotic stresses (Delaeter et al. 2024, Kakabouki et al. 2021, Khalediyan et al. 2021, Mota et al. 2020, Spagnoletti et al. 2017). AMF offer another key disease control strategy through induced systemic resistance (ISR) (Kalamulla et al. 2022). ISR in this context involves a combination of factors: nutritional benefits to the plant, root system alterations, competition with pathogens for infection sites, changes in the microbial community around the roots, and modifications in plant chemical defenses (Khaliq et al. 2022, Wahab et al. 2023). Notably, interactions between arbuscular mycorrhizal fungi and *Trichoderma* spp. can have either stimulatory or inhibitory effects on each other's populations. Understanding these interactions is crucial for developing successful biological control strategies that replace harmful chemical fungicides and fertilizers (Nicolás et al. 2024). This study evaluated *Trichoderma koningii* and *Rhizophagus irregularis* as biological controls against FOL on tomato plants, assessing their effects on disease severity, chlorophyll content, and plant defense under greenhouse conditions.

MATERIALS AND METHODS

Inoculum preparation

Fusarium oxysporum f.sp. *lycopersici* (FOL), *Trichoderma koningii*, and *Rhizophagus irregularis* used in the present study were obtained from the laboratory of Plant Protection and Biological Sciences, Regional Centre for Agricultural Research (Sidi Bouzid, Tunisia). This phytopathogen was isolated from infested tomato roots cultivated in a greenhouse (Regueb, Sidi Bouzid, Tunisia). However, *T. koningii* and *R. irregularis* were isolated from the rhizosphere of the same greenhouse. Spores of FOL and *T. koningii* were produced on Potato Dextrose Agar (PDA) media at 25 °C and quantified using a hemocytometer, revealing a density of 10^7 spores ml^{-1} (Matrood and Rhouma 2021). To cultivate *R. irregularis* (locally sourced), wheat seeds were used as a growth substrate. After a 12-hour soak in distilled water and subsequent air-drying, the seeds were sterilized through three consecutive autoclave cycles at 120 °C for one hour within one-liter flasks. These flasks were then incubated at 25 °C for 16 weeks to allow for complete colonization of the wheat seeds by the arbuscular mycorrhizal fungi. To prevent the formation of inoculum clumps, the flasks were subjected to manual shaking once a week. The resulting colonized wheat seeds served as the inoculum source for subsequent experiments (Dehariya et al. 2015, Egberongbe et al. 2010). Following colonization, wheat seeds served as inoculum for further studies. Spores of *R. irregularis* were extracted from the colonized seeds using a method adapted from Jmal et al. (2015) involving sieving and microscopic enumeration. One hundred grams of seeds were mixed with 200 ml of water, agitated for 5 min on a magnetic stirrer, and then sieved through nested sieves of 1.6 mm, 0.25 mm, and 0.038 mm.

The fraction retained on the 0.038 mm sieve was collected, suspended in distilled water in 60 mm Petri dishes, and enumerated under a stereomicroscope at 40x magnification (Jmal et al. 2015).

In vivo evaluation of *Trichoderma koningii* and *Rhizophagus irregularis on tomato plants inoculated with Fusarium oxysporum* f.sp. *lycopersici*.

Tomato (cv. Crimson Sweet) seedlings were transplanted into pots containing a 1:1 (v/v) mixture of peat and vermiculite. The experiment was divided into three blocks. Within each block, the 45 pots were randomly assigned to the different treatments. This randomization helps to minimize the impact of potential variations between blocks (e.g., differences in light, temperature conditions). For each treatment, three replicates were included within each block. This provides multiple measurements for each treatment condition, improving the precision and reliability of the results. After 15 days of growth in these pots, the seedlings received their designated treatments (Hajji-Hedfi et al. 2024).

Within each block, tomato seedlings received five treatments: T1: seedlings were inoculated only with FOL (positive control), T2: seedlings were treated with sterilized water only (negative control), T3: seedlings were dipped in *T. koningii* conidial suspension for 30 min and watered with FOL (10 ml) 24 h later, T4: 30 grams of wheat seeds containing approximately 130 spores of *R. irregularis* were placed at 5-10 cm deep in the pot. 24 h later, the seedlings were watered with FOL, and T5: Seedlings were dipped in a suspension of *T. koningii*. Then 30 grams of wheat seeds (130 spores of *R. irregularis*) were placed at 5-10 cm deep in the pot. 24 h later, the seedlings were watered with FOL. Following treatments, all pots were incubated in a growth chamber for 60 days under controlled conditions with an 8-hour dark /16-hour light photoperiod and a temperature range of 20-22 °C.

Disease assessment

Disease severity (DS) was evaluated using a visual scoring system from 0 to 5 (Table 1) (Popoola et al. 2015). These scores were then converted into Disease Severity Index (DSI) using McKinney's formula: DSI (%) = (Σvn)/(NV) × 100, where Σvn is the sum of all disease scores, N is the total number of plants, and V is the maximum possible score (Rhouma et al. 2024). Treatment efficacy was assessed based on disease severity (DSI) as described by Wang et al. (1995): EE: Extremely effective (DSI = 0%), HE: Highly effective (DSI = 0.1 to 5%), E: effective (DSI = 5.1 to 25%), I: Ineffective (DSI = 25.1 to 50%) and HI: Highly ineffective (DSI = 50.1 to 100%).

Plant growth parameters

Tomato leaf tissue (0.5 g) was ground in a mortar under low light and homogenized with a 2:1 (v/v) acetone/ethanol solution (4 ml acetone, 2 ml ethanol) in 10 ml tubes. The mixture was stirred for 1 minute and incubated in darkness at -20 °C for 30 min. Centrifugation (2000 rpm, 10 min) separated the extract, which was then collected and re-suspended in fresh 2:1 acetone/ethanol solution (5 ml). After another minute of stirring, the final extract was used for chlorophyll quantification by measuring absorbance at 663 nm and 645 nm. Acetone/ethanol solution served as the blank control (Hajji-Hedfi et al. 2023). Chlorophyll quantification was assessed on 10 tomato leaves per treatment and block. Chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (Chl T) contents were calculated using established formulas (Hajji-Hedfi et al. 2023).

$$\text{Chl a} = 12.41 \times \text{absorbance}_{663} - 2.59 \times \text{absorbance}_{645}$$

$$\text{Chl b} = 22.90 \times \text{absorbance}_{645} - 4.68 \times \text{absorbance}_{663}$$

$$\text{Chl T} = \text{Chl a} + \text{Chl b}$$

Biochemical evaluation of tomato roots

Tomato root biochemical properties, including superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), and total phenolic content (TPC), were evaluated following established protocols. Three tomato roots per treatment and block were homogenized, and enzyme extracts were prepared. SOD activity was measured at 560 nm following a reaction mixture containing 0.5 ml enzyme extract, 1.5 ml phosphate buffer, 227 µl Na_2EDTA (disodium

Table 1. Disease severity scale for tomato Fusarium wilt (Popoola et al. 2015)

DS Score	Symptom Description
0	Symptomless plants
1	Very limited wilting, 5% leaves yellowed and wilted
2	Limited wilting, 6-10% leaves yellowed and wilted
3	Moderate wilting, 11-20% leaves yellowed and wilted
4	Severe wilting, 21-50% leaves yellowed and wilted
5	Very severe wilting, above 50% leaves yellowed and wilted

DS: Disease severity.

ethylenediaminetetraacetic acid), 630 µl nitro blue tetrazolium (NBT), 13 µlL riboflavin, and 130 µl methionine. POX activity was measured at 470 nm following a reaction mixture containing 0.1 ml enzyme extract, 0.5 ml H_2O_2, 0.9 ml distilled water, 1 ml phosphate buffer, and 0.5 ml guaiacol. CAT activity was measured at 240 nm following a reaction mixture containing 0.05 ml enzyme extract, 0.5 ml H_2O_2, 0.95 ml distilled water, and 1.5 ml phosphate buffer. TPC was determined using the Folin-Ciocalteu method with absorbance measured at 765 nm, following the protocol described by Matrood and Rhouma (2021).

Statistical analysis

Statistical analysis was conducted using the mean values of replicated data. ANOVA was employed to analyze the data using SPSS version 20.0 statistical software (SPSS, SAS Institute, USA). Duncan's Multiple Range Test was utilized to evaluate differences between treatments, homogeneity of variances, and normality. All statistical tests were performed at a significance level of 1% ($p \leq 0.01$).

RESULTS AND DISCUSSION

Table 2 presented the findings of an *in vivo* experiment investigating the effectiveness of *T. koningii* and *R. irregularis* in mitigating Fusarium wilt disease caused by FOL in tomato plants. The positive control group, inoculated only with FOL, represents the full impact of the disease without any intervention. As expected, these plants suffered the highest DS (4.83) and were classified as highly susceptible with a DSI of 98.33%. The negative control group, with no pathogen inoculation, served as the healthy plant benchmark, showing no disease and a DSI of zero. The remaining groups explore the protective effects of the beneficial fungi. When applied with FOL, *T. koningii* offered significant disease resistance, reducing DS to 0.83 and DSI to 29.33% compared to the positive control. Besides, combining both fungi (*T. koningii* + *R. irregularis*) provided the most effective protection. This treatment group displayed disease severity comparable to the healthy control group (DS = 0.33) and the lowest DSI (14.33%). The treatment groups with *T. koningii*, either alone or combined with *R. irregularis*, were classified as "Effective" indicating their success in suppressing disease development. *Rhizophagus irregularis* partially mitigated the negative effects of FOL, resulting in moderate resistance (DS = 2.17; DSI = 43%) (Table 2).

This *in vivo* experiment investigated the potential of *T. koningii*, alone or combined with *R. irregularis*, as biocontrol agents against Fusarium wilt in tomato. The findings demonstrated that both single and combined inoculations significantly mitigated the disease. This corroborates previous research highlighting the efficacy of these fungi. For instance, Li et al. (2016) reported that the combined application of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) enhanced cucumber growth and reduced wilt disease in field conditions. Similarly, Merina Prem Kumari and Prabina (2019) observed increased plant growth and decreased wilt incidence in tomato pot cultures when AMF inoculation preceded pathogen introduction. *Trichoderma* spp. have a well-established reputation for combating various plant diseases, including Fusarium wilt in tomato (Ramesh and Singh 2017, Taribuka et al. 2017, Zhang et al. 2022). Furthermore, Kubiak et al. (2023) identified *T. koningii* as an effective biocontrol agent against *F. oxysporum*. Greenhouse experiments by Porteous-Álvarez et al. (2023) and Brizuela et al. (2023) demonstrated that *T. koningii* reduced melon Fusarium wilt occurrence.

Table 3 displayed the average chlorophyll a, chlorophyll b, and total chlorophyll content measured in each treatment. It is important to note that Table 3 presents statistical analysis indicating that the observed differences in chlorophyll content between treatments are highly significant ($p < 0.01$). The data revealed that FOL infection (Chl T = 1.52

Table 2. *In vivo* evaluation of *Trichoderma koningii* and *Rhizophagus irregularis* on disease severity (DS), resistance level of treatment (RLT), and disease severity index (DSI) in tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici*

Treatments	DS	RLT	DSI(%)
Positive control	4.83±0.58a[a]	Highly ineffective	98.33±1.91a
Negative control	0±0d	Extremely effective	0±0e
T. koningii + FOL	0.83±0.17c	Ineffective	29.33±1.07c
R. irregularis + FOL	2.17±0.28b	Ineffective	43±1.82b
T. koningii + *R. irregularis* + FOL	0.33±0.01cd	Effective	14.33±1.14d
P-value[b]	< 0.01	Nd	< 0.01

[a] Duncan's Multiple Range Test, values followed by various superscripts differ significantly at $p \leq 0.01$.
[b] Probabilities associated with individual F tests.
FOL: *F. oxysporum* f.sp. *lycopersici*; Nd: Not determined.
Average values ± standard deviation.

mg g^{-1} Fresh Weight) considerably decreased the chlorophyll content compared to the healthy control (Chl T = 4.17 mg g^{-1} Fresh Weight). Interestingly, inoculating the infected plants with either *T. koningii* or *R. irregularis* alongside FOL partially counteracted these reductions. The most prominent chlorophyll restoration was observed in plants treated with the combination of both fungi. These treatments exhibited the highest Chl a (5.62 mg g^{-1} Fresh Weight), Chl b (3.11 mg g^{-1} Fresh Weight), and Chl T (8.74 mg g^{-1} Fresh Weight), suggesting that the combined application of *T. koningii* and *R. irregularis* might be more effective in defending against FOL infection and preserving chlorophyll content in tomato plants compared to using either fungus alone (Table 3).

to all other treatments. This implies that tomato plants were most susceptible to oxidative stress caused by FOL infection in the absence of beneficial fungal inoculation. In contrast, tomato plants inoculated with both *T. koningii* and *R. irregularis* together displayed the most robust antioxidant response. This is reflected in the significantly higher activity of all three antioxidant enzymes (SOD = 46.17 units g^{-1} ml^{-1} min^{-1}, POX = 5.66 units g^{-1} ml^{-1} min^{-1}, and CAT = 104.42 units g^{-1} ml^{-1} min^{-1}) and the substantially greater TPC (3.14 mg g^{-1}) compared to the controls and other treatments. These findings suggest that the combined application of *T. koningii* and *R. irregularis* effectively alleviated oxidative stress in tomato roots challenged by FOL infection,

Table 3. *In vivo* evaluation of *Trichoderma koningii* and *Rhizophagus irregularis* on chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll content (Chl T) in tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici*

Treatments	Chl a (mg g^{-1} Fresh Weight)	Chl b (mg g^{-1} Fresh Weight)	Chl T (mg g^{-1} Fresh Weight)
Positive control	1.18±0.85e[a]	0.34±0.74d	1.52±0.29e
Negative control	2.73±0.54d	1.44±0.81c	4.17±0.38d
T. koningii + FOL	4.75±0.28b	2.24±0.36b	6.99±0.69b
R. irregularis + FOL	3.18±0.34c	1.70±0.12c	4.88±0.69c
T. koningii + *R. irregularis* + FOL	5.62±0.69a	3.11±0.34a	8.74±0.94a
P-value[b]	< 0.01	< 0.01	< 0.01

[a] Duncan's Multiple Range Test, values followed by various superscripts differ significantly at $p \leq 0.01$.
[b] Probabilities associated with individual F tests.
FOL: *F. oxysporum* f.sp. *lycopersici*
Average values ± standard deviation.

Supporting the observed disease mitigation, previous research by Merina Prem Kumari and Jeberlin Prabina (2019) and Diagne et al. (2020) suggests that mycorrhizal inoculation enhanced the physiological state of host plants. This includes improved chlorophyll content and nutrient uptake, ultimately leading to increased tolerance against pathogen stress. Additionally, *Trichoderma* spp. are well-recognized for their plant growth promotion capabilities. They enhance seedling vigor and crop yields in various plant species (Liu et al. 2017, Zhang et al. 2019). He et al. (2019) specifically reported that *T. asperellum* granules improved growth and resistance to *F. graminearum* in maize. Similarly, Zhang et al. (2022) observed a significant increase in tomato vine length with *T. asperellum* compared to the control treatment.

Table 4 investigated the impact of *T. koningii* and *R. irregularis* on the antioxidant defense system and total phenolic content of tomato roots infected with FOL. Untreated tomato plants exhibited the weakest antioxidant defense system, as evidenced by the lowest activity of SOD (14.83 units g^{-1} ml^{-1} min^{-1}), POX (0.86 units g^{-1} ml^{-1} min^{-1}), and CAT (19.95 units g^{-1} ml^{-1} min^{-1}), and the lowest TPC (1.10 mg g^{-1}) compared

potentially by stimulating the plant's natural antioxidant defense mechanisms and enhancing the production of phenolic compounds. Furthermore, inoculation with either *T. koningii* or *R. irregularis* alone resulted in increased antioxidant activity and TPC compared to the controls. However, the magnitude of this increase was lower than that observed in the combined treatment. This suggests that both fungi can independently induce an antioxidant response in tomato roots, but their combined application has a synergistic effect, leading to a more pronounced enhancement of the plant's defense system (Table 4).

This study demonstrated that both *T. koningii* and *R. irregularis* application significantly enhance the antioxidant defense system in tomato roots infected with FOL. The combined treatment resulted in the highest activities of SOD, POX, and CAT, along with increased TPC. This finding aligns with previous research by Zhang et al. (2022), who reported that *Trichoderma* spp. and AMF treatments elevated the activities of defense-related enzymes in plants under stress conditions. These increased enzyme activities and phenolic content suggest that the biocontrol agents

Table 4. *In vivo* evaluation of *Trichoderma koningii* and *Rhizophagus irregularis* on superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) activity, and total phenolic content (TPC) in tomato roots in the presence of *Fusarium oxysporum* f.sp. *lycopersici*

Treatments	SOD (units g^{-1} mL^{-1} min^{-1})	POX (units g^{-1} mL^{-1} min^{-1})	CAT (units g^{-1} mL^{-1} min^{-1})	TPC (mg g^{-1})
Positive control	17.22±0.33d[a]	2.16±0.71d	15.29±0.89d	1.13±0.14c
Negative control	14.83±1.09e	0.86±0.01e	19.95±1.08d	1.10±0.03c
T. koningii + FOL	40.03±1.92b	4.34±0.24b	79.33±0.98b	2.28±0.04b
R. irregularis + FOL	25.40±0.39c	3.38±0.28c	57.99±1.28c	1.89±0.02b
T. koningii + *R. irregularis* + FOL	46.17±1.81a	5.66±0.16a	104.42±2.59a	3.14±0.04a
P-value[b]	< 0.01	< 0.01	< 0.01	< 0.01

[a] Duncan's Multiple Range Test, values followed by various superscripts differ significantly at $p \leq 0.01$.
[b] Probabilities associated with individual F tests.
FOL: *F. oxysporum* f.sp. *lycopersici*
Average values ± standard deviation.

not only directly suppress the pathogen but also stimulate the plant's intrinsic defense mechanisms against oxidative stress caused by FOL infection. Similar observations were reported by Abbaspour et al. (2012) and Wang et al. (2017) who found that AMF inoculation enhanced antioxidant enzyme activity and reduced stress markers in plants facing drought or pathogen challenges. These findings collectively highlight a potential mechanism by which *Trichoderma* and AMF treatments contribute to disease resistance in plants.

The correlation coefficients revealed strong positive or negative associations between most measures, indicating that changes in one factor often coincide with changes in others. Notably, a particularly pronounced positive correlation (0.973) was observed between DS and DSI, indicating a strong and consistent co-variation between these two specific measures. Chlorophyll content (a, b, and total) demonstrated strong positive correlations amongst themselves (> 0.9) and a negative correlation with disease severity measures (≈-0.7). This suggests that decreased chlorophyll levels, essential for photosynthesis, are linked to increased disease presence. Plants experiencing disease stress might allocate resources away from chlorophyll production, leading to lower chlorophyll content and potentially reduced photosynthetic activity. Similarly, the antioxidant enzyme activities and TPC showed strong positive correlations within themselves (>0.8) and a moderate negative correlation with disease severity (≈-0.4). These enzymes play a crucial role in plant defense mechanisms by scavenging harmful free radicals produced during disease stress. Phenolic compounds are another line of plant defense, and their increased presence might be associated with the plant's response to disease. The positive correlations suggest coordinated activity among these enzymes and TPC, and the negative correlation with disease severity indicates their potential role in mitigating disease impact (Table 5).

Table 5. Correlation coefficients between disease severity (DS), disease severity index (DSI), chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll content (Chl T), superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) activity, and total phenolic content (TPC)

	DSI	Chl a	Chl b	Chl T	SOD	POX	CAT	TPC
DS	0.973**	0.724**	0.770**	0.745**	-0.429	-0.243	-0.489	-0.409
DSI		-0.652**	0.702**	0.674**	-0.331	-0.144	-0.417	-0.367
Chl a			0.977**	0.997**	0.917**	0.832**	0.941**	0.887**
Chl b				0.991**	0.871**	0.792**	0.917**	0.864**
Chl T					0.905**	0.822**	0.937**	0.883**
SOD						0.955**	0.971**	0.937**
POX							0.949**	0.928**
CAT								0.958**

**Significant at level of 1%.

Trichoderma species likely exert its biocontrol effects through a combination of mechanisms. One potential mechanism involves the production of antibiotic substances that inhibit the growth of phytopathogens (Guzmán-Guzmán et al. 2023). Additionally, *Trichoderma* spp. can directly parasitize pathogenic fungi through mycoparasitism (Hajji-Hedfi et al. 2023, Zhang et al. 2022). Beyond direct pathogen antagonism, *Trichoderma* spp. can also indirectly benefit plants by promoting growth and stimulating intrinsic defense mechanisms (Hajji-Hedfi et al. 2023, Zhang et al. 2022). This growth promotion may be partly due to competition for available nutrients and the production of inhibitory compounds against other fungi (Hajji-Hedfi et al. 2023, Kubiak et al. 2023, Tyśkiewicz et al. 2022). Notably, *Trichoderma* spp. can also stimulate plant growth independently of disease presence, suggesting additional mechanisms beyond just pathogen suppression (Nicolás et al. 2024).

Chitinolytic enzyme activity is a well-established mechanism by which AMF and *Trichoderma* spp. contribute to plant disease control (Bisht and Garg 2024, Chen et al. 2020). These biocontrol agents directly target fungal pathogens by secreting lytic enzymes, primarily chitinases, which hydrolyze the pathogen's cell wall components, including chitin and other glycans. This enzymatic breakdown dramatically affects the structural integrity and viability of the pathogenic fungi. These findings align with previous research by Jmal et al. (2015) and Kalamulla et al. (2022), which highlighted the multifaceted role of AMF in plant disease control. While nutrient uptake enhancement is a known benefit of AMF colonization, these studies emphasize the production of phenolic and other secondary inhibitory compounds during plant-AMF interactions as another crucial factor contributing to disease resistance and plant growth.

AMF are a class of beneficial soil microorganisms that form symbiotic relationships with the roots of terrestrial plants (Shi et al. 2023). Beyond their well-known role in enhancing plant nutrition, particularly phosphorus uptake, *R. irregularis* has emerged as promising biocontrol agents against a wide range of fungal diseases. This biocontrol activity arises from a combination of direct and indirect mechanisms (Adriano-Anaya et al. 2006, Kakabouki et al. 2021, Merina Prem Kumari and Jeberlin Prabina 2019, Mota et al. 2020,).

Directly, *R. irregularis* can compete with pathogenic fungi for space and nutrients within the root system, limiting their colonization and growth. Some AMF species produce antifungal compounds that inhibit the growth of pathogens, while others may even parasitize the pathogenic fungi (Jiang et al. 2021, Pan et al. 2020). Indirectly, *R. irregularis* enhance plant growth and vigor by improving nutrient uptake, which strengthens the plant's natural defenses against diseases (Adriano-Anaya et al. 2006, Andrino et al. 2021, Chen et al. 2020, Wang et al. 2017). Furthermore, *R. irregularis* can trigger systemic resistance in plants, activating defense pathways and leading to the production of defense-related compounds. This systemic resistance provides broader protection against a range of pathogens. *R. irregularis* also influence the composition and activity of the rhizosphere microbial community, favoring the growth of beneficial microorganisms that can suppress pathogens (Burke and Carrino-Kyker 2021, Emmett et al. 2021, Müller et al. 2019).

The effectiveness of AMF as biocontrol agents has been demonstrated against various fungal diseases. Their use offers several advantages, such as being environmentally friendly, promoting sustainable agriculture by reducing reliance on chemical fungicides, and improving overall plant health. However, the effectiveness of AMF can vary depending on factors such as the specific AMF species, plant host, pathogen, and soil conditions (Bisht and Garg 2024, Delaeter et al. 2024, Diagne et al. 2020, Kalamulla et al. 2022, Khalediyan et al. 2021, Khaliq et al. 2022).

The study demonstrated the potent antagonistic effects of combined *R. irregularis* and *T. koningii* against FOL regardless of application method. These biocontrol agents likely employ a multifaceted approach to suppress the pathogen. Mechanisms potentially involved include: enhanced nutrient uptake by plants due to AMF colonization (Kalamulla et al. 2022, Zhang et al. 2022); direct mycoparasitism of the pathogen by *Trichoderma* spp. (Zhang et al. 2022); competition with FOL for space and nutrients (Kubiak et al. 2023, Tyśkiewicz et al. 2022); and plant growth promotion by both AMF and *Trichoderma* spp. (Zhang et al. 2022). Additionally, *Trichoderma* spp. may contribute through the secretion of antibiotics (Guzmán-Guzmán et al. 2023), phenolic compounds (Kalamulla et al. 2022), and cell wall degrading enzymes (Bisht and Garg 2024). Furthermore, *Trichoderma* spp. can produce plant growth regulators (Nicolás et al. 2024), potentially enhancing plant health and resilience. The combined effects of these mechanisms likely contribute to improved translocation of macronutrients from roots to shoots (Zhang et al. 2022), ultimately leading to a healthier plant with increased resistance to FOL infection.

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Authors declare the contribution of the authors is equal.

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The authors have declared no conflict of interest.

ÖZET

Bu çalışmada, sera koşullarında domateste Fusarium solgunluk hastalığının kimyasal mücadelesine alternatif olarak çevre dostu *Trichoderma koningii* ve *Rhizophagus irregularis*'in potansiyeli araştırılmıştır. Araştırma, *T. koningii* ve *R. irregularis*'in nasıl etkileşime girdiğine ve bitki büyümesi ve hastalık direnci üzerindeki etkilerine odaklanmıştır. *T. koningii* tek başına hastalık şiddetini *Fusarium oxysporum* f. sp. *lycopersici* (FOL) ile enfekte edilmiş kontrol grubuna kıyasla önemli ölçüde azaltmıştır (DS = 0.83; DSI = %29.33). Ancak, en etkili koruma *T. koningii* ve *R. irregularis*'in birlikte kullanımından elde edilmiş (DS = 0.33; DSI = %14.33) ve sağlıklı kontrollerle karşılaştırılabilir bir seviyeye ulaşılmıştır. Bu kombine uygulama sadece etkili hastalık direnci göstermekle kalmamış, aynı zamanda en yüksek klorofil içeriğini de göstermiştir (Chl a = 5.62 mg g^{-1} Taze Ağırlık; Chl b = 3.11 mg g^{-1} Taze Ağırlık; Chl T = 8.74 mg g^{-1} Taze Ağırlık), bu da FOL enfeksiyonunun neden olduğu klorofil bozunmasına karşı koymada daha güçlü bir yeteneğe işaret etmektedir. Ek olarak, *T. koningii* ve *R. irregularis* ile birlikte aşılanan domates bitkileri en güçlü antioksidan tepkiyi sergileyerek, antioksidan enzimlerinin (süperoksit dismutaz = 46.17 birim g^{-1} ml^{-1} dk^{-1}, peroksidaz aktivitesi = 5.66 birim g^{-1} ml^{-1} dk^{-1} ve katalaz aktivitesi = 104.42 birim g^{-1} ml^{-1} dk^{-1}) ve toplam fenolik içeriğinin (3.14 mg g^{-1}) belirgin şekilde daha yüksek olduğunu göstermiştir. Bu bulgular, *T. koningii* ve *R. irregularis*'in birlikte uygulanmasının, *T. koningii* veya *R. irregularis*'in tek başına kullanılmasına kıyasla, domateste Fusarium solgunluk hastalığının yönetimi ve genel bitki sağlığının geliştirilmesi açısından daha etkili ve çevre dostu bir strateji olma potansiyeline sahip olduğunu göstermektedir.

Anahtar kelimeler: arbusküler mikorizal fungus, *Solanum lycopersicum*, *Fusarium oxysporum* f. sp. *lycopersici*, sera denemesi

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