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Evaluation of pathogenic variation among *Rhizoctonia solani* isolates infecting different crops and potential biocontrol agents

Farklı ürün türlerini enfekte eden *Rhizoctonia solani* izolatları arasındaki patojenik varyasyonun ve potansiyel biyokontrol ajanlarının değerlendirilmesi

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ABSTRACT

Rhizoctonia solani is an important broad-spectrum fungal pathogen that infects over 200 plant species including tomato, melon, and watermelon. This study evaluated the pathogenicity of various *R. solani* isolates (Rs26, Rs94, Rs13, Rs57, and Rs123) and the efficacy of biological agents (*Trichoderma harzianum*, *T. viride*, *Metarhizium* sp., *Gliocladium* sp.) under laboratory and greenhouse conditions for eco-friendly disease management. The results of the pathogenicity assay confirmed the varying aggressiveness of the isolates, with Rs94 and Rs13 causing the most severe disease in watermelon (disease severity (DS) = 3.80 and 3.83, disease severity index (DSI) = 90.43% and 95.75%, respectively). Similarly, isolate Rs26 displayed the highest pathogenicity in tomatoes (DS = 3.84; DSI = 94.86%). Melon exhibited high susceptibility across all isolates, with consistently high DS and DSI values exceeding 2.59 and 80.97%, respectively. Subsequent *in vitro* and *in vivo* assays demonstrated the antifungal potential of all tested agents against *R. solani* isolates. Notably, *Trichoderma* spp. displayed the most consistent and significant inhibition (mycelial growth reduction 82.97%-94.67%), with *T. harzianum* demonstrating superior performance. Greenhouse trials confirmed the effectiveness of *T. harzianum* as a preventative treatment, enhancing plant enzyme activity [peroxidase = 4.97-5.29 units g^{-1} ml^{-1} min^{-1} for tomato and watermelon, respectively; catalase = 99.93-101.22 units g^{-1} ml^{-1} min^{-1} for watermelon and melon, respectively] and significantly reducing disease severity index (DSI < 12.43%). These findings highlight the potential of *T. harzianum* as a sustainable and eco-friendly strategy for managing *R. solani* damping-off disease in tomato, melon, and watermelon crops.

INTRODUCTION

Rhizoctonia solani JG Kühn (teleomorph: *Thanatephorus cucumeris* (AB Frank) Donk) is a complex of soil-borne fungal plant pathogens encompassing over 100 distinct species (Abbas et al. 2023, Li et al. 2021). This necrotrophic fungal pathogen thrives by deriving nutrients from dead or dying host tissues (Li et al. 2021). One of its key survival mechanisms is the formation of sclerotia, hardened structures that allow the fungus to persist in the soil during unfavorable conditions (Erper et al. 2021). This complex has a broad host range and global distribution, causing diseases in various economically important agricultural and horticultural crops and trees (Erper et al. 2021). The wide range of plants it can infect allows it to cause numerous diseases. For instance, it causes sheath blight in key field crops like corn and rice and it also causes root rot in vegetables and legumes (Canpolat and Tülek 2019, Canpolat et al. 2023, Dubey et al. 2012, Ozan and Aşkın 2006, Ozan and Maden 2004, Yücel and Çolak 2008). R. solani exhibits varying degrees of virulence towards different crops. It is particularly destructive to seedlings and seeds of vegetables like eggplant, pepper, lettuce, and zinnia (Abbas et al. 2023). This fungus causes stem canker and black scurf in potatoes, significantly reducing tuber yield and quality (Naqvi et al. 2024). However, one of the most critical diseases caused by *R. solani* is cotton root rot, posing a substantial threat to this economically vital crop. In greenhouses, it is a primary pathogen responsible for root and crown rot in tomatoes. This fungus extends its destructive reach to numerous vegetables, including cucurbits and tomatoes, causing various seedling diseases such as seed rot, root rot, pre-emergence damping-off, and post-emergence damping-off. The economic impact of *R. solani* is substantial, causing significant yield losses across more than a hundred crop and horticultural species annually. Moreover, its emergence as a significant problem is ongoing, with recent observations highlighting its ability to cause stem rot in sweet potatoes (Abbas et al. 2023, Naqvi et al. 2024).

Rhizoctonia solani is a ubiquitous and cosmopolitan soilborne fungal plant pathogen exhibiting a multifaceted lifestyle (Naqvi et al. 2024). This fungus can exist as both a saprophyte, decomposing dead organic matter in the soil, and a pathogen capable of infecting living plants (Li et al. 2021). The *R. solani* complex is further classified into fourteen genetically distinct anastomosis groups (AG1 to AG13 and AGBI) (Abbas et al. 2023). These groups exhibit host specificity, meaning they preferentially infect certain plant species and are unable to reproduce sexually with each other (Erper et al. 2021). As a whole, the complex has a very broad host range, encompassing numerous plant species crucial to agriculture, forestry, and the bioenergy sector (Yang et al. 2024). This includes prominent crops such as tomato, wheat, rice, barley, potato, melon, watermelon, and sugar beet. The extensive host range and diverse lifestyles within the *R. solani* complex highlight its significant role as a plant pathogen with a broad economic impact (Dubey et al. 2012). The increasing pathogenicity of *R. solani* strains underscores the urgent need for the development of effective control strategies to mitigate the widespread damage it causes (Abdelghany et al. 2022, Dubey et al. 2011).

Rhizoctonia solani is a major fungal pathogen responsible for significant pre- and post-emergence damping-off and root rot diseases in various vegetables (Yang et al. 2024). Unfortunately, effective fungicides for *Rhizoctonia* control are limited for many vegetables, with some chemicals like chlorothalonil, thyophanate methyl, and iprodione showing some efficacy but with growing concerns about their environmental impact (Agrios 1988, Hajji-Hedfi et al. 2023). This necessitates exploring alternative, more sustainable solutions. Biocontrol programs using fungal and bacterial mycoparasites offer a promising approach for managing soilborne pathogens like *R. solani* (Albastawisi and Kotan 2024, Mohamed et al. 2020, Ruiz-Cisneros et al. 2018). Among various biocontrol agents, *Trichoderma* spp. have emerged as particularly effective antagonists against *R. solani* (Behiry et al. 2023). *Trichoderma* spp. employ a multifaceted biocontrol strategy, including production of antibiotics and hydrolytic enzymes, direct mycoparasitism of *R. solani* hyphae, and hyphal disruption (Hajji-Hedfi et al. 2023). The specific mechanisms likely vary depending on the fungal strains involved, potentially involving a combination of these strategies acting independently or synergistically during microbial interactions (Almaghasla et al. 2023). Besides, *Trichoderma* spp. may influence the viability of *R. solani* sclerotia, offering an additional layer of control (Behiry et al. 2023). Overall, research on *Trichoderma* spp. and other biocontrol agents presents a promising and environmentally friendly approach for mitigating the detrimental effects of *R. solani* on vegetable crops (Shalaby et al. 2022).

The present study aimed to (i) investigate the pathogenicity of five *R. solani* isolates on tomato, melon, and watermelon, (ii) evaluate the *in vitro* and *in vivo* efficacy of *Trichoderma* spp., *Metarhizium* sp., and *Gliocladium* sp. as a potential biocontrol agent for managing *R. solani* infections.

MATERIALS AND METHODS

Pathogenicity test

A study investigated the virulence of five *R. solani* isolates (Rs26, Rs94, Rs13, Rs57, and Rs123) on three crops [tomato (cv. Firenze), melon (cv. Badii), and watermelon (cv. Crimson Sweet)]. The experiment aimed to identify variations in virulence among the isolates. Each isolate originated from a different soil source (watermelon, tomato, melon, tomato, and watermelon). Disinfested potting mix (clay:sand, 2:1 v/v) was added to sterilized pots (20 cm diameter) at 2.5 kg per pot. Inoculum for each fungal isolate was prepared by culturing them in sterilized sorghum grain medium for 15 days at 25 °C ± 2 °C. To infest the soil, the inoculum was mixed with the upper potting mix layer at a rate of 2% (w/w). The infested potting mix was thoroughly mixed and irrigated every other day for a week before planting to stimulate fungal growth and ensure proper distribution throughout the soil. Five healthy seeds from the Regional Centre of Agricultural Research of Sidi Bouzid, Tunisia were sown in each pot. Three replicate pots were used for each isolate-crop combination, with an additional three un-infested pots serving as controls (negative control). Plants were grown in a greenhouse chamber under a 16 h/8 h light/dark cycle at 23–25 °C with regular irrigation (Matrood and Rhouma 2021, Rhouma et al. 2024). Disease severity (DS) was evaluated after 60 days using a 0-4 scale adapted from Carling et al. (1999): 0 - no visible damage, 1 - minor hypocotyl discoloration, 2 - discoloration with small necrotic lesions (<1 mm diameter), 3 - discoloration with larger necrotic lesions (≥1 mm diameter), and 4 - plant death. 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 (Okon et al. 2023).

Antagonistic action of antagonistic fungi toward Rhizoctonia solani

A dual culture assay on potato dextrose agar (PDA) plates was conducted to evaluate the antagonistic interaction between four biocontrol agents (*T. harzianum*, *T. viride*, *Metarhizium* sp., and *Gliocladium* sp.) and *R. solani* isolates (Rs26, Rs94, Rs13, Rs57, and Rs123). Biocontrol agents were obtained from the Laboratory of Plant Protection's collection (CRRA, Sidi Bouzid, Tunisia) and were isolated from the rhizosphere soil of tomato plants collected in the Regueb agricultural fields of Sidi Bouzid. The assay employed 0.5 cm diameter agar plugs, one containing a four-day-old culture of the biocontrol agent and the other containing the target *R. solani* isolate. Following a standardized protocol, these plugs were placed on opposite sides of a single 9 cm PDA plate: the antagonist plug was positioned 2 cm from the edge towards the center, maintaining a 5 cm gap between the plugs. Control plates included only a blank PDA plug on one side and the *R. solani* isolate plug on the opposite side. Each treatment was replicated three times, with each replicate consisting of five plates. All plates were incubated for seven days at 28 °C ± 2 °C (Hajji-Hedfi et al. 2023, Rhouma et al. 2024). After incubation, the percentage inhibition of *R. solani* radial growth was determined using the formula established by Matrood and Rhouma (2021): I (%) = (1 - Cn/C0) × 100, where Cn represents the radial growth of the *R. solani* colony in the presence of the biocontrol agent and C0 represents the radial growth of the *R. solani* colony in the control plate without an antagonist.

In vivo evaluation of antagonistic fungi on tomato, melon, and watermelon plants inoculated with Rhizoctonia solani

The experiment employed a randomized complete block design with three replicate blocks, each containing 135 pots. Each pot held three seedlings of a single crop species: tomato (cv. Firenze), melon (cv. Badii), or watermelon (cv. Crimson Sweet). The potting mix consisted of a 1:1 (v/v) mixture of peat and vermiculite. Seedlings received designated treatments and inoculations after 15 days of growth (Hajji-Hedfi et al. 2023). Within each block, seedlings received five treatments: T1 (positive control) - inoculation with *R. solani*, T2 (negative control) - treatment with sterilized water only, T3 - dipping in *T. harzianum* conidial suspension for 30 min followed by *R. solani* inoculation (10 ml) 24 hours later, T4 - dipping in *T. viride* conidial suspension for 30 min followed by *R. solani* inoculation (10 ml) 24 hours later, and T5 - dipping in *Metarhizium* sp. conidial suspension for 30 min followed by *R. solani* inoculation (10 ml) 24 hours later. Specific *R. solani* isolates were used for each crop: Rs26 for tomato, Rs57 for melon, and Rs13 for watermelon. Following treatment, all pots were incubated in a growth chamber under controlled conditions for 60 days with an 8-hour dark/16-hour light photoperiod and a temperature range of 20-22 °C.

To prepare the fungi for the antagonism assays, each strain was grown individually on PDA media at a constant temperature of 25 °C for four days. This incubation period allows for sporulation. Following incubation, four colonized agar plugs of each fungal strain were used to inoculate separate flasks containing 50 ml of Potato Dextrose Broth (PDB) media. The use of liquid media in this step further promotes fungal growth and spore production. These flasks

were incubated on an orbital shaker for seven days to achieve even distribution and enhanced spore release. After this incubation period, spores were harvested from each fungal culture using a filtration technique. The concentration of spores in each fungal suspension was then quantified using a hemocytometer. This quantification process revealed a final spore density of 10^6 spores ml^{-1} (Matrood and Rhouma 2021).

A visual scoring system (0-4) was employed to assess DS according to Popoola et al. (2015). McKinney's formula then converted these scores into a DSI expressed as a percentage (Okon et al. 2023, Thakur and Tripathi 2015). Peroxidase (POX) and catalase (CAT) activity were assessed in plant root tissues following established protocols. Three root samples from each treatment and block were homogenized, and enzyme extracts were prepared. POX activity was determined spectrophotometrically at 470 nm. The reaction mixture contained 0.1 ml enzyme extract, 0.5 ml hydrogen peroxide, 0.9 ml distilled water, 1 ml phosphate buffer, and 0.5 ml guaiacol. CAT activity was also measured spectrophotometrically at 240 nm. The reaction mixture included 0.05 ml enzyme extract, 0.5 ml hydrogen peroxide, 0.95 ml distilled water, and 1.5 ml phosphate buffer (Rhouma et al. 2024).

Statistical analysis

The analysis employed a one-way ANOVA on the mean values of replicated data. This was performed using version 20.0 of the SPSS statistical software package (SPSS, SAS Institute, USA) to assess for significant differences between treatment groups. The homogeneity of variances and normality of the data were verified before the ANOVA. All statistical tests were conducted at a significance level of alpha = 0.01 ($P \leq 0.01$).

RESULTS AND DISCUSSION

Pathogenicity test

Analysis of DS scores revealed variations in the pathogenic potential of five *R. solani* isolates towards tomato, melon, and watermelon ($P < 0.01$). All isolates successfully infected all three crops compared to the control group, which exhibited no disease development. This confirmed the inherent pathogenicity of all tested *R. solani* isolates. Besides, a closer examination of the severity scores unveiled interesting patterns. Isolates Rs94 and Rs13 demonstrated the strongest virulence on watermelon (DS = 3.80 and 3.83, respectively), suggesting potential variations in isolate-specific virulence. However, isolate Rs26 exhibited the highest pathogenicity on tomato (DS = 3.84), further supporting the hypothesis of differential virulence among isolates. Interestingly, all isolates caused significant disease development on melon (DS > 2.72), suggesting a high level of susceptibility in this particular crop to all tested *R. solani* isolates (Table 1).

Table 2 investigated the impact of five *R. solani* isolates on the disease severity index in tomato, melon, and watermelon plants. Without any fungal introduction, the control group exhibited minimal disease in all three crops (DSI = 0%). All fungal isolates significantly increased disease severity compared to the control in each plant species ($P < 0.01$). However, the isolates' effect varied across crops. Rs57 caused the most severe disease in melon (97.52%), while Rs26 caused the most severe disease in tomato (94.86%). Interestingly, Rs13 caused the most severe disease in watermelon (95.75%) but ranked lower in tomato and melon disease severity (Table 2).

R. solani isolates exhibit intraspecific diversity in their virulence, as evidenced by this study's investigation into their pathogenicity on various crops (Abdelghany et al. 2022, Eken et al. 2024, Mustafa et al. 2021, Porto et al. 2019). All isolates tested caused damping-off and root rot diseases, albeit with varying degrees of severity. This finding aligns with prior research demonstrating the broad host range of *R. solani* isolates obtained from diverse environments (Abbas et al. 2023, Dubey et al. 2012, Erper et al. 2021, Porto et al. 2019, Yang et al. 2024). The extensive pathogenicity of *R. solani* is likely attributed to its production of polygalacturonase enzymes, which degrade plant cell wall pectate, as suggested by Naqvi et al. (2024). *R. solani* preferentially targets the hypocotyl region of seedlings at the soil line due to the heightened vulnerability of meristematic tissues to its cell wall degrading enzymes. As seedlings mature, they develop resistance mechanisms that counteract the fungus's virulence. These mechanisms include thickening the cuticle, which limits the amount of exudates the fungus needs to form infection cushions, and converting pectin into a form resistant to *R. solani*'s enzymes (calcium pectate) (Naqvi et al. 2024).

Antagonistic action of antagonistic fungi toward Rhizoctonia solani

This study employed a controlled laboratory setting to evaluate the potential application of antagonistic fungal isolates for managing the growth of *R. solani*. The experiment specifically focused on the impact of these antagonists on the mycelial development of five distinct *R. solani* isolates. The obtained results revealed promising antifungal activity from all four antagonists. Statistical analysis confirmed

Table 1. Effect of *Rhizoctonia solani* isolates (Rs26, Rs94, Rs13, Rs57, and Rs123) on disease severity in tomato, melon, and watermelon

Treatments	Disease severity		
	Tomato	Melon	Watermelon
Control	0±0d[a]	0±0c	0±0d
Rs26	3.84±0.07a	2.72±0.11b	1.91±0.06c
Rs94	2.59±0.04b	3.70±0.03a	3.80±0.04a
Rs13	2.02±0.01c	3.68±0.01a	3.83±0.05a
Rs57	1.90±0.02c	3.83±0.05a	3.55±0.14ab
Rs123	1.74±0.09c	3.59±0.06a	3.42±0.04b
P-value[b]	< 0.01	< 0.01	< 0.01

[a]Duncan's Multiple Range Test, values followed by various superscripts differ significantly at P≤0.01.
[b]Probabilities associated with individual F tests.

Table 2. Effect of *Rhizoctonia solani* isolates (Rs26, Rs94, Rs13, Rs57, and Rs123) on disease severity index in tomato, melon, and watermelon

Treatments	Disease severity index (%)		
	Tomato	Melon	Watermelon
Control	0±0e[a]	0±0d	0±0d
Rs26	94.86±1.51a	80.97±1.37c	63.65±1.18c
Rs94	76.61±1.27b	95.50±1.51ab	90.43±1.24b
Rs13	65.51±1.84c	94.35±1.68b	95.75±1.33a
Rs57	59.24±1.05d	97.52±1.54a	94.07±1.95ab
Rs123	56.43±1.87d	92.60±1.35b	90.96±1.07b
P-value[b]	< 0.01	< 0.01	< 0.01

[a]Duncan's Multiple Range Test, values followed by various superscripts differ significantly at P≤0.01.
[b]Probabilities associated with individual F tests.

significant inhibition of mycelial growth for most *R. solani* isolates compared to the negative control (P < 0.05). *T. harzianum* displayed the most broad-spectrum inhibition, ranging from 83% (Rs57) to 94.67% (Rs26). Interestingly, the effectiveness of the other antagonists varied. Thus, *T. viride* exhibited consistent inhibition across all *R. solani* isolates (P ≥ 0.05). *Metarhizium* sp. also demonstrated significant inhibition for most isolates, ranging from 65.57% (Rs26) to 75.57% (Rs94). *Gliocladium* sp., however, showed a more variable effect, with significant suppression observed for some isolates (51.35% for Rs13 and 66.75% for Rs26) but less consistent results for others. This observed variation in effectiveness among both the antagonistic fungi and the *Rhizoctonia* isolates suggests potential differences in how they interact (Table 3).

Multiple studies have demonstrated the efficacy of *T. harzianum* as a biological control agent against *R. solani*. In vitro dual culture experiments consistently reported significant reductions in *R. solani* linear growth following incubation with *T. harzianum*, with inhibition rates ranging from 55.55% to 65.18% (Abd-El-Khair et al. 2011, Ban et al. 2022, Brindhadevi et al. 2023, Elsheshtawi et al. 2012, Naeimi et al. 2010). These findings suggest that *T. harzianum* exerts its antagonistic effect through the secretion of diffusible non-volatile inhibitory compounds before hyphal contact, potentially including exochitinases, as reported by Brunner et al. (2005). Furthermore, Abbas et al. (2017) and Paula Junior et al. (2007) proved that *T. harzianum* can promote plant growth, diminish disease severity, and protect seedlings from *R. solani*-induced pre-emergence damping-off.

Table 3. Evaluation of mycelial growth inhibition in *Rhizoctonia solani* isolates (Rs26, Rs94, Rs13, Rs57, Rs123) by four antagonistic fungal isolates under *in vitro* conditions

Treatments	*T. harzianum*	*T. viride*	*Metarhizium* sp.	*Gliocladium* sp.
Rs26	94.67±±0.92a[a]	89.55±1.01a	65.57±0.62b	66.75±1.86a
Rs94	90.67±0.67ab	86.46±0.96a	75.57±0.54a	53.51±1.19b
Rs13	91.80±0.21a	82.97±0.72a	75.17±0.38a	51.35±1.27b
Rs57	83±0.76b	83.60±1.17a	71.75±1.51ab	51.90±1.35b
Rs123	86.34±0.75ab	89.48±0.86a	74.11±0.69ab	59.78±1.73ab
P-value[b]	< 0.05	< 0.05	< 0.05	< 0.05

[a]Duncan's Multiple Range Test, values followed by various superscripts differ significantly at P≤0.01.
[b]Probabilities associated with individual F tests.

In vivo evaluation of antagonistic fungi on tomato, melon, and watermelon plants inoculated with Rhizoctonia solani

Table 4 evaluated the potential of antagonistic fungal isolates to control damping-off disease caused by *R. solani* in tomato, melon, and watermelon plants. The experiment was conducted under controlled conditions. Table 4 presents the disease severity index, a numerical measure of disease intensity, for each crop-fungus combination. The positive control group represents plants infected only with *R. solani*. As expected, this group suffered the most severe symptoms in all three crops, with disease severity indexes close to 100%, indicating extensive disease development. In contrast, the negative control group, where no fungi were introduced, showed minimal to no disease (DSI = 0%). The data reveals a significant reduction in disease severity for all three beneficial fungi compared to the positive control in each plant type (P < 0.01). This confirmed that these fungi could effectively control *R. solani* infection. However, the extent of protection varies depending on the specific beneficial fungus and the crop. *T. harzianum* emerges as the most effective agent, significantly reducing disease severity across all crops. Tomato plants treated with *T. harzianum* showed the lowest disease severity index (10.26%), followed by melon (10.32%) and watermelon (12.43%). While all fungi bring benefits, *T. viride* offers a moderate level of protection, followed by *Metarhizium* sp. (Table 4).

The study examined the impact of fungal treatments on two enzyme activities within the root systems of tomato, melon, and watermelon plants (Tables 5 and 6). Peroxidase activity, an indicator of a plant's defense response against pathogens, was significantly enhanced (P <0.01) across all three plant species when treated with the fungal strains *T. harzianum*, *T. viride* and *Metarhizium* sp. compared to the control groups. Notably, *T. harzianum* (4.97, 5.29, and 5.27 units' g^{-1} ml^{-1} min^{-1}, respectively) consistently induced the highest level of peroxidase activity in all three plant roots, followed by *T. viride* and *Metarhizium* sp. (Table 5).

Table 4. *In vivo* evaluation of antagonistic fungal isolates on disease severity index (%) in roots in the presence of *Rhizoctonia solani* under controlled conditions

Treatments	Disease severity index (%)		
	Tomato	Melon	Watermelon
Positive control	93.58±1.05a[a]	97.7±0.91a	96.7±0.87a
Negative control	00±00e	00±00e	00±00d
T. harzianum + R. solani	10.26±0.85d	10.32±0.27d	12.43±0.54c
T. viride + R. solani	26.62±0.54c	18.13±0.18c	13.58±0.33c
Metarhizium sp. + R. solani	36.39±0.47b	23.47±0.67b	23.68±0.77b
P-value[b]	< 0.01	< 0.01	< 0.01

[a]Duncan's Multiple Range Test, values followed by various superscripts differ significantly at P≤0.01.
[b]Probabilities associated with individual F tests.

Table 5. *In vivo* evaluation of antagonistic fungal isolates on peroxidase activity in roots in the presence of *Rhizoctonia solani* under controlled conditions

Treatments	Peroxidase activity (units g^{-1} ml^{-1} min^{-1})		
	Tomato	Melon	Watermelon
Positive control	1.96±0.07d[a]	2.1±0.14c	1.98±0.05c
Negative control	0.89±0.01e	0.81±0.02d	0.88±0.06d
T. harzianum + *R. solani*	4.97±0.08a	5.29±0.11a	5.27±0.14a
T. viride + *R. solani*	3.72±0.11b	3.35±0.43b	3.2±0.03b
Metarhizium sp. + *R. solani*	2.92±0.13c	2.97±0.05b	2.92±0.02b
P-value[b]	< 0.01	< 0.01	< 0.01

[a]Duncan's Multiple Range Test, values followed by various superscripts differ significantly at P≤0.01.
[b]Probabilities associated with individual F tests.

Table 6. *In vivo* evaluation of antagonistic fungal isolates on catalase activity in roots in the presence of *Rhizoctonia solani* under controlled conditions

Treatments	Catalase activity (units g^{-1} ml^{-1} min^{-1})		
	Tomato	Melon	Watermelon
Positive control	15.52±1.71e[a]	14.64±1.32e	15.13±1.37e
Negative control	20.19±1.42d	18.99±1.24d	19.9±1.44d
T. harzianum + *R. solani*	100.15±1.85a	101.22±1.54a	99.93±1.72a
T. viride + *R. solani*	68.51±1.56b	66.89±1.89b	68.12±1.28b
Metarhizium sp. + *R. solani*	48.29±0.92c	47.44±1.67c	44.08±1.69c
P-value[b]	< 0.01	< 0.01	< 0.01

[a]Duncan's Multiple Range Test, values followed by various superscripts differ significantly at P≤0.01.
[b]Probabilities associated with individual F tests.

Similarly, all three plant species showed a significant increase (P<0.01) in catalase activity, another enzyme implicated in plant defense responses, when treated with the fungal strains as compared to the controls. Interestingly, *T. harzianum* (100.15, 101.22, and 99.93 units' g^{-1} ml^{-1} min^{-1}, respectively) caused the most substantial increase in catalase activity in all plants, followed by *T. viride* and *Metarhizium* sp. These findings suggested that the tested fungi might stimulate defense mechanisms against *R. solani* infection (Table 6).

This research investigated the efficacy of *T. harzianum*, *T. viride* and *Metarhizium* sp. in controlling *R. solani* infection in tomato, melon, and watermelon. The results revealed that *T. harzianum* and *T. viride* significantly reduced the DSI caused by *R. solani* across the tested crops. Additionally, the application of these strains was associated with an increase in plant enzyme activity. *T. harzianum* shows the most significant disease protection effect. This information is valuable for developing biocontrol strategies using these beneficial fungi to manage *R. solani* infection in various crops. These results aligned with prior greenhouse studies by Ali and Taha (2016), Devi et al. (2017), and Huang et al. (2011) who demonstrated the effectiveness of *T. harzianum* in controlling *R. solani*-induced tomato damping-off disease. Furthermore, Ban et al. (2022) reported that pre-seeding application of *T. harzianum* (five days before planting) in tomato and bean crops yielded significantly better disease control compared to simultaneous application. Additionally, Sreenivasaprasad and Manibhushanrao (1990) reported that *T. virens* were successfully used as a biocontrol agent against groundnut root rot and *R. solani*-induced damping-off in cotton and tomato.

Studies have explored various formulations and applications of *Trichoderma* spp. for disease control and plant growth promotion. Rehman et al. (2011) reported improved protection against damping-off disease and enhanced cauliflower seedling growth using a combination of farm

yard manure, *T. harzianum* and *T. viride* as seed treatments. Lewis and Lumsden (2001) demonstrated the effectiveness of a biocontrol formulation containing vermiculite, powdered wheat bran, and *Trichoderma* / *Gliocladium* biomass in controlling pepper and cucumber damping-off in greenhouse settings. Smolinska et al. (2007) further confirmed the efficacy of four *Trichoderma* strains against *R. solani* in lettuce and cucumber, with *T. harzianum* strain PBG notably increasing plant mass in both crops. Beyond disease control, *Trichoderma* has also been linked to improved and faster seed germination in various plant species, including silverweed (Oyarbide et al. 2001), cotton (Hanson 2000), rice (Mishra and Sinha 2000), chili (Asaduzzaman et al. 2010), and muskmelon (Kaveh et al. 2011).

Studies have shown that plant colonization by *Trichoderma* spp. is associated with reduced disease development in both roots and aboveground tissues. This phenomenon is likely attributed to the interactions between *Trichoderma* and the plant itself (Amer and Abou-El-Seoud 2008, Biam and Majumder 2019, Cai et al. 2013, Gajera et al. 2016).

Beyond its antagonistic interactions with plant pathogens, *Trichoderma* spp. also participated in competitive interactions with other soil microorganisms. This competition primarily revolves around securing essential resources like nutrients and space within the soil environment (Baghani et al. 2012, Bailey et al. 2008, Motesharrei and Salimi 2014, Segarra et al. 2010). Notably, *Trichoderma* spp. can compete for root exudates released by seeds. These exudates, while beneficial to plant growth, can also inhibit the germination of fungal propagules belonging to plant pathogens present in the soil (Howell 2003).

Research by Abd-El-Khair et al. (2011), Hajji-Hedfi et al. (2023), Kobori et al. (2015) and Rhouma et al. (2024) investigated the impact of *Trichoderma* application on enzyme activity in various plants. Their findings revealed a significant increase in the activity of several key enzymes, including polyphenol oxidase, chitinase, catalase, and peroxidase, in plants treated with *Trichoderma* compared to the untreated control group. These specific enzymes are essential for strengthening plant defense mechanisms against the invasion of pathogens.

The many advantages of *T. harzianum* have made it a leading biocontrol and biostimulant. This fungus exhibits remarkable antifungal activity against a broad spectrum of plant pathogens, including *R. solani*. Previous research has unequivocally demonstrated that *T. harzianum* can effectively suppress the growth of isolates of *R. solani*. The prevailing hypothesis suggests that this inhibitory effect stems from the production of antibiotic secondary metabolites. These bioactive compounds are believed to disrupt the growth and function of the target fungal pathogens. By unraveling the intricate mechanisms employed by *T. harzianum*, this study aims to provide a more comprehensive understanding of how microbial biocontrol agents and biostimulants operate. This knowledge is paramount to improving the effective and sustainable integrated plant disease management strategies that reduce reliance on chemical fungicides.

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Author's Contributions

Authors declare that each author's contribution is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Rhizoctonia solani, domates, kavun ve karpuz dahil olmak üzere 200'den fazla bitki türünü enfekte eden önemli ve geniş spektrumlu bir fungal patojendir. Bu çalışmada, farklı *R. solani* izolatlarının (Rs26, Rs94, Rs13, Rs57 ve Rs123) patojenitesi ve çevre dostu hastalık yönetimi için *Trichoderma harzianum*, *T. viride*, *Metarhizium* sp., *Gliocladium* sp. gibi biyolojik ajanların etkinliği laboratuvar ve sera koşullarında değerlendirilmiştir. Patojenite testlerinin sonuçları, izolatların değişen virülensini doğrulamıştır. Rs94 ve Rs13 izolatları karpuzda en ciddi hastalığa neden olmuştur (hastalık şiddeti (HS)= 3.80 ve 3.83, hastalık şiddeti indeksi (HSİ)= %90.43 ve %95.75). Benzer şekilde, Rs26 izolatı domateste en yüksek patojenisiteyi sergilemiştir (HS= 3.84; HSİ= %94.86). Kavun, tüm izolatlara karşı yüksek hassasiyet göstermiş olup, sürekli olarak 2.59'dan yüksek HS ve %80.97'yi aşan HSİ değerleri kaydedilmiştir. Daha sonra yapılan *in vitro* ve *in vivo* denemeler, test edilen tüm ajanların *R. solani* izolatlarına karşı antifungal potansiyelini ortaya koymuştur. Özellikle *Trichoderma* spp., en tutarlı ve anlamlı inhibisyonu göstermiştir (miselyal büyüme azalması %82.97-%94.67). Bu konuda en iyi performansı ise *T. harzianum* göstermiştir. Sera denemeleri, *T. harzianum*'un önleyici bir tedavi olarak etkinliğini doğrulamış, bitki enzim aktivitesini artırmış (peroksidaz = domates ve karpuz için sırasıyla 4.97-5.29 birim g^{-1} ml^{-1} dk^{-1}; katalaz = karpuz ve kavun için sırasıyla 99.93-101.22 birim g^{-1} ml^{-1} dk^{-1}) ve hastalık şiddeti indeksini önemli ölçüde azaltmıştır (HSİ < %12.43). Bu bulgular, *T. harzianum*'un domates, kavun ve karpuz bitkilerinde *R. solani* fide yanıklığı hastalığının yönetimi için sürdürülebilir

ve çevre dostu bir strateji olarak kullanım potansiyelini vurgulamaktadır.

Anahtar kelimeler: Fide yanıklık hastalığı, *Trichoderma* spp., domates, kavun, patojenisite, karpuz

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