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Rhizophagus irregularis as a Biocontrol Agent against Fusarium oxysporum in Tomato (Solanum lycopersicum), with a Focus on Systemic Resistance Mechanisms

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ABSTRACT

Fusarium wilt, caused by Fusarium oxysporum f. sp. lycopersici (Fol), is a devastating soil-borne disease affecting tomato production worldwide. This study investigated the biocontrol potential of the arbuscular mycorrhizal fungus Rhizophagus irregularis against Fusarium wilt in tomato, emphasizing systemic resistance mechanisms. Greenhouse experiments evaluated four treatments: untreated control, F. oxysporum alone, R. irregularis + F. oxysporum, and R. irregularis + Trichoderma harzianum + F. oxysporum. Pre-inoculation with R. irregularis significantly reduced disease incidence and severity compared to pathogen-only controls. Mycorrhizal colonization enhanced shoot height, root length, and biomass. Biochemical analyses revealed substantial upregulation of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), β-1,3-glucanase, and chitinase in mycorrhizal challenged plants. Quantitative PCR confirmed primed expression of jasmonic acid (JA) and salicylic acid (SA) pathway genes, including lipoxygenase (LOX), allene oxide cyclase (AOC), pathogenesis-related protein 1 (PR1), and NPR1. These findings indicate that R. irregularis induces systemic resistance through hormonal signaling and defense enzyme activation, offering a sustainable alternative to chemical fungicides.

KEYWORDS
Comparative anatomy, histomorphology, avian kidney, mammalian kidney, nephron, Gallus domesticus, Rattus norvegicus

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Introduction

Tomato (*Solanum lycopersicum* L.) is among the most economically important vegetable crops globally; however, production faces severe constraints from soil-borne pathogens, particularly *Fusarium oxysporum* f. sp. *lycopersici* (Fol), the causal agent of Fusarium wilt (Chitwood-Brown *et al.*, 2021). This hemibiotrophic fungus invades root vascular tissues, disrupting water transport and causing yield losses exceeding 80% in severely infected fields (Weng *et al.*, 2022). Conventional management relies on chemical fungicides and soil fumigants, which pose environmental risks and promote pathogen resistance (Dey & Ghosh, 2022).

Arbuscular mycorrhizal fungi (AMF) establish mutualistic symbioses with approximately 80% of terrestrial plant species, enhancing nutrient acquisition and stress tolerance (Pozo & Azcón-Aguilar, 2007). *Rhizophagus irregularis* is widely studied for its ability to colonize diverse hosts and confer bioprotection against soil-borne pathogens. Beyond direct nutritional benefits, AMF activate mycorrhiza-induced resistance (MIR), a form of induced systemic resistance characterized by primed defense responses (Pozo & Azcón-Aguilar, 2007; Pozo de la Hoz *et al.*, 2021). MIR involves hormonal crosstalk, primarily between jasmonic acid (JA) and salicylic acid (SA) pathways, and upregulation of pathogenesis-related genes and defense enzymes (Nair *et al.*, 2015; Kumar *et al.*, 2022). Despite this evidence, the specific systemic resistance mechanisms activated by *R. irregularis* against *F. oxysporum* in tomato remain incompletely characterized. This study evaluated *R. irregularis* as a biocontrol agent and elucidated underlying systemic defense mechanisms.

Materials and Methods

Tomato seeds (cv. Rio Grande) were surface-sterilized and germinated in sterile peat moss. *R. irregularis* (DAOM 197198) inoculum was applied at transplanting. *F. oxysporum* f. sp. *lycopersici* conidial suspension (1×10^6 CFU mL^{-1}) was used for root-drench inoculation 14 days after AMF establishment. The greenhouse experiment followed a completely randomized design with four treatments: (T1) untreated control, (T2) *F. oxysporum* alone, (T3) *R. irregularis* + *F. oxysporum*, and (T4) *R. irregularis* + *T. harzianum* + *F. oxysporum*, with 15 replicates each. Disease incidence and severity were recorded at 21 and 35 days post-pathogen inoculation. Root tissues were harvested for spectrophotometric assays of PAL, PPO, β-1,3-glucanase, and chitinase activities. Total RNA was extracted for qPCR analysis of *LOX*, *AOC*, *PR1*, *NPR1*, and *PDF1.2*, normalized against the actin gene. Data were analyzed using one-way ANOVA followed by Tukey's HSD test ($P < 0.05$).

Results

Disease Incidence and Plant Growth Parameters

Pre-inoculation with *R. irregularis* significantly reduced Fusarium wilt incidence and severity. Plants inoculated with *F. oxysporum* alone exhibited 78.5% disease incidence and 65.2% severity, whereas *R. irregularis* reduced these to 32.4% and 24.8%, respectively (Table 1; Figure 1). The combined *R. irregularis* and *T. harzianum* treatment provided the highest protection, reducing incidence and severity to 18.7% and 14.3%. Mycorrhizal colonization improved growth: shoot height increased 28.4% and root dry weight 42.6% over the pathogen-only treatment.

Table 1: Effect of *Rhizophagus irregularis* on Fusarium Wilt Disease and Growth Parameters in Tomato

Parameter	Control	Fo only	Ri + Fo	Ri + Th + Fo
Disease incidence (%)	0.0 ± 0.0c	78.5 ± 5.2a	32.4 ± 4.1b	18.7 ± 3.5c
Disease severity (%)	0.0 ± 0.0c	65.2 ± 4.8a	24.8 ± 3.6b	14.3 ± 2.9c
Shoot height (cm)	45.2 ± 3.1a	28.4 ± 2.5c	42.6 ± 3.3ab	46.8 ± 3.8a
Root dry weight (g)	2.85 ± 0.22a	1.42 ± 0.18c	2.64 ± 0.24ab	3.12 ± 0.28a
Mycorrhizal colonization (%)	0.0 ± 0.0c	0.0 ± 0.0c	68.4 ± 4.2a	64.2 ± 5.1a

Values are means ± SD ($n = 15$). Different lowercase letters within rows indicate significant differences (Tukey's HSD, $P < 0.05$). Fo = *Fusarium oxysporum*; Ri = *Rhizophagus irregularis*; Th = *Trichoderma harzianum*.

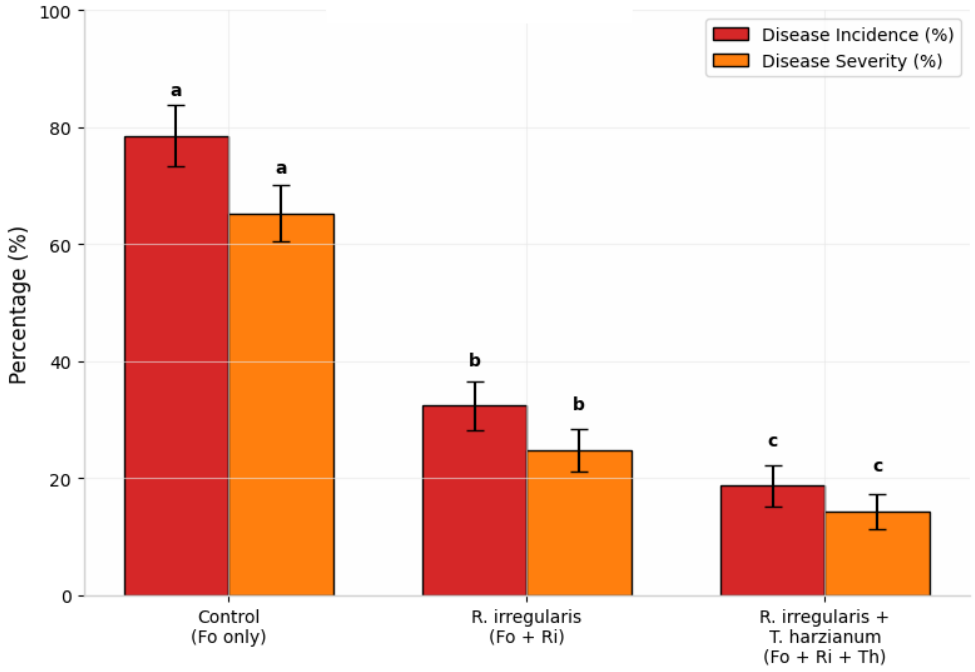

Figure 1. Effect of *Rhizophagus irregularis* on Fusarium wilt disease parameters in tomato (*Solanum lycopersicum*) under greenhouse conditions. Bars represent means ± SD (n = 15). Different letters above bars indicate significant differences (Tukey's HSD, $P < 0.05$).

Defense-Related Enzyme Activities

Biochemical analyses revealed marked differences among treatments (Figure 2). In Ri + Fo roots, PAL activity reached 34.8 µg trans-cinnamic acid mg^{-1} protein h^{-1}, a 91.2% increase over Fo-only and 180.6% over control. PPO activity reached 2.65 ΔOD_{420} min^{-1} mg^{-1} protein, significantly exceeding both control and pathogen-only plants. β-1,3-glucanase and chitinase activities were substantially elevated in mycorrhizal challenged plants, reaching 46.2 and 38.7 units mg^{-1} protein versus 28.4 and 22.5 in the Fo-only treatment

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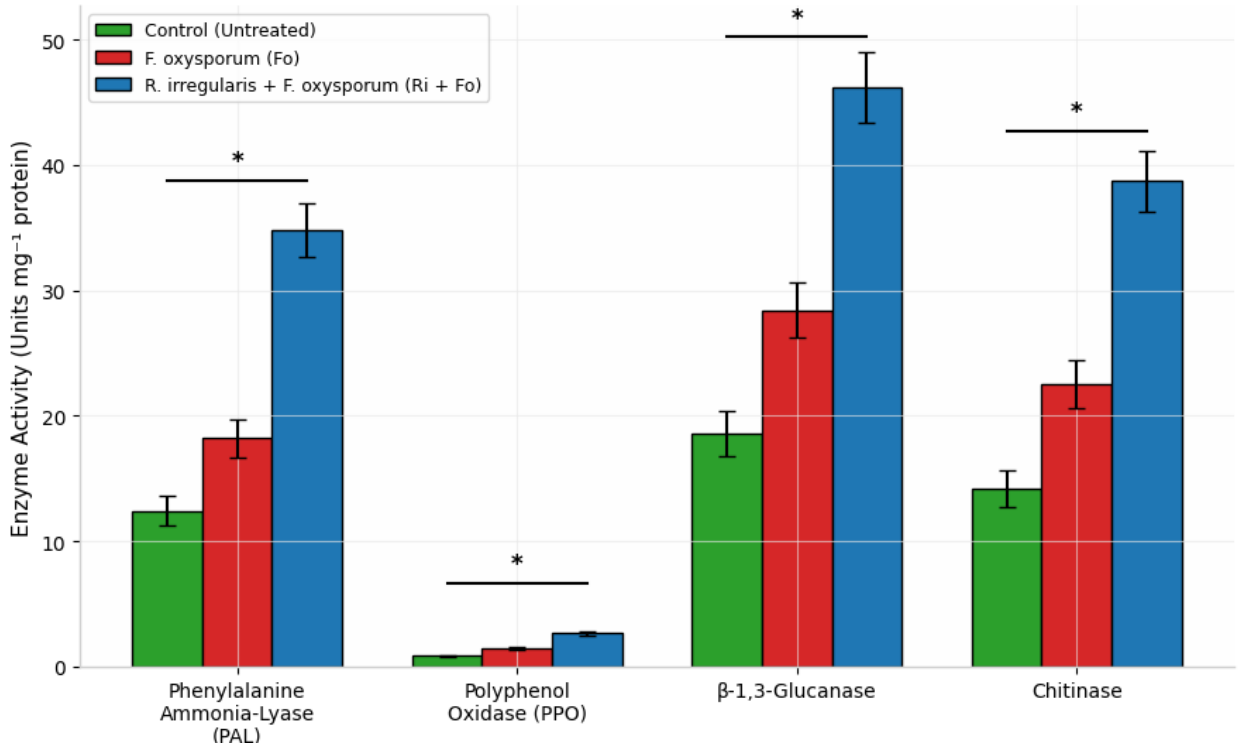

Figure 2. Activities of defense-related enzymes in tomato roots following *Rhizophagus irregularis* colonization and *Fusarium oxysporum* challenge. Bars represent means ± SD (n = 9). Asterisks indicate significant differences between treatments ($P < 0.05$).

Gene Expression Analysis

qPCR demonstrated that *R. irregularis* primed defense gene expression upon challenge (Table 2). JA-biosynthesis genes *LOX* and *AOC* showed 4.8- and 5.2-fold upregulation in Ri + Fo relative to control. SA-responsive genes *PR1* and *NPR1* exhibited 6.4- and 3.9-fold increases, indicating activation of both pathways. The JA-marker *PDF1.2* was upregulated 3.7-fold, suggesting balanced hormonal interplay.

Table 2: Relative Expression of Defense-Related Genes in Tomato Roots at 7 Days Post-Inoculation

Gene	Pathway	Control	Fo only	Ri + Fo	Ri + Th + Fo
LOX	JA	1.0 ± 0.1b	2.4 ± 0.3a	4.8 ± 0.5a	5.1 ± 0.6a
AOC	JA	1.0 ± 0.1b	2.1 ± 0.2a	5.2 ± 0.4a	5.6 ± 0.5a
PR1	SA	1.0 ± 0.1c	1.8 ± 0.2b	6.4 ± 0.6a	7.2 ± 0.8a
NPR1	SA	1.0 ± 0.1b	1.5 ± 0.2b	3.9 ± 0.4a	4.3 ± 0.5a
PDF1.2	JA/ET	1.0 ± 0.1b	1.6 ± 0.2b	3.7 ± 0.3a	4.1 ± 0.4a

Values represent fold change relative to untreated control ± SD (n = 9). Different lowercase letters within rows indicate significant differences ($P < 0.05$). JA = jasmonic acid; SA = salicylic acid; ET = ethylene.

Discussion

This study provides compelling evidence that *R. irregularis* functions as an effective biocontrol agent against Fusarium wilt through multiple systemic resistance mechanisms. The significant disease reduction aligns with previous reports of

AMF-mediated protection (Kumar *et al.*, 2022; Weng *et al.*, 2022). Enhanced growth parameters under pathogen challenge suggest nutritional and bioprotective benefits act synergistically (Pozo de la Hoz *et al.*, 2021).

Upregulation of PAL and PPO indicates phenylpropanoid pathway activation, leading to lignin deposition and antimicrobial compound synthesis. These enzymes serve as critical biochemical markers of induced resistance, consistent with findings in mycorrhizal systems (Ponsankar *et al.*, 2023; Cordier *et al.*, 1998). Elevated β-1,3-glucanase and chitinase reflect enhanced capacity to degrade fungal cell walls, directly inhibiting pathogen proliferation (Dey & Ghosh, 2022).

Molecular data reveal nuanced hormonal interplay underlying MIR. While traditional models posit JA-SA antagonism, our results demonstrate concurrent upregulation of *LOX/AOC* and *PR1/NPR1* in mycorrhizal plants upon attack. This supports the emerging view that MIR against hemibiotrophic *F. oxysporum* involves both pathways through primed sensitivity to damage signals (Pozo & Azcón-Aguilar, 2007; Nair *et al.*, 2015). Priming allows faster, stronger defenses without constitutive metabolic costs. The combined *R. irregularis* and *T. harzianum* treatment showed the lowest disease levels but did not significantly exceed *R. irregularis* alone in all parameters, suggesting overlapping mechanisms where both organisms modulate similar defense networks (Weng *et al.*, 2022).

Conclusion

Rhizophagus irregularis effectively suppresses Fusarium wilt in tomato by inducing systemic resistance characterized by enhanced defense enzyme activities and coordinated JA-SA hormonal signaling. Combined with growth promotion, *R. irregularis* represents a promising component of integrated disease management for sustainable tomato production. Future research should optimize inoculation protocols and validate field-scale efficacy under diverse agroecological conditions.

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