Efficacy of Biofungicides and Botanical Extracts Against Wilt Disease in Tomato (*Solanum lycopersicum* L.)

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Abstract: *Three biological treatments (Trichoderma harzianum, Bacillus subtilis, Pseudomonas fluorescens) and three plant extracts (garlic, neem, turmeric) were tested against tomato wilt in the lab and in field trials at Kanpur, India (Kharif 2023–24), using a randomized block design with 8 treatments × 3 replications. Bacillus subtilis (T3) performed best overall largest inhibition zone (16.2 mm) and lowest disease incidence (28.6%), close to the chemical standard (T8, 22.4%). T. harzianum (T2, 32.1%) and P. fluorescens (T4, 36.4%) were also effective. Neem (38.9%) beat garlic (44.7%) and turmeric (51.2%) among the extracts. All biological agents boosted growth and yield, with T3 and T2 giving the highest yields (40.2 and 38.6 t/ha vs. 18.4 t/ha for untreated). B. subtilis and T. harzianum are recommended as the core of an eco-friendly wilt-control programme, with plant extracts as organic-friendly backups.*

Keywords: *Fusarium wilt,, Trichoderma harzianum, Bacillus subtilis, botanical antifungals.*

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Introduction

Tomato is a major global crop (5.2 million ha; 186 million t/year). India is the second-largest producer (0.84 million ha; 20.7 million t), and Uttar Pradesh contributes about 12% of this. Soil-borne wilt pathogens *Fusarium oxysporum* f. sp. lycopersici (Fol) and *Ralstonia solanacearum* cause 10–30% yield loss normally, and over 80% in severe outbreaks (Jones et al., 2014; Denny, 2006), by infecting roots and blocking water transport.

Biofungicides fight these pathogens through enzymes, antimicrobial compounds, and induced plant immunity: *T. harzianum* via cell-wall-degrading enzymes (Harman et al., 2004), B. subtilis via lipopeptides that damage fungal membranes (Ongena & Jacques, 2008), and *P. fluorescens* via antimicrobial metabolites and induced resistance (Pieterse et al., 2014). Garlic (allicin) and neem (azadirachtin) extracts offer plant-based alternatives with known antifungal activity.

This study aimed to: (i) test lab activity of these agents against Fol and R. solanacearum; (ii) compare field efficacy against wilt; (iii) measure effects on growth and yield; and (iv) test whether combining treatments improves control.

2. Materials and Methods

Wilted tomato plants were collected from Kanpur farms (Kharif 2023) and Fol/R. solanacearum isolated on selective media, identified by spore morphology and biochemical tests.

Field Design

The field trial (Kharif 2023–24, Kanpur; sandy loam, pH 7.6) used a randomized block design with 8 treatments (T1–T8) × 3 replications (24 plots, 20 m² each). Cv. Pusa Ruby was planted at 60 × 45 cm spacing. Treatments were soil-drenched at 0, 15, and 30 days after transplanting (DAT); extracts were also sprayed every 15 days from 15–75 DAT.

3. Results and Discussion

Copper oxychloride (T8) gave the strongest inhibition (18.4 mm *Fusarium*; 71.6% *Ralstonia*), followed by *B. subtilis* (T3: 16.2 mm; 62.1%) and *T. harzianum* (T2: 14.8 mm; 58.4%); P. fluorescens (T4) was moderate (12.6 mm; 51.8%). Among extracts, neem (T6) led (11.4 mm; 47.2%), ahead of garlic (T5: 10.1 mm) and turmeric (T7: 8.6 mm). T3 needed the least amount to work (MIC 1.8 mg/mL) and showed the highest chitinase activity (41.2 U/mL), an enzyme that breaks down fungal cell walls.

Table 1. Lab activity against Fusarium and Ralstonia. Shared letters within a column = not significantly different (Tukey's HSD, P ≤ 0.05).

Treatment	***Fusarium* (mm)**	***Ralstonia* (%)**	**MIC (mg/mL)**	**Enzyme (U/mL)**
T1	0.00e	0.00e	>100	—
T2	14.8b	58.4b	2.4	34.6
T3	16.2a	62.1a	1.8	41.2
T4	12.6c	51.8c	3.6	28.4
T5	10.1d	43.6d	8.2	—
T6	11.4cd	47.2cd	6.4	—
T7	8.6d	36.4e	14.8	—
T8	18.4a	71.6a	0.6	—

These results fit known mechanisms *B. subtilis* lipopeptides (iturin A, surfactin) damage fungal membranes, and fengycin blocks spore germination (Ongena & Jacques, 2008); its high chitinase/peroxidase activity here also reflects induced plant immunity (Ryu et al., 2004). T. harzianum combines mycoparasitism, antifungal volatiles, and root growth promotion

(Harman et al., 2004) its 68.2% root colonization confirms strong establishment. *P. fluorescens* was weaker, likely because its key compound (2,4-DAPG) is sensitive to soil conditions. Among extracts, neem's edge comes from azadirachtin disrupting fungal cell walls and membranes; garlic's active compound (allicin) degrades quickly in heat/UV, limiting field performance despite good lab activity, while turmeric's curcumin is poorly soil-mobile, limiting its reach to the pathogen.

All treatments reduced disease versus the untreated control (T1: 87.3% incidence, severity 3.68). T8 gave the best control (22.4%; 74.8% reduction); *B. subtilis* (T3) matched it statistically (28.6%; 67.1% reduction), ahead of *T. harzianum* (T2: 32.1%) and *P. fluorescens* (T4: 36.4%). AUDPC values confirmed this ranking (T3: 174.8 vs. 412.6 for control). Among extracts, neem (38.9%) beat garlic (44.7%) and turmeric (51.2%).

Table 2. Disease incidence, severity, AUDPC, and reduction. Shared letters = not significantly different (P ≤ 0.05).

Treatment	Incidence (%)	Severity (0–4)	AUDPC	% Reduction	PDI
T1	87.3a	3.68a	412.6a	—	92.0
T2	32.1d	1.74d	189.3d	63.2	43.5
T3	28.6d	1.52d	174.8d	67.1	38.0
T4	36.4c	1.93c	208.4c	57.5	48.3
T5	44.7c	2.31c	251.2c	47.5	57.8
T6	38.9c	2.08c	228.6c	52.1	52.0
T7	51.2b	2.64b	288.4b	35.5	66.0
T8	22.4e	1.18e	138.2e	74.8	29.5

B. subtilis and *T. harzianum* thus offer near-chemical-level wilt control under field conditions, while extracts though weaker still give meaningful protection, useful where synthetic fungicides cannot be used.

B. subtilis (T3) gave the best growth among biocontrol agents 79.1 cm height, 204.2 g weight, 49.4 SPAD chlorophyll, 40.2 t/ha yield statistically on par with T8 (42.6 t/ha) and T2 (38.6 t/ha). Neem (T6) led the extracts (68.4 cm; 33.8 t/ha) but stayed below all biocontrol agents. Untreated plants (T1) were lowest throughout (48.2 cm; 18.4 t/ha). Disease severity correlated strongly and negatively with height, weight, and yield (r = −0.92, −0.89, −0.94).

Table 3. Growth and yield of tomato cv. Pusa Ruby. Shared letters = not significantly different ($P \leq 0.05$). Root Col. recorded for T. harzianum only.

Treatment	Height (cm)	Fresh Wt (g)	Chlorophyll	Yield (t/ha)	Root Col. (%)
T1	48.2e	124.3e	32.1d	18.4e	—
T2	76.4b	198.6b	47.8b	38.6b	68.2
T3	79.1ab	204.2ab	49.4ab	40.2ab	—
T4	72.3bc	188.4bc	45.6bc	36.1bc	—
T5	66.8c	172.1c	43.2c	32.7c	—
T6	68.4c	176.8c	44.1c	33.8c	—
T7	61.2d	158.4d	40.7d	29.4d	—
T8	81.6a	211.8a	51.2a	42.6a	—

This growth boost reflects the plant growth-promoting activity of *B. subtilis* and *P. fluorescens* phosphate solubilization, nitrogen fixation, and auxin production on top of disease suppression.

Defense enzymes and combined treatment

Biological treatments raised peroxidase (POX) far more than extracts or the control: T3 (41.2 U/mg) > T2 (34.6) > T6 neem (22.8) > T5 garlic (19.4) > T7 turmeric (16.1) > T1 control (9.2). Combining *B. subtilis* + neem at half-strength each pushed POX to 48.6 U/mg and cut disease to 24.1% — better than either alone, pointing to a synergistic or additive effect. This supports combining or rotating biocontrol agents with plant extracts as a practical strategy for organic tomato production.

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