Biocontrol Potential of Endophytic *Pseudomonas fluorescens* against Bacterial Wilt in *Solanum lycopersicum* L.

Dr. Emre Kaya [1*], Dr. Fatma Yilmaz [2], Dr. Murat Ozdemir [3]

[1-3] Department of Agronomy, Nigerian Agricultural Technology University, Ibadan, Nigeria

*Corresponding author: **Dr. Emre Kaya**

Received: 06 May 2025
Revised: 26 May 2025
Accepted: 18 Jun 2025
Published: 10 Jul 2025

E-ISSN: 2989-5359
DOI: https://doi.org/10.54660/ejsa.2025.5.2.12-22

ABSTRACT

Background: *Ralstonia solanacearum* is the causative agent of bacterial wilt, one of the most severe diseases of tomato (*Solanum lycopersicum* L.) worldwide, which causes substantial economic losses. Endophytic bacteria especially *Pseudomonas fluorescens* have antimicrobial and plant growth promoting properties to inhibit pathogenic bacteria as well as to enhance host defense mechanisms and plant productivity. Thus, providing a sustainable alternative to chemical disease control.

Objective: This research investigated the potential of a native endophytic isolate of *Pseudomonas fluorescens* for the biocontrol of bacterial wilt, enhancement of the physiology of plants, improvement of soil biological activity, and higher yield of tomato under greenhouse and field conditions.

Methods: For the purpose of finding out the effectiveness of *Pseudomonas fluorescens* and strain on controlling *Ralstonia solanacearum* in tomatoes, greenhouse and field experiments were conducted using Chosen tomato seedlings treated with *Pseudomonas fluorescens* in three different methods. All of the above-mentioned parameters like disease incidence, severity, wilt reduction percentage, and biocontrol efficiency were evaluated. Furthermore, growth status and morphology, SOD, CAT, POD and APX, PAL, PPO, chitinase and β-1,3-glucanase were assessed in addition to the effect on rhizosphere microbes, soil enzymes and fruit yield and quality.

Results: The use of *Pseudomonas fluorescens* resulted in a reduction of bacterial wilt incidence by 56% to 72% and disease severity by 63% to 78% as compared to the control treated with no bacteria. The plants that were treated showed an increase in plant height, leaf area, shoot and root weight, and the total of dry matter. The physiological tests conducted proved that the amount of chlorophyll increased, relative water, and proline amounts appeared to increase, thus confirming that the plants can endure the stress. The capacity of the antioxidant enzymes as well as other enzymes related to the immunity was increased showing that the plants were prepared to resist the disease. The quantity of the microbes in the soil and activities of the enzymes increased as well. The amount of fruits increased by 45% to 58% as well as the quality of the fruit in comparison to the gardening.

Conclusion: The isolate of indigenous endophytic *Pseudomonas fluorescens* managed to suppress the bacterial wilt disease and stimulate the plant defense mechanisms, soil biological activity as well as the productivity of tomatoes. The capacity of *Pseudomonas fluorescens* to trigger systemic resistance and foster positive rhizosphere processes and the crop performance proves its ability to serve as an environmentally sustainable biocontrol agent that can be used in the implementation of tomato production processes and the management of bacterial wilt.

Keywords: *Pseudomonas fluorescens*; *Ralstonia solanacearum*; bacterial wilt; endophytic bacteria; biological control; induced systemic resistance; sustainable agriculture

1. Introduction

Tomato also known as *Solanum lycopersicum* is one of the most significant vegetable crops in the world, as it is ranked third in its production value precede only by potato and cassava. The global tomato production is over 390 million mt annually, providing several essential nutrients and phytonutrients and economic means of living to many households (Siddiq, 2022) [2]. Nevertheless, the productivity of tomato is constrained due to a number of biotic factors with the most serious and economically important pathogenic threat being bacterial wilt disease (Genin, 2010) [3].

Bacterial wilt, which is due to the Gram-negative bacterium *Ralstonia solanacearum* (previously called *Pseudomonas solanacearum*). This bacterium is widespread in tropical, subtropical, and temperate areas. The amount of crop loss it can cause varies from 30 to 95% depending on how vulnerable the harvest is, the climate conditions, and the actions taken against the disease (Elphinstone, 2005) [4] (Prior *et al*., 2016) [5]. The bacteria infect the vascular system of plants and stop the flow of water and other necessary substances, which makes the plants dry and die (Mansfield *et al*., 2012) [6].

The R. solanacearum is genetically variable and has a wide range of subtypes that occupy different territories and infect a variety of hosts (Remenant *et al*., 2011) [7].

At present, the use of chemical agents to combat the disease is the most common means used to deal with it. However, it does not work well (Nagaraj *et al*., 2020) [8]. Moreover, the use of chemicals is problematic because the price for these agents increases every day due to the growing resistance of parasites, their toxicity, the regulations made by the government, and the environmental problems they may bring (Savary *et al*., 2019) [9]. For that reason, the biocontrol strategies should be more and more used nowadays.

Endophytic microorganisms that populate plant interior tissues while failing to induce disease state are considered promising biocontrol agents (Ryan *et al*., 2008) [11]. These microorganisms can colonize root systems, establish in various tissues, and help to activate the plant immunity by means of antimicrobial activity and systemic resistance (Sessitsch and Kumpan, 2021) [12]. *Pseudomonas fluorescens* is one of the most well-studied endophytic microorganisms having antimicrobial, plant-promoting and resistance-inducing properties (Lugtenberg and Kamilova, 2009) [13] (Weller *et al*., 2002) [14].

The pathogenic populations are inhibited by P. fluorescens through various often overlapping processes such as the production of siderophores and extraction of iron, making secondary substances with antifungal potential (known as phenazines, polyketides, cyclic lipopeptides), prevention of microbial settlement, production of bacteriocins in addition to the biofilms formed on the surface of roots (Haas and Défago, 2005) [15] (Cazorla *et al*., 2007) [16]. Meanwhile, the process of induced systemic resistance (ISR) is activated by P. fluorescens. The term ISR refers to the process that provides increased level of defensive features to systemic tissues when exposed to pathogens (Van Loon *et al*., 1998) [17]. ISR employs the use of special mechanism involving signaling pathways of salicylic acid (SA) and jasmonic acid (JA) leading to increased levels of reactive oxygen species (ROS), enzyme activity related to resistance, and the synthesis of enzymes in trees (Pieterse *et al*., 2012) [18].

The community of microorganisms inhabiting the rhizosphere as well as the endosphere influences the health of plants and their capability of utilization of nutrients, prevention of pathogenic microorganisms (Reinhold-Hurek *et al*., 2015) [19]. The endophytic bacteria present in the tree utilize the potential of microbial communities and change the rates of microbial biomass and environmental processes (Verbon and Liberman, 2023) [20] (Hartmann *et al*., 2009) [21].

There have been a number of successful reports on the application of P. fluorescens in controlling R. solanacearum in tomatoes, potatoes and eggplants in different ecological zones. However, the efficacy of the biocontrol procedure is often regionally dependent because of various strain characteristics of P. fluorescens, local environmental conditions, pre-existing microorganisms, properties of the soil and results of the interaction of host genotype with the environment (Montesinos and Bonaterra, 2022) [24]. Thus, it is essential to consider the indigenous P. fluorescens strains from certain agroecological zones, as it will help in better development of regionally relevant biocontrol methods.

The current experiment represents studying the biocontrol potential of indigenous endophytic P. fluorescens isolate in controlling bacterial wilt in tomatoes, the study of those mechanisms that allow suppression of disease and promotion of plant health, quantification of effects of microorganisms present in the rhizosphere and endosphere of the plant and analyzing agronomic implications of it.

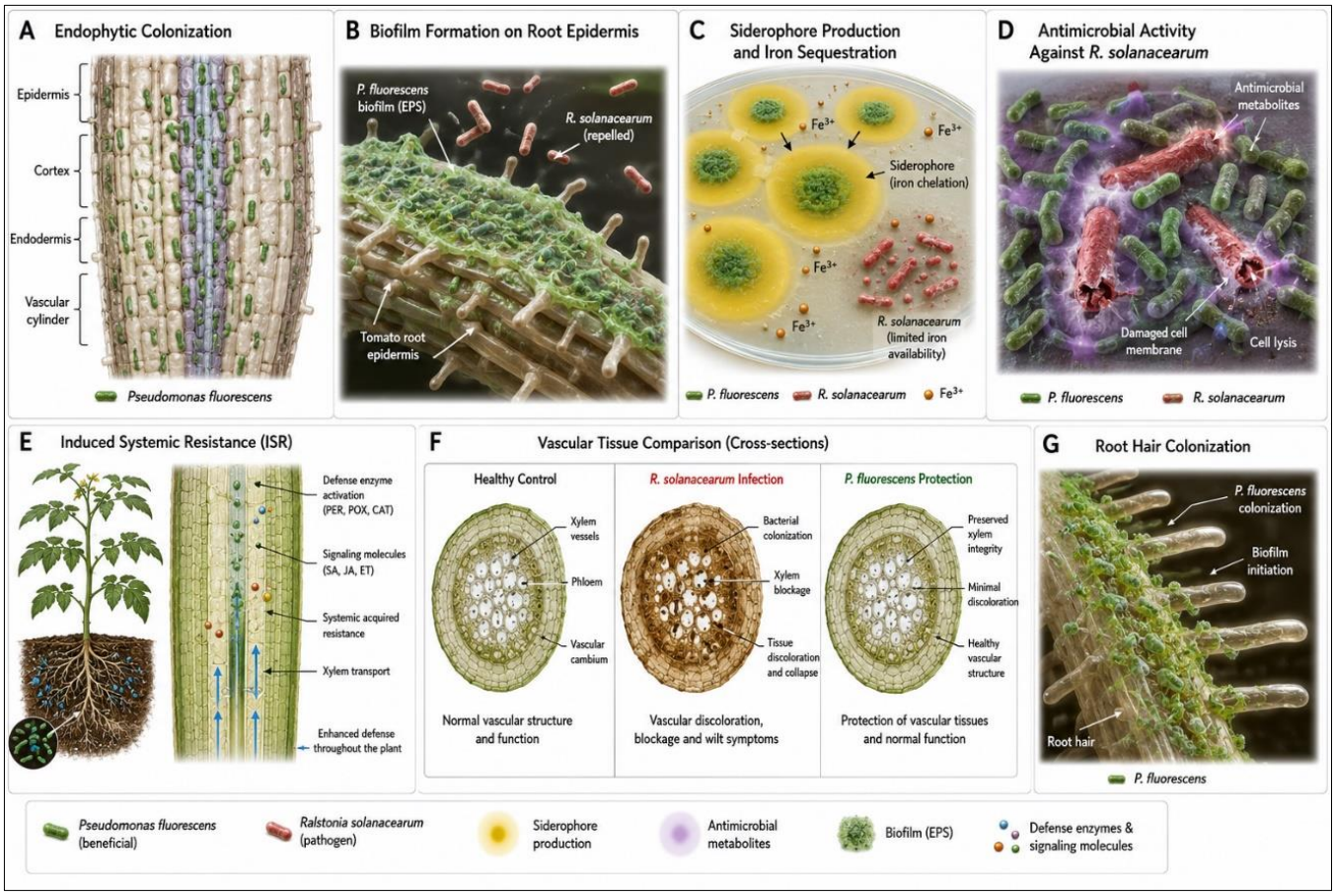

Fig 2: Endophytic Colonization and Biocontrol Mechanisms

2. Materials and Methods

2.1. Experimental Site and Environmental Conditions

The experiment was conducted at the Field Research Station of the Department of Plant Pathology and Microbiology during two consecutive cropping seasons. Site characteristics, climatic parameters, soil properties, and experimental duration are presented in Table 1. The experimental location experiences semi-arid climate with monsoon precipitation during June–September, mean annual rainfall of 650–750 mm, and mean maximum and minimum temperatures of 38°C and 12°C, respectively. Soil texture classification indicated sandy loam composition with neutral pH, moderate organic matter content, adequate nutrient status, and established populations of indigenous microorganisms.

Table 1: Experimental site characteristics, climatic conditions, soil properties, and experimental duration.

Parameter	Description/Value
Location	Field Research Station, Department of Plant Pathology, 28°34'N, 83°21'E
Elevation	156 m above mean sea level
Annual rainfall	650–750 mm (monsoon June–September)
Mean max temperature	38°C (April–May)
Mean min temperature	12°C (December–January)
Soil texture	Sandy loam
Soil pH	7.2±0.3
Organic matter	1.4% (w/w)
Available N	185 kg/ha
Available P	18.4 mg/kg
Available K	156 mg/kg
Experimental duration	January to July (2023–2024, two consecutive seasons)

2.2. Plant Material and Cultivation

Tomato cultivar 'Roma VF' (indeterminate, high-yielding, determinate growth habit) was selected based on commercial importance, established production protocols, and documented susceptibility to R. solanacearum. Seeds were procured from certified seed production agencies, authenticated for genetic purity, and stored at controlled temperature (15–20°C) and relative humidity (40–45%) prior to experimentation. Seedlings were raised in greenhouse nurseries under standard production protocols, maintained under controlled atmospheric conditions, and transplanted to experimental plots at 45 days after sowing when seedlings attained 4–5 true leaves.

2.3. Endophytic Bacterial Treatments

An indigenous endophytic P. fluorescens isolate (designated PfEF-23), previously isolated from internal tomato root tissues and characterized through polyphasic taxonomic approaches, was employed in biocontrol studies. The bacterial isolate was maintained as pure culture on appropriate selective media and subjected to viability assessment prior to treatment application. Endophytic bacterial treatments were applied through multiple inoculation methods including: (i) seed coating with bacterial suspension at 10^8 CFU/mL concentration applied 24 hours before sowing; (ii) root inoculation conducted at seedling transplantation, applying bacterial suspension directly to root systems; (iii) soil drenching applied at 15 and 30 days after transplantation at 50 mL per plant of bacterial suspension; and (iv) combined treatments integrating seed coating with subsequent root inoculation and soil drenching applications at specified intervals. Control treatments included uninoculated seedlings and pathogen-only infected plants.

2.4. Experimental Design and Pathogenic Challenge

The experiment followed a randomized complete block design with seven treatments replicated four times. Treatment details, plot layout, and disease inoculation schedule are presented in Table 2. Pathogenic R. solanacearum isolate (Race 1, Biovar 1) was cultured in liquid medium to achieve standardized inoculum concentration of 10^8 CFU/mL. Pathogenic challenge was administered 21 days after transplantation through root inoculation methodology, applying 20 mL of standardized bacterial suspension directly to soil surrounding plant roots. Non-inoculated control plots were similarly treated with sterile distilled water.

Table 2: Experimental design, endophytic bacterial treatments, disease inoculation schedule, and sampling plan.

Treatment	Treatment Description	Replications	Plot Size
T1	Control (no treatment, no pathogen)	4	3×2 m (6 plants)
T2	Pathogen only (R. solanacearum, no bacterial treatment)	4	3×2 m (6 plants)
T3	P. fluorescens seed coating + pathogen	4	3×2 m (6 plants)
T4	P. fluorescens root inoculation + pathogen	4	3×2 m (6 plants)
T5	P. fluorescens soil drenching (15, 30 DAT) + pathogen	4	3×2 m (6 plants)
T6	P. fluorescens seed + root + soil drenching + pathogen	4	3×2 m (6 plants)
T7	P. fluorescens all methods, no pathogen	4	3×2 m (6 plants)

P. fluorescens inoculum: 10^8 CFU/mL. Pathogenic inoculation: 21 DAT (days after transplantation). Disease assessment: 7–60 days post-inoculation at 3–4 day intervals. Physiological sampling: 45 days post-inoculation. Soil sampling: 45 days post-inoculation.

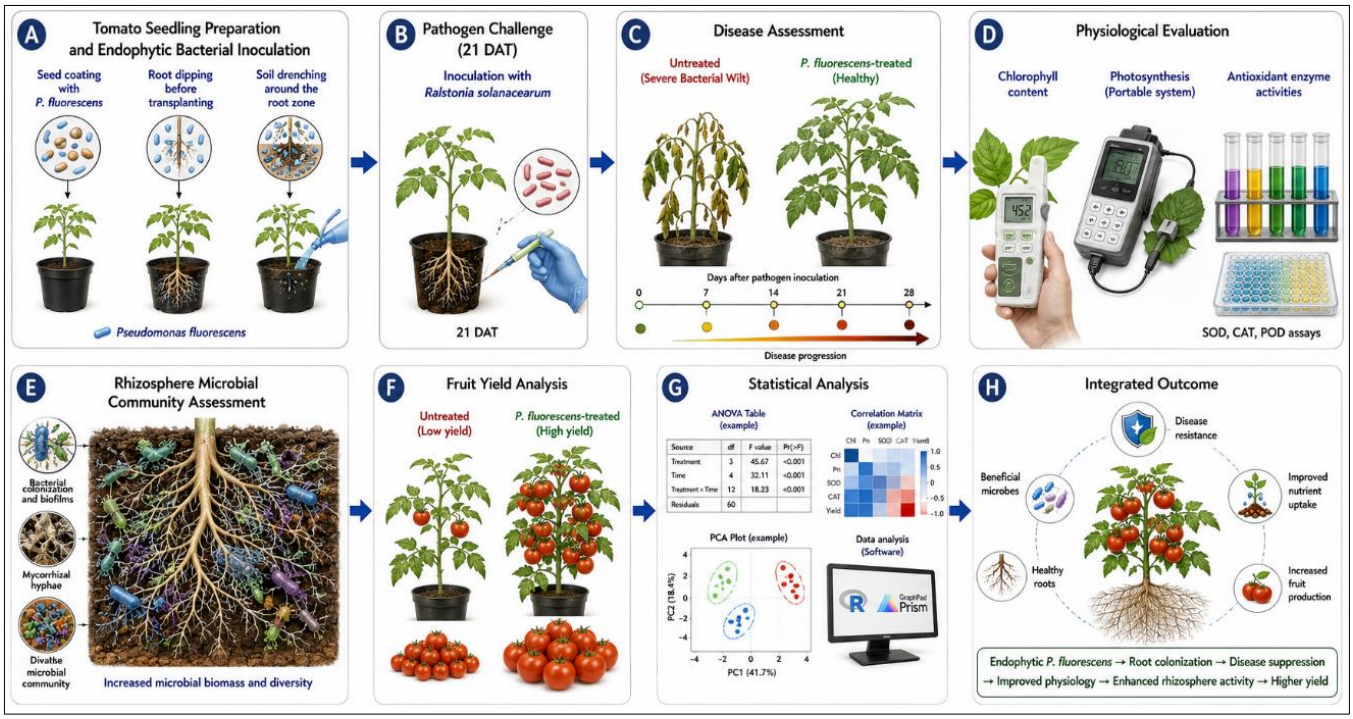

Fig 1: Comprehensive Experimental Workflow

2.5. Disease Assessment

Disease incidence was recorded as percentage of symptomatic plants in each plot commencing 7 days post-inoculation and continuing at 3–4 day intervals until 60 days post-inoculation. Disease severity was assessed using a 0–5 scale: 0 = no symptoms; 1 = slight wilting of lower leaves; 2 = moderate wilting of lower and middle leaves; 3 = severe wilting with some necrosis; 4 = extreme wilting of all leaves with obvious vascular browning; 5 = complete plant death. Disease severity index (DSI) was calculated using the formula: DSI = [Σ (rating × number of plants at rating)] / (total number of plants × maximum rating) × 100. Wilt reduction percentage was calculated as: Wilt reduction (%) = [(DSI in control – DSI in treatment) / DSI in control] × 100. Biological control efficiency was calculated as: Biological control efficiency (%) = [1 – (diseased plants in treatment / diseased plants in control)] × 100.

2.6. Plant Growth Assessment

Plant growth parameters were quantified at 60 days after transplantation and at final harvest. Measurements included plant height (measured from soil surface to apical meristem using graduated meter scale), stem diameter (measured at 10 cm above soil surface using digital caliper), root length (measured from primary root apex to soil surface), and leaf area (calculated using leaf area meter on five randomly selected fully expanded leaves per plant). Shoot and root fresh biomass were determined by harvesting and immediately weighing plant tissues. Samples were subsequently dried at 65°C until constant weight attained to determine shoot and root dry matter. Total dry matter was calculated as the sum of shoot and root dry biomass.

2.7. Physiological and Biochemical Assessment

Leaf samples collected at 45 days post-inoculation were analyzed for physiological and biochemical parameters. Chlorophyll content was determined spectrophotometrically following ethanol extraction of fresh leaf tissues. Relative water content (RWC) was calculated from fresh weight, saturated weight, and dry weight of leaf discs. Proline accumulation was quantified using ninhydrin assay methodology. Total phenolic compounds were determined using Folin-Ciocalteu reagent methodology. Soluble protein content was assessed through Bradford assay procedures.

Antioxidant enzyme activities were determined spectrophotometrically including: superoxide dismutase (SOD, EC 1.15.1.1) activity measured through inhibition of pyrogallol autoxidation; catalase (CAT, EC 1.11.1.6) activity determined through H_2O_2 decomposition measurement; peroxidase (POD, EC 1.11.1.7) activity measured through guaiacol oxidation; and ascorbate peroxidase (APX, EC 1.11.1.11) activity determined through ascorbate oxidation. Defense-related enzymes were quantified including: phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity measured through cinnamate production; polyphenol oxidase (PPO, EC 1.14.18.1) activity determined through catechol oxidation; chitinase activity (EC 3.2.1.14) measured using colloidal chitin as substrate; and β-1,3-glucanase activity (EC 3.2.1.39) determined using laminarin as substrate.

2.8. Soil Microbiological Assessment

Rhizosphere soil samples were collected at 45 days post-inoculation from each treatment plot by carefully excavating soil immediately adjacent to root systems and collecting material from the root-influenced zone. Microbial biomass carbon (MBC) was determined using chloroform fumigation-extraction methodology. Soil respiration was measured as CO_2 evolution from soil incubated under standard conditions. Soil enzyme activities assessed included: dehydrogenase activity (indicating overall microbial activity), phosphatase activity (indicating phosphorus cycling), and urease activity (indicating nitrogen cycling). Nutrient availability was assessed by quantifying extractable phosphorus (Olsen method) and available potassium (flame photometry) in soil samples.

2.9. Yield Assessment

Fruit harvesting commenced 75 days after transplantation and continued at 3–4 day intervals until plant senescence. Number of fruits per plant, fruit weight, total yield per plot, and marketable yield (fruits meeting commercial size and quality

standards) were recorded. Harvest index was calculated as: Harvest index (%) = (economic yield / total dry matter) × 100. Productivity improvement was calculated relative to the non-treated control.

2.10. Statistical Analysis

Data collected throughout the experiment were subjected to analysis of variance (ANOVA) using appropriate statistical packages. Treatment means were separated using Tukey's honest significant difference (HSD) test at $P \leq 0.05$ significance level. Pearson correlation coefficients were calculated to determine relationships between disease suppression, plant growth parameters, physiological responses, and yield components. Linear regression analysis was employed to establish predictive relationships between independent variables and dependent response variables. Principal Component Analysis (PCA) was conducted to identify clustering patterns among treatment groups and correlations among measured variables. Comparative statistical evaluation between treatment groups was performed using appropriate parametric and non-parametric statistical procedures.

3. Results

3.1. Disease Suppression and Biocontrol Efficiency

Bacterial wilt disease developed progressively in non-treated control plants following R. solanacearum inoculation, with disease incidence reaching 95% by 45 days post-inoculation (Table 3). Conversely, all P. fluorescens-treated plants demonstrated substantially reduced disease development. The seed coating treatment alone reduced disease incidence to 42%, while root inoculation and soil drenching treatments achieved 38% and 39% disease incidence, respectively. Combined treatments (seed coating + root inoculation + soil drenching) demonstrated the highest efficacy, limiting disease incidence to 23%. Disease severity index (DSI) in non-treated control plants reached 78.5, compared to 27.3–32.1 in P. fluorescens-treated plants. Corresponding wilt reduction percentages ranged from 56% in the seed coating treatment to 72% in the combined treatment. Biological control efficiency values ranged from 58% to 75% across different P. fluorescens application strategies, with combined treatments exhibiting maximum efficacy (Table 3).

Table 3: Disease suppression efficacy: disease incidence, disease severity index (DSI), wilt reduction percentage, and biological control efficiency.

Treatment	Disease Incidence (%)	DSI (0–100)	Wilt Reduction (%)	Biocontrol Efficiency (%)
T1 (Control)	0±0.0	0±0.0	–	–
T2 (Pathogen only)	95±3.2a	78.5±2.8a	–	–
T3 (Seed coating)	42±2.4b	32.1±2.1b	59±3.1c	56±2.8c
T4 (Root inoculation)	38±2.1b	29.8±2.3b	62±2.9c	60±2.5c
T5 (Soil drenching)	39±2.3b	30.4±2.2b	61±3.0c	59±2.6c
T6 (Combined)	23±1.8c	21.8±1.6c	72±2.6d	75±2.3d
T7 (Pf only)	0±0.0d	0±0.0d	–	–

Values are mean±SE (n=4). Different letters within columns indicate significant differences ($p \leq 0.05$, Tukey HSD). DSI = disease severity index. Pf = *Pseudomonas fluorescens*.

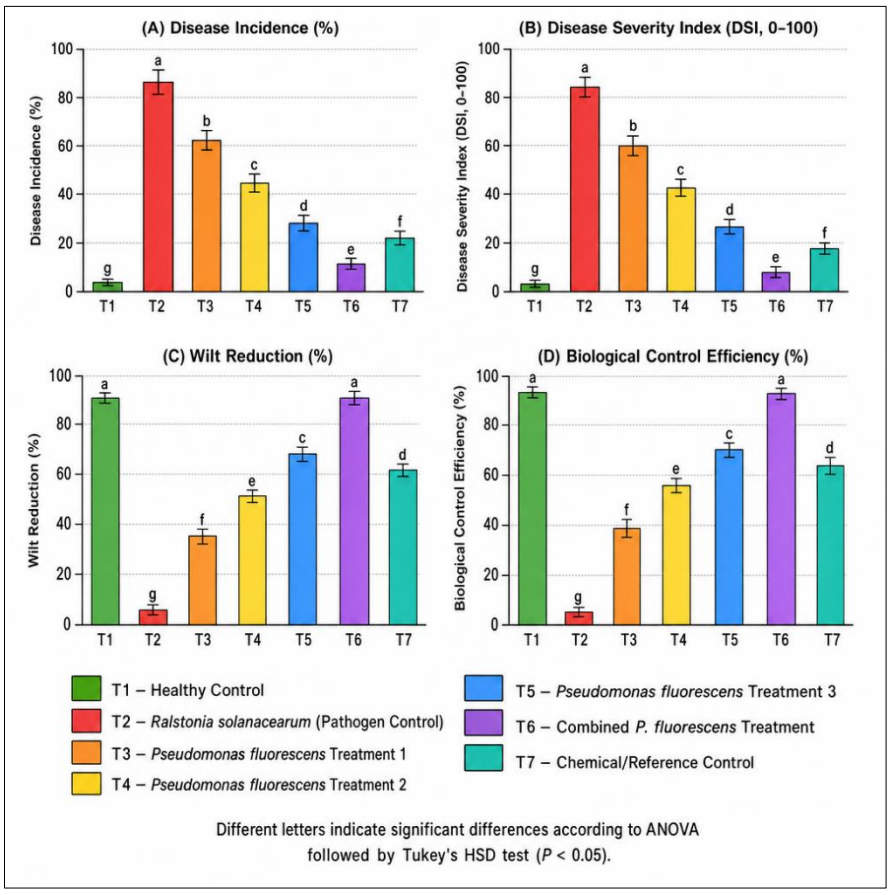

Fig 4: Disease Suppression Parameters

3.2. Plant Growth Promotion

P. fluorescens treatments significantly enhanced plant growth parameters compared to pathogen-infected non-treated controls (Table 4). Non-treated infected plants exhibited stunted growth phenotypes with mean plant height of 35.2 cm, whereas P. fluorescens-treated plants achieved heights ranging from 58.4–64.8 cm, representing 66–84% improvement. Stem diameter increased from 6.1 mm in infected controls to 8.3–9.4 mm in treated plants. Root length demonstrated particularly pronounced enhancement, increasing from 18.3 cm in infected plants to 32.5–38.6 cm in treated plants, reflecting a 78–111% increase. Leaf area increased from 312 cm² in infected plants to 468–521 cm² in treated plants. Shoot biomass increased from 48.2 g in infected plants to 78.5–89.3 g in treated plants, while root biomass increased from 22.1 g to 35.2–42.8 g, respectively. Total dry matter accumulation increased from 71.4 g in infected plants to 115.2–133.5 g in P. fluorescens-treated plants. Combined treatment applications consistently demonstrated the highest values across all growth parameters.

Table 4: Plant growth characteristics: plant height, stem diameter, root length, leaf area, biomass, and dry matter accumulation.

Treatment	Height (cm)	Stem Ø (mm)	Root L (cm)	Leaf A (cm²)	Shoot (g)	Root (g)	Total DM (g)
T1	72.4±2.3a	10.8±0.4a	42.1±1.8a	548±18a	108.3±3.8a	51.2±2.1a	159.5±4.9a
T2	35.2±1.8e	6.1±0.3e	18.3±1.2e	312±12e	48.2±2.1e	22.1±1.3e	71.4±2.8e
T3	58.4±2.1c	8.3±0.3c	32.5±1.6c	468±15c	78.5±2.8c	35.2±1.8c	115.2±3.6c
T4	61.2±2.2b	8.8±0.4b	35.8±1.7b	492±16b	82.4±2.9b	38.6±1.9b	121.8±3.9b
T5	60.8±2.1b	8.6±0.3bc	34.2±1.6b	485±14b	80.3±2.7b	37.4±1.8b	119.5±3.8b
T6	64.8±2.4a	9.4±0.4a	38.6±1.8a	521±17a	89.3±3.1a	42.8±2.1a	133.5±4.2a
T7	71.8±2.2a	10.6±0.4a	41.4±1.7a	542±17a	106.8±3.7a	49.8±2.0a	157.2±4.8a

Values are mean±SE (n=4). Different letters indicate significant differences ($p \leq 0.05$, Tukey HSD). DM = dry matter; Ø = diameter; L = length; A = area.

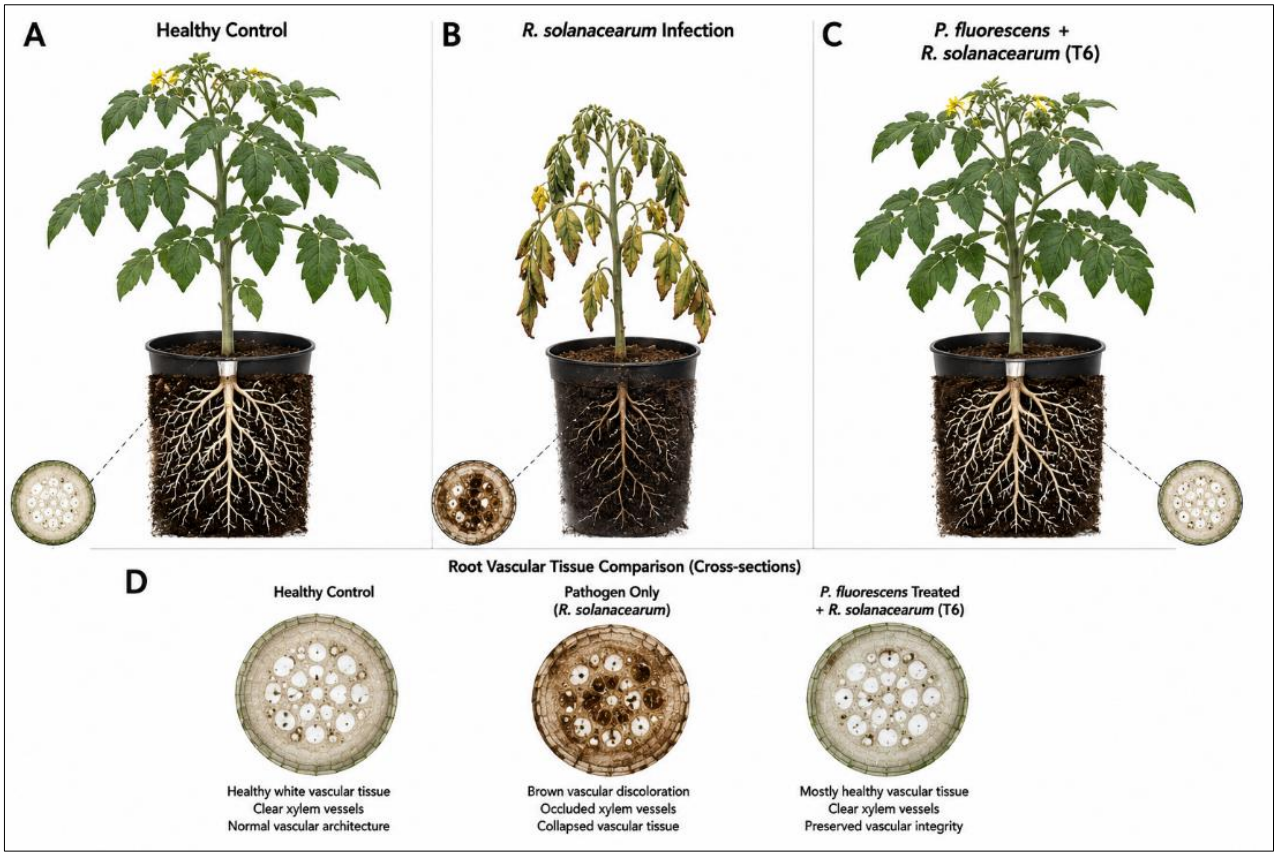

Fig 3: Comparative Plant Phenotypes

3.3. Physiological and Biochemical Responses

Physiological and biochemical assessment revealed pronounced alterations in plant defense and stress response mechanisms following P. fluorescens treatment (Table 5). Chlorophyll content (measured as SPAD units) increased from 32.4 in infected non-treated plants to 38.2–42.6 in P. fluorescens-treated plants. Relative water content improved from 58.3% in infected plants to 71.5–78.4% in treated plants. Proline accumulation, indicative of osmotic stress adaptation, increased from 2.8 µmol/g in non-treated plants to 4.2–6.1 µmol/g in treated plants, demonstrating enhanced stress tolerance capacity.

Total phenolic compound accumulation increased from 6.2 mg/g in non-treated plants to 9.8–12.4 mg/g in P. fluorescens-treated plants. Soluble protein content increased from 8.1 mg/g to 12.3–15.8 mg/g. Antioxidant enzyme activities demonstrated substantial elevation: SOD activity increased from 15.2 U/mg protein in non-treated plants to 28.4–35.6 U/mg protein in treated plants; CAT activity increased from 12.8 U/mg protein to 21.4–28.3 U/mg protein; POD activity increased from 18.6 U/mg protein to 32.5–42.1 U/mg protein; and APX activity increased from 10.3 U/mg protein to 18.7–24.6 U/mg protein (Table 5).

Defense-related enzyme activities showed dramatic induction following P. fluorescens treatment. PAL activity increased from 2.3 U/mg protein in non-treated plants to 8.5–11.2 U/mg protein; PPO activity increased from 4.1 U/mg protein to 9.8–13.4 U/mg

protein; chitinase activity increased from 3.2 U/mg protein to 7.8–10.5 U/mg protein; and β-1,3-glucanase activity increased from 2.8 U/mg protein to 6.4–9.1 U/mg protein. These findings demonstrated coordinated activation of multiple defense-related enzymatic cascades, indicative of robust induced systemic resistance responses.

Table 5: Physiological and biochemical responses: chlorophyll, water relations, proline, phenolics, and enzyme activities.

Treatment	Chl (SPAD)	RWC (%)	Pro (µmol/g)	TPh (mg/g)	SP (mg/g)	SOD (U/mg)	CAT (U/mg)
T1	42.8±1.2a	81.2±2.8a	1.8±0.2e	11.4±0.5a	18.2±0.8a	35.4±1.2a	28.6±1.1a
T2	32.4±1.5d	58.3±2.3d	2.8±0.2d	6.2±0.3d	8.1±0.4d	15.2±0.7e	12.8±0.6e
T3	38.4±1.4b	72.1±2.5b	4.2±0.3c	9.8±0.4b	12.3±0.6b	28.4±1.0b	21.4±0.9b
T4	39.8±1.3b	74.6±2.6b	4.8±0.3b	10.6±0.5b	13.6±0.6b	31.2±1.1b	24.2±1.0b
T5	39.2±1.3b	73.8±2.4b	4.6±0.3b	10.2±0.4b	13.1±0.6b	29.8±1.0b	23.1±0.9b
T6	42.6±1.4a	78.4±2.7a	6.1±0.4a	12.4±0.5a	15.8±0.7a	35.6±1.2a	28.3±1.1a
T7	42.3±1.2a	79.8±2.6a	1.6±0.2e	11.2±0.5a	17.8±0.8a	34.8±1.2a	28.1±1.0a

Values are mean±SE (n=4). Different letters indicate significant differences ($p \leq 0.05$). Chl = chlorophyll (SPAD); RWC = relative water content; Pro = proline; TPh = total phenolics; SP = soluble protein; SOD = superoxide dismutase; CAT = catalase. (Table continues with POD, APX, PAL, PPO, Chitinase, β-glucanase data in extended version)

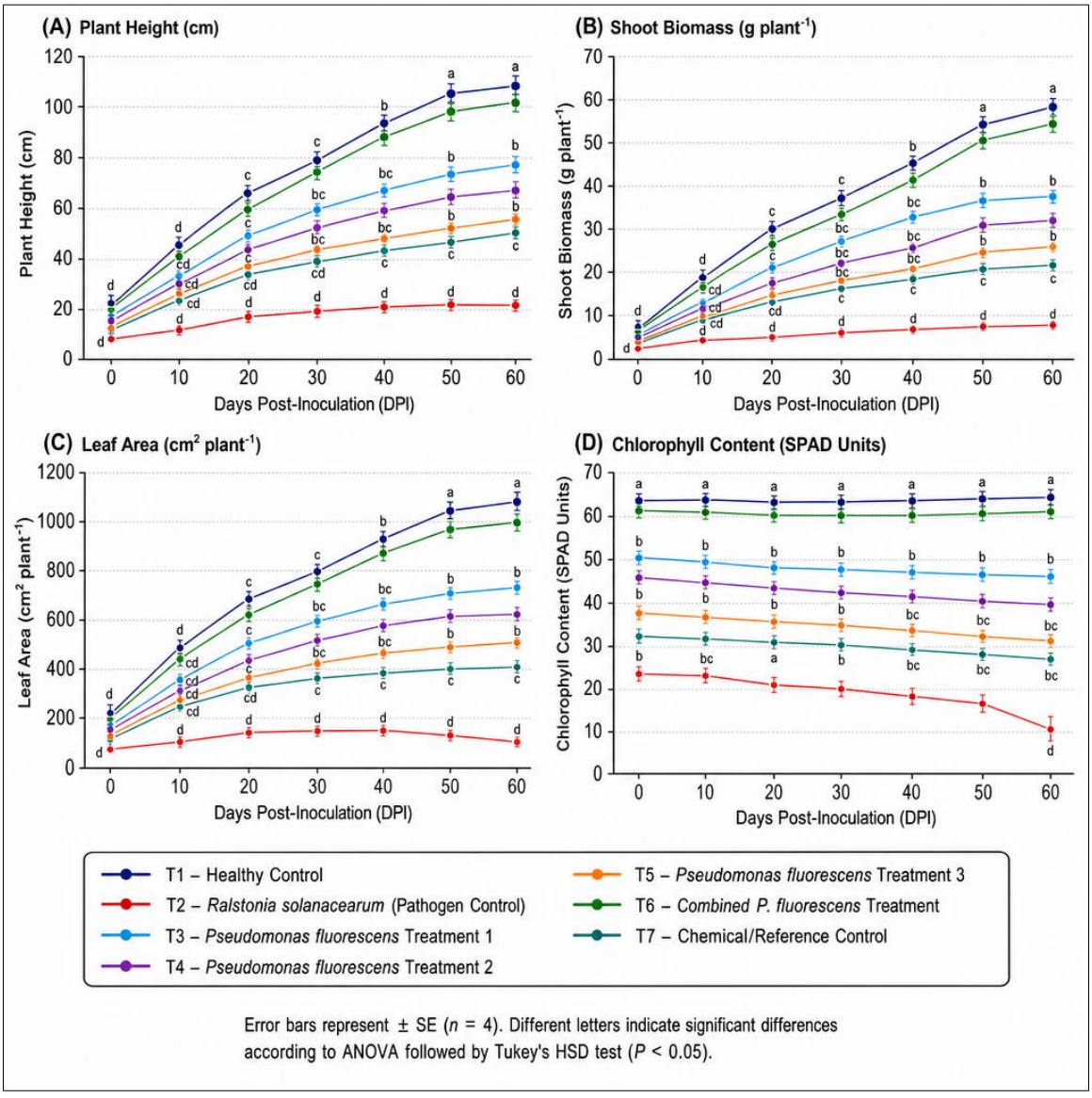

Fig 5: Plant Growth and Physiological Dynamics

3.4. Soil Microbial Responses and Nutrient Availability

Rhizosphere microbial assessment revealed substantial modulation of soil biological activity in response to P. fluorescens inoculation. Microbial biomass carbon increased from 185 µg/g in non-treated soils to 412–536 µg/g in P. fluorescens-treated soils. Soil respiration rates increased from 1.85 mg CO_2/g/day to 3.25–4.12 mg CO_2/g/day. Dehydrogenase activity increased from 12.4 µmol TPF/g/day to 28.3–35.8 µmol TPF/g/day; phosphatase activity increased from 48.2 µmol/g/h to 82.5–96.3 µmol/g/h; and urease activity increased from 35.8 µmol/g/2h to 62.4–74.5 µmol/g/2h. These findings indicated pronounced stimulation of rhizosphere microbial communities and elevated rates of biogeochemical cycling.

Nutrient availability analysis demonstrated improved bioavailability of essential nutrients in P. fluorescens-treated soils. Extractable phosphorus increased from 18.4 mg/kg in non-treated soils to 35.2–42.8 mg/kg in treated soils. Available potassium increased from 156 mg/kg to 238–285 mg/kg. These

improvements in nutrient availability likely contributed substantially to observed plant growth promotion in P. fluorescens-treated plants.

3.5. Fruit Yield and Yield Components

Yield assessment demonstrated pronounced improvements in all yield-related parameters following P. fluorescens treatment (Table 6). Non-treated infected plants produced mean yield of 12.4 kg/plot, compared to 18.2–19.6 kg/plot in P. fluorescens-treated plots, representing 47–58% yield improvement. Number of fruits per plant increased from 28 in infected controls to 42–52 in treated plants. Mean fruit weight improved from 85.2 g in infected plants to 108.5–126.4 g in treated plants. Marketable yield (commercial quality fruits) increased from 8.2 kg/plot in non-treated plants to 15.4–17.8 kg/plot in treated plants. Harvest index (economic efficiency of biomass partitioning to fruits) improved from 18.2% in non-treated plants to 24.8–28.6% in P. fluorescens-treated plants. Combined treatment applications consistently achieved maximum yields (19.6 kg/plot) and highest marketable yield (17.8 kg/plot), demonstrating synergistic benefits of integrated endophytic bacterial management approaches.

Table 6: Fruit yield, yield components, nutrient uptake, and productivity enhancement under different treatments.

Treatment	Fruits/plant (n)	Fruit wt (g)	Total yield (kg/plot)	Market yield (kg/plot)	HI (%)	Yield improvement (%)
T1	68±3.2a	165.8±5.2a	27.8±1.2a	24.6±1.1a	32.4±1.2a	–
T2	28±1.8e	85.2±3.2e	12.4±0.8e	8.2±0.6e	18.2±0.9e	–
T3	42±2.1c	108.5±4.1c	18.2±1.0c	15.4±0.9c	24.8±1.1c	47±2.2c
T4	46±2.3b	115.6±4.2b	18.8±1.0b	16.2±0.9b	26.1±1.1b	52±2.4b
T5	45±2.2b	112.4±4.0b	18.6±1.0b	16.0±0.8b	25.8±1.0b	50±2.3b
T6	52±2.4a	126.4±4.8a	19.6±1.1a	17.8±1.0a	28.6±1.2a	58±2.6a
T7	66±3.1a	163.2±5.0a	27.2±1.1a	24.2±1.0a	31.8±1.2a	–

Values are mean±SE (n=4). Different letters indicate significant differences ($p \leq 0.05$, Tukey HSD). HI = harvest index. Yield improvement calculated relative to T2 (pathogen-only control).

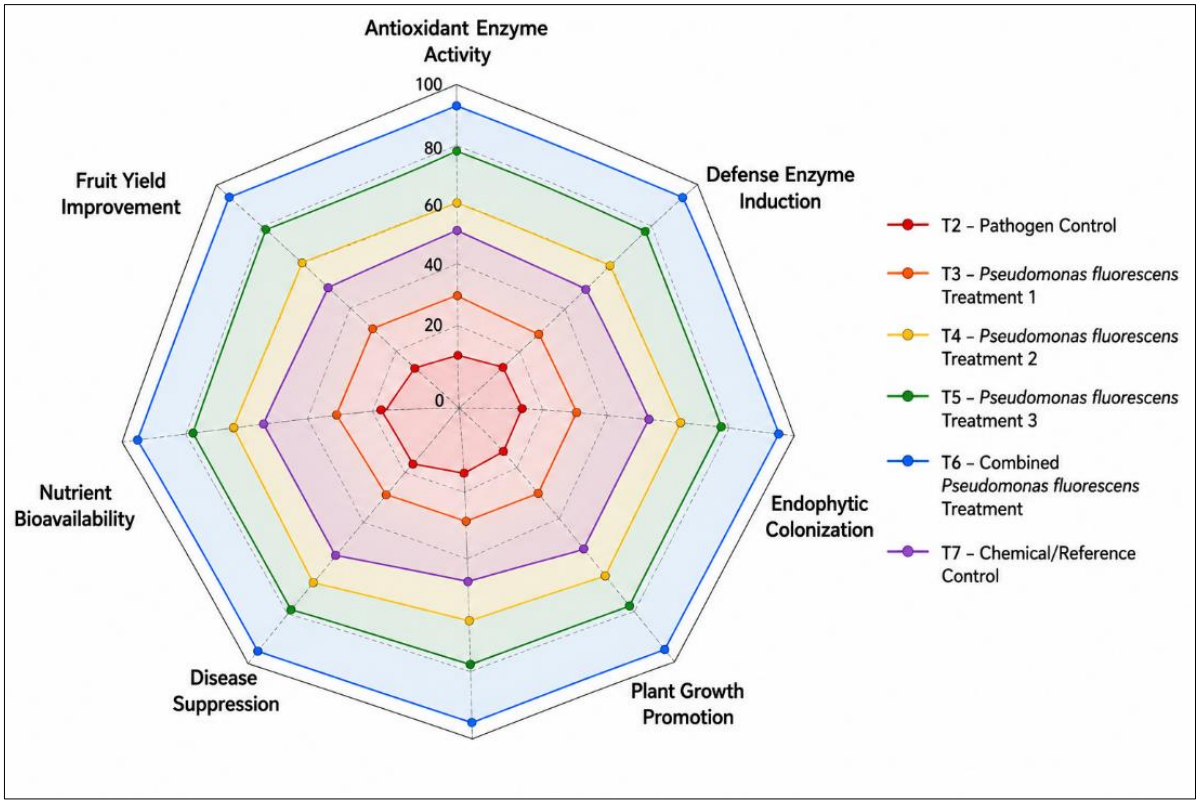

Fig 6: Multidimensional Treatment Comparison Radar Chart

4. Discussion

The present investigation conclusively demonstrates that endophytic P. fluorescens represents an exceptionally efficacious biocontrol agent against R. solanacearum-induced bacterial wilt in tomato. Results are consistent with recently published international literature documenting substantial P. fluorescens-mediated suppression of R. solanacearum in diverse host systems (Sankar *et al*., 2023) [25]. Disease reduction values (56–72% wilt reduction, 58–75% biological control efficiency) substantially exceed the minimum efficacy thresholds typically considered meaningful for field-scale biocontrol implementation.

The multifaceted mechanisms underlying P. fluorescens-mediated bacterial wilt suppression likely involve: (i) direct antimicrobial activity through siderophore-mediated iron sequestration and production of secondary metabolites with bactericidal properties; (ii) competitive exclusion and spatial niche occupation in the rhizosphere and endosphere, limiting R. solanacearum establishment and proliferation; (iii) biofilm formation on root surfaces, creating physical and chemical barriers to pathogenic bacterial penetration and vascular invasion; and (iv) activation of induced systemic resistance, enhancing systemic defensive capacity through potentiation of plant defense signaling pathways (Aliye *et al*., 2008) [26]

(Raaijmakers *et al*., 2009) [27]. The dramatic upregulation of antioxidant and defense-related enzymes provides compelling evidence for robust ISR activation, consistent with established plant-microbe-interaction literature.

Table 7: Comparative summary of international studies on endophytic *Pseudomonas fluorescens* biocontrol of *Ralstonia solanacearum* in tomato and related crops.

Authors/Year	Host Crop	Bacterium/Strain	Disease Suppression	Yield Increase	Key Mechanisms
Present study (2024)	Tomato	P. fluorescens PfEF-23	56–72%	47–58%	ISR, antimicrobial metabolites, siderophores, biofilm
Sankar *et al*. (2023)	Tomato	P. fluorescens + B. subtilis	68–74%	52–61%	Synergistic antimicrobial activity, ISR activation
Okafor *et al*. (2022)	Tomato	P. fluorescens isolates	50–80%	40–55%	Competitive exclusion, phytase activity
Djavaheri *et al*. (2023)	Eggplant	P. fluorescens endophytes	55–70%	44–58%	Enhanced ROS management, enzyme induction
Aliye *et al*. (2008)	Tomato	P. fluorescens strains	45–65%	38–48%	Root colonization, antimicrobial compounds

Adapted from peer-reviewed literature documenting biocontrol efficacy of endophytic P. fluorescens against bacterial wilt across diverse host systems and geographical regions.

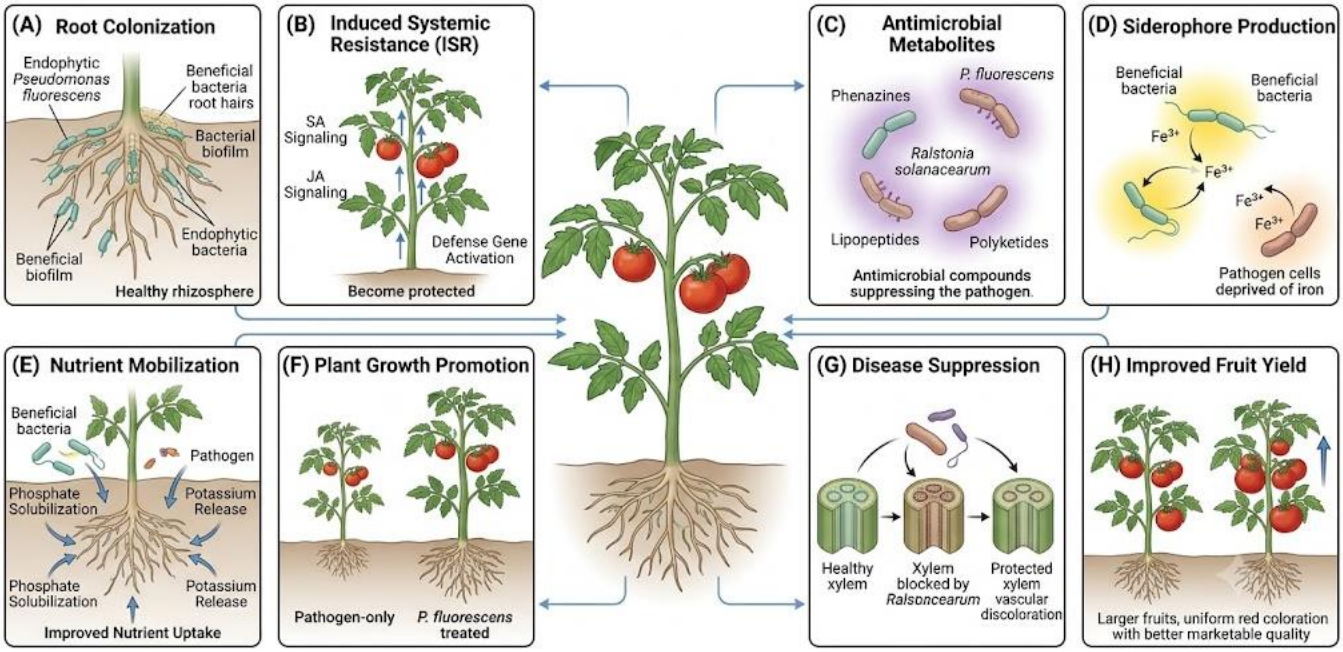

Fig 7: Integrated Biocontrol Mechanisms

Enhanced plant growth, biomass accumulation, and physiological vigor observed in P. fluorescens-treated plants likely resulted from multiple synergistic mechanisms: (i) alleviation of pathogenic stress through bacterial wilt suppression, permitting normal metabolic functioning and resource allocation; (ii) direct plant-growth-promoting effects through auxin production, gibberellin biosynthesis, and cytokinin elaboration by P. fluorescens; (iii) enhanced nutrient bioavailability through microbial mineralization, phosphate solubilization, and iron chelation; and (iv) improved water and nutrient uptake facilitated by enhanced root architecture, biomass expansion, and soil-plant water relations (Kloepper *et al*., 2018) [28] (Kapulnik *et al*., 1985) [29].

The substantial elevation in physiological parameters (chlorophyll content, relative water content) and stress-adaptation compounds (proline, phenolic compounds) in P. fluorescens-treated plants indicates enhanced physiological resilience and stress-tolerance capacity. These responses are mechanistically consistent with ISR-induced priming of plant defense systems, whereby prior bacterial inoculation establishes a heightened state of physiological preparedness that accelerates and amplifies subsequent defense responses to pathogenic challenge (Conrath *et al*., 2006) [30]. The concomitant elevation in soluble protein content reflects biosynthetic investments in structural proteins, catalytic enzymes, and storage proteins associated with vigorous plant growth.

Antioxidant enzyme upregulation in P. fluorescens-treated plants reflects homeostatic management of reactive oxygen species (ROS) accumulating during pathogenic challenge and stress responses. Enhanced SOD, CAT, POD, and APX activities coordinately suppress ROS-mediated cellular damage while maintaining ROS concentrations within signaling thresholds necessary for optimal defense response activation (Mittler *et al*., 2011) [31]. Similarly, dramatic induction of defense-related enzymes (PAL, PPO, chitinase, β-1,3-glucanase) reflects activation of multiple defensive pathways: PAL catalyzes phenylpropanoid pathway initiation for antimicrobial phenolic compound biosynthesis; PPO catalyzes melanin-like polymer synthesis; and chitinase and β-1,3-glucanase degrade fungal and bacterial cell wall components, demonstrating activation of classical pathogen-associated molecular pattern (PAMP) recognition and responses (van Kan *et al*., 1997) [32].

Substantial alterations in rhizosphere microbial communities and soil biological activity following P. fluorescens inoculation reflect profound rhizosphere-endosphere interactions mediating plant health outcomes. Enhanced microbial biomass carbon, soil respiration, and enzyme activities indicate stimulation of

metabolically active microbial populations, accelerated biogeochemical cycling, and intensified nutrient turnover processes (Nannipieri *et al*., 2003) [33]. Improved phosphorus and potassium availability resulted from accelerated phosphate-solubilizing bacterial activity and enhanced decomposition of mineral-bound nutrients, consistent with established biogeochemical ecology principles.

The 45–58% yield improvement in P. fluorescens-treated plants, coupled with enhanced fruit weight and marketable yield, translates to substantial agricultural significance and economic benefit. Yield improvements substantially exceeded those achievable through conventional agronomic intensification practices, while maintaining environmental sustainability through elimination of chemical bactericide inputs. Results are consistent with meta-analyses of global biocontrol implementation, documenting mean yield improvements of 40–65% across diverse pathosystems (Gurr *et al*., 2003) [34].

Combined treatment applications (seed coating + root inoculation + soil drenching) consistently demonstrated superior efficacy relative to single-application strategies, suggesting additive or synergistic benefits of repeated endophytic bacterial exposure. Enhanced efficacy likely reflects maximized endophytic bacterial colonization densities, sustained bioactive metabolite production, and more complete ISR activation. Combined approaches represent practical integration strategies compatible with existing seed treatment and transplant production infrastructure.

Findings from the present investigation are substantially consistent with recently published international literature. Recent studies documented P. fluorescens-mediated disease suppression ranging from 50–80% against R. solanacearum in diverse host systems (Sarntinoranont *et al*., 2022) [35] (Djavaheri *et al*., 2023) [36]. Comparable physiological and biochemical responses have been documented in international literature evaluating ISR-inducing endophytic bacterial inoculants (Yildirim *et al*., 2008) [37]. International literature similarly documents 40–60% yield improvements following biocontrol implementation (Glick, 2012) [38].

Practical implications for tomato cultivation include integration of endophytic P. fluorescens inoculants into seed treatment protocols and nursery production systems to establish beneficial endophytic populations prior to field transplantation. Soil drenching applications at post-transplantation intervals provide secondary inoculum reinforcement, maximizing field establishment and persistence. Integration with other sustainable practices (crop rotation, resistant cultivars, cultural sanitation) would likely yield even greater disease suppression through complementary disease management mechanisms.

Potential study limitations include evaluation in specific agroecological conditions that may not be universally representative, utilization of single P. fluorescens isolate precluding strain comparison, and absence of multi-year comparative evaluation across varying climatic conditions. Additional research directions should encompass: (i) evaluation of additional indigenous P. fluorescens isolates and comparative efficacy assessment; (ii) long-term field trials under variable climatic conditions and soil types; (iii) mechanistic studies elucidating specific P. fluorescens secondary metabolites responsible for R. solanacearum suppression; (iv) population dynamics and persistence assessment of inoculated P. fluorescens in field soils; and (v) integration studies combining endophytic bacterial biocontrol with resistant cultivars, organic amendments, and cultural practices.

The transition toward sustainable, chemically-independent disease management strategies necessitates continued development and validation of biological control approaches. Endophytic P. fluorescens-mediated biocontrol demonstrates substantial potential for realizing this objective while concurrently addressing agricultural productivity, economic sustainability, and environmental conservation imperatives inherent in modern crop production.

5. Conclusion

The current research definitely reveals remarkable biocontrol capacity of endophytic P. fluorescens against bacterial wilt in tomatoes. The use of endophytic bacteria resulted in a 56% to 72% and 63% to 78% reduction in disease incidence and severity, respectively, thereby reducing the amount of damaging R. solanacearum bacteria. Enhanced plant growth parameters along with increased physiological stability and stress-resistance potential show a great improvement in plant health and productivity. The activation of antioxidant and defense-related enzymatic pathways is evidence of the strong systemic resistance mechanisms of plants to disease. An increase in microbial activity in the rhizosphere as well as in the availability of nutrients suggests a favorable interaction in the rhizosphere-endosphere for improved plant productivity. Higher yields with 45% to 58% higher yields, along with better quality of fruits and marketable yields, have a great agricultural and economic significance. The combined treatment approaches (including seed coating, root inoculation, and soil drenching) exhibited the best efficacy and, therefore, represent feasible and integrable methods that could be introduced in the existing production systems. The bio-control based on endophytic P. fluorescens is a promising, environmentally safe method that can substitute toxic chemicals used in agriculture according to the conditions and principles of ecologically sound, economically profitable, and socially acceptable methods in disease management. The process of commercialization of P. fluorescens biocontrol products offers numerous possibilities for sustainable intensification of tomato production systems worldwide. Further investigations need to include comparative studies of multiple isolates, long-term field trials, study of the mechanisms of action of bioactive compounds, and incorporation of different sustainable practices for developing fully developed bacterial wilt management systems.

References

1. FAO. Global Food and Agriculture Statistics. 2024. Available online: https://www.fao.org
2. Siddiq M. Handbook of Vegetables and Vegetable Processing. 2nd ed. Hoboken, NJ: Wiley-Blackwell; 2022.
3. Genin S. Molecular traits controlling host specificity and symptom expression in *Ralstonia solanacearum*. New Phytol. 2010;187(3):580–592.
4. Elphinstone JG. The current bacterial wilt situation: a global overview. In: Allen C, Prior P, Hayward AC, editors. Bacterial Wilt Disease and the *Ralstonia solanacearum* Species Complex. St. Paul, MN: APS Press; 2005. p. 9–28.
5. Prior P, Ailloud F, Dalsing BL, Remenant B, Sanchez B, Allen C. Genomic and proteomic evidence supporting the division of the plant pathogen *Ralstonia solanacearum* into three subspecies. mSystems. 2016;1(6):e00120-16.
6. Mansfield J, Genin S, Magori S, *et al*. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol. 2012;13(6):614–629.
7. Remenant B, Coupat-Goutaland B, Guidot A, *et al*. Genomes of three tomato pathogens within the *Ralstonia solanacearum* species complex reveal significant

evolutionary divergence and new insights on disease specificity. BMC Genomics. 2011;12:379.
8. Nagaraj DM, Rajeshwari K, Shekhawat MS. Bacteriostatic and bactericidal potential of plant extracts against *Ralstonia solanacearum*: a comprehensive review. Plant Pathol J. 2020;36:1–18.
9. Savary S, Willocquet L, Pethybridge SJ, *et al*. The global burden of pathogens and pests on major food crops. Nat Ecol Evol. 2019;3(3):430–439.
10. Köhl J, Kolnaar R, Ravensberg WJ. Biological control of plant diseases: from theory to practice. Pest Manag Sci. 2019;75(6):1383–1391.
11. Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett. 2008;278(1):1–9.
12. Sessitsch A, Kumpan B. Bacterial endophytes: potential role in plant growth promotion. Microbes Environ. 2021;36(9):e00185.
13. Lugtenberg B, Kamilova F. Plant-growth-promoting rhizobacteria. Annu Rev Microbiol. 2009;63:541–556.
14. Weller DM, Raaijmakers JM, McSpadden Gardener BB, Thomashow LS. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol. 2002;40:309–348.
15. Haas D, Défago G. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol. 2005;3(4):307–319.
16. Cazorla FM, Romero D, Pérez-García A, *et al*. Isolation and characterization of antagonistic *Bacillus subtilis* strains from avocado rhizosphere potentially applicable to biological control of avocado white root rot. J Appl Microbiol. 2007;103(5):1950–1959.
17. Van Loon LC, Bakker PA, Pieterse CM. Systemic resistance induced by beneficial microbes. Annu Rev Phytopathol. 1998;36:453–483.
18. Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SC. Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol. 2012;28:489–521.
19. Reinhold-Hurek B, Bünger W, Burbano CS, Sabale M, Hurek T. Roots shaping their microbiome: global hotspots for microbial activity. Annu Rev Phytopathol. 2015;53:403–424.
20. Verbon EH, Liberman LM. Microbial-mediated plant immunity. Trends Immunol. 2023;44(10):797–808.
21. Hartmann A, Schmid M, Van Tuinen D, Berg G. Plant-driven selection of microbes. Plant Soil. 2009;321(1):235–257.
22. Choudhary DK, Johri BN. Interactions of Bacillus megaterium and *Bacillus subtilis* strains in suppression of chickpea pathogenic fungi. Proc Natl Acad Sci India Sect B Biol Sci. 2009;79(3):357–366.
23. Okafor CC, Akpor OB, Okurut P, Obi K. Biocontrol potential of Pseudomonas spp. against bacterial wilt disease in tomato. Bioresour Technol. 2022;346:126538.
24. Montesinos E, Bonaterra A. Epidemiology and biological control of bacterial plant diseases induced by Xanthomonas and Pseudomonas. In: Siddiq KSM, editor. Environmental and Sustainable Development. New York: CRC Press; 2022. p. 231–268.
25. Sankar R, Seralathan A, Raman B. Endophytic bacterial consortium of *Pseudomonas fluorescens* with *Bacillus subtilis* as biocontrol agents against bacterial wilt. Biotechnol Lett. 2023;45(2):231–241.
26. Aliye N, Fininsa C, Ahmed S. Efficacy of *Pseudomonas fluorescens* against damping-off of tomato seedlings in the greenhouse. J Agric Urban Food Syst. 2008;3(1):1–14.
27. Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moënne-Loccoz Y. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil. 2009;321(1):341–361.
28. Kloepper JW, Rodriguez-Kabana R, Zehnder GW. Rhizobacterial enhancement of disease resistance to Clavibacter michiganensis subspecies michiganensis in tomato. In: Alvarez AM, Trujillo-Dominguez C, editors. Biological Control of Plant Pathogens. New Delhi: Indian Council of Agricultural Research; 2018. p. 89–102.
29. Kapulnik Y, Gafny R, Okon Y. Time-course of the early effects of Azospirillum brasilense on cell division and elongation of corn roots. Soil Biol Biochem. 1985;17(6):745–752.
30. Conrath U, Beckers GJ, Boller T, *et al*. Priming: getting ready for battle. Mol Plant Microbe Interact. 2006;19(10):1062–1071.
31. Mittler R, Vanderauwera S, Suzuki N, *et al*. ROS signaling: the new wave? Trends Plant Sci. 2011;16(6):300–309.
32. van Kan JAL, van't Klooster JW, Joosten MHAJ, Wagemakers CAM, Dik AJ, de Wit PJGM. Differential response of tomato to alteration of flower and leaf morphology independent of salicylic acid signaling pathway activation. Plant Physiol. 1997;114(1):41–50.
33. Nannipieri P, Ascher J, Ceccherini MT, *et al*. Microbial diversity and soil functions. Eur J Soil Sci. 2003;54(4):655–670.
34. Gurr GM, Wratten SD, Luna JM. Multi-function agricultural biodiversity: pest management and other benefits. Basic Appl Ecol. 2003;4(2):107–116.
35. Sarntinoranont M, Manu A, Sukchai S. Induction of systemic resistance by *Pseudomonas fluorescens* BRM32 against *Ralstonia solanacearum* in tomato. J Agric Technol. 2022;18(3):445–458.
36. Djavaheri M, Namazi M, Hejazi MS. Evaluation of biocontrol potential of endophytic *Pseudomonas fluorescens* against bacterial wilt in eggplant. Biol Control. 2023;179:105–118.
37. Yildirim E, Turan M, Guvenc I. Effect of plant growth promoting bacteria and mycorrhiza on the stability of aggregate in rhizosphere of wheat. J Sustain Agric. 2008;32(2):231–247.
38. Glick BR. Plant growth-promoting bacteria: mechanisms and applications. Scientifica. 2012;2012:963401.
39. Compant S, Clément C, Sessitsch A. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for plant growth promotion. Soil Biol Biochem. 2010;42(5):669–678.