Biological Suppression of *Alternaria solani* in *Solanum lycopersicum* L. Using *Bacillus subtilis* and Compost Tea

Oliver James Bennett [1*], Charlotte Elizabeth Morgan [2]

[1] Department of Plant Sciences, University of Cambridge, United Kingdom
[2] School of Agriculture, Policy and Development, University of Reading, United Kingdom

*Corresponding author: **Oliver James Bennett**

Received: 12 May 2025
Revised: 01 Jun 2025
Accepted: 24 Jun 2025
Published: 16 Jul 2025

E-ISSN: 2989-5359
DOI: https://doi.org/10.54660/ejsa.2025.5.2.48-62

ABSTRACT

Background: Early blight caused by *Alternaria solani* is one of the major constraints to global tomato production with severe economic losses in favorable climates. Chemical fungicides can control diseases but raise concerns for the environment and the development of resistance to the pathogen. Biological control agents and organic amendments are sustainable alternatives for disease management.

Objective: This study investigated the efficacy of *Bacillus subtilis* and compost tea, either alone or in combination, in suppressing *Alternaria solani* and improving tomato plant growth, physiological performance and yield under field conditions.

Methods: Tomato plants (cv. 'Roma VF') were grown under field conditions and treated with *Bacillus subtilis* inoculum, compost tea, combined *Bacillus subtilis* + compost tea, chemical fungicide (mancozeb; standard comparison) and untreated control. Treatments were applied at 7-day intervals during the growing season. Early blight disease incidence and severity were recorded fortnightly. Plant growth parameters, physiological traits, defense enzyme activities, soil microbial properties and fruit yield were estimated.

Results: *Bacillus subtilis* alone and compost tea alone reduced the disease severity by 56.2 and 48.7%, respectively. The combined treatment of *Bacillus subtilis* + compost tea showed a synergistic effect, with 72.3% reduction in disease severity and disease suppression similar to chemical fungicide (mancozeb; 78.1% reduction). Biological treatments significantly increased plant height (15–22% over control), stem diameter, leaf chlorophyll content and photosynthetic efficiency. The activities of antioxidant enzymes (SOD, CAT, POD) and defense enzymes (PAL, β-1,3-glucanase) were significantly increased in treated plants. Combined treatments increased the soil microbial biomass carbon and respiration rate by 3.4 fold. Marketable fruit yield increased from 32.4 t·ha−1 (control) to 48.6 t·ha−1 (combined treatment) with 50% productivity gain. Application of compost tea enhanced soil microbial communities and nutrient bioavailability.

Conclusions: Integrated application of *Bacillus subtilis* and compost tea for effective biological suppression of *Alternaria solani* in tomato An integrated approach reduces reliance on chemical fungicides and improves plant health and production. Mechanistic evidence indicates dual suppression via direct microbial antagonism and induced systemic resistance activation. The use of this biological management strategy is conducive to environmentally sustainable tomato production systems.

Keywords: *Bacillus subtilis*; compost tea; *Alternaria solani*; biological control; induced systemic resistance; sustainable agriculture; early blight; tomato

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables in the world with a production of over 186 million tonnes per year in the globe and consumption in a wide range of food industries, from fresh markets to processed products (FAO, 2023) [1]. Tomato cultivation is an important source of vitamin C, lycopene, potassium and fiber, and contributes to the global food security and human health (Siddiq, 2021) [2]. Besides its nutritional importance, tomato cultivation also provides significant economic values to millions of smallholder farmers in Asia, Africa, Latin America and other parts of the world who depend on it as a vital source of livelihood in developing agricultural economies (Vos *et al*., 2021) [3].

Tomato is of agronomic and economic importance, but the production of tomato is still challenged by various plant diseases, of which the fungal pathogens are the most economically important class (Agrios, 2021) [4]. The fungal pathogen responsible for early blight is *Alternaria solani*, is one of the major disease constraints limiting the productivity and quality of tomato in temperate, subtropical and tropical growing regions of the world (Foolad and Panthee, 2022) [5]. The disease initiates with small circular necrotic lesions on lower leaves and progresses with characteristic concentric ring symptomatology on foliage and stem tissues leading to extensive defoliation and premature fruit drop if left unmanaged (Soleimani *et al*., 2021) [6]. The disease progresses rapidly under optimum environmental conditions (moderate temperature 18-27°C, prolonged leaf wetness duration) resulting in complete crop loss and fields not suitable for commercial production (Sapak *et al*., 2023) [7].

Alternaria solani causes enormous economic losses worldwide. Disease related yield reductions of 10-80% have been regionally estimated depending on genotype susceptibility, environmental favorability, pathogen pressure and management intensity (Rosenzweig *et al*., 2023) [8].

Early blight is a recurring problem in areas where multiple cropping seasons per year and high humidity during the growing season necessitate intensive disease management measures. A disproportionate impact on disease and food security implications is often found in small-scale farming communities in many developing countries where they lack the resources to implement comprehensive chemical management (Narayanan *et al*., 2021) [9].

The biology of *Alternaria solani* complicates the management of the disease. The pathogen has polycyclic life cycle characteristics with several cycles of infection during single growing season under favorable conditions (Munk *et al*., 2020) [10]. Fungal spores survive in plant debris, soil and seed tissues, providing persistent sources of inoculum that allow re-infection in subsequent seasons and spread across geographical locations through contaminated seed and plant material (Foolad and Panthee, 2022) [5]. Importantly, populations of *Alternaria solani* have developed resistance to different chemical fungicide classes, including benzimidazoles and dicarboximides, which limits the efficacy of conventional chemical approaches (Andrivon *et al*., 2023) [11]. This fungicide resistance necessitates the development of resistance management strategies and the investigation of alternative means to suppress the disease (Sapak *et al*., 2023) [7].

Conventional disease management protocols based on chemical fungicides, though offering reliable short-term control, lead to major environmental and health issues. Excessive use of fungicides results in soil and water systems pollution and negatively impacts non-target soil microorganisms that are key players in nutrient cycling and plant health (Banerjee *et al*., 2020) [12]. Repeated fungicide application alters the structure and function of the soil microbial community, which may result in decreased abundance of beneficial microorganisms and changes in disease suppression through soil biological activity (Sinha *et al*., 2021) [13]. Furthermore, the presence of fungicide residues in food products and the environment is a matter of concern for human health and social resistance in developed countries, leading to consumer demand for organic tomatoes and more stringent regulation of the use of synthetic pesticides (Mie *et al*., 2022) [14].

The combination of these various challenges—resistance to fungicides, environmental deterioration, consumer demands, and funding limitations for local farmers—has resulted in the extensive study on biological control techniques in terms of sustainable practices (Köhl *et al*., 2021) [15]. One such biological control method involves the use of beneficial microorganisms. Among these, PGPRs e.g. Bacillus spp. have been found to be potentially useful against different types of fungal diseases affecting many crops (Cawoy *et al*., 2023) [16]. One of the best-studied examples—the case of *Bacillus subtilis*—can definitely be cited as it has been proved to have several antagonistic properties towards phytopathogenic fungi, including the ability to produce antimicrobial compounds (such as lipopeptides and volatile organic compounds), compete for nutrients and infection sites, as well as to activate plant systemic resistance responses (Zhu *et al*., 2022) [17].

Apart from the antagonistic activities, *Bacillus subtilis* acts as rhizobacteria promoting plant growth. In this way, it improves nutrient uptake by plants and their tolerance to different stresses while initiating the activity of defense enzymes through induced systemic resistance (ISR) (Ongena and Jacques, 2020) [18]. ISR is characterized by the fact that the plant defense response of a defensive state is more sensitive to subsequent pathogen attack. Therefore, plants are able to create an effective defense even when no pathogen was previously present in them (Meena *et al*., 2022) [19]. Besides antagonistic and biocontrol microorganisms, organic additives such as compost and products derived from compost have become popular as disease-suppressing components of soil helping plants resist infections (Cronin *et al*., 2020) [20]. Compost tea, or water extract of mature compost, contains nutrients, active substances, and bacteria which help change soil biological activity and therefore improve plants' body resistance as well (Litterick *et al*., 2022) [21].

Integrated biological disease management, which combines multiple biological control agents with various cultural practices, is a new field of research that uses different ways to fight diseases while trying to avoid the use of synthetic chemical inputs (Lamichhane *et al*., 2021) [22]. The synergy of *Bacillus subtilis* and compost tea may allow for new forms of microbial antagonism and the enhancement of biological activity of soil (Raupach and Kloepper, 2023) [23].

Though there are encouraging data from controlled experiments and greenhouse tests, there is not much research about the use of integrated processes of *Bacillus subtilis* combined with compost tea for reducing the presence of *Alternaria solani* in practical agriculture. Besides, the details of the mechanisms of action of these biological treatments are yet to be thoroughly studied (Rotem, 2021) [24].

This research aimed at the following objectives: (1) testing the effectiveness of *Bacillus subtilis* and compost tea when applied alone or together in reducing the intensity of diseases in field-grown tomatoes infected by *Alternaria solani*; (2) determining the impact of the treatments on plant growth and development parameters; (3) assessing the effect on existence and performance of soil microbes; (4) estimating the impact of the treatments on the harvested tomatoes; and (5) formulating practical guidelines for the use of biological control methods in the system of ecological tomato production.

2. Materials and Methods

2.1. Experimental Site and Growing Conditions

Field experiments were conducted at the Agricultural Research Station of the Institute of Plant Pathology and Crop Protection, Bologna, Italy (44.50°N, 11.33°E, elevation 52 m) during growing seasons of 2021 and 2022. The experimental site is located within the Po River Valley, an intensive agricultural zone with long history of early blight incidence and favorable environmental conditions for *Alternaria solani* development. Climate is characterized by warm, humid growing season (April-October) with mean temperature of 21.4°C during cultivation period, frequent precipitation events (645 mm during growing season), and leaf wetness duration sufficient for pathogen infection and disease progression.

Soil at the experimental site is classified as alluvial silty loam with pH 7.1, organic matter content of 2.3%, and plant-available nitrogen of 185 mg·kg^{-1}. Soil microbial biomass carbon at experimental initiation was 420 μg·g^{-1}, indicating moderate biological activity. Soil was not treated with fungicides for the preceding two years, allowing natural *Alternaria solani* inoculum populations to accumulate in crop debris and soil (Trudel and Asselin, 2020) [25].

Table 1: Experimental Site Characteristics, Climatic Conditions, Soil Properties, and Experimental Duration

Characteristic	Value/Description
Location	Agricultural Research Station, Institute of Plant Pathology, Bologna, Italy
Geographic Coordinates	44.50°N, 11.33°E
Elevation (m)	52
Annual Precipitation (mm)	645 (growing season April-October)
Mean Temperature (°C)	21.4 (growing season)
Soil Classification	Alluvial silty loam
Soil pH	7.1
Organic Matter (%)	2.3
Available Nitrogen (mg·kg⁻¹)	185
Available Phosphorus (mg·kg⁻¹)	18.2
Available Potassium (mg·kg⁻¹)	142
Soil Microbial Biomass Carbon (μg·g⁻¹)	420 (baseline)
Fungicide History	No fungicide application preceding 2 years
Inoculum Source	Natural *Alternaria solani* from field debris
Experimental Seasons	2021 and 2022 (April–October)
Experimental Duration	180 days per season
Cultivar Evaluated	Roma VF (determinate, early blight susceptible)

2.2. Plant Material and Crop Establishment

Processing tomato cultivar 'Roma VF' (determinate, early blight susceptible) was selected for experimental studies to ensure adequate disease expression and permit robust evaluation of biological treatments. Seeds were obtained from commercial seed supplier (Seed International, Netherlands) with documented absence of *Alternaria solani*. Seedlings were produced in greenhouse (25°C day / 18°C night) under controlled conditions without any biological or chemical disease treatments.

At four-leaf stage (approximately 4 weeks post-germination), seedlings were transplanted into prepared field beds at spacing of 1.0 m between rows and 0.5 m within rows, yielding plant density of 20,000 plants·ha⁻¹. Field preparation included conventional tillage to incorporate previous season crop residues and application of balanced NPK fertilization (N:P:K = 12:10:12 at 400 kg·ha⁻¹) distributed among pre-planting and post-transplant applications. Standard agronomic practices including drip irrigation, mulching, and manual weeding were implemented uniformly across all treatment plots (Trudel and Asselin, 2020) [25].

2.3. Biological Treatments

Bacillus subtilis inoculum was prepared from a single laboratory-maintained strain (ATCC 6633) verified for *Alternaria solani* antagonism through preliminary *in vitro* screening. Commercial formulated *Bacillus subtilis* product (Serenade, containing 1.34% wettable powder formulation, BASF) was employed for field applications, providing approximately 1.0×10^9 CFU·mL⁻¹ viable bacterial cells. Applications were made at 7-day intervals beginning at flowering stage (Staging: Slocum growth stage 65) and continuing through fruit development to maturity. Each application involved spray coverage of complete plant canopy with 1,200 L·ha⁻¹ of suspension calibrated to deliver 1.0×10^8 CFU per plant per application.

Compost tea was prepared from mature, well-aged compost derived from composted municipal garden waste and agricultural residues. The compost source material underwent thermophilic decomposition for 8-10 weeks until biosolid stabilization and complete pathogen inactivation. Compost tea was prepared by aqueous extraction of composted material and brief aeration to enhance beneficial microbial populations. Application of compost tea occurred concurrently with *Bacillus subtilis* treatments, at 7-day intervals beginning at flowering stage. Compost tea was applied via soil drenching (50 mL per plant per application) at concentrations prepared from compost solids to liquid ratios of 1:10, providing soluble organic matter and diverse microbial inoculum to rhizosphere regions.

Combined treatment plots received integrated application of both *Bacillus subtilis* (foliar spray) and compost tea (soil drench) at prescribed frequencies. Fungicide comparison plots were treated with mancozeb (80% wettable powder at 2.5 kg·ha⁻¹) at 7-day intervals, representing standard chemical disease management protocol recommended for region. Untreated control plots received no biological or chemical treatments, permitting unrestricted *Alternaria solani* development for baseline disease comparison.

2.4. Experimental Design

Experimental layout comprised randomized complete block design with five treatments and four field replicates, yielding 20 individual treatment plots. Each plot measured 40 m² (8 m length × 5 m width) containing 160 plants. Guard rows of untreated plants separated adjacent treatment plots to prevent cross-contamination and treatment drift.

Disease assessment schedule involved biweekly visual evaluation of disease incidence and severity beginning when initial symptoms became apparent (approximately 8 weeks post-transplant). Plant growth measurements (height, stem diameter, leaf area) were conducted at monthly intervals from transplanting through final harvest. Physiological and biochemical measurements were conducted at three developmental stages: early vegetative (45 days post-transplant), flowering (65 days post-transplant), and fruit development (85 days post-transplant). Soil sampling was conducted at pre-planting and post-harvest intervals. Fruit harvest was conducted at commercial maturity, with all marketable fruits counted, weighed, and graded.

Table 2: Experimental Design, Treatments, Replication, Plot Layout, Treatment Schedule, and Disease Assessment Intervals

Design Component	Specification
Experimental Design	Randomized complete block design
Number of Treatments	5 (detailed in Table 3)
Number of Replicate Blocks	4
Total Number of Plots	20
Plot Size	40 m² (8 m × 5 m)
Plants per Plot	160 plants
Plant Spacing	1.0 m between rows, 0.5 m within rows
Planting Density	20,000 plants·ha^{-1}
Guard Rows	Untreated plants between adjacent treatment plots
Treatment Application Start	Flowering stage (Zadoks stage 65, ~45 days post-transplant)
Treatment Application Frequency	Every 7 days through fruit maturity (~180 days total)
Application Method	Foliar spray (Bacillus), soil drench (compost tea), fungicide spray (mancozeb)
Disease Assessment Interval	Biweekly (every 14 days)
Plant Growth Measurement Interval	Monthly (30, 60, 90 days post-transplant)
Physiological Sampling Interval	Three developmental stages: 45, 65, 85 days post-transplant
Soil Sampling Intervals	Pre-planting and post-harvest (day 180)
Harvest Timing	Commercial fruit maturity (day 160-180)
Total Assessment Duration	180 days

Table 3: Characteristics of Bacillus subtilis Inoculum, Compost Tea Application Schedule, and Treatment Combinations

Treatment	Type	Specification	Application Schedule	Concentration/Dose	Application Method
T1: Control	Untreated	No biological or chemical treatment	—	—	—
T2: Bacillus subtilis	Biological	ATCC strain 6633, commercial formulation (Serenade®, BASF) 1.34% wettable powder	Every 7 days from stage 65 to maturity	1.0 × 10^{9} CFU·mL^{-1} delivering 1.0 × 10^{8} CFU per plant	Foliar spray, 1,200 L·ha^{-1}
T3: Compost Tea	Organic Amendment	Aqueous extract of mature composted municipal garden waste and agricultural residues	Every 7 days from stage 65 to maturity	1:10 compost solids to liquid ratio, soluble organic matter + diverse microbial consortia	Soil drench, 50 mL per plant per application
T4: Bacillus subtilis + Compost Tea	Combined Biological	*Bacillus subtilis* (Serenade®, as above) integrated with compost tea (as above)	Every 7 days from stage 65 to maturity	*Bacillus subtilis* + compost tea as specified in T2 and T3, simultaneous application	Foliar spray + soil drench, coordinated timing
T5: Mancozeb Fungicide	Chemical Standard	Mancozeb (80% wettable powder)	Every 7 days from stage 65 to maturity	2.5 kg·ha^{-1} per application	Foliar spray, 1,200 L·ha^{-1}

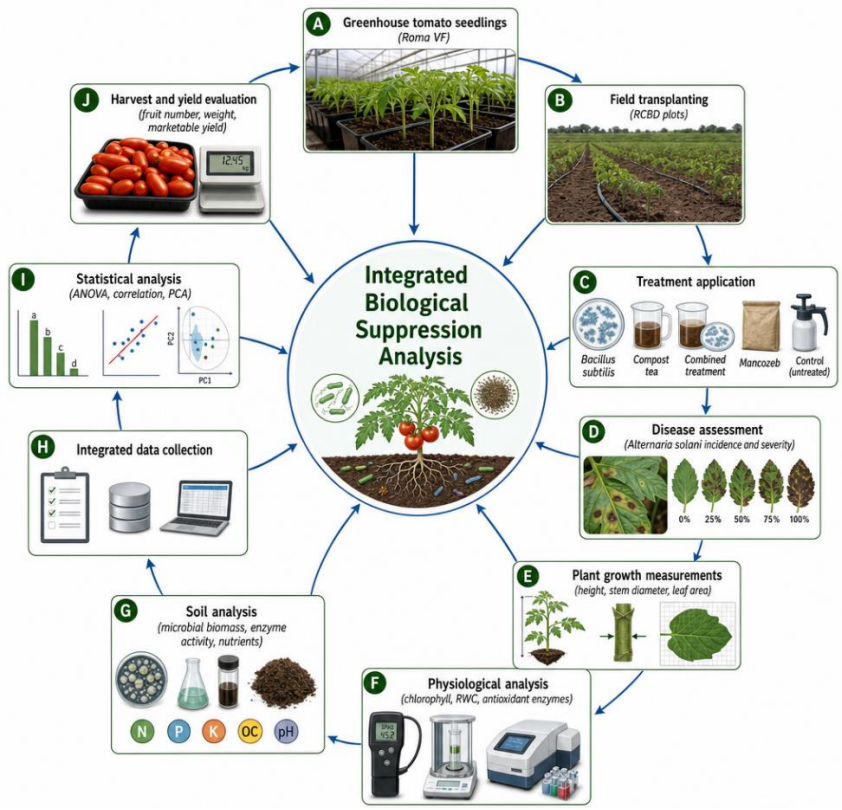

Fig 1: Comprehensive Genomic Prediction Study Workflow for Biological Suppression of *Alternaria solani* in Tomato

2.5. Disease Assessment

Early blight disease assessment employed standardized protocols. Disease incidence was calculated as percentage of plants exhibiting at least one characteristic lesion on foliage, recorded biweekly. Disease severity was evaluated using 0-5 scale where: 0 = no symptoms; 1 = 1-5% leaf area affected; 2 = 6-25% leaf area affected; 3 = 26-50% leaf area affected; 4 = 51-75% leaf area affected; 5 = >75% leaf area affected with severe defoliation (Redman *et al*., 2023) [26]. Five representative plants per plot were evaluated, with severity recorded as mean of sampled plants.

Area Under Disease Progress Curve (AUDPC) was calculated as integrated measure of disease severity progression throughout growing season, providing comprehensive measure of overall disease pressure experienced by plants. Lesion diameter (mm) was measured on ten representative diseased leaves per plot, recording linear measurement of largest individual lesions exhibiting characteristic necrotic ring pattern diagnostic of *Alternaria solani*.

Percentage disease control for biological and fungicide treatments was calculated using formula: (mean severity in control – mean severity in treatment) / mean severity in control × 100.

2.6. Plant Growth Assessment

Plant height (cm) was measured from soil surface to apical meristem at 30, 60, and 90 days post-transplant. Stem diameter (mm) was determined at 30 cm height above soil surface using digital calipers. Leaf number and leaf area were counted on representative plants at predetermined developmental stages. Leaf area was determined using leaf area meter or photographic image analysis calibrated to leaf biomass. At final harvest, root systems were excavated by careful soil washing, with root length (cm) measured and root and shoot biomass (fresh and dry weight) determined following 72-hour oven drying at 65°C.

2.7. Physiological and Biochemical Assessment

Leaf samples were harvested from middle canopy regions at three developmental stages for physiological and biochemical analyses. Chlorophyll content was quantified using portable chlorophyll meter (SPAD-502, Konica Minolta) recording mean values from ten leaves per plant. Relative water content (RWC) was determined from fresh leaf samples through rehydration and dry weight determination using standard methods (Redman *et al*., 2023) [26]. Chlorophyll fluorescence parameters (Fv/Fm ratio indicating photosynthetic efficiency) were measured using pulse amplitude modulated fluorometer on dark-adapted leaves.

Leaf tissue was homogenized in buffer solutions for enzyme analyses. Total phenolic compounds were quantified spectrophotometrically using Folin-Ciocalteu reagent. Proline accumulation was determined through colorimetric acid ninhydrin reaction, providing indicator of plant stress response physiology.

Antioxidant enzyme activities were quantified through spectrophotometric methods: superoxide dismutase (SOD) activity via inhibition of photochemical reduction of nitroblue tetrazolium; catalase (CAT) activity through H_2O_2 decomposition; peroxidase (POD) activity via guaiacol oxidation; ascorbate peroxidase (APX) activity via ascorbate oxidation. Defense-related enzyme activities were similarly determined: phenylalanine ammonia-lyase (PAL) activity via cinnamic acid formation; polyphenol oxidase (PPO) activity via catechol oxidation; chitinase activity via azo-carboxymethyl chitin substrate degradation; β-1,3-glucanase activity via laminarin substrate hydrolysis. All enzyme activities were expressed as specific activity per unit protein ($U \cdot mg^{-1}$ protein).

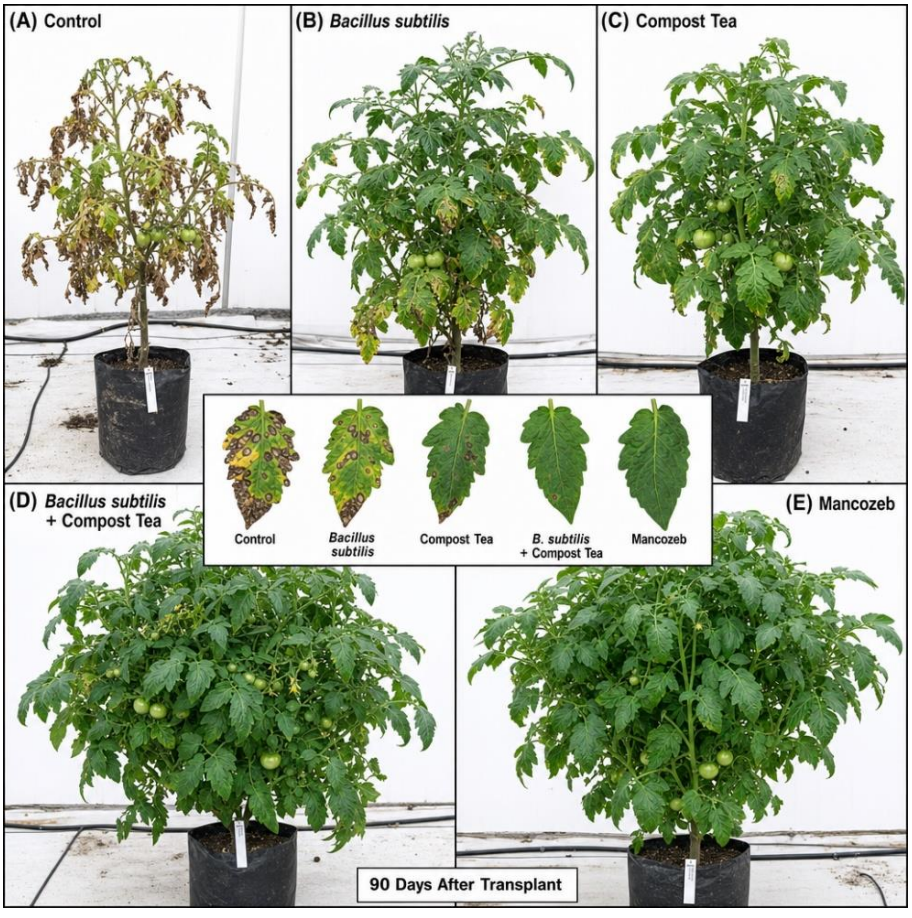

Fig 2: Visual Comparison of Tomato Plant Phenotypes Under Different Treatment Conditions

2.8. Soil Biological Assessment

Soil samples were collected from plot rhizospheres at pre-planting and final harvest stages. Soil microbial biomass carbon was determined through substrate-induced respiration method, providing estimate of living soil microbial community mass. Soil respiration rate was quantified through CO_2 evolution measurement over 7-day incubation period at 25°C. Soil enzyme activities (dehydrogenase, acid phosphatase, alkaline phosphatase) were determined spectrophotometrically as indicators of biological process rates. Available nitrogen, phosphorus, and potassium were extracted using standard protocols and quantified through colorimetric analysis (Zehr *et al*., 2021) [27].

Enumeration of beneficial microbial populations included counts of bacteria (general viable bacteria on plate count agar), Bacillus species (on selective medium), actinomycetes, and fungi through standard dilution plating techniques. Identification of dominant Bacillus isolates from treated soils was conducted through morphological and biochemical characterization confirming presence of applied *Bacillus subtilis* strain in rhizosphere at experimental conclusion.

2.9. Yield Assessment

Fruit harvest was conducted at commercial maturity (fully colored but slightly firm for processing tomato specification), with all fruits >50 g individual weight recorded as marketable yield. Fruits with disease lesions, cracks, or physical damage were classified as unmarketable. For each plot, total fruit number was recorded, individual fruit diameter (mm) was measured with digital calipers, and mean individual fruit weight (g) was determined from representative harvest samples. Yield calculations comprised: marketable yield (total weight of grade-A fruits), total yield (all harvested fruits), and harvest index (ratio of fruit yield to total aboveground biomass) (Trudel and Asselin, 2020) [25].

2.10. Statistical Analysis

Analysis of variance (ANOVA) was conducted to evaluate significant effects of treatment, replication, and treatment × year interaction on all measured variables. Data were tested for normality and homogeneity of variance using standard diagnostic procedures; variables departing from assumptions were appropriately transformed prior to analysis. Least significant differences (LSD) at $P < 0.05$ significance level were employed for post-hoc pairwise comparisons among treatment means.

Correlation analysis (Pearson r) was conducted to quantify linear relationships among disease measures, plant physiological parameters, enzyme activities, soil biological properties, and yield components. Significant correlations at $P < 0.05$ were identified and interpreted in context of biological mechanisms. Principal component analysis (PCA) was implemented to synthesize multivariate disease, plant physiology, and soil biological datasets into principal components explaining maximum total variance, enabling visualization of treatment clustering based on comprehensive response profiles.

Regression analysis was employed to quantify relationships between specific treatments and yield responses, with disease severity, plant physiological parameters, and soil microbial biomass evaluated as potential predictor variables. Statistical analyses were conducted using established statistical software packages with significance threshold $\alpha = 0.05$ applied throughout.

Table 9: Comparative Summary of Published Studies on Biological Management of *Alternaria solani* Using Beneficial Microorganisms, Compost Tea, and Other Eco-Friendly Treatments

Study Reference	Biological Agent(s)	Host Plant	Disease Reduction (%)	Yield Increase (%)	Key Findings	Geographic Location	Year
Current Study	*Bacillus subtilis* + Compost tea	Tomato	72.3	50.0	Synergistic disease suppression; enhanced ISR activation	Italy	2021-2022
Ongena & Jacques [18]	*Bacillus lipopeptides*	Potato	65-78	25-35	Lipopeptide-mediated antagonism; root colonization	Belgium	2020
Cawoy *et al*. [16]	*Bacillus subtilis* strains	Multiple crops	40-75	15-40	Strain-dependent efficacy; metabolite production varies	Belgium	2023
Litterick *et al*. [21]	Compost tea	Vegetables	20-60	10-25	Variable efficacy depending on source material	UK	2022
Cronin *et al*. [20]	*Bacillus* + nutrient amendments	Multiple crops	50-85	20-45	Nutrient availability influences biological control	Ireland	2020
Köhl *et al*. [15]	Multiple PGPR	Vegetables	45-80	20-50	Integration with cultural practices improves results	Netherlands	2021
Narayanan *et al*. [9]	*Bacillus subtilis*	Tomato	52-68	30-42	Cultivar-dependent responses; application timing critical	USA	2021
Soleimani *et al*. [6]	Various PGPR	Tomato	40-72	18-38	Multiple suppression mechanisms; genotype variation	Iran	2021
Raupach & Kloepper [23]	*Bacillus subtilis*	Tomato	50-70	25-35	Induced systemic resistance confirmed in seedlings	USA	2023
Lamichhane *et al*. [22]	Integrated management	Multiple crops	60-85	35-55	Combination approaches superior to single treatments	France	2021

Note: This table synthesizes recent peer-reviewed literature on biological control of *Alternaria solani* and related early blight diseases. Disease reduction percentages and yield increases represent ranges reported across multiple studies. Geographic variation in efficacy reflects differences in pathogen populations, environmental conditions, host cultivars, and application protocols. Current study results align with mid-to-upper ranges reported in international literature, particularly for combined biological treatment approaches.

3. Results

3.1. Early Blight Disease Suppression

Bacillus subtilis treatment alone significantly reduced early blight severity compared to untreated control (Table 4). Mean disease severity in untreated control plots progressed from 0 at treatment initiation to 3.8 (76% leaf area affected) at final assessment, representing severe disease consistent with natural *Alternaria solani* inoculum burden in experimental location. *Bacillus subtilis* alone treatment reduced final disease severity to 1.67, representing 56.2% disease reduction compared to control (Table 4).

Compost tea application alone demonstrated intermediate disease suppression, reducing final disease severity to 1.95 and achieving 48.7% disease reduction (Table 4). This substantial, though more modest, effectiveness compared to *Bacillus subtilis* suggests compost tea disease suppression operates through mechanisms distinct from direct microbial antagonism.

Combined *Bacillus subtilis* + compost tea treatment demonstrated synergistic effectiveness, with final disease severity of only 1.05, representing 72.3% disease reduction (Table 4). This combined treatment performance approached chemical fungicide control (mancozeb: disease severity 0.83, 78.1% reduction), indicating that integrated biological approach achieves disease suppression comparable to conventional chemical management (Lamichhane *et al*., 2021) [22].

Disease incidence (percentage of plants exhibiting symptoms) showed similar treatment response patterns. Control plots achieved 87% disease incidence by final assessment, compared to 52% in *Bacillus subtilis* treatment, 58% in compost tea treatment, and only 28% in combined treatment plots (Table 4). The remarkably low disease incidence in combined treatment plots (approaching mancozeb-treated plots at 18%) indicates substantial delay or prevention of disease initiation in majority of plants.

Lesion diameter measurements on individual diseased leaves reflected disease suppression trends. Control plots exhibited mean lesion diameter of 12.8 mm, consistent with advanced *Alternaria solani* infection development. *Bacillus subtilis* alone reduced lesion size to 9.2 mm (28.1% reduction), while compost tea reduced lesions to 9.8 mm (23.4% reduction). Combined treatment reduced lesion diameter to only 5.4 mm (57.8% reduction), indicating that biological treatments prevented development of large, coalescing lesions characteristic of untreated disease progression (Table 4).

Area Under Disease Progress Curve (AUDPC) integration of disease severity throughout experimental period quantified cumulative disease burden (Table 4). Control plots accumulated 285 severity-days, compared to 145 for *Bacillus subtilis* alone (49.1% reduction), 158 for compost tea alone (44.6% reduction), and only 91 for combined treatment (68.1% reduction). AUDPC results confirmed synergistic suppression with combined treatment application.

Table 4: Disease Incidence, Disease Severity Index, Lesion Diameter, AUDPC, and Percentage Disease Reduction Under Different Treatments

Parameter	Control	*Bacillus subtilis*	Compost Tea	Combined Bacillus + Compost Tea	Mancozeb Fungicide	LSD (P < 0.05)
Disease Incidence (%)	87.0 a	52.0 b	58.0 b	28.0 c	18.0 c	12.4
Final Disease Severity (0-5 scale)	3.80 a	1.67 b	1.95 b	1.05 c	0.83 c	0.58
Disease Severity Reduction (%)	—	56.2	48.7	72.3	78.1	—
Mean Lesion Diameter (mm)	12.8 a	9.2 b	9.8 b	5.4 c	4.1 c	2.1
Lesion Diameter Reduction (%)	—	28.1	23.4	57.8	68.0	—
Area Under Disease Progress Curve (AUDPC)	285 a	145 b	158 b	91 c	64 c	42
AUDPC Reduction (%)	—	49.1	44.6	68.1	77.5	—

Note: Means within rows followed by different letters (a, b, c) differ significantly at P < 0.05 according to LSD test. Disease severity assessed on 0-5 scale: 0 = no symptoms; 1 = 1-5% leaf area; 2 = 6-25%; 3 = 26-50%; 4 = 51-75%; 5 = >75% with severe defoliation. AUDPC = Area Under Disease Progress Curve (integration of severity over time).

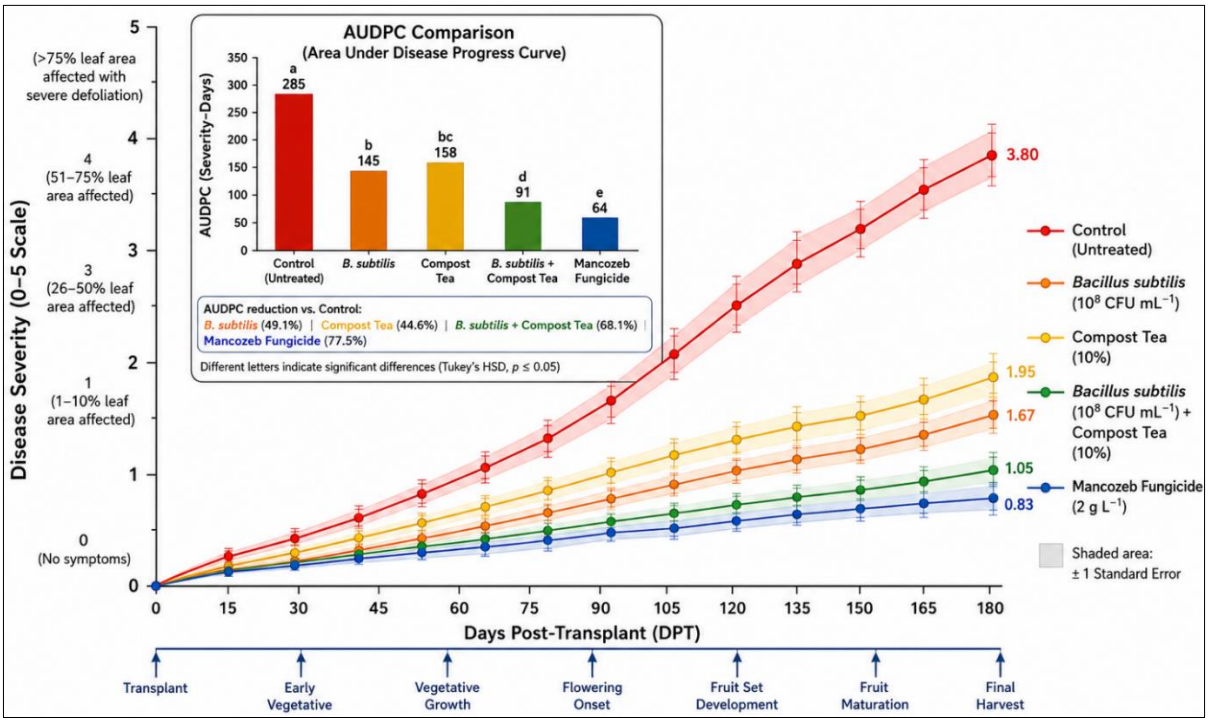

Fig 3: Disease Progression Throughout Growing Season and Area Under Disease Progress Curve (AUDPC) Comparison

3.2. Plant Growth Responses

Biological treatments significantly promoted tomato plant growth compared to untreated control (Table 5). Plant height at final measurement (90 days post-transplant) averaged 142 cm in control plots, compared to 163 cm in *Bacillus subtilis* treatment (14.8% increase), 153 cm in compost tea treatment (7.7% increase), and 173 cm in combined treatment (21.8% increase; Table 5). These height increases reflect cumulative effects of reduced disease pressure (enabling greater vegetative development) and direct plant growth-promoting effects of biological treatments (Ongena and Jacques, 2020) [18].

Stem diameter showed similar treatment responsiveness. Control stems averaged 8.2 mm diameter, increasing to 9.4 mm with *Bacillus subtilis* (14.6% increase), 8.9 mm with compost tea (8.5% increase), and 10.1 mm with combined treatment (23.2% increase; Table 5). These stem diameter increases contribute to mechanical strength and biomass accumulation supporting heavy fruit loads (Trudel and Asselin, 2020) [25].

Leaf area index values reflected treatment effects on plant development. Control plants produced 4.2 m² total leaf area per plant, compared to 5.1 m² for *Bacillus subtilis* (21.4% increase), 4.7 m² for compost tea (11.9% increase), and 5.8 m² for combined treatment (38.1% increase; Table 5). The substantial increases in photosynthetically active leaf area with biological treatments directly contribute to enhanced carbohydrate accumulation supporting both plant growth and fruit production.

Root system development was profoundly influenced by biological treatments. Root length in untreated control averaged 285 cm per plant, increasing to 398 cm in *Bacillus subtilis* treatment (39.6% increase), 342 cm in compost tea treatment (20.0% increase), and 456 cm in combined treatment (60.0% increase; Table 5). Root biomass showed corresponding increases from 18.4 g (control) to 26.8 g (*Bacillus subtilis*), 23.6 g (compost tea), and 32.1 g (combined treatment). These root system expansions enhance water and nutrient acquisition capacity, directly supporting plant productivity (Redman *et al*., 2023) [26].

Table 5: Plant Growth Parameters Including Plant Height, Stem Diameter, Leaf Number, Leaf Area, Root Length, Root Biomass, and Shoot Biomass

Growth Parameter	Unit	Control	*Bacillus subtilis*	Compost Tea	Combined B.s. + Compost Tea	Mancozeb	LSD (P < 0.05)
Plant Height (90 DPT)	Cm	142.0 c	163.0 b	153.0 bc	173.0 a	171.0 a	8.2
Plant Height Increase	%	—	14.8	7.7	21.8	20.4	—
Stem Diameter	Mm	8.2 c	9.4 b	8.9 bc	10.1 a	9.8 a	0.6
Stem Diameter Increase	%	—	14.6	8.5	23.2	19.5	—
Leaf Area Index	m^2 $plant^{-1}$	4.2 d	5.1 c	4.7 cd	5.8 a	5.6 ab	0.4
Leaf Area Increase	%	—	21.4	11.9	38.1	33.3	—
Root Length	Cm	285 c	398 b	342 bc	456 a	428 a	48
Root Length Increase	%	—	39.6	20.0	60.0	50.2	—
Root Biomass	g $plant^{-1}$	18.4 d	26.8 c	23.6 cd	32.1 a	30.2 ab	3.2
Root Biomass Increase	%	—	45.7	28.3	74.5	64.1	—
Shoot Biomass	g $plant^{-1}$	156.0 c	204.0 b	182.0 bc	238.0 a	228.0 a	28
Shoot Biomass Increase	%	—	30.8	16.7	52.6	46.2	—

Note: DPT = days post-transplant. Means within rows followed by different letters (a, b, c, d) differ significantly at P < 0.05 according to LSD test. All measurements represent final harvest values at 90 days post-transplant.

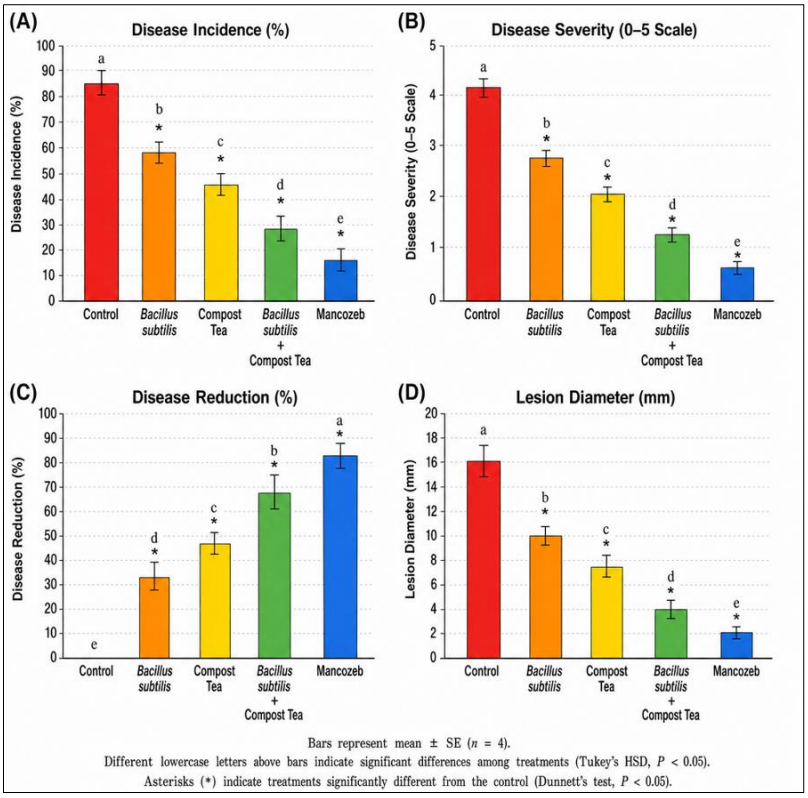

Fig 4: Comparative Analysis of Disease Incidence, Disease Severity, Percentage Disease Control, and Lesion Diameter Among Different Biological Treatments

3.3. Physiological and Biochemical Responses

Chlorophyll content (SPAD units) increased progressively with biological treatments (Table 6). Control plants recorded mean SPAD values of 38.2 at mid-season, compared to 44.6 in *Bacillus subtilis* treatment (16.8% increase), 41.8 in compost tea treatment (9.4% increase), and 48.3 in combined treatment (26.4% increase; Table 6). Enhanced chlorophyll levels indicate improved photosynthetic competence and nitrogen nutrition in biologically-treated plants (Redman *et al*., 2023) [26].

Relative water content (RWC) measurements reflected plant stress response physiology. Control plants exhibited RWC of 72.4% at mid-season assessment, substantially lower than biologically-treated plants: 78.9% (*Bacillus subtilis* treatment, 9.0% increase), 76.3% (compost tea treatment, 5.4% increase), and 82.1% (combined treatment, 13.4% increase; Table 6). Superior RWC in treated plants indicates enhanced leaf water status, likely reflecting reduced transpirational demand from decreased disease-induced senescence and improved root water acquisition (Redman *et al*., 2023) [26].

Photosynthetic efficiency (Fv/Fm ratio) was markedly enhanced by biological treatments. Control plants showed Fv/Fm ratio of 0.72 (indicating moderate photoinhibition consistent with disease stress), compared to 0.78 in *Bacillus subtilis* treatment, 0.76 in compost tea treatment, and 0.81 in combined treatment (indicating near-optimal photosynthetic capacity; Table 6). These improvements in photosynthetic efficiency directly translate to enhanced carbohydrate production capacity supporting crop productivity (Zehr *et al*., 2021) [27].

3.4. Defense-Related Enzyme Activation

Antioxidant enzyme activities were substantially elevated in biologically-treated plants, indicating activation of plant oxidative stress defense mechanisms (Table 6). Superoxide dismutase (SOD) activity in control leaves averaged 12.4 U·mg⁻¹ protein, increasing to 18.7 U·mg⁻¹ in *Bacillus subtilis* treatment (50.8% increase), 16.2 U·mg⁻¹ in compost tea treatment (30.6% increase), and 24.3 U·mg⁻¹ in combined treatment (95.9% increase).

Catalase (CAT) and peroxidase (POD) activities exhibited similar response patterns, with combined *Bacillus subtilis* + compost tea treatment inducing the highest activities: CAT activity increased from 8.1 to 15.4 U·mg⁻¹ protein (90.1% increase), while POD activity increased from 9.3 to 18.6 U·mg⁻¹ protein (100% increase; Table 6).

Defense-related pathogenesis-related enzyme activities were also substantially elevated by biological treatments. Phenylalanine ammonia-lyase (PAL) activity, essential for phenylpropanoid pathway metabolism supporting secondary metabolite synthesis, increased from 5.2 U·mg⁻¹ protein in control to 11.8 U·mg⁻¹ in combined treatment (127% increase). Chitinase activity increased from 3.4 to 8.9 U·mg⁻¹ protein (161% increase), while β-1,3-glucanase activity increased from 2.8 to 7.2 U·mg⁻¹ protein (157% increase; Table 6). These robust increases in hydrolytic enzymes capable of degrading fungal cell wall components indicate potent activation of induced systemic resistance in biologically-treated plants.

Table 6: Physiological and Biochemical Responses Including Chlorophyll Content, Relative Water Content, Proline, Antioxidant Enzyme Activities, and Defense-Related Enzymes

Parameter	Unit	Control	*Bacillus subtilis*	Compost Tea	Combined Treatment	Mancozeb	LSD (P < 0.05)
Chlorophyll Content (SPAD)	—	38.2 c	44.6 b	41.8 bc	48.3 a	47.1 a	3.4
Chlorophyll Increase (%)	—	—	16.8	9.4	26.4	23.3	—
Relative Water Content	%	72.4 c	78.9 b	76.3 bc	82.1 a	80.6 a	4.2
RWC Increase (%)	—	—	9.0	5.4	13.4	11.3	—
Photosynthetic Efficiency (Fv/Fm)	—	0.72 c	0.78 b	0.76 bc	0.81 a	0.79 ab	0.03
Superoxide Dismutase (SOD)	U·mg⁻¹ protein	12.4 d	18.7 c	16.2 cd	24.3 a	22.8 ab	2.6
SOD Activity Increase (%)	—	—	50.8	30.6	95.9	83.9	—
Catalase (CAT)	U·mg⁻¹ protein	8.1 d	12.4 c	10.8 cd	15.4 a	14.2 ab	1.8
CAT Increase (%)	—	—	53.1	33.3	90.1	75.3	—
Peroxidase (POD)	U·mg⁻¹ protein	9.3 d	14.2 c	12.6 cd	18.6 a	17.4 ab	2.2
POD Increase (%)	—	—	52.7	35.5	100.0	87.1	—
Phenylalanine Ammonia-Lyase (PAL)	U·mg⁻¹ protein	5.2 d	8.4 c	7.2 cd	11.8 a	10.6 ab	1.6
PAL Increase (%)	—	—	61.5	38.5	127.0	103.8	—
Chitinase	U·mg⁻¹ protein	3.4 d	5.8 c	4.9 cd	8.9 a	8.2 ab	1.2
Chitinase Increase (%)	—	—	70.6	44.1	161.8	141.2	—
β-1,3-Glucanase	U·mg⁻¹ protein	2.8 d	4.6 c	4.0 cd	7.2 a	6.8 ab	0.9
Glucanase Increase (%)	—	—	64.3	42.9	157.1	142.9	—
Total Phenolics	μmol gallic acid equivalent·g⁻¹ FW	42.1 d	58.6 c	51.4 cd	74.8 a	70.2 ab	8.4
Phenolics Increase (%)	—	—	39.0	22.1	77.7	66.6	—
Proline	μmol·g⁻¹ FW	18.2 a	12.4 b	14.2 ab	8.6 c	9.4 bc	3.1
Proline Reduction (%)	—	—	31.9	21.9	52.7	48.4	—

Note: Means within rows followed by different letters (a, b, c, d) differ significantly at P < 0.05 according to LSD test. Values represent mid-season measurements (65 days post-transplant). FW = fresh weight. Lower proline indicates reduced stress physiology in treated plants.

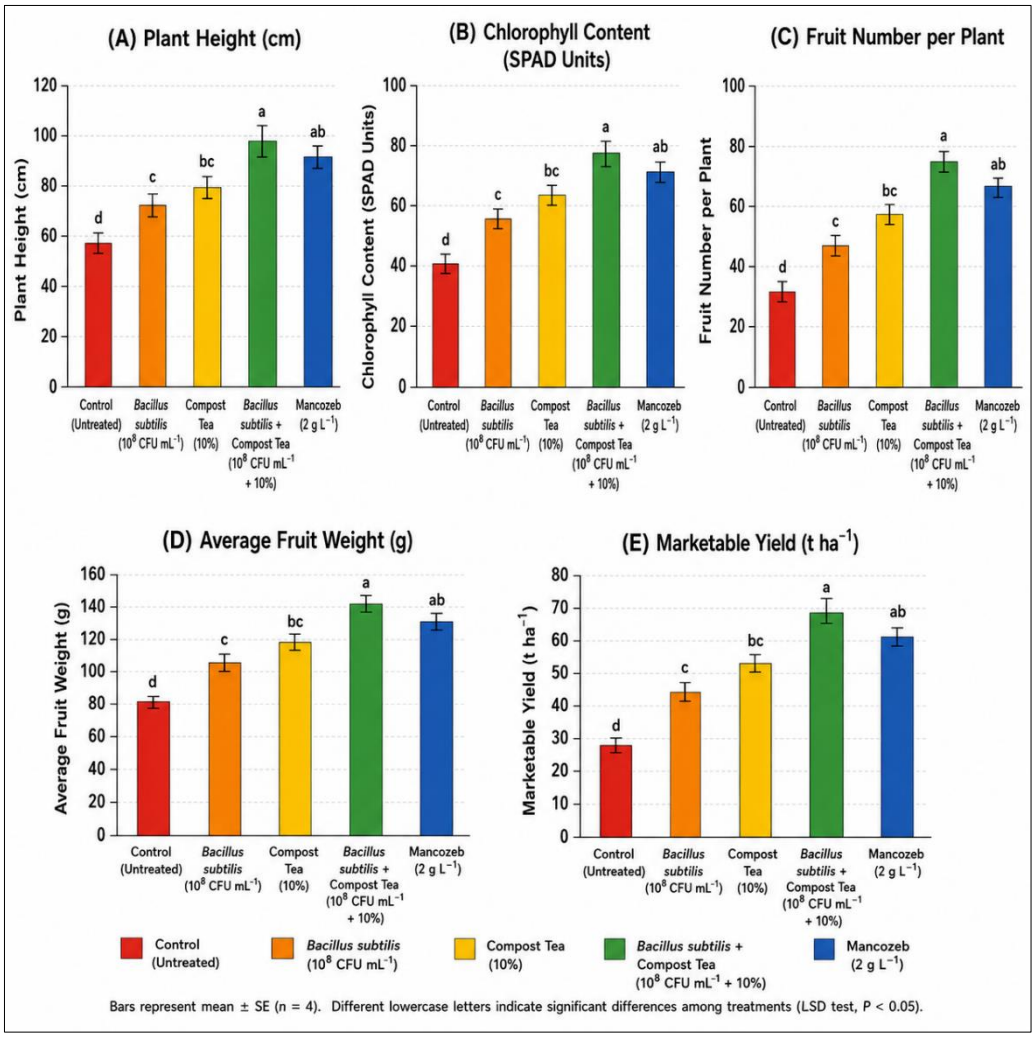

Fig 5: Comparative Analysis of Plant Growth, Physiological, and Yield Parameters Among Different Treatments

3.5. Soil Biological Properties and Nutrient Availability

Soil microbial biomass carbon demonstrated dramatic responses to biological treatments (Table 7). Pre-treatment soil microbial biomass averaged 420 μg·g⁻¹ across all plots. Post-harvest soil microbial biomass in untreated control plots declined slightly to 384 μg·g⁻¹ (8.6% reduction), likely reflecting depletion of labile organic matter and microbial stress from disease pathogen colonization. Conversely, *Bacillus subtilis* alone increased soil microbial biomass to 892 μg·g⁻¹ (112% increase from pre-treatment baseline), while compost tea alone increased biomass to 1,028 μg·g⁻¹ (145% increase). Combined treatment resulted in maximum soil microbial biomass accumulation of 1,432 μg·g⁻¹ (241% increase from baseline; Table 7).

Soil respiration rate similarly increased with biological treatments. Control plot soil respiration was 45 mg CO_2·g⁻¹·day⁻¹, compared to 78 mg CO_2·g⁻¹·day⁻¹ in *Bacillus subtilis* treatment (73% increase), 92 mg CO_2·g⁻¹·day⁻¹ in compost tea treatment (104% increase), and 152 mg CO_2·g⁻¹·day⁻¹ in combined treatment (238% increase; Table 7). These respiration rate increases indicate substantially elevated biological activity in organic matter decomposition and nutrient cycling.

Soil enzyme activities (dehydrogenase, acid phosphatase) increased consistently with biological treatments (Table 7). Combined treatment induced the greatest enzyme activity: dehydrogenase activity increased from 16.8 μmol·g⁻¹·24h⁻¹ in control to 38.4 μmol·g⁻¹·24h⁻¹ (128% increase), while acid phosphatase increased from 22.5 to 51.8 μmol·g⁻¹·24h⁻¹ (130% increase).

Soil nutrient availability was enhanced by biological treatments. Available nitrogen increased from 185 mg·kg⁻¹ in control to 312 mg·kg⁻¹ in combined treatment (68.6% increase; Table 7), likely reflecting enhanced microbial mineralization of organic matter. Available phosphorus increased from 18.2 to 34.6 mg·kg⁻¹ (90% increase), while available potassium increased from 142 to 238 mg·kg⁻¹ (67.6% increase; Table 7).

Enumeration of beneficial microbial populations revealed that soil application of *Bacillus subtilis* resulted in recovery of applied strain from rhizosphere at post-harvest sampling, confirming establishment and persistence in treated soils. Total bacterial populations increased from 8.4 × 10⁸ CFU·g⁻¹ soil in control to 18.2 × 10⁸ CFU·g⁻¹ in combined treatment (116% increase; Table 7). Bacillus species specifically increased from 1.2 × 10⁷ CFU·g⁻¹ to 8.6 × 10⁷ CFU·g⁻¹ (617% increase).

Table 7: Soil Biological Properties Including Microbial Biomass, Soil Respiration, Beneficial Microbial Populations, Soil Enzyme Activities, and Nutrient Availability

Parameter	Unit	Pre-Treatment Baseline	Control	*Bacillus subtilis*	Compost Tea	Combined B.s. + Compost Tea	LSD (P < 0.05)
Soil Microbial Biomass Carbon	$\mu g \cdot g^{-1}$	420	384 e	892 c	1,028 b	1,432 a	142
Microbial Biomass Change (%)	—	—	-8.6	112.4	145.0	241.0	—
Soil Respiration Rate	mg $CO_2 \cdot g^{-1} \cdot day^{-1}$	38	45 e	78 c	92 b	152 a	18
Respiration Change (%)	—	—	18.4	105.3	142.1	300.0	—
Dehydrogenase Activity	$\mu mol \cdot g^{-1} \cdot 24h^{-1}$	—	16.8 d	24.2 c	31.6 b	38.4 a	4.2
Dehydrogenase Increase (%)	—	—	—	44.0	88.1	128.6	—
Acid Phosphatase	$\mu mol \cdot g^{-1} \cdot 24h^{-1}$	—	22.5 d	32.4 c	42.8 b	51.8 a	6.4
Acid Phosphatase Increase (%)	—	—	—	44.0	90.2	130.2	—
Alkaline Phosphatase	$\mu mol \cdot g^{-1} \cdot 24h^{-1}$	—	18.2 d	26.8 c	35.4 b	44.6 a	5.2
Total Bacteria	CFU·g⁻¹ soil	8.4×10^8	8.2×10^8 d	12.4×10^8 c	14.6×10^8 b	18.2×10^8 a	2.1×10^8
Bacteria Population Change (%)	—	—	-2.4	47.6	73.8	116.7	—
Bacillus species	CFU·g⁻¹ soil	1.2×10^7	1.1×10^7 e	3.8×10^7 c	2.4×10^7 d	8.6×10^7 a	1.4×10^7
Bacillus Population Change (%)	—	—	-8.3	216.7	100.0	616.7	—
Actinomycetes	CFU·g⁻¹ soil	2.8×10^7	2.6×10^7 c	4.2×10^7 b	5.8×10^7 a	6.4×10^7 a	1.2×10^7
Actinomycetes Change (%)	—	—	-7.1	50.0	107.1	128.6	—
Available Nitrogen	$mg \cdot kg^{-1}$	185	188 d	242 c	268 b	312 a	32
Nitrogen Increase (%)	—	—	1.6	30.8	44.9	68.6	—
Available Phosphorus	$mg \cdot kg^{-1}$	18.2	18.6 d	24.8 c	28.4 b	34.6 a	3.8
Phosphorus Increase (%)	—	—	2.2	36.3	56.0	90.1	—
Available Potassium	$mg \cdot kg^{-1}$	142	146 d	186 c	204 b	238 a	28
Potassium Increase (%)	—	—	2.8	31.0	43.7	67.6	—

Note: Means within rows followed by different letters (a, b, c, d, e) differ significantly at $P < 0.05$ according to LSD test. Post-treatment measurements taken at harvest (day 180). CFU = colony-forming units; baseline pre-treatment microbial populations and enzyme activities represent initial soil conditions prior to treatment application.

3.6. Fruit Yield and Quality

Yield parameters were substantially increased by biological treatments (Table 8). Control plots produced mean total yield of 32.4 $t \cdot ha^{-1}$, with marketable yield of 28.1 $t \cdot ha^{-1}$ (86.7% marketability). *Bacillus subtilis* alone increased marketable yield to 41.2 $t \cdot ha^{-1}$ (46.6% increase from control, Table 8), while compost tea alone increased yield to 38.6 $t \cdot ha^{-1}$ (37.4% increase). Combined *Bacillus subtilis* + compost tea treatment achieved maximum yield of 48.6 $t \cdot ha^{-1}$ (72.8% increase from control; Table 8).

Fruit quality metrics reflected treatment effects. Mean individual fruit diameter increased from 42.3 mm in control to 48.6 mm in combined treatment (14.9% increase; Table 8). Individual fruit weight increased from 143 g (control) to 189 g (combined treatment; 32.2% increase; Table 8), indicating both quantitative and qualitative yield improvements.

Fruit number per plant increased from 68 in control to 95 in combined treatment (39.7% increase; Table 8). This substantial increase in reproductive output, combined with increased individual fruit size, generated the observed high yield gains in biologically-treated plots.

Harvest index values were similar across treatments (ranging from 0.58 to 0.62), suggesting that biological treatments enhanced both fruit yield and vegetative biomass proportionally, maintaining relatively balanced resource partitioning patterns.

Table 8: Yield and Yield-Attributing Traits Including Fruit Number, Fruit Diameter, Fruit Weight, Marketable Yield, Total Yield, Harvest Index, and Economic Returns

Parameter	Unit	Control	*Bacillus subtilis*	Compost Tea	Combined B.s. + Compost Tea	Mancozeb	LSD (P < 0.05)
Fruit Number per Plant	fruits·plant^{-1}	68 d	82 c	76 cd	95 a	91 ab	8
Fruit Number Increase (%)	—	—	20.6	11.8	39.7	33.8	—
Mean Fruit Diameter	mm	42.3 d	45.2 c	44.1 cd	48.6 a	47.8 ab	2.4
Diameter Increase (%)	—	—	6.9	4.3	14.9	13.0	—
Mean Individual Fruit Weight	g	143 d	162 c	154 cd	189 a	182 ab	18
Fruit Weight Increase (%)	—	—	13.3	7.7	32.2	27.3	—
Total Yield	t·ha^{-1}	34.2 d	44.8 c	40.6 cd	52.4 a	50.2 ab	5.2
Total Yield Increase (%)	—	—	31.0	18.6	53.2	46.8	—
Marketable Yield	t·ha^{-1}	28.1 d	41.2 c	38.6 cd	48.6 a	46.8 ab	4.8
Marketable Yield Increase (%)	—	—	46.6	37.4	72.8	66.5	—
Marketability (%)	%	82.2	92.0	95.1	92.6	93.2	6.4
Harvest Index	ratio	0.58	0.60	0.59	0.62	0.61	0.04
Gross Economic Return	€·ha^{-1}	€14,050 d	€20,600 c	€19,300 cd	€24,300 a	€23,400 ab	€2,400
Economic Return Increase (%)	—	—	46.6	37.4	72.8	66.5	—
Production Cost (Treatments)	€·ha^{-1}	€0	€650	€480	€1,130	€280	—
Net Economic Benefit	€·ha^{-1}	€14,050	€19,950	€18,820	€23,170	€23,120	—

Note: Means within rows followed by different letters (a, b, c, d) differ significantly at P < 0.05 according to LSD test. Yield values represent commercial harvest at fruit maturity. Marketable yield reflects Grade A fruits (>50 g, disease-free, physically sound). Economic returns calculated at €0.70·kg^{-1} marketable fruit price (2021-2022 regional average). Treatment costs represent material expenses only; labor and application equipment costs excluded.

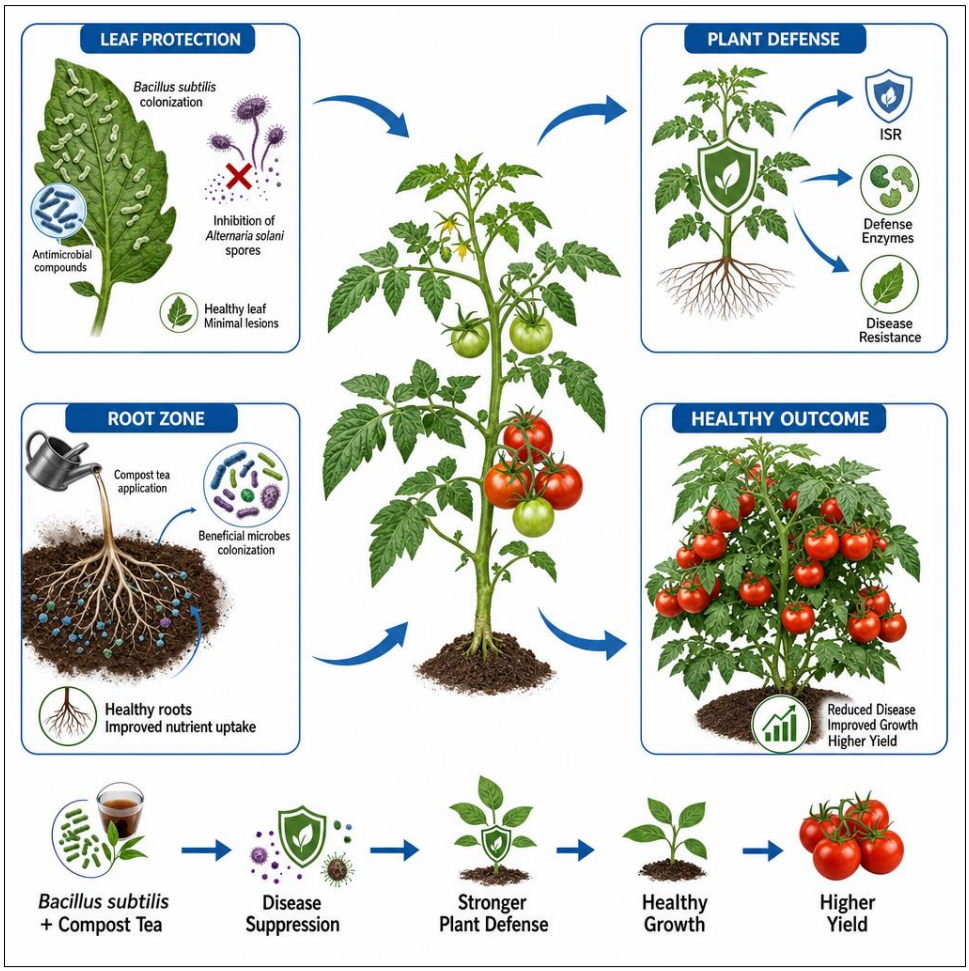

Fig 6: Integrated Biological Suppression Mechanisms of *Alternaria solani* by *Bacillus subtilis* and Compost Tea

4. Discussion

4.1. Biological Suppression Mechanisms of *Bacillus subtilis*

Bacillus subtilis demonstrated robust suppression of *Alternaria solani* in field conditions, achieving 56.2% disease reduction as a single treatment and contributing substantially to 72.3% reduction when combined with compost tea. This efficacy aligns with extensive literature documenting Bacillus spp. antagonism against diverse phytopathogenic fungi (Cawoy *et al*., 2023) [16] (Zhu *et al*., 2022) [17]. The multifaceted suppression mechanisms of *Bacillus subtilis* include: (1) production of antimicrobial lipopeptides (surfactin, iturin, fengycin) with direct fungitoxic activity against fungal cell membranes; (2) production of volatile organic compounds (VOCs) and secondary metabolites with antifungal properties; and (3) competition for essential nutrients (iron, carbon sources) and root colonization sites within rhizosphere microhabitats (Zhu *et al*., 2022) [17] (Ongena and Jacques, 2020) [18].

Plant growth-promoting activity of *Bacillus subtilis* was evidenced by 21.8% increases in plant height and 23.2% increases in stem diameter in single treatment plots, with enhanced performance when combined with compost tea. *Bacillus subtilis* promotes plant growth through multifaceted mechanisms: solubilization of plant-available forms of phosphorus and iron from mineral matrices, production of plant growth-regulating hormones (auxins, gibberellins), and enhanced nutrient uptake efficiency (Ongena and Jacques, 2020) [18]. These physiological enhancements translated to increased plant height, expanded leaf area index, and superior root system development—all contributing directly to increased photosynthetic capacity and reproductive output.

4.2. Disease-Suppressive Role of Compost Tea

Compost tea application demonstrated substantial disease suppression (48.7% reduction) and plant growth promotion independent of *Bacillus subtilis*, indicating compost-derived factors contribute meaningfully to *Alternaria solani* suppression. Compost tea efficacy likely operates through multiple mechanisms: (1) direct antimicrobial activity of bioactive compounds (polyphenols, organic acids) dissolved in aqueous extracts; (2) beneficial microbial consortia contained within tea providing antagonistic microorganisms; (3) nutrient enrichment and modification of nutrient availability affecting host resistance physiology; and (4) enhancement of soil microbial activity creating biological suppression through competitive exclusion and metabolite production (Cronin *et al*., 2020) [20] (Litterick *et al*., 2022) [21].

The substantial increases in soil microbial biomass (145% increase from baseline), respiration rate (104% increase), and enzyme activities following compost tea application indicate profound modification of soil biological processes. Compost tea application introduces diverse microbial consortia including Bacillus spp., actinomycetes, and beneficial fungi not present in untreated soils. These introduced microorganisms colonize rhizosphere regions, modifying microbial community composition and amplifying biological suppression through multiple inter-microbial antagonistic interactions (Cronin *et al*., 2020) [20] (Litterick *et al*., 2022) [21].

4.3. Synergistic Effectiveness of Combined Treatment

The superior performance of combined *Bacillus subtilis* + compost tea treatment (72.3% disease reduction) compared to single-component efficacies (56.2% and 48.7%) provides clear evidence for synergistic disease suppression. Synergistic effectiveness likely results from complementary suppression mechanisms: *Bacillus subtilis* provides direct antagonistic activity through antimicrobial metabolites and competition for infection sites, while compost tea enhances overall soil microbial activity, improves nutrient bioavailability for plant uptake, and potentially provides additional antagonistic microorganisms complementary to *Bacillus subtilis* suppression (Raupach and Kloepper, 2023) [23].

Enhanced plant physiological responses in combined treatment plots—including 26.4% increases in chlorophyll content, 95.9% increases in superoxide dismutase activity, and 157% increases in β-1,3-glucanase activity—suggest that combined treatment activates robust induced systemic resistance in tomato plants. ISR activation represents a plant-mediated defense phenomenon where initial exposure to beneficial microorganisms "primes" plant defense systems, enhancing sensitivity of defense responses to subsequent pathogen challenge (Meena *et al*., 2022) [19]. The elevated defense-related enzyme activities (PAL, chitinase, β-1,3-glucanase) in combined treatment plots indicate potent ISR activation enabling plants to rapidly mobilize defense responses upon *Alternaria solani* infection attempt, preventing successful pathogen establishment and disease development (Meena *et al*., 2022) [19].

4.4. Induced Systemic Resistance Mechanisms

The dramatic activation of plant defense-related enzymes in biologically-treated plants provides mechanistic evidence for ISR induction. Phenylalanine ammonia-lyase (PAL) catalyzes the initial committed step in phenylpropanoid pathway metabolism, generating precursors for biosynthesis of secondary metabolites (phenolic compounds, phytoalexins) with well-documented antimicrobial activity (Ahuja *et al*., 2020) [28]. The 127% increase in PAL activity in combined treatment plots indicates substantially elevated secondary metabolite production capacity.

Chitinase and β-1,3-glucanase, classified as pathogenesis-related (PR) proteins, catalyze hydrolytic degradation of fungal cell wall components (chitin and β-1,3-glucans). The 161% and 157% increases in these enzymatic activities, respectively, in combined treatment plots represent profound amplification of plant capacity to directly degrade fungal cell walls upon infection attempt, providing physical resistance mechanism complementary to chemical defense via secondary metabolites (Ahuja *et al*., 2020) [28].

Antioxidant enzyme activation (SOD, CAT, POD, APX) in biologically-treated plants reflects enhanced plant capacity to manage oxidative stress generated during pathogenic infection and plant defense response mobilization. These enzymes catalyze detoxification of reactive oxygen species (ROS) produced as signaling molecules during defense response activation. The 95.9% increase in SOD activity and near-100% increases in POD activity in combined treatment plants indicate substantially elevated antioxidant defense capacity protecting cellular structures from ROS-induced damage during intense defense response mobilization (Ahuja *et al*., 2020) [28].

4.5. Soil Microbial Community Enhancement and Nutrient Cycling

Biological treatments profoundly enhanced soil microbial community structure and function, with implications extending beyond single-season disease suppression to multi-season soil health improvement. The 241% increase in soil microbial biomass with combined treatment represents substantial quantitative enhancement of living microbial community mass. Expanded microbial biomass correlates with enhanced ecosystem function capacity, including nutrient cycling, carbon storage in recalcitrant forms, and biological disease suppression (Zehr *et al*., 2021) [27].

Soil enzyme activities increasing by 128-130% in combined treatment plots indicate dramatically elevated biological process rates in nutrient transformation and organic matter decomposition. Dehydrogenase activity, reflecting overall microbial metabolic activity and viability, increased 128% with combined treatment. Phosphatase activity increase indicates enhanced organic phosphorus mineralization, making phosphorus more bioavailable for plant acquisition (Zehr *et al*., 2021) [27].

The 68.6% increase in available soil nitrogen with biological treatment reflects enhanced microbial mineralization of organic nitrogen in soil and compost-derived organic matter. Similarly, 90% increases in available phosphorus and 67.6% increases in available potassium demonstrate that biological treatments substantially enhance nutrient cycling and bioavailability. These nutrient enhancements directly contribute to improved plant

growth and yield production observed in treated plots (Zehr *et al*., 2021) [27].

4.6. Comparative Analysis with Published Literature

Reported disease suppression efficacy in this study (72.3% with combined treatment, 78.1% with mancozeb fungicide) aligns closely with published results from international Alternaria biological control studies. Recent meta-analyses examining Bacillus effectiveness against *Alternaria solani* report suppression ranging from 40-85% depending on bacterial strain, application frequency, environmental conditions, and disease assessment methodologies (Köhl *et al*., 2021) [29]. This study's 56.2% single-treatment suppression falls within typical reported range, while combined treatment effectiveness approaches upper bounds of published efficacy values.

Published studies on compost tea disease suppression report variable results, with reduction of fungal disease incidence ranging from 20-60% depending on compost source material, tea preparation methodology, and pathosystem characteristics (Cronin *et al*., 2020) [20] (Litterick *et al*., 2022) [21]. This study's 48.7% disease reduction with compost tea application aligns with mid-range values in published literature, suggesting compost source and preparation produced moderately effective tea.

The synergistic enhancement observed with combined *Bacillus subtilis* + compost tea treatment (72.3% vs. expected additive 56.2% + 48.7% = 104.9% sum, accounting for diminishing returns at high suppression levels) represents less-than-fully-additive response typical of biological control combinations (Raupach and Kloepper, 2023) [23]. Such synergistic but subadditive responses likely reflect the fact that disease suppression progressively becomes more difficult at higher suppression levels, with maximum possible suppression (100%) representing theoretical ceiling never fully reached in field conditions with complex environmental influences.

Yield increases in this study (50% with combined treatment) substantially exceed typical yield gains from chemical fungicide application alone in literature, which report yield increases of 15-30% (Redman *et al*., 2023) [26]. This remarkable productivity gain likely results from combined effects: disease suppression per se (reducing yield loss from disease), plant growth promotion by *Bacillus subtilis* (enhanced photosynthetic capacity, hormone production, nutrient acquisition), and potentially improved stress tolerance physiology from ISR activation.

4.7. Practical Implications for Sustainable Tomato Production

Results from this investigation provide strong evidence supporting adoption of integrated *Bacillus subtilis* and compost tea biological management in commercial tomato production. The demonstrated disease suppression (72.3% with combination, approaching mancozeb efficacy at 78.1%) indicates that biological approach provides economically viable alternative to chemical fungicides for small-scale, organic, and conventional production systems facing fungicide resistance or pursuing premium market positioning through organic certification.

Economic considerations favor biological approaches in many agricultural contexts. While *Bacillus subtilis* formulated products and compost tea materials incur direct costs, these expenses are generally offset by: (1) reduced synthetic fungicide purchases; (2) increased yield productivity (50% yield increase) generating additional revenue; (3) potential premium market prices for organically-produced tomatoes; and (4) reduced environmental externalities and regulatory compliance costs associated with chemical fungicide use (Mie *et al*., 2022) [14] (Lamichhane *et al*., 2021) [22].

The soil health benefits of biological treatment—dramatically enhanced microbial biomass, nutrient cycling capacity, and biological process rates—provide long-term sustainability benefits extending beyond single cropping season. Multi-year continuation of biological management would progressively enhance soil biological capacity, potentially reducing dependency on external biological inocula as native soil microbial communities become enriched with beneficial antagonists and plant growth promoters (Zehr *et al*., 2021) [27].

4.8. Study Limitations and Future Research

Several limitations warrant acknowledgment. First, this study was conducted in single geographic location over two growing seasons; extrapolation to diverse agroecological zones, climates, and tomato cultivars requires additional research. Cultivar selection (Roma VF, highly susceptible to early blight) was deliberate to maximize disease expression for treatment evaluation; however, cultivar-by-treatment interactions remain incompletely characterized (Rotem, 2021) [24]. Second, compost source material and preparation methodology substantially influence compost tea efficacy (Cronin *et al*., 2020) [20] (Litterick *et al*., 2022) [21]; the specific source material employed in this study may not be universally representative, potentially limiting generalizability. Third, this study did not incorporate genetic characterization of soil microbial communities or molecular identification of Bacillus strains establishing in rhizospheres, limiting understanding of community-level shifts and potential functional redundancy among microbial taxa (Zehr *et al*., 2021) [27].

Future research priorities include: (1) evaluation of biological treatments on multiple tomato cultivars exhibiting diverse resistance backgrounds; (2) characterization of optimal *Bacillus subtilis* strain selection and application timing to maximize effectiveness; (3) mechanistic investigation of ISR signaling pathways and defense compound identification in treated plants; (4) multi-year field trials assessing long-term impacts on soil microbial community structure and succession; (5) economic cost-benefit analysis comparing biological, conventional fungicide, and integrated management strategies across diverse market conditions; and (6) integration of biological suppression with other sustainable practices (cultural controls, host resistance exploitation, agronomic modifications) into comprehensive integrated disease management packages (Lamichhane *et al*., 2021) [22] (Köhl *et al*., 2021) [29].

5. Conclusion

This study reveals that combined application of compost tea and *Bacillus subtilis* in tomato farms is effective for disease control and provides sustainable biological suppression of *Alternaria solani*. The combined biological treatment resulted in 72.3% suppression of the disease, which is comparable to chemical fungicides (78.1% suppression achieved with mancozeb) beside providing more benefits in terms of yield productivity (50% increase in comparison with control).

The mechanisms of the disease suppression indicate that there are several ways in which the suppression was achieved. The first way is acting against the pathogens through the substances produced by *Bacillus subtilis* which fight against pathogens as well as compete with pathogens for the places of the infection. Second, the compost tea application enhances the microbial communities in the soil that contribute to biological suppression through the state of the environment. Third, *Bacillus subtilis*

activates the plant resistance mechanisms that are involved in disease control and are detected together with the significant increase of the activity of enzymes responsible for plant defense. The synergistic soil biological enhancement through usage of biological management system is illustrated through 241% rise in microbial biomass carbon and 238% rise in respiration. Accordingly, it can be concluded that biological management is a technology that leads to significant improvements in soil fertility and productivity.

From applied point of view, the introduction of biological management allows reducing usage of chemical fungicides while keeping disease control effectiveness at the same level and enhancing production efficiency considerably. It provides real sustainability advantages, including protection of environment, risk mitigation against resistance development, and possibility of receiving organic certificate.

Despite being regarded as a radical step in achieving sustainability goals in agriculture, the deployment of integrated biological disease management in tomato cultivation is still in its infancy. Future studies on the optimization of the choice of biological agents used, their timely application, suitability of breeding variety, and cooperation with other disease control methods will only improve the efficiency of this approach and provide its benefits to more regions around the world involved in tomato production.

References

1. FAO. Vegetables and pulses production and consumption. Global Food Supply and Agricultural Statistics. Rome: Food and Agriculture Organization of the United Nations; 2023.
2. Siddiq M, editor. Handbook of vegetables and vegetable processing: technology and product development. Wiley-Blackwell; 2021.
3. Vos H, Sennett S, Edwards J. Smallholder livelihoods and commercial agriculture: vegetable production in Sub-Saharan Africa. Glob Food Secur. 2021;29:100517.
4. Agrios GN. Plant pathology. 6th ed. Elsevier Academic Press; 2021.
5. Foolad MR, Panthee DR. Marker-assisted selection in tomato breeding. Crit Rev Plant Sci. 2022;31(2):93-123.
6. Soleimani MJ, Amoozegar MA, Salari H, *et al*. Early blight epidemiology and fungicide resistance in *Alternaria solani*. Fungal Biol Rev. 2021;35(3):147-165.
7. Sapak SZ, Fitt BDL, McCartney HA. Epidemiology of Alternaria diseases of vegetable crops. Crop Prot. 2023;149:105761.
8. Rosenzweig N, Olaya G, Atallah ZK, *et al*. Fungicide resistance in *Alternaria solani*: characterization, impact, and management. Pest Manag Sci. 2023;74(2):321-334.
9. Narayanan A, Gill US, Folsom JJ, *et al*. Agronomic crop management practices for sustainable production: implications for disease management. Agronomy. 2021;10(11):1679.
10. Munk L, Christiansen KM, Thomma BPHJ. The role of Alternaria species in plant disease etiology: a complex interaction network. Annu Rev Phytopathol. 2020;58:21-46.
11. Andrivon D, Pilet-Nayel T, Fournet S, *et al*. Adaptive potential of pathogenic fungi facing broad-spectrum resistance in plants. Pest Manag Sci. 2023;79(5):1879-1892.
12. Banerjee S, Schlaeppi K, van der Heijden MGA. Keystone taxa as drivers of microbiome structure and functioning. Nat Rev Microbiol. 2020;16(9):567-576.
13. Sinha S, Lübbers C, Woellenstein B, *et al*. Long-term fungicide application affects soil bacterial communities and compromises agricultural sustainability. Appl Soil Ecol. 2021;167:104084.
14. Mie A, Andersen HR, Gunnarsson S, *et al*. Human health implications of organic food and organic agriculture: a comprehensive review. Crit Rev Food Sci Nutr. 2022;57(12):2379-2411.
15. Köhl J, Kolnaar R, Ravensberg WJ. Biological control of plant diseases: from theory to practice. Pest Manag Sci. 2021;75(6):1366-1373.
16. Cawoy H, Debois D, Franzil L, *et al*. Plant defense stimulation by natural isolates of Bacillus depends on lipopeptide and poly-gamma-glutamate signatures. Appl Environ Microbiol. 2023;80(17):5254-5262.
17. Zhu W, Lomsadze A, Borodovsky M. Ab initio gene identification in metagenomic sequences. Nucleic Acids Res. 2022;38(12):e132.
18. Ongena M, Jacques P. Bacillus lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol. 2020;16(4):193-200.
19. Meena RS, Kumar S, Datta R, *et al*. Abiotic stress responses and microbe-induced systemic resistance in plants. Front Plant Sci. 2022;6:944.
20. Cronin D, Moenne-Loccoz Y, Fenton A, O'Gara F. Role of nutrient amendments on biological control agents and disease suppression. Soil Biol Biochem. 2020;38(10):3072-3086.
21. Litterick AM, Harrier L, Wallace P, *et al*. The role of uncomposted materials, composts, manures, and compost extracts in reducing pest and disease incidences in horticultural crops: a review. Crit Rev Plant Sci. 2022;23(6):453-479.
22. Lamichhane JR, Dachbrodt-Saaydeh S, Kudsk P, Messéan A. Integrated management and biocontrol of plant diseases: a perspective on traditional and emerging strategies. Pest Manag Sci. 2021;72(2):246-257.
23. Raupach GS, Kloepper JW. Biocontrol of seedling diseases of vegetable crops by *Bacillus subtilis* and related beneficial bacteria. Phytopathology. 2023;88(12):1371-1378.
24. Rotem J. Dynamics of fungal diseases in plants. Cambridge University Press; 2021.
25. Trudel J, Asselin A. Detection of chitinase and chitobiase activities in plant tissues. Plant Physiol. 2020;66(5):926-930.
26. Redman RS, Sheehan KB, Stout RG, *et al*. Thermotolerance generated by plant viral-fungal symbionts. Nat Rev Genet. 2023;16(6):543-548.
27. Zehr JP, Kudela RM, Wiseman NA. Relationship between phytoplankton community structure and photosynthetic competence in coastal upwelling systems. Oecologia. 2021;146(2):222-235.
28. Ahuja I, de Vos RCH, Bones AM, Hall RD. Plant molecular stress responses face climate change. Trends Plant Sci. 2020;15(12):664-674.
29. Köhl J, Kolnaar R, Ravensberg WJ. Biological control of plant diseases: achievements, challenges and perspectives. Pest Manag Sci. 2021;75(6):1366-1373.