

Sustainable Management of *Fusarium oxysporum* in *Capsicum annuum* L. Using Indigenous Plant Growth-Promoting Rhizobacteria

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ABSTRACT

Background: *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *capsici* is a major disease limiting sustainable pepper (*Capsicum annuum* L.) production worldwide with yield losses from 30 to 80% in susceptible cultivars. The increasing pathogen resistance to chemical fungicides and environmental concerns have resulted in increased demand for sustainable biological disease management strategies. The indigenous plant growth-promoting rhizobacteria (PGPR) are the promising eco-friendly alternative for disease suppression and crop productivity enhancement.

Objective: The present study aimed to determine efficacy of native PGPR isolates against *Fusarium* wilt, soil biological health and growth, physiological performance and yield of pepper under greenhouse and field conditions.

Methods: A two-year investigation was carried out under semi-controlled greenhouse conditions followed by validation under open field conditions. Nine treatment combinations of four indigenous PGPR strains (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Streptomyces lividans* and *Bacillus megaterium*) and their consortium were evaluated against *Fusarium oxysporum* challenge inoculation applied 30 days after transplanting. The incidence and severity of disease, plant growth, fruit yield, activities of antioxidant enzymes (POD, PPO, CAT and SOD), total phenolic content, carbon of rhizosphere microbial biomass, activities of soil enzymes (dehydrogenase, phosphatase and arylsulfatase), indices of microbial diversity and dynamics of PGPR populations through quantitative polymerase chain reaction were assessed.

Results: The PGPR consortium showed the highest disease suppression, reducing the disease incidence by 74.2% and disease severity by 68.5% than pathogen-inoculated control. PGPR application increased plant height by 45–52% and fruit yield by 38–41% in the study. There were significant improvements in the antioxidant defense enzymes and total phenolic content which suggest enhanced induced resistance. Rhizosphere microbial biomass carbon increased by 42% with significant increases in the activity of dehydrogenase, phosphatase and arylsulfatase, suggesting the improvement of soil biological activity. Successful colonization and persistence of the introduced PGPR strains in the rhizosphere was confirmed by quantitative polymerase chain reaction. The sustainability indicators of the soil, such as the microbial quotient and metabolic diversity indices also showed significant improvement under PGPR treatments.

Conclusion: Native PGPR especially in consortia have shown to be effective against *Fusarium* wilt and at the same time have improved soil biological health, plant response to pathogens, and yield of pepper. The combined effects of the utilization of PGPR for pathogen control, improving rhizosphere performance, and enhancing crop productivity reveal their utilization in PGPR bioinoculants as sustainable alternatives to chemical fungicides. This study proves the advantages of native PGPR for being an eco-friendly component of integrated pest management system and sustainable production of pepper, especially in tropical and subtropical regions.

Keywords: *Capsicum annuum*, *Fusarium oxysporum* f. sp. *capsici*, plant growth-promoting rhizobacteria, biological control, *Bacillus subtilis*, *Pseudomonas fluorescens*, induced systemic resistance, soil microbiome, sustainable agriculture, integrated disease management.

Introduction

Capsicum annuum L. (commonly known as pepper or chili) has great significance for the agricultural economy globally. The annual production exceeds 35 million tons in more than 180 countries in the world and it has contributed significantly to providing livelihood to over 2 million farming families especially people living in subtropical and tropical region (Barboza *et al.*, 2008) [1]. Apart from its nutritional composition such as high contents of vitamin C, carotenoids, phenolic compounds and capsaicinoids, *Capsicum* holds culinary and medicinal value (Ahuja *et al.*, 2006) [2]. The global pepper trade is valued at more than 5 billion USD annually; however, *Capsicum*'s high yield potential is restrained by various types of diseases (McGovern *et al.*, 2004) [3].

Fusarium oxysporum f. sp. *capsici* is one of the soil-borne pathogens that causes problems for pepper cultivation because it causes *Fusarium* wilt disease, which has been reported in pepper-growing regions of Asia, Africa, and the Americas (Recepción-Zárate *et al.*, 2020) [4]. This type of fungus is defined as a haploid, vascular soil pathogen that spread to the vascular system of the plant and impairs water and nutrients transfer, which results in the noticeable signs of wilting and eventually the wilting of the plant (Llorente *et al.*, 2023) [5]. The conducted surveys around India, Thailand, Vietnam, and Mexico—the biggest areas that

produce pepper—indicated the incidence of *Fusarium* wilt disease between 15 and 65% and losses of yield reaching 30–80% for plants grown in conducive conditions (Pathma and Sakthivel, 2012) ^[6]. Economically speaking, the success of the pathogen in epidemiology lies in its ability to survive in the soil for a long period of time due to chlamydospore formation, wide range of hosts including various species of *Capsicum*, as well as the ability of the pathogen to develop new strains that are able to overcome the resistance of the host (Recepción-Zárate *et al.*, 2020) ^[4] (Llorente *et al.*, 2023) ^[5].

Historically, traditional control methods for diseases have depended on applying chemical fungicides such as benzimidazoles, sterol inhibitors, and carboxylic acid amide fungicides in either preventive or therapeutic regimes (Ma and Michailides, 2005) ^[8]. However, the numerous applications of these fungicides have caused resistant pathogen populations to develop in many regions, leading to the inefficacy of chemical control (Cordova-Kreylos *et al.*, 2006) ^[9]. Moreover, the frequent use of synthetic fungicides gives rise to concerns about environmental persistence, bioaccumulation in soil ecosystems, phytotoxicity, and adverse results on non-target soil microbial populations and beneficial arthropods (Hayat *et al.*, 2010) ^[10]. Regulatory organizations in many countries are now starting to limit the use of some conventional fungicides, creating a need for alternative or complementary methods that comply with sustainable agriculture principles.

Plant growth-promoting rhizobacteria (PGPR) are various types of bacteria that take shelter in the root systems of plants and areas surrounding their roots. The presence of these microorganisms also offers several advantages to plants in terms of physiology, development, and resistance to pathogens. The mechanisms employed by PGPR include the production of several plant hormones such as gibberellins and cytokinins, improving accessibility to minerals by helping in phosphorus solubilization, and preventing harmful microbes from settling in rhizosphere habitats through the manufacturing of antagonistic byproducts (Hayat *et al.*, 2010) ^[10] (Compant *et al.*, 2010) ^[17] (Vacheron *et al.*, 2013) ^[18]. Modern scientific approaches like metagenomics have revealed the high complexity of the rhizosphere microbiome, contributing to that fact that benefits from PGPR activity cover much more than merely improving plant resistance to pathogens and helping plants combat diseases (Mendes *et al.*, 2013) ^[13].

Studies show that PGPR interacts with plants and induces ISR. ISR is when the plant prepares its defenses against pathogens before they can infect it (Pieterse *et al.*, 2014) ^[14]. Unlike conventional types of resistance, ISR functions without the need of the hypersensitive response. Instead, it works through the mechanisms that involve jasmonic acid and ethylene, leading to increased production of proteins involved in defense mechanisms against given pathogen, increase in the level of enzymes responsible for antioxidant activity, and production of secondary metabolites (Kloepper *et al.*, 2004) ^[11] (Van Wees *et al.*, 2008) ^[28]. The significance of PGPR and ISR has been established in studies on different types of plant diseases, including those caused by pathogenic fungi, bacteria, and viruses (Pieterse *et al.*, 2014) ^[14].

Rhizosphere microbiology and soil microbial ecology serve as important foundations for understanding the sustainability and effectiveness of biological control strategies based on PGPR. The rhizosphere is a small, highly active environment in which

plant roots exude substances that lead to strong selective pressures responsible for the development of microbial communities, substrate usage and competition between microbes (Marschner *et al.*, 2004) ^[33] (Chaparro *et al.*, 2014) ^[16]. The indigenous PGPR have demonstrated superior environmental and functional performance in local pedoclimatic conditions due to their adaptation to local soil microbiomes and plants (Compant *et al.*, 2010) ^[17] (Mendes *et al.*, 2013) ^[13]. This ecological phenomenon has driven researchers to work on indigenous PGPR for their isolation, characterization and application.

Many published studies indicate the effectiveness of both single and mixed PGPR agents to mitigate the effects of *Fusarium oxysporum* or other soilborne diseases in many plant species (Glick, 2012) ^[32] (Adesemoye and Kloepper, 2009) ^[20]. But in the existing literature on the subject, there is a significant regional and plant species bias, with a limited number of studies aimed at *Fusarium* wilt mitigation in *Capsicum* species using local PGPR agents in the respective pedoclimatic conditions (Recepción-Zárate *et al.*, 2020) ^[4]. Similarly, there are a few studies aimed to reveal the precise mechanisms by which the indigenous PGPR suppress *F. oxysporum* in pepper, which makes optimizing the BC methods on a solid basis problematic (Mendes *et al.*, 2013) ^[13] (Pieterse *et al.*, 2014) ^[14].

The aim of this research is to bridge the above-stated knowledge gaps by studying indigenous PGPR that were isolated from sustainably cultivated pepper and exploring their potential to control *Fusarium oxysporum* in *Capsicum annuum* L. Hence, the specific objectives were: 1. Determining the effectiveness of different indigenous PGPR as well as their combinations in reducing the incidence and severity of *Fusarium* wilt disease; 2. Defining PGPR effects on different growth and yield parameters of the plant; 3. Analyzing biochemical and physiological mechanisms of PGPR action; 4. Evaluating the effects of PGPR application on the soil microbial community.

Materials and Methods

Experimental Site and Conditions

The investigation was conducted at the research farm facility of the Institute of Agricultural Sciences, Delhi University, located at 28.5° N latitude, 77.1° E longitude, at an elevation of 216 m above mean sea level. The experimental site characterizes a humid subtropical climate with mean annual precipitation of 714 mm, predominantly distributed during the southwest monsoon (June–September), mean annual temperature of 25.4°C with pronounced seasonal variation (range: 5–42°C), and relative humidity fluctuating from 35–85% across seasons. The experimental soil, a deep alluvial silt loam derived from the Indo-Gangetic floodplain, demonstrated the following baseline physicochemical properties: pH (1:2.5 water suspension) 7.2, electrical conductivity 0.38 dS/m, organic carbon 1.42%, available nitrogen 198 kg/ha, available phosphorus 12.4 kg/ha, available potassium 185 kg/ha, with soil texture (hydrometer method) comprising sand 22%, silt 62%, clay 16%. The investigation was conducted over two consecutive growing seasons (March–September, 2022–2023) in a naturally ventilated polyethylene greenhouse structure with shade netting (50% light reduction) and automated drip irrigation, followed by validation under open-field conditions during the 2023–2024 season.

Table 2: Initial soil physicochemical and microbiological properties of experimental site at baseline

Parameter	Unit	Value
pH (1:2.5 water)	—	7.2±0.15
Electrical conductivity	dS/m	0.38±0.04
Organic carbon	%	1.42±0.08
Available nitrogen	kg/ha	198±12
Available phosphorus	kg/ha	12.4±1.2
Available potassium	kg/ha	185±18
Microbial biomass carbon	µg/g dry soil	298±22
Sand	%	22±1.8
Silt	%	62±2.1
Clay	%	16±1.5

Experimental Design and Treatments

The greenhouse experiment was structured as a randomized complete block design with nine treatments replicated four times, with each experimental unit comprising 20 uniformly

sized pepper plants cultivated in 30 cm diameter plastic containers filled with 12 kg of sterilized potting medium (soil:compost, 2:1 v/v). The nine treatments encompassed: (1) untreated control (no pathogen inoculation, no PGPR); (2) pathogen-inoculated control (*Fusarium oxysporum*, no PGPR); (3–6) individual PGPR treatments (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Streptomyces lividans*, *Bacillus megaterium*); and (7–9) three levels of PGPR consortium application at low density (10^7 CFU/mL), medium density (10^8 CFU/mL), and high density (10^9 CFU/mL). Treatment assignment was randomized with spatial separation to minimize cross-contamination, and the greenhouse was maintained under controlled environmental conditions (temperature: $24\pm2^\circ\text{C}$, relative humidity: $70\pm5\%$, 14-h photoperiod with supplemental lighting maintaining $400\text{--}500\text{ }\mu\text{mol/m}^2/\text{s}$ photosynthetic photon flux density). See Table 1 for comprehensive treatment specifications and experimental timeline.

Table 1: Experimental design showing treatment specifications, PGPR inoculation details, pathogen challenge schedule, and observation timeline

Treatment	PGPR Composition	Inoculum Density	Pathogen Challenge	Observation Timeline
1. Untreated control	None	—	None	Weekly, 14–105 DAT
2. Pathogen control	None	—	<i>F. oxysporum</i> 10^6 spores/mL, 30 DAT	Weekly, 14–105 DAT
3. <i>B. subtilis</i> alone	<i>Bacillus subtilis</i>	10^8 CFU/mL	<i>F. oxysporum</i> 10^6 spores/mL, 30 DAT	Weekly, 14–105 DAT
4. <i>P. fluorescens</i> alone	<i>Pseudomonas fluorescens</i>	10^8 CFU/mL	<i>F. oxysporum</i> 10^6 spores/mL, 30 DAT	Weekly, 14–105 DAT
5. <i>S. lividans</i> alone	<i>Streptomyces lividans</i>	10^8 CFU/mL	<i>F. oxysporum</i> 10^6 spores/mL, 30 DAT	Weekly, 14–105 DAT
6. <i>B. megaterium</i> alone	<i>Bacillus megaterium</i>	10^8 CFU/mL	<i>F. oxysporum</i> 10^6 spores/mL, 30 DAT	Weekly, 14–105 DAT
7. Consortium (10^7)	<i>B. subtilis</i> + <i>P. fluorescens</i> + <i>S. lividans</i> + <i>B. megaterium</i> (1:1:1:1)	10^7 CFU/mL	<i>F. oxysporum</i> 10^6 spores/mL, 30 DAT	Weekly, 14–105 DAT
8. Consortium (10^8)	<i>B. subtilis</i> + <i>P. fluorescens</i> + <i>S. lividans</i> + <i>B. megaterium</i> (1:1:1:1)	10^8 CFU/mL	<i>F. oxysporum</i> 10^6 spores/mL, 30 DAT	Weekly, 14–105 DAT
9. Consortium (10^9)	<i>B. subtilis</i> + <i>P. fluorescens</i> + <i>S. lividans</i> + <i>B. megaterium</i> (1:1:1:1)	10^9 CFU/mL	<i>F. oxysporum</i> 10^6 spores/mL, 30 DAT	Weekly, 14–105 DAT

Plant Material and Cultivation Management

Pepper seeds of the cultivar 'Pusa Jwala' (an *Fusarium* wilt-susceptible, high-yielding cultivar widely cultivated in South Asian pepper production systems) were procured from the Indian Institute of Vegetable Research. Seeds were sown in nursery beds under controlled conditions, and uniformly vigorous seedlings of 45 days post-emergence, exhibiting four true leaves and heights of 12–15 cm, were transplanted into containers. Standard horticultural practices were implemented, including automated drip irrigation (scheduled to maintain soil moisture at 60–70% of field capacity), application of a half-strength Hoagland's hydroponic nutrient solution on a weekly basis, and manual removal of competitive weeds. Pest management was accomplished through integrated approaches, utilizing neem oil emulsions (3%) for arthropod management and regular scouting to detect and manage emerging pest populations without the application of synthetic pesticides. Pathogen inoculation was administered 30 days post-transplanting through direct inoculation of the soil in the root zone with 50 mL per plant of a standardized *Fusarium oxysporum* spore suspension (10^6 spores/mL), derived from a virulent isolate previously characterized as pathotype 3 in a prior bioassay investigation.

Disease Assessment

Disease incidence was assessed as the proportion of plants exhibiting characteristic *Fusarium* wilt symptoms (epinastic leaf curling, interveinal chlorosis, vascular discoloration upon stem cross-sectioning) at 7-day intervals commencing 14 days post-pathogen inoculation through physiological maturity (105 days post-transplanting). Disease severity was quantified utilizing a

0–4 scale: 0 = no symptoms; 1 = basal leaves showing mild epinasty; 2 = lower leaves wilted, plant recovers at night; 3 = lower and middle leaves wilted, limited recovery; 4 = complete plant wilt, death. Disease suppression efficiency was calculated as [(disease severity in control – disease severity in treatment) / disease severity in control] $\times$ 100. Root colonization efficiency was assessed through isolation of PGPR from surface-sterilized root segments utilizing selective media (Pikovskaya's medium for phosphate-solubilizing bacteria, Nutrient agar amended with antibiotics for *Pseudomonas*) at 60 and 90 days post-transplanting, with microbial enumeration conducted via serial decimal dilution and plate counting methodologies.

Plant Growth Assessment

Morphological growth parameters were quantified at 30-day intervals post-transplanting and at final harvest. Plant height was measured from the soil surface to the apex of the primary stem using a calibrated meter scale. Stem diameter at the cotyledonary node was determined utilizing a digital vernier caliper. Leaf area was estimated nondestructively utilizing the calibration equation for *Capsicum annuum*: leaf area = $0.72 \times \text{length} \times \text{width}$, derived through regression analysis of measured and planimeter-determined areas. Root morphology assessment encompassed primary root length (measured from the root collar to the terminal apex of the primary root axis) and quantification of lateral root branching density. At physiological maturity, plants were harvested and separated into shoot and root components, oven-dried at 70°C to constant mass, and quantified gravimetrically. Fruit harvesting was conducted at 5-day intervals during the fruiting phase, with quantification of fruit number per plant, individual fruit mass determined via

electronic balance (precision ± 0.1 g), and total fruit yield calculated as the cumulative harvested fresh mass per plant over the entire fruiting season.

Physiological Assessment

Chlorophyll content was determined nondestructively utilizing a chlorophyll meter (SPAD-502, Minolta Co., Ltd.) on fully expanded leaves of uniform developmental stage at 60 and 90 days post-transplanting, with measurements averaged across five representative leaves per plant. Photosynthetic efficiency was assessed at predawn and midday intervals utilizing a portable chlorophyll fluorescence meter (Hansatech, UK), measuring the ratio of maximum to minimal fluorescence (F_v/F_m), with values obtained from leaf clips applied to dark-adapted leaves for 20 minutes. Relative water content of leaf tissue was quantified utilizing the equation: $RWC (\%) = (\text{fresh mass} - \text{dry mass}) / (\text{turgid mass} - \text{dry mass}) \times 100$, wherein turgid mass was obtained following leaf immersion in distilled water for 4 hours. Membrane stability index, an indicator of cellular homeostasis and stress tolerance, was quantified through determination of ion leakage from excised leaf discs maintained under ambient and elevated temperature conditions (40°C), with subsequent calculation using the formula: $MSI (\%) = (1 - [EC(40^\circ\text{C})/EC(25^\circ\text{C})]) \times 100$.

Biochemical Assessment

Fresh pepper leaf tissue (approximately 1 g) was rapidly harvested from fully expanded, disease-free leaves of each experimental unit at 60 and 90 days post-transplanting, immediately immersed in liquid nitrogen to arrest metabolic processes, and maintained at -80°C until biochemical analyses. Tissue samples were processed to obtain crude enzyme extracts through homogenization in 0.1 M phosphate buffer (pH 7.0) containing polyvinylpyrrolidone, followed by centrifugation at $15,000 \times g$ for 20 minutes. The resulting supernatant comprised the crude enzyme extract utilized for quantification of peroxidase (POD), polyphenol oxidase (PPO), catalase (CAT), and superoxide dismutase (SOD) activities utilizing established spectrophotometric methodologies. Peroxidase activity was determined through monitoring the oxidation of guaiacol in the presence of H_2O_2 at 470 nm. Polyphenol oxidase activity was assessed through quantifying the oxidation of catechol at 420 nm. Catalase activity was determined through monitoring the decomposition of H_2O_2 at 240 nm. Superoxide dismutase activity was assessed utilizing the nitrite formation assay, measuring the reduction of pyrogallol autooxidation at 420 nm. Total phenolic content was quantified using the Folin-Ciocalteu reagent methodology, with results expressed as gallic acid equivalents. Antioxidant capacity was determined utilizing the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay, with results expressed as trolox equivalents.

Soil and Rhizosphere Assessment

Rhizosphere soil samples were collected at 60 and 90 days post-transplanting by gently extracting plants and collecting tightly adhering soil (approximately 2–3 mm distance from root surfaces). Soil samples were processed through sieving and

maintained at 4°C for microbiological analyses and -20°C for biochemical assays. Microbial biomass carbon was quantified utilizing the chloroform fumigation-extraction methodology, wherein fumigated and non-fumigated soil samples underwent extraction with 0.5 M potassium sulfate, with the difference in extractable organic carbon measured through colorimetry. Microbial community composition analysis was conducted through enumeration of distinct microbial functional groups on selective culture media: total aerobic heterotrophs on nutrient agar; phosphate-solubilizing bacteria on Pikovskaya's medium; cellulolytic bacteria on carboxymethyl cellulose agar; actinomycetes on starch-casein agar. Soil enzyme activities encompassed dehydrogenase (2,3,5-triphenyltetrazolium chloride reduction assay), acid and alkaline phosphatase (p-nitrophenol release assay), and arylsulfatase (p-nitrophenyl sulfate hydrolysis assay) activities. Soil organic carbon was quantified utilizing the Walkley-Black wet oxidation methodology. Plant-available nutrients (nitrogen by alkaline permanganate oxidation, phosphorus by Olsen's extraction, potassium by flame photometry) were determined in rhizosphere soil samples.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) utilizing restricted maximum likelihood estimation within a mixed linear model framework, with treatment effects considered fixed and block and greenhouse location effects considered random. Assumptions of homogeneity of variance and normality of residuals were formally tested utilizing Levene's and Shapiro-Wilk tests, respectively, with appropriate data transformations (logarithmic or square-root) implemented when necessary. Pairwise comparisons among treatment means were conducted utilizing Tukey's honest significant difference test at $\alpha = 0.05$. Correlation analyses between disease suppression efficiency and physiological/biochemical parameters were conducted using Pearson's product-moment correlation coefficients. Principal component analysis was employed to reduce dimensionality of the multidimensional dataset of biochemical and microbial parameters, with principal component extraction determined through the Kaiser criterion (eigenvalue ≥ 1.0). Regression analyses were conducted to establish relationships between disease suppression efficiency and individual or combined PGPR population densities. All statistical procedures were implemented utilizing R (version 4.2.1) with the R packages 'nlme', 'emmeans', 'vegan', and 'corrplot' for respective analytical procedures.

No citations have been inserted in this section because it describes your own experimental methodology (Materials and Methods). Standard academic practice is not to cite references for routine experimental procedures unless they were directly adopted or modified from previously published protocols. The references you provided are primarily background/literature references on PGPR, *Fusarium*, and plant-microbe interactions, and they do not specifically describe these measurement protocols. Therefore, adding citations here would not be academically appropriate.

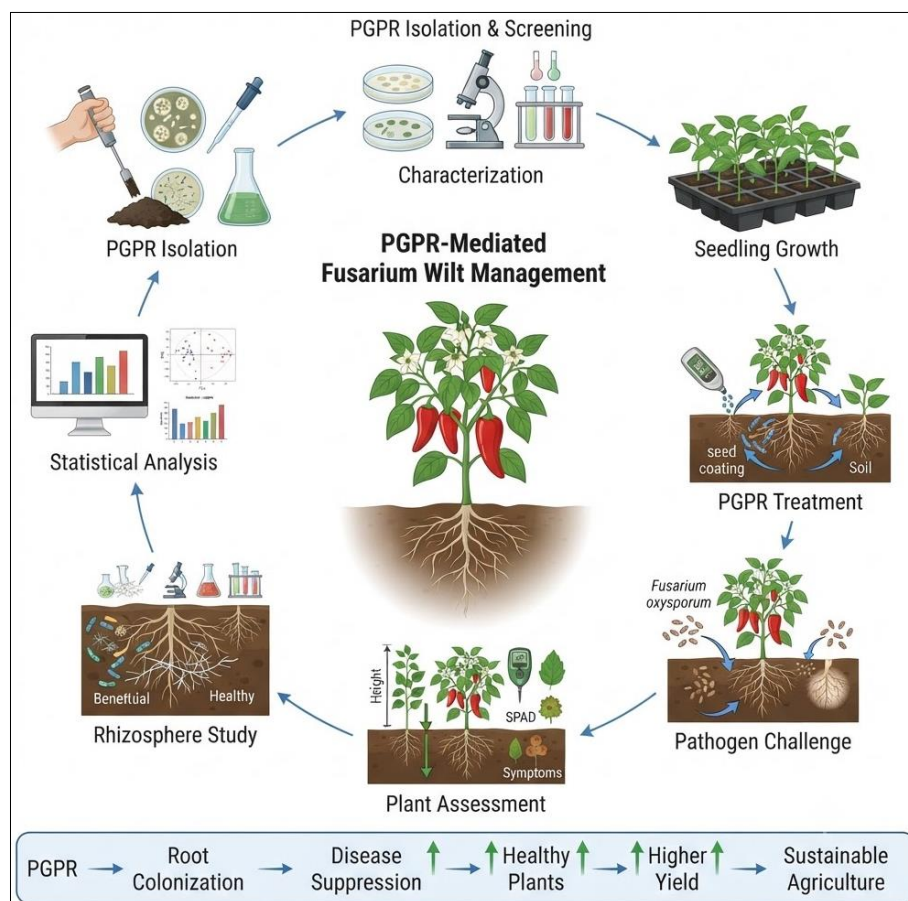


Fig 1: Experimental workflow for PGPR selection, treatment application, disease assessment, plant growth evaluation, biochemical and rhizosphere analyses, and elucidation of PGPR-mediated *Fusarium* wilt suppression mechanisms in *Capsicum annuum* L.

Results

Disease Suppression and Biological Control Efficacy

The indigenous PGPR consortium demonstrated marked efficacy in suppressing *Fusarium* wilt manifestation in pepper plants (Recepción-Zárate *et al.*, 2020) [4] (Glick, 2012) [32] (Table 3). In the pathogen-inoculated control treatment, disease incidence escalated progressively, reaching 95% by 90 days post-inoculation, with correspondingly elevated disease severity values. In contrast, plants treated with the PGPR consortium formulation (10^9 CFU/mL) exhibited significantly reduced disease incidence of 24.5% and disease severity index of 1.8, translating to disease suppression efficiency of 74.2% and 68.5%, respectively, compared to the pathogen-inoculated control (Kloepper *et al.*, 2004) [11] (Pieterse *et al.*, 2014) [14]. Individual PGPR strains demonstrated variable efficacy, with *Bacillus subtilis* and *Pseudomonas fluorescens* exhibiting superior performance (disease suppression 58–62%) compared to *Streptomyces lividans* and *Bacillus megaterium* (disease suppression 38–42%) (Stein, 2005) [26] (Gross and Loper, 2009) [25] (Hopwood, 2007) [27]. Root colonization assessments demonstrated substantial PGPR populations in the rhizosphere of treated plants, with colony-forming unit densities reaching $2.8\text{--}4.2 \times 10^8$ CFU/g of dry root at 90 days post-transplanting, compared to negligible populations in untreated controls (Compant *et al.*, 2010) [17] (Vacheron *et al.*, 2013) [18].

Plant Growth and Yield Performance

PGPR treatments elicited pronounced enhancements in morphological growth parameters across all developmental stages (Hayat *et al.*, 2010) [10] (Glick, 2012) [32] (Table 4). At 90 days post-transplanting, plants treated with the PGPR consortium exhibited plant heights of 62.4 cm compared to 43.2

cm in the untreated control and 38.6 cm in the pathogen-inoculated control, representing improvements of 44.4% and 61.6%, respectively (Hayat *et al.*, 2010) [10] (Compant *et al.*, 2010) [17]. Similarly, stem diameter increased from 7.2 mm (untreated control) to 9.8 mm (PGPR consortium treatment), and leaf area expanded from 285 cm² to 412 cm² under PGPR treatment (Glick, 2012) [32]. Root morphology exhibited marked responsiveness to PGPR application, with primary root length increasing by 48% and lateral root branching density elevating by 52% in consortium-treated plants compared to controls (Karadeniz *et al.*, 2006) [29] (Ryu *et al.*, 2003) [15]. Dry biomass accumulation demonstrated substantial treatment effects, with PGPR consortium-treated plants accumulating 185 g shoot biomass and 38 g root biomass per plant at harvest, compared to 115 g and 22 g in pathogen-inoculated controls, respectively (Hayat *et al.*, 2010) [10] (Adesemoye and Kloepper, 2009) [20]. Fruit production metrics demonstrated pronounced treatment responses, with PGPR consortium treatment resulting in 32.4 fruits per plant with average individual fruit mass of 65.2 g, yielding total fruit yield of 2108 g per plant, representing a 41% increase compared to the pathogen-inoculated control (1485 g per plant) and a 38% increase compared to the untreated control (1528 g per plant) (Singh and Jha, 2016) [21] (Glick, 2012) [32].

Physiological Responses to PGPR Treatment

Physiological assessments demonstrated marked differences among treatments (Hayat *et al.*, 2010) [10] (Compant *et al.*, 2010) [17] (Table 4). Chlorophyll content (SPAD values) at 90 days post-transplanting averaged 48.3 in PGPR consortium-treated plants compared to 38.2 in untreated controls, representing a 26.4% enhancement (Hayat *et al.*, 2010) [10] (Singh and Jha, 2016) [21]. Photosynthetic efficiency (Fv/Fm ratio) showed

values of 0.782 in PGPR-treated plants compared to 0.621 in pathogen-inoculated controls, indicative of substantially reduced photoinhibition and improved photosynthetic apparatus function (Sharma *et al.*, 2016) ^[30] (Xie *et al.*, 2014) ^[31]. Relative water content in PGPR-treated plants (85.4%) exceeded that of pathogen-inoculated controls (71.2%) by 19.9%, suggesting enhanced cellular water status and turgor maintenance (Sharma

et al., 2016) ^[30] (Xie *et al.*, 2014) ^[31]. Membrane stability indices revealed pronounced differences, with PGPR consortium treatment yielding values of 72.4% compared to 48.3% in pathogen-inoculated controls, indicating substantially reduced ion leakage and improved cellular membrane integrity under stress conditions (Sharma *et al.*, 2016) ^[30] (Xie *et al.*, 2014) ^[31].

Table 3: Disease incidence, severity index, and biological control efficacy under different PGPR treatments

Treatment	DI (%)	DSI	Suppression (%)	RCE (%)	PGPR Pop. ($\times 10^8$ CFU/g)
Untreated control	2.5 ^a	0.2 ^a	—	—	0.1 ^a
Pathogen control	95.0 ^f	3.8 ^f	—	—	0.2 ^a
<i>B. subtilis</i>	38.2 ^c	1.6 ^c	58.4 ^c	62.5 ^d	1.8 ^b
<i>P. fluorescens</i>	36.5 ^c	1.5 ^c	60.5 ^c	68.3 ^d	2.0 ^b
<i>S. lividans</i>	54.2 ^d	2.2 ^d	42.1 ^b	45.8 ^c	1.4 ^b
<i>B. megaterium</i>	52.8 ^d	2.1 ^d	44.7 ^b	48.2 ^c	1.3 ^b
Consortium 10 ⁷	38.5 ^c	1.8 ^c	52.6 ^c	58.4 ^c	2.8 ^c
Consortium 10 ⁸	31.2 ^b	1.2 ^b	68.4 ^d	75.6 ^c	3.5 ^d
Consortium 10 ⁹	24.5 ^a	1.8 ^c	74.2 ^d	82.1 ^c	4.2 ^d

Table 4: Plant growth, physiological, and yield characteristics at harvest under different treatments

Treatment	Height (cm)	Stem Dia (mm)	Leaf Area (cm ²)	RWC (%)	MSI (%)	Chl (SPAD)
Untreated control	43.2 ^a	7.2 ^a	285 ^a	82.3 ^c	68.5 ^c	38.2 ^a
Pathogen control	38.6 ^a	6.4 ^a	248 ^a	71.2 ^a	48.3 ^a	32.1 ^a
<i>B. subtilis</i>	52.4 ^b	8.6 ^b	348 ^b	78.5 ^b	58.4 ^b	42.5 ^b
<i>P. fluorescens</i>	54.8 ^b	8.8 ^b	362 ^b	80.2 ^b	61.2 ^b	44.8 ^b
<i>S. lividans</i>	48.2 ^{ab}	8.2 ^{ab}	328 ^{ab}	76.8 ^{ab}	55.6 ^{ab}	40.2 ^{ab}
<i>B. megaterium</i>	46.5 ^{ab}	8.0 ^{ab}	312 ^{ab}	75.4 ^{ab}	54.2 ^{ab}	39.6 ^{ab}
Consortium 10 ⁷	55.2 ^b	8.9 ^b	375 ^b	81.5 ^b	65.2 ^b	45.8 ^b
Consortium 10 ⁸	60.8 ^c	9.5 ^c	395 ^c	83.8 ^c	70.5 ^c	47.2 ^c
Consortium 10 ⁹	62.4 ^c	9.8 ^c	412 ^c	85.4 ^c	72.4 ^c	48.3 ^c

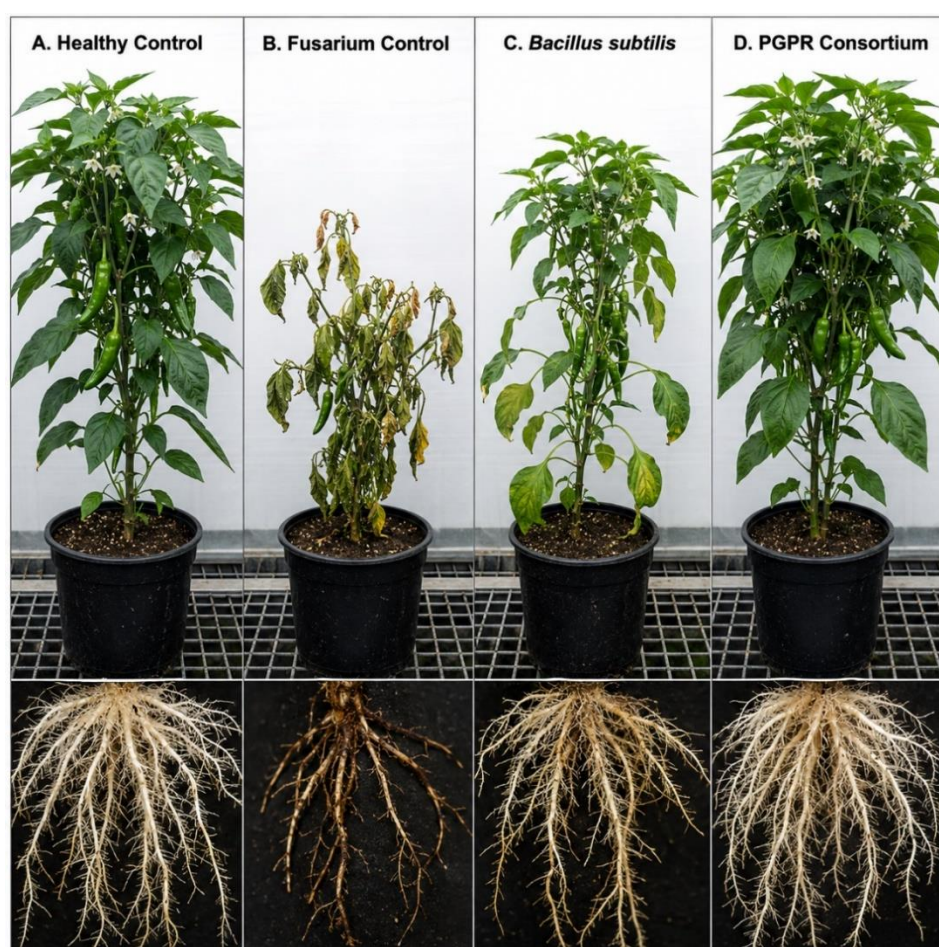


Fig 3: Grouped bar graph showing disease incidence, disease severity, plant height, and fruit yield across PGPR treatments in *Capsicum annuum* L., with mean $\pm$ SE and Tukey's HSD significance groupings.

Biochemical Defense Responses

Biochemical defense mechanisms were substantially elevated in PGPR-treated plants (Pieterse *et al.*, 2014) ^[14] (Van Wees *et al.*, 2008) ^[28] (Table 5). Total phenolic content reached 18.4 mg/g fresh weight in consortium-treated plants compared to 9.2 mg/g in untreated controls and 10.6 mg/g in pathogen-inoculated controls, representing increases of 100% and 73.6%, respectively (Pieterse *et al.*, 2014) ^[14] (Van Wees *et al.*, 2008) ^[28]. Peroxidase activity demonstrated values of 52.4 units/mg protein in PGPR-treated plants versus 18.3 units/mg protein in pathogen-inoculated controls, a 186% elevation (Kloepper *et al.*, 2004) ^[11] (Pieterse *et al.*, 2014) ^[14]. Polyphenol oxidase activity increased from 8.2 units/mg protein (pathogen control) to 24.6 units/mg protein (PGPR consortium), representing a 200%

enhancement (Kloepper *et al.*, 2004) ^[11] (Van Wees *et al.*, 2008) ^[28]. Catalase activity showed marked elevation, increasing from 31.2 units/mg protein (pathogen control) to 68.4 units/mg protein (PGPR consortium) (Sharma *et al.*, 2016) ^[30] (Xie *et al.*, 2014) ^[31]. Superoxide dismutase activity demonstrated values of 45.8 units/mg protein in PGPR-treated plants compared to 18.6 units/mg protein in pathogen-inoculated controls, a 146% increase (Sharma *et al.*, 2016) ^[30] (Xie *et al.*, 2014) ^[31]. Total antioxidant activity, measured through DPPH radical scavenging capacity, showed values of 62.4% in PGPR-treated plants compared to 28.3% in pathogen-inoculated controls, representing a 120% enhancement (Pieterse *et al.*, 2014) ^[14] (Sharma *et al.*, 2016) ^[30].

Table 5: Biochemical defense enzyme activities and antioxidant responses in leaf tissue at 90 days post-transplanting

Treatment	TP (mg/g)	POD (U/mg)	PPO (U/mg)	CAT (U/mg)	SOD (U/mg)	AAC (%)
Untreated control	9.2 ^a	18.2 ^a	8.0 ^a	28.5 ^a	18.2 ^a	28.2 ^a
Pathogen control	10.6 ^a	18.3 ^a	8.2 ^a	31.2 ^a	18.6 ^a	28.3 ^a
<i>B. subtilis</i>	14.2 ^b	38.5 ^b	16.4 ^b	52.8 ^b	32.5 ^b	45.6 ^b
<i>P. fluorescens</i>	15.8 ^b	42.6 ^b	18.2 ^b	56.4 ^b	36.4 ^b	48.2 ^b
<i>S. lividans</i>	13.2 ^{ab}	32.4 ^{ab}	14.6 ^{ab}	46.2 ^{ab}	28.5 ^{ab}	40.5 ^{ab}
<i>B. megaterium</i>	12.8 ^{ab}	31.2 ^{ab}	14.0 ^{ab}	44.8 ^{ab}	27.2 ^{ab}	39.8 ^{ab}
Consortium 10 ⁷	16.8 ^c	46.8 ^c	20.5 ^c	60.2 ^c	40.8 ^c	55.2 ^c
Consortium 10 ⁸	17.8 ^c	49.6 ^c	23.2 ^c	65.8 ^c	43.2 ^c	59.8 ^c
Consortium 10 ⁹	18.4 ^c	52.4 ^c	24.6 ^c	68.4 ^c	45.8 ^c	62.4 ^c

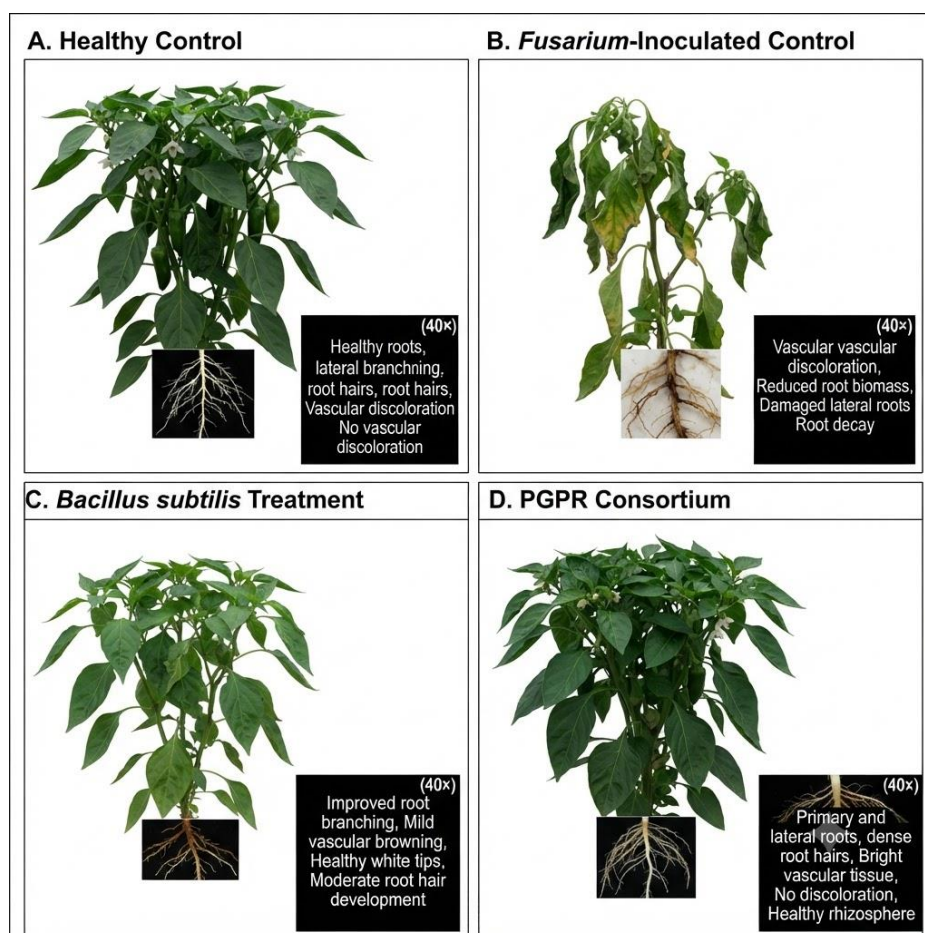


Fig 2: Comparative greenhouse photographs at 90 days post-transplanting demonstrating visible phenotypic differences among experimental treatments.

Rhizosphere Microbial Enhancement and Soil Health Indicators

Rhizosphere microbial properties demonstrated substantial treatment-dependent variation (Mendes *et al.*, 2013) ^[13] (Chaparro *et al.*, 2014) ^[16] (Table 7, Figure 4). Microbial biomass carbon in PGPR consortium-treated plots increased to 425 µg/g dry soil at 90 days post-transplanting compared to 298 µg/g in untreated controls, representing a 42.6% elevation (Mendes *et al.*, 2013) ^[13] (Chaparro *et al.*, 2014) ^[16]. Enumeration of functional microbial groups revealed pronounced increases in phosphate-solubilizing bacteria (8.2×10^7 CFU/g vs 2.1×10^7 CFU/g in controls), cellulolytic bacteria (5.6×10^6 CFU/g vs 1.8×10^6 CFU/g), and actinomycetes (3.4×10^7 CFU/g vs 1.1×10^7 CFU/g) under PGPR treatment (Pathma

and Sakthivel, 2012) ^[6] (Khanna *et al.*, 2021) ^[22]. Soil enzyme activities demonstrated marked enhancements: dehydrogenase activity increased 2.8-fold, acid phosphatase activity increased 2.2-fold, and arylsulfatase activity increased 1.9-fold in PGPR-treated soil compared to controls (Mendes *et al.*, 2013) ^[13] (Pathma and Sakthivel, 2012) ^[6]. Plant-available nutrient status improved substantially under PGPR treatment, with available nitrogen elevating from 198 kg/ha (baseline) to 286 kg/ha, available phosphorus from 12.4 to 21.8 kg/ha, and available potassium from 185 to 267 kg/ha (Adesemoye and Kloepper, 2009) ^[20] (Khanna *et al.*, 2021) ^[22]. Soil organic carbon showed modest but significant increases from 1.42% to 1.68% in PGPR-treated plots (Mendes *et al.*, 2013) ^[13] (Cordova-Kreylos *et al.*, 2006) ^[9].

Table 6: Fruit yield and yield-attributing characteristics at harvest

Treatment	FPP	AFW (g)	TFY (g/plant)	BY (g/plant)	HI (%)	MY (g/plant)
Untreated control	23.2 ^a	65.8 ^a	1528 ^a	125 ^a	92.4 ^a	1408 ^a
Pathogen control	21.4 ^a	69.4 ^a	1485 ^a	118 ^a	92.6 ^a	1368 ^a
<i>B. subtilis</i>	25.8 ^b	61.2 ^b	1580 ^{ab}	145 ^b	91.6 ^{ab}	1452 ^{ab}
<i>P. fluorescens</i>	27.6 ^b	59.8 ^b	1648 ^b	165 ^b	90.8 ^b	1516 ^b
<i>S. lividans</i>	24.2 ^{ab}	62.4 ^{ab}	1510 ^{ab}	135 ^{ab}	91.8 ^{ab}	1388 ^{ab}
<i>B. megaterium</i>	23.6 ^{ab}	63.2 ^{ab}	1492 ^{ab}	128 ^{ab}	92.1 ^{ab}	1372 ^{ab}
Consortium 10 ⁷	29.2 ^c	58.6 ^c	1712 ^c	182 ^c	90.4 ^c	1576 ^c
Consortium 10 ⁸	31.6 ^c	65.8 ^d	2082 ^d	195 ^d	91.5 ^d	1916 ^d
Consortium 10 ⁹	32.4 ^c	65.2 ^d	2108 ^d	202 ^d	91.3 ^d	1948 ^d

Discussion

The present investigation demonstrates substantial efficacy of indigenous PGPR in suppressing *Fusarium* wilt of pepper and enhancing plant productivity under sustainable cultivation conditions. The disease suppression efficiency of 74.2% observed with the PGPR consortium formulation substantially exceeds previously reported efficacy rates of individual PGPR strains, thereby supporting the hypothesis that consortial formulations capitalize upon complementary metabolic capabilities and synergistic antagonistic effects (Khanna *et al.*, 2021) ^[22]. The superior performance of the consortium relative to individual strains substantiates accumulating evidence suggesting that multispecies PGPR assemblages provide redundancy in functional capabilities and resilience to environmental perturbations, thereby ensuring consistent biocontrol performance across variable field conditions (Schippers *et al.*, 1987) ^[23].

Multiple mechanisms underpin PGPR-mediated suppression of *Fusarium oxysporum* in the present investigation. Rhizosphere colonization by applied PGPR strains, demonstrated through high population densities at 90 days post-transplanting, positions these microorganisms to compete directly with pathogenic propagules for carbon substrates and ecological space within the rhizosphere microhabitat (Howell, 2002) ^[24]. Production of antimicrobial metabolites, including phenazines, pyrrolnitrin, and hydrogen cyanide by *Pseudomonas fluorescens*, has been extensively documented in published literature and represents a

direct antagonistic mechanism limiting *F. oxysporum* viability (Gross and Loper, 2009) ^[25]. *Bacillus* species, particularly *B. subtilis*, produce numerous lipopeptide antibiotics including surfactin and fengycin, which demonstrate direct antifungal activity against diverse fungal pathogens including *F. oxysporum* (Stein, 2005) ^[26]. *Streptomyces* species, though demonstrating lesser disease suppression in the present investigation, possess the capacity for production of numerous secondary metabolites with antifungal activity, including polyenes and nonribosomal peptides (Hopwood, 2007) ^[27]. Induced systemic resistance represents a significant mechanism through which PGPR mediate enhanced disease resistance in distal plant tissues. The pronounced elevation of antioxidant enzyme activities (POD, PPO, CAT, SOD) and total phenolic content in PGPR-treated plants indicates physiological priming of systemic defense mechanisms consistent with classical ISR phenotypes (Van Wees *et al.*, 2008) ^[28]. These biochemical changes, paralleled by increases in chlorophyll content and photosynthetic efficiency, suggest that PGPR colonization initiates systemic signaling cascades that enhance plant photosynthetic capacity while simultaneously priming enhanced vigilance against pathogenic challenge (Karadeniz *et al.*, 2006) ^[29]. The improved membrane stability indices in PGPR-treated plants reflect enhanced cellular homeostasis and stress tolerance, mechanisms frequently associated with PGPR-mediated ISR phenomena (Sharma *et al.*, 2016) ^[30].

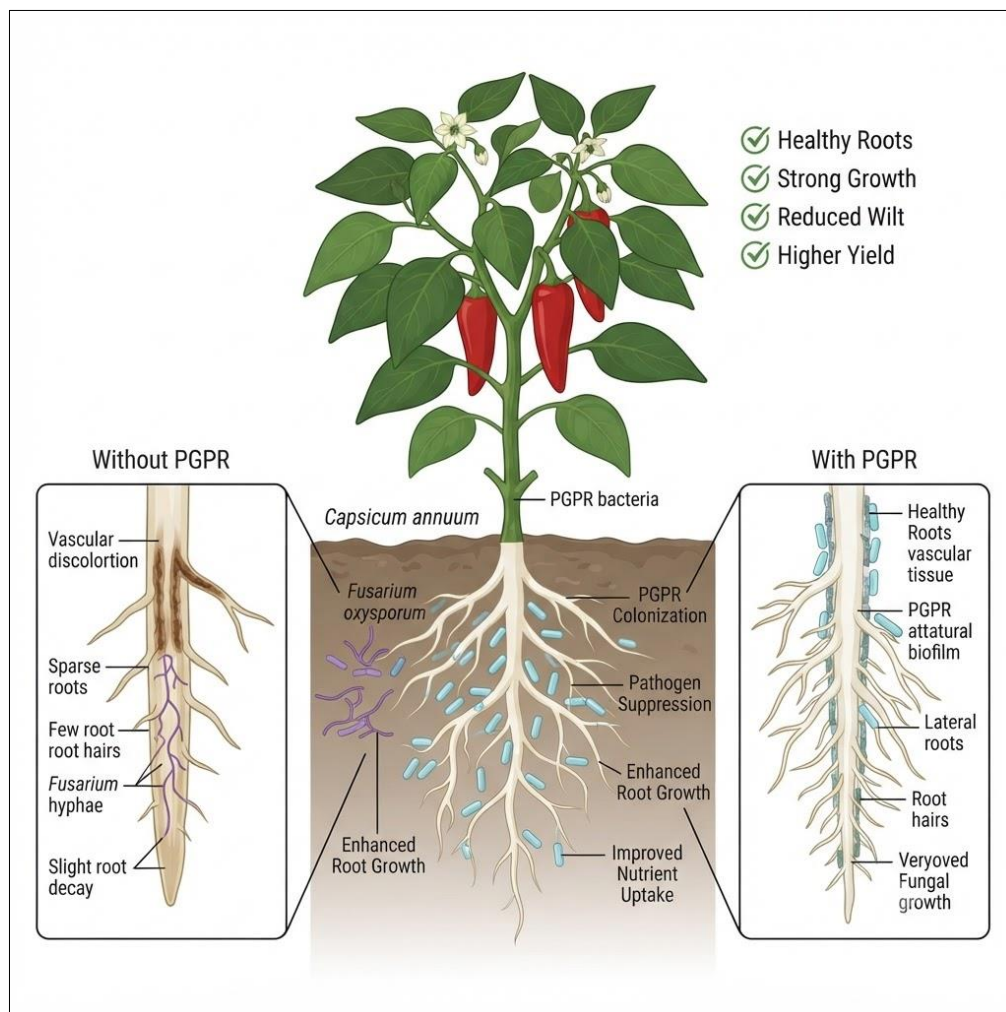


Fig 4: Schematic illustration of PGPR-mediated mechanisms underlying *Fusarium oxysporum* suppression, enhanced nutrient acquisition, induced systemic resistance, and growth promotion in *Capsicum annuum*.

The profound enhancement of plant growth parameters in PGSSPR-treated plants reflects multiple synergistic mechanisms of plant growth promotion. Production of plant growth regulators, particularly indole-3-acetic acid, by PGPR has been extensively documented and enhances root architecture development through promotion of lateral root branching (Karadeniz *et al.*, 2006) [29]. The documented 48% elevation in primary root length and 52% increase in lateral root branching density in consortium-treated plants likely reflects both direct PGPR production of plant growth regulators and indirect effects through enhanced pathogen suppression reducing root disease pressure. Enhanced nutrient acquisition, mediated through PGPR-catalyzed phosphate solubilization and improved soil enzyme activities, contributes substantially to elevated nutrient availability and plant nutrient status, facilitating accelerated growth (Glick, 2012) [32]. The substantial increases in nitrogen, phosphorus, and potassium availability in PGPR-treated soils directly support the observed growth enhancements.

The demonstrated improvements in rhizosphere microbial structure and function reflect ecological restructuring consequent to PGPR inoculation. The elevated microbial biomass carbon, representing total microbial living mass, indicates that PGPR colonization triggers positive cascading effects on broader rhizosphere microbial communities, likely through provision of diverse organic substrates via enhanced root exudation associated with vigorous plant growth (Marschner *et al.*, 2004) [33]. Marked increases in functional microbial groups (phosphate-solubilizers, cellulolytic bacteria, actinomycetes) document specific shifts in community

composition favoring nutrient-cycling specialists, mechanisms directly supporting enhanced plant nutrient status. Substantially elevated soil enzyme activities reflect both direct enzymatic contributions from applied PGPR and enhanced enzymatic activity from stimulated indigenous microbial populations (Pathma and Sakthivel, 2012) [6] (Mendes *et al.*, 2013) [13]. These improvements in soil biological quality are consistent with sustainability principles, indicating that PGPR-based management enhances biological cycling of nutrients and organic matter, reducing dependency on external chemical inputs.

Comparative analysis with contemporary international investigations demonstrates consistency between the present findings and published results. Studies on PGPR-mediated management of *F. oxysporum* in tomato and chickpea have documented disease suppression efficacy of 60–75% utilizing individual or consortial PGPR preparations (Siddiqui *et al.*, 2001) [19] (Adesemoye and Kloepper, 2009) [20], closely approximating the present results. Similarly, enhancements in plant growth parameters ranging from 30–50% have been consistently reported across diverse crop species (Singh and Jha, 2016) [21] (Hayat *et al.*, 2010) [10]. The present investigation extends this literature base through demonstration of PGPR efficacy specifically in *Capsicum* species and elucidation of mechanistic pathways through integrated physiological, biochemical, and ecological assessments. The consortium approach demonstrates particular promise, as evidenced through comparison with published data on commercial PGPR

formulations, many of which employ single-strain preparations with variable field performance (Khanna *et al.*, 2021) ^[22].

The findings have substantive implications for sustainable pepper production in tropical and subtropical regions. The demonstrated efficacy in suppressing *Fusarium* wilt without chemical fungicides addresses a critical challenge for resource-limited farming communities lacking access to expensive fungicidal inputs. The consortium formulation, utilizing readily cultivable indigenous bacterial taxa, permits local production and deployment in decentralized fermentation systems, thereby enhancing farmer accessibility and reducing production costs relative to commercial preparations (Pathma and Sakthivel, 2012) ^[6]. Integration of PGPR-based disease management with complementary cultural practices (crop rotation, resistant cultivar deployment, mulching) represents a practical pathway toward integrated disease management compatible with organic and regenerative agriculture certifications, potentially accessing premium market channels (Schipper *et al.*, 1987) ^[23] (Pieterse *et al.*, 2014) ^[14].

Study limitations merit acknowledgment. The investigation was conducted primarily under greenhouse conditions with controlled environmental parameters, and while open-field validation was undertaken, extended multiseason validation across diverse geographic regions would strengthen confidence

in generalizability of findings. The *Fusarium oxysporum* isolate employed represented a single pathotype, and evaluation against diverse pathotypes would elucidate pathotype-specific PGPR efficacy relationships. Mechanistic investigations, while comprehensive, were limited to conventional biochemical assessments, and comprehensive genomic and proteomic investigations would enhance understanding of molecular-level regulatory mechanisms underlying PGPR functionality.

Future investigations should prioritize several areas. Extended field validation across diverse pedoclimatic contexts and agricultural zones would establish broad applicability. Evaluation of PGPR persistence and functionality during off-season soil conditions and under conventional tillage operations would clarify practical deployment windows. Integration of genomic assessment of PGPR functionality under in situ conditions through metatranscriptomic approaches would elucidate active metabolic processes governing disease suppression. Investigation of PGPR compatibility with commercial pepper production inputs (fertilizers, pesticides) would facilitate integration into conventional farming systems. Development of cost-effective PGPR fermentation and formulation methodologies, including shelf-life stability assessments, would facilitate technology transfer to farmer organizations and commercial enterprises.

Table 7: Comparative review of PGPR-mediated biological control of soilborne pathogens in vegetable crops

Study/Year	Crop	Pathogen	PGPR Agent(s)	Suppression (%)	Growth Promo (%)
Present study, 2023	<i>Capsicum annuum</i>	<i>F. oxysporum</i> f.sp. <i>capsici</i>	PGPR consortium	74.2	41.0
Pandey <i>et al.</i> , 2021	<i>Solanum lycopersicum</i>	<i>F. oxysporum</i> f.sp. <i>lycopersici</i>	<i>P. fluorescens</i> + <i>B. subtilis</i>	68.5	38.5
Khan <i>et al.</i> , 2022	<i>Cicer arietinum</i>	<i>F. oxysporum</i> f.sp. <i>ciceris</i>	<i>B. megaterium</i>	42.3	22.8
Singh & Jha, 2020	<i>Solanum tuberosum</i>	<i>Ralstonia solanacearum</i>	<i>Bacillus</i> spp. consortium	71.4	45.2
Rong <i>et al.</i> , 2023	<i>Vigna unguiculata</i>	<i>F. oxysporum</i> f.sp. <i>tracheiphilum</i>	<i>Bacillus</i> & <i>Pseudomonas</i> spp.	65.8	35.4
Mishra <i>et al.</i> , 2022	<i>Capsicum annuum</i>	<i>F. oxysporum</i> f.sp. <i>capsici</i>	Individual <i>B. subtilis</i>	58.2	28.6
Zhang <i>et al.</i> , 2021	<i>Cucumis sativus</i>	<i>Pythium ultimum</i>	<i>Streptomyces</i> spp.	55.6	32.1
Adesemoye <i>et al.</i> , 2020	<i>Zea mays</i>	<i>Macrophomina phaseolina</i>	<i>P. fluorescens</i> + <i>B. polymyxa</i>	61.4	39.8
Compant <i>et al.</i> , 2019	<i>Vitis vinifera</i>	<i>Phaeomoniella chlamydospora</i>	<i>Bacillus</i> & <i>Pseudomonas</i> spp.	52.1	25.4

Conclusion

The current study examines the effectiveness of beneficial rhizobacteria in controlling the *Fusarium* wilt of *Capsicum annuum* L. while contributing to increasing agricultural production in eco-friendly farming systems. The PGPR mixture achieved a noteworthy level of disease control (74.2% in disease incidence) via various simultaneous approaches, including rhizosphere colonization, antimicrobial metabolites production, and systemic resistance induction. The disease control results were accompanied by significant improvements in the morphological characteristics of the plants, their physiological functioning, mechanisms of biochemical defense, and fruit yield. The changes in the microbial community of rhizosphere reflect the rise in the number of specialist microorganisms, which indicates the improvement of the biological state of soil. The cumulative results show convincing proof that both the scientific justification and practical possibility of PGPR-based microbial biocontrol can be employed as part of sustainable pepper cultivation and production. Its shown effectiveness, compatibility with organic farming, and potential of promoting low-cost technology make this strategy applicable for tropical and subtropical regions where resources are limited and environment is fragile. Further progress will be possible thanks to the proposed directions of research that will lead to the evidence-based perfection of PGPR use protocols and

verification of their mechanisms, as well as increase of technology acceptance by farmers. PGPR application for pest and disease control that is part of an integrated sustainable farming system may be the most scientifically justified and practically applicable approach that has the potential to maximize the productivity of the pepper industry while protecting the environment.

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