

Pseudomonas fluorescens Mediated Induced Systemic Resistance: A Sustainable Approach to Manage Tomato Early Blight Disease of Tomato

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Abstract

Tomato plants were evaluated for growth enhancement and disease resistance using the plant growth promoting rhizobacterium *Pseudomonas fluorescens* (*Pf*). The study focused on early blight caused by *Alternaria alternata* (*Aa*). *Pseudomonas fluorescens* (*Pf*) application significantly improved shoot and root development. When combined with pathogen inoculation, it minimized growth suppression compared to infected controls. All tested *Pf* isolates demonstrated salicylic acid (SA) production in King's B medium and triggered SA accumulation in tomato leaves within 24 hours of treatment. Endogenous SA levels increased progressively from day 1 to day 5, indicating activation of defence signalling. The treatment also enhanced pathogenesis-related (PR) proteins and defence enzymes, confirming induction of systemic resistance. Results suggest *Pf* can serve as an effective biocontrol agent against *A. alternata* in tomato.

Keywords: Tomato, *Alternaria alternata*, PGPR, *Pseudomonas fluorescens*, ISR, Salicylic Acid, Early Blight disease.

Introduction

Tomato (*Solanum lycopersicum* L.) is a globally significant vegetable crop valued for its nutritional content, including vitamins, antioxidants, and lycopene. Despite its economic importance, production is frequently limited by fungal diseases. Early blight, caused by *Alternaria alternata* and *Alternaria solani*, is among the most destructive. It produces characteristic necrotic lesions with concentric rings on leaves, stems, and fruits, leading to defoliation and yield losses of 30–80% under severe conditions. Conventional management relies on repeated fungicide use, which raises concerns regarding resistance development, environmental contamination, and residue levels. This has increased interest in eco-friendly alternatives. Plant growth promoting rhizobacteria, especially *Pseudomonas fluorescens*, are recognized for improving plant Vigor and triggering induced systemic resistance (ISR). ISR primes the plant's defence system through signalling molecules like salicylic acid and activation of PR proteins and defence enzymes such as peroxidase, polyphenol oxidase, PAL, and catalase. Integrating PGPR into disease management aligns with sustainable agriculture goals and reduces chemical dependency.

Multiple studies have documented the role of PGPR in crop protection and growth promotion. Early work established that *Alternaria* species cause major economic losses in tomato and emphasized the need for non-chemical control methods. Researchers have described PGPR mechanisms including nutrient solubilization, phytohormone synthesis, and pathogen antagonism. PGPR are reported to reduce fertilizer and pesticide requirements while maintaining productivity. Their metabolites such as IAA, ACC deaminase, and siderophores enhance stress tolerance. Beneficial microbes are also known to elevate defence enzyme activity and improve disease resistance through microbial elicitors. Greenhouse studies showed that *P. fluorescens* application increased SA accumulation and lowered early blight severity in tomato. Enhanced chitinase and α -1,3-glucanase activities have been linked to fungal resistance. Recent reviews highlight PGPR potential in sustainable agriculture for improving yield and minimizing ecological impact. Field trials further confirm that PGPR application improves growth, activates defence responses, and suppresses pathogen infection effectively.

Materials and Methods

The study was conducted under greenhouse conditions to assess *Pseudomonas fluorescens* (Pf) efficacy against *Alternaria alternata* in tomato. Surface-sterilized tomato seeds were treated with *P. fluorescens* (Pf) suspension before sowing. Four treatments were established with three replications in a Completely Randomized Design: RNC (Control), RNPf (*P. fluorescens*), RNAa (*A. alternata*), RNPfAa (*P. fluorescens* + *A. alternata*). Growth parameters including shoot length, root length, fresh weight, and dry weight were recorded periodically. Disease severity was evaluated using a standard Disease Severity Index based on foliar symptoms. Leaf SA content was quantified spectrophotometrically after extraction. Defence response was measured through PR proteins: PR-1, α -1,3-glucanase, and chitinase. Activities of peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and catalase (CAT) were determined using standard biochemical assays. Data were analysed by one-way ANOVA and means compared using Duncan's Multiple Range Test at p d" 0.05. Values are presented as mean $\pm$ standard error.

Results & Discussion

Growth Parameters

Pseudomonas fluorescens (Pf) inoculation significantly promoted tomato growth. RNPf -99 plants showed maximum shoot length, root length, fresh weight, and dry weight. Pathogen-only treatment RNAa-99 caused growth reduction due to disease stress. RNPfAa-99 improved growth over RNAa-99 but remained slightly below RNPf-99, indicating PGPR mitigated pathogen damage.

Table 1 : Showing Growth parameters of shoot length and root length

Treatment	Shoot length (cm)	Root length (cm)
RNC-99 (Control)	28.4 $\pm$ 1.2	12.6 $\pm$ 0.7
RNPf-99 (<i>P.fluorescens</i>)	38.7 $\pm$ 1.5	18.9 $\pm$ 0.8
RNAa-99 (<i>A. alternata</i>)	21.5 $\pm$ 1.1	8.7 $\pm$ 0.5
RNPfAa-99 (<i>P.fluorescens</i> + <i>A. alternata</i>)	34.2 $\pm$ 1.3	15.4 $\pm$ 0.6

Table 2 : Showing Growth parameters of Biomass (gm)

Treatment	Fresh Weight (gm)	Dry Weight (gm)
RNC-99 (Control)	24.5 $\pm$ 1.1	4.8 $\pm$ 0.2
RNPf-99 (<i>P.fluorescens</i>)	36.8 $\pm$ 1.5	7.9 $\pm$ 0.3
RNAa-99 (<i>A. alternata</i>)	17.5 $\pm$ 0.8	3.7 $\pm$ 0.5
RNPfAa-99 (<i>P.fluorescens</i> + <i>A. alternata</i>)	32.2 $\pm$ 1.3	6.4 $\pm$ 0.6

Disease Suppression

Pseudomonas fluorescens (Pf) treatment reduced early blight severity. RNPfAa-99 recorded lowest disease severity, confirming ISR-mediated protection. Disease reduction was 93.8% in RNPf-99 and 66.6% in RNPfAa-99.

Table 3 : Disease Severity in Tomato plants treated with *Pseudomonas fluorescens*

Treatment	Disease Severity (%)
RNC-99 (Control)	00.00
RNPf-99 (<i>P. fluorescens</i>)	4.5 ± 0.4
RNAa-99 (<i>A. alternata</i>)	72.8 ± 2.4
RNPfAa-99 (<i>P. fluorescens</i> + <i>A. alternata</i>)	24.3 ± 1.5

Salicylic Acid Production and Accumulation

All *Pseudomonas fluorescens* (Pf) isolates produced Salicylic Acid (SA) in Kings-B broth medium. Isolate PfA3-99 showed highest production at $27.8 \pm 1.3 \mu\text{g ml}^{-1}$. In plants, SA accumulated progressively: Day 1: 5.4 ± 0.3 , Day 2: 8.2 ± 0.4 , Day 3: 11.8 ± 0.6 , Day 4: 15.6 ± 0.8 , Day 5: $18.9 \pm 0.9 \mu\text{g g}^{-1}$ Fresh weight (FW). This indicates activation of SA-dependent defence signalling.

Table 4: Salicylic acid (SA) accumulation in plants ($\mu\text{g ml}^{-1}$).

	SA accumulation ($\mu\text{g ml}^{-1}$).
Day 1	6.4 ± 0.3
Day 2	8.4 ± 0.2
Day 3	12.4 ± 0.3
Day 4	15.4 ± 0.3
Day 5	18.9 ± 0.3
PfA3-99	18.4 ± 0.3

PR Proteins and Defence Enzymes

PR proteins and enzyme activities were highest in RNPfAa-99, showing synergistic effect of PGPR and pathogen challenge.

Table 5 : Showing PR-Protein α -1,3-Glucanase & Chitinase activity (Units mg^{-1})

Treatment	PR-1 Proteins	β -1,3-Glucanase	Chitinase
RNC-99 (Control)	0.42	0.38	0.35
RNPf-99 (<i>P. fluorescens</i>)	0.95	0.83	0.79
RNAa-99 (<i>A. alternata</i>)	0.65	0.68	0.57
RNPfAa-99 (<i>P. fluorescens</i> + <i>A. alternata</i>)	1.38	1.21	1.22

Activities of peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and catalase (CAT) were highest in *Pseudomonas fluorescens* treated and challenged with pathogen *A. alternata*.

Table 6: Showing enzymatic expression of Peroxidase (PO), Polyphenol Oxidase (PPO), Phenylalanine Ammonia Lyase (PAL) & Catalase (CAT) ($\text{U min}^{-1} \text{g}^{-1}$):

Treatment	PO	PPO	PAL	CAT
RNC-99 (Control)	12.60	8.40	10.70	6.80
RNPf-99 (<i>P. fluorescens</i>)	22.70	16.90	19.50	12.60
RNAa-99 (<i>A. alternata</i>)	16.4	12.40	14.80	09.70
RNPfAa-99 (<i>P. fluorescens</i> + <i>A. alternata</i>)	29.60	22.40	28.40	17.90

Correlation Analysis

Strong positive correlations were observed: SA vs PR proteins $r = 0.91^*$, SA vs Disease Reduction $r = 0.88^*$, PR proteins vs Disease Reduction $r = 0.94^*$. This confirms that SA-mediated defence activation directly contributes to disease suppression.

Pseudomonas fluorescens enhanced tomato growth and triggered ISR against *A. alternata*. Improved shoot length, root length and biomass in RNPf-99 and RNPfAa-99 support earlier reports on PGPR-mediated nutrient uptake and hormone regulation. Reduced disease severity in PGPR treatments indicates both direct antagonism and host defence activation. Elevated SA levels post-inoculation align with studies showing SA as a key signal in ISR. Increased PR proteins and defence enzymes in RNPfAa-99 suggest primed plants respond more strongly to infection. Correlation data validate that SA accumulation, PR protein expression, and enzyme activity collectively reduce early blight. Thus, *P. fluorescens* (PfA3-99) offers a viable, sustainable biocontrol strategy for tomato, decreasing reliance on chemical fungicide.

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