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Research Article

Evaluating the biocontrol efficacy of selected plant growth promoting rhizobacteria against tomato bacterial wilt caused by *Ralstonia solanacearum*

Hassam Qayyum[1], Basharat Mahmood[1], Shameen Arif[2]*, Mujeeb ur Rehman[1], Kinza Tasneem[1], Bilal Abdullah[3], Ejaz Hussain[4], Abdul Wahab[1], Adnan[1], Wajahat Ullah[5]

[1] *Département of Plant Pathology, University of Poonch Rawalakot AJK, Pakistan.*
[2] *Department of Plant Pathology, Yangzhou University, China.*
[3] *Département of Entomology, University of Poonch Rawalakot AJK, Pakistan.*
[4] *Department of Horticulture, University of Poonch Rawalakot AJK, Pakistan.*
[5] *Department of Agronomy, University of Poonch Rawalakot AJK, Pakistan.*

*Correspondence: shamiarif346@gmail.com

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Abstract

Tomato bacterial wilt, caused by the pathogen *Ralstonia solanacearum*, poses a significant threat to tomato production worldwide. The management of this devastating disease often relies on chemical pesticides, which can have adverse environmental and health impacts. In recent years, there has been growing interest in the use of plant growth promoting rhizobacteria (PGPR) as eco-friendly alternatives for disease control. This study aimed to evaluate the biocontrol efficacy of selected PGPR strains against tomato bacterial wilt. A total of ten PGPR strains were purified from soil samples and were assessed for their potential to promote tomato growth through various plant growth-promoting traits, such as production of indole-3-acetic acid (IAA), phosphate solubilization, and siderophore production. All the ten bacterial strains were screened for their ability to suppress the growth of *R. solanacearum in-vitro* using dual-culture assays. In greenhouse experiments, selected PGPR strains were applied as seed treatments and soil drenches to tomato plants infected with *R. solanacearum*. Disease incidence and biocontrol efficacy by PGPR were monitored throughout the study. Results revealed that several PGPR strains exhibited strong antagonistic activity against *R. solanacearum in-vitro* dual culture technique ranging from 0-34 mm zone of inhibition. The greenhouse trials demonstrated that the application of selected three PGPR strains alone and in consortium significantly reduced the disease incidence up to 5% as compared with control 71% of tomato bacterial wilt and have up to 64% biocontrol efficacy to inhibit *R. solanacearum* under greenhouse conditions. Overall, this study highlights the promising biocontrol potential of selected PGPR strains against tomato bacterial wilt caused by *R. solanacearum*. These findings suggest that the integration of PGPR-based biocontrol strategies into tomato production practices has the potential to reduce the reliance on chemical pesticides, promote sustainable agriculture, and minimizing the environmental impact of disease management.

Keywords: Biocontrol; Bacterial wilt; Greenhouse; PGPRs; Sustainable agriculture.

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Introduction

Tomatoes (*Solanum lycopersicum*) are one of the most economically important and widely cultivated vegetable crops worldwide, contributing significantly to global food security and agricultural economies. However, the production of this staple crop is severely threatened by various pathogens, with tomato bacterial wilt, caused by the soilborne bacterium *Ralstonia solanacearum*, being a particularly devastating and challenging disease. *R. solanacearum*, also known as the "southern bacterial wilt," is responsible for substantial yield losses, rendering affected fields unproductive and causing economic hardship for growers (Morais *et al.*, 2019). The bacterium's ability to persist in soil for extended periods and its broad host range further compound the difficulties associated with its control (Nion and Toyota, 2015).

Traditional management strategies for tomato bacterial wilt typically rely on chemical pesticides, such as copper-based compounds and fumigants, which can have detrimental effects on the environment and human health (Palilo, 2019). Moreover, the continuous use of these chemicals has led to the emergence of resistant strains of *R. solanacearum*, making disease management even more challenging (Mekonnen *et al.*, 2022a). As a result, there is a growing urgency to develop sustainable and eco-friendly alternatives for the control of tomato bacterial wilt.

One promising approach involves harnessing the beneficial interactions between plants and certain soil-dwelling microorganisms, particularly plant growth promoting rhizobacteria (PGPR). PGPR are a diverse group of bacteria that colonize the rhizosphere, the region of soil surrounding plant roots, and exert beneficial effects on plant growth and health through various mechanisms (Meng, 2013; Berg, 2009). Many PGPR strains are known for their ability to suppress soilborne pathogens by directly inhibiting their growth or inducing systemic resistance in plants (Najeeb *et al.*, 2019; Bibi *et al.*, 2017). Additionally, PGPR can enhance nutrient uptake, improve soil structure, and mitigate various abiotic stresses, making them valuable allies in sustainable agriculture (Aysan *et al.*, 2003; Bashir *et al.*, 2020).

In recent years, the biocontrol potential of PGPR against tomato bacterial wilt has gained attention from researchers and agricultural practitioners. These bacteria have shown promise in suppressing the growth and spread of *R. solanacearum* while simultaneously promoting tomato growth (Yendyo *et al.*, 2017; Doolotkeldieva and Bobusheva, 2016). However, the efficacy of PGPR strains can vary widely depending on factors such as bacterial species, strain specificity, and environmental conditions, necessitating a systematic evaluation of their biocontrol potential.

This manuscript presents the results of a study aimed at evaluating the biocontrol efficacy of selected PGPR strains against tomato bacterial wilt caused by *R. solanacearum*. Through a series of *in vitro* and greenhouse experiments, we assess the ability of these PGPR strains to inhibit pathogen growth, promote tomato growth, and reduce disease incidence and severity. Our findings contribute to the growing body of knowledge on sustainable disease management strategies for tomato bacterial wilt and offer insights into the potential of PGPR-based biocontrol as an environmentally friendly alternative to chemical pesticides.

Methodology

Pathogenic isolates

Isolates of pathogenic bacteria that had been previously documented were obtained from the bacteriology laboratory at PMAS-Arid Agriculture University Rawalpindi. These

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isolates were then revived by culturing them on nutrient agar and 2,3,5-triphenyltetrazolium chloride (TZC)-casein-peptone-gulcose agar media and allowing them to incubate at a temperature of 27±2 °C for a period of 24 to 48 hours. All the revived isolates were subsequently maintained and stored for future research purposes.

Pathogenicity test

Tomato seedlings, aged one month, were subjected to bacterial inoculation via soil drenching approximately two weeks after being transplanted. These inoculated seedlings were then maintained in a greenhouse until the development of symptoms, following the methodology described by Pradhanang *et al.* (2005). As a negative control, sterile water was used. The isolates under examination were subsequently retrieved and cultivated on TZC medium.

Isolation of Plant Growth Promoting Rhizobacteria (PGPR)

PGPR strains were collected from rhizospheric soil samples obtained from healthy tomato plants. Isolation was performed by serial dilution and plating on selective media, such as nutrient agar and King's B media. Isolated colonies were screened for PGPR traits, including phosphate solubilization, indole-3-acetic acid (IAA) production, and siderophore production (Kheirandish and Harighi, 2015; Sarwar and Kremer, 1995; Beneduzi *et al.*, 2008).

In Vitro Antagonistic Activity

The antagonistic activity of PGPR strains against *R. solanacearum* was assessed using dual-culture assays. The selected PGPR isolates were streaked on one side of agar plates, and *R. solanacearum* was streaked on the other side. Plates were incubated at an appropriate temperature and observed for inhibition zones around the PGPR colonies (Nair and Anith, 2009; Sharma, 2011).

Green house evaluation of recovered rhizobacterial isolates

A greenhouse study was carried out to evaluate the efficacy of three candidate antagonist isolates, namely Rh-AK-3, Rh-AK-7, and Rh-AK-9, for managing bacterial wilt in tomato plants. The antagonistic bacterial inoculum was prepared following the technique outlined by Islam *et al.* (2016), utilizing fresh bacterial cultures grown for 24 hours. Tomato seeds were immersed in each bacterial suspension and allowed to stand at room temperature overnight. Subsequently, these treated seeds were planted in sterilized soil within trays. The experiment followed a Completely Randomized Design (CRD) with a total of nine treatments including positive and negative control including alone and in consortium of biocontrol agents with three replications.

Two weeks after the tomato plants were planted, an evaluation was conducted, and data regarding disease incidence and the biocontrol efficacy percentage were recorded on a weekly basis for a period of three weeks.

The disease incidence (DI) was calculated using this formula (Cao *et al.*, 2011);

$$Disease\ Incidence\ (\%) = \frac{No.of\ wilted\ plants}{Total\ number\ of\ plants} \times 100$$

The biocontrol efficacy was calculated using the following formula (Chen *et al.*, 2019);

$$Biocontrol\ Efficacy\ (\%) = \frac{DIC - DIAT}{DIC} \times 100$$

Where, DIC = disease incidence of the control, and DIAT = disease incidence of antagonist treated.

Statistical analysis

The data collected were subjected to analysis using Statistical Package for Social Sciences (SPSS) version 20.0. Additionally, an Analysis of Variance (one-way ANOVA) was performed, and Duncan's multiple range test was utilized to separate the means. The significance level used for the analysis was set at 5%.

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Results and Discussion

When the previously revived pathogenic isolates were introduced to tomato seedlings, they exhibited symptoms of wilt. On a susceptible tomato variety, the onset of wilt was rapid, leading to the complete wilting of the seedlings. These symptoms became evident approximately two weeks after inoculation and were represented by the drooping of leaves, without any yellowing or necrosis. Furthermore, the identical pathogen was re-isolated from the symptomatic plants, confirming the virulence of the isolates.

Isolation and characterization of the PGPR for growth promotion

From the soil samples a total of 10 different bacterial isolates were recovered on the basis of their colony color and texture. All the 10 isolates were purified on King's B medium and were subjected to the evaluation of their rhizobacterial properties.

The plant growth promotion traits by PGPR showed that the isolate Rh-AK-3, Rh-AK-7, and Rh-AK-9 was positive for phosphate solubilization, indole-3-acetic acid (IAA) production, and siderophore production, while other isolates showed negative results for one or more of growth promotion character (Table 1).

Similarly, Kheirandish and Harighi (2015) documented that strains such as *Pseudomonas putida*, *Pseudomonas fluorescens*, and *Pseudomonas fluorescens* exhibited the production of siderophore, hydrogen cyanide (HCN), and protease enzyme. The multifaceted mechanisms of action have been identified as the primary factors contributing to the plant growth-promoting and disease-suppressing capabilities of PGPR. The effectiveness of *Pseudomonas fluorescens* strains as biocontrol agents is attributed to their versatility in utilizing various substrates across diverse conditions, rapid growth rates, and their ability to move, which enhances their capacity for root colonization (Bashan and De-Bashan, 2010; Zhou *et al.*, 2012).

Table 1. Production of hydrolytic enzymes and plant promoting properties of potential plant growth promoting rhizobacteria antagonists.

S. No.	Isolates	Phosphate solubilization	Indole-3-acetic acid	Siderophore production
1	Rh-AK-1	-	+	+
2	Rh-AK-2	+	-	-
3	Rh-AK-3	+	+	+
4	Rh-AK-4	-	+	+
5	Rh-AK-5	+	-	-
6	Rh-AK-6	-	-	-
7	Rh-AK-7	+	+	+
8	Rh-AK-8	+	-	+
9	Rh-AK-9	+	+	+
10	Rh-AK-10	-	+	-

***In Vitro* Antagonistic Activity**

The results of the *in-vitro* antibacterial activity of all the 10 recovered rhizobacterial isolates revealed that the zones of inhibition ranged from 0.0mm to 34.0 mm. Rh-AK-7 and Rh-AK-3 were the most potent isolates followed by Rh-AK-9. While Rh-AK-6 had no antibacterial activity against *R. solanacearum* (Figure 1).

Similar results were reported by Mohammed *et al.* (2020) that the *in vitro* assessments of the antibacterial potential of *Pseudomonas* species against *R. solanacearum* indicated that *P.*

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aeruginosa and *P. syringae* exhibited robust antibacterial properties against this pathogen. *P. aureofaciens*, on the other hand, did not demonstrate any inhibitory effects. Meanwhile, *P. alcaligenes* and *P. fluorescens* showed moderate inhibition, with inhibition rates of 21.0% and 20.0%, respectively, against the pathogen. These findings also align closely with the research reported by Maji and Chakrabartty (2014) regarding the utilization of *Pseudomonas* species for the biocontrol of bacterial wilt in tomato plants.

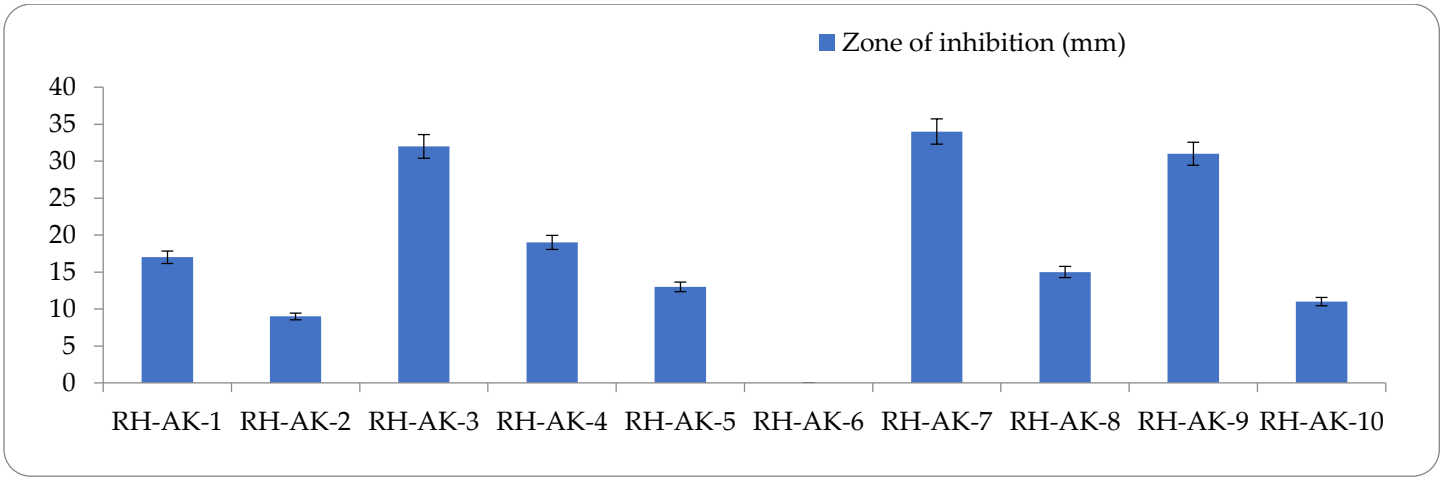

Figure 1. *In vitro* antibacterial activity of rhizobacterial isolates against *Ralstonia solanacearum*.

Green house evaluation of recovered rhizobacterial isolates

The effects of selected antagonistic bacterial isolates (Rh-AK-3, Rh-AK-7 and Rh-AK-9) alone and in consortium on the incidence of bacterial wilt and biocontrol efficacy of tomato plants in the greenhouse showed that the disease incidence was highest in positive control with *R. solanacearum* excluding any biocontrol agent that was 71% while the least disease incidence was recorded in treatment having two antagonists (Rh-AK-3 + Rh-AK-7) in consortium that was 5% and in negative control (0%) (Figure 2).

These results are in line with the findings reported by Sharma and Kumar (2009), who determined that soil drenching with chemical treatments resulted in a mere 36.2% reduction in the population of bacterial pathogens in the soil after a span of 90 days. Additionally, plots subjected to a combination of treatments T1, T2, and T4 exhibited an average increase of 2% in the population of *R. solanacearum*, both at the Kabete and Mwea locations. The findings of current study closely resemble the outcomes of the earlier investigation conducted by Kumar *et al.* (2015) concerning the impact of *Pseudomonas* species on promoting the growth of *Lycopersicon esculentum* (tomato). Moreover, past research has consistently documented the effective use of strains from *Pseudomonas, Stenotrophomonas*, and *Bacillus* genera in both plant pathogen control and the enhancement of plant growth (Bibi *et al.*, 2017; Shahzaman *et al.*, 2016; Shakoor *et al.*, 2015).

The treatment involving a consortium of Rh-AK-3 and Rh-AK-7 exhibited the highest biocontrol efficiency at 64%, whereas the treatment with Rh-AK-9 showed the lowest biocontrol efficiency at 34.4% (Figure 3). These findings are similar with the findings of Mekonnen *et al.* (2022b) that the combined treatment involving cyanobacteria RZ2AB2.1, *Bacillus subtilis* BSn5 RBI IPBL 2.3, and *Bacillus cereus* strain APSB-03 RBI 2AB 2.2 exhibited the most effective capacity to mitigate the progression of bacterial wilt disease in tomato plants. While the results are contradict with the treatment including consortium of all the three biocontrol agents showed less effectiveness that could be attributed to the incompatibility of the all three bacterial isolates with each other.

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Numerous mechanisms have been documented to play a role in the promotion of plant growth by various strains of plant growth-promoting rhizobacteria. These mechanisms include the production of phytohormones, inhibition of harmful organisms, stimulation of phosphate solubilization, and facilitation of the uptake of essential mineral nutrients (Yanti and Hamid, 2021).

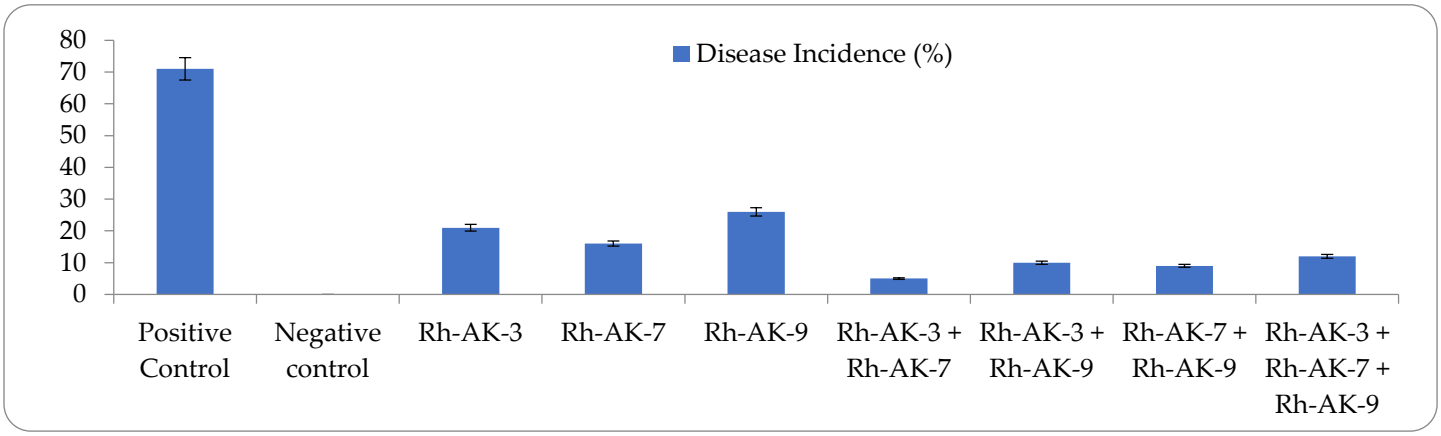

Figure 2. Disease incidence percentage using selected antagonistic bacterial isolates against *R. solanacearum*.

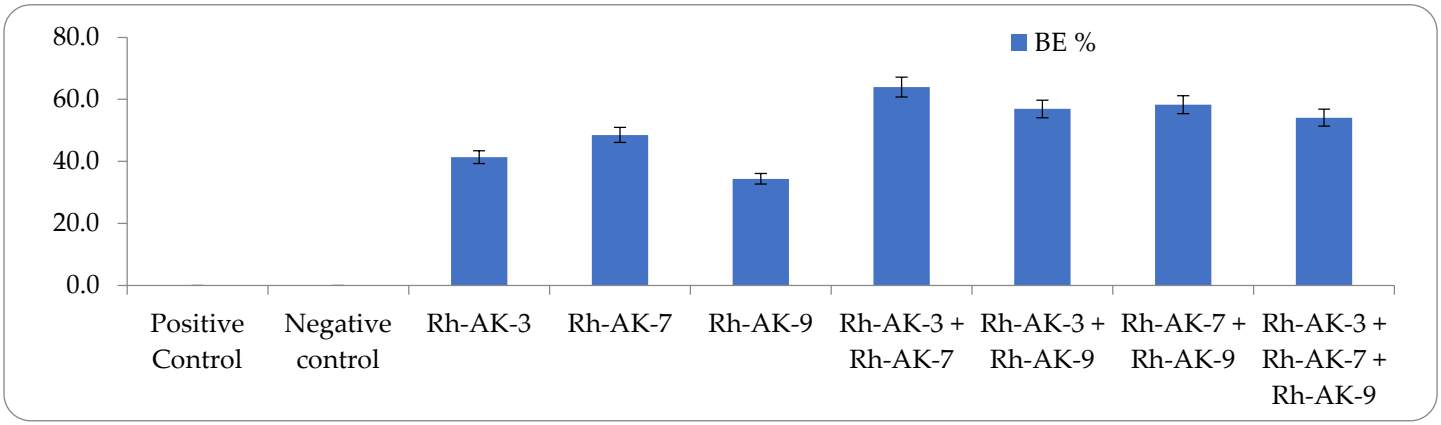

Figure 3. Biocontrol efficiency percentage using selected antagonistic bacterial isolates against *R. solanacearum*.

Conclusion

The research affirmed that plant growth-promoting rhizobacteria exhibited several traits conducive to plant growth promotion. These isolates notably reduced the incidence of bacterial wilt in tomatoes within a controlled greenhouse environment. In summary, the study's outcomes suggest that Rh-AK-3 and Rh-AK-7 hold potential for both enhancing plant growth and serving as effective biocontrol agents for managing bacterial wilt disease in tomatoes, pending validation of their effectiveness under real field conditions.

Conflict of Interest

The authors have not declared any conflict of interest.

Authors Contributions

All the authors contributed equally in the manuscript.

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