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Research Article

# EVALUATION OF RHIZOSPHERIC *PSEUDOMONAS* SPP. FOR THE MANAGEMENT OF *FUSARIUM* WILT OF TOMATO IN CHOLISTAN, PAKISTAN

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**ABSTRACT**

Agriculture plays a crucial role in national development, food security, and poverty reduction. Despite its importance, the agricultural sector's contribution to GDP is gradually decreasing. Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *lycopersici*, has significantly hindered tomato production globally. Tomato (*Lycopersicon esculentum* Mill.), a widely grown vegetable, is threatened by various pathogens, with Fusarium wilt being particularly damaging due to yield and quality loss. Synthetic chemicals are commonly used to control Fusarium wilt in tomatoes, but these chemicals are harmful to the environment and human health due to their toxicity and persistence. This has prompted researchers to develop eco-friendly alternatives. In preliminary tests, 10 isolates of *Pseudomonas* spp. were evaluated *in vitro* against *Fusarium oxysporum*. Four isolates that effectively inhibited the growth of *F. oxysporum* were chosen for further experimentation. A greenhouse study revealed that two bacterial isolates, IUB310 and Mad1230, significantly reduced disease severity by 69.2% and 65%, respectively. Plant growth-promoting rhizobacteria from the *Pseudomonas* family not only combat pathogens but also induce systemic resistance in plants by strengthening cell walls and triggering defensive proteins and compounds. The results clearly highlight the significance of *Pseudomonas* strains in suppressing *F. oxysporum* and promoting plant growth, indicating their potential use in managing Fusarium wilt in tomato cultivation.

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## INTRODUCTION

Tomato (*Solanum lycopersicum* L. Moench) is an edible vegetable, frequently red, belonging to the nightshade family, Solanaceae (Gafforov et al., 2024). The use of tomatoes as food began in Mexico, with the crop originating from the South American Andes, from where it spread around the globe (Gondal et al., 2012).

Vegetables are crucial in the human diet, and the tomato has become particularly important due to its widespread use in daily life (Chavan et al., 2015). It is believed to play a critical role in national food security and safety because of its extensive use (Khokhar, 2013). The tomato crop is financially appealing due to its high production and relatively short growing period, leading to an increasing amount of land dedicated to its cultivation over time (Zahedi et al., 2012).

According to statistical data from the Food and Agriculture Organization (FAO) for the year 2020, China led global tomato production with 64,768,158 tons, followed by India with 20,573,000 tons and Turkey with 13,204,015 tons (FAO, 2020). In Pakistan, all four provinces are engaged in tomato cultivation. As of 2020, the area allocated for tomato cultivation in Pakistan spanned 57,838 hectares, yielding a total production of 594,210 tons. Despite this, Pakistan does not hold a significant position in global tomato production rankings (GOP, 2020; FAO, 2020).

Worldwide, tomato production experienced stable and uninterrupted growth throughout the 20th century. According to Jayasuriya et al. (2021), tomatoes are cultivated year-round over an area of 150,000 hectares in Pakistan. The predominant high-yielding tomato varieties grown in the country include Money Maker, Roma, and Riogrande. Despite the numerous benefits of tomato cultivation, it has been observed that the tomato crop is highly susceptible to adverse weather conditions. Specifically, flower development is impeded when temperatures drop below 15°C or rise above 35°C. In Pakistan, the optimal temperature range for the growth and development of tomatoes is documented to be between 21°C and 24°C (Khokhar, 2013).

Although tomato cultivation can be economically rewarding, it is characterized by seasonality, which leads to price volatility (Das and Jahan, 2022). Additionally, tomato crops are vulnerable to insect pest infestations and diseases, which can substantially affect both yield and overall production (Aslam et al., 2017; Mukhtar, 2018; Stephenson et al., 2020; Azeem et al., 2021). Pakistan ranks 37th as the largest producer in the world, with an output of 0.566 million tons. According to the FAO, the top ten producers of tomatoes include China, the USA, India, Turkey, Egypt, and Italy, with the top fifteen nations accounting for about 82.21% of the total world production. China is considered the main supplier, contributing 31.47% of worldwide production and 53% of the tomatoes delivered in Asia.

Tomato crops play a major role in agribusiness and industry, serving as a vital source of popular supplements and minerals for human consumption. Nowadays, Pakistan is focusing on trading tomatoes with Afghanistan and Middle Eastern markets. Therefore, it is essential to enhance tomato trade in other markets during periods of surplus supply (Bergougnoux, 2014). Various ecological conditions, including abiotic and biotic stresses, adversely affect tomato plant production (Aslam and Mukhtar, 2023a, b, 2024; Aziz et al., 2024; Saeed and Mukhtar, 2024). Fluctuations in climate and the continuous increase in global temperatures have also reduced the crop's production levels. These environmental changes, particularly heat stress, further decrease production or cause harvest failures in sensitive plants (Singh et al., 2007).

The growth of tomato plants is notably impacted by abiotic stresses, such as salinity, drought, and temperature extremes, which are recognized globally as critical limiting factors (Pervez et al., 2009). Tomato wilt primarily caused by the soil-borne pathogen *Fusarium oxysporum* f.sp. *lycopersici*, is a significant disease affecting tomatoes worldwide. This disease, first identified in England in 1895, is especially problematic in warm climates and has been reported in 32 countries. Fusarium wilt disrupts the xylem vessels of plants, impeding the transportation of water and nutrients and leading to wilting symptoms while the plant's exterior remains green. The fungus *F. oxysporum* is soil-borne, persisting in soils for long periods and affecting plants under suitable environmental conditions (Zeeshan et al., 2023; Yaseen et al., 2024). The pathogen's life cycle begins with a saprophytic stage. Chlamydospores are a form of fungal presence in the soil (Beckman and Roberts, 1995). The main entry point of this pathogen into plants is through root tips. This pathogen can survive in the soil for up to 30 years under favorable environmental conditions (Thangavelu et al., 2004). The fungus has also been found to be implicated with root-knot nematodes, resulting in disease complexes that escalate the incidence and severity of wilts in plants (Saeed et al., 2023; Yaseen et al., 2023).

Managing Fusarium wilt is challenging. Strategies include using resistant tomato cultivars and chemical fungicides such as benomyl and carbendazim (Mukhtar et al., 2023). However, these chemicals pose environmental risks due to their toxicity and accumulation. An alternative, environmentally friendly approach involves using antagonistic microorganisms, like certain strains of *Fusarium* that can inhibit pathogenic strains, and applying plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas fluorescens*. PGPR not only reduces disease incidence but also promotes plant growth and induces systemic resistance in plants against pathogens (Shahzaman et al., 2015).

This study aimed to explore local strains of *Pseudomonas* for their effectiveness in managing Fusarium wilt of tomato. It assessed their impact on disease management, field viability, and potential to enhance tomato growth.

## MATERIALS AND METHODS

### Test plant

The tomato variety 'Nagina', which is susceptible to Fusarium wilt, was obtained from the Punjab Seed Corporation in Bahawalpur. Healthy seeds were first separated and then surface sterilized using a 0.5% NaOCl (sodium hypochlorite) solution.

### Collection of *Pseudomonas* spp. strains from different rhizospheres

*Pseudomonas* spp. was isolated from the healthy root tissue (90 days old) or rhizosphere of tomato, potato, radish, and rice plants collected from the Horticulture Research Area at the Islamia University of Bahawalpur as mentioned in Table 1. Ten strains that were effective in reducing Fusarium wilt in tomatoes were selected. The strains were stored at -80°C in 50% glycerol.

### Procurement of pathogen

Pure cultures of *Fusarium oxysporum* were procured from the Fungal Culture Bank (FCB) at the Plant Pathology Department, Faculty of Agriculture and Environmental Science (FA&ES), Islamia University of Bahawalpur. For sub-culturing the fungus, 2% MEA media (20.0 g malt extract and 20.0 g agar in 1000 ml water) was used, and the cultures were kept at 4°C. Freshly prepared fungal cultures were used for each experiment.

### Preparation of bacterial culture

To prepare the growth medium, 28 g of agar powder was dissolved in 100 ml of water, boiled, and sterilized by autoclaving at 121°C for 15 min. After cooling, the medium was poured into Petri dishes and further sterilized under UV light for 10 min. The bacterial isolates were re-cultured by adding nutrient agar to a sterile plate, selecting a single colony or a small number of bacteria from the mother culture, inoculating with a wooden stick, and incubating at 37°C for 16-24 h.

Table 1. *Pseudomonas* spp. strains selected for study.

| Strain | Origin | Area |
|---|---|---|
| *Iub310* | Tomato rhizosphere | Horticulture research area |
| *Ck2020* | Potato rhizosphere | Horticulture research area |
| *Iubf12* | Radish rhizosphere | Horticulture research area |
| *Iubc20* | Rice root tissue | Agronomy field area |
| *Lalsoh20* | Tomato root tissue | Horticulture research area |
| *Chist20* | Potato rhizosphere | Horticulture research area |
| *Haroon2020* | Tomato rhizosphere | Horticulture research area |
| *Lodh2020* | Maize root tissue | Agronomy field area |
| *Karr1220* | Cotton rhizosphere | PBG research area |
| *Mad1230* | Tomato rhizosphere | Horticulture research area |

### Antagonistic Interaction

The antagonistic effect of *Pseudomonas* strains against *Fusarium oxysporum* was tested *in vitro* using PDA (potato dextrose agar) media in petri dishes. The poisoned food technique described by Perrucci et al. (1994) was employed for the assessment.

### Calculation of percentage inhibition

The inhibition zone in the antagonistic interaction between bacteria and fungi was measured using the following formula:

$$\text{Inhibition percentage} = \frac{\text{Growth of pathogen in control} - \text{Growth of pathogen in treatment}}{\text{Growth of pathogen in control}} \times 100$$

### Inoculum preparation

To inoculate soil with *F. oxysporum*, millet seeds were first partially boiled and then air-dried to remove moisture. The seeds were placed in plastic bags and autoclaved at 121°C for 30 minutes. After cooling to room temperature, *F. oxysporum* was cultured on the seeds at 27°C for 7 days. For the inoculation experiment, clay pots (35 cm in height and 27 cm in diameter) were filled with 6 kg of soil, a mixture of sand, field soil, and farmyard manure in a 1:2:1 ratio. The inoculated millet seeds were incorporated into the soil at a rate of 5 g per kg and left for 7 days. Negative control pots were prepared similarly but without the fungus, using autoclaved seeds for comparison.

**Cultivation and transplantation of tomato seedlings**

Tomato seedlings were grown in earthen pots containing 1000 g of sterilized soil, which was a mixture of sand, field soil, and farmyard manure (FYM) in a 1:2:1 ratio. The soil was irrigated to soften it. Then, 8-10 tomato seeds were sown in each pot. The experiment utilized 10 pots, with 8-10 seeds per pot. Polythene sheets were used to cover the pots to maintain a temperature range of 18-30°C. Tomato seedlings that were 2-3 weeks old were then transplanted into the pots, with one plant per pot.

**Roots inoculation with *Pseudomonas* culture**

The bacterial culture was thoroughly mixed with sterile wheat straw and then integrated into the root zone. Soil was added to cover the roots in the pots. The seedlings were allowed to interact with the bacterial culture for approximately two weeks. Morphological traits and yield components, including plant height, number of flowers per plant, and yield for each treatment, were assessed, along with the percentage of disease severity.

$$\text{Disease index (\%)} = \sum \frac{\text{(Grade of disease severity + diseased plants of this grade)}}{\text{Total plants} \times \text{Highest grade of disease severity}} \times 100$$

**Statistical analysis**

A Completely Randomized Design (CRD) was employed in the laboratory settings. The experimental data were analyzed statistically using Fisher's analysis of variance at a 5% probability level (Steel et al., 1997).

**RESULTS**

**Biocontrol screening of ten bacterial strains against Fusarium wilt in Nagina tomatoes**

*In vivo*, the Nagina variety of tomato, which is susceptible to Fusarium, was used throughout the entire experiment. Ten selected bacterial strains were tested against Fusarium wilt of tomato as shown in Figure 1. These strains were screened in preliminary tests consisting of three trials. The best results were obtained with isolates/strains *IUB 310*, *MAD1230*, *CK2020*, *IUBMAD*, and *KAR1220*. When compared to the control, these strains reduced disease severity by 69.2%, 65%, 57%, 55.5%, and 50%, respectively. From these ten strains, four isolates were selected for further trials as potential biological control agents against Fusarium wilt of tomato. The bacterial strains *IUB310* and *MAD1230* were observed to be the most effective, significantly reducing disease severity in tomato plants. They were followed by *CK2020*, *IUBMAD*, and *KAR1220*. The effects of bacterial strains *LODH2020*, *LALSOH20*, and *HAROON2020* were observed to be the least effective compared to the other isolates/strains. All ten strains significantly ($P<0.10$ or $P<0.05$) reduced symptom expression when compared to the control.

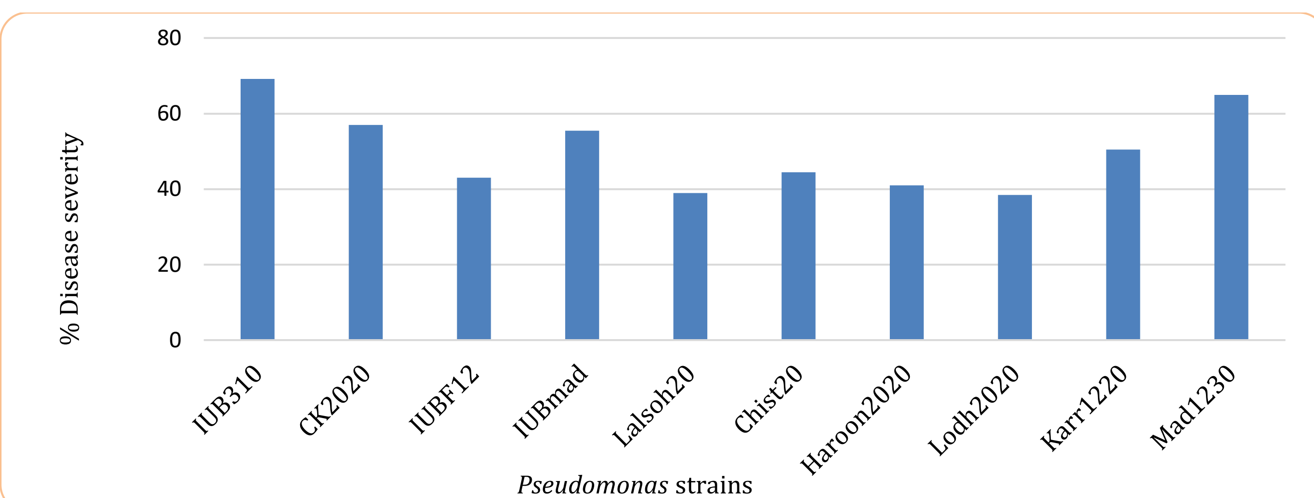


Figure 1. Effectiveness of ten endophytic bacterial strains in controlling Fusarium wilt in tomato plants.

### *In vitro* control of *F. oxysporum* f.sp. *lycopersici* using poisoned food technique

All ten strains of *Pseudomonas* (*IUB310*, *Ck2020*, *IUBmad*, *Karr1220*, *Mad1230*, *Lodh2020*, *Haroon2020*, *Chist20*, *Lalsoh20*, and *IUBC2020*) tested *in vivo* effectively suppressed *F. oxysporum* f.sp. *lycopersici* and increased tomato yield, as shown in Figure 2. Among the ten strains, *IUB310* exhibited the highest growth inhibition, with a diameter of 57.5% against *F. oxysporum* f.sp. *lycopersici*. *Mad1230* was the second most effective antagonistic bacterial strain, resulting in 55.5% inhibition of the growth of *F. oxysporum* f.sp. *lycopersici*. Other bacterial strains, such as *IUBF12* and *Karr1220*, resulted in 55%, 50%, and 45% inhibition of the growth of the fungal pathogen, respectively.

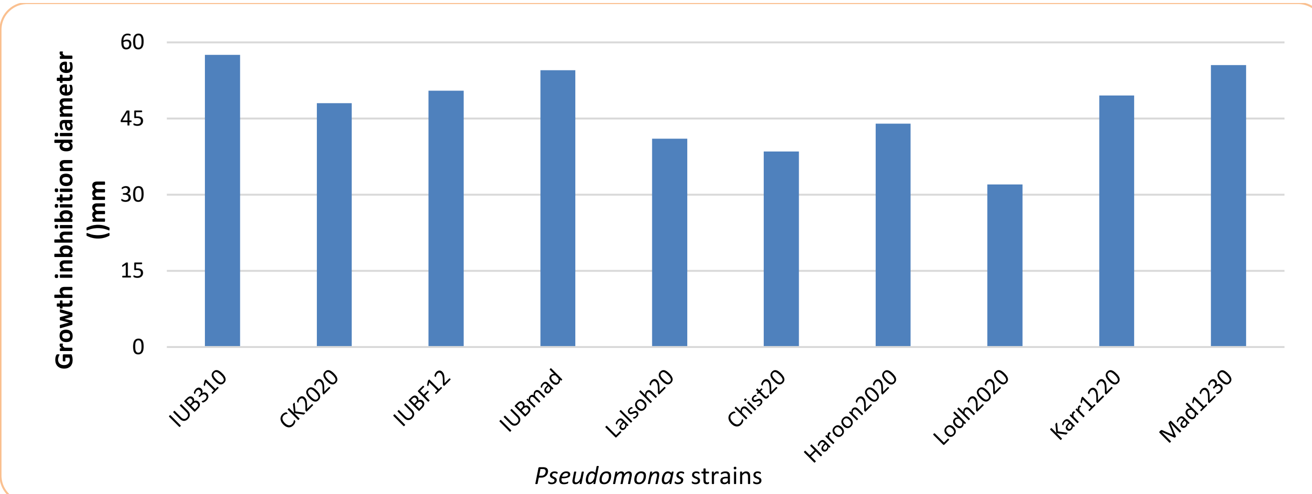


Figure 2. Effect of *Pseudomonas* strains on the *in vitro* growth inhibition percentage of *F. oxysporum*.

### Efficacy of *Pseudomonas* strains against root wilt of tomato

The objective of this experiment was to evaluate the efficacy of different strains of *Pseudomonas* in combating root wilt in tomato plants. All the bacterial isolates significantly reduced the incidence of root wilt disease in tomatoes. The bacterial strain *IUB310* was the most effective, reducing the root wilt infection percentage to 6.7% as shown in Figure 3. It was followed by strain *CK2020* with an infection percentage of 12.0%, and strain *IUB310/Mad1230* with 12.6%. Conversely, the bacterial strain *Mad1230* was the least effective, with an infection percentage of 35%, which was higher than all other strains tested.

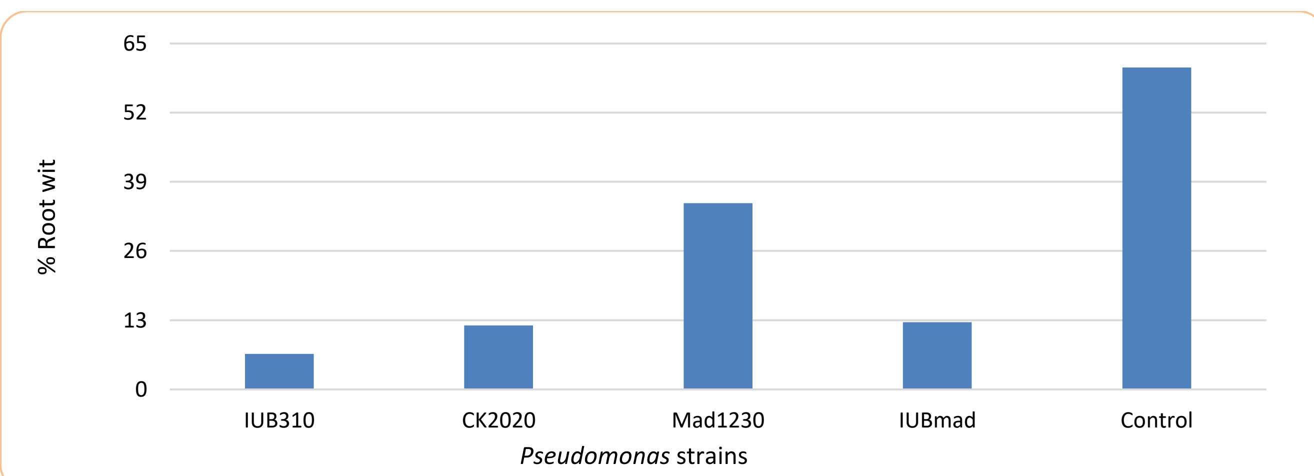


Figure 3. Effect of *Pseudomonas* strains on tomato root wilt infection.

**Effect of bacterial strains on fungal transmission in tomato seed**

All bacterial strains were applied to tomato seeds before sowing by soaking the seeds in bacterial suspensions. The colonization of *F. oxysporum* in the tomato seeds was significantly reduced or inhibited in plants grown from treated seeds. Moreover, the yield from treated seeds was higher compared to the yield from non-treated seeds. The *IUB310/Mad1230* combination was the most effective treatment, completely inhibiting fungal transmission in tomato seeds (0.00%). *Mad1230* alone also significantly suppressed the colonization of *F. oxysporum* in tomato seeds (7.53%), followed by *IUB310* (5.5%) and *CK2020* (9.33%) as shown in Figure 4. Additionally, *CK2020*, *Mad1230*, and *IUB310* showed similar results, moderately reducing *F. oxysporum* in tomato seeds 30 days after storage.

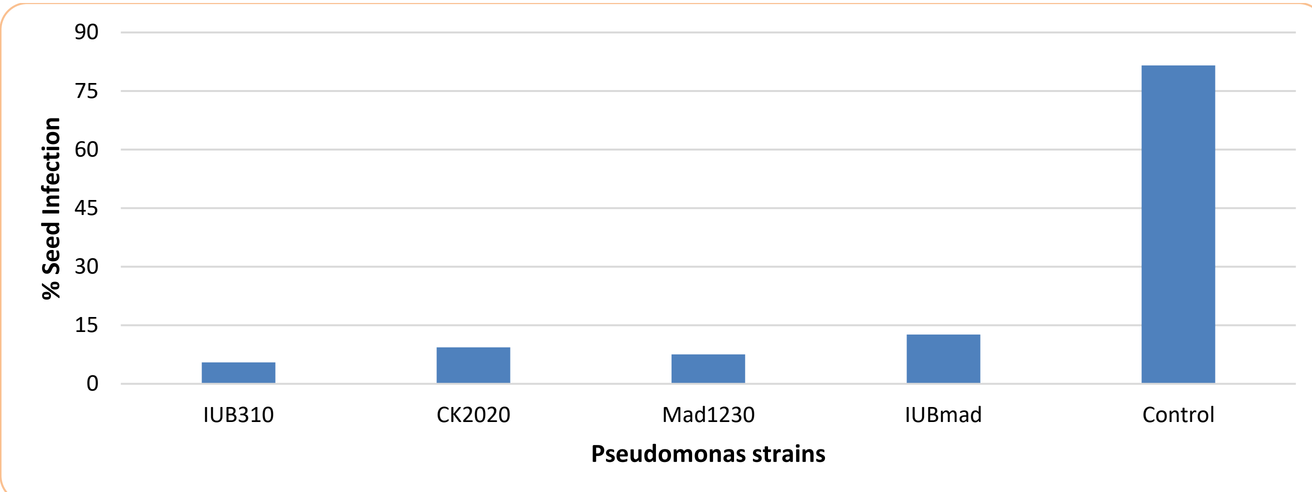


Figure 4. Effect of *Pseudomonas* strains on fungal transmission in tomato seed.

**Effect of *Pseudomonas* strains on tomato growth**

The objective of this experiment was to evaluate the effect of different bacterial strains on the growth of tomato plants. The impact of various bacterial strains on the growth and yield of tomato plants was significant as shown in Table 2. Tomato plants treated with bacterial isolates exhibited a considerable increase in the number of flowers and fresh fruit diameter compared to untreated (control) plants. The bacterial strain *IUB310* showed the most promising results, with treated plants producing an average of 8 flowers per plant, while untreated plants produced only 4 flowers. The fresh fruit diameter in *IUB310*-treated plants was 3.05 cm, compared to 2.2 cm in control plants. Plants treated with *Pseudomonas* strain *CK2020* and *Mad1230* produced 6 and 7 flowers, respectively. The fruit diameters in *CK2020* and *Mad1230* treated plants were 2.9 cm and 2.6 cm, respectively. Plant height was measured after 45 and 90 days. The maximum height of 50.5 cm was observed in plants treated with the *Pseudomonas* strain *IUBmad*. Heights of 47.5 cm and 47 cm were observed in plants treated with strains *IUB310* and *CK2020*, respectively. After 90 days, the maximum height of 105.5 cm was observed in *IUB310*-treated plants, while the minimum height of 95 cm was observed in *CK2020* and *Mad1230* treated plants. Control plants had a height of 88.5 cm after 90 days.

Table 2. Effect of four *Pseudomonas* strains on some growth parameters of tomato.

| *Pseudomonas* strain | Number of flowers per plant | Fruit diameter (cm) | Plant height (cm) 45 days | Plant height (cm) 90 days |
|---|---|---|---|---|
| *IUB310* | 67 | 3.5 | 47.5 | 105 |
| *CK2020* | 50 | 2.9 | 47 | 95 |
| *Mad1230* | 53 | 2.6 | 49 | 95 |
| *IUBmad* | 57 | 2.8 | 50.5 | 102.5 |
| Control | 52 | 2.2 | 46.5 | 88.5 |

## DISCUSSION

The present study highlights the potential of rhizospheric *Pseudomonas* spp. as effective biocontrol agents against Fusarium wilt in tomatoes. Our findings showed that selected *Pseudomonas* strains significantly reduced disease severity and promoted plant growth, offering a promising alternative to chemical fungicides for managing this devastating disease.

Agricultural yield losses due to plant diseases can have severe economic repercussions, impacting economic growth of a state. Therefore, it is crucial to address the role of plant protection in the economy as well as in safeguarding plants (Savary et al., 2012). Fungal pathogens cause numerous diseases in various plants, and while chemical control methods are commonly used, their intensive application can harm both plants and humans (Dellavalle et al., 2011; Iqbal and Mukhtar, 2020). Employing natural organisms and beneficial microbes as biological control systems offers a more environmentally friendly approach, protecting the environment, soil, plants, and humans. This method is considered effective for pathogen control (Gerbore et al., 2014; Afzal and Mukhtar, 2024).

Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *lycopersici*, is a major disorder affecting tomato growth. Utilizing natural compounds, such as biocontrol agents and other beneficial microorganisms, presents sustainable and eco-friendly alternatives for disease management (Bibi et al., 2023). The trend is shifting towards biological controls rather than traditional methods (El Hassni et al., 2007; Rauf et al., 2007). In this study, various *Pseudomonas* strains were evaluated as biocontrol agents for managing Fusarium wilt in tomatoes.

Among the ten *Pseudomonas* strains evaluated, *IUB310* and *Mad1230* emerged as the most effective, reducing disease severity by 69.2% and 65%, respectively, in greenhouse trials. These results are comparable to those reported by Fatima and Anjum (2017), who found that *P. aeruginosa* and *P. putida* controlled tomato Fusarium wilt by 65.5% to 78.89%. The variations in effectiveness among the strains tested may be attributed to differences in their metabolic capabilities, colonization efficiency, or mechanisms of action against the pathogen. All ten *Pseudomonas* strains were effective in suppressing the growth of *F. oxysporum* f.sp. *lycopersici*, with five strains (*IUB310*, *CK2020*, *IUBmad*, *Karr1220*, and *Mad1230*) proving to be particularly effective. *In vivo*, each of the ten strains was tested in three separate trials. The strains *IUB310*, *Mad1230*, *CK2020*, *IUBmad*, and *Karr1220* achieved the best results, reducing disease severity by 69.2%, 65%, 57%, 55.5%, and 50%, respectively, compared to the control.

Plants treated with the *Pseudomonas* strains exhibited increased height compared to the untreated control in both greenhouse trials. The experiment assessed the impact of various bacterial isolates on tomato plant growth and yield. The results demonstrated significant growth, an increased number of flowers, and a larger fruit diameter. The strain *IUB310* showed the most promising results, producing an average of 8 flowers per plant. *Pseudomonas* strains *CK2020* and *Mad1230* also yielded positive outcomes. Plant height measurements taken after 45 and 90 days revealed that *IUB310*-treated plants reached a maximum height of 105.5 cm, while *CK2020* and *Mad1230* plants reached heights of 95 cm and 95 cm, respectively. In contrast, untreated plants reached a height of 88.5 cm after 90 days. *P. fluorescens* is recognized as an effective soil-borne endophyte, extensively used against tomato Fusarium wilt. It also plays a positive role in enhancing plant yield and growth by controlling pathogens (Bora et al., 2004).

Different antifungal compounds are produced by *P. fluorescens*. The parasitic effects of *P. fluorescens* are widely utilized to combat pathogens. The roles of various enzymes, such as β-glucanase and chitinase, in degradation are complex (Velazhahan et al., 1999). *P. fluorescens* affects different soil-borne pathogens due to its lytic enzymes (Lim et al., 1991). Under iron deficiency, *P. fluorescens* strains primarily produce water-soluble siderophores. These siderophores suppress the growth of chlamydospores of *F. oxysporum* (Bakker et al., 1986).

Recent studies have demonstrated the effectiveness of *P. fluorescens* as a control agent against *F. oxysporum* f.sp. *lycopersici*. Additionally, it plays a role in the mechanism of induced systemic resistance (ISR) in the host (Maurhofer et al., 1998). The antibiotic pyrolnitrin, produced by *P. fluorescens*, is widely used to combat damping-off and provides effective protection (Mercado-Blanco and Bakker, 2007).

Several mechanisms are known through which these endophytes manage Fusarium wilt. These mechanisms include the production of antifungal compounds, siderophore production, competition for resources, niche exclusion, and the induction of systemic resistance (Cook and Baker, 1983).

Nonpathogenic *F. oxysporum* Fo47 slightly reduced the incidence of Fusarium wilt in split plants, though this reduction was not statistically significant. This finding supports the previously described ability of Fo47 to induce systemic resistance in tomatoes (Olivain et al., 1995; Fuchs et al., 1997). Both nonpathogenic and pathogenic strains of *F. oxysporum* from different formae speciales have been shown to induce resistance (Tamietti and Matta, 1991; Olivain et al., 1995; Fuchs et al., 1997). However, the effectiveness of this induced resistance varies depending on the fungal biocontrol strain, with Fo47 being less efficient at inducing resistance compared to another nonpathogenic *F. oxysporum* strain (Olivain et al., 1995).

Several mechanisms may contribute to the biological control observed with the ten endophytes studied. While the research does not rule out any specific mechanisms, the results suggest that induced systemic resistance might play a significant role in disease control. Although siderophore production is important in some soils that suppress Fusarium wilt, it appears to affect chlamydospore germination and is unlikely to be a key factor in biological control by endophytes (Kloepper et al., 1980; Elad and Baker, 1985). The limited dispersal of the bacterial strains tested also suggests that other mechanisms, such as antifungal compound production, competition, and niche exclusion, are less likely to be involved. Enhanced host defenses through induced resistance may explain the reduction in symptom severity. Notably, one of the ten strains that reduced wilt severity in both final screens, strain *IUB310*, is a rhizosphere-colonizing *Pseudomonas* strain.

## CONCLUSION

The aim of this study was to demonstrate the significant potential of rhizospheric *Pseudomonas* spp. in managing Fusarium wilt in tomatoes. The identified strains, particularly *IUB310* and *Mad1230*, offer a promising alternative to chemical fungicides, aligning with the increasing demand for sustainable agricultural practices. By providing effective disease control and promoting plant growth, these biocontrol agents could enhance food security and reduce environmental impact in tomato cultivation. Developing these strains for commercial use represents an important step towards more sustainable and eco-friendly tomato production systems.

## AUTHORS' CONTRIBUTIONS

MTM and AH designed, formulated and laid out the study; AH, FR, and SUR conducted the experiments; MTM and AH collected, arranged and analyzed the data; WA and SUR provided technical assistance; AH and FR supervised the work; MTM and AH wrote the manuscript; FR proofread the paper.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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