Plant growth promoting and biocontrol efficacy of *Pseudomonas* spp. for tomato root rot disease caused by *Fusarium solani*

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ABSTRACT

Root rot disease in tomatoes caused by *Fusarium solani* is one of the most serious diseases and causes severe problems in all tomato growing regions. *Pseudomonas* is a genus of bacteria capable of synthesizing valuable active compounds, promoting plant growth, and controlling disease-causing fungi. This study aimed to evaluate the plant growth- promoting potential of selected bacterial strains from the *Pseudomonas* genus and their ability to prevent the occurrence of *Fusarium solani*, the fungus responsible for tomato root rot disease. *In vitro* experiments were conducted to assess the characteristics and effects related to plant growth promoting of the tested *Pseudomonas* strains. The antagonistic capability of the bacteria against the fungi was also evaluated by measuring mycelial growth inhibition. Greenhouse conditions were used to evaluate the effectiveness of *Pseudomonas* as a soil treatment for plant growth promoting and reducing the severity of tomato root rot disease. The results demonstrated a positive influence of *Pseudomonas* spp. on the growth and development of tomato plants, with *Pseudomonas* spp. PN05 exhibited the most significant impact. Moreover, both strains *Pseudomonas* spp. PN02 and PN05 exhibited inhibitory effects on *F. solani in vitro* and under greenhouse conditions, limiting root rot disease in tomato plants with a control efficiency ranging from 48.3 to 62.2%.

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1. Introduction

In the context of an increasing population that seriously impacts the environment, ensuring the quality of agricultural products is a significant challenge for both farmers and researchers. The use of biofertilizers and biotic agents, in conjunction with appropriate farming management practices, is a suitable approach that has received significant attention from the community. Among the biological solutions applied in agriculture, the use of plant growth-promoting bacteria (PGPB) has been reported. The PGPB are freely distributed in soil, on root surfaces, or endophytically within roots, and can promote plant growth and inhibit plant diseases. The mechanisms of action of PGPB bacteria include (i) direct mechanisms related to various processes such as phosphorus solubilization, nitrogen fixation, siderophore production, HCN, ammonia, vitamins, and phytohormone production (such as auxin, cytokinin, and gibberellin) and (ii) indirect mechanisms including 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, antibiotic production, cell wall degradation enzyme production, and induced systemic resistance (ISR) (Jaizme-Vega et al., 2004).

Among PGPBs, *Pseudomonas* spp. have been widely distributed in soil, water, and agricultural ecosystems because of their diversity in nutritional and physiological processes, making them highly adaptable to adverse environmental factors such as temperature, humidity, oxygen content, nutrient availability, and the presence of toxic substances. In addition, their ability to synthesize various valuable nutrients, clean the environment from pollutants, and their extremely simple cultivation conditions, along with their rapid growth rate, make them potential candidates for agricultural applications. Some species of *Pseudomonas* genus produce 2,4-diacetylphloroglucinol (2,4-DAPG), hydrogen cyanide, pyoluteorin (PLT), phenazine (including *P. chlororaphis*, *P. fluorescens*, and *P. aeruginosa*), and pyocyanin…, secrete cell wall-degrading enzymes such as chitinase, protease inhibiting the development of fungal diseases (Gupta et al., 2005).

Fusarium root rot caused by *Fusarium solani* is a significant problem in tomato production regions (Simões & de Andrade, 2023). *Pseudomonas fluorescence* and *P. aeruginosa* have antagonistic effects on *F. solani*. These *Pseudomonas* strains could inhibit the mycelial growth of *F. solani* in *in vitro* experiments (Kumar et al., 2015; Hofny et al., 2022). In addition, the volatile metabolites produced by *P. aeruginosa* were found to be efficient in reducing the mycelial growth of *F. solani* (Alsudani & Al-Awsi, 2020). Using of *P. fluorescens* as a biocontrol agent decreased disease severity under greenhouse conditions (Cai et al., 2021).

This study aimed to elucidate the characteristics related to the ability of some *Pseudomonas* spp. to promote plant growth and control *Fusarium solani*, the agent causing tomato root rot disease, to provide a basis for the selection and formulation of bio-products for sustainable agriculture.

2. Materials and Methods

2.1. Materials

Six *Pseudomonas* spp. and *Fusarium solani* strains were isolated, identified, and preserved at the Plant Integrated Biology (PIB) Laboratory of Nong Lam University Ho Chi Minh City, Vietnam. Phu Nong F1 (T-11) tomato seeds were used in the present study.

2.2. Determination of the ability of *Pseudomonas* spp. strains to promote plant growth

Pseudomonas spp. were cultured in Tryptone Soya Broth (TSB) for 24 h at 30°C with shaking at 150 rpm. Preparation of the bacterial suspension with an optical density of 0.1 at 600 nm. The experiments were designed in a completely randomized design with 4 replicates. The ability to dissolve phosphate was observed by the appearance of a clear zone of $Ca_3(PO_4)_2$ on Pikovskaya's agar medium after 3 days of cultivation at 30°C (Nautiyal, 1999). Indole-3-acetic acid (IAA) production was detected and quantified using the Salkowski reagent colorimetric method at 520 nm (Sasirekha & Shivakumar, 2012). Gibberellic acid (GAs) production was quantified using the extraction method with ethyl acetate, and absorbance was measured at 254 nm (Sasirekha & Shivakumar, 2012). The ability to form biofilms was determined using the method of O'Toole et al. (2000), siderophore production was determined on CAS agar medium according to Srivastava et al. (2018), and nitrogen fixation of the bacterial strains was determined according to Bashan et al. (1993).

2.3. Investigation of the influence of *Pseudomonas* spp. on tomato plant growth

In vitro conditions: Tomato seeds were sterilized and soaked in bacterial suspension (10^9 CFU/mL), and after 3 h, seeds were placed on a plate covered with a layer of sterile filter paper. In each treatment, 30 seeds were sown in Petri dishes containing moistened absorbent cotton and incubated at 25 ± 1°C. Seed germination was monitored after 2 and 3 days, and the germination rate was calculated.

Greenhouse conditions: Healthy tomato seedlings with 3 fully developed leaves were transplanted into pots (25 x 21 cm) containing clean soil, manure, lime, and phosphate fertilizer at a ratio of 4:1:1:1. When the plants had 5 true leaves, 5 mL of bacterial culture (10^7 CFU/mL) was irrigated at the base of each plant, once a week for 4 weeks. Plant height, leaf number, branch number, cluster number, flower number, and fruit quality were monitored.

2.4. Evaluation of the antagonistic ability of bacteria against *Fusarium solani*

The dual culture method was performed according to Ferreira et al. (1991) by measuring the fungal colony radius and calculating the antagonistic efficacy according to the formula of Moayedi et al. (2009) and the rating scale of Soytong (1988). The agar well diffusion method was performed according to Sharma et al. (2017), and the antagonistic activity of bacteria was demonstrated through the formation of inhibition zones after a 48-h incubation time, calculated using the formula of Azman et al. (2017). The experiments were arranged in a completely randomized design, with each bacterial strain as a treatment, repeated 3 times with four petri dishes in each replication.

Evaluation of disease control ability against *Fusarium solani*: seven-day-old tomato seedlings were immersed in a suspension of *F. solani* spores at a density of 10^6 spores/mL and a bacterial suspension at a density of 10^9 CFU/mL (Fs + PN) or in the reverse order (PN + Fs), and then transferred to 1.5 mL tubes. All seedlings were placed at 28°C with a 12-h light cycle. Disease progression was monitored after 7 days. The experiments were arranged in a completely randomized design, with each treatment consisting of 3 seedlings. The experiments were repeated twice.

Healthy tomato seedlings with 3 fully developed leaves were transplanted into pots (25 x 21 cm) containing clean soil, manure, lime, and phosphate fertilizer in a ratio of 4:1:1:1. When the plants had 5 true leaves, their roots were injured, and 5 mL of *F. solani* spore suspension (10^6 spores/mL) was applied. Additionally, 5 mL of bacterial suspension (10^9 CFU/mL) was applied around the plant base, either 24 h before, at the same time, or 24 h after the fungal inoculation, depending on the treatment. The experiments were arranged in a randomized complete block design, with each treatment replicated 3 times, with 4 pots per treatment. The pots were placed in a greenhouse, and watering was provided as per the plant requirements. Disease incidence (%) and disease index (%) after infection at 7, 14, 21, and 28 days were recorded and classified according to Ateka et al. (2001). The efficacy of disease control was calculated using the Abbott (1925) formula.

2.5. Data analysis

The experimental data were analyzed using analysis of variance (ANOVA) to compare means based on Duncan test. The data were transformed to ensure compliance with the normal distribution, if necessary.

3. Results and Discussion

3.1. Characteristics related to the plant growth-promoting ability

All six strains of *Pseudomonas* spp. exhibited the ability to solubilize phosphate, synthesize IAA, form biofilms, and produce siderophores, but did not produce GAs or fix nitrogen. Among them, strain PN04 showed the highest phosphate solubilization, measuring 21.13 mm, while strain PN01 produced the highest concentration of IAA at 23.15 µg/mL after 120 h in TSB supplemented with tryptophan (Table 1, Figure 1).

Table 1. Characteristics related to the plant growth-promoting ability of *Pseudomonas* strains

Strains	Phosphorus solubilization (mm)	IAA production (mg/mL)	Biofilm formation	Siderophore production	GAs production	Nitrogen fixation
PN01	20.90[a]	23.15[a]	+	+	-	-
PN02	14.67[d]	12.89[b]	+	+	-	-
PN03	16.97[c]	7.09[c]	+	+	-	-
PN04	21.13[a]	15.00[b]	+	+	-	-
PN05	19.63[b]	12.90[b]	+	+	-	-
PN06	10.97[e]	3.03[c]	+	+	-	-
CV (%)	8.87	8.74				
F	5.69[**]	7.92[**]				

In the same column, numbers with the same letters represent statistically insignificant differences; (**) α = 0.01. IAA: indole-3-acetic acid; Gas: gibberellic acid.

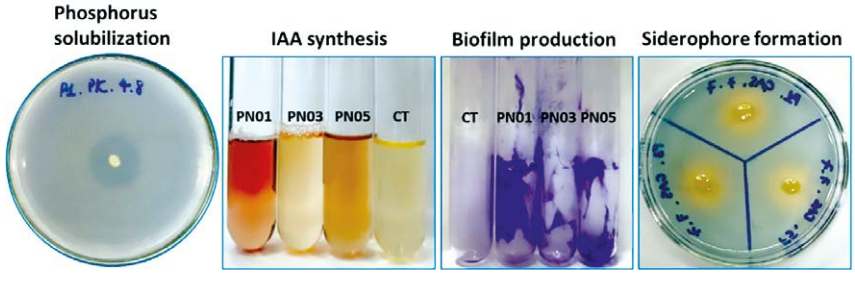

Figure 1. Characteristics related to the plant growth promotion ability of *Pseudomonas* spp.

3.2. Influence of *Pseudomonas* spp. on tomato seed germination *in vitro* condition

After 72 h of cultivation, tomato seeds treated with PN03 strain had the highest germination rate (75.0%), equivalent to the control (71.3%), whereas the seeds treated with PN04 strain had the lowest germination rate (49.67%). Seeds treated with the remaining four bacterial strains had germination rates ranging from 53.00% to 64.33% ($P < 0.01$) (Figure 2).

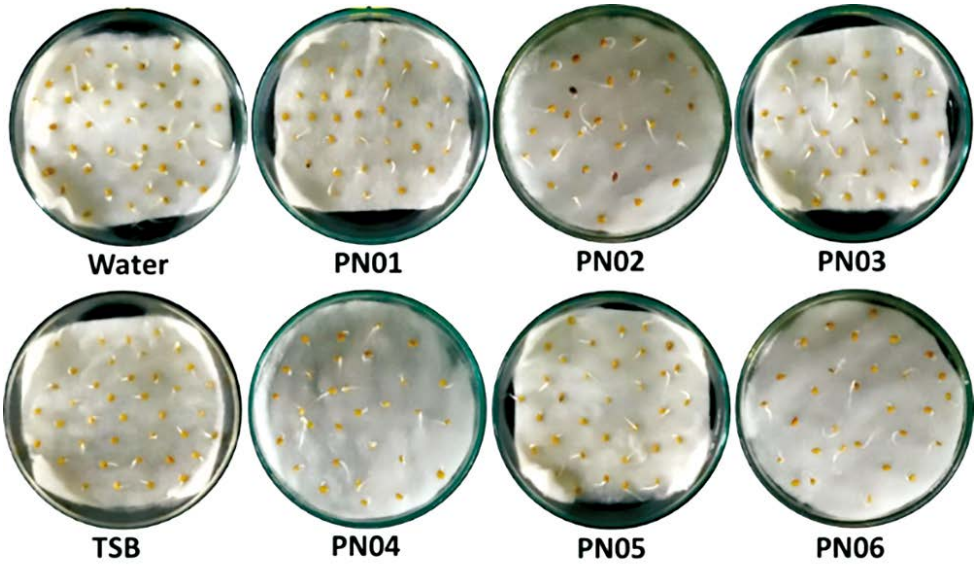

Figure 2. Influence of bacteria on tomato seed germination.

3.3. Growth promotion effect of *Pseudomonas* spp. on tomato plants in greenhouse condition

The results showed that at the 14-day stage, the height of plants treated with strain PN03 reached the highest value of 12.88 cm. Control treatments and strains PN02, PN05, and PN06 had heights ranging from 9.00 cm to 11.75 cm. At the 28-day stage, treatment with strain PN05 had a significant impact on plant growth and development, with a height of 39.88 cm, the highest number of leaves (13.00), branches (2.59), flower clusters (3.50), and flowers (3.25) ($P < 0.05$) (Figure 3, Table 2). After 56 days of inoculation, the highest number of fruits per plant was observed in the treatment with the PN05 strain (8 fruits per plant), whereas the other bacterial strains did not show any significant difference compared with the control. However, parameters such as fruit length, fruit diameter, and fruit flesh thickness were significantly different compared with the control ($P < 0.05$) (Table 3). The results showed that strain PN05 stands out for its ability to significantly promote both vegetative and reproductive growth in tomato plants, leading to increased plant height, leaf number, branching, flower production, and fruit yield. The other strains also contributed positively to specific growth parameters and fruit quality, highlighting their potential as part of integrated plant growth-promoting strategies. Further research is needed to fully understand the mechanisms behind these effects and to validate the findings in field conditions.

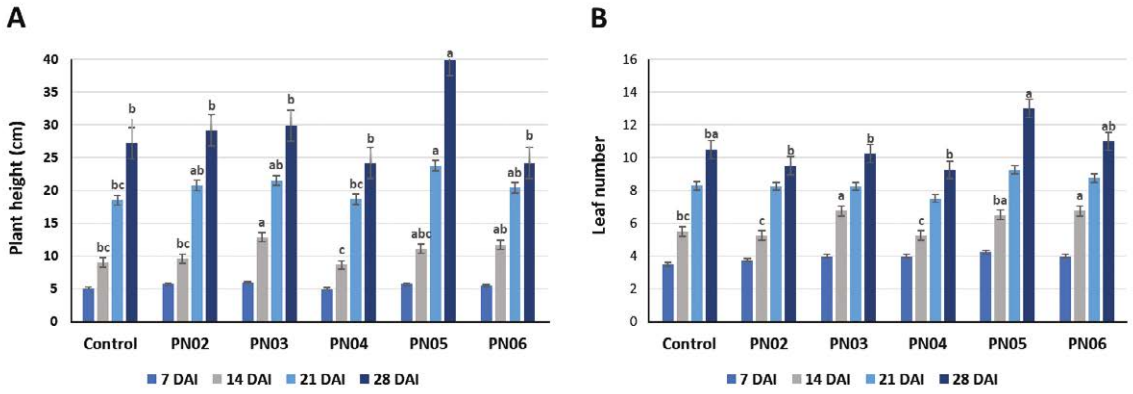

Figure 3. Plant height (A) and leaf number (B) of tomato plants treated with bacteria. DAI: days after inoculation.

Table 2. Influence of bacteria on the growth of tomato plants in greenhouse conditions 28 days after inoculation

Treaments	Branch number	Flower clusters	Flower numbers
Control	0,75^{b}	1,75^{ba}	8,00^{b}
PN02	0,25^{b}	1,25^{c}	5,75^{b}
PN03	0,75^{b}	1,75^{bc}	7,00^{b}
PN04	0,25^{b}	1,00^{c}	4,50^{b}
PN05	2,59^{a}	3,50^{a}	17,25^{a}
PN06	1,25^{b}	3,00^{ba}	10,50^{ba}
CV (%)	24.16	18.33	19.99
F	5.74**	4.37*	5.67**

In the same column, numbers with the same letters represent statistically insignificant differences; (*) α = 0.05; (**) α = 0.01.

Table 3. Fruit quality at 56 days after inoculation

Treatments	Number	Fresh weight (g)	Length (cm)	Diameter (cm)	Fruit flesh thickness (mm)
Control	4,00^{b}	77,9	3,70^{b}	4,90^{b}	43^{c}
PN02	5,50^{b}	79,8	4,13^{a}	5,37^{ba}	50^{bac}
PN03	6,25^{b}	80,9	4,20^{a}	5,50^{a}	57^{ba}
PN04	4,50^{b}	78,4	4,06^{a}	5,06^{ba}	47^{bc}
PN05	8,00^{a}	83,1	4,23^{a}	5,63^{a}	60^{a}
PN06	6,50^{b}	80,5	4,10^{a}	5,30^{ba}	50^{bac}
CV (%)	19.9	14.8	3.01	4.01	11.3
F	5.67**	0.86^{ns}	7.41**	4.93**	3.47*

In the same column, numbers with the same letters represent statistically insignificant differences; (ns) no significant difference; (*) α = 0.05; (**) α = 0.01.

3.4. Antagonistic ability against *Fusarium solani* by *Pseudomonas* strains under *in vitro* conditions

Using the dual culture method, all bacterial strains except PN06 showed varying degrees of antagonism against *F. solani*. Strain PN01 had the lowest resistance rate (48.1%), while strains PN02 and PN05 had the highest resistance rates (60.3% and 58.9%, respectively) ($P \leq 0.05$). In the agar well diffusion method, all six bacterial strains exhibited resistance against *F. solani*. PN06 had the lowest antagonistic efficiency (32.5%), while PN02 and PN05 had the highest efficiencies (45.8% and 45.3%, respectively) ($P \leq 0.05$) (Figure 4). Strains PN02 and PN05 were further evaluated for their ability to control the disease in tomato plants under *in vitro* and greenhouse conditions.

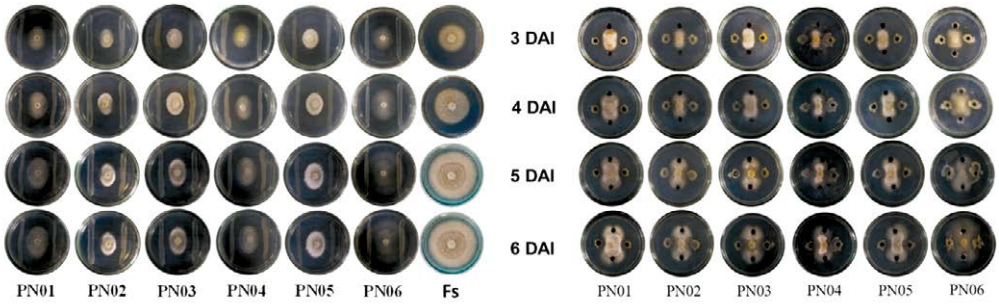

Figure 4. Inhibitory ability against *Fusarium solani* of the six *Pseudomonas* strains. Dual culture method (left) and agar well diffusion method (right). DAI: days after inoculation. Fs: *Fusarium solani*.

3.5. Antifungal activity of *Pseudomonas* strains against *Fusarium solani* under *in vitro* conditions

After 72 h of inoculating the tomato seedlings with *F. solani*, symptoms of withering appeared, and by 7 DAI, the plants had completely dried up. Treatments with PN02 and PN05 whether applied before fungi (PN02 + Fs, PN05 + Fs), or after (Fs + PN02, Fs + PN05) showed no symptom of yellowing and exhibited healthy growth (Figure 5). The experimental results indicated that two *Pseudomonas* strains could limit the impact of *F. solani* on young tomato plants *in vitro*. This lays the foundation for further investigation into the disease control abilities of two strains, PN02 and PN05, on mature tomato plants in greenhouse conditions.

Figure 5. Tomato seedlings treated with *Pseudomonas* bacteria and *Fusarium solani*, 72 h after inoculation (top panel) and 7 days after inoculation (bottom panel). CT: control, Fs: *Fusarium solani*. P2, P5: PN02, PN05 strains.

3.6. Ability to control tomato root rot caused by *Fusarium solani* by *Pseudomonas* spp. strains under greenhouse conditions

At 28 DAI, the disease incidence showed a difference between the two groups. The group with a high disease incidence ranging from 77.8% to 88.9% was treated with fungal infection before bacterial treatment (*F. solani* + PN02; *F. solani* + PN05; *F. solani* simultaneous with PN05), and the group with a low disease incidence of 55.6% was treated with bacterial inoculation before fungal infection (PN02 + *F. solani*; PN05 + *F. solani*; PN02 simultaneous with *F. solani*) (Table 3).

Table 3. Disease index (%) and control efficacy (%) of tomato root rot caused by *Fusarium solani* of two bacterial strains in greenhouse conditions

Treatments	Height (cm)	Root length (cm)	Disease Incidence (%)	Disease Index (%)			Control efficacy (%)
	28 DAI	28 DAI	28 DAI	14 DAI	21 DAI	28 DAI	28 DAI
Control	41.7^{ba}	23.3^{b}	0.00^{e}	0.00	0.00^{c}	0.00^{d}	-
F. solani	36.5^{c}	20.1^{c}	100^{a}	6.66	22.2^{a}	68.9^{a}	-
F. solani + PN02	45.0^{a}	26.7^{a}	88.9^{b}	2.22	11.1^{b}	42.3^{b}	38.8
F. solani + PN05	44.7^{ba}	25.8^{ba}	88.9^{b}	4.45	20.0^{ab}	35.6^{bc}	48.5
F. solani - PN02	41.0^{bac}	23.3^{b}	55.6^{d}	2.22	15.6^{ab}	26.7^{c}	61.2
F. solani - PN05	39.7^{bc}	23.1^{b}	77.8^{c}	2.22	17.8^{ab}	35.6^{bc}	48.3
PN02 + F. solani	40.7^{bac}	23.4^{b}	55.6^{d}	4.45	11.1^{b}	40.0^{bc}	41.9
PN05 + F. solani	41.2^{bac}	23.1^{b}	55.6^{d}	2.22	22.2^{a}	42.2^{b}	38.7
CV (%)	10.59	6.09	31.7	9.46	34.6	35.9	
F	2.88*	7.64**	4.53**	0.73^{ns}	13.3**	15.9**	

In the same column, numbers with the same letters represent statistically insignificant differences; (ns) no significant difference; (*) α = 0.05; (**) α = 0.01. DAI: days after inoculation.

At day 28 after inoculation, the disease index increased by 68.9% in the fungal treatment whereas in the bacterial treatments, the disease index ranged from 26.7 - 42.3% and the disease control efficacy ranged from 38.7% (PN05 + *F. solani*) to 61.2% (*F. solani* - PN02) (Table 3, Figure 6).

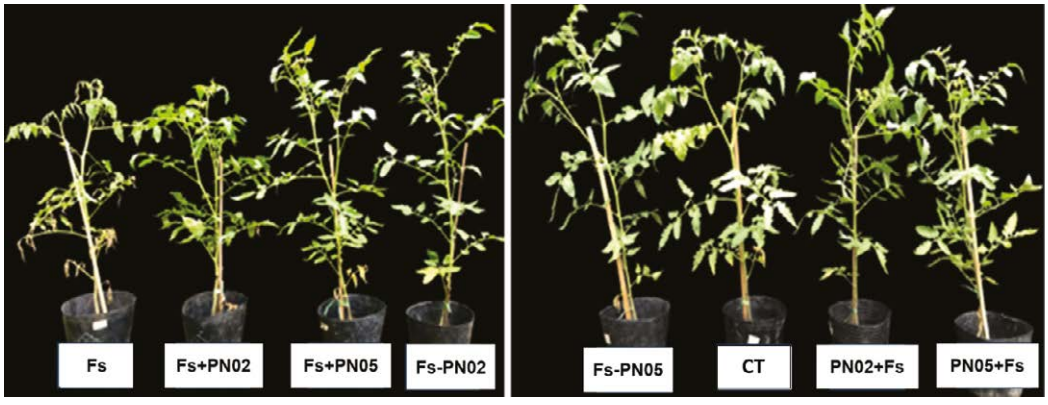

Figure 6. Ability to control root rot disease by *Pseudomonas* sp. strains PN02 and PN05**.** CT: control, Fs: *Fusarium solani*.

Some PGPR species found worldwide could promote growth in various crops. They can be used as biofertilizers, biopesticides, root bacterial treatments, and bio-stimulants (Ryu et al., 2003). *Pseudomonas* and *Bacillus* are the most extensively studied and widely used PGPRs. The mechanisms by which PGPR promotes plant growth involve the synthesis of certain metabolites (auxin, cytokinin, and gibberellin), the production of siderophores, hydrocyanic acid (CN), antibiotics, and volatile compounds. Competition, inducing systemic resistance, and mineral solubilization (such as phosphorus or potassium) are other factors that *Pseudomonas* uses to enhance crop productivity (de Andrade et al., 2023). This bacterial species also exhibited biological control activity against plant pathogens. In this study, all *Pseudomonas* strains showed the ability to solubilize phosphate, synthesize IAA, form biofilms, and produce siderophores, but did not produce GAs or fix nitrogen. *In vitro* conditions, these strains did not improve tomato seed germination rate after 3 days of incubation. Reports have shown that phytohormones produced by bacteria may reduce plant seed germination (Tabatabaei et al., 2016; Pirog, 2018; Mohammed et al., 2023). The bacterial density and treatment duration for each type of plant seed need to be determined before application. All five tested bacterial strains showed positive effects on plant growth through growth and fruit quality parameters, with *Pseudomonas* sp. strain PN05 having the most pronounced effect.

Pseudomonas spp. can colonize the roots of plants and produce antifungal metabolites represent a real alternative chemical fungicide (Walsh et al., 2001). Studies selecting *Pseudomonas* bacteria using antagonism against plant pathogens have also been conducted. The two bacterial strains PN02 and PN05 showed effective antagonism against *Fusarium solani*. Strain PN02 was identified as *Pseudomonas aeruginosa* (Tran et al., 2023) whereas PN05 has not been identified. Yasmin et al. (2014) reported an antagonistic *P. aeruginosa* strain that showed effectiveness against *F. oxysporum*, *F. moniliforme*, *F. solani*, and *Rhizoctonia solani* causing diseases in cotton; it inhibited the *in vitro* development of *Colletotrichum gloeosporioides* and *Rhizoctonia solani* (Sasirekha & Srividya, 2016). To date, there have been few reports on the biological control potential of *P. aeruginosa* under field conditions, possibly because of biosafety concerns. *Pseudomonas* strains have been evaluated as biocontrol agents against *Fusarium solani* in multiple studies. Abo-Zaid et al. (2023) found that *P. aeruginosa* F2 and *P. fluorescens* JY3 produced siderophores with high efficiency and showed antagonistic activity against *F. oxysporum* and *R. solani*. Kedia and Sharma isolated *Pseudomonas* strains from tomato rhizosphere soil and found that two strains, *Pseudomonas* VK1 and VK2, showed antagonistic activity against *F. solani* (Kedia et al., 2023). Hu et al. (2021) isolated *P. aeruginosa* strain YY322 from saffron rhizosphere soil and found that it exhibited highly effective antagonistic activity against *F. oxysporum* and *F. solani*. These findings demonstrate the potential of *Pseudomonas* strains as biocontrol agents for plant diseases in Vietnam, offering sustainable alternatives to chemical fertilizers and pesticides.

4. Conclusions

All *Pseudomonas* spp. exhibited no negative effects on tomato growth and development, with *Pseudomonas* sp. strain PN05 having the most pronounced impact. Strains PN02 and PN05 inhibited *F. solani* and reduced root rot disease under *in vitro* and greenhouse conditions.

Further research could assess the persistence of these biocontrol effects over multiple growing seasons and investigate the mechanisms by which *Pseudomonas* inhibits pathogen growth and induces biochemical changes in treated tomato plants.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgements

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