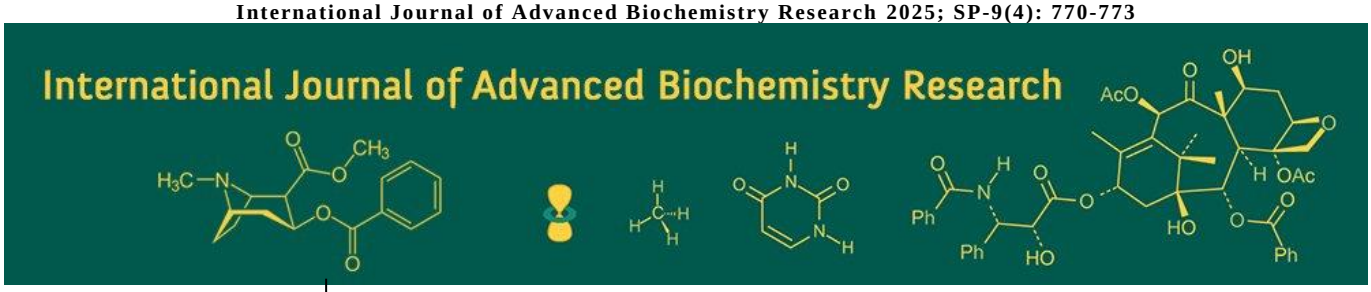

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; SP-9(4): 770-773
www.biochemjournal.com
Received: 19-01-2025
Accepted: 23-02-2025

Rashmi Nigam
Department of Plant Pathology, Janta Vedic College, Baraut, Baghpat, Uttar Pradesh, India

Joginder Singh
Department of Horticulture, Janta Vedic College, Baraut, Baghpat, Uttar Pradesh, India

Anupam Tiwari
Department of Horticulture, Janta Vedic College, Baraut, Baghpat, Uttar Pradesh, India

MS Rathi
Department of Horticulture, Janta Vedic College, Baraut, Baghpat, Uttar Pradesh, India

Corresponding Author:
Rashmi Nigam
Department of Plant Pathology, Janta Vedic College, Baraut, Baghpat, Uttar Pradesh, India

Assessment of *Pseudomonas* spp. against *Rhizoctonia solani, Fusarium oxysporum* and *Pythium ultimum* pathogens of pea

Rashmi Nigam, Joginder Singh, Anupam Tiwari and MS Rathi

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i4Si.4260

Abstract
Pea *(Pisum sativum)* cultivation is frequently threatened by soilborne pathogens, notably *Rhizoctonia solani, Fusarium oxysporum,* and *Pythium ultimum*. This research paper explores the potential of *Pseudomonas* spp. as biocontrol agents to manage these pathogens. In the present study, an attempt was made to isolate *Pseudomonas* spp., a potent plant growth promoting rhizobacteria in the rhizosphere. The study demonstrated the presence of fluorescent and nonfluorescent *Pseudomonads* in the rhizosphere of pea through appropriate microbiological and biochemical methods. Fifteen isolates of *Pseudomonas* were isolated from rhizosphere and identified by biochemical tests. The present study deals with series of *in vitro* experiments were conducted to evaluate the efficacy of different *Pseudomonas* strains in inhibiting soil borne pathogen growth. Five strains were tested for their antagonistic potential against *Rhizoctonia solani, Fusarium oxysporum* and *Pythium ultimum*. The study exhibited that all *Pseudomonas* strains significantly inhibited the growth of *Fusarium oxysporum* f. sp. *pisi, Rhizoctonia solani* and *Pythium ultimum* as compared to control. Findings of the study indicates that *Pseudomonas* spp. exhibit significant antagonistic activity against the targeted pathogens, suggesting their potential for integrated pest management in pea crops.

Keywords: *Pseudomonas spp., Biocontrol, Rhizoctonia solani, Fusarium oxysporum, Pythium ultimum,* pea, soil borne pathogens

Introduction
Peas are an important legume crop cultivated globally, valued for their nutritional content and role in crop rotation. However, their productivity is severely affected by soilborne pathogens such as *Rhizoctonia solani, Fusarium oxysporum,* and *Pythium ultimum*. These pathogens cause diseases that result in significant yield losses. Traditional chemical control methods are not only costly but also detrimental to the environment. Increasing knowledge and growing concern of pesticide applications on environment have aroused interest in alternative methods of plant protection. Plant growth promoting rhizobacteria (PGPR) are the important group of microorganisms, which play a major role in the biocontrol of plant pathogens. PGPR can profoundly improve seed germination, root development, and water uptake by plants (Siddiqui and Akhtar, 2010) [12]. Out of different organisms used for biocontrol, rhizosphere microorganisms may provide a front line defense against pathogen attack and are ideal for use as biocontrol agents (Weller 1988; Siddiqui 2006) [19, 13]. Biocontrol using beneficial microbes, particularly *Pseudomonas* spp., offers a sustainable alternative. *Pseudomonas* spp. are known for their diverse mechanisms of pathogen suppression, including competition, antibiosis, and induced systemic resistance. Biocontrol agents in general and *Pseudomonas fluorescens* in particular have gained importance as a component of Integrated Pest Management for sustainable agriculture (Mukhopadhyay, 1987) [9]. *Pseudomonas fluorescens* belong to Plant Growth Promoting Rhizobacteria (PGPR), the important group of bacteria that play a major role in the plant growth promotion, induced systemic resistance, biological control of plant pathogens *etc*. This study aims to evaluate the efficacy of various Pseudomonas strains against these pathogens and assess their potential for practical application.

Materials and Methods

Pathogen Isolates and Pseudomonas Strains

- **Pathogens:** *Rhizoctonia solani, Fusarium oxysporum,* and *Pythium ultimum* were isolated from infected pea plants and identified based on morphological and molecular characteristics. Pure cultures of the pathogens were obtained by the single hyphal tip method (Rangaswami,1972) [11]
- ***Pseudomonas* Strains:** *Pseudomonas* strains were isolated from the rhizosphere of pea by serial dilution method. One gram of rhizosphere soil was collected and transferred in 9ml of sterilized water and shaken thoroughly to get the soil particle uniformly dispersed in the suspension. After shaking for 15 minutes dilutions were prepared. One ml of suspension from the first dilution ($1:10^{-1}$) was aseptically transferred to another tube (10^{-2}) and this procedure further repeated till the dilution 10^{-6} was obtained. Transfer 0.1ml of sample from each dilution in King's B medium. Spread it by sterilized glass spreader. The plates were then incubated for 3 days at 30 ±1°C. The growth of rhizobacterial colonies on King's B medium plates were observed and recorded. The selected isolates of rhizobacteria were subjected to Plant growth promoting traits like HCN and ammonia production. In order to identify volatile toxicity in the strains HCN production test was conducted by using filter paper pre-soaked in picric acid solution (Wei, *et al.*, 1991) [18]. A total of 5 *Pseudomonas* isolates, Ps1, Ps2, Ps5, Ps9, Ps10 were selected based on their known biocontrol properties.

In vitro antagonistic assays

Dual Culture Technique: *In vitro* antagonistic efficacy of *Pseudomonas* isolates on inhibition of test pathogens *i.e. Fusarium oxysporum* f. sp. *pisi, Rhizoctonia solani* and *Pythium ultimum* was studied, through dual culture technique. Four discs of the test fungus were placed in the periphery of petriplate at equal distance there after the blotting paper discs having the diameter of 10mm dipped in bacterial suspension and placed in the centre of petriplates. In control, no blotting paper was placed and petri plates were incubated for five days at 30ºC. Each treatment had three replications. Radial growth inhibition of test pathogens was measured at an interval of 24h for five days to record different stages of antagonism. The observations on radial growth inhibition of test pathogens *i.e*. *Rhizoctonia solani*, *Fusarium oxysporum,* and *Pythium ultimum* were recorded after 120 hrs. The percent inhibition over control, noted after 5 days of incubation. The percent inhibition over control, noted after 5 days of incubation was calculated by the following formula (Vincet, 1947; Nigam *et al*.,2016) [, 10].

$$I = \frac{C\text{-}T}{C} \times 100$$

Where,
I = Percent Inhibition
C = Colony diameter in control
T =Colony diameter in treated petriplate.

Statistical Analysis Data were analyzed using ANOVA to determine the significance of differences between treatments.

Results and Discussion

Identification of plant pathogens

Three major fungal pathogens *viz Rhizoctonia solani, Fusarium oxysporum,* and *Pythium ultimum* were found associated with roots of pea plant based on their cultural and morphological charactersticts.

Identification and characterization of Pseudomonas isolates

The rhizospheric region of pea yielded 35 different bacterial colonies. Among these, fifteen isolates (Ps1 to Ps15) were identified as Pseudomonads. These isolates of *Pseudomonas* were characterized on the basis of their morphological and biochemical characteristics. *Pseudomonas* isolates grown on medium produced pale yellow and mucoid colonies. All the isolates of *Pseudomonas* were found to be gram negative, chaemohetrotrophic motile rods with polar flagella. In this study, the *Pseudomonas* isolates were also screened for their capacity to fix nitrogen and excrete ammonia and HCN production. The observations revealed that all the isolates except Ps7 and Ps8, produce ammonia, though, two of them (Ps2 and Ps5) exhibited higher production of ammonia (Table-1). All *Pseudomonas* isolates (Ps1 to Ps15) were also tested for their PGPR activity such as production of HCN. Among the 15 isolates of *Pseudomonas*, Ps1, Ps2, Ps5, Ps9, Ps10 and Ps11 produced hydrogen cyanide and turned piric acid paper to brown orange colour (Table 1). Hydrogen cyanide is produced by many rhizobacteria and has been found to play a very significant role in the biological control of soil borne pathogens (Voisard *et al.*, 1989) [17]. Cook (1993) [1] reported that certain plant associated bacteria particularly fluorescent pseudomonads have been exploited for suppression of crop diseases. *Pseudomonas* sp. are known to produce volatile compounds. One such metabolite is HCN (Tripathi and Johri, 2002) [14].

Among the all *Pseudomonas* isolates, five isolated *Pseudomonas* strains exhibited varying degrees of antagonistic activity and showed the largest zones of inhibition. Graphical pattern presented in Figure 1 reveal that all *Pseudomonas* isolates significantly inhibited the growth of test pathogens in comparison to control. However, the maximum growth inhibition of *Fusarium oxysporum, Rhizoctonia solani* and *Pythium ultimum* was resulted due to isolate Ps5 followed by Ps2 and Ps1. The percent inhibition in growth of test pathogens corresponding to the isolates Ps5, was recorded 72.5, 70.2 and 70.2 percent for *Fusarium oxysporum, Rhizoctonia solani* and *Pythium ultimum,* respectively, the differences in the radial growth inhibition of test pathogens due to isolates Ps5, Ps2 and Ps1 were found to be statistically non-significant when compared from one another. On the other hand isolates Ps9 and Ps10 were found to be least zone of inhibition and exhibited insignificant difference in their efficacy when compared from each other. A graphical representation of Figure 1 also indicates that, there was a proportionate increase in the antagonistic potential of all *Pseudomonas* isolates at different interval after inoculation. It was also noted that isolates Ps1, Ps2 and Ps5 exhibited more or less a similar trend having a close pace in inhibiting the growth of test pathogens at each interval of observation. The present findings are similar with the findings of Duffy and Defago 1999 [3]; Nigam *et al*.,2016 [10] and Delany *et al.*, 2000. [2] reported that *P. fluorescens* is very effective antibiotic producer and found that the anti-fungal metabolite 2,4-

diacetyl pphloroglucinol play a major role in the biocontrol capabilities of *P. fluorescens*. Many secondary metabolites of *P. fluorescens* acts as antibiotics against plant pathogens. The *P. fluorescens* produces antifungal compounds like phenazine-1-carboxylic acid (PCA), 2, 4 - diacetylphloroglucinol (DAPG), pyocinine, pyrrolnitrin, pyoluteorin and oomycin-A which are fungistatic, inhibiting spore germination and lysis of fungal mycelia (Karunithi *et al.*, 2000). The first antibiotics clearly implicated in biocontrol by fluorescent pseudomonads were the phenazine derivatives (Handelsman and Stabb 1996) [4]. *P. fluorescens* strain CHA0 produces hydrogen cyanide, 2,4-diacetylphloroglucinol, and pyoluteorin, which directly interferes with the growth of various pathogens and contributes to the disease suppression (Voisard *et al.* 1989; Keel *et al.* 1992; Maurhofer *et al*. 1994b; Duffy and Defago 1999) [17, 6, 8, 3].

Urkade (2010) [15] studied invitro antibiosis of *Pseudomonas fluorescens against Rhizoctonia bataticola* and reported that *Pseudomonas fluorescens* isolates Pf2 and Pf5 were most effective against *R. bataticola* which recorded 30.28% and 28.12% growth inhibition, respectively. Suppression of *Rhizoctonia bataticola* by *Pseudomonas fluorescens* in agar plate might be due to the production of siderophores (Laha *et al.*, 1992) [7]. The study demonstrates that Pseudomonas spp. can effectively manage soilborne pathogens affecting pea plants. The ability of these strains to inhibit pathogen growth through multiple mechanisms, including competition and VOCs production, suggests their potential for use in integrated pest management systems. Ps5, Ps2 and Ps1 emerged as the most promising candidates due to their broad-spectrum activity and significant impact on plant health. *Pseudomona*s spp. exhibit substantial potential as biocontrol agents against *Rhizoctonia solani*, *Fusarium oxysporum*, and *Pythium ultimum*. Their effectiveness in both *in vitro* and *in vivo* settings highlights their suitability for developing sustainable disease management strategies in pea cultivation. Future research should focus on optimizing application methods and understanding the interaction dynamics between Pseudomonas strains and pea plants.

Table 1: HCN and Ammonia Production by Pseudomonas isolates

Pseudomonas isolates	HCN Production	Ammonia Production
Check	-	-
PS1	+	++
PS2	+	+++
PS3	-	+
PS4	-	+
PS5	++	+++
PS6	-	+
PS7	-	-
PS8	-	-
PS9	+	+
PS10	+	+
PS11	+	+
PS12	-	+
PS13	-	+
PS14	-	+
PS15	-	+

+ Low
++ Moderate
+++ Strong
- Not detected

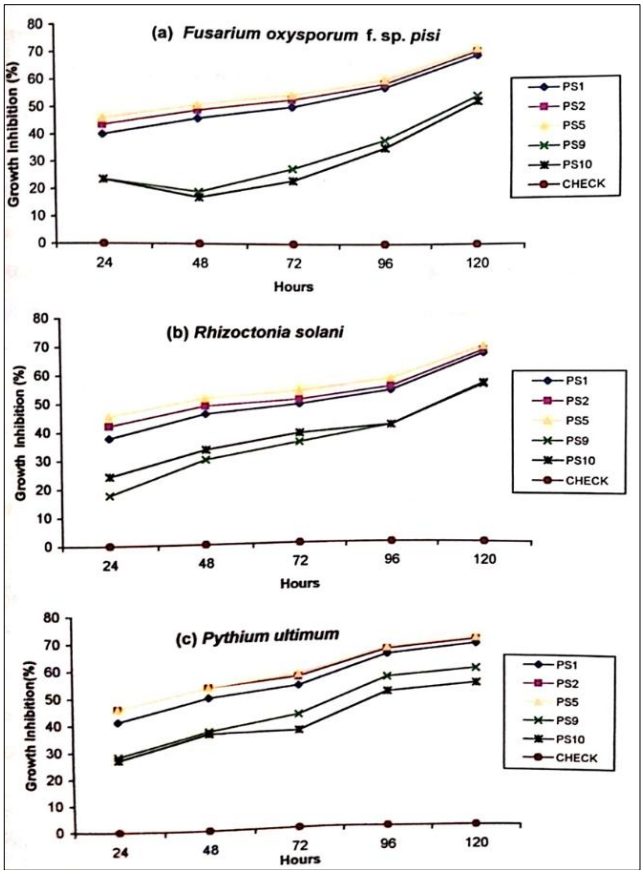

Fig 1: *In vitro* efficacy of *Pseudomonas* isolates against *Fusarium oxysporum*, *Rhizoctonia solani* and *Pythium ultimum*

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