Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(3): 308-311
Received: 05-03-2020
Accepted: 09-04-2020

Nohar Singh
Department of Plant Pathology, College of Agriculture, IGKV Raipur, Chhattisgarh, India

Nirmal Prasad
Department of Plant Pathology, College of Agriculture, IGKV Raipur, Chhattisgarh, India

PK Tiwari
Department of Plant Pathology, College of Agriculture, IGKV Raipur, Chhattisgarh, India

Kamlesh Kumar Mohle
Department of Plant Pathology, College of Agriculture, IGKV Raipur, Chhattisgarh, India

Corresponding Author:
Nohar Singh
Department of Plant Pathology, College of Agriculture, IGKV Raipur, Chhattisgarh, India

In-vitro evaluation of *P. fluorescens* isolates for antagonistic potential against *Rhizoctonia solani* and *Sarocladium oryzae*

Nohar Singh, Nirmal Prasad, PK Tiwari and Kamlesh Kumar Mohle

Abstract
Rice (*Oryza sativa* L.) is second most important cereal and the staple food for more than half of the world's population. *P. fluorescens* is the plant growth-promoting rhizobacteria (PGPR) are the rhizosphere bacteria that can enhance plant growth by a wide variety of mechanisms like phosphate solubilization, siderophore production, biological nitrogen fixation, production of 1-Aminocyclopropane-1- carboxylate deaminase (ACC), phytohormone production, exhibiting antifungal activity, production of volatile organic compounds (VOCs), induction of systemic resistance, promoting beneficial plant-microbe symbioses, interference with pathogen toxin production etc. In the study of Twelve different isolates of *P. fluorescens* were tested for their antagonistic potential against *Rhizoctonia solani* and *Sarocladium oryzae* pathogens. The results revealed that among all isolates the isolate P11exhibited *in-vitro* maximum antagonistic potential and significantly reduced mycelial growth (23.00 mm) and percent inhibition (74.44%) of *Rhizoctonia solani*. Whereas among all isolates maximum reduction in mycelial growth (12.66 mm) and percent inhibition (70.55%) was recorded with isolate P29 against *Sarocladium oryzae.*

Keywords: *P. fluorescens*, *Rhizoctonia solani*, *Sarocladium oryzae*

Introduction
Rice (*Oryza sativa* L.) is second most important cereal and the staple food for more than half of the world's population. It provides 20% of the worlds dietary energy supply followed by Maize and Wheat. In the world at present the area of rice is 162.26 M ha with production of 483.80 million metric tons and productivity of 2.98 Mt ha^{-1}. In India the area of rice is 44.50 M ha with production of 106.50 million metric tons and productivity 3.59 Mt ha^{-1}. In Chhattisgarh state rice occupies an area of 3.68 M ha with the production of 5.22 Mt ha^{-1} and productivity of 1.14 Mt ha^{-1} (Anonymous, 2016) [1].

Sheath blight is one major biotic constraints of rice. The disease is caused by *Rhizoctonia solani* Kuhn (teleomorph: *Thanetophorus cucumeris* (Frank) Donk). The disease has been named as "Sheath blight" because of primary infection on leaf sheath. The symptoms of the disease appear on leaf and leaf sheath as 2-3 cm long greenish gray lesions, turning to straw colour and surrounded by bluish gray narrow bands. The lesions increase in size and girdle the stem. Once the infection is established, it spread through contact between diseased and healthy plants.

P. fluorescens is the plant growth-promoting rhizobacteria (PGPR) are the rhizosphere bacteria that can enhance plant growth by a wide variety of mechanisms like phosphate solubilization, siderophore production, biological nitrogen fixation, production of 1-Aminocyclopropane-1-carboxylate deaminase (ACC), phytohormone production, exhibiting antifungal activity, production of volatile organic compounds (VOCs), induction of systemic resistance, promoting beneficial plant-microbe symbioses, interference with pathogen toxin production etc.

Material of Method
Total twelve numbers of isolates i.e. P11, P12, P13, P15, P19, P20, P21, P22, P23, P26, P27 and P29 were also tested for their efficacy as bio-control agent against the phyto-pathogens *R. solani* and *S. oryzae* of rice. 20 ml melted sterilized potato dextrose agar (PDA) poured in sterilized Petri dishes. A heavy inoculums from an actively growing *P. fluorescens* was streaked at 1 cm away from the edges of the plate and the mycelial disc of the pathogens were placed at the centre of Petri- plates. Control plates were inoculated only with phyto-pathogens but not with *P. fluorescens* isolates and mycelial growth (mm) recorded in three days interval.

Percent inhibition of pathogens by *Pseudomonas* isolates over control was calculated by using the formula of Vincent (1947) [7]:

[(Growth of pathogen in control - Growth of pathogen with *Pseudomonas* isolate)/ Growth of pathogen in control] x 100

Result and discussion

In vitro antagonistic potential of different isolates of *P. fluorescens* was studied on the mycelial growth of *R. solani* of rice following dual culture method. The mycelial growth was assessed after 3 days of inoculation. There were differences in the antagonistic abilities of isolates of *P. fluorescens* against *R. solani.* All the 12 isolates of *P. fluorescens* showed reduction in different rates of mycelial growth and % inhibitions of *R. solani* ranging from 74.44 to 54.48% respectively. The maximum mycelial growth (23.00 mm) inhibition and % inhibition (74.44%) was recorded by *P. fluorescens* isolate P11 which is statistically at par with *P. fluorescens* isolate P27 with reduced mycelial growth of (24.00 mm) and % inhibition of (73.33%), the isolate P15 with mycelial growth of (26.00 mm) and % inhibition of (71.11%) and isolate P26 with mycelial growth of (26.33 mm) and % inhibition (70.77%) were recorded. It is followed by *P. fluorescens* isolate P13 with mycelial growth of (27.33 mm) and % inhibition (69.99%), isolate P21 with mycelial growth of (27.66 mm) and % inhibition (69.33%), isolate P12 with mycelial growth of (28.33 mm) and % inhibition (68.52%), isolate P22 with mycelial growth of (28.66 mm) and % inhibition (68.22%), isolate P23 with mycelial growth of (29.00 mm) and % inhibition (67.77%), isolate P29 with mycelial growth of (29.66 mm) and % inhibition (67.11%), the isolate P19 mycelial growth of (31.00 mm) and % inhibition (65.55%).

Table 1: Efficacy of *Pseudomonas fluorescens* isolates against *Rhizoctonia solani*

Treatment	isolates	Mycelia growth(mm)			Mean mycelial growth	% inhibition
T1	P11	24	22	23	23.00	74.44
T2	P12	30	27	28	28.33	68.52
T3	P13	31	30	21	27.33	69.99
T4	P15	27	25	26	26.00	71.11
T5	P19	30	30	33	31.00	65.55
T6	P20	40	44	38	40.66	54.48
T7	P21	30	27	26	27.66	69.33
T8	P22	28	25	33	28.66	68.22
T9	P23	28	27	32	29.00	67.77
T10	P26	27	28	24	26.33	70.77
T11	P27	22	25	25	24.00	73.33
T12	P29	31	28	30	29.66	67.11
T13	Control	90	90	90	90.00	-
C.D. (5%)					4.298	
SE(m)+					1.471	

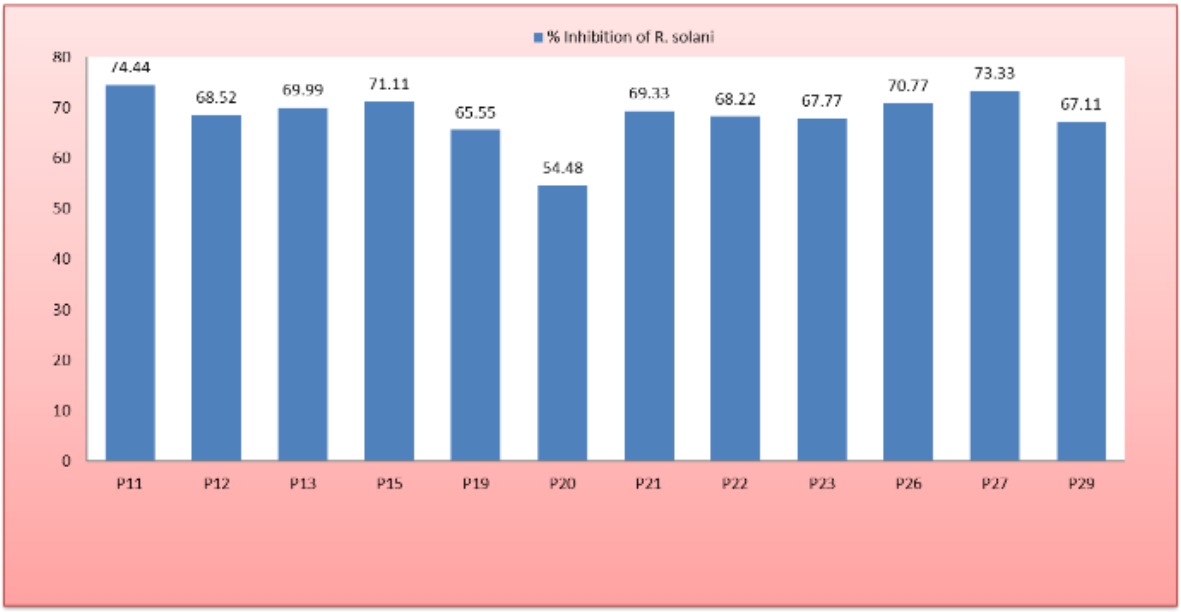

Fig. 1: % Inhibition of *Pseudomonas fluorescens* isolates against *R. solani.*

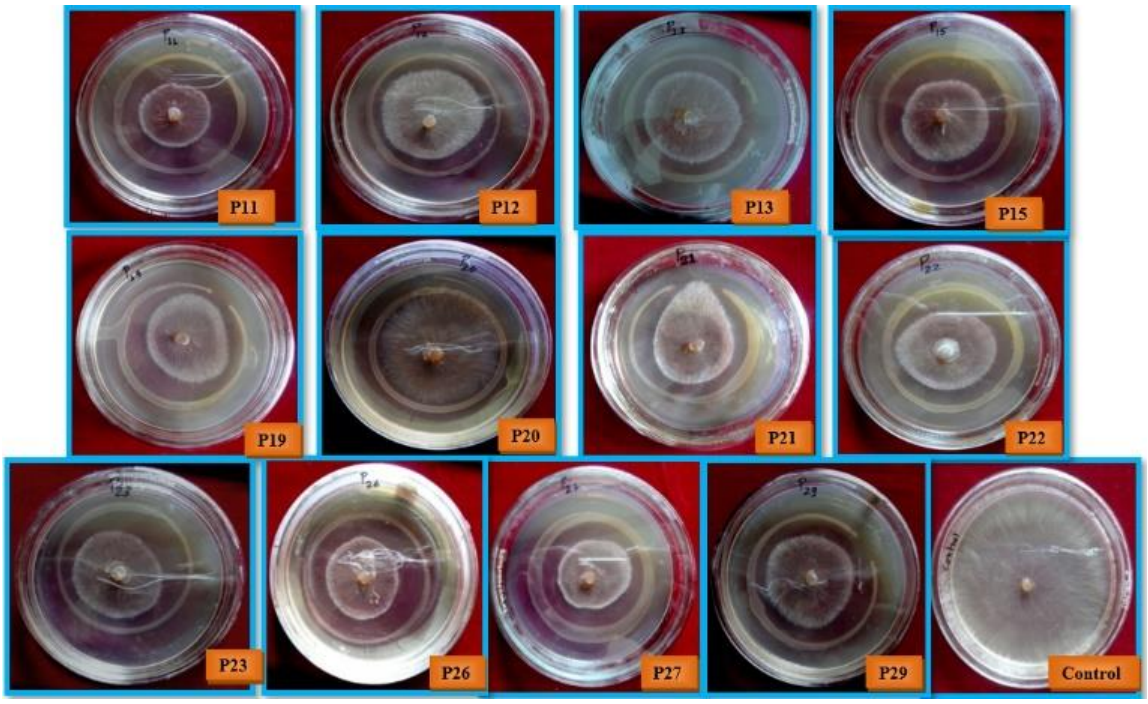

Plate 1: Efficacy of *P. fluorescens* isolates against *R. solani*

The minimum mycelial growth of (40.66 mm) and % inhibition (54.48%) was recorded by *P. fluorescens* isolate P20.

The work carried out by Pande and Chaube (2003) [5] reported that *in vitro* evaluation of *Pseudomonas fluorescens* isolates resulted in reduction of mycelial growth of *R. solani* and the inhibition zone ranged from 1.3 to 22.5 mm in different isolates on King's B medium. Similarly work carried out by Krishnamurthy and Gnanamanickam (1998) [2] reported that fluorescent *Pseudomonads* strains recorded antagonistic to *P. grisea*, *R. solani* and *S. oryzae* were isolated from rice rhizosphere. Maurya *et al*. (2014) [3] also found that *Pseudomonas fluorescens* strain P.f 07 was found most effective with the highest antagonistic activity against three fungal pathogen and show maximum inhibition of mycelial growth of *Rhizoctonia solani* (68.23%), *Fusarium moniliforme* (65.45%) and *Alternaria alternate* (48.13%).

The 12 isolates of *P. fluorescens* are assayed under *in vitro* were evaluated for their antifungal activity on the mycelium growth of *S. oryzae*. At 3rd day after inoculation (DAI) it is clear from the data, (Table 3 and Plate 4) that all isolates of *P. fluorescens* significantly reduced the fungal mycelial growth of *S. oryzae* over untreated (control). The *P. fluorescens* isolate P29 was recorded with minimum mycelium growth (4.33 mm) which is statistically at par with the isolates of P19, P23 (5.00 mm), P12, P27 (5.66 mm), P11 (6.00 mm), P21 and P26 (6.33 mm) and followed by the isolates of P13 and P22 (6.66 mm), P20 (7.00 mm), P15 (8.00 mm). The maximum mycelial growth was recorded with control treatment (9.33 mm).

At 6th DAI, all the *P. fluorescens* isolates significantly reduced the mycelia growth of *S. oryzae* over untreated treatment. *P. fluorescens* isolate i.e P29 was maximum in reduced the fungal mycelial growth (6.00 mm) which is statistically at par with the isolates P11 (7.33 mm), P19 and P21 (7.66 mm), P12 and P23 (8.00 mm) and followed by the isolates of P27 (8.33 mm), P13 (8.66 mm), P20 and P22 (9.00 mm), P26 (9.66 mm) and P15 (11.00 mm). The maximum mycelial growth was recorded with untreated (control) treatment (15.33 mm).

At 9th DAI, all the *P. fluorescens* isolates significantly reduced the mycelial growth of *S. oryzae* over untreated treatment. The *P. fluorescens* isolate P29 was effectively reduced the mycelial growth (7.00 mm) is statistically at par with the isolates of P21 (8.33 mm) and P11 (9.33 mm) are followed by isolates i.e P19 (9.66 mm), P20 and P26 (10.00 mm), P27 (10.33 mm), P12 (10.66 mm), P13 (11.00 mm) P22 (12.00 mm), P23 (13.66) and P15 (15.66). The highest mycelial growth was recorded with untreated (control) treatment (26.00 mm).

At 12th DAI, all the *P. fluorescens* isolates significantly reduced the mycelial growth of *S. oryzae* over untreated treatment. The *P. fluorescens* isolate P29 was observed highly effective and reduced the mycelial growth of (10.66 mm) is statistically at par with the isolates P12 (11.33 mm) and P20 (12.66 mm) which is followed by the isolates of P19 (13.33 mm), P11 (13.66 mm), P22 (14.00 mm), P21 (14.33 mm), P26 (14.66 mm), P13 (15.33 mm) P27 (15.66) and P15 (19.33 mm). The maximum mycelium growth was recorded with untreated (control) treatment (36.33 mm).

At final observation on 15th DAI, all the *P. fluorescens* isolates significantly reduced the mycelial growth of *S. oryzae* over untreated (control) treatment. The minimum mycelial growth (12.66 mm) and maximum % inhibition were recorded with the *P. fluorescens* isolate of P29 (70.55%) is statistically at par with the isolate of P12 with mycelial growth of (15.33 mm) and % inhibition of (64.34%) is followed by the isolates of P20 mycelial growth of (16.00 mm) and % inhibition (62.79%), P26 mycelial growth of (16.66 mm) and inhibition % (61.25%), P27 mycelial growth of (17.00 mm) and % inhibition (60.46%), P11 mycelial growth of (17.66 mm) and % inhibition (58.93%), P13 mycelial growth of (18.00 mm) and % inhibition (58.13%), P19 mycelial growth of (18.33 mm) and % inhibition (57.37%), P21 mycelial growth of (19.00 mm) and % inhibition (55.81%), P23 mycelial growth of (19.66 mm) and % inhibition (54.27%), P22 mycelial growth of (21.33 mm) and % inhibition (50.39%) and P15 mycelial growth (23.00 mm) and % inhibition (46.51%) respectively. The maximum mycelium growth was recorded with untreated (control) treatment (43.00 mm).

Table 2: *In-vitro* evaluation of the *Pseudomonas fluorescens* isolates for antagonistic potential against *Sarocladium oryzae.*

Treatment	Isolates	Mycelia (DAI)*					% inhibition
		3DAI	6DAI	9DAI	12DAI	15DAI	
T1	P11	6.00	7.33	9.33	13.66	17.66	58.93
T2	P12	5.66	8.00	10.66	11.33	15.33	64.34
T3	P13	6.66	8.66	11.00	15.33	18.00	58.13
T4	P15	8.00	11.00	15.66	19.33	23.00	46.51
T5	P19	5.00	7.66	9.66	13.33	18.33	57.37
T6	P20	7.00	9.00	10.00	12.66	16.00	62.79
T7	P21	6.33	7.66	8.33	14.33	19.00	55.81
T8	P22	6.66	9.00	12.00	14.00	21.33	50.39
T9	P23	5.00	8.00	13.66	16.66	19.66	54.27
T10	P26	6.33	9.66	10.00	14.66	16.66	61.25
T11	P27	5.66	8.33	10.33	15.66	17.00	60.46
T12	P29	4.33	6.00	7.00	10.66	12.66	70.55
T13	Control	9.33	15.33	26.00	36.33	43.00	_
C.D. (5%)		2.128	2.111	2.356	2.093	2.935	
SE(m)+		0.728	0.722	0.806	0.716	1.004	

* Day after inoculation (DAI)

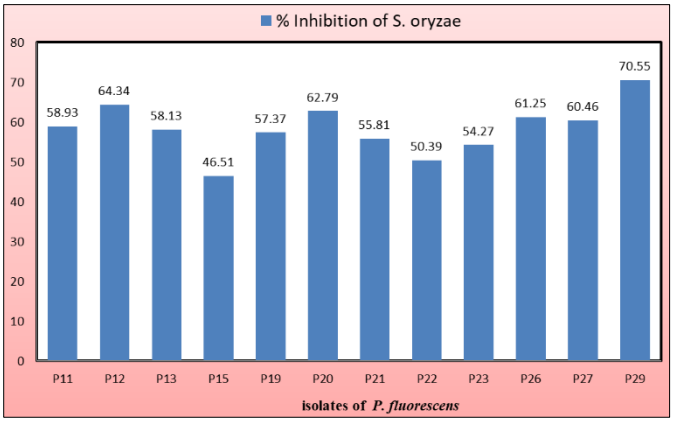

Fig 2: % Inhibition of *Pseudomonas fluorescens* isolates against *S. oryzae*

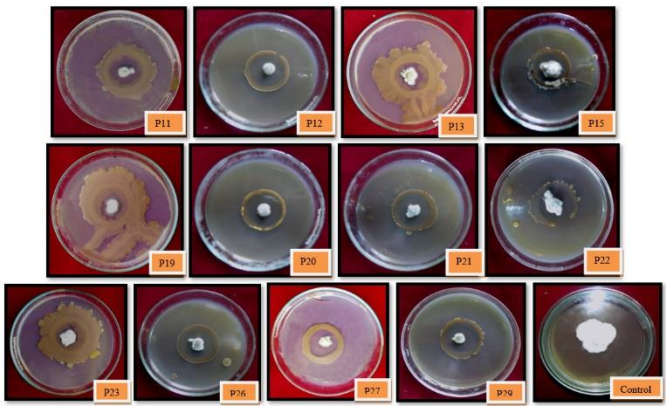

Plate 2: Evaluation of *P. fluorescens* isolates against *S. oryzae* under *in-vitro*

Present finding was supported by Saranya and Sowndaram (2014) revealed that the complete inhibition of mycelia growth of *Rhizotonia solani* (85%) and partial inhibition of *Sarocladium oryzae* (45%) against two rhizobacteria. Antifungal compound extracted from both rhizobacteria were found to exhibit maximum antagonism against rice pathogens. Meera and Balabaskar (2012) [4] also supported this investigation a maximum inhibition 93.3% was recorded by PF 013 and minimum of 68.2% was recorded with the isolate PF 03. PF 04 (89.7%), PF 011 (85.5%), PF 020 (82.4%) PF 06 (78.8%), PF 015 (75.1%) and PF 08 (71.5%) were significantly superior to other isolates against *S. oryzae*.

Conclusion

Tested 12 isolates of *Pseudomonas fluorescens* were screened *in vitro* by dual culture plate method for its ability to show antagonism against *R. solani* the maximum inhibition (74.44%) was recorded by *P. fluorescens* isolate P11. All the *P. fluorescens* isolates significantly reduced the mycelial growth of *S. oryzae* over untreated (control) treatment. The minimum mycelial growth (12.66 mm) and maximum % inhibition were recorded with the *P. fluorescens* isolate of P29 (70.55%).

Reference

1. Anonymous. Krishi Darshika I.G.K.V. Raipur (C.G.), 2016, 4-5.
2. Krishnamurthy K, Gnanamanickam SS. Biological control of rice blast by *Pseudomonas fluorescens* strain Pf7–14: Evaluation of a marker gene and formulations. Biol. Control. 1998; 13(3):158-165.
3. Maurya MK, Singh R, Tomer A. *In vitro* evaluation of antagonistic activity of *Pseudomonas fluorescens* against fungal pathogen. J. Bio-pest. 2014; 7(1):43.
4. Meera T, Balabaskar P. Isolation and characterization of *Pseudomonas fluorescens* from rice fields. Int. J Food Agril. Veterinary Sci. 2012; 2(1):113-120.
5. Pande VS, Chaube HS. Effect of *Pseudomonas fluorescens* isolates on sclerotial viability of *Rhizoctonia solani* (Kuhn). Annal. Plant Protec. Sci. 2003; 11(1):57-60.
6. Saranya DK, Sowndaram S. Biological control of rhizobacteria against rice diseases caused by *Rhizoctonia solani* (*Sheath blight*) and *Sarocladium oryzae* (*Sheath rot*). Int. J Adv. Res. 2014; 2(5):818–823.
7. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947; 159:850.