Research Note

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EVALUATION OF *Pseudomonas fluorescens* AGAINST *Pyricularia oryzae IN VITRO*

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Rice blast caused by *Pyricularia oryzae* is one of the major destructive diseases of rice growing regions of the world including India. It causes yield loss up to 80 per cent (Ou, 1985) but normally can lead to 30 per cent yield loss annually (Talbot, 2003). *P. oryzae* is a hemi biotrophic pathogen, initially grow biotrophically and then switch to necrotrophic growth, killing the infected tissues. *P. oryzae*, however, invades foliar tissues biotrophically and necrotrophically simultaneously (Kankanala *et al*., 2007). The disease can strike all aerial parts of the plant. Most infections occur on the leaves, causing diamond-shaped lesions with a grey or white center to appear, or on the panicles, which turn white and die before being filled with grain.

Pseudomonas fluorescens is a gram negative, rod shaped, non pathogenic bacteria that colonize in soil, water and plant surface environments. Since they are well adapted in soil, *P. fluorescens* strains are being investigated extensively for use in bio control of pathogens in agriculture (Ganeshan and Kumar, 2006). Biological control of plant pathogens by antagonistic micro organisms is a potential non-chemical means and is known to be a cheap and effective eco-friendly method for the management of crop diseases (Cook and Baker, 1983). Many biocontrol agents from *P. fluorescens* and closely related species are well characterized for their ability to produce antimicrobial compounds, including 2, 4- diacetylphloroglucinol (DAPG), phenazines, hydrogen cyanide and surfactants (Haas and Defago, 2005).The use of biological control agents as an alternative to fungicides is increasing rapidly in the present day agriculture, due to the deleterious effects of chemical pesticides on human health and environment. Hence in the present investigation, *Pseudomonas fluorescens* isolates were tested for their antagonistic activity against *P. oryzae.*

Two *Pseudomonas fluorescens* isolates were collected from Department of Plant Pathology, ARS, Nellore (*Pf 1* and *Pf 2*) and eight isolates (*Pf 3* to *Pf 10*) were from department of Agricultural microbiology, COA, Rajendranagar, PJTSAU. In order to find the antagonistic effect of *Pseudomonas fluorescens* against *P. oryzae, in vitro,* dual culture studies were employed (Mortan and Straube, 1955). 20 ml of PDA was poured aseptically into sterilized Petri dishes and allowed to solidify. Mycelial discs of 5 mm diameter from the edge of actively growing culture of *P. oryzae* placed on the periphery about 1 cm from the edge of the Petri dishes and *Pseudomonas fluorescens* was streaked on opposite side. Three replications were maintained for each interaction. The Petri dishes containing potato dextrose agar inoculated with *P. oryzae* (mono culture) alone served as control. All the Petri dishes were incubated at (25 ± 1°C) for fifteen days. At the end of incubation, the colony diameter of the *P. oryzae* was measured and the per cent inhibition of *P. oryzae* was calculated by adopting the formula given by Vincent (1927).

Results indicated that, the isolate *Pf* 2 had highest per cent inhibition of mycelial growth (91.11%) and it was closely followed by *Pf* 1 (89.72%). The isolates *Pf* 3, *Pf* 4 and *Pf* 8 showed the inhibitions at 70, 67.04 and 64.44 per cent, respectively. The mycelial inhibition was very low in case of the isolates *Pf* 9 (10.00%), *Pf* 10 (16.30%), *Pf* 6 (21.85%) and *Pf* 7 (23.70%) and did not sustain as evidenced by mycelial growth of *P. oryzae* which overgrew the above isolates *in vitro* in course of time (Table 1 and Plate 1).

The *in vitro* dual culture test is being used widely by researchers, to measure the mycoparasitic activity of *Pf* isolates against *P. oryzae*. This mycoparasitic activity of *Pseudomonas* spp. was well documented by Keel and Defago (1997). The results from the present study were

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Table 1. *In vitro* screening of *P. fluorescens* isolates against mycelial growth of *P. oryzae*

Isolate	*P. oryzae* Average radial growth (mm)*	Per cent growth inhibition over control*
Pf 1	9.25	89.72(71.27)[a]
Pf 2	8.00	91.11(72.62)[a]
Pf 3	27.00	70.00(56.78)[b]
Pf 4	29.67	67.04(54.95)[bc]
Pf 5	47.00	47.78(43.71)
Pf 6	70.33	21.85(27.84)[d]
Pf 7	68.67	23.70(29.12)[d]
Pf 8	32.00	64.44(53.38)[c]
Pf 9	81.00	10.00(18.31)
Pf 10	75.33	16.30(23.78)
Control	90.00	0.00(0.00)
	C.D.	2.245
	SE(m)	0.761
	SE(d)	1.076
	C.V.	3.208

* Mean of three replications

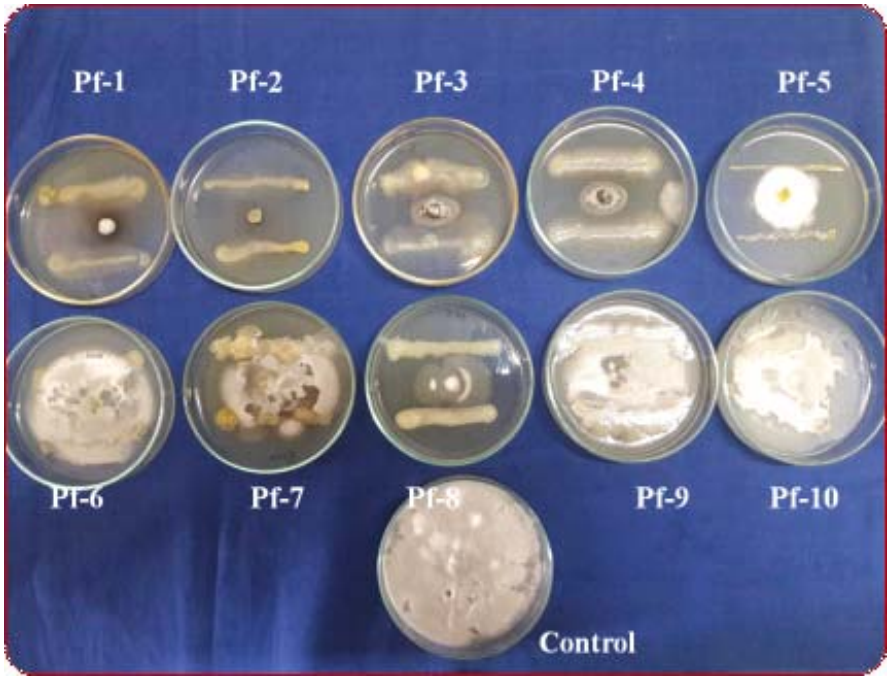

Plate 1. Antagonistic effect of *Pseudomonas fluorescens* isolates against *P. oryzae*

in confirmatory with the findings of many scientists who studied *in vitro* effect of *Pseudomonas fluorescens* against *P. oryzae* growth and reported the inhibition of 50 per cent (Goud and Muralikrishnan, 2008) and 59 per cent (Pandey and Chandel, 2014). Kulmitra *et al*., (2017) reported that some of *Pseudomonas fluorescens* did not show any inhibition of mycelial growth of *P. oryzae* as the pathogen over grew them. The inhibition of mycelial growth of the pathogen by *Pseudomonas fluorescens* may be due to the production of antibiotics. Production of antibiotis *viz.,* HCN, pyrrolnitrin, phenazine and 2, 4-diacetyl phloroglucinol and lytic enzymes by *P. fluorescens* against fungal pathogens were reported by many workers (Ramamoorthy *et al.,* 2001 and Saravanakumar *et al.,* 2008).

Among ten *Pf* isolates, *Pf* 2 and *Pf* 1 had exhibited maximum antagonistic activity against *P. oryzae*, so that these two isolates can be used in future for management of rice blast disease at field level.

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