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Research Note

Effect of *Pseudomonas fluorescens* (Migula) on *Rhizoctonia solani* Kuhn and on the growth of rice plant

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ABSTRACT: ***Pseudomonas fluorescens*** **native isolate and** ***Pseudomonas fluorescens*** **strains of pf 1, pf 27 effectively inhibited the growth of** ***Rhizoctonia solani.*** **Seed treatment with** ***Pseudomonas fluorescens*** **native isolate and** ***Pseudomonas fluorescens*** **strains of pf1 (4 x 10 ^{9}cfu/ml) recorded the maximum germination (98.66% and 86.66%), shoot length (20 and 18cm), root length (15 and 13 cm), dry matter production (60 mg and 57 mg) and vigor index (1973.2 and 1618) compared to control.**

KEY WORDS: Growth inhibition, *Pseudomonas fluorescens*, *Rhizoctonia solani*, rice, sheath blight

Rice (*Oryza sativa* L.) is the major staple food crop in India. One of the major constraints in achieving the potential yield is the occurrence of diseases. Among the diseases of rice, sheath blight caused by *Rhizoctonia solani* accounts for severe crop loss in all areas. The damage due to this range from 25 to 50 per cent (Feakin, 1987). Management of the disease is difficult in developing countries, where fungicides are very expensive. Biological control appears to be an alternate strategy in the management of disease (Cook And Baker, 1983). Foliar spray with different fungicides gives only limited success. However, biocontrol agents are very cheap, effective and can be used without much skill or additional infrastructure. Use of different strains of *Pseudomonas fluorescens* (Mew and Rosales, 1986; Savithry and Gnanamanickam, 1987; Janiscwz and Raitman, 1988; Gnanamanickam *et al.*, 1992; Vidhyasekaran and Muthamilan, 1995; Manoranjitham *et al.*, 1999) in plant disease management has been well documented. In the present study, attempts were made to assess the effect of antagonists and *P. fluorescens* strains on the growth of *Rhizoctonia solani* and rice plant.

Isolation of pathogen (*Rhizoctonia solani*)

Rice plants showing typical symptom of sheath blight were collected from rice field at Agricultural college and Research Institute, Madurai. The pathogen was isolated by tissue segment method on potato dextrose agar (PDA) medium and purified in plain agar medium by single hyphal tip method (Rangaswamy, 1958).

Isolation of *Pseudomonas fluorescens* native isolates

Pseudomonas was isolated from the

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rhizosphere soil region of rice plant collected from rice fields at different blocks of Agricultural College and Research Institute, Madurai. The antagonists were isolated by dilution plate method on Kings B Medium (King *et al.*, 1954) incubated at room temperature for 24 h and colonies were viewed under UV light at 366 nm.

Identification of *P. fluorescens*

Based on the biochemical tests, *viz.*, oxidase reduction (Kovaces, 1956), nitrate reduction, starch hydrolysis (Dowson, 1957), gelatin hydrolysis and gram reaction and also based on the number of flagella, growth at 4° C, shape and fluorescence, the isolate were identified as *P. fluorescens* (Laskin and Lecheralier, 1977).

Effect of antagonists on the *in vitro* growth of *R. solani*

The cultures of different isolates of *P. fluorescens* obtained from the Department of Plant Pathology, TNAU, Coimbatore were used in the study.

The efficacy of *P. fluorescens* isolates was tested by dual plate technique (Dennis and Webster, 1971) using PDA medium. A three-day-old 9 mm diameter PDA culture disc of the pathogen was placed on sterilized and solidified medium in a sterilized Petri-dish. Approximately at a distance of 3.5mm opposite to the fungal culture disc, the bacterial inoculum was streaked on the culture medium. The plates were incubated at 28° C. Each treatment was replicated thrice and control was also maintained. The width of the inhibition zone was measured after 96h.

Effect of *P. fluorescens* on the growth and vigour of rice

Rice seeds (ADT 36) were treated with *P. fluorescens* strains (4x 10^9 cfu/ml) at the rate of 400 ml/kg of seeds by shaking in a plastic container for 15 min. The untreated seeds served as control. Seeds treated with Carbendazim 2g/kg of seeds were also maintained for comparison. The treated seeds were then used for assessing the germination and seedling vigor. Roll towel method (ISTA, 1985) was used to study the effect of *P. fluorescens* on growth and vigor of the rice seedlings. Roll towels with treated seeds were incubated at 25±5°c. Three replications were maintained for each treatment. The germination was recorded on 7th day and expressed as per cent germination and vigor indices were also calculated by following the procedure suggested by Abdul – Baki and Anderson (1973).

Among nine native isolates of *P. fluorescens*, isolates number 1, 5 and 6 were identified as *P. fluorescens* based on the morphological and biochemical tests. All these three were rod shape and gram negative; grew at 4°c; showed fluorescence against UV light and positive reaction to oxidase test, gelatin liquefaction and nitrate tests but failed to hydrolyze the sucrose. When the native isolates of *P. fluorescens* tested for their antagonistic activity against the pathogen, *P. fluorescens* native isolate number 1 and 6 were the most effective in inhibiting the mycelial growth (55.57 and 30.00 mm of inhibition zone, respectively) (Table 1). The inhibition zone of 0.47, 3.7, 0.1, 17.33, 13.0, 8.0 and 3.5 mm were, respectively recorded by the native isolates 2, 3, 4, 5, 7, 8 and 9.

When the nine *P. fluorescens* isolates were screened for their antagonistic efficacy in suppressing the growth of *R. solani*, most of these inhibited the mycelial growth at varying degrees. The isolate *P. fluorescens* 1, *P. fluorescens* 27 and *P. fluorescens* 2 were the most effective and recorded the maximum inhibition zone of 48.33, 41.27 and 33.33 mm, respectively. The other isolates, *viz.*, *P. fluorescens* 7, *P. fluorescens* 10, *P. fluorescens* 24, *P. fluorescens* 127, and *P. fluorescens* 20 recorded 25.6, 20.5, 18.66, 13.8 and 4.0 inhibition zone, respectively (Table 1).

The effect of different isolates on growth and vigour of rice was evaluated (Table1). All the treatments recorded increased germination, shoot length, root length, dry matter production and seedling vigour as compared to control. Seed treatment with *P. fluorescens* native isolate exerted the maximum germination of 98.66 per cent, shoot length 20.0cm, root length 15.0cm, dry matter production 60.0 mg and vigor index 1973.2 as against

Table 1. Identification, Efficacy and seed treatment with *P.fluorescens*

Sl. No.	Native isolate	Growth at 4° c	Shape	Fluor-escens	Nitrate reduc-tion	Oxidase reac-tion	Starch hydro-lysis	Gelatin hydro-lysis	Growth inhibition zone (mm) *	Pf strains	Growth inhibition zone (mm) *	Seed treatment	Germina-tion (%)*	Shoot length (cm) *	Root length (cm) *	Dry matter production (mg)*	Vigour index*
1.	PfNi 1	+	Rod	+	+	+	-	+	55.57 (5.90)	*Pf* 1	48.33(6.95)	Pf Ni (400ml/kg)	98.66	20.00	15.00	60	1973.2
2.	Pf Ni2	+	Rod	-	+	+	-	+	0.47 (0.68)	*P f* 2	33.33(5.80)	Pf 1	86.66	18.67	13.33	57	1618.0
3.	Pf Ni3	-	Rod	-	-	-	+	-	3.70 (1.60)	*P f* 7	25.60(5.06)	Pf 2	68.00	15.66	11.66	47	1064.8
4.	PfNi4	-	Rod	-	-	-	+	-	0.10 (0.71)	*Pf* 10	20.50(4.53)	Pf 27	74.66	17.00	12.33	51	1296.2
5.	PfNi5	+	Rod	+	+	+	-	+	17.33 (4.16)	*P f* 14	0.34(0.71)	Pf cf * *	80.00	17.00	12.00	54.3	13.60
6.	PfNi6	+	Rod	+	+	+	-	+	30.00 (5.52)	*P f* 20	4.00(2.00)	Carbendazim 2g/kg	86.66	18.00	13.00	56.3	1559.8
7.	PfNi7	-	Rod	-	-	-	+	-	13.00 (3.60)	*P f* 24	18.66(4.28)	Control	61.33	10.50	8.00	40.0	643.9
8.	PfNi8	-	Rod	-	-	-	+	-	8.00 (2.76)	*P f* 27	41.27(6.41)						
9.	PfNi9	-	Rod	-	-	-	-	-	3.50 (1.87)	*Pf*127	13.80(3.70)						
10.	Control	-	-	-	-	-	-	-	0.00 (0.71	Control	0.00(0.71)						
CD P=(0.05)		-	-	-	-	-	-	-	0.91		0.87		7.34	0.62	1.10	2.15	177.5

* = Figures in parentheses represent transformed x + 0.5 values.
* * = Commercial formulation
Pf = Pseudomonas fluorescens
Ni = Native isolate
\+ = Positive

61.33 per cent 10.5cm, 8.0cm, 40mg and 643.9, respectively in control. Mew and Rosales (1986) reported that seed treatment with fluorescent Pseudomonas suppressed the rice sheath blight infection. Harris *et al.* (1994) also reported that seed treatment with *P. fluorescens* reduced damping off of capsicum and increased shoot length. Present study revealed that *P. fluorescens* native isolate and pf 1 can be used for effective control of sheath blight of rice and also to increase seedling vigour.

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