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Prevalence of *Pseudomonas fluorescens* in Different Cropping Systems and Soil Types

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ABSTRACT

Twenty eight isolates of *Pseudomonas fluorescens* were isolated from rhizosphere of eleven cropping systems in Guntur district. All the isolates produced pigment on King's B medium and showed fluorescence under UV light. Highest number of isolates was obtained from cotton and turmeric rhizospheres. Population and frequency of obtaining *P. fluorescens* was much higher in black soils than in sandy soils. Frequency of *P. fluorescens* isolates was highest at pH 8.0 followed by pH 7.5 and the least number of isolates was obtained at pH 7.0. All the isolates showed antagonistic activity against *Sclerotium rolfsii* in dual culture with four of them showing >75% inhibition besides inhibiting sclerotial production. Cell free culture filtrates of the four *P. fluorescens* isolates also inhibited growth and the sclerotial germination of *S. rolfsii* at concentrations higher than 40%.

Key words : Cropping systems, Dual culture, Population dynamics, *Pseudomonas fluorescens*, *Sclerotium rolfsii*, Soil types.

Fluorescent pseudomonads are ubiquitous soil microorganisms and are common inhabitants of the rhizosphere of several plant species. The population of these bacteria in rhizosphere varies based on plant species, crop stage, soil type and physico-chemical properties of soil (Van *et al.,* 1986; Bergsma-Vlami *et al.,* 2005; Houlden *et al.,* 2008). *Pseudomonas fluorescens* is nonspecific in its ability to protect plants from soil phytopathogens. Each strain can protect more than one plant species from often distinct pathogens (Coulliorot *et al.,* 2009). *Sclerotium rolfsii* is a facultative, soil-borne pathogen that causes several destructive plant diseases of different crop species. Chemical control of this soil-borne disease is ineffective, costlier and can completely pollute the environment (Mostapha, 2004). In this regard biocontrol of collar rot by fluorescent pseudomonads is likely to be an effective approach that is safe to environment and ecological balance. Present study was conducted to assess the prevalence of *P. fluorescens* in different cropping systems and evaluate them for their biocontrol efficacy with a view to exploiting them in the management of collar rot/stem rot caused by *S. rolfsii* in different economically important crops.

MATERIAL AND METHODS

Isolation of Fluorescent Pseudomonads

Rhizosphere soil samples were collected from 11 different cropping systems from the farmers' fields in Guntur district, Andhra Pradesh. For each cropping system four locations were chosen. In each location samples were collected from three fields. The samples collected from different cropping systems were assessed for fluorescent pseudomonad population by isolating on King's B medium following dilution plate technique. The bacterial colonies which showed pigment production and fluorescence on UV illuminator were counted to arrive at the number of colony forming units (cfu). The fluorescing colonies maintained on King's B medium in slants for further investigations.

Screening of *P. fluorescens* isolates against *S. rolfsii in vitro*

The antagonistic potential of *P. fluorescens* isolates was tested against *S. rolfsii in vitro* by dual culture plate method. Mycelial discs of *S. rolfsii* (5- mm) were inoculated at the centre of the Petri plate containing solidified PDA medium. The *P. fluorescens* isolates were streaked

triangularly at a distance of two cm from the edge of the culture disc of the pathogen (Prasadji *et al.,* 2012). The plates with pathogen alone served as control. All the inoculated plates were incubated at 28±1^{0}C and periodical observations were recorded on radial growth of *S. rolfsii.*

Effect of culture filtrates of *P. fluorescens* isolates on sclerotial germination of *S. rolfsii in vitro*

Cell free culture filtrates of four *P. fluorescens* isolates selected in dual culture were obtained by centrifuging their culture broths (36 h old) at 10,000 rpm for 15 min. The filtrates were passed through G3 filter under vacuum. The cell free filtrates were mixed in PDA at different concentrations and the sclerotial bodies were inoculated on the medium.

RESULTS AND DISCUSSION

Prevalence of *P. fluorescens*

Pseudomonas fluorescens isolates were obtained from 64% of soil samples, *i.e*., 28 isolates from 44 soil samples and were designated based on the cropping system and location from which they were isolated. All the isolates produced pigment in King's B medium and showed fluorescence under UV light. But they varied in cultural characters and colour, and intensity of fluorescence which could be attributed to production of different types of pigment by the isolates (Paramageetham and Prasad Babu, 2012). Most of the isolates were transluscent with fine margins and some of them produced brown growth on King's B medium. Isolates obtained from same cropping system also showed variation in culture growth and fluorescence.

The frequency of *P. fluorescens* was much higher in black soils (75.76%) than in sandy soils (27.27%). Population of the antagonist isolates was also markedly higher in black soils (0 to 10.0 x 10^5 cfu g^{-1} soil) than in sandy soils (0 to 0.7 x 10^5 cfu g^{-1} soil) (Table: 1). High clay content in black soils which affects the rhizobacteria by different mechanisms like buffering the pH, retaining the soil organic matter and soil moisture for longer periods at desirable level could be the reasons for higher population as found out by Kurek and Jaroszuk-Sciesel (2003) through enrichment of soil with clay in rye rhizosphere. In some locations though the soils were clayey textured no pseudomonas population was recorded which indicated that the population dynamics of fluorescent pseudomonads may also be influenced by factors other than soil type *viz.,* crop, crop stage and physico-chemical properties of soil (Loper *et al.,* 1985; Bergsma-Vlami *et al.,* 2001; Houlden *et al.,* 2008).

Soil pH was found to have no substantial influence as the differences in population at different pH levels were only marginal. Frequency of obtaining *P. fluorescens* isolates was highest at pH 8.0 (66.66%) followed by pH 7.5 (63.15%) and the least number of isolates was obtained at pH 7.0 (63.15%). But the population of fluorescent pseudomonads was the highest at pH 7.0 (0.3 to 10.0 x 10^5 cfu g^{-1} soil) followed by 7.5 (0 to 8.3 x 10^5 cfu g^{-1} soil) and least at pH 8.0 (0 to 7.0 x 10^5 cfu g^{-1} soil)

Frequency of pseudomonad isolation was maximum in turmeric, cotton, rice and sorghum cropping systems (four isolates in each). The least number of isolates was obtained from banana and blackgram cropping systems. Turmeric and cotton cropping systems recorded the maximum population of fluorescent pseudomonads. The number of *P. fluorescens* isolates from black soil with clay texture was substantially more than that in sandy textured soils. Among 28 isolates obtained, 25 were obtained from clay soils and only three were from sandy soils (Table: 1).

Many cropping systems were reported to support fluorescent pseudomonads at varying frequencies. Manjunatha *et al.* (2012) isolated 92 isolates of fluorescent pseudomonads from rhizosphere of chilli, sunflower, redgram, brinjal, groundnut, fieldbean, greengram, tomato, beans, sorghum, paddy and sesame. Tiwari and Thrimurthy (2007) isolated 21 *P. fluorescens* isolates from rhizosphere of rice, maize, wheat, chickpea, mungbean, urdbean, soybean and sunflower.

The number of isolates obtained and also the population of *P. fluorescens* was high in turmeric and cotton cropping systems. In rhizosphere, root exudates decide the composition of microbial population (Loper *et al.,* 1985). The root exudates released in to cotton and turmeric rhizosphere might have encouraged the growth of these beneficial bacteria.

Table 1. Population of *P. fluorescens* in the soil samples collected from different cropping systems.

S. No.	Cropping System	Location	Number of colonies ($\times 10^5$ cfu g^{-1} soil)	Texture	Soil pH	Designation of isolates	Frequency of *P. fluorescens* isolation
1	Rice	Bapatla	0.7	Clay	7.5	RB	
2		Nidubrolu	0.7	Clay	7.5	RN	100%
3		Kondabotlapalem	0.3	Sandy	7.0	RKBP	
4		Paruchuru	2.3	Clay	7.0	RPA	
5	Betelvine	Machavaram	1.3	Clay	7.5	BVMV	
6		Ponnuru	0.0	Clay	8.0	-	
7		Chinthalapudi	1.3	Clay	7.0	BVCHP	50%
8		Kottapalem	0.0	Clay	7.5	-	
9	Turmeric	Nidubrolu	10.0	Clay	7.0	TN	
10		Ponnuru	1.7	Clay	7.0	TP	100%
11		Bapatla	0.7	Clay	7.5	TB	
12		Kolakaluru	3.0	Clay	7.0	TK	
13	Groundnut	Bapatla	0.0	Sandy	7.0	-	
14		Kavuripalem	0.3	Sandy	7.5	GNKVP	50%
15		Vedullapalli	0.0	Sandy	7.0	-	
16		Karlapalem	0.7	Sandy	7.0	GNKRP	
17	Tobacco	Vedullapalli	0.0	Sandy	7.0	-	
18		Kavuripalem	0.0	Sandy	7.5	-	50%
19		Paruchuru	1.7	Clay	7.5	TBPA	
20		Ammanabotlapalem	1.3	Clay	7.0	TBABP	
21	Cotton	Lam	7.0	Clay	8.0	CL	
22		Chebrolu	8.3	Clay	7.5	CC	100%
23		Yedlapadu	2.3	Clay	8.0	CY	
24		Timmarajupalem	3.3	Clay	7.5	CTP	
25	Blackgram	Lam	0.3	Clay	8.0	BGL	25%
26		Ananthavarmpadu	0.0	Clay	7.5	-	
27		Bapatla	0.0	Clay	7.5	-	
28		Vatticherukuru	0.0	Sandy	7.5	-	
29	Sorghum	Timmarajupalem	0.7	Clay	7.5	STP	
30		Yedlapadu	0.7	Clay	8.0	SY	100%
31		Paruchuru	2.0	Clay	7.5	SPA	
32		Ammanabotlapalem	1.0	Clay	7.0	SABP	
33	Maize	Bapatla	2.0	Clay	7.5	MB	
34		Nidubrolu	0.3	Clay	7.0	MN	
35		Machavarm	0.0	Clay	7.5	-	50%
36		Kavuripalem	0.0	Sandy	7.0	-	
37	Chilli	Karlapalem	0.0	Sandy	7.0	-	
38		Kavuripalem	0.0	Sandy	7.0	-	
39		Paruchuru	4.3	Clay	7.5	CHPA	50%
40		Pericharla	0.3	Clay	7.0	CHPE	
41	Banana	Bapatla	0.0	Clay	7.5	-	
42		Nidubrolu	0.3	Clay	7.0	BN	25%
43		Modukuru	0.0	Clay	8.0	-	
44		Kolakaluru	0.0	Clay	7.0	-	

Table 2. Radial growth (cm) and number of sclerotial bodies of *S. rolfsii* in dual culture with *P. fluorescens* isolates.

S.No.	Isolates	Radial growth of *S. rolfsii* (cm) (4-DAI)	Per cent inhibition of *S. rolfsii* growth	Number of sclerotial bodies (8-DAI)	% inhibition of sclerotial bodies
1	TK	8.97 (3.16)*	0.37	65 (1.82)**	81.03
2	TP	7.99 (3.00)	11.26	104 (2.02)	69.88
3	TB	8.63 (3.10)	4.07	121 (2.09)	64.79
4	TN	8.95 (3.15)	0.56	89 (1.95)	74.19
5	BN	1.85 (1.69)	79.44	0 (0.00)	100.00
6	SY	8.97 (3.16)	0.37	174 (2.24)	49.67
7	SPA	1.93 (1.71)	78.52	1 (0.30)	99.81
8	STP	6.77 (2.79)	24.81	77 (1.89)	77.77
9	SABP	7.51 (2.92)	16.59	176 (2.25)	49.06
10	MN	9.00 (3.16)	0.00	153 (2.19)	55.45
11	MB	7.12 (2.85)	20.93	164 (2.22)	52.63
12	CHPA	8.95 (3.15)	0.56	186 (2.27)	45.98
13	CHPE	2.02 (1.74)	77.59	0 (0.00)	100.00
14	BGL	8.95 (3.15)	0.56	240 (2.38)	30.40
15	TBABP	8.95 (3.15)	0.56	172 (2.24)	50.28
16	TBPA	8.93 (3.15)	0.74	132 (2.12)	61.76
17	BVCHP	5.57 (2.56)	38.15	152 (2.18)	55.76
18	BVMV	8.97 (3.16)	0.37	54 (1.74)	84.16
19	RPA	7.35 (2.89)	18.33	96 (1.99)	72.07
20	RKBP	6.97 (2.82)	22.59	117 (2.07)	66.20
21	RN	3.07 (2.02)	65.93	123 (2.09)	64.50
22	RB	9.00 (3.16)	0.00	99 (2.00)	71.41
23	GNKRP	6.15 (2.67)	31.67	144 (2.16)	58.27
24	GNKVP	5.82 (2.61)	35.37	183 (2.26)	46.85
25	CTP	8.97 (3.16)	0.37	116 (2.07)	66.32
26	CY	2.82 (1.95)	68.70	69 (1.85)	79.91
27	CC	2.23 (1.75)	77.04	2 (0.48)	99.41
28	CL	9.00 (3.16)	0.00	113 (2.06)	67.20
CHECK		9.00 (3.16)		347 (2.54)	
CD (P=0.05)		0.05		0.13	
CV%		1.02		4.28	

DAI- Days after inoculation

* Figures in parentheses are logarithmic transformed values

** Figures in parentheses are square root transformed values

Evaluation of Bio-efficacy of *P. fluorescens* against *S. rolfsii in vitro*

All the twenty eight *P. fluorescens* isolates inhibited growth of *S. rolfsii* in dual culture. Per cent inhibition of *S. rolfsii* radial growth four days after inoculation (DAI) was highest in BN followed by SPA, CHPE and CC *i.e.*, 79.44, 78.52, 77.59 and 77.04%, respectively (Table: 2). When incubated further *S. rolfsii* was observed to overgrow CC in dual culture 10 DAI. However, there was no difference in the growth of *S. rolfsii* dual cultured with BN, SPA and CHPE isolates even after one month of incubation.

Inhibitory effects of *P. fluorescens* on different pathogens, particularly *S. rolfsii* was well established. Umamaheswari *et al.* (2002) observed 87.22% inhibition in *S. rolfsii* growth by *P. fluorescens* in dual culture technique. Ganesan and Sekar (2012) reported that six isolates of *Pseudomonas* showed above 68% of inhibition in growth of *S. rolfsii*.

Pseudomonas fluorescens isolates exhibited an inhibitory effect on sclerotial production by *S. rolfsii*. Number of sclerotial bodies produced by *S. rolfsii* varied with the *P. fluorescens* isolate in dual culture. The highest number of sclerotia was produced (347) in control plate on 8-DAI while sclerotial production was completely inhibited by BN and CHPE isolates and the other two isolates, SPA (99.81%) and CC (99.41%) also caused a significant reduction in sclerotial production. More than 80% inhibition was recorded by BVMV (84.16%) and TK (81.03%) isolates. The sclerotial production was delayed for two days in dual culture with TK and BVMV. Least inhibition was observed in BGL *i.e.*, 30.40%. Highly significant positive correlation was observed between *S. rolfsii* radial growth and sclerotial production in dual culture with *P. fluorescens* isolates (Table: 2). The results were in agreement with the results obtained by Arunasri *et al*. (2013), which showed 71% of inhibition of sclerotial production with *Pseudomonas* sp. Production of antifungal metabolites by fluorescent pseudomonads was well demonstrated (Rakh *et al.,* 2011; Manjunatha *et al.*, 2012; Sen *et al.,* 2012) which might have inhibited not only growth but also sclerotial production of *S. rolfsii*.

Fig 1. Effect of cell free culture filtrates of *P. fluorescens* isolates on *S. rolfsii* sclerotial germination

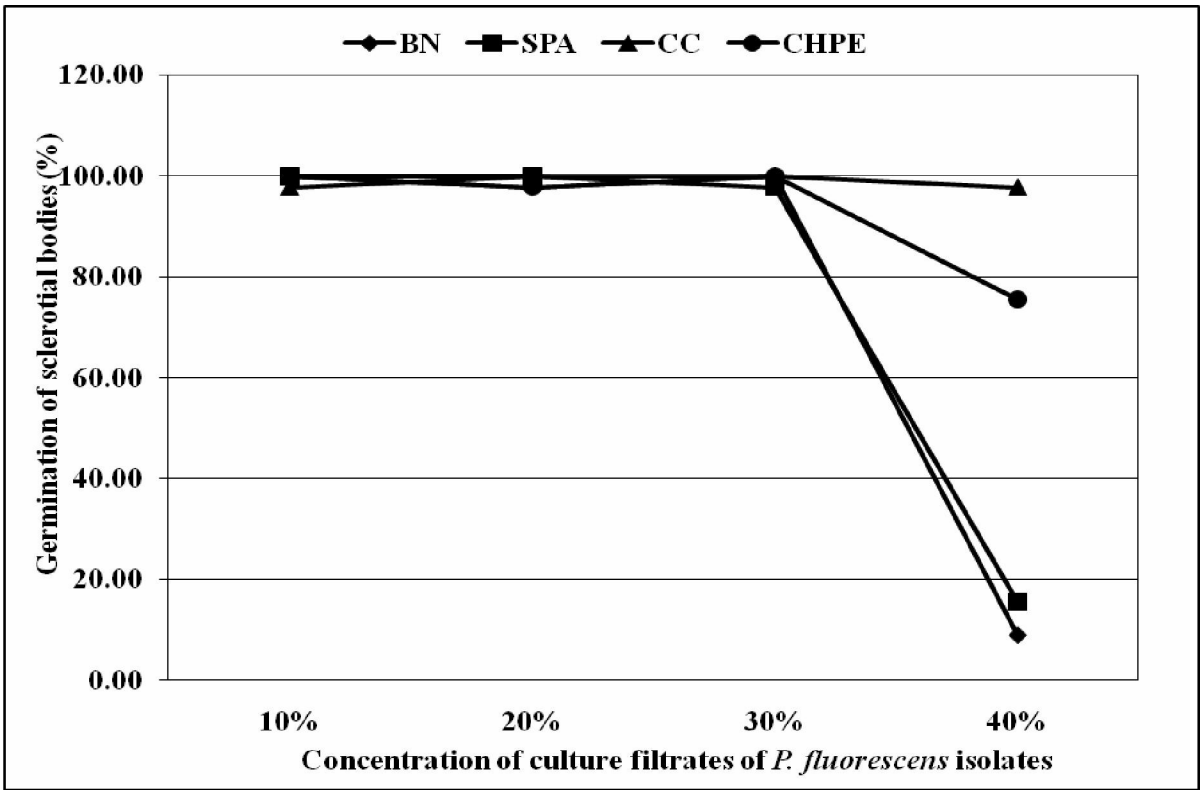
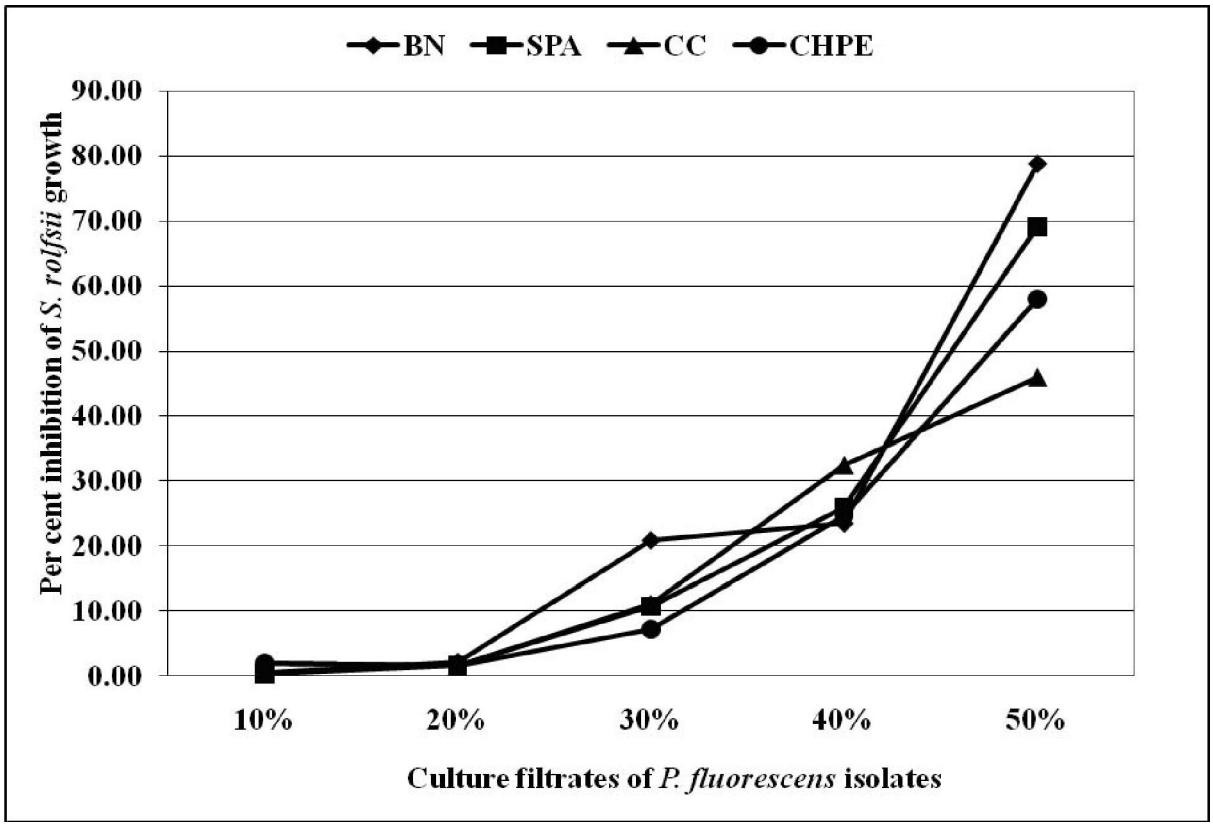


Fig 2. Per cent inhibition of radial growth of *S. rolfsii* by culture filtrates of *P. fluorescens* isolates three days after incubation.



Only six isolates that showed more than 50% reduction of *S. rolfsii* growth were reckoned as effective. The pathogen is known and found to reduce the pH of the medium by secreting oxalic acid, an adaptive advantage, (Kishore *et al*., 2005; Madhava Rao, 1979) which perforce rendered most of the antagonistic bacterial isolates barring six (CY, RN, BN, CHPE, SPA and CC) ineffective on continued incubation. The six isolates have shown more than 50% inhibition of the pathogen on PDA medium even though pH was decreased from 7.02 to 3.30 by *S. rolfsii* through production of oxalic acid (data not presented).

Effect of cell free culture filtrates of four isolates *viz.,* BN, SPA, CHPE and CC that have shown more than 75% inhibition in dual culture was studied on sclerotial germination and radial growth of *S. rolfsii*. Cell free culture filtrates of the four selected *P. fluorescens* isolates reduced the sclerotial germination at higher concentrations. At all concentrations of culture filtrates CC isolate could not inhibit sclerotial germination. However, the other three isolates at 40% concentration significantly reduced sclerotial germination with BN (8.89% germination) and SPA isolates (15.56% germination) being most effective (Fig 1). Post sclerotial germination mycelial growth was slower at 10, 20, 30 and 40% culture filtrate concentrations of all isolates than in check. At 50% concentration maximum inhibition of *S. rolfsii* growth was observed in all the isolates with the highest inhibition (78.70%) by BN isolate (Fig 2).

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