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Biocontrol and plant growth promotion activity of indigenous isolates of *Pseudomonas fluorescens*

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One fifty isolates of *Pseudomonas jfluorescens were* obtained from soil samples collected from the rhizosphere of chilli, sunflower and cotton from Raichur, Karnataka. Twenty five colonies showing yellow pigmentation on King's B medium from different rhizosphere soil were picked up. Based on the pigment formation and fluorescens under UV light, finally six isolates from chilli, one from sunflower and an isolate from Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore, were used for further study and comparison. The different isolates of *Pseudomonas fluorescens* were designated as Pfl, Pf2, Pf3, Pf4, Pf5, Pf6, Pf7 and Pf8 were characterized. Further, the efficacy of eight isolates of *Pseudomonas fluorescens* was evaluated against four different plant pathogenic fungi *viz., Fusarium solani (wilt of* chilli), *Rhizoctonia solani* (cotton root rot), *Colletotrichum gloeosporioides* (anthracnose of pomegranate) and *Alternaria alternata* (leaf spot of cotton) through dual culture technique. Among different *P. fluorescens* isolates, an indigenous isolate Pf4 showed maximum inhibition of mycelial growth of *F. solani, C. gloeosporioides, A. alternata* and *R. solani.* The efficacy of *P. fluorescens* isolates in inducing systemic resistance was further tested against *F. solani* isolate (F121) causing wilt of chilli. The germination and vigour index were considered as indices of systemic induced resistance. Even in vigour index an indigenous isolate Pf4 showed highest induction of resistance showing higher seed germination per cent, mean shoot length and root length and vigour.

Key words: *Alternaria alternata, Colletotrichum gloeosporioides, Fusarium solani, Pseudomonas fluorescens, Rhizoctonia solani,* systemic induced resistance, vigour index

INTRODUCTION

Modern agriculture is heavily dependent upon monoculture of crops and varieties, which has out weighed the balance in favour of pathogen, thus ending up in epidemics. This is particularly true for soil borne plant pathogens, in which repeated culture of mono crop or variety making soil extremely sick. Under such circumstances, use of chemical fungicides is environmentally hazardous and impracticable to the farming community. The cultural practices such as crop rotation, mixed cropping etc. are quite passive and are often not attaining the results to desired level. Biological methods offer an excellent alternative strategy not only for effective management of various diseases but also augmentation of nutrients in the rhizosphere. The biological control employing phylloplane, rhizospheric microflora or more recently by indigenous endophytic bacterial flora seems to hold a great promise (Chen, 1995). These bacteria are abundant in number, quick in reproduction and strong in activity. They select and regulate function of roots in screening and absorbing nutrients.

Soil has enormous untapped potential of antagonistic microbes, which are helpful in reducing pathogen population through different mode of action such as competition for nutrients and space, antibiosis, mycoparasitism, production of siderophores and lytic enzymes (Sharma and Sain, 2005). *Pseudomonas flucrescens* bacteria play a major role in plant growth promotion, induced systemic resistance and biological control of

pathogen. (Krishnamurthy *et al*, 1999). In this context, the Plant Growth Promoting Rhizobia (PGPR)offer an excellent combination of traits useful both in disease control and increased nutrient availability. Among the PGPR, fluorescent pseudomonads stand out because of high level of genetic variability and competitiveness in soil. Hence, they have been advocated as effective and economical bio-inoculants for use in the integrated nutrient and pest management systems (Appanna, 1997).

MATERIALS AND METHODS

Collection and isolation of indigenous Pseudomonas fluorescens

Soil samples were collected from the rhizosphere of chilli, sunflower and cotton from Raichur, Karnataka. In chilli, as many as fifty soil samples from the rhizosphere of healthy plants in the vicinity of sick plants were collected by uprooting the plants from the location viz., Gabbur, Attanur, Sankeshwaral, Masaḍkal, Budur, Yatagal and Yermaral. Around 80-100 g of rhizosphere soil was collected, shade dried and sieved to remove root debris. Fifty each rhizosphere soil samples of sunflower Regional Agricultural Research Station (RARS) [Raichur, Sankeshwaral and Attanur] and cotton [Sankeshwaral and RARS] were also similarly collected. The serial dilution of 10^{-5} was placed on King's B medium to isolate the colonies. Then the plates were incubated at 28° C for 48 hrs. About 25 colonies showing yellow pigmentation on King's B medium from different rhizosphere soil were picked up. The isolates thus collected (150) were inoculated on King's B medium containing 1% tyrosine and incubated at 28±2° C for 48 hrs and development of zone of fluorescent pigmentation around colonies were recorded for identifying them as *P. fluorescens* isolates. Based on the pigment formation and fluorescens under UV light finally six isolates from chilli, one from sunflower and an isolate from Department of Plant Pathology, Tamilnadu Agricultural University, Coimbatore (TNAU) were used for further study and comparison.

Characterization of indigenous Pseudomonas fluorescens isolates

Characterization of the different isolates of *Pseudomonas fluorescens* rhizobacteria was carried out according to the Laboratory Guide for Identification of Plant Pathogenic Bacteria published by the American Phytopathological Society (Schaad, 1992). Gram staining, potassium hydroxide solubility test, pigment production test, anaerobic growth test, oxidase test, levan formation from sucrose, starch hydrolysis, growth at 4° and 45°C and Arginine dehydrolase test were carried out. For each test, 24 to 48 hrs old cultures were used.

Dual culture test

Efficacy of eight isolates of *Pseudomonas fluorescens* were evaluated against *Fusarium solani* (wilt of chilli), *Rhizoctonia solani* (cotton root rot), *Colletotrichum gloeosporioides* (anthracnose of pomegranate) and *Alternaria alternata* (leaf spot of cotton) to see the spectrum of plant pathogens targeted through dual culture technique. The bioagent and the test fungus were inoculated side by side on a single Petri plate containing solidified PDA medium. The bacterial bioagents were streaked one day earlier to the test pathogen. Three replications were maintained for each isolate with one control by maintaining only pathogen. They were incubated at 28° C. Observations were recorded when there was a full growth of pathogen in the control plate (6-7 days). The diameter of the colony of the pathogen was measured in both directions and average was recorded and the per cent inhibition on growth of the test pathogen was calculated by using the formula given below by Naik and Sen (1995), as follows :

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

Where; I = Per cent inhibition; C= Radial growth of the pathogen in control; and T = Radial growth of pathogen in treatment

Systemic Induced resistance under in vivo (pot culture)

The efficiency of different isolates of Pseudomonas fluorescens was studied in this experiment. The surface sterilized chilli seeds of cultivar Byadagi Kaddi were soaked in suspension of different isolates of *P. fluorescens* for 4 hrs followed by thirty minutes shade dried and the seeds were challenge

inoculated with spore suspension of 1×10^6 ml^{-1} of *F. solani* (F121), the wilt causing pathogen in chilli. The seeds treated with distilled water alone (control) and challenge inoculated with spore suspension of *F. solani* (F121) were maintained for comparison. Fifty seeds were sown separately and seedling vigour was calculated three weeks after sowing. The formula proposed by Abdulbaki and Anderson (1976) was used for calculating the seedling vigour, as follows : Seedling vigour = (Mean shoot length + Mean root length) × Per cent of germination

RESULTS AND DISCUSSION

Preliminary collection and isolation of indigenous P. fluorescens isolates

About one fifty isolates of *P. fluorescens* were collected from the rhizosphere soil of chilli, sunflower and cotton using specific medium. The isolates of P. fluorescens thus obtained are inoculated on King's B medium supplemented with 1 % tyrosine. Based on the zone of fluorescent pigmentation around the colonies and fluorescens under UV light, seven isolates were identified for further works. Finally six *P. fluorescens* isolates from chilli (Pfl, Pf2, Pf3, Pf4, Pf5 and Pf6), one from sunflower (Pf8) and an isolate obtained from Department of Plant Pathology, TNAU, Coimbatore (Pf7) were used for further study and comparison.(Table. 1).

Characterization of indigenous P. fluorescens isolates

The source and identity of different *P. fluorescens* isolates are presented in Table 1. Characterization of different isolates was carried out according to the Laboratory Guide for Identification of Plant Pathogenic Bacteria published by The American Phytopathological Society (Schaad, 1992). All the isolates showed Gram-negative reaction, positive for oxidase test, negative for levan formation from sucrose and positive for starch hydrolysis. All the isolates produced pigmentation on both King's A and King's B medium and showed growth at 4° and 45° C. Further all isolates showed positive for KOH and arginine dehydrogenase and negative for anaerobic growth test. The summary of biochemical characterization of eight isolates of *P. fluorescens* is presented in Table 2.

Spectrum of biocontrol of P. fluorescens against test plant pathogens

In vitro evaluation against F. solani

Among different *P. fluorescens* isolates, Pf4 showed maximum inhibition of mycelial growth of *F. solani* (78.09%), causal agent of wilt of chilli which significantly differed from other isolates tested, followed by Pf6 (72.35%), Pf5 (65.17%), Pf3 (53.19%) and least inhibition was noticed by Pf2 41.13%o) (Table 3). Alabouvette *et al.* (1993) suggested the practical application of antagonistic microorganisms particularly non-pathogenic *Fusarium oxysporum* and *Pseudomonas* spp. as biological control agent against *Fusarium* wilt. Gehlot and Purohit (2002) observed increase in plants biomass of yield and biological control of *Fusarium solani* on chilli using *Pseudomonas fluorescens*. Ramesh Kumar *et al.* (2002) isolated 40 strains of *Pseudomonas fluorescens* from rhizosphere of rice and sugarcane, of which 18

Table 1: Identity of *P. fluorescens* (Pf) isolates used for biocontrol and PGPR.

Isolate No.	Place of collection*	Crop rhizosphere	Cultivar
Pfl	Raichur (Gabbur)	Chilli	Guntur
Pf2	Raichur (Masadkal)	Chilli	Guntur
Pf3	Raichur (Yatagal)	Chilli	Byadagi kaddi
Pf4	Raichur (Sankeshwaral)	Chilli	Byadagi kaddi
Pf5	Raichur (Sankeshwaral)	Chilli	Byadagi kaddi
Pf6	Raichur (Yermara)	Chilli	Byadagi kaddi
Pf7	TNAU, Coimbatore	Department of Plant Pathology	-
Pf8	RARS, Raichur	Sunflower	Modern

* Names in the parenthesis idicate village from where isolates were collected

Table 2 : Summary of biochemical characterization of *P. fluorescens* isolates.

Biochemical tests	*Pseudomonas fluorescens* isolates*							
	Pfl	Pf2	Pf3	Pf4	Pf5	Pf6	Pf7	Pf8
Gram reaction	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
Pigment production in king's A media	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Pigment production in king's B media	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Oxidase test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Levan formation from sucrose	- ve	- ve	- ve	- ve	-ve	- ve	-ve	- ve
Starch hydrolysis	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Growth at 4° c	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Growth at 45° c	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
KOH test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Anaerobic growth	- ve	-ve	-ve	-ve	-ve	-ve	-ve	- ve
Arginine dehydrogenase	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve

* Age of the culture = 24 h

Table 3. *In vitro* screening of *P. fluorescens* (Pf) isolates against some target pathogens

Treatments	Per cent inhibition of mycelial growth			
	Fusarium solani (F121)	*Colletotrichum gloeosporioides*	*Alternaria alternata*	*Rhizoctonia solani*
pn	44.19(41.66)	61.74(51.79	44.92 (42.08)	64.93 (53.70)
PC	41.13 (39.89)	52.97(46.71)	40.33 (39.43)	55.56(48.19)
Pf3	53.19 (46.83)	73.96(59.32)	64.00 (53.14)	79.62(63.18)
Pf4	78.09 (62.10)	81.43(64.50	70.52 (57.14)	84.37 (66.73)
Pf5	65.17 (53.84)	65.70(54.17)	67.01 (54.95)	74.89 (59.94)
Pf6	72.35 (58.30)	74.08(59.45)	49.61 (44.78)	82.18(65.08)
Pf7	51.51 (45.87)	59.21(50.32)	58.24 (49.75)	73.63(59.11)
Pf8	48.77 (44.30)	47.02(43.29)	42.53 (40.70)	71.22(57.58)
S. Em±	0.62	1.09	0.90	0.77
C. D. at 0.01	2.62	4.62	3.81	3.09

Figures in the parenthesis are arc sign transformed value

strains exhibited antifungal activity against *Fusarium oxysporum* mainly through production of antifungal metabolites. Inhibition of growth of *F. solani* by *P. fluorescens* was also observed by Devika Rani *et al.* (2006).

In vitro evaluation against C. gloeosporioides

A significantly higher inhibition of mycelial growth of *C. gloeosporioides*, causal agent of anthracnose of pomegranate, was noticed in Pf4 isolate (81.43%), followed by Pf6 (74.08%), Pf3 (73.96%), Pf5 (65.70%o) and least inhibition was noticed in Pf8 (47.02%). The bacterial antagonists such as B. subtilis isolate (BSCBE4) *P. fluorescens* isolate (ENPfl) and *P. chlororaphis* isolate (PA23) were found to be effective in inhibiting the mycelial growth of *Colletotrichum cassicola* causing stem blight of *Phyllanthus amarus* (Mathiyazhagan *et al*, 2004).

In vitro evaluation against A. alternata

The isolate Pf4 showed maximum inhibition of mycelial growth of *A. alternata*, inducing cotton leaf

Table 4. Induction of systemic resistance in chilli seed/seedling by different *P. fluorescens* (Pf) isolates challenge inoculated with *F. solani* (F121).

Treatments	Per cent germination	Mean shoot Length (cm)	Mean root Length (cm)	Vigour index
PF1+F121	89.51	6.80	5.43	1223
Pf2+F121	89.54	6.53	5.17	1170
Pf3+F121	92.73	7.97	6.90	1487
Pf4+F121	96.50	9.00	8.03	1703
Pf5+F121	94.41	8.50	7.57	1607
Pf6+F121	95.67	8.37	7.03	1540
Pf7+F121	91.25	7.80	7.00	1480
Pf8+F121	90.47	7.43	6.60	1404
Uninoculated control	87.00	5.83	4.80	1061
Inoculated control (F121)	37.33	4.17	3.33	735
SEm±	0.54	0.11	0.12	30.50
CD. at 0.01	2.20	0.46	0.56	124.13

spot (70.52%), which was on par with Pf5 (67.01%). This was followed by Pf3 (64%), Pf7 (58.24%) and the least was in Pf2 (40.33%). The study made by Jeyalakshmi and Seetharaman (1999) showed that where in the antagonist *B. subtilis* inhibited mycelial growth of *C. capsici* followed by *P. fluorescens*. Similar studies were made by Ramamurthy and Samiyappan (2001) both under *in vitro and in vivo* conditions Ramegowda *et al.* (2007) also showed the effectiveness of *P. fluorescens* bioagents against *Alternaria* spp. infecting Bt cotton under *in vitro* conditions. Incidence of *Alternaria*-leaf spot of sesame (*Alternaria sesame*) was reduced significantly with the use of *P. fluorescens* spray under field condition (Naik *et al.*, 2002).

In vitro evaluation against R. solani

Again the isolate Pf4 showed maximum inhibition of mycelial growth of *R. solani* (84.37%) which was on par with Pf6 (82.18%). The isolates PD (79.62%), Pf5 (74.89%) and Pf7 (73.63%o) were the next in the order. The least inhibition was noticed in Pf2 isolate (55.56%). Loper (1988) isolated *P. fluorescens* Migula strain 3551, from cotton rhizosphere soil which protected cotton from seed colonizaition and pre-emergence damping off caused by *Pythium ultimum*. Selvarajan and Jeyarajan (1996) reported that *P. fluorescens* formed inhibition zones against chickpea root rot pathogens, *Fusarium solani* and *Macrophomina phaseolina*. They reduced sporulation of *F. solani* and sclerotial size, germination and germ tube number of *M. phaseolina*.

Induction of systemic resistance and plant growth promotion activity in chilli

The efficacy of *P. fluorescens* isolates in inducing systemic resistance was tested against *F. solani* isolate causing wilt of chilli (F121) as the target pathogen. The germination and vigour index were considered as indices of systemic induced resistance. The indigenous isolate Pf4 once again showed highest induction of resistance showing higher seed germination of 96.5 per cent, mean shoot length of 9.0 cm and mean root length of 8.03 cm, with vigour index of 1703 which was on par with Pf5 (vigour index of 1607) and was significantly superior over all other isolates. That was followed by Pf 6 (vigour index of 1540), Pf3 (vigour index of 1487) and Pf 7 (vigour index 1480). The vigour index in uninoculated control (vigour index of 1060) significantly differed from inoculated control (vigour index of 735) (Table 4). Sivamani and Gnanamanickam (1988) noticed that application of *P. fluorescens* gave better root growth and plant height in banana. Similarly, Bhatia *et al.* (2005)

noticed that seed treatment of sunflower with *P. fluorescens* I and *P. fluorescens* II resulted in increase in total root biomass in sunflower.

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