Studies on Compatibility of *Pseudomonas putida* with Fungicides, Insecticides and Plant Extracts

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In vitro studies on compatibility of an indigenous strain of *Pseudomonas putida* (RFP-13) were undertaken with the commonly used fungicides, insecticides and plant extracts for plant protection at different concentrations by poisoned food technique. Results revealed that there was minimum inhibition of *P* putida with carbendazim (Bavistin 50WP), carbofuran (Furadan 3G) and neem seed kernel extract whereas complete inhibition was observed with mancozeb (Dithane M45), captan (Captaf 50WP), indoxacarb (Avaunt 14.5SC), novaluron (Rimon 100EC), Nimbicidine® (0.03% Azadirachtin) and eucalyptus leaf extract.

Key words: Pseudomonas putida, Fungicides, Insecticides, Plant extracts, Colony forming unit (cfu).

Plant diseases are to be managed to maintain the quality and abundance of food, feed and fibre produced by growers all round the world. Modern agriculture is heavily relied on chemical fertilizers and pesticides besides monoculture of crops and varieties. No doubt such inputs to agriculture have contributed significantly to the spectacular improvements in crop productivity and quality over the past 100 years. However, raising ecological problems, viz. environmental pollution, development of pesticide resistant strains of plant pathogens and their detrimental effects on beneficial organisms caused by excessive use of agro chemicals are all the issues of great concern today. In such a scenario, biological control of plant diseases has come to the fore front as an excellent alternative strategy to the chemicals not only for

effective management of plant diseases but also augmentation of nutrients in the rhizosphere and induction of systemic resistance in the plants.

Fluorescent pseudomonads play an important role in biological control of plant diseases. Many of them promote plant growth by suppressing pathogenic microorganisms, synthesizing growth-stimulating plant hormones and promoting increased disease resistance (Choudhary et al., 2009)¹. Fluorescent pseudomonads are known to produce a large array of antibiotics of which 2,4-Diacetylphloroglucinol (DAPG) is an important because of its antifungal, antibacterial, nematicidal and antiviral nature. Since modern agriculture is highly dependent on chemicals for plant disease management, it is necessary to have strains of fluorescent Pseudomonas spp. possessing 2,4-DAPG gene that are compatible with chemical pesticides and can be successfully integrated in integrated disease management (IDM) along with the chemical pesticides without any reduction in their antagonistic nature. With an increasing interest in

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organic farming, there is a necessity of evaluating compatibility of fluorescent *Pseudomonas* spp., with the plant products. The present investigation was therefore undertaken to determine *in vitro* compatibility of a native strain of *P. putida* (RPF-13) isolated from brinjal rhizosphere and detected to possess broadspectrum antibiotic gene, 2,4-Diacetylphloroglucinol (DAPG) with the commonly used fungicides, insecticides and plant extracts.

MATERIALAND METHODS

Determination of compatibility of Pseudomonas putida (RFP-13) was done with commonly used fungicides, viz. mancozeb (Dithane M45), carbendazim (Bavistin 50WP), captan (Captaf 50WP), hexaconazole (Contaf 5EC) and propiconazole (Tilt 25EC) and insecticides, viz. chlorpyriphos (Dursban 30EC), carbofuran (Furadan 3G), indoxacarb (Avaunt 14.5SC), novaluron (Rimon 100EC) and imidacloprid (Confidor 20SL) at 0.1 and 0.2 per cent concentrations and plant extracts, viz. neem seed kernel extract (NSKE), Nimbicidine® (0.03% Azadirachtin), pongamia leaf extract (Pongamia *pinnata*), eucalyptus leaf extract (Eucalyptus spp.) and Bellary jali leaf extract (Prosofis juliflora) at 2.5 and 5.00 per cent concentrations by poisoned food technique (Nene and Thapliyal, 1982)². The calculated quantity of fungicide, insecticide and

 Table 1. Compatibility of Pseudomonas putida (RPF-13) with different fungicides

Concentration (%)		
0.1	0.2	
cfu (10 ¹⁰)		
0.00 (100.00)	0.00 (100.00)	
88.00 (5.38)	35.67 (61.65)	
0.00 (100.00)	0.00 (100.00)	
35.00 (62.33)	16.33 (82.44)	
44.33 (52.33)	26.33 (71.69)	
93.00		
S.Em±	CD at 1%	
0.53	2.10	
0.31	1.21	
0.75	2.97	
	Concent 0.1 cfu (0.00 (100.00) 88.00 (5.38) 0.00 (100.00) 35.00 (62.33) 44.33 (52.33) 93 S.Em± 0.53 0.31 0.75	

*Figures in parentheses indicate per cent reduction over control, ** Mean of four replications

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plant extract was added differently to King's B medium (King *et al.* 1954)³, mixed thoroughly and poured into sterilized Petri plates and allowed to solidify. After solidification about 36 hour old culture of *Pseudomonas putida* (RPF-13) was inoculated on Petri plates by spread plate technique. Four replications were maintained for each treatment. Controls were maintained without any fungicide, insecticide and plant extract respectively. The inoculated plates were incubated at $28+2^{\circ}$ C in a BOD incubator and colony forming unit (cfu) count was taken after 48 hours of inoculation.

RESULTS AND DISCUSSION

Among the fungicides tested, *P. putida* was found to be compatible carbendazim, hexaconazole and propiconazole at both 0.1 and 0.2 per cent concentrations. Control recorded a cfu count of 93.00 X10¹⁰. A cfu count of 88.00X10¹⁰ and 35.67X10¹⁰ with carbendazim with 5.38 and 61.65 per cent reduction over control at 0.1 and 0.2 per cent concentrations respectively. With hexaconazole, it recorded cfu of 35.00X10¹⁰ and 16.33X10¹⁰ and 62.37 and 82.44 per cent reduction over control at 0.1 and 0.2 per cent respectively. Propiconazole recorded cfu of 44.33X10¹⁰ and 26.33X10¹⁰ cfu and 52.33 and 71.69 per cent respectively. There was no compatibility with

 Table 2. Compatibility of Pseudomonas putida (RPF-13) with different insecticides

	Concentration (%)		
Insecticide	0.1	0.2	
	cfu (10 ¹⁰)		
Chlorpyriphos	41.67 (56.14)	19.00 (80.00)	
Carbofuran	70.00 (26.32)	39.00 (58.95)	
Indoxacarb	0.00 (100.00)	0.00 (100.00)	
Imidacloprid	62.00 (34.37)	28.33 (70.18)	
Novaluron	0.00 (100.00)	0.00 (100.00)	
Control	95.00		
Comparing of means	S.Em±	CD at 1%	
Insecticide (I)	0.54	2.12	
Concentration (C)	0.31	1.22	
Interaction (I x C)	0.76	3.00	

*Figures in parentheses indicate per cent reduction over control, ** Mean of four replications

	Concentration (%)		
Plant product	0.1	0.2	
	cfu (10 ¹⁰)		
Neem Seed	71.00 (21.11)	30.67 (65.92)	
Kernel Extract			
Nimbicidine	0.00 (100.00)	0.00 (100.00)	
Pongamia leaf	47.33 (47.41)	13.33 (85.19)	
extract			
Bellary jali	41.00 (54.44)	17.67 (80.37)	
leaf extract			
Eucalyptus	0.00 (100.00)	0.00 (100.00)	
leaf extract			
Control	90.00		
Comparing of means	S.Em±	CD at 1%	
Plant product (P)	0.40	1.57	
Concentration (C)	0.23	0.91	
Interaction (P x C)	0.56	2.22	

 Table 3. Compatibility of Pseudomonas putida (RPF-13) with different plant extracts

*Figures in parentheses indicate per cent reduction over control, ** Mean of four replications

mancozeb and captan (Table. 1).Similar reports were reported by Khan and Gangopadhyay (2008)⁴. They reported that carboxin, chlorothalonil and carbendazim were least toxic to *P. fluorescens* strain PFBC-25, while captan was most inhibitory. Laha and Venkataraman (2001)⁵ have also reported compatibility of *P. fluorescens* with carbendazim while studying sheath blight in rice.

Among the different insecticides evaluated, indoxacarb and novaluron were found to incompatible with P. putida at both 0.1 and 0.2 per cent concentrations in which no colonies were recorded. P. putida recorded 95.00 X1010 cfu in control. 41.67X10¹⁰ and 19.00X10¹⁰ cfu with chlorpyriphos resulting in 56.14 and 80.00 per cent reduction over control, 70.00X1010 and 36.33X1010 cfu with carbofuran which resulted in 26.32 and 58.95 per cent reduction over control and 62.00X1010 and 28.33X1010 cfu resulting in 34.74 and 70.18 per cent reduction over control with imidacloprid at 0.1 and 0.2 per cent respectively (Table. 2). The results are similar to those obtained by Jayakumar et al. (2004)6 wherein P. fluorescens was compatible with carbofuran 3G and avermectin for management of Rotylenchulus reniformis in okra. Combined application of P. fluorescens and carbofuran 3G significantly improved the plant

growth Senthilkumar and Ramakrishnan, (2004)⁷. Kumar *et al.* (2008)⁸ reported compatibility of *P. fluorescens* with imidacloprid and carbofuran.

Among the different plant extracts tested, P. putida showed incompatibility with nimbicidine and eucalyptus leaf extract at both 2.5 and 5.00 per cent concentrations since no cfu developed. P. putida gave 92.00 X1010 cfu in control. 71.00X1010 and 30.67X1010 cfu with NSKE resulting in 21.11 and 65.92 per cent reduction over control, 47.33X1010 and 13.33X1010 cfu in pongamia leaf extract with 47.41 and 85.19 per cent reduction over control and 41.00X1010 and 17.67X1010 cfu with Bellary jali leaf extract which resulted in 54.44 and 80.37 per cent reduction over control at 2.5 and 5.00 per cent respectively (Table. 3). Similar results were reported by Kumar and Palakshappa (2009)9 wherein compatibility of P. fluorescens with castor cake, pongamia cake, neem cake, safflower cake was observed. Manjunatha et al. (2010)10 reported compatibility of P. fluorescens with carbendazim, thiram (fungicides), imidacloprid, carbofuran (insecticides) at both 0.1 and 0.2 percent concentration and with plant extracts, viz. NSKE, garlic bulb extract and tulasi leaf extract at 2.5 and 5.00 per cent concentrations.

REFERENCES

- Choudhary, D. K., Prakash, A., Wray, V., Joshi, B. N. Insights of the fluorescent pseudomonads in plant growth regulation. *Curr. Sci.*, 2009; (2): 170-179.
- Nene, Y.L., Thapliyal, P.N. Fungicides in Plant Diseases Control. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, 1982; pp. 163.
- King, E. O., Ward, M. K., D. E. Raney. Two simple media for demonstration of pyocyanin and fluorescin. *J. Lab. Clin. Med.*, 1954; 44: 301–307.
- Khan, M. A., Gangopadhyay, S. Efficacy of *Pseudomonas fluorescens* in controlling root rot of chickpea caused by *Macrophomina phaseolina. J. Mycol. Pl. Pathol.*, 2008; **38**: 580-587.
- Laha, G. S., Vekataraman, S. Sheath blight management in rice with biocontrol agents. *Indian Phytopathol.*, 2001; 54(4): 461-464.
- Jayakumar, J. S., Ramakrishnan., Rajendran, G. Evaluation of *Pseudomonas fluorescens* strains isolated from cotton rhizosphere against *Rotylenchus reniformis. SAARCJ. Agric.*, 2003;

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2: 153-156.

- 7. Senthilkumar, T., Ramakrishnan, S. Studies on the compatibility of *Pseudomonas fluorescens*, *Trichoderma viride* and carbofuran 3G and their influences on *Meloidogyne incognita* in okra. *Annu. Pl. Protec. Sci.*, 2004; **12**(1): 140-142.
- Kumar, K., Santharam, G., Sridhar, R. Compatibility of imidacloprid with plant pathogenic antagonistic microorganisms, *Trichoderma viride* (Pers) and *Pseudomonas fluorescens* (Migula) in cotton. *Asian Bio. Sci.*, 2008; 3(1): 171-175.
- Kumar, T. P., Palakshappa, M. G. Effect of organic amendments on antagonists and pathogen causing purple blotch of onion. *Karnataka J. Agril. Sci.*, 2009; 22(1): 229-230.
- Manjunatha, H., Naik, M. K., Rangeshwaran, R., Vajid, N. V. Biological and molecular characterization of indigenous fluorescent pseudomonads for biocontrol activity. *National* symposium on "Molecular approaches for management of fungal diseases of Crop Plants" IIHR, Bangalore, December 27-30, 2010; pp. 93-94.