Compatibility of fluorescent pseudomonads with different pesticides under in vitro conditions

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ABSTRACT

In vitro study was conducted to find out the compatability of fluorescent pseudomonads with fungicides, insecticides and botanicals using poisoned food technique. Fluorescent pseudomonads isolates EP5 (*Pseudomonas fluorescens*) and RP46 (*P putida*) were compatible with hexaconazole, propiconazole, carbendazim, chlorpyriphos and imidachloprid at 0.1 and 0.2 per cent. Isoprothiolane, fipronil and buprofezin were not compatible with *P fluorescens* and *P putida*. Among the botanicals fluorescent pseudomonads were compatible with NSKE, neem leaf and nimbicidine whereas neem oil garlic emulsion showed incompatibility.

Keywords: Botanicals; fungicides; insecticides; botanicals; poisoned food technique

INTRODUCTION

In commercial agriculture, crop protection against phytopathogens relies heavily on agro-chemicals. Use of commercial insecticides and pesticides offers an effective control strategy but the approach is not only expensive but also poses adverse effects on human health and environment and is lethal to other beneficial bacteria. At this juncture environment-friendly approach to control pathogens for agricultural sustainability is the need of the hour. Biological control employing phylloplane, rhizospheric microflora or indigenous endophytic bacterial flora seems to be promising against plant pathogens without adverse environmental effects. The cost, convenience, efficacy and reliability of biological control are important considerations in comparison to the alternative disease control strategies and hence are expected to play an important role in integrated pest management (IPM) systems. Biological control of soil-borne diseases by plant growth-promoting rhizobacteria is a well-established phenomenon and has been shown to play a major role in suppression of several plant pathogens (Handelsman and Stabb 1996). Plant growth promoting rhizobacteria (PGPR) are rhizosphere-competent bacteria that aggressively colonize plant roots and have ability to multiply and colonize across the ecological niches found on the roots at all stages of plant growth in the presence of a competing microflora (Antoun and Kloepper 2001). Among the various PGPR, fluorescent *Psuedomonas* is considered as the most important as it has both plant growth promotion activity and production of antifungal secondary metabolite. In recent years several plant root-colonizing *Pseudomonas* spp have shown to be potent biocontrol agents in various plant-pathogen systems (Thomashow and Weller 1996). The production of antifungal secondary metabolites like 2,4-diacetylphloroglucinol (2,4-DAPG/ DAPG or Phl), pyoluteorin (Plt), hydrogen cyanide, phenazines or pyrrolnitrin (PRN) is a prominent feature of many biocontrol fluorescent *Pseudomonas* spp (Raaijmakers and Weller 1998). Compatibility study of biocontrol agent with commonly used fungicides, insecticides and plant extracts can be useful for the successful integration of these formulations as an essential component in IPM in an agro-ecosystem.

MATERIAL and METHODS

Under compatibility studies two isolates of fluorescent *Pseudomonads* from the collections of the Department of Plant Pathology, University of Agricultural Sciences, Raichur, Karnataka were used.

The isolates used were EP5 (*Pseudomonas fluorescens*) and RP46 (*P putida)* whose identity and details are presented in Table 1 along with NCBI accession number. The list of fungicides, insecticides and botanicals which were used in the present study is given in Tables 2 and 3. Compatibility study was done using poison food technique (Shravelle 1961).

Required quantities of the fungicides and insecticides were added aseptically into 100 ml King's B medium just before pouring in sterilized Petri dishes. Petri dish containing King's B medium without fungicide and insecticide was served as control. For study of compatibility of botanicals the extraction was done in a sterilized pestle and mortar by adding ethanol and sterile distilled water (1:1 w/v). The extracts were filtered through double-layered cheese cloth, allowed for ethanol evaporation and kept at 5°C in refrigerator for further usage. These extracts were added to sterilized 100 ml KB medium. The medium without extracts served as control.

RESULTS and DISCUSSION

Among the fungicides tested using poisoned food technique it was noted that *P fluorescens* and *P putida* were compatible with hexaconazole, carbendazim and propiconazole at both 0.1 and 0.2 concentrations but incompatible with isoprothiolane at these concentrations. *P fluorescens* (EP5) gave 58.40 x 10^{10} cfu and 23.80 x 10^{10} cfu with propiconazole and thus gave 35.10 per cent and 73.50 per cent reduction over control at 0.1 and 0.2 concentrations respectively (Table 4). In the case of *P putida* highest compatibility was noted with propiconazole with a cfu count of 75.40 x 10^{10} and 25.80 x 10^{10} at 0.1 and 0.2 concentrations respectively (Table 5).

Joseph et al (2003) worked on the compatibility of *Pseudomonas* (PS1) culture with mancozeb and hexaconazole and showed that the antagonist was not inhibited even at the highest concentration of the fungicides. Khan and Gangopadhyay (2008) studied

Table 1. Identity of fluorescent pseudomonads isolates used for compatibility study

Isolate	Identification	Host (rhizosphere/endophyte)	NCBI accession number
EP5	*Pseudomonas fluorescens*	Chickpea (endophyte)	JN624291
RP46	*Pseudomonas putida*	Pigeonpea (rhizosphere)	JN624287

Table 2. List of fungicides and insecticides used for in vitro evaluation against fluorescent pseudomonads

Trade name	Chemical name	Active ingredient	Concentration (%)
Contaf	Hexaconazole	5 EC	0.1, 0.2
Bavistin	Carbendazim	50 WP	0.1, 0.2
Fujione	Isoprothiolane	40 EC	0.1, 0.2
Tilt	Propiconazole	25 EC	0.1, 0.2
Dursban	Chlorpyriphos	20 EC	0.1, 0.2
Confidor	Imidachloprid	17.8 SL	0.1, 0.2
Regent	Fipronil	80 WG	0.1, 0.2
Applaud	Buprofezin	25 SC	0.1, 0.2

EC: Emulsifiable concentrate, WP: Wettable powder, SL: Soluble liquid, WG: Wettable concentrate, SC: Suspended concentrate

Table 3. List of botanicals used for in vitro evaluation against fluorescent pseudomonads

Botanical	Concentration (%)
Neem seed kernel extract (NSKE)	2.5, 5.0
Nimbicidine (0.03% azadiractin)	2.5, 5.0
Neem leaf extract	2.5, 5.0
Neem oil garlic emulsion	2.5, 5.0

Table 4. Compatibility of *P fluorescens* (EP5) isolate with different fungicides

Fungicide	cfu (x 10^{10}) at concentration		Reduction in cfu (%) at concentration	
	0.1%	0.2%	0.1%	0.2%
Propiconazole	58.40	23.80	35.10	73.50
Carbendazim	21.60	10.40	76.00	88.40
Hexaconazole	42.20	14.00	53.10	84.40
Isoprothiolane	0.00	0.00	100.00	100.00
Control	90.00			
	SEm±	$CD_{0.01}$		
Fungicide (A)	0.66	1.70		
Concentration (B)	0.46	1.18		
Interaction (A x B)	0.93	2.39		

Table 5. Compatibility of *P putida* (RP46) isolate with different fungicides

Fungicide	cfu (x 10^{10}) at concentration		Reduction in cfu (%) at concentration	
	0.1%	0.2%	0.1%	0.2%
Propiconazole	75.40	25.80	18.90	72.20
Carbendazim	33.20	12.40	64.30	86.60
Hexaconazole	53.20	16.20	42.70	82.50
Isoprothiolane	0.00	0.00	100.00	100.00
Control	93.00			
	SEm±	$CD_{0.01}$		
Fungicide (A)	1.25	3.20		
Concentration (B)	0.88	2.27		
Interaction (A x B)	1.77	4.56		

the in vitro sensitivity of *P fluorescens* towards fungicides and reported that carboxin, chlorothalonil and carbendazim were least toxic to *P fluorescens* strain PFBC-25 while captan was most inhibitory to this strain. Laha and Venkatraraman (2001) noted the compatibility of *P fluorescens* with carbendazim while studying sheath blight management in rice.

P fluorescens and *P putida* were compatible with chlorpyriphos and imidachloprid but fipronil and buprofezin were incompatible. The EP5 isolate of *P fluorescens* gave 72.80 x 10^{10} and 41.40 x 10^{10} cfu with chlorpyriphos and 14.30 and 51.20 per cent reduction over control at 0.1 and 0.2 per cent respectively. It gave 52.00 x 10^{10} and 26.80 x 10^{10} cfu with imidachloprid resulting in 38.80 and 68.40 per cent reduction over control at 0.1 and 0.2 per cent respectively (Table 6). *P putida* RP46 isolate on the other hand recorded 76.40 x 10^{10} and 36.80 x 10^{10} cfu with chlorpyriphos resulting in 19.50 and 61.20 per cent reduction over control at 0.1 and 0.2 concentrations as against 95.00 x 10^{10} cfu in control. This was followed by imidachloprid which gave a cfu count of 56.60 x 10^{10} and 17.00 x 10^{10} at 0.1 and 0.2 per cent concentrations respectively (Table 7).

Both *P fluorescens* and *P putida* showed incompatibility with neem oil garlic emulsion at both concentrations. *P fluorescens* isolate (EP5) gave 54.60 x10^{10} cfu and 24.80 x 10^{10} cfu at 2 and 5 per cent respectively with neem seed kernel extract (Table 8). *P putida* isolate RP46 also showed highest compatibility with neem seed kernel extract recovering 72.20 x10^{10} and 28.80 x 10^{10} cfu at 2 and 5 per cent and 19.70 and 68.00 per cent reduction over control respectively (Table 9). Manjunath et al (2011) reported the compatibility of *P fluorescence* with carbendazim and thiram among fungicides, imidachloprid and carbofuran among insecticides at both 0.1 and 0.2 concentrations and among plant products fluorescent

Table 6. Compatibility of *P fluorescens* (EP5) isolate with different insecticides

Insecticide	cfu (x 10^{10}) at concentration		Reduction in cfu (%) at concentration	
	0.1%	0.2%	0.1%	0.2%
Chlorpyriphos	72.80	41.40	14.30	51.20
Imidachloprid	52.00	26.80	38.80	68.40
Fipronil	0.00	0.00	100.00	100.00
Buprofezin	0.00	0.00	100.00	100.00
Control	85.00			
	SEm±	$CD_{0.01}$		
Insecticide (A)	0.67	1.72		
Concentration (B)	0.47	1.21		
Interaction (A x B)	0.94	2.42		

Table 7. Compatibility of *P putida* (RP46) isolate with different insecticides

Insecticide	cfu (x 10^{10}) at concentration		Reduction in cfu (%) at concentration	
	0.1%	0.2%	0.1%	0.2%
Chlorpyriphos	76.40	36.80	19.50	61.20
Imidachloprid	56.60	17.00	38.40	82.10
Fipronil	0.00	0.00	100.00	100.00
Buprofezin	0.00	0.00	100.00	100.00
Control	95.00			
	SEm±	$CD_{0.01}$		
Insecticide (A)	0.68	1.21		
Concentration (B)	0.33	0.85		
Interaction (A x B)	067	1.72		

Table 8. Compatibility of *P fluorescens* (EP5) isolate with different botanicals

Botanical	cfu (x 10^{10}) at concentration		Reduction in cfu (%) at concentration	
	2.5%	5.0%	2.5%	5.0%
NSKE	54.60	24.80	39.30	72.40
NOGE	0.00	0.00	100.00	100.00
Nimbicidine	48.60	14.20	46.00	84.20
Neem leaf extract	25.60	11.20	71.50	87.50
Buprofezin	0.00	0.00	100.00	100.00
Control	90.00			
	SEm±	$CD_{0.01}$		
Botanical (A)	0.70	1.80		
Concentration (B)	0.50	1.29		
Interaction (A x B)	1.00	2.58		

Table 9. Compatibility of *P putida* (RP46) isolate with different botanicals

Botanical	cfu (x 10^{10}) at concentration		Reduction in cfu (%) at concentration	
	2.5%	5.0%	2.5%	5.0%
NSKE	72.20	28.80	19.70	68.00
NOGE	46.40	13.20	48.40	85.30
Nimbicidine	0.00	0.00	0.00	0.00
Neem leaf extract	39.80	16.20	55.70	82.00
Control	90.00			
	SEm±	$CD_{0.01}$		
Botanical (A)	0.75	1.93		
Concentration (B)	0.53	1.36		
Interaction (A x B)	1.06	2.73		

Pseudomonas was compatible with neem seed kernel extract, garlic bulb extract and Tulsi leaf extract at 2.00 and 5.00 per cent concentrations.

CONCLUSION

The problems of present day crop protection are multiple and can't be mitigated with single-bullet approach or quick-kill measures. The success of an IPM technology depends upon how best the various components are integrated right from planting to harvesting. Hence it is essential that potential bioagents used for crop protection are compatible with commonly used fungicides, insecticides and plant products so that they can be integrated and practiced in systems approach.

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