



## Research Note

# Evaluation of different fungicides and their compatibility with *Pseudomonas fluorescens* in the control of redgram wilt incited by *Fusarium udum*

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**ABSTRACT:** *In vitro* studies with 36 isolates of *Pseudomonas fluorescens* were tested against *Fusarium udum*, 15 isolates showed maximum percentage of inhibition of *F. udum*. Highest percentage of inhibition (87.40%) was recorded in CPF<sub>4</sub> isolate and the least in KPF<sub>12</sub>. *In vitro* efficacy of four systemic fungicides viz., carbendazim, propiconazole, tebuconazole and hexaconazole and two non-systemic fungicides viz., mancozeb and cheshunt compound were evaluated at 50, 100, 250, 500 and 1000 ppm concentrations, Tebuconazole, carbendazim, propiconazole completely inhibited the mycelial growth of the pathogen even at 50 ppm followed by cheshunt compound at 100 ppm. *In vitro* compatibility of six fungicides against *F. udum* with potential antagonist CPF<sub>4</sub> at different concentrations, indicated carbendazim to be of high compatibility with CPF<sub>4</sub> followed by mancozeb.

**KEY WORDS:** *Fusarium udum*, *Pseudomonas fluorescens*, compatibility

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Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is widely grown in the Indian sub-continent and accounts for almost 90 per cent of the world's pigeonpea area. In Andhra Pradesh, it is grown in an area of 0.462 mha with annual production of 0.301 mt and with an average yield of 652 kg ha<sup>-1</sup>. The wilt caused by *Fusarium udum* (Butler) is one of the most important diseases of pigeonpea in India resulting in yield losses up to 67 per cent at maturity and 100% in case of infection at pre pod stage (Kannaiyan and Nene, 1981). An annual loss of US \$ 36 millions was estimated due to this disease in India alone (Kanaiyan *et al.*, 1984). *F. udum*, the causal organism of redgram wilt is soil borne and is capable of saprophytic survival on crop residues in the soil for up to eight years (Nene, 1980). Chemical control of the disease is therefore difficult, impractical and uneconomical, as the large scale soil application of chemicals required is expensive, hazardous and disturbs the ecological balance. Though, there is lot of information available on bio-control of the wilt disease in redgram, little work has been done on development of bio-formulation, based on *Pseudomonas fluorescens* and its compatibility with different fungicides. Therefore, the efficacy of native bacterial antagonists and four systemic fungicides and two non-systemic fungicides on wilt of redgram in *in vitro* and the compatibility of fungicides with *P. fluorescens* was studied.

The laboratory experiments pertaining to the research work were conducted in the Department of Plant Pathology, S.V. Agricultural College, Tirupati, Chittoor district (A.P.). The general laboratory techniques described by Dingra and Sinclair (1995), Rangaswami and Mahadevan (1999), Nene and Thapliyal (1993) and Aneja (1993) were followed for preparation of media, sterilization, isolation and maintenance of fungal cultures with slight modifications wherever necessary. The pathogen was isolated from the wilt infected redgram plants by using tissue segment method (Rangaswami and Mahadevan, 1999). Dual culture technique was used to identify the potential antagonist from rhizosphere (Mahesh and Mohammad Saifulla, 2006). *In vitro* studies were conducted to test efficacy of different systemic fungicides (carbendazim, hexaconazole, tebuconazole, propiconazole) and non-systemic fungicides (mancozeb, cheshunt compound) at 50, 100, 250, 500 and 1000 ppm concentrations against the test fungus *Fusarium udum*. All the treatments were replicated thrice and suitable control was maintained.

A total of 112 rhizosphere soil samples from healthy plants of disease infested fields were collected during survey in 2010 from Chittoor and Kadapa districts of A.P. for isolation of bacterial antagonist. Bacterial colonies

(*P. fluorescens*) were observed after two days of inoculation on King's B medium. Bacterial antagonists were purified on King's B medium and maintained in NA slants for further experimental studies. To test the efficacy of *P. fluorescens*, a loopful of bacterial culture was taken and streaked longitudinally in a zigzag fashion at one end of the Petriplate, simultaneously 5 mm disc of mycelial growth of *F. udum* was placed on opposite side of the streak and incubated at  $28 \pm 2C$  for 5 d (Narasimha Rao *et al.*, 2004). The per cent reduction in radial growth of test pathogen was calculated after 5 d incubation. Three replications were maintained for each isolate and suitable control was maintained without antagonist. Growth of pathogen was measured 7 days after recording full growth of the test pathogen in control plate. Per cent inhibition of mycelial growth of test pathogen was calculated by using the formula.  $I = (C - T / C) \times 100$  where, I = Per cent reduction in growth of test pathogen, C = Radial growth of test pathogen (mm) in control, T = Radial growth of test pathogen (mm) in treatment.

Poisoned food technique was followed as described by Vyas (2002). Fifty ml double strength PDA was mixed with 50 ml of double concentrated fungicidal solution to obtain required final concentrations of 50, 100, 250, 500 and 1000 ppm. Twenty ml of this medium was plated in 90 mm diam Petriplates. A 6 mm mycelial disc of 5 d old pathogen was inoculated at the centre and incubated at  $28 \pm 2C$  for 10 d. A control was maintained without fungicide. Per cent reduction in radial growth of pathogen over control was calculated using the formula:  $I = (C - T / C) \times 100$  Where, I = Pre cent reduction in growth of test pathogen, C = Radial growth of test pathogen (mm) in control, T = Radial growth of test pathogen (mm) in treatment.

*P. fluorescens* found superior in antagonism was tested for their compatibility with systemic (carbendazim, hexaconazole, Tebuconazole, propiconazole) and non-systemic fungicides (mancozeb, cheshunt compound) by using spectrophotometric method under *in vitro* (Kishore *et al.*, 2005). The antagonistic bacterial culture was grown in 500 ml of nutrient broth (NB) for 16 hours at  $28 \pm 2C$ , rotated gently at 180 rpm on orbital shaker and dispensed 50 ml of it to 250 ml flasks containing different concentration of fungicides. Inoculated flasks were incubated at  $28 \pm 2C$  at 180 rpm on orbital shaker. Bacterial growth was determined in Systronic spectrophotometer at 600 nm after 24 h of incubation. Each treatment was replicated thrice.

The redgram variety "LRG-41" popularly cultivated in Chittoor and Kadapa districts was used for pot culture studies. The pathogen was mass multiplied on sand-pigeonpea flour medium and added to soil at the time of sowing @ 200 g kg<sup>-1</sup> of soil. The potential biocontrol agent and compatible fungicide were evaluated under glasshouse conditions against the pathogen by imposing the treatments *viz*; T<sub>1</sub>: Seed treatment with *P. fluorescens* @ 4g kg<sup>-1</sup> of seed, T<sub>2</sub>: Seed treatment with compatible fungicide @ 2g kg<sup>-1</sup> of seed, T<sub>3</sub>: Soil application with *P. fluorescens* @ 2 g kg<sup>-1</sup> of soil, T<sub>4</sub>: Soil drenching with compatible fungicide @ 20 ml kg<sup>-1</sup> of soil, T<sub>5</sub>: Seed treatment with *P. fluorescens* @ 4g kg<sup>-1</sup> of seed + Seed treatment with compatible fungicide @ 2g kg<sup>-1</sup> of seed, T<sub>6</sub>: Soil application with *P. fluorescens* @ 2 g kg<sup>-1</sup> of soil + Soil drenching with compatible fungicide @ 20 ml kg<sup>-1</sup> of soil, T<sub>7</sub>: Seed treatment with compatible fungicide @ 2g kg<sup>-1</sup> of seed + Soil application with *P. fluorescens* @ 2 g kg<sup>-1</sup> of soil, T<sub>8</sub>: Un inoculated control, T<sub>9</sub>: Inoculated control. Three replications were maintained for each treatment.

Required quantity of seed was taken in polythene bag and treated with talc formulation of *P. fluorescens* @ 4g kg<sup>-1</sup> of seed by uniform shaking for seed treatment with bioagent. For treating the seed with compatible fungicide, required quantity of fungicide was added to the seed and shaking them in a closed vessel for 5 to 15 minutes to facilitate even coating of fungicide over the surface of all seeds. For treatment with potential biocontrol agent and compatible fungicide, initially the seeds were treated with biocontrol agent and followed by compatible fungicide. For soil treatment, the talc formulation was used @ 2g kg<sup>-1</sup> of soil. For drenching the soil with fungicide, the fungicidal solution was prepared by dissolving 2 g of compatible fungicide in one litre of water and applied to soil @ 20 ml kg<sup>-1</sup> of soil. Observations *viz*; percentage of disease incidence (PDI), shoot and root length and dry weight of shoots and roots were recorded.

A total of 36 *P. fluorescens* isolates were obtained from the rhizosphere soil samples collected from Chittoor and Kadapa districts which were evaluated for their efficacy against *F. udum*. Maximum percentage of inhibition (87.40%) was recorded with CPF<sub>4</sub> isolate and fifteen isolates which showed inhibition of >60% were selected for further studies. The isolate CPF<sub>4</sub> showed maximum percentage of inhibition (87.40%) followed by KPF<sub>15</sub> (85.00). However, both were found to be on par with each other

in inhibiting the growth of the pathogen in dual culture (Table 1).

**Table 1. *In vitro* screening of *Pseudomonas fluorescens* against *Fusarium udum* by dual culture technique**

Isolates	Radial growth of <i>F. udum</i> (CFU <sub>2</sub> ) (mm)*	% inhibition over control
CPF <sub>1</sub>	25.20	69.30 (56.36)**
CPF <sub>2</sub>	21.50	73.80 (59.22)
CPF <sub>3</sub>	24.80	69.80 (56.69)
CPF <sub>4</sub>	10.30	87.40 (69.24)
CPF <sub>5</sub>	28.10	65.70 (54.17)
CPF <sub>6</sub>	23.20	71.70 (57.89)
CPF <sub>7</sub>	29.50	64.00 (53.14)
CPF <sub>8</sub>	30.20	63.20 (52.66)
KPF <sub>9</sub>	22.30	72.80 (59.87)
KPF <sub>10</sub>	18.20	77.80 (61.98)
KPF <sub>11</sub>	27.50	66.50 (54.66)
KPF <sub>12</sub>	31.10	62.10 (52.01)
KPF <sub>13</sub>	26.90	67.20 (55.06)
KPF <sub>14</sub>	20.70	74.80 (59.87)
KPF <sub>15</sub>	12.00	85.00 (67.48)
Control	82.00	0.00 (0.00)

\* Mean of three replications

\*\* Figures in parentheses are angular transformed values.

CPF : Chittoor *Pseudomonas fluorescens*; KPF : Kadapa *Pseudomonas fluorescens*

S. Ed.	1.3224	1.7014
CD ( $P = 0.05$ )	2.6939	3.4661
C.V (%)	5.97	3.83
S.Em	0.9350	1.20307

The inhibition percentage of other isolates were CPF<sub>2</sub> (73.80), CPF<sub>6</sub> (71.70), KPF<sub>9</sub> (72.80), KPF<sub>10</sub> (77.80) and KPF<sub>14</sub> (74.80). Least percentage inhibition was observed in the isolate KPF<sub>12</sub> (62.10). The isolate CPF<sub>4</sub> with maximum percentage inhibition (87.40%) was selected as potential native antagonistic bacterium against virulent *Fusarium udum* isolate CFU<sub>2</sub> and it was used for further studies. In a similar study, Gowdars and Srikantkulkarni (2007) reported that *Bacillus subtilis* and *P. fluorescens* produced inhibition zone of 3.5 mm and

7.5 mm respectively against *Fusarium udum* and 81.87% inhibition was recorded with *P. fluorescens*.

Fluorescent pseudomonads can be tested for production of fluorescent pigment on King's B medium and FeCl<sub>3</sub>-amended King's B medium. Fluorescent pseudomonads produce a fluorescent pigment on King's B medium but not on FeCl<sub>3</sub>- amended King's B medium.

All the fungicides at all concentrations reduced mycelial growth of *F. udum* when compared to control (Table 2). Among the fungicides, propiconazole, tebuconazole and carbendazim showed 100 per cent inhibition of mycelial growth at concentration from 50 to 1000 ppm followed by hexaconazole, mancozeb and cheshunt compound which showed 100 per cent inhibition at 500 ppm and 1000 ppm, respectively. Mancozeb showed 58.82, 85.88 and 90.58 per cent inhibition at 50, 100 and 250ppm concentrations, whereas hexaconazole showed 85.88, 90.58 and 92.94 per cent of inhibition at 50, 100 and 250 ppm and cheshunt compound showed 78.82 per cent inhibition of mycelial growth of *F. udum* at 50 ppm. Overall per cent inhibition of mycelial growth of *F. udum* was maximum with carbendazim, propiconazole and tebuconazole followed by cheshunt compound. In a similar study, Sumitha and Gaikwad (1995) reported that *F. udum* was completely inhibited by bavistin (0.1%), topsin-M-70 (0.1%), thiram (0.1%), captan (0.15%) and dithane-Z-78 (0.37) in *in vitro*. Penchal Raju *et al.* (2006) studied the effect of carbendazim, captan, Dithane-Z-78, thiophanatemethyl and thiram against *Fusarium udum* under *in vitro*. Among which, carbendazim was found to be effective at 100, 250 and 500 ppm concentrations. Shah *et al.* (2006) reported that mancozeb showed maximum inhibition of *F. udum* as compared to carbendazim, mancozeb, sulphur and companion (mancozeb 63% + carbendazim 12%) fungicides.

The potential antagonist *P. fluorescens* (CPF<sub>4</sub>) showed maximum inhibition of *F. udum* growth in dual culture studies was used to evaluate the compatibility with different fungicides which indicated high compatibility with specific fungicide. The mean OD values of *P. fluorescens* (CPF<sub>4</sub>) at different fungicide concentrations were *viz.*, propiconazole (0.579), tebuconazole (0.572), hexaconazole (0.601), mancozeb (0.702), carbendazim (0.846), cheshunt compound (0.608) and control (1.230) (Table 3). CPF<sub>4</sub> was more compatible with carbendazim (0.846) followed by mancozeb (0.702) and cheshunt compound (0.608) while, less compatibility was recorded with tebuconazole (0.572). Similar observations were made by Vidhyasekharan and Muthamilan (1995) and

**Table 2. In vitro evaluation of efficacy of fungicides on growth of *Fusarium udum***

Sl. No.	Fungicide	Per cent inhibition of mycelial growth of <i>F. udum</i>					
		Concentration (ppm)					
		50	100	250	500	1000	Mean*
Systemic fungicides							
1	Propiconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
2	Tebuconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
3	Hexaconazole	85.88 (68.17)	90.58 (72.17)	92.94 (74.61)	100.00 (90.00)	100.00 (90.00)	93.88 (78.99)
4	Carbendazim	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Non systemic fungicides							
5	Mancozeb	58.82 (50.09)	85.88 (67.94)	90.58 (72.77)	100.00 (90.00)	100.00 (90.00)	87.05 (74.16)
6	Cheshunt compound	78.82 (62.63)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	95.76 (85.52)
7	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

\*Mean of three replications

\*\*Figures in parentheses are angular transformed values

	S Em ±	CD ( $P = 0.05$ )
Fungicide	0.37	1.05
Concentration	0.31	0.89
Fungicide x concentration	0.83	2.36

reported that carbendazim was not inhibitory to *P. fluorescens* under *in vitro* conditions. Durai (2004) reported that among the five fungicides tested for compatibility, least inhibition (0.14%) of *P. fluorescens* was shown by mancozeb. Khan and Gangopadhyay (2008) tested the compatibility of *P. fluorescens* with fungicides and revealed that carboxin and carbendazim were least toxic to *P. fluorescens* strain PFBC-25, where as captan was most inhibitory to this strain. Present study indicated that the carbendazim was effective on *F. udum* isolate CFU<sub>2</sub> and also compatible with *P. fluorescens* isolate CPF<sub>4</sub>.

All the treatments were significantly superior over control in reducing the disease incidence (Table 4). Maximum disease reduction was observed in treatment T<sub>6</sub> [(soil application of carbendazim @ 20 ml kg<sup>-1</sup> + soil application of effective bacterial antagonist @ 2g kg<sup>-1</sup> (talc based)] in which PDI of 11.1 per cent was noticed when compared to inoculated control (64.9%). Seed

treatment with *P. fluorescens* @ 4g kg<sup>-1</sup> of seed (T<sub>1</sub>) recorded 32.8 PDI and was at par with T<sub>4</sub> treatment (Soil drenching with compatible fungicide @ 20 ml kg<sup>-1</sup> of soil). Seed treatment with compatible fungicide @ 2g kg<sup>-1</sup> of seed (T<sub>2</sub>) was at par with T<sub>3</sub> (soil application with *P. fluorescens* @ 2 g kg<sup>-1</sup> of soil). Treatments T<sub>4</sub> (soil drenching with compatible fungicide @ 20 ml kg<sup>-1</sup> of soil) and T<sub>7</sub> (seed treatment with *P. fluorescens* @ 4g kg<sup>-1</sup> of seed (T<sub>1</sub>) + Seed treatment with compatible fungicide @ 2g kg<sup>-1</sup> of seed (T<sub>2</sub>)) were at par with each other.

Maximum plant height (98.4 cm) was recorded in integrated treatment (T<sub>6</sub>) (Soil application with *P. fluorescens* @ 2 g kg<sup>-1</sup> of soil (T<sub>3</sub>) + Soil drenching with compatible fungicide @ 20 ml kg<sup>-1</sup> of soil (T<sub>4</sub>).

It is evident that least plant height was recorded in inoculated control. Treatment-6 (Soil application with *P. fluorescens* @ 2 g kg<sup>-1</sup> of soil (T<sub>3</sub>) + Soil drenching with

**Table 3. *In vitro* compatibility of potential *Pseudomonas fluorescens* (CPf<sub>1</sub>) with different fungicides**

Fungicide	Growth of bacterial isolate (OD at 600 nm)					*Mean
	50 ppm	100 ppm	250 ppm	500 ppm	1000 ppm	
Propiconazole	0.745	0.630	0.610	0.480	0.430	0.579
Tebuconazole	0.705	0.620	0.560	0.540	0.435	0.572
Hexaconazole	0.720	0.665	0.635	0.520	0.466	0.601
Mancozeb	0.932	0.814	0.720	0.530	0.515	0.702
Carbendazim	0.985	0.920	0.870	0.740	0.715	0.846
Cheshunt compound	0.840	0.745	0.582	0.472	0.402	0.608
Control	–	–	–	–	–	1.230

\* Mean of three replications

	SEm ±	CD ( <i>P</i> = 0.05)
Fungicide	0.0052	0.0147
Concentration	0.0044	0.0124
Fungicide x concentration	0.0116	0.0328

**Table 4. Integrated management of redgram wilt under glasshouse conditions**

Treatment No.	Treatment	*Per cent disease incidence	Plant height (cm)	Root length (cm)	Dry weight (g)	
					Shoot	Root
T1	Seed treatment with <i>P. fluorescens</i> @ 4g kg <sup>-1</sup> of seed	32.80 **(34.93)	82.59	13.92	4.92	0.41
T2	Seed treatment with compatible fungicide @ 2g kg <sup>-1</sup> of seed	27.42 (31.56)	73.08	12.09	3.62	0.35
T3	Soil application with <i>P. fluorescens</i> @ 2 g kg <sup>-1</sup> of soil	25.37 (30.23)	79.25	14.80	5.01	0.51
T4	Soil drenching with compatible fungicide @ 20 ml kg <sup>-1</sup> of soil	28.51 (32.24)	70.37	13.18	4.25	0.39
T5	T1 + T2	19.68 (26.33)	81.64	15.02	5.68	0.60
T6	T3 + T4	11.06 (19.41)	98.42	18.95	7.85	1.26
T7	T2 + T3	16.54 (23.99)	93.69	16.18	6.12	0.92
T8	Un inoculated control	0.00 (0.00)	94.50	17.20	6.57	1.14
T9	Inoculated control	64.89 (53.67)	54.41	9.52	2.18	0.25
SEm±	0.71	2.56	0.68	0.22	0.04	
CD ( <i>P</i> = 0.05)	2.36	7.62	2.02	0.67	0.12	

\* Mean of three replications

\*\*Figures in parenthesis are angular transformed values

compatible fungicide @ 20 ml kg<sup>-1</sup> of soil (T4) stimulated the growth of the plant by obtaining increase in root length (18.95 cm), shoot weight (7.85 g), root weight (1.26 g) and dry weights of both shoot and root compared to inoculated control and was found to be superior to all other treatments.

In the present investigation, maximum disease control was observed in integrated treatment (T<sub>6</sub>). Madhavi *et al.* (2006) utilized carbendazim tolerant mutants of *T. viride* (Tvm<sub>1</sub>) and *T. harzianum* (Thm<sub>1</sub>) and a bacterial biological control agent *P. fluorescens* in combination with different compatible fungicides (carbendazim, fipronil, fluchloralin) for the management of *Fusarium solani* causing wilt in chilli. The effectiveness of these mutants (Tvm<sub>1</sub> and Thm<sub>1</sub>) and *P. fluorescens* increased when used as soil and seed application treatments and also when combined with soil drenching chemicals resulted in zero per cent wilt incidence.

*Pseudomonas fluorescens* was observed to increase germination, shoot length and root length in several crops (Jayalakshmi *et al.*, 2003; Manoranjithan *et al.*, 1999; Sendhilvel *et al.*, 2005) conforming to the present studies.

The present findings corroborate with the reports of other workers that the integration of biocontrol agent with compatible fungicide gave significantly higher disease control in several crops than obtained by use of either biocontrol agent (or) fungicide alone (Henis *et al.*, 1978; Sawant and Mukhopadhyay, 1991).

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