Research Paper :

Survival and effect of *Pseudomonas fluorescens* formulation developed with various carrier materials in the management of late leaf spot of groundnut

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SUMMARY

The effect of various carrier materials *viz*., gypsum, lignite, talc, vermiculite and their combinations in supporting the growth of *Pseudomonas fluorescens* was assessed. The population of *P. fluorescens* at different days after storage was estimated. Among the various formulations tested, talc based and talc + gypsum based formulations supported better survival of *P. fluorescens*. Talc based powder formulation of *P. fluorescens* Pf1 isolate was highly effective in reducing the late leaf spot disease intensity.

Key words : *Pseudomonas fluorescens,* Formulation, Carrier, Groundnut, Late leaf spot

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Late leaf spot caused by *Cercosporidium personatum* is the destructive foliar disease in groundnut. The most obvious effect of this disease is the loss of photosynthetic tissue, which leads to premature defoliation (Kaur *et al*., 1992). Fluorescent pseudomonads have emerged as the largest and potentially most promising group of plant growth promoting rhizobacteria (PGPR) for biocontrol of plant diseases (Liu *et al.,* 1995). Several fluorescent Pseudomonads were known to control soil borne fungal pathogens like *Pythium*, *Fusarium*, *Rhizoctonia* in a wide range of crops (Vidhyasekaran *et al*., 1997a and b). Earlier workers have used bacterial cell suspension for seed treatment, soil application or foliar spray for the control of foliar diseases. Injection or other methods of application of bacterial suspension is impracticable for large scale application to control foliar diseases in field (Capper and Higgins, 1993). Bacterial cell suspension cannot be used for large-scale field use due to difficulty in storage, transport and handling. A powder formulation with a long shelf-life would be beneficial.

The present study was undertaken to develop *P. fluorescens* as a commercial formulation with suitable carrier and to test the efficacy of the developed formulation as seed treatment and foliar spray for the management of diseases under greenhouse condition.

MATERIALS AND METHODS

P. fluorescens was isolated using King's B (KB) medium (King *et al.,* 1954). King's B broth was inoculated with *P. fluorescens* isolate Pf1 and bacterium was grown with constant shaking at 150 rpm for 48 h at room temperature (28±2^{0}C). Centrifugation was done at 6000 g for 10 minutes and the cells were resuspended in 0.01M phosphate buffer, pH 7.0, concentration was adjusted to give 10^9 colony forming units (cfu) per ml (OD_{595}=0.3).

Efficacy of various carrier materials to support the growth of *P. fluorescens* in storage was assessed. Talc, vermiculite, lignite, gypsum and their combinations were used as carrier materials and pH was adjusted to neutral by adding calcium carbonate at the rate of 15 g kg^{-1} substrate. These substrates were taken at the rate of 100 g per polypropylene bag along with 1 g of CMC, sealed and autoclaved at 1.4 kg cm^{-2} for one hour on two successive days. To this, 40 ml of 48 h old bacterial inoculum as described above was added, mixed under aseptic condition and stored at room temperature (28±2^{0}C). At 15 days interval, one gram sample was drawn from each bag and serially diluted in sterile distilled water up to 10^{-8} level and 1 ml aliquot from 10^8 dilution was pipetted out, poured in sterile Petri dishes to which King'B medium was poured, gently rotated and incubated at room temperature (28±2^{0}C). The number of colonies were counted after 24 h.

Groundnut plants (45 days old) were sprayed with Pf1 formulation at the rate of 1 kg ha^{-1} and after two days, the plants were inoculated with conidial suspension of *C. personatum* (5x10^4 spores ml^{-1}). Disease

intensity was assessed at 70 and 90 days after sowing using a modified 9-point scale (Subrahmanyam *et al*., 1995). The number of nodules per plant was recorded at 75 days after sowing. The pod yield per plant was also recorded.

RESULTS AND DISCUSSION

Several formulations of *P. fluorescens* had been developed for large-scale field application (De Freitas and Germida, 1992). The survival capacity of *P. fluorescens* Pf1 isolate in gypsum, lignite, talc, vermiculite and their combinations was determined. The population of *P. fluorescens* was estimated at 0, 15, 30, 45, 60, 75 and 90 days after storage. Among the various formulations tried, talc based and talc + gypsum based formulations supported better survival of *P. fluorescens* followed by gypsum based and gypsum + lignite based formulations. Talc based and talc + gypsum based formulations supported the growth of bacterium efficiently even after 90 days of storage at room temperature. Vermiculite and vermiculite combinations were the least effective in supporting the survival of *P. fluorescens* (Table 1). In general, the survival of *P. fluorescens* in carrier materials decreased during storage.

Among the various formulations of Pf1 isolate tested against late leaf spot, talc based powder formulation significantly reduced the disease intensity followed by gypsum + talc formulation. The talc based formulation of *P. fluorescens* reduced the late leaf spot disease intensity by 42 per cent and increased the pod yield by 60 per cent when applied as foliar spray. Lignite + vermiculite formulation was the least effective in reducing the disease intensity. Nodules per plant was found to be maximum in talc and gypsum + talc formulations. Vermiculite based formulations recorded the lowest nodules per plant. Foliar application of talc based formulation gave the maximum pod yield per plant. Vermiculite based formulations gave the lowest pod yield per plant (Table 2). A talc based powder formulation of *P. fluorescens* was effective for the control of rice blast (Vidhyasekaran *et al*., 1997a).

Various workers had reported different carriers for delivery of various bacteria to the desired target; *P. fluorescens* in peat soil, dried-dust formulation of xanthan gum Arabic, powdered vermiculite, diatomaceous earth,

Table 1 : Survival of *P. fluorescens* Pf1 isolate in different carrier materials

Carrier materials	Population of *P. fluorescens* (cfu x 10^8 g^{-1})						
	Days of storage						
	0	15	30	45	60	75	90
Gypsum	16.0^{bc}	12.3^{b}	9.3^{b}	7.3^{c}	7.0^{bc}	7.0^{cd}	6.3^{b}
	(9.2)	(9.1)	(9.0)	(8.9)	(8.8)	(8.8)	(8.8)
Lignite	12.0^{de}	8.0^{cd}	8.0^{c}	6.3^{c}	4.0^{e}	4.0^{ef}	4.0^{cd}
	(9.1)	(8.9)	(8.9)	(8.8)	(8.6)	(8.6)	(8.6)
Talc	20.0^{a}	14.7^{a}	12.3^{a}	12.0^{a}	11.0^{a}	11.0^{a}	9.3^{a}
	(9.3)	(9.1)	(9.1)	(9.1)	(9.0)	(9.0)	(9.0)
Vermiculite	11.3^{e}	10.0^{bc}	8.3^{c}	6.7^{c}	6.0^{cd}	5.3^{de}	4.0^{cd}
	(9.1)	(9.0)	(8.9)	(8.8)	(8.8)	(8.7)	(8.6)
Gypsum + Lignite (1:1)	17.3^{b}	12.0^{b}	9.7^{b}	9.0^{b}	8.3^{b}	8.0^{bc}	7.3^{b}
	(9.2)	(9.1)	(9.0)	(9.0)	(8.9)	(8.9)	(8.9)
Gypsum + Talc (1:1)	20.7^{a}	15.0^{a}	12.3^{a}	12.0^{a}	12.0^{a}	11.0^{a}	11.0^{a}
	(9.3)	(9.2)	(9.1)	(9.1)	(9.1)	(9.0)	(9.0)
Gypsum + Vermiculite (1:1)	8.3^{f}	7.3^{d}	6.0^{d}	6.0^{cd}	4.3^{e}	4.0^{ef}	3.0^{d}
	(8.9)	(8.8)	(8.8)	(8.8)	(8.6)	(8.6)	(8.5)
Lignite + Talc (1:1)	14.0^{cd}	10.0^{bc}	9.0^{bc}	6.7^{c}	6.0^{cd}	5.3^{de}	5.0^{c}
	(9.1)	(9.0)	(9.0)	(8.8)	(8.8)	(8.7)	(8.7)
Lignite + Vermiculite (1:1)	7.7^{f}	6.7^{d}	6.0^{d}	4.0^{d}	4.0^{e}	3.0^{f}	2.3^{d}
	(8.9)	(8.8)	(8.8)	(8.6)	(8.6)	(8.5)	(8.4)
Talc + Vermiculite (1:1)	8.0^{f}	8.0^{cd}	6.3^{d}	5.0^{d}	5.0^{de}	3.3^{f}	3.0^{d}
	(8.9)	(8.9)	(8.8)	(8.7)	(8.7)	(8.5)	(8.5)
Gypsum + Lignite + Talc + Vermiculite	8.0^{f}	8.0^{cd}	8.0^{c}	6.3^{c}	6.0^{cd}	6.0^{d}	4.0^{cd}
(1:1)	(8.9)	(8.9)	(8.9)	(8.8)	(8.8)	(8.8)	(8.6)

Data represented mean of three replications

Data followed by the same letter in a column are not significantly different (p=0.05) by DMRT

Table 2 : Effect of foliar application of *P. fluorescens* Pf1 formulation with various carrier materials in the management of late leaf spot

Carrier materials	Nodules plant^{-1} (75 DAS)	Disease intensity (grade)		Pod yield plant^{-1} (g)
		70 DAS	90 DAS	
Gypsum	166^{ab}	2.8^{bc}	5.2^{c}	3.6^{b}
Lignite	136^{cd}	3.1^{cd}	5.4^{c}	3.1^{b}
Talc	182^{a}	2.1^{a}	4.1^{a}	5.3^{a}
Vermiculite	125^{d}	3.4^{d}	6.2^{d}	2.8^{bc}
Gypsum + Lignite (1:1)	131^{d}	2.9^{c}	5.4^{c}	3.4^{b}
Gypsum + Talc (1:1)	178^{a}	2.6^{b}	4.8^{bc}	5.1^{a}
Gypsum + Vermiculite (1:1)	134^{d}	3.2^{d}	6.2^{d}	2.6^{c}
Lignite + Talc (1:1)	138^{c}	3.1^{cd}	5.1^{c}	3.2^{b}
Lignite + Vermiculite (1:1)	126^{d}	3.6^{dc}	6.4^{d}	2.8^{bc}
Talc + Vermiculite (1:1)	131^{d}	3.4^{d}	6.2^{d}	3.2^{b}
Gypsum + Lignite + Talc + Vermiculite (1:1)	134^{d}	3.1^{cd}	5.4^{c}	3.6^{b}
Carbendazim (500 g ha^{-1}) + Mancozeb (1 kg ha^{-1})	151^{bc}	2.3^{ab}	4.3^{ab}	3.8^{b}
Control – Pathogen inoculated	84^{e}	4.0^{e}	7.1^{e}	2.1^{c}
Control – Pathogen uninoculated	120^{d}	2.4^{b}	4.9^{c}	2.8^{bc}

Data represented mean of three replications

Data followed by the same letter in a column are not significantly different (p=0.05) by DMRT

dried talc-methyl cellulose and talc based formulation (Capper and Higgins, 1993; Vidhyasekaran *et al.,* 1997 a and b). Seed treatment of winter wheat by fluorescent Pseudomonads multiplied in King's B broth for 72 h mixed with 1 per cent carboxy methyl cellulose and talc improved the plant growth (De Freitas and Germida, 1992).

The present studies indicated the usefulness of talc based powder formulation of Pf1 isolate for the control of late leaf spot of groundnut. The bacterium was observed to survive up to 90 days in the formulation. This formulation has much practical advantage as it can directly be supplied to the farmers for seed treatment.

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