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Evaluation of *Pseudomonas fluorescens* (Pf-1) against blackgram and sesame root-rot disease

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ABSTRACT: ***Pseudomonas fluorescens*** **strain Pf1, effectively inhibited the mycelial growth of** ***Macrophomina phaseolina,*** **the pathogen causing dry root-rot in blackgram and sesame. Application of Pf1 as seed treatment (10g / kg seed) followed by soil application (2.5 kg / ha) against root rot effectively supported higher plant growth, better native** ***Rhizobium*** **nodulation and grain yield. Sclerotial number and root rot incidence were also greatly reduced. The rhizosphere soil recorded a higher number of Pf1 population.**

KEY WORDS: Biocontrol, *Macrophomina phaseolina, Pseudomonas fluorescens,* root- rot, seed treatment, soil application

Macrophomina phaseolina (Tassi) Goid. a serious soil borne pathogen causes seedling rot / dry rot in blackgram (*Vigna mungo* L.) and sesame (*Sesamum indicum* L.) leading to extensive yield loss. Chemical control measures, with broad-spectrum fungicides, create imbalances in the microbial community, which may be unfavourable for the activity of the beneficial organisms and also lead to the development of resistant strains. It is now widely recognized that biological control of plant pathogens using antagonistic fungi and bacteria is a distinct possibility for the future and can be successfully utilized especially within the framework of integrated disease management system (Muthamilan and Jeyarajan, 1996). Use of antagonistic organisms against *Macrophomina* root-rot has been well documented (Lockwood, 1985; Mukhopadhyay, 1987; Raguchander *et al.*, 1995) in several crops. Heterotrophic rhizobacteria of *Pseudomonas fluorescens* types have been successfully used for biological control of several plant pathogens (Howell and Stipanovic, 1979; Weller and Cook, 1983; Ganesan and Gnanamanickam, 1987). In the present study, we investigated the efficacy of an efficient strain of *Pseudomonas fluorescens* as seed treatment and soil application for the control of *Macrophomina* root-rot in blackgram and sesame.

MATERIALS AND METHODS

Macrophomina phaseolina was isolated from infected blackgram and sesame stems and roots, using potato dextrose agar (PDA) medium. The pathogenicity of the fungus was tested by applying the fungus multiplied in sand + maize medium (Riker and Riker, 1936). Eight blackgram (Co 5) and sesame (TMV 6) seeds were sown in each pot (300mm diam) containing sterilized soil incorporated with the fungal culture at a ratio of 1:19 (sand – maize inoculum: soil). The root-rot incidence was assessed and re-isolation of the pathogen was obtained from the rotted plant.

Rhizoplane – colonizing fluorescent pseudomonads were isolated from fresh roots of blackgram, carrot, banana, tapioca, pepper, rice and forest trees grown in several geographic areas of Tamil Nadu. After vigorous shaking of excised roots to remove all but tightly adhering soil, root segments (1g) were shaken in 100ml of sterile distilled water for 15min and serial dilutions were made. Fluorescent pseudomonads were isolated using King's medium B (KMB) (King *et al.*, 1954) and fluorescent colonies were detected by viewing under UV light.

The efficacy of the fluorescent pseudomonad isolates was tested by streaking the bacteria at one side of the Petri-dish containing PDA. A 4mm-mycelial disc from 5day old *M. phaseolina* culture was placed on the opposite side in the Petri-dish, perpendicular to the bacterial streak. The growth of the fungus was inhibited when it grew toward the bacterial colony in PDA. The inhibition zone was measured from the edge of mycelium to the bacterial colony edge after 7 days of incubation and expressed as per cent inhibition over control. The bacterial strains, which showed inhibition against *M. phaseolina*, were identified based on the following biochemical tests *viz.*, production of fluorescent pigment (King *et al.*, 1954), gelatin liquefaction, nitrate reduction, arginine dihydrolase, levan formation and growth at 4 °C and 41 °C and different carbon source utilization (Hildebrand *et al.*, 1992).

One fluorescent pseudomonad strain Pf 1 which effectively inhibited the growth of *M. phaseolina* isolates obtained from blackgram and sesame under *in vitro* condition and the same was used to develop powder formulation, following the method described by Vidhyasekaran and Muthamilan (1995).

Glasshouse trials were conducted with completely randomized design during February–April, 1999. Blackgram and sesame seeds were separately treated with the talc - based formulation of Pf1 at 4g / kg seed and shade dried for 2h. The blackgram (Co 5) and sesame seeds (TMV 6) were sown separately in pots containing unsterilized field soil. Twenty-five mg of the formulated product (2.5 kg talc based formulation mixed with 50 kg of farmyard manure) was given as soil application / pot at 30 days after sowing (DAS). As a check to biocontrol agent, carbendazim and Bavistin (BASF India Limited, Mumbai) were used @ 2g / kg of seed and 0.05 per cent in pots as soil drenching (SD) at 30 DAS. Seeds without treatments served as control. Eight seeds were sown / pot with three replications for each treatment. The entire trial was repeated and the data presented are the means of two trials.

The rhizosphere populations of the strain were assessed at 30 and 60 days after sowings (DAS) by the method described by Papavizas and Davey (1961). The fluorescent colonies were viewed under UV light. Three replications were maintained and the bacterial population was expressed per gram of soil. Observations on seed germination (%), root and shoot length (cm), sclerotial number of *M. phaseolina* (per g of soil), number of nodules (per plant) and root-rot incidence (%) were recorded at 30 and 60 DAS. Grain yield (kg / ha) was recorded separately for each treatment.

RESULTS AND DISCUSSION

Ten fluorescent pseudomonad strains were obtained from rhizoplanes of blackgram, carrot, banana, tapioca, pepper, rice and forest trees. All the strains were identified as *P. fluorescens*. Among the strains, Pf 1 was found to be more effective in inhibiting the mycelial growth of *M. phaseolina* of blackgram (82.6mm) and sesame (84.5mm) (Table 1).

The germination percentage of blackgram and sesame are presented in Table 2. Maximum germination percentage of 95.2 and 97.5 were observed in blackgram and sesame, respectively, when Pf 1 was applied as seed treatment (ST) combined with soil application (SA). The influence of ST + SD of carbendazim and ST of Pf 1 + SD of

Table 1. Efficacy of *P. fluorescens* strains in inhibiting the growth of *M. phaseolina* on blackgram and sesame under *in vitro* condition

P. fluorescens (strain)	Source of isolate	Location	Per cent inhibition over control	
			Blackgram	Sesame
Pf 1	Blackgram	Coimbatore	82.6 i	84.5 h
Pf KO 1	Pepper	Kodaikanal	36.5 c	38.3 c
Pf BS 1	Paddy	Bhavanisagar	23.3 a	21.5 a
Pf ATR 1	Tapioca	Attur	40.7 d	42.8 d
Pf MDU 1	Paddy	Madurai	51.8 f	48.6 e
FP 7	Paddy	Trivandrum	75.3 h	73.1g
Pf NA 1	Banana	Namakkal	47.3 e	53.4 f
Pf NL 1	Forest trees	Nilgiris	30.4 b	33.2 b
Pf KO 2	Pepper	Kodaikanal	57.3 g	55.9 f
Pf RA 1	Carrot	Rajapalayam	21.7 a	20.4 a

In a column, means followed by the same letter do not differ significantly (P = 0.05) by Duncan's multiple range test.

Table 2. Effect of *P. fluorescens* (Pf-1) on germination of blackgram (Co 5) and sesame (TMV 6) under pot culture condition

Treatment	Germination (%)	
	Blackgram	Sesame
ST - Pf 1	90.5^{bc}	92.0^{cd}
ST + SA - Pf 1	95.2^{d}	97.5^{e}
SA - Pf 1	85.5^{a}	89.0^{bc}
ST - Carbendazim	88.2^{ab}	90.2^{bcd}
ST + SD - Carbendazim	93.8^{cd}	94.2^{d}
SD - Carbendazim	86.0^{a}	86.8^{ab}
ST - Pf 1 + SD - Carbendazim	92.3^{cd}	93.5^{d}
ST – Carbendazim + SA - Pf 1	91.5^{bc}	92.5^{cd}
Control	83.6^{a}	82.5^{a}

ST-Seed Treatment; SA-Soil Application; SD-Soil Drenching
In a column, means followed by the same letter do not differ significantly (P = 0.05) by Duncan's multiple range test.

carbendazim on seed germination of blackgram and sesame were on par with each other (Table 2).

The biometrical observations on plant growth were taken at 30 DAS (Table 3). Pf 1 as ST + SA recorded highest root and shoot length for both blackgram (18.2cm, 52.5cm) and sesame (7.5cm, 48.6 cm). ST + SD of carbendazim showed next best in influencing the root growth of both blackgram and sesame and shoot growth of sesame. The trend continued even after 60 DAS (Table 4).

The survival of *Pseudomonas* strain Pf1 was estimated from the rhizosphere of blackgram and sesame (Table 3 and 4). Maximum population (CFU = colony forming units) of Pf1 and reduced sclerotial population of *M. phaseolina* were recorded in the treatment Pf1 - ST + SA (Table 3). Carbendazim application was observed to be the next best treatment in reducing the sclerotial population at 30 and 60 DAS (Table 4). Highest sclerotial population per g of soil was recorded in control.

Table 3. Effect of *P. fluorescens* (Pf-1) treatments on plant biometrics of blackgram and sesame at 30 DAS

Treatment	Crop							
	Blackgram				Sesame			
	Root length (cm)	Shoot length (cm)	*Pseudomonas* population / 100 g of soil (x 10^6)	Sclerotia / g of soil	Root length (cm)	Shoot length (cm)	*Pseudomonas* population / 100 g of soil	Sclerotia/ g of soil
ST - Pf 1	14.5 d	43.0 e	32.2 f	10.57 b	6.0 e	41.4 e	30.5 f	12.42 b
ST + SA - Pf 1	18.2h	52.5 I	65.2I	5.88 a	7.5 I	48.6 h	61.7 I	7.03 a
SA - Pf 1	12.0 c	39.7 c	57.1h	24.31 e	5.3 c	35.0 c	54.3 h	26.11 d
ST– Carbendazim	14.3d	40.5 d	10.0 d	12.36 c	5.7 d	40.5 d	7.5 d	14.32 b
ST+SD–Carbendazim	17.0g	45.6 f	4.5 c	5.31 a	7.0 h	44.2 g	3.2 c	8.97 a
SD– Carbendazim	10.6b	35.0 b	3.5 b	23.89 e	4.9 b	31.8 b	3.0 d	26.12 d
ST - Pf 1 + SD – Carbendazim	15.5e	48.2 h	28.7 e	15.41 d	6.8 g	42.7 f	24.5 e	16.00 bc
ST – Carbndazim + SA - Pf 1	15.9f	46.0 g	50.3 g	16.29 d	6.4 f	41.1 e	46.8 g	18.35 c
Control	9.5a	30.6 a	2.0 a	32.21 f	4.2 a	25.6 a	2.6 a	35.66 e

ST-Seed Treatment; SA-Soil Application; SD-Soil Drenching

In a column, means followed by the same letter do not differ significantly (P = 0.05) by Duncan's multiple range test.

Table 4. Effect of *P. fluorescens* (Pf-1) treatments on plant biometrics of blackgram and sesame at 60 DAS

Treatment	Crop							
	Blackgram				Sesame			
	Root length (cm)	Shoot length (cm)	*Pseudomonas* population / 100 g of soil (x 10^6)	Sclerotia / g of soil	Root length (cm)	Shoot length (cm)	*Pseudomonas* population / 100 g of soil (x 10^6)	Sclerotia / g of soil
ST - Pf 1	19.8 e	69.3 e	41.7 f	9.52 c	15.2d	58.7 d	44.3 e	11.21b
ST + SA - Pf 1	25.0I	85.3I	78.1I	3.33a	18.4h	71.5h	78.3 h	4.35a
SA - Pf 1	18.2c	62.2c	63.4h	20.32e	14.6 c	52.1 c	60.1 g	23.14f
ST– Carbendazim	18.7d	66.1 d	12.5 d	15.73e	15.0d	56.4 d	8.7 c	19.77e
ST+SD– Carbendazim	23.5h	75.2h	7.8 b	4.66b	17.9g	65.8 g	5.2 b	6.05b
SD– Carbendazim	17.9b	61.0b	10.4c	25.66g	13.1b	45.9b	8.7c	28.21g
ST - Pf 1 + SD – Carbendazim	21.6f	72.4g	33.6c	14.25d	16.5f	62.6g	31.3d	14.25d
ST – Carbndazim + SA - Pf 1	22.2g	70.1f	60.3g	17.01e	16.0e	60.2f	57.4f	20.09e
Control	15.4a	55.3a	4.0 a	40.62h	10.3a	40.7a	3.5a	37.45h

ST-Seed Treatment; SA-Soil Application; SD-Soil Drenching

In a column, means followed by the same letter do not differ significantly (P = 0.05) by Duncan's multiple range test.

The number of nodules per plant at 60 DAS along with root rot incidence is presented in Table 5. ST + SA of Pf 1 continued its superiority over other treatments in maintaining *Rhizobium* nodulation and in reducing the disease incidence.

The effect of root rot incidence on yield is presented in Table 5. Among the various methods of Pf 1 application, ST + SA recorded maximum yield in blackgram (1238 kg / ha) and sesame (1200 kg / ha) followed by ST + SD of carbendazim, which recorded grain yield to the extent of 1205 kg / ha for blackgram and 820 kg / ha for sesame. Control recorded the lowest yield of 458 kg / ha (blackgram) and 190 kg / ha (sesame). In general all the treatments were effective in increasing the yield over untreated control (Table 5). In the past also, a combination of seed and soil application of fluorescent pseudomonads for soil-borne disease management was reported in chickpea (Vidhyasekaran and Muthamilan, 1995), pigeonpea (Vidhyasekaran *et al.*, 1996), groundnut (Vidhyasekaran *et al.*, 1996). As indicated in earlier studies (Weller, 1988), due to the colonization of roots by fluorescent pseudomonads systemic movement, an improved capacity to compete for root exudates along with plant pathogen (Gamliel and Katan, 1992) and other added advantages of strain Pf 1 can able to restrain many seed, soil and foliar pathogens. The

Table 5. Effect of *P. fluorescens* (Pf-1) treatments on root nodulation, root rot incidence and yield

Treatment	Crop				
	Blackgram			Sesame	
	Number of Nodules / plant	Root rot incidence (%)	Grain yield (kg / ha)	Root rot incidence (%)	Grain yield (kg / ha)
ST - Pf 1	47.53 b	29.7 abcd	1086 f	38.5 c	320 b
ST + SA - Pf 1	58.60 e	25.1 a	1238 h	23.2 a	1200 g
SA - Pf 1	50.50 bcd	28.9 abc	1125 g	35.4 bc	680 e
ST – Carbendazim	47.26 b	32.8 bcd	1023 e	40.3 c	400 bc
ST + SD – Carbendazim	54.20 d	27.6 ab	1205 h	26.8 ab	820 f
SD – Carbendazim	48.26 bc	39.4 cd	727 b	37.1 c	460 cd
ST - Pf 1 + SD – Carbendazim	52.33 d	35.6 d	830 c	27.9 ab	900 f
ST – Carbendazim + SA - Pf 1	51.60 cd	31.5 bcd	968 d	31.2 abc	560 d
Control	38.86 a	55.8 e	458 a	58.3 d	190 a

ST-Seed Treatment; SA-Soil Application; SD-Soil Drenching
In a column, means followed by the same letter do not differ significantly (P = 0.05) by Duncan's multiple range test.

forgoing studies conclude that among the various methods of Pf 1 and carbendazim application, ST combined with SA of Pf 1 was found to be the most effective treatment in controlling root-rot disease on blackgram and sesame.

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