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Effect of fungal and bacterial biofomulation mixtures on major soil borne diseases of cotton

T Anand
Assistant Professor (Plant Pathology), Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Abstract
Two field experiments were conducted at Cotton Research Station farm, Veppanthattai, Perambalur Dt., Tamil Nadu, India to assess the effect of fungal [*Trichoderma viride* (Tv)] and bacterial antagonistic [*Pseudomonas fluorescens* (Pf1) and *Bacillus subtilis* (Bs)] strains individually and in combinations against major soil borne diseases *viz*., damping off, wilt and root rot. Among the treatments, the plots treated with the mixture of Pf1+Bs+Tv recorded only 3.18% and 1.12% of pre and post emergence damping-off, respectively and 0.00, 0.37 and 0.91% wilt incidence and 0.00, 0.21 and 0.72% root rot incidence at 30, 60 and 90 days after sowing (DAS), respectively. This treatment also recorded higher germination per cent, plant height, number of bolls/plant and seed cotton yield (23.45 q/ha).

Keywords: *Bacillus subtilis*, cotton, *Pseudomonas fluorescens*, *Trichoderma viride*

Introduction
Diseases cause serious loss to cotton production under favourable weather conditions. Among the soil borne diseases, damping off (*Pythium ultimum*), root rot (*Rhizoctonia solani*/*Macrophomina phaseolina*) and *Fusarium* wilt (*Fusarium oxysporum* f.sp. *vasinfectum*) are important and causing considerable yield losses. Biological method of disease management is gaining momentum in recent years due to their advantages over chemical methods. Several antagonistic organisms have been successfully used as biocontrol agents for controlling soil borne pathogens (Deacon, 1991) [2]. In most of the research, to date, biocontrol agents are applied singly to combat the growth of the pathogens. Although the potential benefits of a single biocontrol agent application has been demonstrated in many studies, it may also partially account for the reported inconsistent performance because a single biocontrol agent is not likely to be active in all agricultural ecosystems (Raupach and Kloepper, 1998) [13]. These have resulted in inadequate colonization, limited tolerance to changes in environmental conditions and fluctuations in production of antifungal metabolites (Weller and Thomashow, 1994; Dowling and O' Gara, 1994) [18, 3].
Thus, more emphasis was laid on the combined use of two or more strains of biocontrol agents, which turned out to be more successful than either of them alone, as reported by several workers (Nandakumar *et al*., 2001a [9]; Thilagavathi *et al*., 2007; Senthilraja *et al*., 2010) [15].
Hence, the present investigation was undertaken to study the effectiveness of combinations of bacterial and fungal biocontrol agents against major soil borne diseases (damping off, wilt and root rot) of cotton.

Materials and Methods
Biocontrol agents
The isolates of *Pseudomonas fluorescens*, *Bacillus subtilis*, *Trichoderma viride* were obtained from the Culture Collection Section, Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore, India.

Preparation of talc-based bioformulation
A loopful of *P. fluorescens* and *B. subtilis* was inoculated into sterilized KB and NB, respectively and incubated in a rotary shaker at 150 rpm for 48 h at room temperature (28 ± 2 °C). After 48 h of incubation, the broth containing 9 x 10^8 cfu/ml was used for the preparation of talc-based formulation. To the 400 ml of bacterial suspension, 1 kg of the talc powder (sterilized at 105 °C for 12 h), calcium carbonate 15 g (to adjust the pH to neutral) and carboxymethyl cellulose (CMC) 10 g (adhesive) were mixed under sterile conditions, following the method described by Nandakumar *et al.* (2001b) [10]. After shade drying for overnight, it was packed in polypropylene bag and sealed. At the time of application, the population of bacteria in talc formulation was 2.5–3 x 10^8 cfu/g.
T. viride isolate was multiplied in molasses-yeast broth (30 ml molasses; 5 g yeast; 1 l distilled water). The sterile broth was inoculated with an actively growing mycelial disc (8 mm) and incubated for 10 days. The biomass (3 ± 10^8 cfu/ml) along with the medium was incorporated into the sterilized talc powder at the rate of 50 ml of suspension per 100 g of talc powder and thoroughly mixed with 500 mg CMC as described by Ramakrishnan *et al*. (1994) [12].
The talc-based formulation containing both fungal and bacterial antagonists was prepared by mixing equal amounts of the biocontrol agents at the time of application. For bacterial strain mixtures, the bacterial strains were grown separately each in its respective broth and two strains that are going to make up the mixture were added equally (v/v) and finally mixed with talc powder, $CaCO_3$ and CMC.

Field experiments
Two field trials were conducted to test the efficacy of different microbial consortia against major soil borne diseases *viz*., damping off, wilt and root rot at Cotton Research Station Farm, Veppanthattai, Perambalur, Tamil Nadu during September 2010 – March 2011 and September 2011 – March 2012. The field trials

were laid out in a randomized block design (RBD) with three replications. The cotton hybrid RCH 530 BG II was used and the seeds were sown in 20 m^2 plots with a spacing of 90 x 60 cm. The treatments of experiment were T1- *P. fluorescens* (Pf1), T2- *B. subtilis* (Bs), T3- *T. viride* (Tv), T4- Pf1+Bs, T5- Pf1+Tv, T6- Bs+Tv, T7- Pf1+Bs+Tv, T8- Carbendazim (Standard check) and T9- Untreated check.

Method of application

Seed bacterization

Before sowing, cotton seeds were treated with talc-based formulation (10 g/kg of seeds) of individual or mixture of fungal and bacterial strains. The fungicide carbendazim was applied at the rate of 2 g/kg of seeds. The seeds not treated with bioagents or fungicide served as control.

Soil application

The talc-based formulation of fungal and bacterial strains (2.5 kg/ha) was mixed with 50 kg of farmyard manure (FYM) and applied 30 days after sowing (DAS) as soil application.

Disease assessment

Percentages of pre and post emergence damping-off in each treatment were recorded 10 and 25 days after sowing using the formula

Pre-emergence (%) = (Number of non-germinated seeds/Total number of sown seeds) x 100

Post-emergence (%) = (Number of dead seedlings/ Total number of sown seeds) x 100

The wilt and root rot disease incidence was recorded 30, 60 and 90 days after sowing and % disease incidence was calculated using the formula:

% disease (root rot/wilt) incidence = (No. of infected plants/Total number of plants) x 100.

The growth and yield attributing parameters *viz*., plant height and number of bolls/plant were recorded. The seed cotton yield in different treatments was also recorded.

Statistical analysis

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez, 1984). Prior to statistical analysis of variance (ANOVA) the percentage values of the disease index were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels ($P < 0.05$ and $P < 0.01$) and means were compared by Duncan's Multiple Range Test (DMRT).

Results and Discussion

The talc-based formulation of fungal and bacterial strains either individually or as a mixture significantly reduced the incidence of damping off, wilt and root rot diseases in cotton (Table 1, 2 and 3). The efficacy of the fungal and bacterial strain mixture was better than the standard fungicide (carbendazim) application in controlling the disease severity. Combining fungal antagonistic strain (*T. viride*) with bacterial strains (*P. fluorescens* and *B. subtilis*) resulted in improved suppression of damping off, wilt and root rot relative to individual application of these strains.

Among the different treatments, the mixture of Pf1+Bs+Tv recorded only 3.18% and 1.12% of pre and post emergence damping-off, respectively followed by Pf1+Tv, Pf1+Bs and Bs+Tv. The fungicide carbendazim treated plots recorded the incidence of 6.78 and 5.57%, where as the untreated control recorded 16.78 and 10.70% pre and post emergence damping off, respectively. Similarly, the formulation of fungal and bacterial strain mixture (Pf1+Bs+Tv) was also effective for the control of wilt and root rot diseases compared to other treatments and untreated control. This treatment recorded only 0.00, 0.37 and 0.91% wilt incidence and 0.00, 0.21 and 0.72% root rot incidence at 30, 60 and 90 DAS, respectively. Where as, the untreated control recorded the incidence of 2.41, 9.97 and 13.31% wilt and 2.57, 6.79 and 12.22% root rot at 30, 60 and 90 DAS, respectively. Seed treatment and soil application of biocontrol agents *viz*., *P. fluorescens*, *B. subtilis* and *T. viride* effectively reduced root rot caused by soil borne pathogens in several crops (Thilagavathi *et al*., 2007; Senthilraja *et al*., 2010; Lath *et al*., 2011)[15, 8]. The inhibitory effect of *Trichoderma* species might be due to direct mycoparasitism in addition to competition for nutrients (Elad *et al*., 1982; Henis and Papavizas, 1983) [4, 6]. The *P. fluorescens* strains reduced the root rot infection through several mechanisms including production of lytic enzymes (Velazhahan *et al*., 1999) [17], siderophores (Scher and Baker, 1982) [14] and hydrogen cyanide (Bakker and Schippers, 1987) [1]. *B. subtilis* strains known to inhibit several soil borne diseases such as *Fusariam* wilt of red gram (Podile and Dube, 1985) [11] and *R. solani* (damping off of peppermint) (Kamalakannan *et al*., 2003) [7]. The fungal and bacterial strain mixtures (Pf1+Bs+Tv) recorded significantly higher germination per cent, plant height, no. of bolls/plant and seed cotton yield than the individual strains and untreated control. The number of bolls and seed cotton yield was higher in the plots treated with mixture of strains compared to individual strains. Maximum yield (23.45 q/ha) and BC ratio (1:4.71) was recorded in Pf1+Bs+Tv mixture treated plots (Table 4). The yield was also significantly greater than the plots treated with individual strains and untreated control. Interestingly Pf1+Bs+Tv mixture treated plots recorded significantly higher seed cotton yield and BC ratio over chemical fungicide treatment. Similarly, a combined application of *P.* fluorescens + *B. subtilis* + *T. viride* increased growth and yield attributes in black gram (Thilagavathi *et al*., 2007) and physic nut (Lath *et al*., 2011) [8].

Table 1: Effect of different microbial consortia on the incidence of damping off in cotton (Pooled mean of two seasons)

S. No.	Treatments	Damping off (%)	
		Pre-emergence	Post-emergence
1.	*P. fluorescens* (Pf1)	9.05^{ef}	5.83^{cd}
2.	*B. subtilis* (Bs)	11.85^{f}	7.14^{d}
3.	*T. viride* (Tv)	8.66^{de}	5.92^{cd}
4.	Pf1+Bs	6.87^{bc}	4.27^{bc}
5.	Pf1+Tv	5.39^{b}	3.50^{b}
6.	Bs+Tv	8.62^{cd}	4.30^{bc}
7.	Pf1+Bs+Tv	3.18^{a}	1.12^{a}
8.	Carbendazim (0.1%)	6.78^{bc}	5.57^{c}
9.	Untreated check	16.78^{g}	10.70^{e}

Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 2: Effect of different microbial consortia on the incidence of wilt in cotton (Pooled mean of two seasons)

S. No.	Treatments	Wilt incidence (%)		
		30 DAS	60 DAS	90 DAS
1.	*P. fluorescens* (Pf1)	0.14[a]	4.11[cd]	5.61[cd]
2.	*B. subtilis* (Bs)	0.77[a]	5.17[d]	8.32[e]
3.	*T. viride* (Tv)	0.27[a]	4.10[cd]	5.74[cd]
4.	Pf1+Bs	0.00[a]	1.95[ab]	2.85[b]
5.	Pf1+Tv	0.00[a]	1.59[ab]	2.55[ab]
6.	Bs+Tv	0.15[a]	2.67[bc]	3.92[bc]
7.	Pf1+Bs+Tv	0.00[a]	0.37[a]	0.91[a]
8.	Carbendazim (0.1%)	0.27[a]	2.29[ab]	6.17[d]
9.	Untreated check	2.41[b]	9.97[e]	13.31[f]

Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 3: Effect of different microbial consortia on the incidence of root rot in cotton (Pooled mean of two seasons)

S. No.	Treatments	Root rot incidence (%)		
		30 DAS	60 DAS	90 DAS
1.	*P. fluorescens* (Pf1)	0.46[a]	3.70[bc]	4.53[cd]
2.	*B. subtilis* (Bs)	0.96[a]	5.13[cd]	6.47[d]
3.	*T. viride* (Tv)	0.32[a]	3.68[bc]	4.40[bc]
4.	Pf1+Bs	0.00[a]	1.49[a]	3.13[b]
5.	Pf1+Tv	0.00[a]	0.72[a]	2.16[b]
6.	Bs+Tv	0.24[a]	2.26[ab]	3.29[b]
7.	Pf1+Bs+Tv	0.00[a]	0.21[a]	0.72[a]
8.	Carbendazim (0.1%)	0.49[a]	4.82[cd]	4.94[cd]
9.	Untreated check	2.57[b]	6.79[e]	12.22[e]

Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 4: Effect of different microbial consortia on growth and yield attributes in cotton (Pooled mean of two seasons)

S. No.	Treatments	Germination (%)	Plant height (cm)			No. of bolls/plant	Yield (q/ha)	BCR
			30 DAS	60 DAS	90 DAS			
1.	*P. fluorescens* Pf1	91.0[cd]	26.50[ab]	81.80[c]	122.6[c]	47.45[c]	18.92[e]	2.80
2.	*B. subtilis* (Bs)	88.2[d]	22.35[c]	71.03[e]	107.5[e]	41.99[e]	16.90[f]	2.46
3.	*T. viride* (Tv)	91.1[cd]	24.70[b]	76.91[d]	114.2[d]	44.25[d]	18.15[e]	2.68
4.	Pf1+Bs	93.1[bc]	27.25[ab]	82.68[bc]	124.4[c]	47.80[c]	20.92[cd]	3.40
5.	Pf1+Tv	94.6[ab]	27.88[a]	84.56[ab]	128.4[b]	50.79[b]	21.90[bc]	3.95
6.	Bs+Tv	91.3[b]	25.60[b]	79.64[c]	116.0[d]	45.32[cd]	19.68[de]	2.90
7.	Pf1+Bs+Tv	96.8[a]	28.44[a]	86.71[a]	132.1[a]	54.55[a]	23.45[a]	4.71
8.	Carbendazim (0.1%)	93.2[bc]	20.32[c]	61.68[f]	102.3[f]	36.36[f]	18.17[e]	1.91
9.	Untreated check	83.2[e]	14.31[d]	50.62[g]	83.70[g]	26.68[g]	9.82[g]	-

Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRTs

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