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Research Papers

Combined effects of biocontrol agents and soil amendments on soil microbial populations, plant growth and incidence of charcoal rot of cowpea and wilt of cumin

Vijeta SINGH, Ritu MAWAR and Satish LODHA

Plant Pathology Laboratory, Central Arid Zone Research Institute, Jodhpur - 342 003, India

Summary. Field experiments were conducted for 2 years to determine the effectiveness of combined use of two biocontrol agents, *Bacillus firmus* and *Aspergillus versicolor* for control of *Macrophomina phaseolina* induced charcoal rot of cowpea and *Fusarium oxysporum* f. sp. *cumini* induced wilt of cumin. The lowest level of plant mortality (3–4%) due to charcoal rot of cowpea was recorded when bacterium coated seeds were sown in radish compost amended soil compared to the non-amended soil (13.8–20.5%), but this was not significantly better than some other treatments. Cowpea roots from *B. firmus* coated seeds had better nodulation than any of the individual *A. versicolor* treatments. Although *B. firmus* coated seeds + *A. versicolor* + farmyard manure resulted in maximum nodulation this was not significantly different to *B. firmus* seed coating. Root colonization by the combined biocontrol agent treatments was better than the individual biocontrol agent treatments. Combining *A. versicolor* with farmyard manure supported the maximum populations of total fungi and actinomycetes. In both winter seasons, the lowest incidence of wilt (1.0–5.2%) on cumin was recorded when *A. versicolor* was amended with neem compost compared to the non-amended soil (5.7–10.5%). Maximum colonization of *A. versicolor* on roots was observed in *B. firmus* + *A. versicolor* + farmyard manure amended plots. During both years, the treatment combination of *A. versicolor* in neem compost amended plots resulted in maximum populations of fungi, bacteria and *A. versicolor* in the soil, which was greater than in the non-amended soil. Significant increases in disease control were not recorded after single or repeated delivery of *A. versicolor*. These results suggest that combining *B. firmus* as seed coatings with *A. versicolor* as soil applications gives improved control of *M. phaseolina* and *Fusarium* induced diseases on legume and seed spice crops in arid soils.

Key words: neem compost, radish compost, *Macrophomina phaseolina*, *Fusarium oxysporum* f. sp. *cumini*.

Introduction

In the Indian arid region, cumin (*Cuminum cyminum* L.), a seed spice, is grown during the winter season (November–March) in irrigated areas. Wilt caused by *Fusarium oxysporum* f. sp. *cumini* (*Foc*) is the most destructive disease of this crop, and yield losses can often reach 40% (Lodha *et al.*, 1986). During the rainy season (July– October), cowpea (*Vigna unguiculata* (L.) Walp.), an annual legume, is grown in the same fields. Concurrent heat and moisture stress favor development of charcoal or dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid., often making cultivation of cowpea uneconomical. A heat tolerant strain of *Aspergillus versicolor* has been reported as a potential antagonist to *Foc* (Israel and Lodha, 2005), which was also found to be antagonistic to several plant pathogens including *M. phaseolina* (Bhattacharya *et al.*, 1985). A strain of *Bacillus firmus* has also shown specific antagonism to *M. phaseolina* in repeated laboratory and field experiments (unpublished data).

The method of applying biocontrol organisms to a target area is critical in the development of bio-

Corresponding author: S. Lodha
E-mail: satish_lodha@rediffmail.com

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logical control strategies (Stack *et al*., 1988). Various delivery systems have been explored, particularly applications to seed and soil. Conidia of *Trichoderma* and *Gliocladium* spp. have been added to bran-sand mixtures and incubated for 1–3 d prior to application. Commonly, bacterial cells have been incorporated into methyl cellulose and coated directly onto seeds (Milus and Rothrock, 1993; Duffy and Weller, 1995).

Despite many advances made in understanding mechanisms of biological control, use of biocontrol agents (BCAs) have achieved limited success. Failure of introduced BCAs to establish in soil or on host roots has been associated with lack of disease control (Nemec *et al*., 1996). Bahme *et al*., (1988) showed that repetitive applications of *Pseudomonas fluorescens* through drip-irrigation improved colonization of the root system of potatoes compared with seed piece inoculation or incorporation of bacteria-impregnated granules into the soil. Repetitive inoculations increased the nodulation effectiveness of a strain of *Rhizobium luguminosarum* on red clover (Martensson, 1990).

In India, *A. versicolor* and *B. firmus* are newly reported biocontrol agents against soilborne plant pathogens. It was therefore considered worthwhile to study the rate and frequency of application of *A. versicolor* in the field when applied alone or in association with *B. firmus* seed coating during rainy and winter seasons for control of charcoal rot of cowpea and wilt of cumin.

Materials and methods

Location

The experiments were conducted at the Central Arid Zone Research Institute, Jodhpur, India during the rainy and winter seasons of 2005–2007. The loamy sand soil used for experiments had 85% sand, 8.9% clay, 5.5% silt, 0.031% nitrogen, 0.25% organic carbon, 7 μg g^{-1} Olsen P and pH of 8.1. The soil had an electrical conductivity of 0.88 dSm^{-1} (soil: water ratio 1:2.5), bulk density of 1.56 g cm^{-3} and moisture holding capacity (MHC) of 10.4% (w/w).

Crops and pathogens

The test crops grown for experiments were cowpea (cv. CAZC10) during the rainy season (July–October) and cumin (cv. RZ-19) in the winter season (November – March) in the same field. Virulent strains of *M. phaseolina* were isolated from diseased roots of cowpea, and of *Foc* from diseased roots of cumin.

Inoculum preparation

Aspergillus versicolor isolated from native soil of the region was grown in potato dextrose broth; fungal mats were harvested after 7 d on filter paper and blended for 30 s in 200 mL of sterile distilled water before use. The resulting suspension had 3–5 × 10^{10} CFU mL^{-1} of *A. versicolor*. *B. firmus* was multiplied on Czapek dox broth for 3 days.

Preparation of compost

Composts were prepared in separate pits (1 × 1 × 0.75 m) under partially aerobic conditions, adopting the Indore composting method (Howard and Ward, 1931). The residues of leaves of radish (*Rhaphanus sativus* L.) and neem (*Azadirachta indica* A. Juss.) were collected and cut up into small pieces (1–1.5 cm) and placed separately in four layers in each pit in the month of December. Each layer (30 cm) of residues was enriched with 1% gypsum and 2% urea and was covered with a 10 cm thick layer of 2-month-old cow dung. Addition of cow dung compressed residues in each layer, thus approximately 40% of the volume in each pit was occupied by cow dung. Approximately 60% moisture (w/w) was maintained by addition of 6–10 L of water every 7 to 10 d. Two turnings were made at intervals of 2 months. Mature composts were ready by June after about 6 months. Fresh composts were prepared for each year of the study.

Experimental design

The experiment comprised ten treatments in 4 × 3 m plots arranged in a completely randomized block design with three replications, and the experimental treatments were: 1. *B. firmus* seed coating (*Bf* ST); 2. *Bf* ST + radish compost (RC); 3. *Bf* ST + Farm Yard Manure (FYM); 4. *A. versicolor* seed treatment (*Av* ST); 5. *A. versicolor* (*Av*) + neem compost (NC); 6. *Av* + FYM; 7. *Bf* ST + *Av* + FYM; 8. treatment 7 but *Av* again amended before the winter season; 9. FYM; 10. non-amended experimental control.

Aspergillus versicolor was multiplied on potato dextrose broth (30°C in dark), while *B. firmus* was grown on Czapek dox agar for 3 d (30°C at 150 rpm

on an orbital shaker). Cowpea seeds, weighing 270 g were coated with 3-day-old cells and spores of *B. firmus* (8.4×10^9 mL^{-1}) and sprinkled with 1 g of carboxymethyl cellulose. These were then spread in a plastic tray and allowed to dry overnight in shade. After drying, the bacterium coated seeds were separated into 15 lots, each of 18 g, for use in replicated plots of treatments 1, 2, 3, 7 and 8. In treatments 2 and 5, 60 g (50 kg ha^{-1}) of radish and neem compost, respectively, and for treatments 3, 7 and 8 equal amount (50 kg ha^{-1}) of FYM was amended at 0–20 cm soil depth by a hand spade 1 d before planting of seeds. Fifty four grams of cowpea seeds were treated with *A. versicolor* bioformulation in talc in 1:6 ratio (5.3×10^{10} CFU g^{-1} with 1 g of carboxymethyl cellulose and separated in three lots, each of 18 g, for use in plots of treatment 4. Plots of treatment 9 were amended only with FYM (60 g), while untreated seeds in treatment 10 were considered as the non-amended experimental controls. Seeds were sown in 6 rows, 50 cm apart in these plots, adopting standard agronomic practices. During the winter season, cumin seeds were sown in different plots in the above described treatments, except that *B. firmus* coating was not done in treatments 1, 2, 3, 7 and 8. However, *A. versicolor* amended with FYM was again delivered in the plots of treatment 8.

After 20 days of plant growth, three plants were gently uprooted from each plot, nodules were counted and shoot and root length measured. Plants were then each cut at the junction of root and shoot to record fresh weight, and then oven dried at 80°C for 2 h before recording dry weights.

Root colonization

Apparently healthy and *Macrophomina* infected cowpea plants were uprooted from the field after 45 d growth to study root colonization. One centimetre portions were cut at the same place from each root and placed in capped borosilicate glass tubes containing 10 mL of sterilized water. These tubes were gently rotated for 10–15 s. After serial dilutions, 1 mL was spread on *A. versicolor* or *B. firmus* medium (see below). Colony forming units (CFUs) of each BCA were counted after 4–5 d of incubation at 30 ± 1°C in dark in a BOD incubator. In cumin, root colonization data were collected only for *A. versicolor,* using the same procedure described for cowpea. Root samples of partially wilt affected plants were also analyzed to study colonization of *A. versicolor.* Presence of the pathogen in diseased roots was confirmed by plating 1 cm root pieces on PDA slants.

Disease incidence

Data on plant mortality due to dry root rot were recorded from initiation of the disease until harvest of the 2nd – 4th row of plants in each plot. All plants in these rows were counted 10 days after sowing, and then at weekly intervals, healthy and dry root rot affected plants were counted. Percentage mortality due to dry root rot was determined by subtracting plants killed from total plants. After crop harvest (70 d after sowing), soil samples were collected from the 3rd row of each replicate of all the treatments at 0–15 cm depth. Populations of microbes including *A. versicolor* were estimated separately by the same procedure. Data collected from six Petri plates for each microbe represented one replicate.

Data on cumin plant mortality due to wilt were recorded on each plant in the 6th to 8th row of each plot at the initiation of disease and thereafter every week until harvest. Percent mortality due to wilt was calculated. After crop harvest (105 d after sowing), soil samples were collected from 0–15 cm depth from each replicate of all the treatments to estimate the population of all microbes and *A. versicolor* on their specified media as described below.

Biological assays

The populations of *A. versicolor* and total fungi were enumerated by serial dilutions on Martin's rose-bengal agar (Martin, 1950), total bacteria on Thornton's agar (Thornton, 1922) and total actinomycetes on Ken-knight agar (Allen, 1959). Six Petri dishes (90 mm diam.) of each medium were used for enumeration of each category of organisms from one soil sample. Petri dishes having serial dilutions of 10^4–10^5 were incubated at ±30°C in a BOD incubator under a 12 h cycle of light and dark. Bright shining colonies of *A. versicolor* were easily distinguishable from other *Aspergillus* species. The mean of six Petri dishes was considered as one estimation per replicate of each treatment.

Statistical analyses

All data were subjected to analysis of variance (ANOVA) and the treatment means compared by

LSD (P = 0.05) separately for each year (Snedecor and Cochran, 1967). Data on percentage mortality were converted to angular transformed values before analysis.

Results

Growing season rainfall

The seasonal rainfall after planting of cowpea seeds was 133 mm (2005) and 239 mm (2006). There were only three major rain events (>25 mm) in the crop growing season in 2005 compared to six in 2006. In 2005, after initial major rain events in the first fortnight, there were no significant rain events for another 35 d. Cowpea plants thus experienced moderate to severe moisture stress leading to *Macrophomina* infection. Evenly distributed rain events in 2006 favored crop growth in such a way that cowpea did not experience long periods of moisture stress, and this lead to less incidence of charcoal rot.

Cowpea

Disease incidence

All the treatments having BCA or FYM alone significantly (P = 0.05) gave less charcoal rot incidence on cowpea than the non-amended control during both years of the field experiment. Least plant mortality due to charcoal rot was recorded in the treatment *Bf* ST + radish compost in 2005 (Table 1) but this was statistically similar to the treatment with *Bf* ST + *A. versicolor* + FYM. In 2006, the reverse was true, with least plant mortality recorded in *Bf* ST + *A. versicolor* + FYM treatment, which was significantly (P = 0.05) better than *Bf* ST + radish compost. The other two *B. firmus* treatments were similar in 2005, but *Bf* ST + FYM was significantly better than *Bf* ST in the second season. All *A. versicolor* treatments were comparable during 2005, but in 2006, the *A. versicolor* + NC treatment was superior to the other treatments. In both seasons, incidence of charcoal rot was less in *A. versicolor* compared to *Bf* ST seed coating, but a statistically significant difference between these treatments was established only in 2006. Amendment of soil with FYM also reduced charcoal rot incidence during both the seasons compared to the non amended control. Further, combining FYM with BCAs was significantly better than FYM alone except for *Bf* ST + FYM in 2005.

Plant growth parameters

All the amended treatments except FYM and *Av* ST treatment improved nodulation in cowpea roots compared to the non-amended control (Table 2). Maximum nodules were recorded in the *Bf* ST + *A.versicolor* + FYM combination but this was statistically (P = 0.05) comparable to the *Bf* ST treatment. Cowpea roots from *B. firmus* coated seeds had better nodulation than any of the *A. versicolor* treatments.

Shoot and root lengths and shoot and root fresh and dry weights were significantly greater in amended compared to non-amended treatments, except for minor deviations in some treatments viz., *Bf* ST + FYM (root length), *Av* ST and *A. versicolor* + NC (root dry weight). Greatest shoot and root lengths and root fresh weight occurred in the *A. versicolor* + FYM treatment, while shoot fresh weight was maximum in *Bf* ST + radish compost treatment. Shoot dry weight was greatest in the *Bf* ST + *A. versicolor* + FYM treatment.

Root colonization

Maximum root colonization occurred in the *Bf* ST + *A. versicolor* + FYM treatment (Table 3). Combining substrates (neem and radish compost) improved colonization by both BCAs on roots. In general, all the amended treatments had better root colonization than the non amended controls.

Microbial population

There was significant variation between the total fungal populations estimated in 2005 compared to 2006 after the cowpea harvest (Table 4). Fungal counts ranged from 14.7 to 40.7 × 10^3 CFU g^{-1} soil in 2005 and between 5.3 and 9.3 × 10^3 CFU g^{-1} soil in 2006. Maximum fungal counts occurred in the *Bf* ST+ *A. versicolor* + FYM and *A. versicolor* + FYM treatments, which were significantly greater than in the non-amended control. The treatment combination *A. versicolor* + NC did not support fungal populations in the soil which remained significantly less than the non-amended control in 2005.

There was no variation in total bacterial counts in both years of the study. These ranged between 5.6 and 25.7 × 10^4 CFU g^{-1} soil in 2005 and between 9.6 and 19.3 × 10^4 CFU g^{-1} soil in 2006. Maximum bacterial counts were recorded in *A. versicolor* + NC and *A. versicolor* (ST) in 2005 and 2006, respectively, which were significantly greater than the non-amended control. Bacterial counts varied considerably in the

Table 1. Mean incidence of cowpea charcoal rot in two growing seasons, from treatments with the biocontrol agents *Bacillus firmus* and *Aspergillus versicolor* in different soil amendments..

Treatments	Charcoal rot incidence (%)	
	2005	2006
B. firmus seed coating (*Bf* ST)	6.8 (15.1)[c]	7.0 (15.3)
Bf ST+ radish compost	3.0 (9.9)	4.0 (11.5)
Bf ST +farm yard manure (FYM)	6.8 (15.1)	4.5 (12.2)
A. versicolor seed treatment (*Av*ST)	6.3 (14.5)	5.6 (13.7)
A. versicolor + neem compost	6.9 (15.2)	4.6 (12.4)
A. versicolor + FYM	5.2 (13.2)	5.4 (13.5)
A. versicolor one application[a] + *Bf* ST + FYM	5.1 (12.9)	3.4 (10.5)
A. versicolor two applications[b] + *Bf* ST + FYM	4.2 (11.8)	2.8 (9.6)
FYM	9.2 (17.7)	11.7 (20.0)
Non amended control	20.5 (26.9)	13.8 (21.8)
LSD ($P = 0.05$)	3.4	0.33

[a] *A. versicolor* was amended in the plots in the rainy season only.
[b] *A. versicolor* was amended in the plots twice, i.e. before each of the rainy and winter seasons
[c] Angular transformed values.

Table 2. Mean plant parameters for 20 d old cowpea plants from plots treated in 2006 with the biocontrol agents *Bacillus firmus* and *Aspergillus versicolor* and soil amendments.

Treatment	Nodules	Shoot			Root		
		Length (cm)	Fresh Wt (g)	Dry Wt (g)	Length (cm)	Fresh Wt (g)	Dry Wt (g)
B. firmus seed coating (*Bf* ST)	5.7	11.0	0.33	0.10	19.7	2.0	0.41
Bf ST + radish compost	4.3	11.4	0.36	0.11	20.0	2.5	0.48
Bf ST + farm yard manure(FYM)	4.0	10.2	0.23	0.09	16.6	1.5	0.33
A. versicolor seed treatment (*Av*ST)	1.7	12.3	0.22	0.08	19.4	1.6	0.37
A. versicolor + neem compost	2.0	11.0	0.21	0.07	18.3	1.7	0.40
A. versicolor + FYM	2.3	12.7	0.32	0.10	21.6	2.4	0.48
A. versicolor one application[a] + *Bf* ST + FYM	2.7	11.0	0.29	0.11	17.7	1.4	0.46
A. versicolor two applications[b] + *Bf* ST + FYM	6.3	13.1	0.30	0.09	19.6	2.0	0.53
FYM	1.3	11.5	0.29	0.10	20.0	2.3	0.34
Non-amended control	1.3	9.7	0.19	0.07	15	1.4	0.19
LSD ($P = 0.05$)	0.7	0.8	0.1	0.02	0.8	0.1	0.3

[a, b] See Table 1.

Table 3. Mean populations of *Aspergillus versicolor* or *Bacillus firmus* colonizing 45 d old cowpea roots, after application of the biocontrol agents in different soil amendments in 2006.

Treatment	*A. versicolor* (× 10^3 mL^{-1})	Treatment	*B. firmus* (× 10^2 mL^{-1})
A. versicolor seed treatment(*Av* ST)	15.6[a]	*B. firmus* seed coating (*Bf* ST)	6.2*
A. versicolor + neem compost	19.3	*Bf* ST + radish compost	8.7
A. versicolor + FYM	10.6	*Bf* ST + FYM	7.0
A. versicolor + *Bf* ST + FYM	32.3	*Bf* ST + *A. versicolor* + FYM	39.5
Non amended control	10.3	Non amended control	4.5
LSD ($P = 0.05$)	0.3	LSD ($P = 0.05$)	0.5

[a] Mean of six Petri plates per replicate sample.

Table 4. Mean soil populations of *Aspergillus versicolor* and of soil microorganisms in cowpea field plot soils in two growing seasons, after different methods of *A. versicolor* application.

Treatments	*Aspergillus versicolor* (×10^3 g^{-1} soil)		Total bacteria (×10^4 g^{-1} soil)		Total actinomycetes (×10^4 g^{-1} soil)		Total fungi (×10^3 g^{-1} soil)	
	2005	2006	2005	2006	2005	2006	2005	2006
A. versicolor seed treatment (*Av* ST)	10.7[c]	0.6	6.0	19.3	11.3	12.6	26.9	6.0
A. versicolor + neem compost	25	3.0	25.7	16.3	13.3	9.6	14.7	6.6
A. versicolor +FYM	17.3	1.3	10.3	19.0	8.0	13.3	40.3	7.6
A. versicolor one application[a] +*Bf* ST+ FYM	13.6	1.0	15.9	11.0	10.8	6.0	40.7	5.3
A. versicolor two applications[b] +*Bf* ST+ FYM	15.7	1.0	16.7	12.3	11.3	9.6	36	8.3
Non amended control	3.3	1.0	5.6	9.6	6.3	6.6	32.6	9.3
LSD ($P = 0.05$)	12.7	1.5	12.6	7.3	4.4	5.2	17.1	2.65

[a, b] See Table 1.
[c] Mean of six Petri plates per replicate sample.

Av ST treatment, being the least in 2005 but the greatest in 2006.

The actinomycetes populations ranged between 6.0 and 13.3 × 10^4 CFU g^{-1} soil in both years of experimentation. The delivery of *A. versicolor* in any form did not change the population of actinomycetes. Maximum counts of actinomycetes were estimated in the *A. versicolor* + FYM treatment in 2006, and this treatment gave significantly greater populations than the non amended control. In addition, the *Av* ST and *Bf* ST + *A. versicolor* + FYM treatments also gave significantly greater populations to the non amended control in 2005, while only the *Av* ST treatment gave greater populations in 2006.

Considerable variations in *A. versicolor* populations were recorded in both years of study where populations ranged between 3.3 and 25.0 × 10^3 CFU g^{-1} soil in 2005, and 0.6 and 3.0 × 10^3 CFU g^{-1} soil in 2006. The *A. versicolor* + NC treatment was gave better establishment of *A. versicolor* in the soil compared

to any other treatment during both years. Amending the soil with FYM also stimulated *A. versicolor*, but the *A. versicolor* seed treatment on its own failed to promote establishment.

Cumin

Disease incidence

Treatments having BCAs or FYM alone were significantly superior to the non amended control in reducing wilt incidence during both years of field study (Table 5). Lowest wilt incidence was recorded in the *A. versicolor* + NC treatment in both years followed by the *A. versicolor* + FYM treatment. However, *Av* ST performed significantly better in the 2006–2007 season compared to 2005–2006 in reducing wilt incidence.

Root colonization

In general, healthy cumin roots were colonized more by *A. versicolor* than diseased roots. The combined *A. versicolor* + FYM treatment and the *B. firmus* seed coating treatment gave maximum colonization of cumin roots by *A. versicolor* (Figure 1). Other treatments gave almost equal colonization, except for the *A. versicolor* + NC and *Av* ST treatment.

Microbial populations

Total fungal counts ranged between 6.0 and 13.6 × 10^3 CFU g^{-1} soil in 2005–2006 and 4.5 and 16.2 × 10^3 CFU g^{-1} soil in 2006–2007 (Table 6). During both years, maximum fungal counts were obtained with the *A. versicolor* + NC treatment followed by the *A. versicolor* + FYM or FYM alone treatments. Combining *A. versicolor* with FYM and *Bf* ST treatment did not promote fungal counts in the soil in 2005–2006. Total bacterial counts ranged between 10.0 and 35.6 × 10^4 CFU g^{-1} soil in samples analysed after harvest in both seasons, where the *A. versicolor* + NC treatment encouraged maximum bacterial counts. The treatments of *A. versicolor* + FYM and *Bf* ST were next in supporting bacterial counts consistently. Total actinomycetes ranged between 5.6 and 18.5 × 10^4 CFU g^{-1} soil, with little variations in the samples estimated after harvest of crops, with maximum counts in plots receiving the *Av* ST treatment. The *A. versicolor* + NC treatment also stimulated actinomycetes in the soil.

The *A. versicolor* populations ranged between 1.0 and 4.7 × 10^4 CFU g^{-1} soil with non significant variations in both seasons. Maximum *A. versicolor* population was estimated in the *A. versicolor* + NC treatment in both seasons.

Discussion

The results of the present study have shown the potential of native BCAs for control of important soilborne plant pathogens in an arid region. It has been suggested that microorganisms isolated from the root or rhizosphere of a specific crop may be better adapt-

Table 5. Mean incidence of cumin wilt in field plots in two growing seasons, treated with the biocontrol agent *Aspergillus versicolor* applied using different methods and with different soil amendments.

Treatment	Wilt incidence (%)	
	2005–2006	2006–2007
A. versicolor seed treatment (*Av*ST)	7.1 (15.4)[c]	1.2 (6.3)
A. versicolor + neem compost	5.2 (13.1)	1.0 (5.8)
A. versicolor + farm yard manure (FYM)	6.8 (15.0)	1.8 (7.7)
A. versicolor one application[a] +FYM	7.2 (15.5)	2.8 (9.7)
A. versicolor two applications[b] + FYM	6.7 (14.9)	1.8 (7.7)
FYM	7.4 (15.8)	1.7 (7.5)
Non amended control	10.5 (18.9)	5.7 (13.7)
LSD ($P = 0.05$)	1.8	1.1

[a, b, c] See Table 1.

Table 6. Mean soil populations of soil microorganisms and *Aspergillus versicolor* in cumin field plot soils in two growing seasons, after different methods of *A. versicolor* application.

Treatment	Total fungi ($\times10^3 g^{-1}$ soil)		Total bacteria ($\times10^4 g^{-1}$ soil)		Total actinomycetes ($\times10^4 g^{-1}$ soil)		*A. versicolor* ($\times10^3 g^{-1}$ soil)	
	'05–'06	'06–'07	''05–'06	'06–'07	"05–'06	'06–'07	'05–'06	'06–'07
A. versicolor seed treatment (*Av*ST)	9.3[c]	7.7	19.0	18.0	17.6	18.5	3.0	2.2
A. versicolor + Neem compost	11.0	12.0	16.6	16.7	13.6	12.5	2.0	3.0
A. versicolor + FYM	6.3	4.5	15.0	15.5	6.3	7.1	2.0	1.2
A. versicolor one application[a] + FYM	12.0	15.0	10.0	12.0	6.0	6.5	1.5	3.0
A. versicolor two applications[b] + FYM	6.0	10.0	35.0	30.0	5.6	15.5	1.5	3.5
FYM	13.0	14.5	14.0	14.5	15.3	15.0	1.5	1.2
Non amendment control	7.0	8.0	11.3	13.2	6.3	6.5	1.5	1.2
LSD ($P = 0.05$)	4.9	6.9	15.9	13.3	7.9	7.7	1.8	2.0
LSD ($P = 0.05$)	4.9	6.9	15.9	13.3	7.9	7.7	1.8	2.0

[a, b] See Table 1.
[c] See Table 4.

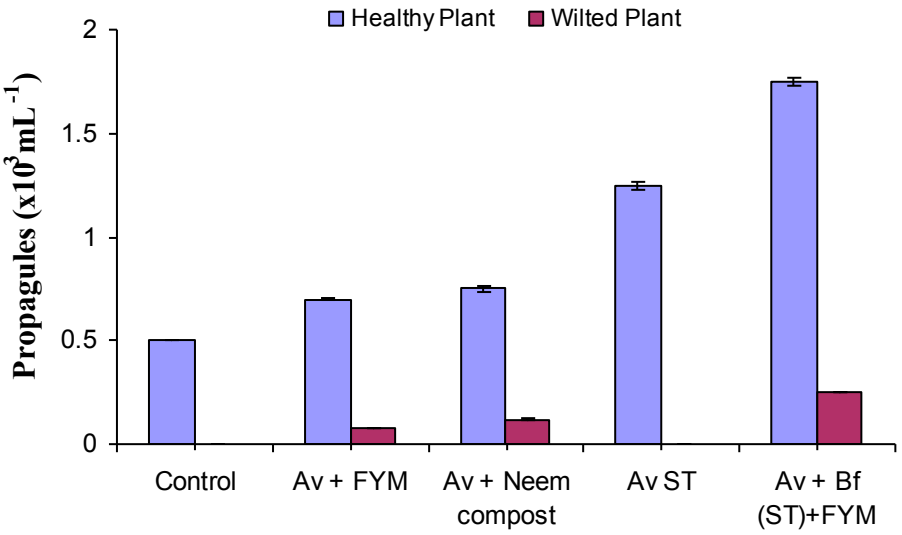

Figure 1. Mean colonization of cumin roots (1 cm) by *Aspergillus versicolor* (Av), 45 d after treatment in 2006 with various biocontrol agents and amendments. FYM = Farm yard manure , ST = Seed treatment, Bf = *B. firmus.*

ed to that crop and may provide better control of diseases than organisms originally isolated from other plant species (Cook, 1993). In the present study, *A. versicolor* has shown adaptation in our soils, perhaps due to its temperature tolerance as soil temperatures often reach between 55 and 60°C during hot summer days.

Lowest levels of plant mortality due to charcoal rot occurred in the *B. firmus* seed treatment planted in radish compost amended soil or when *A. versicolor* was amended with neem compost. This may be attributed to the increased availability of food substrates to the BCAs for their survival and activity

against target pathogens. If food sources are limited, the introduced organism may not be able to grow and proliferate. Urban and agricultural wastes have been used as mulches on avocado and citrus crops and for the delivery of microbial biocontrol agents like *T. harzianum*, *G. virens* and *P. fluorescens* (Casale *et al.*, 1995). The addition of some composts to soil has been reported to increase the incidence of plant growth promoting rhizobacteria (PGPR) in the tomato rhizosphere exhibiting antagonism towards *F. oxysporum* f. sp. *radicis-lycopersici*, *Pyrenochaeta lycopersici*, *Pythium ultimum*, and *Rhizoctonia solani* (Alvarez *et al.*, 1995). Spent compost obtained from *Agaricus bisporus* cultivation increased the populations of *B. subtilis* in a peat-based formulation (Manjula and Podile, 2001).

Improvements in the disease control obtained when both BCAs were used in combination are ascribed to the operation of different mechanisms of antagonism. Similar results have been reported previously. For example, two chitinolytic bacterial strains, *Paenibacillus* sp. 300 and *Streptomyces* sp. 385, suppressed Fusarium wilt of cucumber caused by *F. oxysporum* f. sp. *cucumerinum* in non-sterile soil-less potting medium. A mixture of the two strains gave better control of the disease than each of the strains used individually (Singh *et al.*, 1999).

In our study, improved colonization of *A. versicolor* and *B. firmus* in the rhizosphere, when both were applied together, is an indication that both BCAs are compatible and also that they had synergistic effects in promoting each other's establishment in the rhizosphere. This observation is in close agreement with other findings, where two nonpathogenic strains of *Fusarium* that were reported to give greatest reduction in the proportion of plants infected by the pathogen were also the best root colonizers (Nagao *et al.*, 1990). Raaijmakers *et al.* (1995) studied biocontrol efficacy of *Pseudomonas* strain WLC 358 against Fusarium wilt of radish, and suggested that better colonization of the antagonists in the rhizosphere is an important determinant of ability to suppress the diseases.

Increased nodulation in treatments having *B. firmus* coated seeds is of significance for cultivation of legumes dependent only on limited rainfall. Nitrogen fixing ability of other species of *Bacillus* has also been observed by Bajoria *et al.* (2008). Stimulation of nodulation and plant growth when both BCAs were combined confirmed that *A. versicolor* supported better colonization of *B. firmus* resulting in a small improvement in nodulation and growth of cowpea. Ryder *et al.* (1999) reported similar results with *B. subtilis* B908, which gave consistent shoot growth promotion in addition to reduced severity of take-all of wheat.

Improved disease control and better establishment of *A. versicolor* when it was amended with neem compost has significant implications for the control of pathogens such as *M. phaseolina* and *Foc*, whose populations are well distributed in the soil at 0–30 cm depths (Lodha *et al.*, 1990; Israel and Lodha, 2004). *Aspergillus versicolor* established well in arid soils, being native and because of the availability of a substrate that supported its survival and biocontrol ability. Although repeated application did not significantly stimulate the *A. versicolor* population compared to one application, in situations where substrates are limited then repeated applications may be required.

The method and frequency of application of *A. versicolor* affected the suppression of dry root rot of cowpea and wilt of cumin. One of the biggest problems in biological control is maintaining a high population of an active BCA during the entire period of disease activity; therefore, the timing of application of the BCA is very important. De Cal *et al.*, (1999) reported suppression of Fusarium wilt of tomato by manipulation of timing and method of application of *P. oxalicum*. In many biological control systems, lack of consistency has been associated with low populations of the antagonists. The low population of *A. versicolor* observed in the soil when *A. versicolor* was used as a seed treatment may explain the low level of disease control observed on cowpea.

In a citrus orchard, repeated application increased the population of *P. putida* resulting in reduced populations of *Phytophthora parasitica* and ten repetitive applications of *P. putida* at low concentrations through irrigation water resulted in soil populations similar to those from a single application at a greater concentration (Steddom and Menge 2001; Steddom *et al.*, 2002). In our study, the establishment of an optimum density of *A. versicolor* in field soil, even after one application, confirmed these findings. Microbial populations that are maintained or increased through to harvest time are positive indications that the BCAs have not adversely affected the soil microflora, particularly total fungi.

Our study demonstrates the efficacy of a low input technology for resource deficient growers of the Indian arid region. The use of multiple BCAs with soil amendments provide a potentially useful, low

input strategy for the management of two important soilborne plant pathogens.

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