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RESEARCH PAPERS

Biological control of *Fusarium oxysporum* f. sp. *cumini* with *Aspergillus versicolor*

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Summary. A native heat-tolerant strain of *Aspergillus versicolor* (Vuill.) Tirab. highly antagonistic to *Fusarium oxysporum* f. sp. *cumini* (*Foc*) was isolated from arid soils. In tests performed to ascertain its antagonistic activity against *Foc* as compared to *Trichoderma harzianum*, a 99.2 and 96.4% reduction in *Foc* propagules was achieved in *A. versicolor* and *T. harzianum* infested soil respectively. The reduction of *Foc* propagules in *Foc* and *A. versicolor*-infested soil was also determined. In a liquid-culture test, even at a low concentration of 0.5 ml cell-free filtrate, *A. versicolor* inhibited mycelial growth of *Foc*. Population changes of *A. versicolor* were examined at different soil moisture gradients, where maximum survival and multiplication of *A. versicolor* was estimated at 50% of moisture holding capacity. In general, with increasing concentrations of *A. versicolor* inoculum, soil population densities of *Foc* went down. Studies on thermal resistance showed that *A. versicolor* survived and multiplied even at 65°C. Soil amended with *A. versicolor* alone, or with a combination of *T. harzianum* and *Verbisina enceloides* residues was significantly better at reducing *Foc* than was non-amended control soil. A marked increase in the root length of cumin was observed in soil amended with *A. versicolor* or *T. harzianum* or both. The results suggest that *A. versicolor* has a potential value for use against *Fusarium* in hot arid soils because it can survive under dry and high-temperature conditions.

Key words: soil amendment, cumin, cruciferous residues.

Introduction

Cumin (*Cuminum cyminum* L.) is grown extensively in arid and semi-arid regions of India during the winter season under assured irrigation. Besides India, it is also cultivated in Bulgaria, Egypt, Argentina, Bangladesh and Pakistan (Gaetan and Madia, 1993; Omer *et al*., 1997).

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A wilt caused by *Fusarium oxysporum* f. sp. *cumini* (*Foc*) is responsible for serious economic losses to cumin (Lodha *et al.,* 1986). Growers often abandon cumin cultivation altogether and shift to other, less remunerative crops due to repeated infection of cumin with *Foc* in the same piece of land (Lodha, 1995). Cruciferous residues and oil-cake used as soil amendments have been found effective in reducing *Foc* population densities in the soil, and wilt incidence on cumin (Mawar and Lodha, 2002). Cruciferous residues during decomposition produce many biotoxic volatile compounds in the soil (Brown *et al.,* 1991). The

biotoxic effects of these volatiles, particularly the allyl-isothiocyanates (Mayton *et al*., 1996), at high soil temperatures have been well demonstrated (Lewis and Papavizas, 1971; Gamliel and Stapleton, 1993). However, the amount of pesticidal compounds produced by volatiles during decomposition is often low, and may not be the only cause of the reduction of *Foc* in the soil. Antagonists are also likely to bring about lower levels of weakened propagules. To investigate the role that antagonists play as biocontrol agents of *Foc*, several soil samples with or without residues were analysed to study the population dynamics of any native antagonists. It was found that there was a sudden upsurge in the population of *Aspergillus versicolor* in naturally heated, residue-amended soils, and that this control agent was highly antagonistic to *Foc* in repeated tests. *A. versicolor* is already known as a biological control agent (BCA) against *Macrophomina phaseolina* (Bhattacharya *et al.,* 1985), *Phymatotrichum omnivorum* (Kenerley and Stack, 1987) and *Puccinia helianthi* (Patil *et al*., 2000). However, its antagonistic potential against *Fusarium* has not yet been investigated. Any BCA of *Foc* must have the capacity to survive in a soil which remains warm and dry during most part of the year.

It was therefore thought worthwhile to study the biocontrol ability of *A. versicolor* against *Foc* and the influence of bio-ecological factors on the survival of the antagonist in arid soils.

Materials and methods

All experiments were conducted at the Central Arid Zone Research Institute, Jodhpur, India. A loamy sandy soil was used for the tests (85% sand, 8.9% clay, 5.5% silt), with 0.031% nitrogen, 0.25% organic carbon, 7 μg g^{-1} Olsen P and 8.1 pH.

Fungal cultures and inoculum preparation

A virulent strain of *Foc* was isolated from the roots of naturally infected cumin plants. Mycelial discs (5-mm) from 7-day-old cultures of the pathogen were inoculated in 250-ml Erlenmeyer flasks containing 50-ml Armstrong medium (Booth, 1971) and incubated at 30±2°C. After 15 days, the contents of the flasks, consisting mainly of mycelium and microconidia, were filtered through filter paper and then added to 3 kg field soil. The soil was thoroughly mixed and allowed to dry till only chlamydospores remained.

Aspergillus versicolor and *T. harzianum* isolated from native soil of the region were grown separately in potato dextrose broth (PDB). Fungal mats were harvested after 7 days on filter paper and blended for 30 sec in 200-ml of sterile distilled water, before use in the experiments. Each suspension had $\times 10^{10}$ and $\times 10^{9}$ cfu ml^{-1} of *A. versicolor* and *T. harzianum* respectively.

In-vitro test

Antagonism of *A. versicolor* against *Foc* was repeatedly confirmed by the dual-culture method. One 5-mm disc of *Foc* per Petri dish was cut from culture actively growing on potato dextrose agar (PDA) and placed on the periphery of Petri dishes (9 cm) containing PDA. Opposite to this disc, on the other side of the Petri dish, a 5-mm disc of *A. versicolor* was placed. Six Petri dishes were inoculated each time and kept in the dark at 30±1°C. Observations were recorded from day 3 to day 7 after inoculation.

In a separate experiment, the antagonistic activity of *A. versicolor* was compared with that of *T. harzianum*. Equal amounts (5 ml) of *A. versicolor* and *T. harzianum* were separately mixed in 100 g of *Foc*-infested soil and kept in polyethylene bags. Three bags were taken as three replicates for each treatment. Moisture was provided in all the bags to bring the soil to field capacity (10.4% w:w). One gram of soil was withdrawn from each bag to determine the initial population of *Foc* propagules. All the soil samples were incubated at 30±1°C. After 30 days, 1 g of soil was withdrawn from each replication of all the three treatments, air-dried and processed to determine the quantity of viable *Foc* propagules.

Effect of cell-free filtrate

A laboratory experiment was performed to ascertain whether *A. versicolor* released any metabolites toxic to *Foc* in culture, and to compare it with *T. harzianum* in this respect. Both BCAs were separately cultured in 250-ml Erlenmeyer flasks containing 50-ml of PDB for 7 days. Fungal mats were harvested on a G-5 filter. The spore-free filtrates were inoculated separately at 0.5, 1, 2 and 3 ml in 100-ml flasks containing 30-ml Richard's liquid medium. One 5-mm disc of *Foc* was inoculated in

each flask. Three flasks were kept as three replications for each concentration of filtrate. In the control flasks, only *Foc* discs were inoculated. After 10 days of growth dry mycelial weights were recorded after drying in an oven at 60°C for 48 h.

A separate laboratory experiment examined whether the amount of metabolites increased with the duration of culture of *A. versicolor* and *T. harzianum*. Both BCAs were cultured separately on PDB for 4, 8, 12 and 16 days each time in 3 flasks. A 2-ml spore-free filtrate at each culture duration was inoculated separately in 100-ml flasks containing 30-ml Richard's liquid medium. One 5-mm disc of *Foc* was inoculated in each flask. Three flasks were kept as three replications for culture duration. In the control flasks, only *Foc* discs were inoculated. After 10 days of culture dry mycelial weight was recorded after drying in an oven at 60°C for 40 h.

Soil moisture

Population changes in *A. versicolor* were monitored for 120 days in soils at different moisture gradients. Blended suspensions of *A. versicolor* were added to 1.2 kg soil. Infested soil was divided into four lots of 300 g each. Soil moisture gradients of 30, 50, 70 and 100% of moisture holding capacity (MHC) were separately maintained by adding the requisite amounts of water to each lot. The soil of each lot was then further divided into three portions and kept in 8×4" polyethylene bags. The bags were punctured 10–12 times to allow exchange of gases and were then tied and incubated at 30±1°C. Loss of soil moisture was compensated by adding 1–2 ml sterile water at frequent intervals. After thoroughly shaking for 20 s, soil samples were withdrawn from each bag at 30 day intervals and air-dried for 24 h to determine the population density of *A. versicolor*.

Effect of population density of *A. versicolor*

Aliquots of 2, 4 and 6 ml of 7-day-old *A. versicolor* ($\times 10^{10}$ cfu ml^{-1}) suspensions, were added to 5, 3 and 1 ml of sterile water respectively, and each suspension was added separately to lots of 100 g of *Foc*-infested soil. *Foc*-infested soil without *A. versicolor* served as control, but here 7-ml of sterile water was added to ensure the same soil moisture level (70% of MHC). A 5-g sample was immediately withdrawn from each lot, air-dried and processed for the estimation of the initial population of *A. versicolor* and *Foc*. Lots were kept in punctured polyethylene bags, thoroughly shaken and incubated at 30±1°C. Three bags served as three replications of each treatment. Population changes of *A. versicolor* were recorded at 30-day intervals by withdrawing a 10-g sample from each bag. After 120 days, viable propagules of *Foc* were determined and the percent reduction was calculated.

Time-temperature relationship

Five-gram lots of *A. versicolor*-infested soil were placed aseptically in several 10-ml culture tubes having plastic screw caps. In half the tubes, 0.5 ml sterilized water was added to adjust the soil water content to 10.4% (w:w); the remaining culture tubes contained only infested dry soil. All the tubes were capped and kept in an aluminium test tube stand, which was partially immersed in a thermostatically controlled water bath so that the water level remained above the soil surface level in the tubes. Two tubes each were maintained at 50°C (30, 45, 60, 75, and 90 min); at 55°C (15, 30, 45, 60, and 75 min); at 60°C (6, 9, 12, 15, and 18 min); at 62°C (4, 8 and 12 sec); and at 65°C (4, 8 and 12 sec). However, at this last temperature only dry soil samples were tested. The controls were kept at 37±2°C. Soil withdrawn from the culture tubes for the specified exposure time at each temperature was processed to determine the number of viable colony forming units (cfus) of *A. versicolor* on Martin Rose-bengal agar.

Combination of bio-control agents and residues

The effect of *A. versicolor, T. harzianum* and residues of *Verbisina enceloides* (Cav.) Benth. & Hook. on the survival of *Foc* propagules and on the root and shoot length and weight of cumin seedlings was studied separately and in all combinations. This part of the experiment comprised 6 treatments: 1. *V. enceloides* residues (0.5%); 2. *A. versicolor*; 3. *T. harzianum*; 4. *A. versicolor* + *T. harzianum*; 5. *A. versicolor* + *T. harzianum* + *V. enceloides*; and 6. unamended control, arranged in a completely randomized design. Five 23 cm earthen pots each filled with 5 kg *Foc*-infested soil served as five replications. A 2 g initial sample was withdrawn from each pot, bulked to form one sample for each treatment and processed to determine the initial *Foc* population. Blended suspensions of *A.*

versicolor or *T. harzianum* were mixed separately in 1 kg soil and uniformly mixed in pots for treatments 2, 3, 4 and 5 as required. Ground-up residues of *V. enceloides* were worked into the soil of treatments 1 and 5. Ten cumin seeds were sown on November 27, 2001 in each pot. Pots were irrigated frequently and 5 seedlings from each pot were gently uprooted after 15 days of growth and root and shoot lengths measured. The fresh weight of the roots and shoots was also recorded after cutting seedlings at the root-shoot junction. The final population of *Foc* was determined and the percent reduction calculated.

Determination of viable propagules

Soil samples were air-dried and ground to pass through a 2-mm sieve for quantitative estimation of microbes. The population of *Foc* and *A. versicolor* was determined by a serial dilution technique on modified peptone-PCNB medium (Papavizas, 1967), and on Martin Rose-bengal agar respectively.

Field demonstration

On a farmer's field with a heavy incidence of wilt, one demonstration was undertaken in the Institute's Village Linkage Programme (IVLP). A 1-kg bioformulation of *A. versicolor* prepared in talc and termed 'Maru sena 2' was mixed with 10 kg farmyard manure and worked into a 1600 m^2 field at 20 cm soil depth. An equal area was kept untreated as a control. Wilt incidence was recorded in 8 1×1 m squares in February.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and the treatment means compared with LSD (P=0.05) (Snedecor and Cochran, 1967).

Results

Inhibition tests

In the dual-culture tests, mycelial growth of *Foc* was almost completely inhibited by several colonies of *A. versicolor* within 3–4 days of inoculation. In the following 3 days, mycelium of *A. versicolor* overgrew *Foc* so completely that whitish *Foc* growth was no longer visible. In another set of Petri plates, whitish *Foc* mycelium was visible only during the initial stage, but then a dense hyphal growth of *A. versicolor* overgrew and completely hyperparasitized the *Foc* mycelial mat.

In *Foc*- and *A. versicolor*-infested soil, an initial *Foc* population of 4.2×10^4 cfu g^{-1} drastically declined to 2.8×10^3 cfu g^{-1} soil after 15 days of incubation (a 93.3% reduction). In *Foc*- and *T. harzianum*-infested soil, 1.1×10^4 cfu g^{-1} soil of viable propagules of *Foc* (73.8%) were counted after 15 days under similar conditions. This decline continued, so that after 30 days only 0.3×10^3 and 1.5×10^3 cfu g^{-1} soil of *Foc* were estimated in *A. versicolor* and *T. harzianum*-infested soils respectively.

Effect of cell-free filtrate

In the liquid culture test, a cell-free filtrate even at a low concentration of 0.5 ml of *A. versicolor* and *T. harzianum* inhibited 29 and 13% mycelial growth of *Foc*, respectively. This reduction increased with increasing concentration of both BCAs. However, the percent reduction in *Foc* was much higher with the filtrate of *A. versicolor* than with the filtrate of *T. harzianum,* so much so that at a 3-ml concentration of *A. versicolor* no mycelial growth of *Foc* was visible.

In a separate liquid test, the effect of filtrate age of *A. versicolor* and *T. harzianum* on the mycelial growth of *Foc* was evaluated. The reduction of *Foc* mycelium was significantly greater with 8-day-old than with 4-day-old cell-free filtrates of both BCAs (Table 1), but a further increase in the age of filtrate did not lead to any further significant reduction in *Foc* growth. However, the reduction was higher with *A. versicolor* than with *T. harzianum* filtrate.

Effect of soil moisture

There was a sudden upsurge in the population of *A. versicolor* at all moisture gradients after 30 days of incubation, the maximum increase being in soil at 70% MHC (Table 2). However, the *A. versicolor* population then declined at 60 days in all moisture gradients except at 50% MHC, where there was a further 2-fold increase. At 90 and 120 days populations fluctuated only marginally. Maximum survival and multiplication of *A. versicolor* was recorded at 50% MHC.

Effect of different concentrations of *A. versicolor*

The initial (0 days) cfus of *A. versicolor* so obtained in the soil was not proportional to the con-

Table 1. Effect of cell-free culture filtrates of *Aspergillus versicolor* and *Trichoderma harzianum* on mycelial growth of *Fusarium oxysporum* f. sp. *cumini* (*Foc*)

Treatment	Age of the culture (days)	Dry mycelial weight of *Foc* (mg)	Reduction (%) in mycelial growth
A. versicolor	4	358	33.2
	8	245	54.3
	12	243	54.6
	16	241	55.0
T. harzianum	4	311	41.9
	8	298	44.4
	12	293	45.3
	16	288	46.2
Unamended control		536	0.0

Table 2. Effect of soil moisture gradient on the population of *Aspergillus versicolor* ($\times 10^8$ g^{-1} soil)[a].

Soil moisture gradient (% of MHC)	Sampling time (days)			
	30	60	90	120
30	4.2	1.8	2.4	2.1
50	3.6	7.3	7.7	6.8
70	7.1	3.4	4.0	3.6
100	5.5	3.5	4.2	4.2

LSD (P=0.05): treatment -2.7; interval -2.4; treatment × interval - 5.5.

[a] Initial population of *A. versicolor* was 1.5×10^8 g^{-1} soil.

centration of the aliquots added. When 2 and 4 ml of a 7-day-old suspension of *A. versicolor* were worked into the soil, the population of the antagonist increased for the first 60 days, then declined in the next 60 days; on the whole it remained 60.5–61.9% higher than the original counts (Table 3). However, an initial *A. versicolor* concentration in the 6-ml treatment (2.4×10^7 g^{-1} soil) increased approximately 2.5-fold in 120 days. Amending the soil with different concentrations of *A. versicolor* significantly reduced *Foc* propagules after 30 days of incubation. This reduction increased with increasing concentration of *A. versicolor* in the suspension. Thus, amending 100 g soil with a suspension of the 6-ml concentration of *A. versicolor* brought about a 93.1% reduction in viable propagules of *Foc* (Table 3).

Time-temperature relationship

Aspergillus versicolor survived and multiplied even at 65°C (Fig. 1). An initial count of 6.3×10^4 cfu g^{-1} soil of *A. versicolor* increased manifold over time at 50–55°C under moist conditions. There was a positive correlation between the duration of the experiment and the population of *A. versicolor*. At 60°C, however there was a sharp decline in viable propagules of *A. versicolor* in the first 6 min of exposure, but this was followed by a gradual increase under both moist and dry conditions (Fig. 1). After 18 min of exposure, counts of *A. versicolor* were double and triple the initial count. Similarly, at 62°C, the population of *A. versicolor* declined for the first 4 sec, under both moist and dry conditions, but then increased again: after 12 sec, the counts were higher than at the start, es-

Table 3. Effect of culture concentrations on survival of *Aspergillus versicolor* ($\times 10^7$ g^{-1} soil) and *Fusarium oxysporum* f. sp. *cumini* (*Foc*) ($\times$ 10^3 g^{-1} soil)[a] in soil after 120 days.

Treatment	Sampling time (days)					
	A. versicolor					*Foc*
	0	30	60	90	120	120
2 ml *A. versicolor* + 5 ml sterile water	1.8	4.2	4.2	3.2	2.9	4.0 (75.0)[b]
4 ml *A. versicolor* + 3 ml sterile water	2.1	6.1	6.2	4.7	3.4	2.1 (86.8)
6 ml *A. versicolor* + 1 ml sterile water	2.4	5.0	5.4	6.7	5.8	1.1 (93.1)
Unamended control						13.4 (16.2)
LSD (P=0.05): treatment -0.28; interval -0.32; treatment $\times$ interval -0.56						6.1

[a] Initial population of *Foc* was 1.6×10^4 g^{-1} soil.
[b] Reduction (%) in the No. of *Foc* propagules.

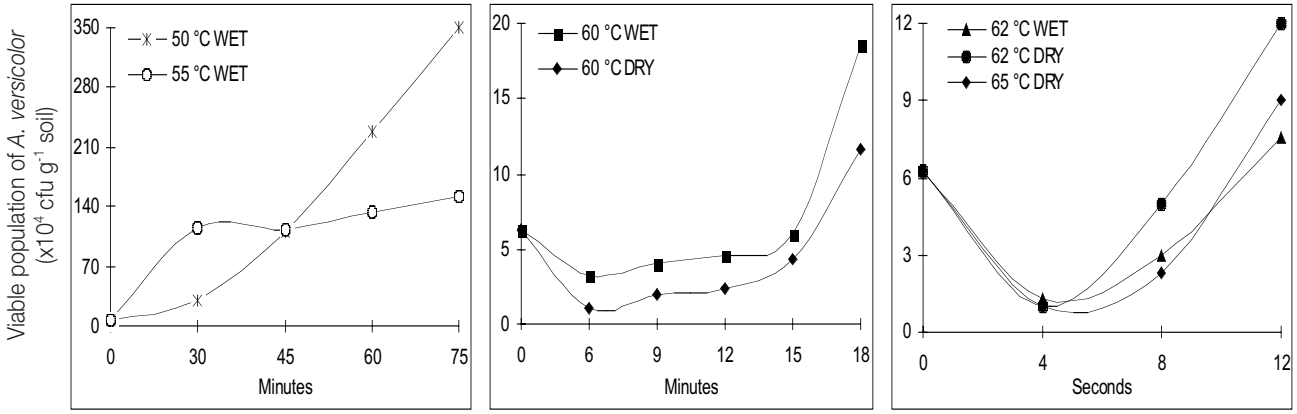

Fig. 1. Time-temperature relationship for survival of *Aspergillus versicolor* in wet and dry soil at 50, 55, 60, 62 and 65°C.

pecially under dry conditions. At 65°C, where the test was run only under dry conditions, populations of *A. versicolor* also increased at 12 sec, after having declined initially.

Combination of bio-control agents with residues

Aspergillus versicolor in the soil alone or in combination with *T. harzianum* and/or *V. enceloides* residues was significantly better at reducing the *Foc* population than was the unamended control soil (Table 4). The maximum reduction in *Foc* was achieved when both BCAs were combined with *V. enceloides* residues. The reduction in *Foc* propagules was significantly greater in *A. versicolor*-amended soil, but a combination of both BCAs was significantly better than one BCA applied alone. Both *A. versicolor* and *T. harzianum* and their combined application significantly increased cumin root length, and the combined use of both BCA's plus *V. enceloides* markedly increased shoot length and weight (Table 4).

Field demonstration

Wilt incidence was significantly lower in the *A. versicolor*-amended field (14.2%) than in the unamended control field (33.3%).

Table 4. Effect of *Aspergillus versicolor, Trichoderma harzianum* and *V. enceloides* residues on growth of cumin and survival of *Fusarium oxysporum* f. sp. *cumini* (*Foc*) propagules.

Treatment	Length (cm)		Weight (mg)		Reduction (%) in *Foc* propagules
	Root	Shoot	Root	Shoot	
V. enceloides	1.4	7.1	6.0	32.3	82.9
A. versicolor	2.3	6.9	2.0	34.0	87.1
T. harzianum	2.3	6.4	3.0	25.4	82.4
A. versicolor + *T. harzianum*	2.1	6.8	4.0	70.3	92.1
V. enceloides + *A. versicolor* + *T. harzianum*	1.6	8.6	4.0	78.5	96.3
Unamended control	1.3	6.5	2.0	21.5	46.3
LSD (*P*=0.05)	0.2	0.1	1.8	5.8	8.1

Discussion

Aspergillus versicolor isolated from heated (naturally or solarized) cruciferous residue-amended soils of this region, parasitized *Foc* in laboratory tests and reduced the *Foc* population density in such soil.

In the analysis of antagonists from heated cruciferous-amended soil, a high number of colonies of *Bacillus firmus,* an antagonistic bacterium, and *A. versicolor* were always found on Czapek's dox and PDA respectively. The specific antagonism of *B. firmus* against *Macrophomina phaseolina* has recently been investigated (Lodha *et al*., 2000; Mawar and Lodha, 2002). The hyperparasitism of *A. versicolor* over *Foc* was comparable to that of *T. harzianum*. A significant reduction in *Foc* propagules in soil containing *A. versicolor* is an indication that mycoparasitism has occurred in that soil. Facultative mycoparasites such as species of *Trichoderma* have frequently been reported in the sclerotia of many pathogens including *Phymatotricum omnivorum, Rhizoctonia* sp., *Sclerotinia* sp., *Sclerotium* sp. and *Verticillium dahliae* (Gladders and Coley-Smith, 1980; Zazzerini and Tosi, 1985; Howell, 1987; Kenerley and Stack, 1987; Keineth *et al*., 1991; Van den Boogert and Saat, 1991). A similar phenomenon may well have been operating with *A. versicolor*, though this remains to be established. *A. versicolor* has also been reported as an antagonist against *M. phaseolina* (Bhattacharya *et al*., 1985) and it inhibits the germination of uredospores of *Puccinia helianthi* (Patil *et al.,* 2000). *A. versicolor* is further known to produce the antibiotic mycoversilin (Samanta *et al*., 1983).

Certain compounds released from crucifers during hydrolysis at high soil temperatures could stimulate the multiplication and activity of *A. versicolor*. Soil amendments that stimulate antagonists have been reported (Mitchell and Alexander, 1961; Weinhold *et al.,* 1969). Nutrients from decomposed residues, and certain compounds such as ethylene, ammonia, acetone, ethanol, methanol, formaldehyde, acetaldehyde, etc. were reported to stimulate the germination of micro-organisms (Harman *et al*., 1980). Many of these and related compounds were isolated from heated cruciferous residues and characterized by Gamiliel and Stapleton (1993). An increase in the populations of certain species of *Aspergillus* and *Penicillium* in soil amended with cruciferous residues was also reported by Ramirez-Villapadua and Munnecke (1988).

The fact that mycelial growth of *Foc* was reduced in the presence of a cell-free filtrate of *A. versicolor*, and that this reduction became more marked as the concentration of the cell-free filtrate increased, was a clear sign that *A. versicolor* released into the culture substances with an antibiotic activity. This was confirmed when it was found that the older cell free-filtrates (up to 8 days old) reduced *Foc* mycelium growth more than did fresh filtrates. The fact that filtrates older than 8 days did not further reduce growth of *Foc* significantly, indicated that *A. versicolor* released its substances within this period. Bhattacharya *et al*. (1985) also reported that *A. versicolor* in a glucose-peptone broth was most effective in the first 10 days. Apart from releasing antibiotic substances, *A. versicolor* also produces volatile metabolites such as various hydrocarbons, alcohols, ketones, ethers,

esters and sulphur-containing compounds (Sunsesson *et al.,* 1995). The most commonly produced substances are 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 3-methylfuran and dimethyl sulphide (Keller *et al*., 1997). Sulphur-containing compounds such as dimethyl sulphide and dimethyl disulphide, both toxic to soil-borne pathogens, have also been reported from decomposing cruciferous residues (Lewis and Papavizas, 1970; 1971). Thus, apart from its hyperparasitic action, a suppressive effect of *A. versicolor* through the release of volatile metabolites into the soil cannot be ruled out.

The good survival and multiplication of *A. versicolor* at low soil moisture and high soil temperatures is an indication that it is well adapted to dry sandy soils, where the temperature often goes up to 50–60°C during the hot summer months. Cholil (1981) also reported the survival of *A. versicolor* at 60°C and postulated a mechanism by which this fungus could survive at such a high temperature. Heat-shock proteins (HSPs) play a crucial role in tolerance to heat (Freeman *et al.,* 1989). Inulinase, an enzyme having thermal stability at 65°C, is produced in high quantities by *A. versicolor* and might be one such protein responsible for heat-tolerance (Kochhar *et al.,* 1998). The dramatic increases in *A. versicolor* counts at 62 and 65°C after an initial decline could be attributed to the cumulative effect of heating that concurrently created a vacuum in the soil and increased the availability of the nutrients, which may have encouraged the surviving heat-tolerant propagules to multiply rapidly. The prevalence of *A. versicolor* in house dust, a nearly dry carrier, has also been reported by Pasanen *et al*., (1997). The increasing survival and multiplication of *A. versicolor* with increasing concentration and a proportionate reduction in *Foc* propagules as recorded in the present study is a further sign of the suitability of *A. versicolor* as a BCA against *Fusarium* in arid soils, where hot dry spells are frequent.

When considering any mixture or consortium of strains for use in bio-control, it is important that no member of a mixture should inhibit any of the others. The greater reduction in the viability of *Foc* propagules found in the present study when both BCAs, with or without *V. enceloides* residues, were used against them, indicated that the BCAs had a synergistic action, improving the effectiveness of on-farm wastes against *Foc*. *V. enceloides* is an obnoxious weed in arid regions, which has shown anti-fungal properties in laboratory and field tests (Israel, 2002). A binary system of *Serratia marcescens* and *Streptomyces anulatus* was effective in controlling tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Toyoda *et al*., 1993). Similarly, *Pythium nunn* and *T. harzianum* in combination reduced Pythium damping-off in cucumber (Paulitz *et al*., 1990). However, further studies are required to better understand the interaction of the two BCAs tested in the present study before any final conclusion can be reached. The plant-growth promoting nature of *A. versicolor* was demonstrated in pot experiments, where it was equally effective as *T. harzianum*. Field experiments are in progress to confirm this aspect of *A. versicolor* as a BCA.

Our studies demonstrated that *A. versicolor* is a potential native BCA against *Foc*, the most important root pathogen in the Indian arid region.

Literature cited

Bhattacharya D., S. Basu, J.P. Chattopadhyay. and S.K. Bose, 1985. Biocontrol of *Macrophomina* root rot disease of jute by an antagonistic organism, *Aspergillus versicolor. Plant and Soil* 87, 435–438.

Booth C., 1971. *The Genus Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England, 237 pp.

Brown P.D., M.J. Morra, J.P. McCaffrey, D.L. Auld and L. Williams, 1991. Alleochemicals produced during glucosinolate degradation. *Journal of Chemical Ecology* 17, 2021–2034.

Cholil A., 1981. Effect of heat treatment on the soil microflora of a potato field. *Agrivita* 4, 30–33.

Freeman S., C. Ginzburg and J. Katan, 1989. Heat shock protein synthesis in propagules of *Fusarium oxysporum* f. sp. *niveum*. *Phytopathology* 79, 1656–1661.

Gamliel A. and J.J. Stapleton, 1993. Characterization of antifungal volatile compounds evolved from solarized soil amended with cabbage residue. *Phytopathology* 83**,** 899–905.

Gladders P. and J.R. Coley-Smith, 1980. Interactions between *Rhizoctonia tuliparum* sclerotia and soil microorganisms. *Transactions of the British Mycological Society* 74, 579–586.

Howell C.R., 1987. Relevance of mycoparasitism in the biological control of *Rhizoctonia solani* by *Gliocladium virens*. *Phytopathology* 77, 992–994.

Israel S., 2002. *Effect of Amendments on Wilt of Cumin caused by* Fusarium oxysporum *f. sp*. cumini *Prasad and Patel in Aridisols*. PhD Thesis, Jai Narain Vyas University, Jodhpur, India, 141 pp.

Keinath A.P., D.R. Fravel and G.C. Papavizas, 1991. Potential of *Gliocladium roseum* for biocontrol of *Verticillium dahliae*. *Phytopathology* 81, 644–648.

Kellar R., R. Sonnichsen and H. Ohgka, 1997. Investigation of microbially volatile organic compounds from moulds (*P. expansum*, *A. versicolor*) by GC-MSD analysis. *Umweltmedizin in Forschung und Praxis* 4, 265–274.

Kenerley C.M and J.P. Stack, 1987. Influence of assessment methods on selection of fungal antagonists of the sclerotium-forming *Phymatotrichum omnivorum*. *Canadian Journal of Microbiology* 33, 632–635.

Kochhar A., N. Kaur, A.K. Gupta, Anuradha-Kochhar and Narinder-Kaur, 1998. Immobilization of inulinase from *Aspergillus versicolor* for preparing fructose from Inulin. *Journal of Scientific and Industrial Research* 57, 184–187.

Lewis J.A. and G.C. Papavizas, 1970. Evolution of volatile sulfur containing compounds from decomposition of crucifers in soil. *Soil Biology and Biochemistry* 2, 239–246.

Lewis J.A. and G.C. Papavizas, 1971. Effect of sulfur containing volatile compounds and vapors from cabbage decomposition on *Aphanomyces euteiches*. *Phytopathology* 61, 208–214.

Lodha S., 1995. Soil solarization, summer irrigation and amendments for the control of *Fusarium oxysporum* f. sp. *cumini* and *Macrophomina phaseolina* in arid soils. *Crop Protection* 14, 215–219.

Lodha S., G.K. Gupta and S. Singh, 1986. Crop disease situation and some new records in Indian arid zone. *Annals of Arid Zone* 25, 311–320.

Lodha S., R. Mawar and S.K. Sharma, 2000. *Bacillus firmus*: a specific biocontrol agent against *Macrophomina phaseolina* isolated from cruciferous residue amended soil. In: *Symposium on Biotechnology of Plant Protection,* Feb 25–27 2000, Banaras Hindu University, Varanasi, India, 91 (abstract).

Mawar R. and S. Lodha, 2002. Brassica amendments and summer irrigation for the control of *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *cumini* in hot arid regions. *Phytopathologia Mediterranea* 41, 45–54.

Mayton H.S., C. Olivier, S.F. Vaughan and R. Loria, 1996. Correlation of fungicidal activity of *Brassica* species with allyl isothiocyanate production in macerated leaf tissue. *Phytopathology* 86, 267–271.

Mitchell R. and M. Alexander, 1961. Chitin and the biological control of *Fusarium* diseases. *Plant Disease Reporter* 45, 487–490.

Omer E.A., M.A. Nofal, S.M. Lashin and W.M.E. Haggaq, 1997. Effect of some growth regulators on the growth parameters and oil content of cumin with disease incidence under two types of soil. *Egyptian Journal of Horticulture* 24, 29–41.

Pansanen P., A. Korpi, P. Lalliokoski and A.L. Pasanen, 1997. Growth and volatile metabolite production of *Aspergillus versicolor* in house dust. *Environment International* 4, 425–432.

Papavizas G.C., 1967. Evaluation of various media and antimicrobial agents for isolation of *Fusarium* from soil. *Phytopathology* 57, 848–852.

Patil P.V., M.R. Kachapur and K.H. Anahosur, 2000. Role of phyllosphere microorganisms in the control of sunflower rust caused by *Puccinia helianthi*. In: *Integrated Plant Disease Management for Sustainable Agriculture,* November 11–15 2000, IARI, New Delhi, 897–900.

Paulitz T.C., J.S. Ahmad and R. Baker, 1990. Integration of *Pythium nunn* and *Trichoderma harzianum* isolate T-95 for the biological control of Pythium damping-off of cucumber. *Plant and Soil* 121, 243–250.

Ramirez-Villapudua J. and D.E. Munnecke, 1988. Effects of solar heating and soil amendments of cruciferous residues on *Fusarium oxysporum* f. sp. *conglutinans* and other micro-organisms. *Phytopathology* 78, 289–295.

Samanta A.K., H.K. Kole, S.K. Goswami and S.K. Bose, 1983. Mycoversilin, a new antifungal antibiotic from a mutant derivative of *Aspergillus versicolor. Indian Journal of Experimental Biology* 21, 577–578.

Snedecor G.E. and W.J. Cochran, 1967. *Statistical Methods*. Oxford & IBH Publishing Co, Calcutta, India.

Sunsesson A.L., W.H.J. Vaes, C.A. Nillson, G. Blomquist, B. Anderson and R. Carlson, 1995. Identification of volatile metabolites from five fungal species cultivated on two media. *Applied and Environmental Microbiology* 61, 2911–2918.

Toyoda H., M. Morimoto, K. Kakutani, M. Morikawa, T. Fukamizo, S. Goto, H. Terada and S. Ouchi, 1993. Binary microbe system for biological control of Fusarium wilt of tomato: enhanced root-colonization of an antifungal rhizoplane bacterium supported by a chitin-degrading bacterium. *Annals of the Phytopathological Society of Japan* 59, 375–386.

Van den Boogert P.H.J.F. and T.A.W.M. Saat, 1991. Growth of the mycoparasitic fungus *Verticillium biguttatum* from different geographical origins at near minimum temperatures. *Netherlands Journal of Plant Pathology* 97, 115–124.

Weinhold A.R., T. Bowman and R.L. Dodman, 1969. Virulence of *Rhizoctonia solani* as affected by nutrition of the pathogen. *Phytopathology* 59, 1601–1605.

Zazzerini A. and I. Tosi, 1985. Antagonistic activity of fungi isolated from sclerotia of *Sclerotinia sclerotiorum*. *Plant Pathology* 34, 415–421.

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