

IN VITRO INTERACTION OF *FUSARIUM* SPP., WITH OTHER FUNGI

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Abstract

In the present study interaction of 10 *Fusarium* spp., namely *Fusarium equiseti*, *F. longipes*, *F. moniliforme*, *F. oxysporum*, *F. proliferatum*, *F. scirpi*, *F. pallidroseum*, *F. sporotrichioides*, *F. solani* and *F. subglutinans* with other fungi viz., *Alternaria alternata*, *Aspergillus niger*, *A. flavus*, *A. terreus*, *A. versicolor*, *Cladosporium herbarum*, *Drechslera hawaiiensis*, *Paecilomyces* sp., *Penicillium digitatum*, *P. funiculosum*, *Rhizoctonia solani* and *Trichoderma hamatum* was studied in vitro. In dual culture plate assays, *Trichoderma hamatum* showed inhibition in growth of *Fusarium* spp., by producing zones of inhibition.

Introduction

Fusarium spp., are known to cause seed rot, damping off, wilting and root rot diseases resulting in severe losses to a variety of crop plants (Miller, 1994). In several crops *Fusarium* diseases are generally controlled by fumigation with methyl bromide (Awuah & Lorbeer, 1991). Considering the cost of pesticides and environmental hazards of the use of these chemicals, the use of microbial antagonists in the control of plant pathogens has received increasing attention throughout the world (Ghaffar, 1988a,b, 1992). Several workers have used antagonistic microorganisms to control plant pathogens (Ghaffar, 1988a,b; Henis *et al.*, 1979). Of the antagonists *Trichoderma* spp., have been commonly used to control *Fusarium* spp., (Marois *et al.*, 1981). Biological control of *Fusarium* spp., with other non- pathogenic fungi has been reported by Dawson *et al.*, (2002), Simon & Sivasithamparam (1989), Butt & Ghaffar (1972), Waksmundzka & Mazur (2001) and Duda (2001). Present study was carried out to investigate the biological control as an alternative strategy for management of *Fusarium* diseases.

Materials and Methods

A number of fungi were isolated from soil by soil dilution plate and soil plate techniques (Waksman & Fred, 1922; Warcup, 1950). Interaction of *Fusarium* spp., with other fungi viz., *Alternaria alternata*, *Aspergillus niger*, *A. flavus*, *A. terreus*, *A. versicolor*, *Cladosporium herbarum*, *Drechslera hawaiiensis*, *Paecilomyces* sp., *Penicillium digitatum*, *P. funiculosum*, *Rhizoctonia solani* and *Trichoderma hamatum* were studied by dual culture plate assays. Growth of fungi and zone of inhibition were measured and inhibition of radial growth was calculated as follows:

$$100 \frac{r_2 - r_1}{r_2}$$

where, r_1 = radial growth of pathogenic fungus on opposed side.

r_2 = radial growth of pathogenic fungus on unopposed side.

Interactions were assessed using a key based on observations of Porter (1924) and Dickinson & Boardman (1971) as given below:

- A.** Mutually intermingling growth where both fungi grew into one another without any microscopic signs of interaction.
- Bi.** Intermingling growth where the fungus being observed was growing into the opposed fungus either above or below its colony.
- Bii.** Intermingling growth where the fungus under observation has ceased growth and is overgrown by another colony.
- C.** Slight inhibition where the fungus approach each other until almost in contact and a narrow demarcation line, 0.1 – 2mm, between the two colonies clearly visible.
- D.** Mutual inhibition at a distance of > 2 mm.

In evaluating interactions, each fungus was assessed for its ability to inhibit growth of another fungus and assessments were made when the fungi had achieved an equilibrium after which there was no further alteration in the growth pattern (Fig. 1).

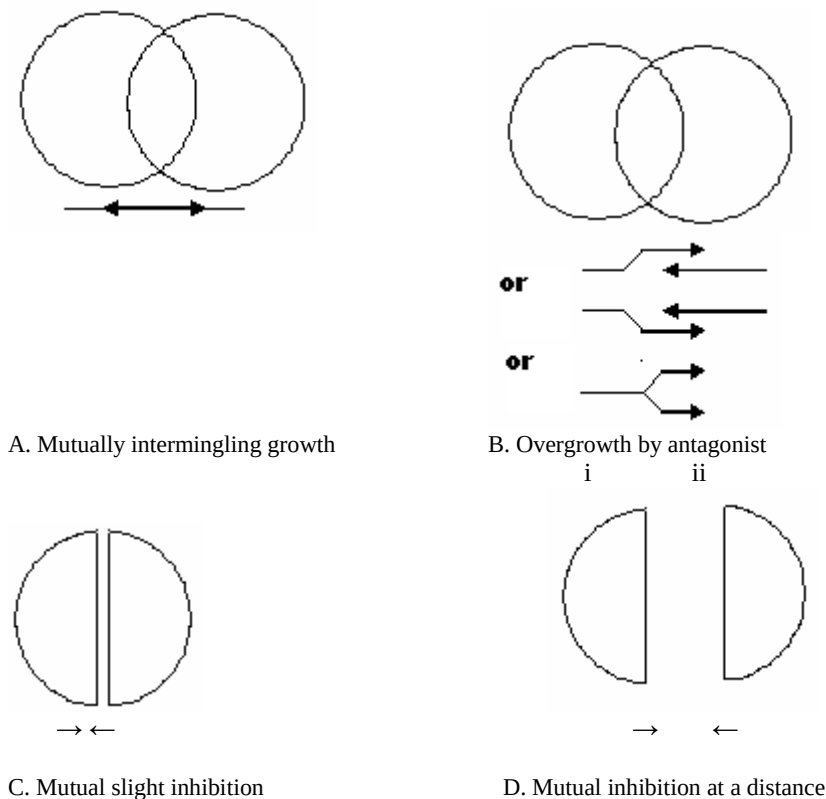


Fig. 1. Interactions observed between adjacent fungal colonies on agar medium.

Results and Discussion

Using dilution plate and soil plate techniques (Waksman & Fred, 1922; Warcup, 1950) a number of fungi viz., *Alternaria alternata*, *Aspergillus niger*, *A. flavus*, *A. terreus*, *A. versicolor*, *Cladosporium herbarum*, *Drechslera hawaiiensis*, *Paecilomyces* sp., *Penicillium digitatum*, *P. funiculosum*, *Rhizoctonia solani* and *Trichoderma hamatum* were isolated from the soil and identified.

In dual culture plate assays, *T. hamatum* showed inhibition in radial growth of *F. oxysporum* (79.97%), *F. pallidoroseum* (57.89%), *F. sporotrichioides* (22.16%), *F. moniliforme* (10.64%), *F. subglutinans* (25%), *F. proliferatum* (57.89%), *F. equiseti* (5.88%), *F. longipes* (32.73%), *F. scirpi* (12%), and *F. solani* (40%) producing “B_{ii}” type reaction (Porter, 1924) and later showed overgrowth on the colonies of *Fusarium* spp. Inhibition in growth of *Fusarium* spp., by *Stachybotrys atra* (Butt & Ghaffar, 1972), *Pythium oligandrum* (Benhamou et al., 1997), *Arachniotus* sp., *Chaetomium globosum*, *Memnoniella echinata*, *Talaromyces flavus* as well as *Trichothecium roseum* (Ghaffar, 1988a,b) and *Trichoderma harzianum* (Sharma & Dohroo, 1991) have been reported.

Similarly, in dual culture plate assay *Alternaria alternata*, *Aspergillus niger*, *A. flavus*, *A. terreus*, *A. versicolor*, *Cladosporium herbarum*, *Drechslera hawaiiensis*, *Paecilomyces* sp., *Penicillium digitatum*, *P. funiculosum* and *Rhizoctonia solani* inhibited the growth of *Fusarium* spp., by producing zone of inhibition (Table 1).

It would suggest that fungi such as *Trichoderma hamatum*, which inhibited the growth of *Fusarium* spp., could be used in the biological control of the diseases caused by *Fusarium* spp.

Table 1. In vitro interaction of *Fusarium* spp. with other fungi.

Test fungi	Radius		Zone of inhibition	Type of reaction
	r ₁	r ₂	r ₁	r ₂
<i>F. equiseti</i>				
<i>Alternaria alternata</i>	25.3	30.3	nil	A
<i>Aspergillus flavus</i>	18.3	27.6	nil	A
<i>A. niger</i>	15.7	31.3	1.0	C
<i>A. terreus</i>	20.0	31.0	2.0	C
<i>Cladosporium herbarum</i>	22.3	26.0	nil	B _i
<i>Drechslera hawaiiensis</i>	19.0	30.7	nil	A
<i>Paecilomyces</i> sp.	19.7	27.7	nil	A
<i>Penicillium digitatum</i>	20.0	31.7	1.0	C
<i>P. funiculosum</i>	21.3	25.7	nil	B _i
<i>Rhizoctonia solani</i>	19.3	28.0	nil	A
<i>Trichoderma hamatum</i>	24.0	25.5	nil	B _i
<i>F. longipes</i>				
<i>Alternaria alternata</i>	15.0	30.7	nil	B _i
<i>Aspergillus flavus</i>	14.7	31.7	nil	A
<i>A. niger</i>	14.0	30.0	5.0	D
<i>A. terreus</i>	20.0	29.7	nil	A
<i>Cladosporium herbarum</i>	19.5	28.0	nil	B _i
<i>Drechslera hawaiiensis</i>	12.3	28.0	nil	A
<i>Paecilomyces</i> sp.	17.3	28.0	nil	A
<i>Penicillium digitatum</i>	22.0	27.7	nil	A
<i>P. funiculosum</i>	20.7	26.0	nil	A
<i>Rhizoctonia solani</i>	17.5	30.0	nil	A
<i>Trichoderma hamatum</i>	18.5	27.5	nil	B _{ii}

Table 1. (Cont'd.)

Test fungi	Radius		Zone of inhibition	Type of reaction
	r ₁	r ₂	r ₁	r ₂
<i>F. moniliforme</i>				
<i>Alternaria alternata</i>	13.0	27.0	nil	A
<i>Aspergillus flavus</i>	15.0	18.0	nil	A
<i>A. niger</i>	10.0	30.5	nil	A
<i>A. terreus</i>	15.0	29.5	nil	A
<i>Cladosporium herbarum</i>	18.0	26.5	7.5	D
<i>Drechslera hawaiiensis</i>	10.0	25.0	nil	A
<i>Paecilomyces</i> sp.	21.5	30.0	nil	A
<i>Penicillium digitatum</i>	20.0	30.0	nil	A
<i>P. funiculosum</i>	25.0	25.0	nil	A
<i>Rhizoctonia solani</i>	16.0	27.5	nil	A
<i>Trichoderma hamatum</i>	21.0	23.5	nil	B _{ii}
<i>F. oxysporum</i>				
<i>Alternaria alternata</i>	12.3	17.0	3.3	D
<i>Aspergillus flavus</i>	8.3	14.7	1.7	C
<i>A. niger</i>	2.7	16.7	8.7	D
<i>A. terreus</i>	8.0	12.0	10.3	D
<i>Cladosporium herbarum</i>	6.7	14.3	13.3	D
<i>Drechslera hawaiiensis</i>	9.3	16.3	99.7	D
<i>Paecilomyces</i> sp.	15.0	15.0	5.0	D
<i>Penicillium digitatum</i>	8.0	18.0	4.0	D
<i>P. funiculosum</i>	6.0	15.5	2.0	C
<i>Rhizoctonia solani</i>	8.0	13.0	nil	A
<i>Trichoderma hamatum</i>	2.7	9.3	nil	B _{ii}
<i>F. proliferatum</i>				
<i>Alternaria alternata</i>	11.0	29.3	nil	A
<i>Aspergillus flavus</i>	15.3	30.0	nil	A
<i>A. niger</i>	15.5	29.0	1.5	C
<i>A. terreus</i>	18.7	28.7	1.0	C
<i>Cladosporium herbarum</i>	51.0	29.3	nil	B _i
<i>Drechslera hawaiiensis</i>	15.0	29.0	nil	A
<i>Paecilomyces</i> sp.	17.0	27.0	nil	A
<i>Penicillium digitatum</i>	26.3	32.3	2.0	C
<i>P. funiculosum</i>	25.0	28.3	nil	A
<i>Rhizoctonia solani</i>	17.3	25.7	nil	A
<i>Trichoderma hamatum</i>	8.0	19.0	nil	B _{ii}
<i>F. scirpi</i>				
<i>Alternaria alternata</i>	18.3	28.3	nil	B _i
<i>Aspergillus flavus</i>	15.3	34.3	nil	A
<i>A. niger</i>	11.0	32.0	nil	A
<i>A. terreus</i>	20.3	26.7	2.0	C
<i>Cladosporium herbarum</i>	20.0	30.0	nil	B _i
<i>Drechslera hawaiiensis</i>	15.0	25.7	nil	A
<i>Paecilomyces</i> sp.	15.7	26.0	nil	A
<i>Penicillium digitatum</i>	22.3	27.0	nil	A
<i>P. funiculosum</i>	21.7	25.0	nil	A
<i>Rhizoctonia solani</i>	20.0	27.5	nil	A
<i>Trichoderma hamatum</i>	22.0	25.0	nil	B _{ii}

Table 1. (Cont'd.)

Test fungi	Radius		Zone of inhibition	Type of reaction
	r ₁	r ₂	r ₁	r ₂
<i>F. pallidoroseum</i>				
<i>Alternaria alternata</i>	16.0	28.0	nil	A
<i>Aspergillus flavus</i>	16.5	21.0	1.8	C
<i>A. niger</i>	15.5	20.0	10.5	D
<i>A. terreus</i>	10.5	13.0	12.5	D
<i>Cladosporium herbarum</i>	11.5	16.0	19.8	D
<i>Drechslera hawaiiensis</i>	16.5	21.5	10.0	D
<i>Paecilomyces</i> sp.	12.0	18.0	2.0	C
<i>Penicillium digitatum</i>	13.5	18.0	19.5	D
<i>P. funiculosum</i>	10.5	14.5	26.5	D
<i>Rhizoctonia solani</i>	11.0	12.0	14.5	D
<i>Trichoderma hamatum</i>	14.0	19.5	nil	B _{ii}
<i>F. solani</i>				
<i>Alternaria alternata</i>	16.7	29.7	nil	A
<i>Aspergillus flavus</i>	12.3	30.0	nil	A
<i>A. niger</i>	13.0	28.5	9.0	D
<i>A. terreus</i>	16.0	27.7	2.0	C
<i>Cladosporium herbarum</i>	24.0	27.0	nil	B _i
<i>Drechslera hawaiiensis</i>	10.0	26.7	nil	A
<i>Paecilomyces</i> sp.	15.3	26.0	2.0	C
<i>Penicillium digitatum</i>	19.0	27.0	nil	B _i
<i>P. funiculosum</i>	22.7	27.0	nil	A
<i>Rhizoctonia solani</i>	20.0	25.5	nil	A
<i>Trichoderma hamatum</i>	15.0	25.5	nil	B _{ii}
<i>F. sporotrichioides</i>				
<i>Alternaria alternata</i>	19.0	21.3	8.0	D
<i>Aspergillus flavus</i>	12.7	16.3	9.0	D
<i>A. niger</i>	11.7	15.3	19.3	D
<i>A. terreus</i>	15.0	13.7	20.7	D
<i>Cladosporium herbarum</i>	15.0	20.3	25.3	D
<i>Drechslera hawaiiensis</i>	20.3	25.0	nil	D
<i>Paecilomyces</i> sp.	8.5	17.5	2.0	C
<i>Penicillium digitatum</i>	3.8	16.0	29.7	D
<i>P. funiculosum</i>	15.7	19.0	17.7	D
<i>Rhizoctonia solani</i>	7.3	11.7	25.3	D
<i>Trichoderma hamatum</i>	13.0	16.7	nil	B _{ii}
<i>F. subglutinans</i>				
<i>Alternaria alternata</i>	24.7	21.7	nil	A
<i>Aspergillus flavus</i>	20.7	30.0	nil	A
<i>A. niger</i>	19.0	32.0	nil	A
<i>A. terreus</i>	22.1	31.0	nil	A
<i>Cladosporium herbarum</i>	46.3	30.3	nil	B _i
<i>Drechslera hawaiiensis</i>	18.0	26.7	nil	A
<i>Paecilomyces</i> sp.	20.0	26.0	nil	A
<i>Penicillium digitatum</i>	30.3	28.7	nil	A
<i>P. funiculosum</i>	60.0	30.0	nil	B
<i>Rhizoctonia solani</i>	18.5	27.5	1.0	C
<i>Trichoderma hamatum</i>	21.0	28.0	nil	B _{ii}

r₁ = Radial growth of pathogenic fungus on opposed side.r₂ = Radial growth of pathogenic fungus on unopposed side.

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