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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. pallidoroseum, 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:

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

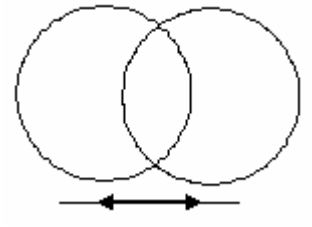
where, r_1 = radial $_{growth}$ of pathogenic fungus on opposed side.

r_2 = radial $_{growth}$ of pathogenic fungus on unopposed side.

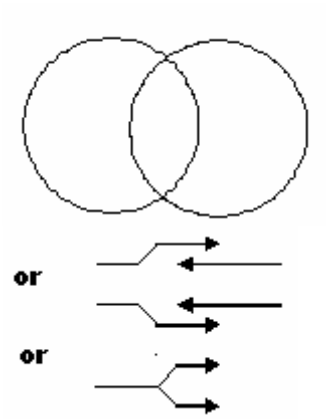
Interactions were assessed using a key based on observations of Porter (1924) and Dickinson & Boardman (1971) as given below:

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.

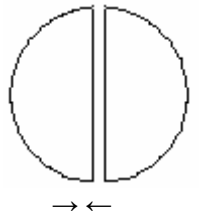
In evaluating interactions, each fungus was assessed for its ability to inhibit growth of another fungus and assessments were made when the fungi had achieved an equilibrium after which there was no further alteration in the growth pattern (Fig. 1).



A. Mutually intermingling growth



B. Overgrowth by antagonist



C. Mutual slight inhibition

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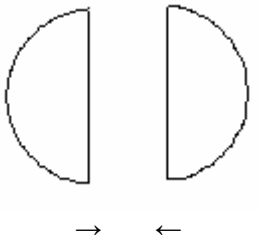

D. Mutual inhibition at a distance

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
	F. equiseti			
Alternaria alternata	25.3	30.3	nil	A
Aspergillus flavus	18.3	27.6	nil	A
A. niger	15.7	31.3	1.0	C
A. terreus	20.0	31.0	2.0	C
Cladosporium herbarum	22.3	26.0	nil	B_i
Drechslera hawaiiensis	19.0	30.7	nil	A
Paecilomyces sp.	19.7	27.7	nil	A
Penicillium digitatum	20.0	31.7	1.0	C
P. funiculosum	21.3	25.7	nil	B_i
Rhizoctonia solani	19.3	28.0	nil	A
Trichoderma hamatum	24.0	25.5	nil	B_i
	F. longipes			
Alternaria alternata	15.0	30.7	nil	B_i
Aspergillus flavus	14.7	31.7	nil	A
A. niger	14.0	30.0	5.0	D
A. terreus	20.0	29.7	nil	A
Cladosporium herbarum	19.5	28.0	nil	B_i
Drechslera hawaiiensis	12.3	28.0	nil	A
Paecilomyces sp.	17.3	28.0	nil	A
Penicillium digitatum	22.0	27.7	nil	A
P. funiculosum	20.7	26.0	nil	A
Rhizoctonia solani	17.5	30.0	nil	A
Trichoderma hamatum	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
			F. moniliforme	
Alternaria alternata	13.0	27.0	nil	A
Aspergillus flavus	15.0	18.0	nil	A
A. niger	10.0	30.5	nil	A
A. terreus	15.0	29.5	nil	A
Cladosporium herbarum	18.0	26.5	7.5	D
Drechslera hawaiiensis	10.0	25.0	nil	A
Paecilomyces sp.	21.5	30.0	nil	A
Penicillium digitatum	20.0	30.0	nil	A
P. funiculosum	25.0	25.0	nil	A
Rhizoctonia solani	16.0	27.5	nil	A
Trichoderma hamatum	21.0	23.5	nil	B_{ii}
			F. oxysporum	
Alternaria alternata	12.3	17.0	3.3	D
Aspergillus flavus	8.3	14.7	1.7	C
A. niger	2.7	16.7	8.7	D
A. terreus	8.0	12.0	10.3	D
Cladosporium herbarum	6.7	14.3	13.3	D
Drechslera hawaiiensis	9.3	16.3	99.7	D
Paecilomyces sp.	15.0	15.0	5.0	D
Penicillium digitatum	8.0	18.0	4.0	D
P. funiculosum	6.0	15.5	2.0	C
Rhizoctonia solani	8.0	13.0	nil	A
Trichoderma hamatum	2.7	9.3	nil	B_{ii}
			F. proliferatum	
Alternaria alternata	11.0	29.3	nil	A
Aspergillus flavus	15.3	30.0	nil	A
A. niger	15.5	29.0	1.5	C
A. terreus	18.7	28.7	1.0	C
Cladosporium herbarum	51.0	29.3	nil	B_i
Drechslera hawaiiensis	15.0	29.0	nil	A
Paecilomyces sp.	17.0	27.0	nil	A
Penicillium digitatum	26.3	32.3	2.0	C
P. funiculosum	25.0	28.3	nil	A
Rhizoctonia solani	17.3	25.7	nil	A
Trichoderma hamatum	8.0	19.0	nil	B_{ii}
			F. scirpi	
Alternaria alternata	18.3	28.3	nil	B_i
Aspergillus flavus	15.3	34.3	nil	A
A. niger	11.0	32.0	nil	A
A. terreus	20.3	26.7	2.0	C
Cladosporium herbarum	20.0	30.0	nil	B_i
Drechslera hawaiiensis	15.0	25.7	nil	A
Paecilomyces sp.	15.7	26.0	nil	A
Penicillium digitatum	22.3	27.0	nil	A
P. funiculosum	21.7	25.0	nil	A
Rhizoctonia solani	20.0	27.5	nil	A
Trichoderma hamatum	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
			F. pallidoroseum	
Alternaria alternata	16.0	28.0	nil	A
Aspergillus flavus	16.5	21.0	1.8	C
A. niger	15.5	20.0	10.5	D
A. terreus	10.5	13.0	12.5	D
Cladosporium herbarum	11.5	16.0	19.8	D
Drechslera hawaiiensis	16.5	21.5	10.0	D
Paecilomyces sp.	12.0	18.0	2.0	C
Penicillium digitatum	13.5	18.0	19.5	D
P. funiculosum	10.5	14.5	26.5	D
Rhizoctonia solani	11.0	12.0	14.5	D
Trichoderma hamatum	14.0	19.5	nil	B_{ii}
			F. solani	
Alternaria alternata	16.7	29.7	nil	A
Aspergillus flavus	12.3	30.0	nil	A
A. niger	13.0	28.5	9.0	D
A. terreus	16.0	27.7	2.0	C
Cladosporium herbarum	24.0	27.0	nil	B_i
Drechslera hawaiiensis	10.0	26.7	nil	A
Paecilomyces sp.	15.3	26.0	2.0	C
Penicillium digitatum	19.0	27.0	nil	B_i
P. funiculosum	22.7	27.0	nil	A
Rhizoctonia solani	20.0	25.5	nil	A
Trichoderma hamatum	15.0	25.5	nil	B_{ii}
			F. sporotrichioides	
Alternaria alternata	19.0	21.3	8.0	D
Aspergillus flavus	12.7	16.3	9.0	D
A. niger	11.7	15.3	19.3	D
A. terreus	15.0	13.7	20.7	D
Cladosporium herbarum	15.0	20.3	25.3	D
Drechslera hawaiiensis	20.3	25.0	nil	D
Paecilomyces sp.	8.5	17.5	2.0	C
Penicillium digitatum	3.8	16.0	29.7	D
P. funiculosum	15.7	19.0	17.7	D
Rhizoctonia solani	7.3	11.7	25.3	D
Trichoderma hamatum	13.0	16.7	nil	B_{ii}
			F. subglutinans	
Alternaria alternata	24.7	21.7	nil	A
Aspergillus flavus	20.7	30.0	nil	A
A. niger	19.0	32.0	nil	A
A. terreus	22.1	31.0	nil	A
Cladosporium herbarum	46.3	30.3	nil	B_i
Drechslera hawaiiensis	18.0	26.7	nil	A
Paecilomyces sp.	20.0	26.0	nil	A
Penicillium digitatum	30.3	28.7	nil	A
P. funiculosum	60.0	30.0	nil	B
Rhizoctonia solani	18.5	27.5	1.0	C
Trichoderma hamatum	21.0	28.0	nil	B_{ii}

r_1 = Radial growth of pathogenic fungus on opposed side.
r_2 = Radial growth of pathogenic fungus on unopposed side.

References

Awuah, R.T. and J.W. Lorbeer. 1991. Methyl bromide and steam treatment of an organic soil for control of *Fusarium* yellows of celery. *Plant Dis*., 75: 123-125.

Benhamou, N., P. Rey, M. Cherif, J. Hockenhull and Y. Trilly. 1997. Treatment with the mycoparasite *Pythium oligandrum* triggers induction of defense related reactions in tomato roots when challenged with *Fusarium oxysporum, f.* sp. *radicis-lycopersici*. *Phytopathol*., 87: 108-122.

Butt, Z.L. and A. Ghaffar. 1972. Inhibition of fungi, actinomycetes and bacteria by *Stachybotrys atra*. Mycopath. *Mycol. Appl*., 34: 196-201.

Dawson, W.A.J., G.L. Bateman, M. Jestoi and A. Rizzo. 2002. Biological control of ear blight of wheat caused by *Fusarium culmorum. Journal of Applied genetics*, 43A: 217-222.

Dickinson, C.H. and F. Boardman. 1971. Physiological studies of some fungi isolated from peat. *Tanns. Brit. Mycol. Soc*., 55: 293-305

Duda, B. 2001. *Trichoderma viride* Pers. ex Gray against Damping-off in Forest Nursery. Bulletin of the Polish Academy of Sciences: Biological Sciences vol. 3.

Ghaffar, A. 1988a. Biological control of sclerotial diseases In: *Biocontrol of plant diseases*. (Eds.): K.G. Mukerji and K.L. Garg. CRC Press Inc.Boca Raton, Florida, USA, pp.153-175

Ghaffar, A. 1988b. Soil-borne Disease Research Centre. Final research report, 1st January 1986-30 June 1988, pp.110, Univ. Karachi, Karachi, Pakistan.

Ghaffar, A. 1992. Achievements in biological control of soil-borne pests and parasites in Pakistan.pp.. 287-293. In: *Proceedings of COMSTECH-NIAB International workshop on agroclimatology pests and parasites and their control*. (Eds.): F.F. Jamil and S.H.M. Naqvi. NIAB, Faisalabad, Pakistan.

Henis, Y.A., A. Ghaffar and R. Baker. 1979. Induction of soil suppressive to *Rhizoctonia solani. Phytopathology*, 69: 1164-1169.

Marois, J.J., D.J. Mitchell and R.M. Sonoda. 1981. Biological control of *Fusarium* crown rot of tomato under field conditions. *Phytopath*., 71: 1257-1260.

Miller, J.D. 1994. Epidemiology of *Fusarium graminearum* diseases of wheat and corn. In: *Mycotoxins in grain: Compounds other than aflatoxin*. (Eds.): J.D. Miller and H.L. Trenholm. Eagan Press, St. Paul, MN.pp.19-36.

Porter, C.L. 1924. Concerning the characters of certain fungi as exhibited by their growth in the presence of other fungi. *Am. J. Bot*., 11: 168-188.

Sharma, S.K. and N.P. Dohroo. 1991. Post –harvest management of rhizome rot (*Fusarium oxysporum* f.sp. *zingiberi* Trujillo) of ginger through chemical and antagonist. *Indian Cocoa Arecanut and Spices Journal*, 14: 150-152.

Simon, A and K. Sivasithamparam. 1989. Pathogen suppression: a case study in biological suppression of *Gaeumannomyces graminis* var. *tritici* in soil. *Soil Biology and Biochemistry*, 21: 331±7.

Waksman, S.A. and E.B. Fred. 1922. A tentative outline of the plate method for determining the number of micro-organisms in the soil. *Soil Sci*., 14: 27-28.

Waksmundzka, A. and S. Mazur. 2001. Polyversum and Chitosan activity against Pathogenic Fungi of Sweet Basil (*Ocimum basilicum* L.). *Bulletin of the Polish Academy of Sciences: Biological Sciences*, vol. 3.

Warcup, J.H. 1950. The soil plate method for isolation of fungi from soil. *Nature*, 166: 117.

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