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In Vitro Effects of Antagonistic Microorganisms on *Fusarium oxysporum* [Schlecht. Emend. Synd & Hans] Infecting *Arachis hypogaea* L.

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Summary

In an attempt to develop biocontrol system for management of *Fusarium* wilt in groundnut, *Trichoderma viride, Trichoderma harzianum,* and *Pseudomonas fluorescens* were evaluated for their antagonistic activity against *Fusarium oxysporum in vitro* .The conidia of *Fusarium oxysporum* were found to be inhibited by all the three antagonistic microorganisms. Among them, highest percent inhibition of conidial germination was brought out by *Trichoderma viride* [89.4%] *followed by Trichoderma harzianum* [85.7%] *and Pseudomonas fluorescens* [83.15%]and inhibition of radial mycelial growth were 86.6%, 84.0%, 60.0% respectively.This inhibition is due to the volatile and non volatile metabolites and cell wall degrading enzymes produced by *Trichoderma spp* . Antagonistic activity of *Pseudomonas spp.*against *Fusarium oxysporum* is mainly due to the antibiotics, Fe-chelating siderophores and hydrogen cyanide.

Key Words: *Arachis hypogaea, Fusarium oxysporum, in vitro* inhibition, *Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens*

Introduction

Arachis hypogaea is an important legume crop in many tropical and sub tropical areas of the world. *Fusarium* is a genus of harmful fungi that cause vascular diseases in plants such as water melon, cucumber, tomato, pepper, musk melon, bean, cotton, and groundnut [1 - 6]. *Trichoderma* spp. are effective in the control of soil/seed-borne fungal diseases in several crop plants [7] including groundnut [8]. Major mechanisms involved in the biocontrol activity of *Trichoderma* spp. were reported to be competition for space and nutrients, production of diffusible and/or volatile and non-volatile antibiotics and hydrolytic enzymes which partially degrade the pathogen cell wall and leads to its parasitization [7]. *Pseudomonas* spp. are effective root colonizers and biocontrol agents by production of antibiotics and other antifungal metabolites hydrogen cyanide and Fe- chelating siderophores [9] There appears to be no report on the effect of *Trichoderma species* and *Pseudomonas fluorescens* on the inhibition of *Fusarium oxysporum* infecting *Arachis hypogaea*. Hence the present study was undertaken to study the inhibitory effects of two *Trichoderma species* and *Pseudomonas fluorescens* on the conidial germination and mycelial growth of *Fusarium oxysporum in vitro.*

Materials and Methods

*Trichoderma viride, Trichoderma harzianum,*and *Pseudomonas fluorescens* were obtained from Institute of Microbial Technology (IMTECH), Chandigarh and were used for the present study. The pathogen *Fusarium oxysporum* was obtained from the infected leaves of *Arachis hypogaea* and was purified by single conidium isolation method. The purified culture was stored in the slants of PSA.

Medium & Growth

Fusarium oxysporum was grown on PSA medium for 30 days and further grown in Czapeks medium for 7 days and filtrate was taken. *Trichoderma viride*, *Trichoderma harzianum and Pseudomonas fluorescens* were grown on Malt Extract agar and ABM Medium simultaneously for 30 days and further grown in Czapeks medium for 7 days and culture filtrate was taken

Conidial Germination Studies

The culture filtrates of biocontrol agents were prepared in six concentrations [0.5 ,1.0,1.5,2.0, 2.5,3.0] using sterile distilled water. Conidial suspension of *Fusarium oxysporum was* added to the different concentrations of the biocontrol agents so as to make the final count adjusted to 8000-12000 conidia/ml with the help of haemocytometer.Conidial germination studies were carried out in cavity slides. Triplicate slides were maintained for each concentration. For control, conidial suspension was added to the sterile water. The slides were incubated in moist chamber at 30°C and conidial germination was observed after 24 h.

Radial Mycelial Growth Studies

The prepared culture filtrates of the biocontrol agents in six concentrations [0.5,1.0,1.5, 2.0,2.5,3.0] were added through a sietz filter to the PSA medium separately. The PSA plates were inoculated by placing 9 mm disc cut from growing tip of 7 days old culture of *Fusarium oxysporum*. The PSA plates without any biocontrol agent served as control. The triplicates were maintained and kept in BOD incubator at

28± 0.2°C for 7 days and the radial growth of pathogen was measured in cm after 7 days.

Percentage of conidial germination and radial mycelial growth studies calculated by the formula of Vincent 1927.

The percentage of Inhibition over control:

I= (C- T) / C x100

Conidial germination Radial mycelial growth

Where I=Inhibition over control Where I= Inhibition of mycelial growth (%)

C=% germination in control C=Growth of pathogen in control

T=% germination in treated T=Growth of pathogen in treatment

Results & Discussion

The effects of antagonistic microorganisms, *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* on the conidial germination and radial mycelial growth of *Fusarium oxysporum* are presented in the table1.

Table1

Treatments	Concentration	Conidial germination (24Hrs) %of conidial germination	%of inhibition over control	Mycelial growth(7days) (cms)	% of inhibition over control
Control		95.0± 0.70	0	7.5± 0.12	0
Trichoderma viride	0.5	27.0± 0.28	71.5	4.5± 0.07	40.0
	1	10.0± 0.21	89.4	1.0± 0.14	86.6
	1.5	11.2± 0.14	88.2	1.4± 0.09	81.3
	2	15.0± 0.07	84.2	2.2± 0.02	70.6
	2.5	13.0± 0.42	86.3	2.0± 0.04	73.3
	3	12.2± 0.35	87.1	1.8± 0.09	76.0
Trichoderma harzianum	0.5	28.8± 0.11	69.6	4.5± 0.04	40.0
	1	24.0± 0.09	74.7	4.0± 0.18	46.6
	1.5	13.5± 0.02	85.7	1.2± 0.14	84.0
	2	16.0± 0.04	83.1	2.0± 0.32	73.3
	2.5	17.5± 0.02	81.5	2.5± 0.09	66.6
	3	15.2± 0.07	84.0	1.7± 0.07	77.3
Pseudomonas fluorescens	0.5	50.0± 0.21	47.3	6.5± 0.09	13.3
	1	46.5± 0.02	51.05	6.0± 0.14	20.0
	1.5	18.0± 0.2 1	81.05	4.5± 0.16	40.0
	2	16.0± 0.42	83.15	3.0± 0.07	60.0
	2.5	22.3± 0.21	76.52	5.2 ± 0.14	30.6
	3	20.6±0.07	78.31	4.7± 0.04	37.3

The results obtained, reveal that the maximum inhibition of conidial germination of *Fusarium oxysporum* was brought out by 1 % *Trichoderma viride* [89.4%], 1.5% *Trichoderma harzianum* [85.7%], and 2% *Pseudomonas fluorescens* [83.15 %]and the highest inhibition of radial mycelial growth of pathogen was effected by 1% *Trichoderma viride* [86.6], 1.5% *Trichoderma harzianum* [84.0], 2% *Pseudomonas fluorescens* [60.0%]

Discussion

All concentrations of culture filtrate of *Trichoderma* species and *Pseudomonas fluorescens* were found to inhibit conidial germination and mycelial growth of F*usarium oxysporum* . However, 1% conc. of *T.viride* brought out the maximum inhibition while 1.5% conc. of culture filtrate of *T. harzianum* and 2% *Pseudomonas fluorescens* showed maximum inhibition. All the conc. of *T. species* and *P. fluorescens* inhibited the germination of *Fusarium oxysporum* in the range of 71.5% to 89.4% over control. Mycelial growth showed greater inhibition when treated with culture filtrate of *Trichoderma spp* compared to that of *Pseudomonas fluorescens.*

Inhibition of colony growth of *F.oxysporum* was earlier reported [10]. Fakhrunnisa et al. [11] confirmed that *T. harzianum* inhibited radial growth of *F. oxysporum* to the extent of 79.97%.

Inhibition of germination and mycelial growth of pathogenic fungi *in vitro* was attributed to the antifungal properties of volatile compounds (alkyl pyrones) produced by *T. harzianum* [12]. Considerable reduction in the biomass and synthesis of DNA, RNA and protein of *Colletotrichum capsici* were obtained in the treatments with NVAC (non volatile antibiotic extracted in chloroform [13]. Role of diffusible volatile compounds produced by *T. viride and T. harzianum* in the inhibition of germination and mycelial growth of *Fusarium oxysporum in vitro* was reported [14,15]. Extra cellular lytic enzymes produced by *Trichoderma* species were found to inhibit certain fungal pathogens. The inhibitory potential of *Trichoderma species* in the present study may be due to the volatile compounds and non-volatile antibiotics and extra cellular enzymes, as reported earlier.

In vitro inhibition by *Pseudomonas fluorescens* might be due to the production of Fe- chelating siderophores and hydrogen cyanide which is toxic to pathogenic fungi [16].

Conclusion

Volatile and non-volatile compounds and hydrolytic enzymes produced by *Trichoderma species* and antibiotics, Fe- chelating siderophores and hydrogen cyanide produced by *Pseudomonas fluorescens* inhibited the conidial germination and mycelial growth of *Fusarium oxysporum* infecting *Arachis hypogaea*.

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