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IN VITRO EVALUATION OF MICROBIAL ANTAGONISTS AGAINST *SCLEROTIUM ROLFSII*

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Abstract

Trichoderma harzianum and *T. longibrachiatum* were found to inhibit the *in vitro* growth of *S. rolfsii* and produced coiling around mycelium of *S. rolfsii* resulting in lysis of hyphae. *T. pseudokoningii, T. polysporum* and *Gliocladium virens* also inhibited the growth of *S. rolfsii*. Where *Aspergillus niger, A. fumigatus, A. flavus, A. terreus, A. nidulans, A. sulphureus, A. parasiticus, A. tamarii, A. versicolar, A. versiclar* and *A. wentii* were used, colonies of *Aspergillus* spp., and *S. rolfsii* met each other but *S. rolfsii* later overgrew the colonies of *Aspergillus* spp.

Introduction

Sclerotium rolfsii Sacc., is an economically important pathogen in warm, moist climate worldwide, causing disease on more than 500 species of plants (Aycock, 1966). Ahmed *et al.*, (1984) made the first report of *S. rolfsii* from Pakistan on maize (*Zea mays*). The fungus was subsequently reported from oat (*Avena sativa*) and mash bean (*Vigna mungo*) by Shahzad & Ghaffar (1995), apple (*Malus sylvestris*) by Jahangir *et al*., (1995), lentil (*Lens culinaris*) by Iqbal *et al*., (1995) and seed of sugarbeet (*Beta vulgaris*) by Ruqia (2001).

Of the various methods used to control plant diseases, use of chemical fungicides is very common and very effective. However in view of the complexities arising from the use of chemical pesticides, such as harmful effect on environment and non-target organisms including man, domestic animals, beneficial insects, wild life, the use of microorganism as biocontrol agents has provided a very promising alternative and less hazardous method for plant disease control. *In vitro* interaction of *S. rolfsii* with microorganisms was therefore, evaluated to find effective antagonists against *S. rolfsii*.

Materials and Methods

Microorganisms used: Cultures of antagonists used in the present studies were obtained from Karachi University culture collection (KUCC) or isolated from soil using serial dilution technique (Waksman & Fred, 1922) where 0.1% water agar was used instead of distilled water. Isolate of *S. rolfsii* used in the present studies was isolated from sugar-beet seed by Ruqia (2001).

***In vitro* interaction of *S. rolfsii* with microorganisms:** In dual culture plate assay, a 5 mm diam., inoculum disc of *S. rolfsii* was placed near the edge of a Petridish containing Potato Sucrose Agar (PSA) medium. A similar inoculum disc of a test fungus was placed at the opposite end of the Petri dish. There were 6 replicates of each treatment. Plates

Table 1. Effect of different antagonists on *in vitro* growth of *S. rolfsii*.

S. No.	Microorganisms	Days of incubation	Diameter of test fungi (mm)	Diameter of pathogen (mm)	Type of interaction
1.	*Aspergillus flavus*	3	60	30	A
2.	*A. fumigatus*	3	43	45	A
3.	*A. nidulans*	3	45	35	A
4.	*A. niger*	3	45	35	A
5.	*A. parasiticus*	3	45	35	A
6.	*A. sulphureus*	3	45	43	A
7.	*A. tamarii*	3	40	50	A
8.	*A. versicolor*	3	60	30	A
9.	*A. terreus*	3	70	20	A
10.	*A. wentii*	3	50	40	A
11.	*Alternaria alternata*	4	20	70	D
12.	*Lasiodiplodia theobromae*	3	35	50	D
13.	*Chaetomium globosum*	4	43	45	D
14.	*Curvularia lunata*	4	35	50	D
15.	*Drechslera australiensis*	4	35	50	D
16.	*Fusarium solani*	3	26	35	D
17.	*F. semitectum*	4	30	45	D
18.	*Gliocladium virens*	4	35	50	C
19.	*Macrophomina phaseolina*	3	43	45	D
20.	*Myrothecium* sp.	3	15	75	D
21.	*Penicillium* sp.	3	40	45	D
22.	*Pythium aphanidermatum*	4	40	35	D
23.	*Rhizoctonia solani*	4	30	60	D
24.	*Stachybotrys atra*	4	55	40	D
25.	*Trichoderma harzianum*	4	45	40	B
26.	*T. longibrachiatum*	3	55	35	B
27.	*T. pseudokoningii*	3	53	37	C
28.	*T. polysporum*	3	50	40	C

were incubated at 25°C, colony diameter of the pathogen and the test organism were recorded. The following types of interaction were recorded:

A: Colonies of test organism and pathogen met each other; The Pathogen overgrew the colony of test organism
B: Growth of pathogen inhibited; Test fungus produced coiling around mycelium of the pathogen
C: Colonies of test organism and pathogen met each other; No further growth of either the pathogen or the test fungus was observed
D: Colonies of pathogen and test fungi intermingled.

Results and Discussion

Of the microorganisms tested, only *Trichoderma harzianum* and *T. longibrachiatum* showed inhibition in growth of *S. rolfsii* and produced coiling around its mycelium. *T. pseudokoningii, T. polysporum* and *Gliocladium virens* also inhibited the growth of *S. rolfsii* but no further growth and coiling were observed. (Table 1). Species of *Aspergillus viz., A. niger, A. flavus. A. fumigatus. A. wentii, A. sulphurus, A. tamarii. A nidulans, A.*

terreus, A. parasiticus and *A. versicolor* showed 'A' type of interaction with *S. rolfsii* where the pathogen inhibited the growth of *Aspergillus* species and overgrew the colonies of test organisms. *Alternaria alternata, Lasiodiplodia theobromae, Chaetomium globosum, Curvularia lunata, Drechslera australiensis, Fusarium solani, F. semitectum, Macrophomina phaseolina, Myrothecium sp., Penicillium sp., Pythium aphanidermatum, Rhizoctonia solani, Stachybotrys atra* showed type 'D' interaction where growth of the pathogen and test organisms were intermingled (Table 1).

There are reports where the growth of *S. rolfsii* was inhibited by *T. harzianum* (Henis, 1984; Jomduang, 1995), *T. koningii* (Latunde-Dada, 1993), *T. longibrachiatum* (Sreenivasaprasad & Manibhushanrao, 1990) and *G. virens* (Sreenivasaprasad & Manibhushanrao, 1990; Mukherjee & Raghu 1997; Jomduang, 1995). Formulated *T. ressei, T. harzianum* and *T. koningii* effectively controlled *S. rolfsii* of pine seedling (Widyastuti *et al*., 2003). Similarly, use of *Trichoderma* species effectively reduced the viability of sclerotia of *S. rolfsii* (Artigues and Davet, 1984; Khattabi, *et al*., 2001) provided good control of damping-off of beans caused by *S. rolfsii* in the greenhouse (Henis, 1984). No previous report of antagonistic activity of *T. polysporum* and *T. pseudokoningii* against *S. rolfsii* is available. Use of *T. harzianum* and *T. longibrachiatum, T. pseudokoningii, T. polysporum* and *G. virens* in the control of *S. rolfsii* needs further elucidation.

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