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Detection of Presumptive Mycoparasites in Soil Placed on Host-Colonized Agar Plates in Riyadh Region, Saudi Arabia

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Abstract: The presumptive mycoparasites *Trichoderma* sp., *Pythium* sp., *Gliocladium* sp. and *Verticillium* sp. were detected in 42 (51.2%), 2 (2.5%), 23 (28.4%) and 1 (1.2%) of a total 81 soils, respectively, when soil samples were placed on sectors of potato dextrose agar colonized by the appropriate host fungi. Most (81) of the soils in the study contained three or more mycoparasities, but the frequency of detection on replicate host sectors suggested that *Trichoderma* sp. and *Gliocladium* sp. were the more abundant species in all soils in which they occurred. The type of host fungus markedly influenced the efficiency of detection of the different mycoparasities: *Fusarium* sp. was most efficient for *Trichoderma* sp. and *Gliocladium* sp. and *Rhizoctonia solani* for *Trichoderma* sp. Only a single host was suitable for consistent detection of each of the mycoparasite species.

Key words: *Trichoderma* sp., *Gliocladium* sp., mycoparasite, Saudi Arabia

INTRODUCTION

Soil-borne pathogens produce serious to the yield of losses in different plant groups (Iftikhar *et al*., 2003). Micro-organisms that colonized in the rhizosphere were classified according to their effects on plants, some of these micro-organisms considered plant pathogens, whereas others trigger beneficial effects (Mantellin and Touraine, 2004). Fungi with antagonistic activity toward plant pathogens had an essential role in plant growth and health. A plant and site dependent specificity of the composition of antagonistic morphotypes and their genotypic diversity was found by Berg *et al*. (2005).

Mycoparasites and presumptive mycoparasites have biocontrol potential some are responsible for natural suppressiveness of soils to certain plant pathogens, examples being *Pythium nunn* in *Pythium*-suppressive soils (Lifshitz *et al*., 1984; Martin and Hancock, 1986) and *Trichoderma hamatum* in *Rhizoctonia* suppressive soils (Liu and Baker, 1980). Many studies showed that root colonization by specific fungi was found recently gave increases in plant growth, yield or a measurable control of known root pathogens (Cook, 1993; Cook and Baker, 1983; Linderman, 1986). *Fusarium* species were economically important as pathogens on many agricultural, horticultural and field crops grown in the world. Chet (1987) showed that several species of *Trichoderma* were used successfully against certain pathogenic fungi. Similarly, Harman (2000) showed that *Trichoderma* sp. was used as commercial bio-fungicides to control a range of economically important soil-borne fungal plant pathogens. The objective of this study was to isolate the mycoparasitic fungi from soils of the region, Saudi Arabia, that might be used as biological control agents as an alternative to chemical control methods.

MATERIALS AND METHODS

This study was conducted in 2004-2005 at the Plant Protection Department, College of Food and Agriculture Science, King Saud University, Riyadh, Saudi Arabia.

Isolation of the Host Fungi

Initial studies (not described) involved comparisons of many potential host fungi which were isolated from different host plants. Then 6 hosts were selected to isolate the mycoparasities populations in 81 soils. These hosts were *Fusarium proliferatum*, *Fusarium* sp., *Botrytis cinerea*, *Sclerotinia* sp., *Rhizoctonia solani* and *Pythium* sp. Identification of the different types of developed fungal isolates colonies were carried out and identified based on microscopic morphologies according to Ellis (1971), Kirk and Ansell (1992) and Samson and Pitt (2000).

Isolation of Mycoparasites from Soil

Eighty one soils were collected from arable fields (47), garden sites (19) and woodlands (15) in the Riyadh region of Saudi Arabia (Table 1, 2). Each sample was bulked from usually 2 or 3

Table 1: Occurrence of different mycoparasites in 81 soils as determined by detection on agar previously colonized by appropriate host fungi in Al-Dereia, Al- Oiaina and Al Wasil wa Al Amaria regions, Saudi Arabia

			Mycoparasites			
Soil	Location	Cropping/ vegetation	*Trichoderma* sp.	*Pythium* sp.	*Gliocladium* sp.	*Verticillium* sp.
	Al-Dereia					
1	Farm 1	Arable	+	+	-	-
2	Farm 2	Arable	+	-	-	-
3	Farm 3	Woodland	-	-	+	-
4	Farm 4	Arable	+	-	-	-
5	Farm 5	Arable	+	-	-	-
6	Farm 6	Woodland	+	-	-	-
7	Farm 7	Woodland	+	-	-	-
8	Farm 8	Garden	-	-	+	-
9	Farm 9	Arable	-	-	+	-
10	Farm 10	Arable	+	-	-	-
	Al-Oeuina					
11	Farm 1	Woodland	+	-	-	-
12	Farm 2	Garden	-	-	-	-
13	Farm 3	Woodland	+	-	+	-
14	Farm 4	Woodland	+	-	-	-
15	Farm 5	Arable	+	-	-	-
16	Farm 6	Arable	-	-	-	-
17	Farm 7	Arable	-	-	-	-
18	Farm 8	Arable	-	-	-	-
19	Farm 9	Arable	-	-	+	-
20	Farm 10	Arable	-	-	-	-
21	Farm 11	Arable	-	-	-	-
22	Farm 12	Arable	-	-	-	-
23	Farm 13	Arable	-	-	+	-
24	Farm 14	Arable	-	-	-	-
25	Farm 15	Arable	+	-	+	-
	Al-Wasil					
26	Farm 1	Arable	+	-	-	-
27	Farm 2	Arable	+	-	-	-
28	Farm 3	Arable	+	-	-	-
29	Farm 4	Arable	-	-	+	-
30	Farm 5	Arable	-	-	-	-
31	Farm 6	Arable	-	-	+	-
32	Farm 7	Arable	-	-	+	-
33	Farm 8	Arable	-	-	-	-
34	Farm 9	Arable	+	-	-	-
35	Farm 10	Arable	+	-	-	-
36	Farm 11	Arable	-	-	+	-
37	Farm 12	Arable	+	-	-	-
38	Farm 13	Arable	-	-	-	-
39	Farm 14	Arable	-	-	+	-
40	Farm 15	Arable	-	-	+	-
41	Farm 16	Arable	+	-	-	-
42	Farm 17	Arable	+	-	-	-
Total occurrence			20	1	13	-

+: Present; -: Absent

Table 2: Occurrence of different mycoparasites in 81 soils as determined by detection on agar previously colonized by appropriate host fungi in Al- Kharj, Dayrab and Wadi Hanifa regions, Saudi Arabia

Soil	Location	Cropping/ vegetation	Mycoparasites			
			Trichoderma sp.	*Pythium* sp.	*Gliocladium* sp.	*Verticillium* sp.
	Al-Kharj					
1	Farm 1	Woodland	-	-	+	+
2	Farm 2	Woodland	+	-	-	-
3	Farm 3	Woodland	+	-	+	-
4	Farm 4	Garden	+	-	-	-
5	Farm 5	Woodland	-	-	+	-
6	Farm 6	Arable	+	-	-	-
7	Farm 7	Arable	-	-	-	-
8	Farm 8	Arable	+	-	-	-
9	Farm 9	Arable	+	-	-	-
10	Farm 10	Arable	-	-	+	-
11	Farm 11	Arable	-	+	-	-
12	Farm 12	Arable	+	-	-	-
13	Farm 13	Arable	+	-	-	-
	Dayrab					
14	Farm 1	Woodland	+	-	-	-
15	Farm 2	Woodland	-	-	-	-
16	Farm 3	Garden	-	-	+	-
17	Farm 4	Garden	+	-	-	-
18	Farm 5	Garden	+	-	-	-
19	Farm 6	Woodland	+	-	-	-
20	Farm 7	Garden	+	-	-	-
21	Farm 8	Garden	-	-	-	-
22	Farm 9	Garden	-	-	-	-
23	Farm 10	Garden	-	-	-	-
24	Farm 11	Garden	+	-	+	-
	Wadi Hanifa					
25	Farm 1	Garden	-	-	+	-
26	Farm 2	Garden	+	-	-	-
27	Farm 3	Garden	-	-	-	-
28	Farm 4	Garden	+	-	-	-
29	Farm 5	Woodland	-	-	-	-
30	Farm 6	Woodland	-	-	-	-
31	Farm 7	Arable	+	-	+	-
32	Farm 8	Arable	-	-	-	-
33	Farm 9	Arable	+	-	-	-
34	Farm 10	Garden	+	-	-	-
35	Farm 11	Garden	-	-	+	-
36	Farm 12	Garden	+	-	-	-
37	Farm 13	Garden	+	-	-	-
38	Farm 14	Arable	-	-	+	-
39	Farm 15	Arable	+	-	-	-
Total occurrence			22	1	10	1

+: Present; -: Absent

subsamples which mixed thoroughly and stored for up to 1 week in a polyethylene bags at room temperature. Plates of Potato-Dextrose Agar (PDA, 15 mL per 9 cm diam. plate) were inoculated in the centrally with the different host fungi and incubated at 25°C for different time periods, until the colony margin just reached the edge of the plate. Then, each agar was cut into 6 equal sectors, which were placed separately in sterilized plastic petri dishes. A sample of soil (0.4 mL, ca. 0.4 g), was placed on the oldest part of each host fungal sector (Mullingan and Deacon, 1992). The sectors were incubated again at 25°C and examined after 7, 14 and 21 days of incubation. Mycoparasities were detected by the presence of sporulation or other fungal structures on the host colony. Identification were confirmed with pure cultures, obtained by subculturing of the mycoparasities from representative host sectors onto plates of fresh PDA. The host sectors were selected at randomized, so for any one soil they were usually from different colonies.

RESULTS AND DISCUSSION

The presumptive mycoparasities *Trichoderma* sp., *Pythium* sp., *Gliocladium* sp. and *Verticillium* sp. were detected in 42, 2, 23 and 1 of a total 81 soils, respectively of a total 81 soils when samples were placed on sectors of agar colonized by the appropriate host fungi (Table 1, 2). *Trichoderma* sp. and *Gliocladium* sp. were the more abundant species in all soils in which they occurred for the soils. Of the 6 hosts used, *Fusarium* sp. was more efficient for *Trichoderma* sp., *Rhizoctonia solani* for *Trichoderma* sp., *Fusarium* sp., for *Gliocladium* sp. Mullingan and Deacon (1992) reported that, all earlier studies researchers have used a single host to identify presumptive mycoparasites and thus probably underestimated the presence of mycoparasities in soils (Foley and Deacon, 1985). Also, mycoparasites could be detected and identified to at least generic level without the need for subculturing (Mullingan and Deacon, 1992). *Fusarium* sp., *Botrytis cinerea* and *Rhizoctonia solani* were gave different patterns of detection of mycoparasities (Table 3). *Trichoderma* sp. and *Gliocladium* sp., were detected on all areas and 2 hosts. *Trichoderma* sp. was seen mostly on all hosts except *Pythium* sp., *Verticillium* sp. was seen only on *Rhizoctonia solani*. Comparison of Table 1 and 2 shows that *Pythium* sp. was reported only in Al-Dereia and Al- Kharj regions for one time. Only a single host was suitable for consistent detection of one single mycoparasitie. These results cannot be ascribed to chance, because in several soils a mycoparasities that was not detected. Table 3 shows that the greatest likelihood of detecting all the mycoparasities in any one soil was by the use of *Fusarium* sp., for *Trichoderma* sp., *Rhizoctonia solani* for *Trichoderma* sp., *Fusarium* sp., for *Gliocladium* sp. The hosts in this study were selected for rapid, uniform growth on PDA and for the known abilities of mycoparasities to grow across them in culture (Laing and Deacon, 1991). Theses abilities were precisely matched by the results of the soil survey. The hosts for future surveys could be selected on this basis, or specific pathogens could be used to detected potential biocontrol agents (Van den Boogert *et al*., 1990). But the best host for detection of a mycoparasities may not be the best for its isolation into pure culture. *Trichoderma* was detected in all 42 soils, *Gliocladium* sp. in
23 soils, *Pytuhium* sp. in 2 soils and *Verticillium* sp. in one soil (Table 3). Moreover, all soils had more than one mycoparasities: 8 soils contained three types, 33 contained 3 types and 4 contained 1 type.

Table 3: Incidence of detection of different mycoparasites in soils samples were placed on agar colonized by different host fungi

Mycoparasites	Host	No. soils samples in which mycoparasites was detected
Trichoderma sp.	*Fusarium proliferatum*	4
	Fusarium sp.	18
	Botrytis cinerea	4
	Sclerotinia sp.	4
	Rhizoctonia solani	12
	Pythium sp.	0
Pythium sp.	*Fusarium proliferatum*	1
	Fusarium sp.	1
	Botrytis cinerea	0
	Sclerotinia sp.	0
	Rhizoctonia solani	0
	Pythium sp.	0
Gliocladium sp.	*Fusarium proliferatum*	3
	Fusarium sp.	11
	Botrytis cinerea	0
	Sclerotinia sp.	0
	Rhizoctonia solani	8
	Pythium sp.	1
Verticillium sp.	*Fusarium proliferatum*	0
	Fusarium sp.	0
	Botrytis cinerea	0
	Sclerotinia sp.	0
	Rhizoctonia solani	1
	Pythium sp.	0

As an estimate of the abundance of each mycoparasities in each soil, Table 1 and 2 shows, the cases in which a mycoparasite was detected on at least half of the replicate sectors of any 1 host. Overall, *Trichoderma* sp. was detected in 51% of the samples of soil placed on host colonies and *Gliocladium* sp. in 28% of all samples. In present study, only some of previously reported soil-borne mycoparasites were detected (Deacon and Henry, 1978; Foley and Deacon, 1985). Further study, with different hosts and a broader geographical or ecological rang of sites should resolve these issues.

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