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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

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### 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.

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### 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 mycoparasites 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- Oaina and Al Wasil wa Al Amaria regions, Saudi Arabia

| Soil                    | Location | Cropping/<br>vegetation | Mycoparasites          |                    |                        |                         |
|-------------------------|----------|-------------------------|------------------------|--------------------|------------------------|-------------------------|
|                         |          |                         | <i>Trichoderma</i> sp. | <i>Pythium</i> sp. | <i>Gliocladium</i> sp. | <i>Verticillium</i> sp. |
| <b>Al-Dereia</b>        |          |                         |                        |                    |                        |                         |
| 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                  | +                      | -                  | -                      | -                       |
| <b>Al-Oeina</b>         |          |                         |                        |                    |                        |                         |
| 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                  | +                      | -                  | +                      | -                       |
| <b>Al-Wasil</b>         |          |                         |                        |                    |                        |                         |
| 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                  | +                      | -                  | -                      | -                       |
| <b>Total occurrence</b> |          |                         | <b>20</b>              | <b>1</b>           | <b>13</b>              | <b>-</b>                |

+: 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/<br>vegetation | Mycoparasites          |                    |                        |                         |
|-------------------------|----------|-------------------------|------------------------|--------------------|------------------------|-------------------------|
|                         |          |                         | <i>Trichoderma</i> sp. | <i>Pythium</i> sp. | <i>Gliocladium</i> sp. | <i>Verticillium</i> sp. |
| <b>Al-Kharj</b>         |          |                         |                        |                    |                        |                         |
| 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                  | +                      | -                  | -                      | -                       |
| <b>Dayrab</b>           |          |                         |                        |                    |                        |                         |
| 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                  | +                      | -                  | +                      | -                       |
| <b>Wadi Hanifa</b>      |          |                         |                        |                    |                        |                         |
| 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                  | +                      | -                  | -                      | -                       |
| <b>Total occurrence</b> |          |                         | <b>22</b>              | <b>1</b>           | <b>10</b>              | <b>1</b>                |

+: 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 (Mulligan 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 mycoparasites *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. Mulligan 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 mycoparasites in soils (Foley and Deacon, 1985). Also, mycoparasites could be detected and identified to at least generic level without the need for subculturing (Mulligan and Deacon, 1992). *Fusarium* sp., *Botrytis cinerea* and *Rhizoctonia solani* were gave different patterns of detection of mycoparasites (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). These 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, *Pythium* 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 |
|-------------------------|------------------------------|---|
| <i>Trichoderma</i> sp.  | <i>Fusarium proliferatum</i> | 4   |
|                         | <i>Fusarium</i> sp.          | 18  |
|                         | <i>Botrytis cinerea</i>      | 4   |
|                         | <i>Sclerotinia</i> sp.       | 4   |
|                         | <i>Rhizoctonia solani</i>    | 12  |
|                         | <i>Pythium</i> sp.           | 0   |
| <i>Pythium</i> sp.      | <i>Fusarium proliferatum</i> | 1   |
|                         | <i>Fusarium</i> sp.          | 1   |
|                         | <i>Botrytis cinerea</i>      | 0   |
|                         | <i>Sclerotinia</i> sp.       | 0   |
|                         | <i>Rhizoctonia solani</i>    | 0   |
|                         | <i>Pythium</i> sp.           | 0   |
| <i>Gliocladium</i> sp.  | <i>Fusarium proliferatum</i> | 3   |
|                         | <i>Fusarium</i> sp.          | 11  |
|                         | <i>Botrytis cinerea</i>      | 0   |
|                         | <i>Sclerotinia</i> sp.       | 0   |
|                         | <i>Rhizoctonia solani</i>    | 8   |
|                         | <i>Pythium</i> sp.           | 1   |
| <i>Verticillium</i> sp. | <i>Fusarium proliferatum</i> | 0   |
|                         | <i>Fusarium</i> sp.          | 0   |
|                         | <i>Botrytis cinerea</i>      | 0   |
|                         | <i>Sclerotinia</i> sp.       | 0   |
|                         | <i>Rhizoctonia solani</i>    | 1   |
|                         | <i>Pythium</i> sp.           | 0   |

As an estimate of the abundance of each mycoparasites 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.

## REFERENCES

- Berg, G., C. Zachow, J. Lottmann, M. Gotz, R. Costa and K. Smalla, 2005. Impact of plant species and site on rhizosphere associated fungi antagonistic to *Verticillium dahliae* Kleb. Applied Environ. Microbiol., 71: 4203-4213.
- Chet, I., 1987. *Trichoderma*-Application, Mode of Action and Potential as a Bio Control Agent of Soil Borne Plant Pathogenic Fungi. In: Innovative Approaches to Plant Disease Control, Chet, I. (Ed.). Wiley/Interscience, New York, pp: 137-160.
- Cook, R.J. and K.F. Baker, 1983. The Nature and Practice of Biological Control of Plant Pathogens. 1st Edn., American Phytopathological Society, St. Paul, MN., pp: 539.
- Cook, R.J., 1993. Making greater use of introduced microorganisms for biological control of plant pathogens. Ann. Rev. Phytopathol., 31: 53-80.
- Deacon, J.W. and C.M. Henry, 1978. Mycoparasitism by *Pythium oligandrum* and *P. acanthicum*. Soil Biol. Biochem., 10: 409-415.
- Ellis, M.B., 1971. Dematiaceus Hyphomycetes. Commonwealth Mycological Institute, Kew. Surry, UK., pp: 608.
- Foley, M.F. and J.W. Deacon, 1985. Isolation of *Pythium oligandrum* and other necrotrophic mycoparasities from soil. Trans. British Mycol. Society, 85: 631-639.
- Harman, G.E., 2000. Myths and dogmas of biocontrol: Changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Dis., 84: 377-393.
- Iftikhar, S., A. Sultan, A. Munir, S. Iram and I. Ahmed, 2003. Fungi associated with rice, wheat cropping system in relation to zero and conventional tillage technologies. J. Biol. Sci., 3: 1076-1083.
- Kirk, P.M. and A.E. Ansell, 1992. Authors of Fungal Names Index of Fungi Supplement. International Mycological Institute, Institute of CAB, Kew. Surry, UK., pp: 95.
- Laing, S.A.K. and J.W. Deacon, 1991. Video microscopical comparison on mycoparasities by *Pythium oligandrum*, *P. nunn* and an unnamed *Pythium* species. Mycol. Res., 95: 469-479.
- Lifshitz, R., B. Sneh and R. Baker, 1984. Soil suppressiveness to *Pythium ultimum* induced by antagonistic *Pythium* species. Phytopathology, 74: 1054-1061.
- Linderman, R.G., 1986. Managing rhizosphere microorganisms in the production of horticultural crops. Hort Science, 21: 1299-1302.
- Liu, S. and R.R. Baker, 1980. Mechanism of biological control in soil suppressive to *Rhizoctonia solani*. Phytopathology, 70: 404-412.
- Mantellin, S. and B. Touraine, 2004. Plant growth-promoting bacteria and nitrate availability: Impacts on root development and nitrate uptake. J. Exper. Bot., 55: 27-34.
- Martin, F.N. and J.G. Hancock, 1986. Association of chemical and biological factors in soil suppressive to *Pythium ultimum*. Phytopathology, 76: 1221-1331.
- Mulligan, D.F.C. and J.W. Deacon, 1992. Detection of presumptive mycoparasites in soil placed on host-colonized agar plates. Mycol. Res., 96: 605-608.
- Samson, R.A. and J.I. Pitt, 2000. Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* Classification. 1st Edn., Harwood Academic Publishers, Amsterdam, pp: 510.
- Van den Boogert, P.H.J.F., G. Jager and H. Velvis, 1990. *Verticillium biguttatum*, an Important Mycoparasite for the Control of *Rhizoctonia solani* in Potato. In: Biological Control of Soil-Borne Plant Pathogens, Homby, D. (Ed.). CAB International, Wallingford, pp: 77-91.