Turk J Biol
35 (2011) 457-462

doi:10.3906/biy-1004-98

In vitro antagonistic activity of fungi isolated from sclerotia on potato tubers against *Rhizoctonia solani*

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Received: 08.04.2010

Abstract: Forty-five fungal isolates were obtained from sclerotia of *Rhizoctonia solani* on potato tubers in Erzurum, Turkey. The interaction between fungal isolates and *R. solani* was studied in dual culture technique. Some fungal isolates affected *R. solani* by antibiosis and/or parasitism. Results of the antagonism tests showed that *Acremonium* sp., *Gliocladium viride*, *Paecilomyces marquandii*, *Paecilomyces sulphurellus*, *Penicillium camemberti*, *Penicillium expansum*, *Penicillium frequentans* (ME-50), *Penicillium nigricans*, *Penicillium olsonii*, *Penicillium phialosporum*, *Sporothrix* sp. (MCY-4), *Sporothrix schenckii*, and *Verticillium dahliae* isolates produced an inhibition zone in front of the *R. solani* colony to a varying degree. *Trichoderma harzianum* isolates were able to overgrow the mycelium of *R. solani*. Physical colony contact was observed between the remaining 21 fungal isolates and *R. solani*. Furthermore, coiling of hyphae of *Acremonium* sp., *Acremonium strictum*, *Gliocladium catenulatum*, *G. viride*, and *T. harzianum* around those of *R. solani* was commonly observed.

Key words: Biocontrol, potato, antibiosis, parasitism, *Rhizoctonia solani*

Patates yumrularındaki sklerotiumlardan izole edilen fungusların *Rhizoctonia solani*'ye in vitro antagonistik etkileri

Özet: Patates yumruları üzerinde bulunan *Rhizoctonia solani*'nin sklerotiumlarından 45 fungal izolat elde edilmiştir. Fungal izolatlar ve *R. solani* arasındaki etkileşim ikili kültür yöntemi ile çalışılmıştır. Bazı fungal izolatlar *R. solani*'ye antibiosis ve/veya parazitizm yolu ile etki yapmıştır. Antagonism test sonuçlarına göre, *Acremonium* sp., *Gliocladium viride*, *Paecilomyces marquandii*, *Paecilomyces sulphurellus*, *Penicillium camemberti*, *Penicillium expansum*, *Penicillium frequentans* (ME-50), *Penicillium nigricans*, *Penicillium olsonii*, *Penicillium phialosporum*, *Sporothrix* sp. (MCY-4), *Sporothrix schenckii* ve *Verticillium dahliae* izolatları ile *R. solani* kolonileri arasında değişen derecelerde inhibisyon zonu oluşmuştur. *Trichoderma harzianum* izolatları ise *R. solani* miselyumunun üzerinde gelişmiştir. Geri kalan 21 izolat ile *R. solani* kolonileri arasında fiziksel koloni teması gerçekleşmiştir. Ayrıca, *Acremonium* sp., *Acremonium strictum*, *Gliocladium catenulatum*, *G. viride* ve *T. harzianum* izolatlarına ait hiflerin *R. solani* hiflerini sarması yaygın olarak gözlenmiştir.

Anahtar sözcükler: Biyokontrol, patates, antibiosis, parazitizm, *Rhizoctonia solani*

Introduction

Rhizoctonia solani Kühn [teleomorph: *Thanatephorus cucumeris* (Frank) Donk.] is an important pathogen responsible for serious damage in many crops including potato (*Solanum tuberosum* L.) (1). *R. solani* causes stem canker and black scurf of potato and occurs in all potato growing areas of the world (2). The pathogen is also found to be widely distributed on the potato plants and tubers in Erzurum province (3-5). Rhizoctonia disease of potato is mainly caused by *R. solani* anastomosis group (AG)-3, but isolates that belong to other AGs, such as AG-2 type 1, AG-2 type 2, AG-4, and AG-5, also infect potato stems and/or tubers (4).

Both soil-borne and tuber-borne inoculum of *R. solani* is important in disease development on potato (6,7). Present chemical and cultural control methods have reduced the soil-borne and tuber-borne inoculum, although research has been directed toward the use of antagonists for biocontrol of *R. solani* on potato. A biocontrol agent may act against pathogens by using one or more of the following mechanisms: competition, antibiosis, and parasitism as well as activating host defense mechanisms (8). In fact, several fungi have been reported to be effective biocontrol agents of *R. soloni* on potato. Among these are species of *Glioclodium* (9-11), *Trichoderma* (9-16), and *Verticillium* (17,18). *Chaetomium olivaceum, Cylindrocarpon destructans, Epicoccum nigrum, Fusarium culmorum, Fusarium moniliforme, Gliocladium viride* (syn. *Gliocladium deliquescens*), *Gliocladium roseum, Penicillium cyclopium, Penicillium nigricans, Trichoderma harzianum*, and *Trichothecium roseum* were frequently isolated from sclerotia of *R. solani* (12). In another study, fungal isolates from the sclerotia of *R. solani* were identified as *Alternaria*, *Aspergillus*, *Cladosporium*, *Coniothyrium*, *Curvularia*, *Gliocladium*, *Fusarium*, *Metarhizium*, *Penicillium*, *Phoma*, *Phytophthora*, and *Trichoderma* genera (19). Additionally, *Verticillium biguttatum* was reported from sclerotia of *R. solani* on potato tubers in Turkey (20).

The objective of the present study was to isolate and identify fungi from sclerotia of *R. solani* on potato tubers, and to determine the efficacy of their potential as biocontrol agents on interaction with *R. solani* in pure culture.

Materials and methods

Isolation and identification of fungal isolates

Potato tubers of cv. Marfona affected with black scurf were obtained from 2 randomly selected potato storage facilities in Erzurum, Turkey, and 100 sclerotia from each sample were divided into 5 sub-samples having 20 sclerotia. Isolation of fungi was studied using a method modified from Chand and Logan (12). Each sub-sample was placed on autoclaved moist coarse sand in petri dishes. After incubation at 20 °C for 4 weeks, the sclerotia of each sub-sample were blended together in 200 mL of sterile distilled water together with agar (2%). Serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) were made and 1 mL aliquots of each dilution were pipetted into 10 petri dishes. Each of them was mixed with 15-20 mL of cooled (40-45 °C) potato dextrose agar (PDA) by gentle swirling. The plates were incubated at 25 °C in the dark for 7 days or longer and then emerging fungi were repeatedly sub-cultured to obtain pure culture. The fungi isolates were identified using cultural and morphological features (21,22). Single-spore isolates were transferred to PDA medium in tubes for preservation at 4 °C.

Dual culture tests

Fungal isolates from sclerotia on potato tubers were tested in a dual culture assay against *R. solani* representing AG-3 (isolate R-99), which was recovered from potato tubers with black scurf. A mycelial disc (4 mm diam.) of each fungal isolate was transferred on fresh PDA, 1 cm from the wall of each petri dish, immediately or after 2-4 days, depending upon the growth rate of the fungus, a 4 mm disc of *R. solani* removed from the colony margin of actively growing cultures on PDA was placed 6 cm away from the disc of fungal isolate in the same petri dish. All petri dishes were incubated in the dark at 25 °C for 7 days or longer according to the growth rate of fungi. The percentage inhibition of radial growth [100 × (r1 – r2)/r1] of *R. solani* and the width of the zone of inhibition between both colonies were recorded as described by Royse and Ries (23). Mode of inhibition was assessed on a scale from 1 to 4, in which 1 = mycelial growth of *R. solani* ceased due to overgrowth of interacting fungus, 2 = partial inhibition of *R. solani* and interacting fungus but both grow slowly over each other, 3 = mutual inhibition at a distance <

2 mm, 4 = growth of *R. solani* inhibited at a distance > 2 mm (12). The mean of the 4 measurements was recorded for each fungus.

Hyphal interaction between fungal isolates and *R. solani*

Hyphal interactions were observed in 5-day-old *R. solani* cultures on 2% water agar (WA) in 9 cm petri dishes, which were inoculated with a 4 mm disc removed from actively growing cultures on PDA of each fungal isolate. Plates were incubated at 25 °C in a sterile humidity chamber (100% r.h.). After 7-14 days, 3 rectangular blocks (about 4 cm × 2 cm) from each plate were cut, mounted on glass slides, and examined for hyphal interaction between fungal isolates and *R. solani* by a phase contrast microscope at 400× magnification.

Results and discussion

Totally, 45 fungal isolates (Table) were obtained from tuber-borne sclerotia of *R. solani* in Erzurum province. They were identified by cultural and morphological characteristics and tested for their potential as biocontrol agents.

The isolates were identified as *Acremonium* sp. (1 isolate), *Acremonium strictum* (1 isolate), *Alternaria alternata* (1 isolate), *Chaetomium* sp. (1 isolate), *Cladosporium cladosporioides* (1 isolate), *Fusarium equiseti* (3 isolates), *Fusarium oxysporum* (1 isolate), *Fusarium solani* (2 isolates), *Fusarium verticillioides* (1 isolate), *Gliocladium catenulatum* (1 isolate), *Gliocladium viride* (2 isolates), *Paecilomyces* sp. (2 isolates), *Paecilomyces marquandii* (2 isolates), *Paecilomyces sulphurellus* (1 isolate), *Penicillium camemberti* (1 isolate), *Penicillium expansum* (7 isolates), *Penicillium frequentans* (2 isolates), *P. nigricans* (1 isolate), *Penicillium olsonii* (1 isolate), *Penicillium phialosporum* (2 isolates), *Plectosporium tabacinum* (5 isolates), *Sporothrix* sp. (2 isolates), *Sporothrix schenckii* (1 isolate), *T. harzianum* (2 isolates), and *Verticillium dahliae* (1 isolate).

All isolates were tested against *R. solani* AG-3 isolate in dual culture. Colony and hyphal interactions of these fungal isolates with *R. solani* on PDA are listed in the Table. Results of the antagonism tests showed that *T. harzianum* isolates were able to overgrow (Mode 1) the mycelium of *R. solani*. Physical colony contact (Mode 2) was observed between the 21 isolates of fungi and *R. solani*. No physical contact was observed between 22 isolates of fungi and *R. solani*; moreover, *Acremonium* sp., *G. viride*, *P. marquandii*, *P. sulphurellus*, *P. camemberti*, *P. expansum*, *P. frequentans* (ME-50), *P. nigricans*, *P. olsonii*, *P. phialosporum*, *Sporothrix* sp. (MCY-4), *S. schenckii*, and *V. dahliae* isolates produced an inhibition zone (Mode 3 and 4) in front of the *R. solani* colony (Table). An inhibition zone was observed, which indicates the presence of fungistatic metabolites secreted by these fungi. Different isolates of the same fungus species showed the same mode of inhibition to the pathogen, except for *P. expansum*, *P. frequentans*, and *P. phialosporum* isolates, which showed different inhibition modes, with interaction modes 3 and 4, 2 and 3, and 3 and 4, respectively.

A biocontrol agent may act against pathogens by using one or more of the following mechanisms: competition, antibiosis, and parasitism as well as activating host defense mechanisms (8). Antagonistic activity by *Penicillium* species against *R. solani* has been observed, and it has been reported in relation to the production of toxic metabolites (12,24-27). According to reports in the literature, all *Penicillium* species in the present study except *P. expansum* and *P. nigricans* are new records as possible antagonists of *R. solani*.

Coiling of hyphae of *Acremonium* sp., *A. strictum*, *G. catenulatum*, *G. viride*, and *T. harzianum* around those of *R. solani* was commonly observed (Table). Of these results, *Acremonium* sp. and *G. viride* also affected *R. solani* by antibiosis and parasitism. *R. solani* can be parasitized by parasites such as *Gliocladium* spp. (11,12,18), *Trichoderma* spp. (11,12,15), and *Verticillium* spp. (11,17,18,20). *A. strictum* is notable for parasitizing *Helminthosporium solani* (28) and *Botrytis cinerea* (29).

Several microorganisms including fungi have been shown to be effective antagonists of *R. solani*. The genera *Glioclodium* and *Trichoderma* contain many mycoparasitic species that are considered good biocontrol agents against soil-borne pathogens (9). Within the genus *Trichoderma*, species such as *T. hamatum*, *T. harzianum, T. reesei*, *T. virens*, and *T. viride* have demonstrated excellent antagonistic activity against *R. solani* on potato pot and/

Table. Colony and hyphal interactions of fungal isolates with *Rhizoctonia solani* in vitro.

Fungal species	Isolate number	Mode of inhibition*	Width of the zone (mm)	Inhibition of *R. solani* (%)	Hyphal interaction[x]
Trichoderma harzianum	MEY-1	1	-	69	+
Trichoderma harzianum	MCY-1	1	-	72	+
Acremonium strictum	MEY-10	2	-	20	+
Alternaria alternata	MCY-2	2	-	29	-
Chaetomium sp.	ME-8	2	-	30	-
Cladosporium cladosporioides	AE-4	2	-	13	-
Fusarium equiseti	MEY-4	2	-	47	-
Fusarium equiseti	AE-9	2	-	48	-
Fusarium equiseti	MC-9	2	-	37	-
Fusarium oxysporum	MEY-6	2	-	35	-
Fusarium solani	AE-8	2	-	42	-
Fusarium solani	AE-7	2	-	40	-
Fusarium verticillioides	MEY-3	2	-	52	-
Gliocladium catenulatum	MEY-2	2	-	37	+
Paecilomyces sp.	MC-1	2	-	22	-
Paecilomyces sp.	AE-3	2	-	50	-
Penicillium frequentans	ME-57	2	-	26	-
Plectosporium tabacinum	MC-10	2	-	33	-
Plectosporium tabacinum	MC-4	2	-	34	-
Plectosporium tabacinum	MC-11	2	-	38	-
Plectosporium tabacinum	MEY-5	2	-	35	-
Plectosporium tabacinum	AE-6	2	-	27	-
Sporothrix sp.	MC-5	2	-	34	-
Paecilomyces marquandii	MEY-8	3	1.0	39	-
Paecilomyces marquandii	MEY-9	3	1.0	37	-
Penicillium expansum	MC-61	3	0.8	17	-
Penicillium frequentans	ME-50	3	0.9	24	-
Penicillium nigricans	AE-62	3	0.5	21	-
Penicillium phialosporum	ME-51	3	1.1	14	-
Acremonium sp.	AE-5	4	3.9	47	+
Gliocladium viride	ME-7	4	2.3	35	+
Gliocladium viride	ME-10	4	2.1	31	+
Paecilomyces sulphurellus	MC-2	4	2.9	21	-
Penicillium camemberti	AE-63	4	6.1	31	-
Penicillium expansum	ME-56	4	5.5	35	-
Penicillium expansum	MC-60	4	5.3	29	-
Penicillium expansum	ME-52	4	3.8	33	-
Penicillium expansum	AE-64	4	7.3	28	-
Penicillium expansum	ME-53	4	12.0	40	-
Penicillium expansum	ME-59	4	7.8	19	-
Penicillium olsonii	ME-58	4	7.8	35	-
Penicillium phialosporum	ME-55	4	8.3	29	-
Sporothrix sp.	MCY-4	4	3.5	32	-
Sporothrix schenckii	MCY-3	4	4.0	25	-
Verticillium dahliae	ME-2	4	2.5	33	-

* 1 = Mycelial growth of *R. solani* ceased due to overgrowth of interacting fungus, 2 = Partial inhibition of *R. solani* and interacting fungus but both grow slowly over each other, 3 = Mutual inhibition at a distance < 2 mm, 4 = Growth of *R. solani* inhibited at a distance > 2 mm (12).

[x] +: Coiling of *R. solani* hyphae, -: No coiling of *R. solani* hyphae.

or field tests (10,12-16). *T. harzianum,* the most commonly studied species, was tested with varying degrees of success against *R. solani* on potato. *G. virens* successfully controlled *R. solani* on potato in greenhouse and field tests (10). Currently, commercial biocontrol products including several *Trichoderma* and *Glioclodium* species are used for the control of soil-borne pathogens (e.g. *R. solani*) in the United States (30,31) and some other countries (31).

In another study (20), *V. biguttatum* was isolated from sclerotia of *R. solani* on potato tubers in Erzurum, and it also significantly reduced the disease severity of *R. solani* on potato sprouts in pot experiments. As a matter of fact, the mycoparasite *V. biguttatum* is an effective biocontrol agent against Rhizoctonia disease of potato in field experiments (17). It was shown to quickly establish itself on the host fungus by colonizing the sclerotia, and killing the moniliod cells (32). This is the first observation except *V. biguttatum* on potential biocontrol agents from sclerotia of *R. solani* on potato tubers in Erzurum province.

Some of the fungal species encountered in this study (e.g. *G. catenulatum*, *G. viride*, *P. expansum*, *P. nigricans*, and *T. harzianum*) have previously been studied as antagonists of *R. solani*. Other species, such as *Acremonium* sp., *A. strictum*, *Paecilomyces marquandii*, *P. sulphurellus*, *Penicillium camemberti*, *P. frequentans* (ME-50), *P. olsonii*, *P. phialosporum*, *Sporothrix* sp. (MCY-4), and *S. schenckii* appear to be candidates for in vivo investigations to check their suitability as biocontrol agents. Other fungi (e.g. *A. alternata*, *Chaetomium* sp., *C. cladosporioides*, *F. equiseti*, *F. oxysporum*, *F. solani*, *F. verticillioides*, *Paecilomyces* sp., *P. frequentans* (ME-57), *P. tabacinum*, and *Sporothrix* sp. (MC-5)) are not antagonists of *R. solani*, and some of them and *V. dahliae* are pathogens on potato.

Acknowledgements

The authors would like to thank the Atatürk University Scientific Research Projects Unit for its financial support. We are also grateful to Professor İsmet Hasenekoğlu (Kazım Karabekir Education Faculty, Atatürk University, 25240 Erzurum, Turkey) for identification of some of the fungi species.

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