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Full Length Research Paper

Biological control of *Rhizoctonia solani* on potato by *Verticillium biguttatum*

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Ten isolates of *Verticillium biguttatum* were obtained from sclerotia of *Rhizoctonia solani* on potato tubers in Erzurum, Turkey. The interaction between *V. biguttatum* and *R. solani* was studied *in vitro* and *in vivo*. *V. biguttatum* isolates affected *R. solani* by antibiosis and parasitism. All isolates of *V. biguttatum* inhibited the growth of *R. solani* colony. After the coiling around of *R. solani* hyphae, *V. biguttatum* hyphae penetrated host cell walls and grew within the hyphae. Viability of hyphae and sclerotia of *R. solani* was reduced by *V. biguttatum* isolates. *V. biguttatum* also significantly reduced the disease severity of *R. solani* on potato sprouts in pot experiments. This is the first report of *V. biguttatum* from sclerotia of *R. solani* in Turkey.

Key words: Bio-control, potato, *Rhizoctonia solani*, *Verticillium biguttatum*.

INTRODUCTION

Rhizoctonia solani Kühn [teleomorph: *Thanatephorus cucumeris* (Frank) Donk.] is an important fungal pathogen that causes stem canker and black scurf of potato (*Solanum tuberosum* L.), and widespread in all potato growing areas of the world (Frank, 1986). Rhizoctonia disease of potato is mainly caused by *R. solani* anastomosis group 3 (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 tubers (Demirci and Döken, 1993). Both soilborne and tuberborne inoculum of *R. solani* is important in disease development on potato (Frank and Leach, 1980; Demirci and Eken, 1995).

R. solani can be parasitized by mycoparasites such as *Gliocladium* spp., *Trichoderma* spp. and *Verticillium biguttatum* Gams (Van den Boogert, 1996). The fungus *V. biguttatum* is a mycoparasite with biological activity against the important plant pathogen *R. solani.* The biotrophic mycoparasite *V. biguttatum* was first isolated from sclerotia of *R. solani* on potato tubers in the Netherlands (Jager et al., 1979). This fungus is able to destroy sclerotia (Velvis and Jager, 1983; Jager and Velvis, 1988) and hyphae (Van den Boogert and Deacon, 1994) of *R. solani*. When applied to potato tubers as a spore suspension, mycoparasite *V. biguttatum* has the ability to suppress stem canker and black scurf of potato (Velvis and Jager, 1983; Jager and Velvis, 1986; Jager et al., 1991; Van den Boogert and Velvis, 1992; Van den Boogert and Luttikholt, 2004).

The objective of this investigation was to determine the efficacy of *V. biguttatum* in the biological control of Rhizoctonia disease of potato. Specific objectives were to determine the effect of *V. biguttatum* on viability of hyphae and sclerotia of *R. solani,* and on disease severity causing by the pathogen.

MATERIALS AND METHODS

Isolation and identification of isolates

V. biguttatum isolates were obtained from sclerotia of *R. solani* on potato tubers (cv. Marfona) collected from two storages in Erzurum, Turkey. One hundred sclerotia from each sample were divided into five sub-samples having twenty sclerotia. Isolation of *V. biguttatum* was studied using a method modified from Chand and Logan (1984). Twenty sclerotia of *R. solani* were placed on autoclaved moist coarse sand in Petri plates (9 cm diam.). After incubation at 20°C for 4 weeks, the sclerotia were blended in 200 ml of sterile

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distilled water together with agar (2%). The serial dilutions (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) were made and 1 ml aliquots of each dilution were pipetted into ten Petri plates. 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 *Verticillium*-like colonies were repeatedly sub-cultured to obtain pure cultures. The identity of the fungi was confirmed by macroscopic observation of the colonies and microscopic observation of phialides and conidia (Gams and Van, 1982). Single-spore isolates were transferred to PDA medium in tubes for preservation at 4°C. Isolate R-99 of *R. solani* representing AG-3 was isolated from potato tubers with black scurf.

Dual culture tests

Ten isolates of *V. biguttatum* were tested in a dual culture assay against *R. solani* AG-3 (isolate R-99) on PDA. A mycelial disc (4 mm diam.) of *V. biguttatum* was removed from the margin of actively growing colony on PDA and placed on the surface of fresh PDA plate. After four days, a 4 mm disc of *R. solani* was placed 6 cm apart from *V. biguttatum* on the same PDA plate. Dual cultures were incubated in the dark for 3-4 days at 25°C. The percentage inhibition of radial growth of *R. solani* and the width of the zone of inhibition between both colonies were recorded as described by Royse and Ries (1978). The mean of the four measurements were recorded for each isolate of *V. biguttatum*.

Hyphal interaction between *V. biguttatum* and *R. solani*

Hyphal interactions were studied in 5-day old *R. solani* cultures on 2% water agar (WA) in 9 cm Petri plates, which were inoculated with a 4 mm disc removed from actively growing cultures on PDA of each *V. biguttatum* isolate. Plates were incubated at 25°C in a sterile humidity chamber (100% rh). After 2 weeks, 3 rectangular blocks (about 4 x 2 cm) from each plate were cut, mounted on glass slides, and examined for hyphal interaction between *V. biguttatum* and *R. solani* by a phase contrast microscopy (400x).

Effect of *V. biguttatum* on viability of *R. solani* hyphae

For the test of hyphae viability, mature cultures of *R. solani* in 9 cm Petri plates containing PDA were inoculated with three disks (4 mm diam.) of *V. biguttatum* and incubated for 3 weeks at 25ºC in a sterile humidity chamber (100% rh). PDA plates inoculated with *R. solani* alone were used as the control. Four mycelial discs (6 mm diam.) per plate were placed on fresh WA plates for 48 h at 25°C and counted the number of emerging hyphae of *R. solani* with phase contrast microscopy (100x). The hyphae viability was assessed on a scale from 0 to 4; 0 = without emerging hyphae, 1 = with 1 - 5 emerging hyphae, 2 = with 6-10 emerging hyphae, 3 = with 11 - 25 emerging hyphae, 4 = with more than 25 emerging hyphae (Jager and Velvis, 1988). Each experiment was repeated three times with three samples per replicate.

Effect of *V. biguttatum* on germination of *R. solani* sclerotia

Effect of *V. biguttatum* on the germination of *R. solani* sclerotia was determined according to Jager and Velvis (1988). Sclerotia from tubers were dipped into a spore suspension (1 x 10^6 conidia ml^{-1}) of *V. biguttatum* isolates (ME-3 and MC-7), and dried on filter paper in an air current for two hours. Untreated sclerotia were used as the control. The sclerotia were placed on the bottom of Petri plates, and plates were incubated at 20°C in a sterile humidity chamber (100% rh). After 30 days, the viability of *R. solani* sclerotia was estimated by placing them on WA for 48 h at 25°C and counting the number of emerging hyphae with phase contrast microscopy (100x). The sclerotia viability was assessed on a scale from 0 to 4, and sclerotia viability index was calculated according to Jager and Velvis (1988). Fifty sclerotia were used for each isolate and control.

Effect of *V. biguttatum* on disease severity of *R. solani*

The ability of *V. biguttatum* isolate MC-7 to control *R. solani* was determined on potato sprouts (cv. Marfona) in growth chamber. Seed tubers were prepared: 1 = clean seed tubers, 2 = disinfected seed tubers with *R. solani* sclerotia (disinfected in 2% formaldehyde for 5 min), 3 = seed tubers with *R. solani* sclerotia, 4 = seed tubers with *R. solani* sclerotia inoculated with *V. biguttatum*, 5 = clean seed tubers inoculated with three mycelial discs (8 mm diam.) of *R. solani*, 6 = clean seed tubers inoculated with *V. biguttatum* and three mycelial discs (8 mm diam.) of *R. solani*. The seed tubers were sprayed with a spore suspension of the *V. biguttatum* MC-7 (1 x 10^6 conidia ml^{-1}), dried for two hours, and stored 3 days before planting at 20°C in a humidity chamber (100% rh). Three mycelial discs (8 mm diam.) of *R. solani* R-99 isolate were removed from the margin of actively growing colony on PDA and placed on seed tuber for inoculation. Each inoculation treatment was replicated four times. For sprout tests, plants were grown in 14 cm diameter pots containing sterile soil mix of topsoil and sand (2:1, v/v) in growth chamber maintained at 20°C with a 12-h photoperiod. Potato tuber was placed to a depth of 8 cm in each pot and covered with sterile soil mix. Eighty days after planting, plants were harvested, washed and rated for stem canker on a scale 0-4 as follows; 0 = no lesions, 1 = one to several lesions less than 1 mm in diam., 2 = several lesions 1 - 3 mm in diam., 3 = lesions larger than 3 mm in diam. and sprout girdling, and 4 = sprout girdling and sprout death (Carling and Leiner, 1986). Data from the experiment were subject-ed to variance analysis (ANOVA), and differences are presented by the results of LSD multiple range test.

RESULTS AND DISCUSSION

Isolates of *V. biguttatum*

Ten isolates of *V. biguttatum* were obtained from tuber-borne sclerotia of *R. solani* in Erzurum, Turkey (Table 1). Colonies of *V. biguttatum* on PDA reaching 43 - 53 mm diameter in 14 days at 25°C, white or yellow, very thinly velvet; conidiophores erect; phialides 20 - 45 µm long; conidia cylindrical, 4 - 9 x 2 µm size, with 1 - 7 guttules. These cultural and microscopic characteristics of *V. biguttatum* isolates agreed with the description of the species (Gams and Van Zaayen, 1982). The occurrence of *V. biguttatum* from sclerotia of *R. solani* on potato tubers in Turkey was determined for the first time in this study.

Dual culture tests

All isolates of *V. biguttatum* caused large inhibition zones (3.3-4.8 mm) in front of the *R. solani* colony (Table 1). No physical contact was observed between any isolates of *V. biguttatum* and *R. solani* in dual culture. An inhibition

Table 1. Colony and hyphal interactions of *Verticillium biguttatum* isolates with *Rhizoctonia solani in vitro*.

Fungal species	Isolate number	Width of the zone (mm)	Inhibition of *R. solani* (%)	Hyphal interaction*	Viability of *R. solani* hyphae**
V. biguttatum	MC-6	3.3	33	+	0
V. biguttatum	MC-7	3.4	35	+	0
V. biguttatum	MC-12	4.8	23	+	0
V. biguttatum	MC-13	4.0	32	+	0
V. biguttatum	ME-1	3.8	26	+	0
V. biguttatum	ME-3	3.3	31	+	0
V. biguttatum	ME-4	4.3	31	+	0
V. biguttatum	ME-5	4.8	37	+	0
V. biguttatum	ME-6	3.3	32	+	0
V. biguttatum	ME-9	3.5	32	+	0
R. solani	R-99				4

* +: Coiling and penetration of *R. solani* hyphae. ** Viability of *R. solani* hyphae was assessed on a scale from 0 to 4; 0 = without emerging hyphae, 1 = with 1 - 5 emerging hyphae, 2 = with 6-10 emerging hyphae, 3 = with 11 - 25 emerging hyphae, 4 = with more than 25 emerging hyphae (Jager and Velvis, 1988).

Table 2. Viability of *Rhizoctonia solani* sclerotia inoculated with *Verticillium biguttatum*.

Treatment	Isolate number	Percentage of sclerotia according to the number of emerging hyphae					Viability index of sclerotia
		0	1-5	6-10	11-25	> 25	
V. biguttatum	ME-3	54	28	10	8	0	18.0
	MC-7	94	4	2	0	0	2.0
Control		8	6	4	8	74	83.5

zone was observed which indicates the presence of fungistatic metabolites secreted by the *V. biguttatum* isolates. As a matter of fact, production of antifungal hydroxymethyl phenols (bigutol and methylbigutol) by *V. biguttatum* is known suggesting that antibiosis may play a role during biocontrol of R. solani by this mycoparasite (Morris et al., 1995). The results of dual culture studies showed that *V. biguttatum* isolates inhibited the growth of *R. solani* mycelium by antibiosis.

Hyphal interaction between *V. biguttatum* and *R. solani*

After the coiling around of *R. solani*, *V. biguttatum* hyphae penetrated host cell walls and grew within the hyphae (Table 1). Similar results were obtained by Van den Boogert and Deacon (1994). According to authors, chitinase, *β*-1,3-glucanase and protease were produced by *V. biguttatum*, and these enzymes may play role in dissolving and penetrating the cell walls of *R. solani* (McQuilken and Gemmell, 2004).

Effect of *V. biguttatum* on viability of *R. solani* hyphae

V. biguttatum completely reduced viability of hyphae of *R. solani* on PDA (Table 1). *V. biguttatum* is a biotrophic mycoparasite; it penetrated the hyphae of *R. solani*, later the mycoparasite sporulated, and the infected host cells died (Van den Boogert and Deacon, 1994).

Effect of *V. biguttatum* on germination of *R. solani* sclerotia

Germination of *R. solani* sclerotia was drastically reduced by *V. biguttatum* (Table 2). The viability index of sclerotia inoculated with ME-3 and MC-7 isolates of *V. biguttatum* were 18 and 2%, respectively, while non-inoculated sclerotia showed 83.5% viability index. MC-7 isolate was shown the strongest inhibition of sclerotia germination. *R. solani* sclerotia were inactivated after inoculation of infected tubers with a suspension of conidia and hyphal fragments of *V. biguttatum* (Velvis and Jager, 1983;

Table 3. Effect of *Verticillium biguttatum* on disease severity caused by *Rhizoctonia solani* on potato sprouts.

Treatments	Disease severity *
Clean tubers	0.0b **
Tubers with sclerotia disinfected formaldehyde	0.0b
Tubers with sclerotia	3.5a
Tubers with sclerotia + *V. biguttatum*	0.2b
Clean tubers + *R.solani*	3.4a
Clean tubers + *R.solani* + *V. biguttatum*	0.5b
LSD	1.2

*Disease severity was assessed on a scale from 0 to 4; 0 = no lesions, 1 = one to several lesions less than 1 mm in diam., 2 = several lesions 1 - 3 mm in diam., 3 = lesions larger than 3 mm in diam. and sprout girdling, and 4 = sprout girdling and sprout death (Carling and Leiner, 1986). **Within column, means followed by different letters are significantly different (P<0.01; LSD test).

Jager and Velvis, 1988).

Effect of *V. biguttatum* on disease severity of *R. solani*

Based on the results of growth chamber tests, *V. biguttatum* isolate (MC-7) also significantly reduced the disease severity of *R. solani* on potato sprouts in pot experiments. Statistical analysis of data on the stem canker cause by *R. solani*, indicated that there are significant differences between treatments (Table 3). In treatments, the disease severity of tubers with sclerotia, tubers with sclerotia + *V. biguttatum*, clean tubers + *R. solani*, clean tubers + *R. solani* + *V. biguttatum* were 3.5, 0.2, 3.4 and 0.5, respectively, while the clean tubers and tubers with sclerotia disinfected formaldehyde showed no disease symptoms. These results showed that *V. biguttatum* isolate (MC-7) effectively controlled stem canker when it was applied to tubers. *V. biguttatum* was found to be mycoparasite of *R. solani* and had been shown to reduce stem canker (Velvis and Jager, 1983; Jager and Velvis, 1986) and black scurf (Jager et al., 1991; Van den Boogert and Velvis, 1992) of potatoes, caused by *R. solani*, in experimental tests.

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REFERENCES

Carling DE, Leiner RH (1986). Isolation and characterization of *Rhizoctonia solani* and binucleate *R. solani*-like fungi from aerial stems and subterranean organs of potato plants. Phytopathology, 76: 725-729.

Chand T, Logan C (1984). Antagonists and parasites of *Rhizoctonia solani* and their efficacy in reducing stem cancer of potato under controlled conditions. T. Brit. Mycol. Soc. 83: 107-112.

Demirci E, Döken MT (1993). Anastomosis groups and pathogenicity of *Rhizoctonia solani* Kühn isolates from potatoes in Erzurum-TÜRKİYE. J. Turk. Phytopath. 22: 95-102.

Demirci E, Eken C (1995). The effect of tuberborne and soilborne inoculum of *Rhizoctonia solani* Kühn on disease severity on potato. Proceedings of the 7th Turkish Phytopathological Congress, Adana 1995: 39-43 (in Turkish, with English Summary).

Frank JA (1986). Rhizoctonia canker (Black scurf). In Hooker WC (ed) Compendium of Potato Diseases, Am. Phytopathological Soc. USA., pp. 52-54.

Frank JA, Leach SS (1980). Comparison of tuberborne and soilborne inoculum in the Rhizoctonia disease of potato. Phytopathol. 70: 51-53.

Gams W, Van Zaayen A (1982). Contribution to the taxonomy and pathogenicity of fungicolous *Verticillium* species. I. Taxonomy. Neth. J. Plant Pathol. 88: 57-78.

Jager G, Velvis H (1986). Biological control of *Rhizoctonia solani* on potatoes by antagonists. 5. The effectiveness of three isolates of *Verticillium biguttatum* as inoculum for seed tubers and of a soil treatment with allow dosage of pencycuron. Neth. J. Plant Pathol. 92: 231-238.

Jager G, Velvis H (1988). Inactivation of sclerotia of *Rhizoctonia solani* on potato tubers by *Verticillium biguttatum*, a soil-borne mycoparasite. Neth. J. Plant Pathol. 94: 225-231.

Jager G, Ten Hoopen A, Velvis H (1979). Hyperparasites of *Rhizoctonia solani* in Dutch potato fields. Neth. J. Plant Pathol. 85: 253-268.

Jager G, Velvis H, Lamers JG, Mulder A, Roosjen J (1991). Control of *Rhizoctonia solani* in potato by biological, chemical and integrated measures. Potato Res. 34: 269-284.

McQuilken MP, Gemmell J (2004). Enzyme production by the mycoparasite *Verticillium biguttatum* against *Rhizoctonia solani*. Mycopathologia 157: 201-205.

Morris RAC, Ewing DF, Whipps JM, Coley-Smith JR (1995). Antifungal hydroxymethyl-phenols from the mycoparasite *Verticillium biguttatum*. Phytochem. 39: 1043-1048.

Royse DJ, Ries SM (1978). The influence of fungi isolated from peach twigs on the pathogenicity of *Cytospora cincta*. Pyhtopathology 68: 603-607.

Van den Boogert PHJF (1996). Mycoparasitism and biocontrol. In Sneh B, Jabaji-Hare S, Neate S, Dijst G (eds) *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control, Kluwer Academic Publishers, Netherlands, pp. 485-493.

Van den Boogert PHJF, Velvis H (1992). Population dynamics of the mycoparasite *Verticillium biguttatum* and its host *Rhizoctonia solani*. Soil Biol. Biochem. 24: 157-164.

Van den Boogert PHJF, Deacon JW (1994). Biotrophic mycoparasitism by *Verticillium biguttatum* on *Rhizoctonia solani*. Eur. J. Plant Pathol. 100: 137-156.

Van den Boogert PHJF, Luttikholt AJG (2004). Compatible biological and chemical control systems for *Rhizoctonia solani* in potato. Euro. J. Plant Pathol. 110: 111-118.

Velvis H, Jager G (1983). Biological control of *Rhizoctonia solani* on potatoes by antagonists. 1. Preliminary experiments with *Verticillium biguttatum*, a sclerotium-inhabiting fungus. Neth. J. Plant Pathol. 89: 113-123.