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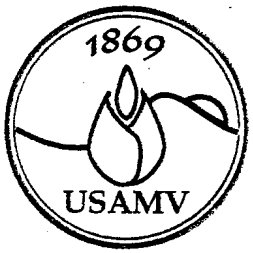

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BIOLOGICAL CONTROL AGAINST *RHIZOCTONIA* DAMPING-OFF DISEASE OF TOMATO BY *TRICHODERMA* STRAINS

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Key words: damping-off, tomato, biological control, *Trichoderma*, *Rhizoctonia solani*, seed treatments, metabolites

Abstract: Tomato seedlings were grown in glasshouse under poor light condition in pots, which contained infested soil. The plants showing damping-off disease symptoms were collected and the causing agent *Rhizoctonia solani* pure cultures were isolated and grown on Ethanol-potassium nitrate medium. Isolation of potential antagonist *Trichoderma* species/strains were made by laboratory procedures on selective medium from the same soil samples. The 47 isolates represented 7 *Trichoderma* species (*T. viride, T. atroviride, T. virens, T. koningii, T. strictipilis, T. spirale, T. tomentosum, T. harzianum*, and *T. hamatum*). Connection between plant pathogenic fungus and the beneficial *Trichoderma* strains can be direct parasitism (hyperparasitism), antibiosis (antibiotic metabolites, lytic enzymes), competition for nutrients, and/or their different combinations. *In vitro* antagonism was studied on PDA in Petri dishes. 10 mm in diam. disc of each *Trichoderma* strains grown on PDA were put in the middle of the surface of 14-day-old culture of *Rhizoctonia solani* colonies. Growing of *Trichoderma* colonies were measured daily and dynamism of colonisation was characterised by diameter of colony. In these trials all the *Trichoderma* strains killed *Rhizoctonia solani* however intensity of colonisation were different. *Trichoderma atroviride, T. harzianum* and *T. koningii* strains were fast growing on colonies of test fungus. However dynamic of colonisation had not allowed far-reaching consequences for efficacy of the beneficial fungus in spite of that these direct contacts resulted death of pathogen. To determine biological activity among beneficial and parasitic fungi we chose the feature of antibiotic metabolite productivity of beneficial fungus. *Trichoderma* strains were grown in Potato broth in shaked culture for a week. Mycelium and conidia free filtrates were pipetting in the hole of agar plates. Surrounding each hole 10-mm in diam. agar discs covered by *Rhizoctonia* fungus were placed (biculture). During this agar-gel diffusion trials the metabolites of *Trichoderma* strains blocked the growing of *Rhizoctonia solani*. There were no differences between efficacy in metabolite activity of beneficial strains. The cellophane-agar diffusion method showed valuable results for making differences among antibiotic metabolite production of strains. On the surface of PDA medium sterile cellophane disks were put and transferred beneficial strains on them. Semipermeabilty of cellophane disks allowed diffusing metabolites into the lower agar-gel. After 3 days growing of *Trichoderma* strains the cellophane disks were gently removed and agar discs covered by *Rhizoctonia solani* was put on the middle. Growing of fungus in diam. was measured daily. Totally inhibition were showed by *Trichoderma hamatum* (Tha-2), *T. viride* (Tv-5), and *T. virens* (Tvr-1). Considerable growing reduction of *Rhizoctonia solani* was observable at treatments by *T. tomentosum* (Tt-44) and *T. harzianum* (Thz-33) caused by diffusible metabolites. *In vivo* pot trials were made by *Trichoderma* strains against soil-borne *Rhizoctonia* damping off disease. Seeds of susceptible tomato variety (cv. Uno /K-652/) were sowed in the depth of 1 cm of pots artificially infested soil. To avoid microbiological interactions all microbe were suppressed by sterilisation of soil. *Rhizoctonia solani*, which had been grown on grinded maize and Czapek-Dox agar mixture, was mixed into the soil.

Suspensions of beneficial fungus strains (107 spores/ml) were spread on the seeds. Efficacy of beneficial *Trichoderma* strains *in vivo* differed from *in vitro* features. However some effective strains (Tt-44, and Tvr-1: 88, Thz-33: 98 percent efficacy, respectively) were selected for biological control against damping off disease caused by *Rhizoctonia solani*.

INTRODUCTION

Nowadays a lot of new environmental-saving or less harmful methods have gained priority in plant protection activities. Intensive research projects on biological control against plant pathogens have started in 1970's in all over the world. Research works on biological control are also very popular fields also in Hungary. Scientific interest has turned toward *Trichoderma* species, as potential agents of biological control since early 1980's. Beside of potential opportunities for practical usage in plant protection it is necessary to examine physiology, ecology, components of bioactivity of these microorganisms, and new effective strains should be isolate.

Rhizoctonia solani KÜHN is a very frequent plurivourus soil-borne fungus causes damping-off disease on different horticultural plants including tomato. As a result of *R. solani* infection insufficient emergence of seedlings, then damping-off occurs which threaten the young plants until 4-6-leaf stage. The most of damping-off cases occurred in seedbeds were caused by *Rhizoctonia solani* in Hungary (SZIRMAI, 1941; KASZONYI, 1956). HADAR et al. (1979a,b) protected their plants successfully using *Trichoderma* strains against *Rhizoctonia solani* in bean, tomato, and eggplant cultures in glasshouses. CHET et al. (1979, 1982) isolated effective *Trichoderma* strains against soil-borne *R. solani*, which were applicable to protect irises (*Iris*) bulbs. ELAD et al. (1981a) protected their strawberry plants against root rot caused by *Rhizoctonia solani* with *Trichoderma harzianum* fungus, and the infection rate reduced by 18-46 percent. Treated plants yielded 21-37 percent more fruits than control ones. ELAD et al. (1981b) examined the efficacy of *Trichoderma harzianum* against carnation disease caused by *Rhizoctonia solani,* and observed 70 percent effectiveness among different methods which was the best when *Trichoderma* was mixed into the nutritive mixture using for making roots of plants. Biological control against *Rhizoctonia solani* at potato plants were made trials by BEAGLE and PAPAVIZAS (1985) using *T. viride* species. Occurrence of disease was decreased by 50 percent after treatment. BELDAN (1988) used *Trichoderma viride* strains against lettuce root rot disease, which were effective after irrigation of plots. The yield was 40 percent more when applied this method. LEWIS and LARKIN (1997) applied biopreparates containing *Trichoderma virens, T. hamatum,* and *T. harzianum* fungi respectively with success on egg-plants for protection against damping-off disease and decreasing inocula of *R. solani* in soil-free culture. Improving seed-dressing biological control methods were developed by HARMAN et al. (1980) and HARMAN (1991) which were reliable and economic and contained *Trichoderma* strains with suitable efficacy. Pea and radish seeds were treated by conidia of *T. hamatum* strains against soil-borne pathogens including *R. solani* and observed the same efficacy like fungicide seed-dressings. In our research aims were finding effective beneficial strains among *Trichoderma* species against the serious soil-borne pathogen *Rhizoctonia solani*.

MATERIALS AND METHODS

Collecting infested soil samples. For isolation pure cultures of both phytopathogenic *Rhizoctonia solani* and beneficial *Trichoderma* fungi we used in all likelihood infested soil samples. Soil samples were collected from seedbeds of Demonstration Farm of Horticulture

Department of Debrecen University. Soils containing organic materials in high rate are favour for *Rhizoctonia* survival if soils had inocula. Isolation of *Rhizoctonia solani* Isolation of pathogen was made by sowing susceptible tomato seeds (*Lycopersicon esculentum* cv. UNO/K-652/) in pots of 10 cm diam. contained infested soil samples. Pots were overwashed and kept poorly lighted circumstances promoting disease occurrence. Seedlings showing symptoms of damping-off disease were removed from soil and after surface sterilisation process the small pieces of chopped stems were put on ethanol-potassium nitrate *Rhizoctonia* selective medium (Trujillo et al. 1987). Developed mycelia of pathogen were transferred on Potato-dextrose-agar (PDA) getting pure cultures of *Rhizoctonia solani.* Isolation of *Trichoderma* strains The isolation of beneficial fungus was made on *Trichoderma* selective medium (Askew and Laing, 1993) from the same soil samples. 16 granules of soil were put on the top of media. From colonies, which had developed on the surface of soil granules or the surrounding media, conidia were collected with sterile needle for gaining monospore colonies. *Trichoderma* conidia were transferred on Petri dishes containing selective media. The isolated 46 strains were representatives of 7 *Trichoderma* species, *Trichoderma* /syn: *Gliocladium/ virens* Miller; *T. atroviride* Karsten; *T. harzianum* Rifai; *T. strictipilis* Bisset; *T. spirale* Bisset, *T. koningii* Oud.; *T. tomentosum* Bisset respectively. We also used in our trials *Trichoderma viride* (TV-5) and *Trichoderma hamatum* (Tha-2) previously selected strains as standards. We appreciate for these to Dr László Vajna, Plant Protection Institute of Hungarian Academy of Sciences, Budapest. Trials for study of direct antagonism. Our experiments were made on totally covered PDA dishes by *Rhizoctonia solani* followed by 7 days growing. Agar discs in 5-mm diam. contained *Trichoderma* strains were cut out and put in the centre of each Petri dish. During the 6 day incubation period the diameter of colonies were measured daily to determine dynamic of colonisation. After the total colonisation of *R. solani* colony by *Trichoderma,* discs were cut out contained both pathogen and beneficial fungi, and were put on 1 cm distances from each other radial on *Rhizoctonia*-selective medium and grown in biculture to examine the efficacy of antagonism.

Testing of indirect antagonism (toxic metabolite production)

By agar-gel diffusion test. Beneficial strains were grown in 100 ml Erlenmeyer flasks on 10 ml liquid media (Potato-dextrose broth) applied continuous shaking (100 rpm) for 7 days. After this incubation period conidium and mycelium free filtrates were made (0.45 μm diam. filter) and dropped in the hole, which had been made in the centre of PDA discs. Surrounding of central whole 4 discs of *R. solani* were placed 3 cm far from the centre point. The presumed metabolite/s could diffuse in the media retarding the growth of the pathogen. On the base of retarded zones we could deduce the strength of antagonism.

By cellophane-disc diffusion test. Applying another method beneficial organisms were transferred to the surface of sterilised cellophane discs on PDA medium. Using the diffusible nutriments the *Trichoderma* colonies started to develop and after 3-day-long incubation the cellophane discs together mycelia were gently removed. Metabolite/s produced by beneficial fungus could diffuse into the agar across the semipermeable cellophane discs. PDA discs with *Rhizoctonia solani* were put on the centre of media and measured the growing of colonies daily.

Seed treatments. Seed germination rate was checked on the top of wet filter paper in Petri dishes. Germinating test was made in according with the international seed testing standard (ICTA, 1976). Origin of soil samples was the same as had been used for isolation process of pathogenic and beneficial fungi. Samples were sterilised in autoclave to eliminate all microbes and interactions. The artificial soil infestation was made by mixture of 250-g ground maize seeds and 5 g Czapek-Dox medium with mycelia of *Rhizoctonia solani* per pots

in 10-cm diam. Tomato test plants (*Lycopersicon esculentum* cv. UNO /K-652/) were used because these ones are susceptible to *R. solani* and easy to promote the development of damping-off disease. Seeds were inoculated by conidial-mycelial suspension of beneficial fungi, which contained 10^7 spores per ml. The treated seeds were sown in the infested soil in 1-cm depth. Each pot contained 50 seeds and treatments were made in four repetitions.

During the evaluation period we counted the symptom-bearing seedlings and measured the high of plants deducing for the colonisation abilities of the *Trichoderma* strains in the rhizosphere and their beneficial effects.

RESULTS AND DISCUSSIONS

Results of direct antagonism tests. *Trichoderma* strains grown very intensively in biculture on the surface of previously colonised medium by Rhizoctonia solani. The radial growing of *Trichoderma* species reached the edge of the total surface of 9-cm Petri dishes by the fifth or sixth day (Fig. 1).

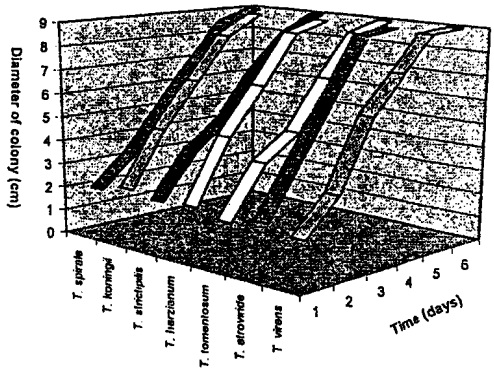

Fig. 1: Colonisation of *Trichoderma* species on *Rhizoctonia solani*

We checked the biculture under light microscope (400-x magnification) and observed the parasitism of *Trichoderma* species on *Rhizoctonia solani*. The narrower hyphae of *Trichoderma* fungi twisted on the *Rhizoctonia* hyphae, resulted mycelium-capsules, and the pathogen started to die. Cells of *R. solani* collapsed with presumably help of antibiotic metabolites and lytic enzymes. In our furthermore trials we experienced that all the biculture discs collapsed and there were no growing at all on the *Rhizoctonia*-selective medium. In these cases both direct antagonism (myco-parasitism) and inhibitors could mutually contribute to the effectiveness of biocontrol results. The direct contact between the pathogen and the beneficial fungus result *in vitro* destroying effects for the latest.

Results of indirect antagonism tests. By agar-gel diffusion. Changes were followed daily after dropping the filtrate of *Trichoderma* metabolites into the central hole on the *Rhizoctonia*-agar discs. After 24-hrs fungus discs have started to grow into the inhibitor-contained medium. On the untreated (control) medium the growing of fungus has reached 1 cm in diam. by that time. Later on *Rhizoctonia solani* was not able to grow on the treated media and hyphae collapsed meantime. By the fifth day the pathogen covered 2/3 of the media at control, as there was hardly observable growing on the treated Petri dishes even on the seventh day without measurable data. Because none of the 9 tested *Trichoderma* metabolite filtrate showed total inhibition, there was no comparable differences among efficacy of strains. The tested antagonist fungi produced toxic metabolites during the growing period and the filtrates contained them presumably in too high concentration. However by the end of 24, 36, and 48 hrs growing there were no sufficient metabolite production to detect them biologically.

By cellophane-disc diffusion. Metabolites of *Trichoderma viride* (Tv-5), *T. hamatum* (Th-2), and *T. virens* (Tvr-1) did not allow growing *Rhizoctonia solani* at all (Fig. 2). *T. harzianum* (Th-33) and *T. tomentosum* (Tt-44) strains showed extraordinary good results (more than 50 percent inhibition rate by the fourth day). The other strains also had some effectiveness but there were no satisfactory (below 50 percent efficacy).

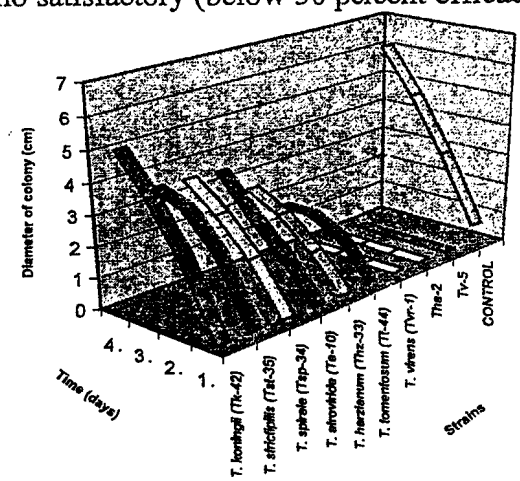

Fig. 2: Effects of the diffusible metabolites of *Trichoderma* strains on growing *Rhizoctonia solani* mycelia in cellophane-disc diffusion test

Results of seed treatments. Germination rate of seeds on the top of filter paper was 96 percent. Emerging rate of seeds in sterilised soil (0 control) was 94 percent. Seedlings emerged in less number from *Rhizoctonia solani* infested soils and showed damping-off symptoms in a high rate (*R. solani* control: more than 50 percent) (Table 1). The efficacy of *Trichoderma* strains was between 67.9 and 98.77 percent. On the base of seed treatment efficacy, the row in decreasing effectiveness: *T. harzianum* (Thz-33), *T. virens* (Tvr-1), *T. tomentosum* (Tt-44), *T. strictipilis* (Tst-35), *T. spirale* (Tsp-34), *T. atroviride* (Ta-10), *T. hamatum* (Tha-2), *T. viride* (Tv-5), and *T. koningii* (Tk-42) in potentially applicability for biocontrol of tomato seeds against the damping-off disease caused by *Rhizoctonia solani.*

Effects of *Trichoderma* seed treatments on emergence and symptoms of damping-off disease caused by *Rhizoctonia solani* (* significantly less emerged plants; **significantly less symptoms)

Table 1:

Treatments	*Average emerged seedlings number*		Average symptom-bearing seedlings		Efficacy of treatments %
	from 50 seeds/pot	%	number from emerged plants	in *Rhizoctonia solani* control %	
0 control	47.00	100	0	0**	-
R. solani control	43.00*	91.49*	27.00	100	-
T. viride (Tv-5)	45.00	95.74	8.00	29.63**	70.37
T. hamatum (Tha-2)	45.33	96.45	7.33	27.16**	72.84
T. virens (Tvr-1)	44.33	94.33	3.00	11.11**	88.89
T. harzianum (Thz-33)	44.33	94.33	0.33	1.23**	98.77
T. spirale (Tsp-34)	41.33*	87.94*	5.67	20.99**	79.01
T. strictipilis (Tst-35)	44.67	95.04	5.00	18.52**	81.48
T. atroviride (Ta-10)	43.67	92.91	6.00	22.22**	77.78
T. koningii (Tk-42)	46.33	98.58	8.67	32.10**	67.9
T. tomentosum (Tt-44)	45.67	97.16	3.33	12.35**	87.65
$LSD_{5\%}$	2.82	6.00	1.68	6.22	

CONCLUSIONS

Efficacy of the tested beneficial *Trichoderma* strains *in vivo* differed from *in vitro* features. However some more effective strains (Tt-44, and Tvr-1: 88, Thz-33: 98 percent efficacy, respectively) were selected based on both *in vitro* and *in vivo* tests for the potentially applicable agents in biological control against damping off disease caused by *Rhizoctonia solani*.

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