CZECH MYCOL. 52 (3), 2000

Root mycoflora of pepper (Capsicum annuum) antagonistic to Verticillium dahliae

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Gherbawy Y. A. M. H. and Prillinger H. (2000): Root mycoflora of pepper (Capsicum annuum) antagonistic to Verticillium dahliae – Czech Mycol. 52: 219–226

Thirty-two species belonging to 19 genera of fungi were collected from 30 soil samples from the rhizosphere of pepper plants. The fungal colonies were characterised using classical morphological methods following identification keys. The most frequently isolated fungi were *Chaetomium globosum, Fusarium oxysporum, Gliocladium roseum, Mucor racemosus, Myrothecium verrucaria, Penicillium aurantiogriseum, P. expansum* and *Trichoderma harzianum*. The crude culture filtrate of *Verticillium dahliae* at 100% concentration caused sharp decrease in pepper seed germination. *Chaetomium globosum, Gliocladium roseum, Myrothecium verrucaria,* and *Trichoderma harzianum* produced a metabolite that retarded radial growth of *Verticillium dahliae*.

Key words: Frequency of root fungi, *Verticillium dahliae*, *Chaetomium globosum Gliocladium roseum, Myrothecium verrucaria, Trichoderma harzianum*, pepper seed germination.

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Bylo odebráno 30 půdních vzorků z rhizosféry papriky, kde bylo nalezeno 32 druhů hub, které patřily k 19 rodům. Kolonie byly určeny klasickými morfologickými metodami za použití určovacích klíčů. Nejčastěji izolovanými druhy byly *Chaetomium globosum, Fusarium oxysporum, Gliocladium roseum, Mucor racemosus, Myrothecium verrucaria, Penicillium aurantiogriseum, P. expansum* a *Trichoderma harzianum*. Surový 100 % filtrát čisté kultury *Verticillium dahliae* působil prudké snížení klíčivosti semen papriky. Druhy *Chaetomium globosum, Gliocladium roseum, Myrothecium verrucaria* a *Trichoderma harzianum* produkovaly metabolit, který retardoval radiální růst *Verticilium dahliae*.

INTRODUCTION

Verticillium dahliae Kleb. (Fungi imperfecti, Moniliales) is a soil-borne fungus with a world-wide distribution. It caused wilting, stunting and early dying of pepper plants in Israel, resulting in a 22 % reduction in yield (Tsror et al. 1998). It has a wide host range and causes tracheomycosis of many economic plants such as tomato (Osman et al. 1991; Dobinson et al. 1996; Madhosingh 1996; Li et al. 1996; Gold and Robb 1996).

Strategies for the biological control of fungal pathogens are commonly based on direct antagonism like antibiosis or hyperparasitism. Retardation of epidemiological spread offers another promising strategy (Lennartz et al. 1998). Competition

from antagonists for nutrients and space can result in reduced pathogen development, especially in reduced spore production (Lennartz et al. 1998).

Antagonistic microorganisms are able to suppress growth and development of phytopathogenic fungi (Huber et al. 1987; Phillipp 1988). Disease suppression and use of antagonistic microorganisms as biocontrol agents depends on their ability to both colonise roots and produce substances inhibitory to the pathogen (Thomashow et al. 1990). Several antagonistic microorganisms to *Verticillium* wilt have been isolated, most of these fungi, e.g. *Aspergillus flavus, Gliocladium roseum, Penicillium griseofulvum, P. vermiculatum, Pythium* sp., *Trichoderma harzianum* and *T. viride* (Keinath et al. 1981; Ghaffer 1988; Zeise 1990; Osman et al. 1991).

The principal aim of this study was to test fungal species isolated from roots of pepper plants as biological agents for the control of *Verticillium dahliae* causing wilt disease of many crops.

MATERIALS AND METHODS

Estimation of rhizosphere fungi

This part of the study was performed to isolate some fungi that are known to be biological agents and grow saprophytically in pepper field soil also. Samples of rhizosphere soil were collected from the root system of 30 healthy pepper plants growing at a private farm in Stockerau near Vienna City (Austria). From these soil samples, fungi were isolated on potato – dextrose agar (PDA) by the dilution plate method as described by Abdel-Hafez et al. (1990a). Five replications were made for each sample and the developing fungi were identified, counted and the numbers were calculated per g dry soil.

Effect of some root's mycoflora on radial growth of *Verticillium dahliae*

Verticillium dahliae (Isolate MD 15) previously isolated from pepper in Burgenland, Austria, was used during this study. Cultures of *Chaetomium globosum, Gliocladium roseum, Myrothecium verrucaria* and *Trichoderma harzianum* (previously isolated from pepper roots) were maintained on PDA at 28 °C. Two agar discs (1.2 cm in diameter) from a 1–2 wk old culture medium (Mizuno et al. 1974) of the above mentioned fungi were used for production of the inhibitory metabolite. Four replicate flasks of these liquid cultures were maintained at 27 °C ± 0.2 °C on a rotary shaker at 120 rpm. After a 8-day incubation period, samples were filtered through a 0.45 mm filter to remove hyphae and spores of *Chaetomium globosum, Gliocladium roseum, Myrothecium verrucaria* and *Trichoderma harzianum*. The resulting cell-free culture filtrates were separated into two groups. The first group was autoclaved for sterilisation (sterile), the second remained without sterilisation

(normal). The dilutions of both types of filtrate (normal and sterile), uninoculated medium (broth) and sterile distilled water were incorporated into Czapek's solution.

Effect of culture filtrates of *Verticillium dahliae* on pepper seed germination

Seeds of pepper were sterilised by soaking them in 0.1% mercuric chloride for 2 minutes. They were rinsed several times in sterile distilled water. Sterilised Petri dishes (10 cm in diameter) containing sterilised filter paper were prepared for seed germination. Twenty seeds were germinated in 10 ml of the normal and autoclaved fungal filtrates (100%, 75%, 50% and 25% concentrations), respectively. Control treatments received 10 ml of sterilised distilled water. All plates were incubated at 25 °C for 5 days, after which the germination rate n each treatment was recorded.

RESULTS AND DISCUSSION

Thirty–two species belonging to 19 fungal genera (Table 1) were isolated from 30 rhizosphere soil samples of pepper plants.

The most prevalent genera were *Chaetomium, Fusarium, Gliocladium, Mucor, Myrothecium, Penicillium* and *Trichoderma*. They were recovered from 56.7, 50, 63.3, 60, 63.3 and 66.7% of the total number of samples comprising 8.95, 8.08, 9.96, 7.88, 21.96 and 15.54% of total fungi, respectively. They were represented by *Chaetomium globosum, Fusarium oxysporum, Gliocladium roseum, Mucor racemosus, Myrothecium verrucaria, Penicillium aurantiogriseum, P. expansum* and *Trichoderma harzianum*. Gherbawy and Abdelzaher (1999) isolated *Chaetomium globosum, Fusarium oxysporum, Mucor racemosus, Myrothecium verrucaria, Penicillium aurantiogriseum* and *Trichoderma harzianum* from the rhizosphere of tomato plants in Egypt. They reported that these fungal species were recovered from 10, 35, 30, 40, 20, and 15% of the samples comprising 1.0, 3.0, 1.5, 3.7, 0.5 and 1.3% of total fungi, respectively. Most of the above mentioned species were previously isolated but with different incidences from the rhizosphere of several plants cultivated or grown in many parts of the world (Abou El-Souod et al. 1988; Abdel-Hafez et al. 1990a,b, 1995; Rajendra and Saxena 1991; Abdelzaher et al. 1999). The remaining genera and species were less frequently or rarely isolated (Table 1).

Effect of the culture filtrates of *Verticillium dahliae* on pepper seed germination

The crude filtrate (100%), either autoclaved or not, showed an inhibitory effect on the germination of pepper seeds (Fig. 1). The pepper seed germination rates were 50%, 56%, 53.1% and 59% when allowed to germinate in 25%, 50%, 75%

Table 1. Average total count (ATC), number of isolation cases (NIC), occurrence remarks (OR) and percentage of total count (TC) of various fungal genera and species recovered from 30 rhizosphere soil samples of pepper plants on PDA medium at 28 °C.

Genera & species	ATC	NIC & OR	TC %
Acremonium strictum	15300	10 M	6.7
Alternaria alternata	2500	5 L	1.09
Aspergillus sp.	1570	5 L	0.69
A. flavus	500	3 R	0.22
A . fumigatus	420	1 R	0.18
A. ochraceus	650	2 R	0.28
Chaetomium globosum	20500	17 H	8.95
Cladosporium cladosporioides	3500	6 L	1.53
Fusarium oxysporum	18500	15 H	8.08
Gliocladium roseum	22800	19 H	9.96
Mucor sp.	18040	18 H	7.88
M. circinelloides	1500	8 M	0.65
M. hiemalis	500	4 L	0.22
M. pusillus	440	2 R	0.19
M. racemosus	15600	16 H	6.81
Myrothecium verrucaria	21400	15 H	9.34
Nectria haematococca	7500	8 M	3.27
Nigrospora spherica	360	1 R	0.16
Paecilomyces variotii	450	2 R	0.20
Penicillium sp.	50300	19 H	21.96
P. aurantiogriseum	15000	15 H	6.55
P. brevicompactum	450	4 L	0.20
P. citreonigrum	6000	7 L	2.62
P. citrinum	7500	9 M	3.27
P. corylophilum	300	2 R	0.13
P. expansum	15400	16 H	6.72
P. griseofulvum	350	1 R	0.15
P. montanense	5000	8 M	2.18
P. waksamanii	300	2 R	0.13
Rhizopus stolonifer	3200	5 L	1.4
Stachybotrys chartarum	400	1 R	0.17
Trichoderma harzianum	35600	20 H	15.54
Gross Total counts	229020		
Number of genera	19		
Number of species	32		

Occurrence remarks: H = high occurrence, between 15–30 cases (out of 30); M = moderate occurrence, between 8–14 cases; L = low occurrence, 4–7 cases; R = rare occurrence, 1–3 cases.

and 100% of the normal filtrate of *Verticillium dahliae*. On the other hand, these percentages were 43.3%, 54.1%, 51.1% and 32.1%, in the case of the autoclaved filtrate. Vilich et al. (1998) reported that barley seeds treatments with *Chaetomium globosum* and *C. funicola* resulted in an increase in root fresh weight. They also reported that after seed inoculation and 3 weeks of cultivation, the fungi were re-isolated initially from the roots and later from leaves without causing any symptoms. Khallil and Ammer (1994) reported that sunflower seed germination rates were 0, 55 and 85% respectively, when allowed to germinate in the normal and autoclaved filtrate of *Fusarium solani* and distilled water. Variation of germination rates is probably due to the fungal filtrate effect on either plant germination enzymes or growth hormones.

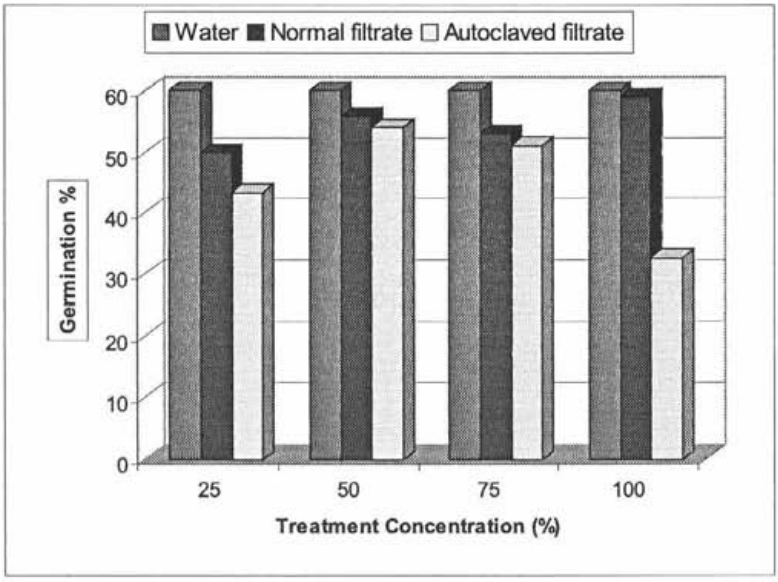

Fig. 1. Effect of *Verticillium dahliae* culture filtrates on the germination rate of pepper seeds.

Effect of root mycoflora on radial growth of *Verticillium dahliae*

The culture filtrate (normal and autoclaved) of all tested fungi showed a great effect on radial growth of *Verticillium dahliae* when they amended with medium. The filtrate of *Chaetomium globoseum* demonstrated the greatest effect in comparison with water and broth. The radial growth of *Verticllium dahliae* was 2.5, 2.3 and 2 cm when the medium was amended with a 25, 50, 100% concentration of the normal filtrate of *Chaetomium globosum,* receptively. On the other hand,

the radial growth was 2.75, 2.4 and 2.2 cm in the case of the autoclaved filtrate. (Fig. 2). Osman et al (1991) reported that filtrates of *Pythium* sp. and *Aspergillus flavus* suppressed radial growth and conidial production of *Verticillium dahliae*. Farvel *et al.* (1987) reported that *Talaromyces flavus* produced a metabolite that not only retarded the radial growth but also killed the sclerotia of *Verticillium dahliae*. This supports the possibility of biocontrol of *Verticillium* wilt of pepper, using other soil inhabiting fungi.

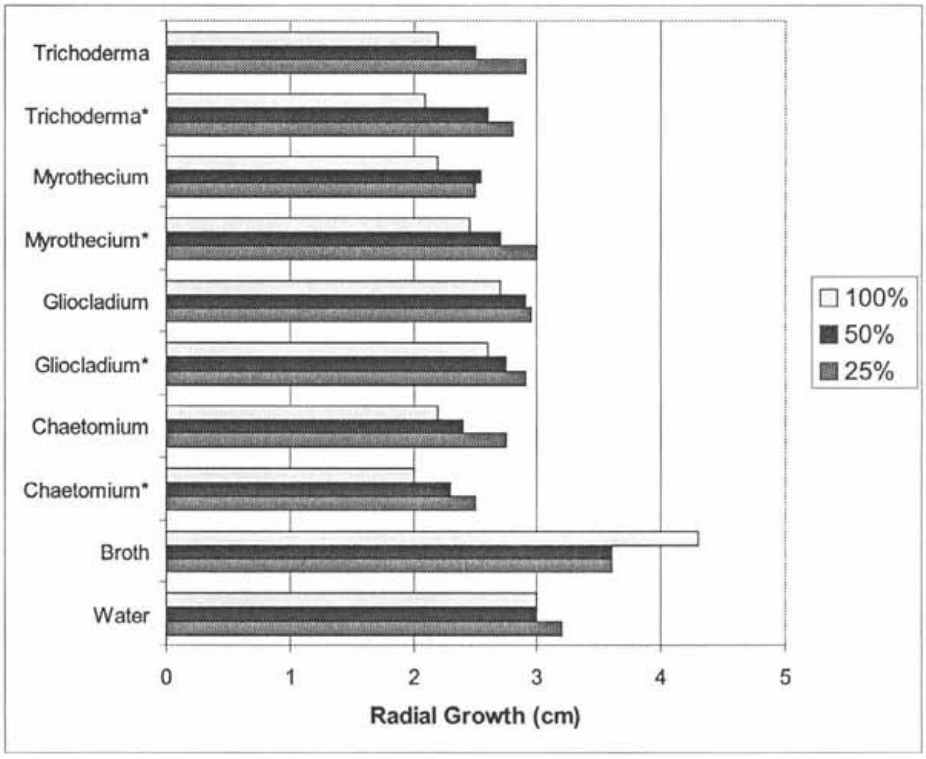

Fig. 2. Radial growth of Verticillium dahliae on Czapek's solution agar amended with 25, 50, 100% dilution of culture filtrate (normal* and autoclaved) of tested fungal species, broth and water.

Vilich et al. (1998) observed that culture filtrates of *Chaetomium globosum* and *C. funicola* inhibited the germination of mildew conidia on solid media in vitro. Mathew and Gupta (1998), during their study on biological control of root rot of French bean caused by *Rhizoctonia solani*, used *Chaetomium globosum*, *Coniothyrium mintans*, *Gliocladium virens*, *Trichoderma hamatum*, *T. harzianum*

and *T. viride*. They reported that *Gliocladium virens* and *Trichoderma harzianum* proved superior to other antagonists in reducing pre-emergence root rot to 6.7% and 13.3% respectively, as compared to 36.7% in the control.

ACKNOWLEDGEMENTS

We wish to thank Dr. K. Lopandic and Mag. J. Kraus for their helpful discussion. This work was supported by ÖAD (Österreichischer Akademischer Austauschdienst).

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