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FUNGI COLONIZING THE SOIL AND ROOTS OF TOMATO (*Lycopersicum esculentum* Mill.) PLANTS TREATED WITH BIOLOGICAL CONTROL AGENTS

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A b s t r a c t

Tomato plants, cv. Rumba Ożarowska, grown in the greenhouse of the University of Warmia and Mazury, were protected in the form of alternate spraying (twice) and watering (twice) with 5% aqueous extracts of the following plant species: *Aloe vulgaris* Lam., *Achillea millefolium* L., *Mentha piperita* L., *Polygonum aviculare* L., *Equisetum arvense* L., *Juglans regia* L. and *Urtica dioica* L. Plants not treated with the extracts served as control. After fruit harvest, samples of roots and soil were collected. The roots were disinfected and next placed on PDA medium. Soil-colonizing fungi were cultured on Martin medium. Fungi were identified microscopically after incubation.

Pathogenic fungal species, *Colletotrichum coccodes, Fusarium equiseti, F. oxysporum* and *F. poae*, accounted for over 60% of all isolates obtained from the roots of tomato plants. The soil fungal community was dominated by yeast-like fungi (75.4%), whereas pathogenic fungi were present in low numbers. The applied 5% aqueous plant extracts effectively reduced the abundance of fungi, including pathogenic species, colonizing tomato plants and soil. The extract from *P. aviculare* showed the highest efficacy, while the extract from *J. regia* was least effective. Fungi showing antagonistic activity against pathogens (*Paecilomyces roseum* and species of the genus *Trichoderma*) were isolated in greatest abundance from the soil and the roots of tomato plants treated with *A. millefolium, M. piperita* and *U. dioica* extracts.

Key words: pathogenic fungi, saprotrophic fungi, roots of tomato plants, soil

INTRODUCTION

Plants extracts show potential for biocontrol of the following pathogenic species: *Colletotrichum capsici* (O b a g w u et al. 1997), *Rhizoctonia solani* (S r i v a s t o v a and L a l , 1997; S e h a j p a l et al. 2009), *Phytophthora infestans* (S c h m i t t et. al. 2005), *Erwinia carotovora* ssp. *carotovora* (B d l i y a and D a h i r u , 2006), *Fusarium oxysporum* f. sp. *ciceris* (S a h a y a r a j et al. 2006) and *Verticillium dahliae* (S h o h o u g et al. 2009). In a pot experiment carried out by B u r g i e ł et al. (2008), extracts from plants of the family Apiaceae provided effective control of powdery mildew *Erysiphe* spp. on marigolds *Calendula officinalis*. S l u s a r e n k o et al. (2008) and P o r t z et al. (2008) demonstrated that greenhouse vegetables may be protected against fungus-like organisms (e.g. *Phytophthora* and *Pseudoperonospora*) with allicin from *Allium sativum.* Extracts from *Paeonia suffruticosa, Hedera helix* (R o h n e r et al. 2004), plants of the genus *Cameroon* (G o u f o et al. 2008) and *A. sativum* (P o r t z et al. 2008) showed high efficacy in late blight control in tomatoes. According to K o n s t a n t i n i d o u - D o l t s i n i s et al. (2006), *Reynoutria sachalinensis* extract has a fungistatic effect on *Leveillula taurica*, the causal agent of powdery mildew of tomatoes.

The aim of this study was to determine the quantitative and qualitative composition of fungal communities colonizing the soil and roots of tomato plants treated with aqueous plant extracts.

MATERIALS AND METHODS

Tomato plants *Lycopersicum esculentum* Mill., cv. Rumba Ożarowska, were grown in the greenhouse of the University of Warmia and Mazury in Olsztyn in 2006-2007. Tomato seedlings were planted in pots (one seedling per pot) filled with peat substrate and garden soil at a ratio of 1:3 (total 9 dm^3 of substrate, without inoculum). The experiment involved biological control

(three weeks after seedling planting) in the form of alternate spraying (twice) and watering (twice) at 10-day intervals with aqueous extracts of the following plant species: *Aloe vulgaris* Lam., *Achillea millefolium* L., *Mentha piperita* L., *Polygonum aviculare* L., *Equisetum arvense* L., *Juglans regia* L. and *Urtica dioica* L. (45 ml/plant). Aqueous extracts were prepared from dry plant material (50 g) mixed with water (1 l), except for aloe whose fresh leaves were crushed in a mortar to obtain 5% extract. The experiment was performed in six replications (six pots). Plants not treated with the extracts served as control. After fruit harvest, samples of roots and soil were collected from each pot**.** Following disinfection (with 50% ethanol and 1% sodium hypochlorite), pieces (0.5 cm) were cut from the roots and placed on PDA medium (in 5 replications per treatment – 5 Petri dishes x 6 pieces of roots). Soil samples collected from under each plant (at a depth of up to 5 cm) were placed in dishes and mixed with a rotary motion. 149 g fine sand was mixed thoroughly with 1 g of the resultant fraction in a flask with a rotary motion (for 10 minutes). 300 mm^3 of the mixture was poured with Martin medium (50°C) (Mańka, 1974). Colonies of yeast-like fungi were counted. After 5 days of incubation at 22°C, fungal colonies were transferred to PDA slants for species identification (Booth, 1971; Ellis, 1971; Skirgiełło et al. 1979).

RESULTS AND DISCUSSION

The fungal soil community was more abundant and diverse than the community colonizing the roots of tomato plants. The applied biological control, which involved alternate spraying and watering with aqueous extracts of seven plant species, effectively reduced the abundance of fungi, including pathogenic species, colonizing tomato plants and soil. Fifteen species of filamentous fungi were isolated from the tomato roots (Table 1). Yeast-like fungi accounted for 6.4% of all isolates (Fig. 1a). Among saprotrophic fungi, species of the order Mucorales were isolated in relatively high numbers (*Mortierella alpina, M. isabelina, M. zonata* and *Rhizopus nigricans* – over 14% of all isolates). Fungi showing antagonistic activity against pathogens were represented by *P. roseum, Trichoderma hamatum* and *T. harzianum*. They were isolated in greatest abundance from the roots of tomato plants treated with *A. millefolium, M. piperita, E. arvense* and *U. dioica* extracts (Fig. 1b).

In a study by Jamiołkowska and Wagner (2007), the essential oil from *Thymus vulgaris*, containing thymol and carvacrol, increased the abundance of fungi of the genera *Trichoderma* and *Mucor* on the roots of pepper plants, but it also inhibited the growth of *Alternaria alternata* and *F. oxysporum* on the aboveground parts of plants. According to Osorio et al. (2009), the development of pathogens such as *A. alternata, C. coccodes, F. oxysporum, F. sambucinum, F. solani* and *R. solani* may be inhibited by phenolic extracts from *Carya illineonsis, Punica granatum* and *Larrea tridentate*.

The abundance of pathogenic fungi isolated from the roots of tomato plants ranged from 32% in the treatment with *P. aviculare* extract (a significantly lower number of pathogens was isolated from the tomato roots in this treatment, compared with other treatments – Table 3) to 72% in the control treatment and in the treatment with *J. regia* extract. In all treatments, the predominant species was *C. coccodes* – the only pathogen that colonized the roots of tomato plants treated with *A. vulgaris, M. piperita, P. aviculare* and *J. regia* extracts. Fungi of the genus *Fusarium* were isolated in highest numbers from the roots of control tomato plants (34.5%). Those pathogens are the causal agents of tomato root rot (Byrne et al. 1997; Vatchev and Hadjidimitrov, 2006). In an *in vitro* experiment, Burgieł et al. (2008) noted an inhibitory effect of extracts from plants of the family Apiaceae on the growth of *A. alternata, Botrytis cinerea* and *Fusarium culmorum*. As demonstrated by Sefidkon et al. (2004), extract from the seeds of *Heracleum sosnowskyi* was characterized by the highest activity against *F. culmorum*, due to high concentrations of esters found also in other species of this genus. In an earlier study, Wolski et al. (1996) attributed the fungistatic effects of *H. sosnowskyi* extracts to the production of secondary metabolites such as *coumarins and furanocoumarins*.

The soil fungal community was dominated by yeast-like fungi, which accounted for 75.4% of all isolates from all treatments (Fig. 2a). Among 27 species of filamentous fungi (Table 2), members of the genus *Penicillium* were found in relatively large numbers, in particular in treatments with *J. regia* and *U. dioica* extracts (approximately 18% of all isolates in each treatment). Fungi of the order Mucorales (*M. alpina, M. isabelina, M. vinacea, Mucor hiemalis* and *R. nigricans*) and antagonists of plant pathogens (*P. roseum, T. hamatum, T. harzianum, T. koningii, T. polysporum* and *T. viride*) had a 3.9% and 4.5% share of all isolates, respectively. The latter were most frequently isolated from soil samples collected from under tomato plants sprayed with *A. millefolium, M. piperita* and *U. dioica* extracts (Fig. 2b).

Pathogenic fungal species, including *B. cinerea, C. coccodes, F. oxysporum* and *F. solani*, were seldom isolated from the soil. They were not found in treatments with *P. aviculare* and *E. arvense* extracts. According to Sas-Piotrowska and Piotrowski (1995, 2003), the fungicidal properties of extracts from plants of the family Polygonaceae against *Fusarium* species result from high concentrations of phenolic acids.

Table 1
Fungi isolated from the roots of tomato plants during the investigation period (% of all isolates)

Species	C	Av	Am	Mp	Pa	Ea	Jr	Ud
Aureobasidium pullulans (de Bary) Arnaud						12.5		
Cladosporium cladosporioides (Fres.) de Vries						12.5		
Colletotrichum coccodes (Wallr.) Hughes*	37.9	58.3	38.5	65.3	31.6	45.8	71.4	44.0
Fusarium equiseti (Corda) Sacc. *	6.9		11.5			4.2		8.0
Fusarium oxysporum Schlecht.*	13.8					12.5		12.0
Fusarium poae (Peck.) Wollenweber*	13.8		3.8					
Mortierella alpina Peyronel			7.8	8.7	15.8		4.8	8.0
Mortierella isabelina Oudemans		10.4	3.8		10.5		4.8	
Mortierella zonata Linn. ex Gams			3.8	4.3	15.8			
Paecilomyces roseum (Thom) Samson**						8.3		
Penicillium spp.		6.3			5.3			8.0
Rhizopus nigricans Ehrenberg			11.5		10.5	4.2		
Sporotrichum olivaceum (Link) Fries	10.4	10.4	11.5	4.3			9.5	8.0
Trichoderma hamatum (Bon.) Bain**	3.4		7.8					
Trichoderma harzianum Rifai**	3.4	4.2		8.7				8.0
Yeast-like	10.4	10.4		8.7	10.5		9.5	4.0
Total number of isolates	58	48	52	46	38	48	42	50

Explanations: C – control, Av – *Aloe vulgaris* Lam., Am – *Achillea millefolium* L., Mp – *Mentha piperita* L., Pa – *Polygonum aviculare* L., Ea – *Equisetum arvense* L., Jr – *Juglans regia* L. i Ud – *Urtica dioica* L. * – pathogenic fungi, ** – antagonistic fungi

Table 2
Fungi isolated from soil samples collected from under tomato plants during the investigation period (% of all isolates)

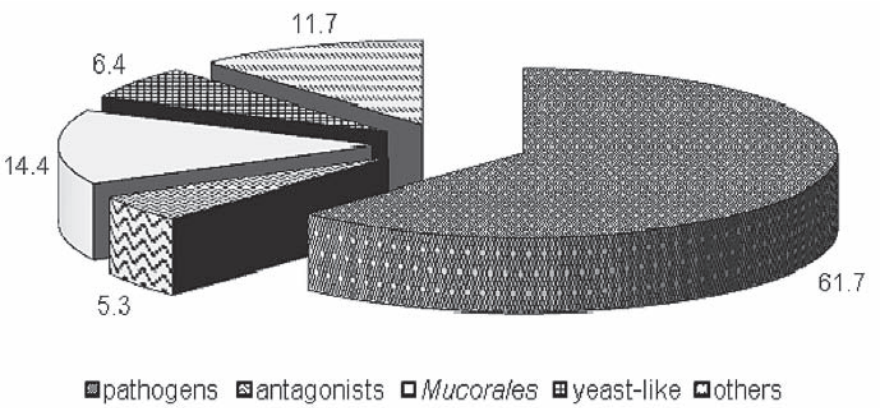
Fungi	C	Av	Am	Mp	Pa	Ea	Jr	Ud
Aureobasidium pullulans (de Bary) Arnaud		1.1		1.0	1.1			
Botrytis cinerea Pers.*	0.7							
Cladosporium cladosporioides (Fres.) de Vries	1.4	1.1						
Colletotrichum coccodes (Wallr.) Hughes*				1.0				
Fusarium equiseti (Corda) Sacc. *	1.4							
Fusarium oxysporum Schlecht.*	1.4	1.1						1.0
Fusarium solani (Mart.) Sacc.*			1.0				3.2	
Gilmaniella humicola Barron	0.7							
Gliomastix murorum (Corda) Hughes	2.1			7.0	1.1	1.3		1.0
Humicola fuscoatra Traaen						1.3		
Humicola grisea Traaen			1.0		1.1	1.3	4.3	
Humicola nigrescens Omvik	2.7	1.1		1.0		1.3		1.0
Mortierella alpina Peyronel	1.4			1.0		1.3	1.1	3.8
Mortierella isabelina Oudemans		1.1	1.0		1.1			1.0
Mortierella vinacea Dixon-Stewart					1.1			
Mucor hiemalis Wehmer							1.1	3.8
Paecilomyces roseum (Thom) Samson**			1.0	5.0				1.0
Papulaspora irregularis Hotson	2.7					1.3		
Penicillium spp.	6.2	1.1	3.0	3.0	11.2	6.3	17.2	18.3
Periconia macrospinosa Lefebvre and Johnson	1.4		1.0				4.3	
Rhizopus nigricans Ehrenberg	0.7	3.4		2.0		2.5	4.3	
Sporotrichum olivaceum Fries	2.1			1.0				
Trichoderma hamatum (Bon.) Bain**			1.0	1.0				4.8
Trichoderma harzianum Rifai**		3.4	3.9		1.1	3.8		1.0
Trichoderma koningii Oudemans**				1.0			2.2	
Trichoderma polyversum (Link ex Pers.) Rifai**			1.0	1.0				
Trichoderma viride Person ex Tries.**			2.9		1.1			
Yeast-like	75.1	85.5	82.2	73.0	81.1	79.6	62.3	63.3
Non-sporulating fungi		1.1	1.0	2.0				
Total number of isolates	292	176	206	198	178	160	186	208

Explanations as in Table 1

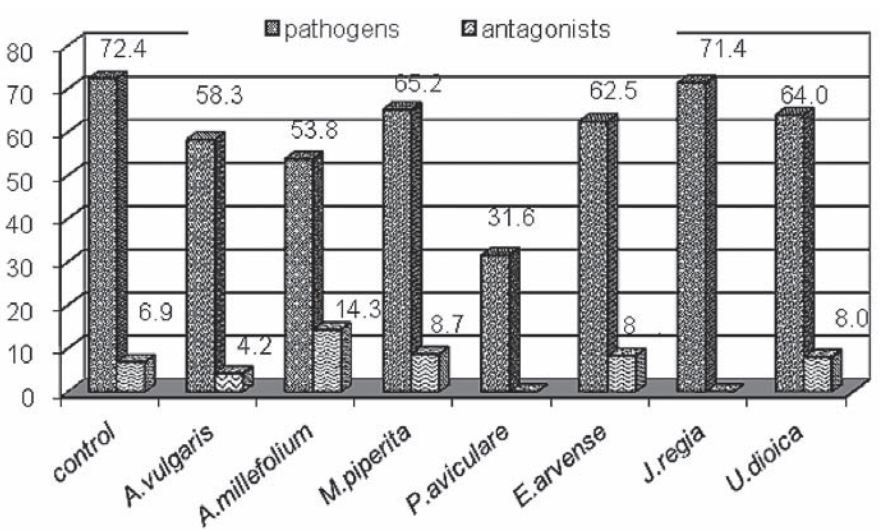
Table 3
Fungi isolated most frequently from tomato organs and soil (mean number of isolates in years)

Treatments	Roots					Soil				
	Path	Antag	Muc	Spor	Yeast	Path	Antag	Muc	Pen	Yeast
Control	21.0a*	2.0a	0c	3.0a	3.0a	5.0a	0d	3.0b	9.0ab	110.0a
Av	14.0ab	1.0a	2.5c	2.5a	2.5a	1.0ab	3.0bcd	4.0ab	1.0b	75.0a
Am	14.0ab	2.0a	7.0ab	3.0a	0a	1.0ab	10.0a	1.0b	3.0b	85.0a
Mp	15.0ab	2.0a	3.0bc	1.0a	2.0a	1.0ab	8.0ab	3.0b	3.0b	72.0a
Pa	6.0b	0a	10.0a	0a	2.0a	0b	2.0cd	2.0b	10.0ab	72.0a
Ea	15.0ab	2.0a	1.0c	0a	0a	0b	3.0bcd	3.0b	5.0b	64.0a
Jr	15.0ab	0a	2.0c	2.0a	2.0a	3.0ab	2.0cd	6.0ab	16.0a	58.0a
Ud	16.0a	2.0a	2.0c	2.0a	1.0a	1.0ab	7.0abc	9.0a	19.0a	66.0a

Explanations as in Table 1, Path – pathogens, Antag – antagonists, Muc – Mucorales, Spor – *Sporotrichum olivaceum*, Yeast – yeast-like, Pen – *Penicillium* spp.
* means with the same letter do not differ significantly (Duncan's test, p=0.01)

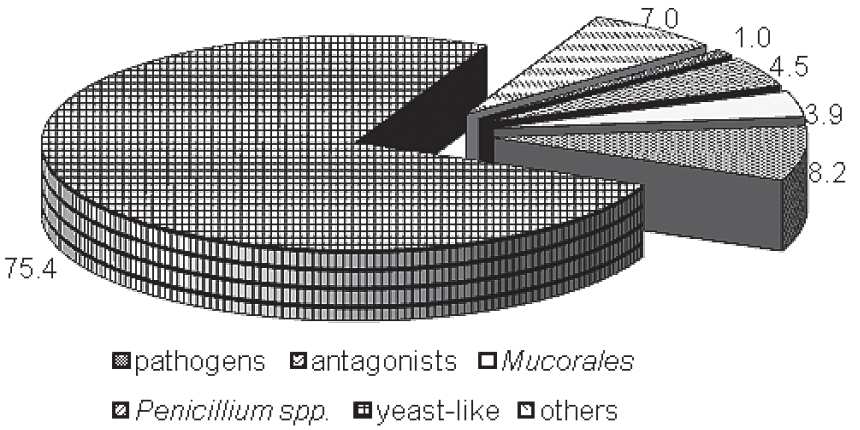


a. fungi isolated most frequently

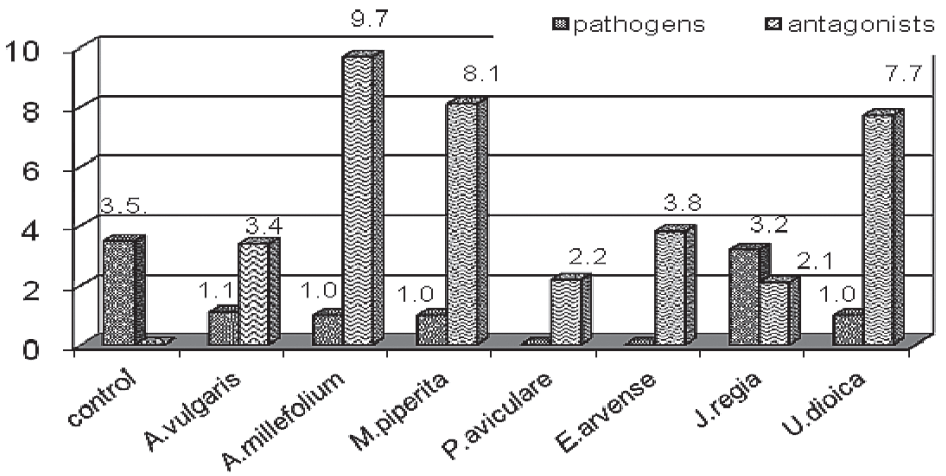


b. pathogenic and antagonistic fungi isolated in individual treatments

Fig. 1. Fungi isolated from the roots of tomato plants during the investigation period (%)



a. fungi isolated most frequently



b. pathogenic and antagonistic fungi isolated in individual treatments

Fig. 2. Fungi isolated from soil during the investigation period (%)

CONCLUSIONS

The applied biological control, which involved alternate spraying and watering with aqueous extracts of seven plant species, effectively reduced the abundance of fungi, including pathogenic species, colonizing tomato plants and soil.

The extract from *Polygonum aviculare* showed the highest efficacy.

C. coccodes and *Fusarium* spp. were isolated in great abundance (over 70%) from the roots of control tomato plants and tomato plants treated with an aqueous extract from *Juglans regia*.

The soil fungal community was dominated by yeast-like fungi, whereas pathogenic fungi were present in low numbers.

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Grzyby kolonizujące korzenie oraz glebę spod uprawy pomidora (*Lycopersicum esculentum* Mill.) chronionego biologicznie

Streszczenie

Pomidor odmiany Rumba Ożarowska uprawiano w szklarni UWM w Olsztynie. Zastosowano ochronę w postaci opryskiwania i podlewania na przemian (po dwa razy) 5% wodnymi wyciągami z następujących gatunków roślin: *Aloe vulgaris* Lam., *Achillea millefolium* L., *Mentha piperita* L., *Polygonum aviculare* L., *Equisetum arvense* L., *Juglans regia* L. i *Urtica dioica* L. W kombinacji kontrolnej rośliny traktowano destylowaną wodą. Po zbiorach owoców pobierano próby korzeni i gleby. Korzenie odkażano i wykładano na podłoże PDA. Hodowlę grzybów glebowych prowadzono na podłożu Martina. Wyrosłe gatunki grzybów identyfikowano mikroskopowo.

Patogeny licznie – ponad 60% udział wśród ogółu izolatów, zasiedlały korzenie pomidora. Wśród nich zidentyfikowano następujące gatunki: *Colletotrichum coccodes, Fusarium concolor, F. oxysporum* i *F. poae*. Udział patogenów w zbiorowisku grzybów glebowych był nieznaczny, natomiast dominowały tam grzyby drożdżopodobne – 75,4% udział. Zastosowane wyciągi wodne w stężeniu 5% ograniczały liczebność grzybów, w tym patogenicznych, zasiedlających korzenie oraz glebę spod uprawy pomidora. Najbardziej skuteczna okazała się ochrona biologiczna z zastosowaniem wyciągu z *P. aviculare*, a najmniej z użyciem wyciągu z *J. regia*. Najliczniejszą populację antagonistów *Paecilomyces roseum* i rodzaju *Trichoderma* w obu omawianych środowiskach stwierdzono w kombinacji z traktowaniem wyciągiem z *A. millefolium, M. piperita* i *U. dioica*.