Fungi colonizing and damaging different parts of peppermint (*Mentha piperita* L.) cultivated in South-Eastern Poland

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S u m m a r y

In 2004–2006 there were studies on fungi colonizing and damaging different plant parts conducted. They were performed on productive plantations of peppermint (*Mentha piperita* L.) grouped in South-Eastern Poland. Fungi were isolated from superficially disinfected roots, rhizomes, stem bases and leaves by means of a mineral medium. Fungi from *Fusarium* spp. including *F. avenaceum, F. equiseti, F. culmorum, F. oxysporum* were obtained in the greatest number from roots showing necrotic symptoms. The *Phoma strasseri* was commonly isolated from rhizomes and stem bases with symptoms of necrosis and tissue disintegration. *Altenaria alternata* was isolated from leaves showing symptoms of necrotic, irregular spots.

Key words: mint, Mentha piperita, pathogenic fungi

INTRODUCTION

The area of herb cultivation in Poland constitutes of about 50% of its cultivated area in the European Union considering the possibilities of our country – an increase of the plantation area of herbs should be taken into account [1]. Peppermint (*Mentha piperita* L.) is one of the leading species cultivated in Poland. Due to the rich and differentiated chemical composition of *folium menthae piperitae* and *herba menthae piperitae*, raw material is used in pharmaceutical, food and perfume industries [2, 3]. Good quality of raw material depends in a large extent on the health state of plants in the conditions of their cultivation. Plant pathogens, espe-

cially fungi, while overgrowing plant tissues, damage secretion cells, which leads to the modification of the composition of volatile fractions in plants [4].

In the United States, the economically important pathogens causing the greatest loss in mint cultivation include *Puccinia menthae, Verticillium daliae, Verticillium albo-atrum* and *Phoma strasseri* [5]. The most dangerous species in India are *Erysiphae cichoracearum, Altenaria alternate* and *Rhizoctonia solani* [3,6]. In former Yugoslavia and the USA *Sphaceloma menthae* causes a premature leaf defoliation [3].

Initial studies carried out in 2003 on a few chosen production plantations of peppermint grouped in the świętokrzyskie province pointed to different fungi which pose a threat to the plant health including species known from literature as being pathogenic to this plant [7]. Considering above mentioned, complex studies were conducted in 2004–2006. The aim was getting to know the fungi colonizing and damaging different parts of peppermint cultivated in South-Eastern Poland.

MATERIAL AND METHODS

The studies conducted in 2004–2006 comprised three plantations of 2-year-old peppermint plants situated in the Fajsławice commune (lubelskie province), while those carried out in 2005–2006 concerned three plantations grouped in the Michałów commune (świętorzyskie province). The forecrop on the examined plantations in the Lublin province most frequently included other herbs such as lemon balm, thyme, marjoram or wheat and beet. On the other hand, in the świętokrzyskie province mint was most often the forecrop. In the period of full vegetation the percentage of plants with necrotic symptoms on the rhizomes, stems and leaves was established. Samples of plants with necrotic symptoms were taken at the beginning of and at full vegetation stage from three places of each plot. Twenty plants were taken for laboratory examinations from each sample and from each plantation. The presence of fungi was assumed on the basis of etiological symptoms observed on the infected plant parts and on the basis of the mycological analysis. Fungi were isolated from the rhizomes, stem base and leaves by means of the method of artificial cultures, with the use of a mineral medium [8]. The plant material was superficially disinfected for 2 min. in 10% sodium hypochlorite and washed 3 times for 3 min. each in sterile distilled water. The obtained fungal colonies, after segregation and after making single-spore cultures, were marked for the species on the maltose medium or on standard media [9-12].

RESULTS

During the studies different disease symptoms on mint plants growing both on the plantations grouped in the Lublin province and those in the świętokrzyskie province were observed. Necrotic, irregular spots, often merged together,

occupying the greater part of the leaf blade occurred on the leaves, especially the lower ones. This symptom increased at full vegetation and then premature defoliation of the leaves took place. Progressing necroses, often accompanied by symptoms of softening and disintegration of tissues were observed on the stems and rhizomes. In the case of stems, necrosis occurred up to height of about 7–10 cm from base, whereas a characteristic narrowing was observed on the border of diseased and healthy tissues. The appearance of these disease symptoms often resulted in disintegration of those parts tissues. Moreover, the reduction of size and quantity of leaves was observed, and those parts were frequently colored red. Etiological signs in the form of picnidia including conidial with the features typical of *Phoma* genus and *P. strasseri* species were found on infected stems and rhizomes. Conidia typical to genera *Fusarium* and *Altenaria* were also observed on the same parts of plants. Besides, symptoms of disease in form of necrotic changes were observed on the main root and on lateral roots of mint. Frequent occurrence of these symptoms, followed by local dying out of plants, was observed especially on two shaded and wet mint plantations in the Świętokrzyskie province which were situated in the lagging of the leafy forests.

The percentage of plants with above-discussed disease symptoms ranged in the studied years from 15 to 35% on plantations in the Lublin province, and from 30 to 60% in Świętokrzyskie province.

In total, 2650 isolates of fungi representing 31 species were isolated from examined plant parts in plantations situated in the lubelskie province. The greatest number of isolates was obtained from the rhizomes and the stem base, slightly fewer from the roots, and the fewest from mint leaves (tab. 1). Fungi from *Fusarium* spp. were obtained from all examined parts and they were isolated in the biggest quantities from roots, rhizomes and stem base, with mean proportion of 60.68%, 43.18% and 48.11%, respectively, of all the fungi obtained from those parts (fig. 1). The fungi also isolated from all examined parts included *Phoma* spp. (tab. 1). *Phoma exigua* var. *exigua* and *Phoma glomerata* were obtained both from roots, rhizomes, as well as stem base and leaves. Within *Phoma* spp., *P. strasseri* was the most frequently occurring species on the rhizomes and the stem base, and the mean proportion of the isolates of this fungus constituted, respectively, 15.15% and 9.50% of all fungi obtained from these parts (fig. 2). Singular isolates of the fungus were also obtained from roots. *Botrytis cinerea* and *Alternaria alternata* were isolated from all examined parts, especially from leaves and stem base, with *A. alternata* as a species isolated most frequently (tab. 1, fig. 1). *Rhizoctonia solani* was isolated from all examined parts, with an exception of leaves (tab. 1, fig. 1). Individual isolates of *Colletotrichum gloeosporioides* were obtained only from peppermint rhizomes (tab. 1).

In total, 2229 isolates of fungi representing 27 species were obtained from examined parts of mint from plantations situated in the Świętokrzyskie province. The greatest number of isolates came from stem base and rhizomes, while slightly fewer from leaves, and the fewest from roots (tab. 1). Fungi from *Fusarium*

spp. were isolated from all examined plant parts. However, they were isolated with the biggest frequency from the roots, the stem base and the rhizomes, with their mean proportion constituting, 66.39%, 41.27% and 38.78%, respectively, of all fungi obtained from these parts (fig. 1). Fungi from *Phoma* spp. were obtained from all analyzed plant parts, while *P. strasseri* was most frequently isolated from rhizomes, stem base and roots. The mean proportion of the isolates of this fungus constituted 35.59%, 29.13% and 4.2% of all fungi obtained from these parts, respectively (fig. 2). *R. solani* was isolated from all studied mint parts, with exception of leaves. Mean proportion of the isolates of this fungus constituted 2.41%, 1.37% and 0.84% of all fungi obtained from stem base, rhizomes and roots, respectively (fig. 2). *A. alternate* was isolated from all examined parts; most frequently isolates of this fungus were obtained from the leaves, and they constituted as much as 94.78% of all fungi obtained from this part (fig. 1). Moreover, singular isolates of *B. cinerea, Cladosporium cladosporoides* and *Epicoccum purpurascens* were obtained from mint leaves (tab. 1). Due to different studies durations plantations in the świętokrzyskie and lubelskie provinces, charts 1 and 2 provide mean proportion of selected fungal species. It was turned out that more *P. strasseri* isolates were obtained from rhizomes, stem base and roots of mint plants coming from the świętokrzyskie province as compared to the lubelskie province, on average. Moreover, more *Fusarium* spp. isolates were obtained from the roots of mint cultivated in the Swiętokrzyskie province than in the Lublin province, on average, but those fungi were isolated similarly from stem base and rhizomes (fig. 1, 2).

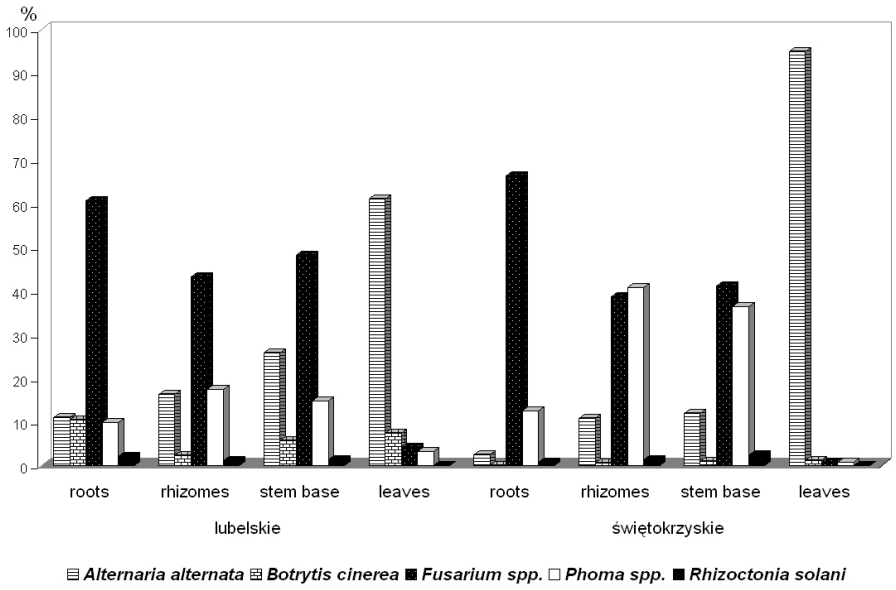

Fig. 1. Means of some fungus isolated from examined organs of mint in 2004-2006

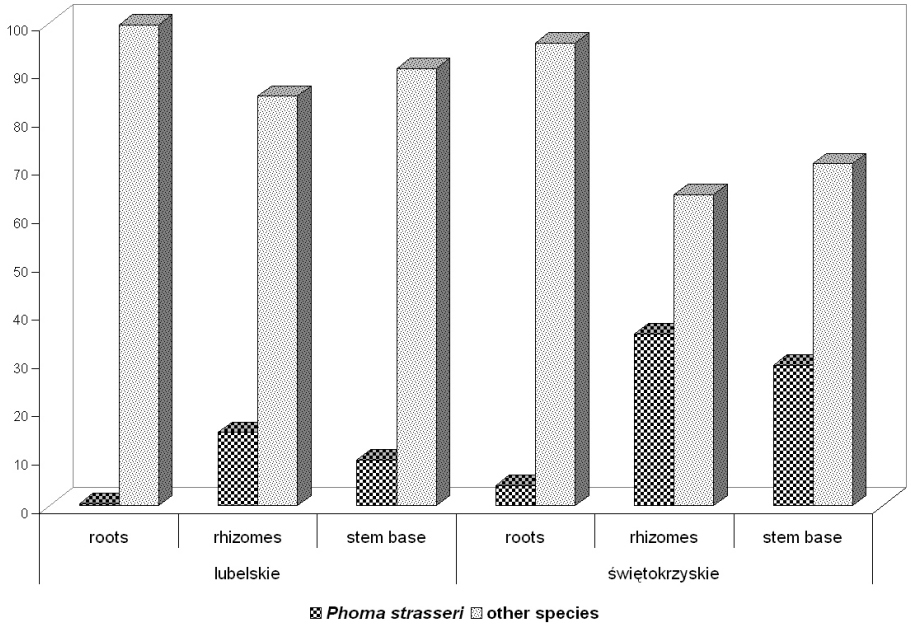

Fig. 2. Mean participation of Phoma strasseri among fungi isolated from mint roots, rhizomes and stem base in 2004-2006

Table 1.

Participation of fungi isolated from various organs of mint in 2004–2006

fungus species	number of isolates									
	lubelskie					świętokrzyskie				
	a	b	c	d	total (%)	a	b	c	d	total (%)
Alternaria alternata (Fr.) Keissler	78	129	204	74	634 (23.92)	12	63	80	471	626 (28.08)
Botrytis cinerea Pers.	3	18	45	27	93 (3.51)		3	5	6	14 (0.63)
Cladosporium cladosporioides (Fres. De Vries)	3		3	18	24 (0.91)		2	8	3	13 (0.58)
Chaetomium globosum Kunze	3				3 (0.11)					
Colletotrichum gloeosporioides (Penz.) Sacc.		6			6 (0.23)					
Cylindrocarpon didymum (Harting) Wollenw.	6	3			9 (0.34)	9	3	3		15 (0.67)
Cylindrocarpon heteronema (Berk. et Br.)	3	3	3		9 (0.34)					
Cylindrocarpon obtusisporum (Cooke et Harkness) Wollenw.	21	3	6		30 (1.13)	25	3	19		47 (2.11)
Epicoccum purpurascens Ehrenberg			6	18	24 (0.91)			2	15	17 (0.76)
Fusarium avenaceum (Fr.) Sacc.	105	90	105	12	312 (11.77)	54	62	51	3	170 (7.63)
Fusarium culmorum (W. G. Smith) Sacc.	102	105	99	3	309 (11.66)	97	68	89		254 (11.39)
Fusarium equiseti (Corda) Sacc.	105	84	108		297 (11.21)	84	56	79		219 (9.83)
Fusarium oxysporum Schlecht.	99	60	69		228 (8.60)	78	42	50		170 (7.63)
Fusarium solani (Mart.) Appel et Wollenw., Snyd et Marasas	15	3			18 (0.68)	3		5		8 (0.36)
Gliocladium catenulatum Gilman et Abbott	6	6	3	6	21 (0.79)	10	13			23 (1.03)
Gliocladium fimbriatum Gilman et Abbott	3	12	9		24 (0.91)	8	5	3		16 (0.72)
Gliocladium roseum Bainier	15	9	6		30 (1.13)					

Mucor hiemalis Bainier	9				9 (0.34)					
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson. Stolk et Hadlok	24	6			30 (1.13)	5	5			10 (0.45)
Phoma capitulum Pawer. Mathur et Thirumalachar	3	6	9		18 (0.68)	3		2		5 (0.22)
Phoma exigua var. *exigua* Desm.	39	6	18	6	69 (2.60)	18	12	10		40 (1.79)
Phoma eupyrena Sacc.								5	1	6 (0.27)
Phoma glomerata (Cda.) Wollenw. et Hochapf.	15	6	15	3	39 (1.47)	3	8	8		19 (0.85)
Phoma herbarum Westend.						4	4	10		18 (0.81)
Phoma heterodera Chen. Dickson & Kimbrough						11	5	13		29 (1.3)
Phoma labilis Sacc.	9				9 (0.34)					
Phoma pereupyrena de Gruyter. Noordel. & Boerema				3	3 (0.11)					
Phoma strasseri Moesz.	3	120	75		198 (7.47)	20	210	194		424 (19.02)
Rhizoctonia solani Kühn	15	9	9		33 (1.25)	4	8	16		28 (1.26)
Trichoderma aureoviride Rifai	12	6		3	21 (0.79)	3				3 (0.13)
Trichoderma harzianum Rifai		9			9 (0.34)	5	4	2		11 (0.49)
Trichoderma koningii Oud.		54		36	90 (3.39)	13	10	11		34 (1.53)
Trichoderma polysporum (Link ex Pers. Rifai)	6	30			36 (1.36)	2				2 (0.09)
Trichoderma viridae Pers. ex Gray		9		6	15 (0.57)	5	3			8 (0.36)
total	**702**	**792**	**792**	**364**	**2650 (100)**	**476**	**589**	**665**	**499**	**2229 (100)**

a – roots, b – rhizomes, c – base of stems, d – leaves

DISCUSSION

Contemporary studies pointed to occurrence of infectious diseases caused by fungi on peppermint plants cultivated in South-Eastern Poland. Disease symptoms characteristic for pathogens of this group of plants were observed in all analyzed parts. Frequent occurrence of necrosis of roots and stem base, especially on mint plants coming from plantations in the świętokrzyskie province suggests the consequence of the pathogenic effect of soil-borne fungi [13, 14]. It should be supposed that – due to lack of rotation in mint cultivation in those plantations – the soil has been dominated by those species [15]. This is indicated by greater percentage of plants with disease symptoms and greater number of isolates, which – in spite of shorter study period – was similar to the number of isolates obtained from plants cultivated in the lubelskie province. Studies on peppermint plantations carried out by Hindu scientists pointed to the important role of rotation in mint cultivation. A five-year-long break in plant cultivation, when maize or a grass mixture grew on the fields, caused a significant improvement of mint healthiness in next years of cultivation [3].

Results of mycological analysis showed that roots and stem base of examined mint plants were colonized by a complex of pathogens, among which the greatest importance should be ascribed to *Fusarium, R. solani* and *Phoma* spp. Within *Fusarium* spp., the species that most frequently occurred on herbs and which were also isolated in present studies included *F. avenaceum, E. equiseti, F. culmorum* and

F. oxysporum [13, 16, 17]. In India, except *V. dahliae*, *F. oxysporum* was considered the cause of mint wilt [3]. Taking into consideration the gradual climate warming in Poland and the thermophilous character of the fungus, it should be expected that it can become a big danger for mint cultivated in our country. However, only pathogenicity tests can accurately establish the pathogenic character of *Fusarium* spp., including *F. oxysporum*. At present, *F. oxysporum* f.sp. *corianderi, F. oxysporum* f.sp. *cumini* [15] and *F. oxysporum* f.sp. *basilici* have been observed on herbs abroad [18]. In Poland, dying out of lemon balm and thyme germs and seedlings was reported to be caused by *F. culmorum, F. equiseti* and *F. avenaceum* [19, 20].

Root, stem base and rhizome colonization by *R. solani,* shown in present studies, can indicate the harmfulness of this fungus to enumerated parts of peppermint. The pertinence of this thesis can be confirmed by the fact that *R. solani* together with *R. bataticola* and *Thielaviopsis basicola* were considered one of the major factors of dying out of mint roots and rhizomes in India shown in yellowing and necrosis of the plants [3]. Extensive harmfulness of *R. solani* was shown in Poland for ginseng roots and stems [21], and – in the regions where basil is cultivated – for this plant seedlings [16].

P. strasseri was considered to be the major cause of necrosis, the next one is rot of rhizomes and stem base. The fact that fungus cultures were isolated often from dying out parts and that etiological symptoms in the form of picnidia and conidia of *P. strasseri* were present made such a conclusion possible. Moreover, it is known from the literature that discussed species causes a disease called black rot of mint stems and rhizomes. It is considered to be the most dangerous pathogen to this plant in different cultivation areas in the world, with losses resulting from dying out reaching as much as 90% of plants [3, 6, 22]. Common isolation of *P. strasseri* from mint plants coming from two wet plantations situated in the vicinity of a leafy forest in the świętokrzyskie province can confirm the results of Melouk and Horner studies[6]. They experimentally proved that air humidity and bedding as well as temperatures from 15°C to 25°C are the conditions that favor infection, and subsequently disease process development. The results of the studies conducted by American scientists and by the Author (unpublished results) on the growth and sporulation of *P. strasseri* in differentiated thermal conditions suggest that development of first disease symptoms has to be considered already at the beginning of the vegetation, whereas their increasing occurrence at full vegetation, which was observed in present studies. Moreover, it is known from the literature that *P. strasseri* infects through damaged tissue which is typical to facultative fungal pathogens [23]. In field conditions most frequently infection is a result of different kinds of mechanical damage of stem and rhizome tissues, whereas in glasshouse cultivation injuries after stem tops cut and traces after picked up leaves are the fungus' way of penetration [6].

The obtained results also point on the presence of other species from genus *Phoma* in the cultivation environment of mint. The occurrence of *P. exigua* var. *exigua* on the examined plant parts should be considered dangerous. It was shown

that this fungus can cause necrosis of sprouts and seedlings of thyme, lemon balm and St. John's wort (unpublished results).

Species *A. alternata* was recognized as a leading cause of leaf spot and then leaf defoliation. The number of fungus isolates obtained from this part and information from the literature on losses in mint cultivation on plantations in India caused by the defoliation of leaves infected by the discussed species point to it [3]. Moreover, the negative effect on the quality of raw material also results from toxin-forming abilities of that fungus [24].

Colonization of the analyzed mint parts by *B. cinerea* found out in the present studies can indicate the role of fungus in causing plant diseases. It is known that in Italy and Israel this fungus is considered to be one of the most dangerous pathogens, causing rot and dying out of basil leaves and stems [14].

On the other hand, the fact that species from genera *Trichoderma* and *Gliocladium* are obtained from examined mint parts should be considered to be a positive phenomenon since these fungi are known for their antagonistic effect towards different plant pathogens [25, 26]. In Japan selected strains of *T. harzianum* were used in production of free of diseases mint seedlings [3].

The present studies made possible finding fungi colonizing and damaging different parts of peppermint cultivated in South-Eastern Poland. Taking into consideration the results of present studies and information from the literature the occurrence of species *P. strasseri* discovered in Poland in recent years on rhizomes and stems should be viewed as especially dangerous. Finding fungus cultures in enumerated plant parts suggests worse quality and lower quantity of raw material and possibility of pathogen's spread through infected rhizomes. Moreover, on the basis of performed studies and research reports, *A. alternata* should be also considered to be economically important species that can cause considerable losses in mint cultivation.

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GRZYBY ZASIEDLAJĄCE I USZKADZAJĄCE RÓŻNE ORGANY MIĘTY PIEPRZOWEJ (*MENTHA PIPERITA* L.) UPRAWIANEJ W POŁUDNIOWO-WSCHODNIEJ POLSCE

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S t r e s z c z e n i e

W latach 2004–2006 na plantacjach produkcyjnych mięty pieprzowej (*Mentha piperita* L.) zgrupowanych w południowo-wschodniej Polsce przeprowadzono badania nad grzybami zasiedlającymi i uszkadzającymi różne organy roślin. Grzyby izolowano z powierzchniowo odkażonych korzeni, rozłogów, podstawy łodygi oraz liści przy zastosowaniu pożywki mineralnej. Z korzeni wykazujących objawy nekrozy najliczniej otrzymywano grzyby z rodzaju *Fusarium*, w tym gatunki *F. avenaceum, F. equiseti, F. culmorum* i *F. oxysporum*. Z rozłogów i podstawy łodygi z objawami nekrozy i dezintegracji tkanek powszechnie izolowano gatunek *Phoma strasseri*. Z liści wykazujących symptomy w postaci nekrotycznych, nieregularnych plam wyosobniono *Alternaria alternata*.

Słowa kluczowe: mięta, Mentha piperita, grzyby patogeniczne