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EXPERIMENTAL PAPER

Fungi colonizing and damaging different parts of some medicinal plants

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Summary

Introduction: Many fungal species infect medicinal plants during their cultivation, causing great damage to the yield and decreasing the quality of raw material.

Objective: Due to the increase in contamination of raw material and the damage caused by pathogenic fungi, the main species of medicinal plants cultivated in Poland were subject to the investigation.

Methods: In 2012–2014, an experiment was conducted on eight medicinal plant species breeding nurseries grown in the field in Plewiska. The following species and cultivars were investigated: lemon balm, peppermint, St. John's wort cv. 'Topaz', lovage cv. 'Amor', valerian cv. 'Polka', caraway cv. 'Kończewicki', sweet basil cv. 'Wala', marjoram cv. 'Miraż'.

Results: Fourteen species of eleven genera of pathogenic fungi were isolated from the investigated medicinal plants: *Fusarium avenaceum, F. culmorum, F. equiseti, F. oxysporum, Botrytis cinerea, Alternaria alternata, Cladosporium* sp., *Rhizoctonia solani, Septoria* sp., *Boeremia exiqua, Golovinomyces cichoracearum, Penicillium* sp., *Ramularia* sp., and *Sclerotinia sclerotiorum*. The most severe infection was caused by *Fusarium* sp. and *Botrytis cinerea*. Also *Cladosporium* sp. (4 species), *A. alternata* and *Septoria* sp. (3 species) infected plant tissue. Leaves and stems were the most infected parts of the tested plants. Lemon balm, lovage and valerian were the most infected species, while marjoram was infected only with *Fusarium* sp.

Key words: ***medicinal plants, pathogenic fungi, infection***

INTRODUCTION

In Poland, there are many species of medicinal plants cultivated and raw material production is on the increase. Many fungal species infect medicinal plants during their cultivation, causing great damage to yield and decreasing the quality of raw material [1-7]. Additionally, pathogenic fungi produce secondary metabolites (mycotoxins) which are harmful to humans and animals [8]. Growing demands of the medicinal plant industry force raw material to meet a high quality standard, among others, microbiological purity. There are many sources of contamination of raw material with bacteria or fungi such as epiphytic flora, flora originating from plant natural environment, i.e. soil, water or air. But pathogenic microorganisms bringing about medicinal plant diseases are also a real cause of microbiological contamination of medicinal plant raw material. Kędzia [9] stated that raw material that comes from cultivation is more infected with pathogenic microorganisms compared with the one collected in the wild. Contamination of medicinal plant raw material caused by bacteria is rare. It is fungi that infect plants most frequently. Numerous fungi have been reported to infect medicinal plants, among which there are *Botrytis*, *Fusarium*, *Alternaria*, *Cladosporium*, *Ramularia*, *Oidium* and most of them are pathogenic. They are the main cause of diseases of medicinal plants [1-10]. Many pathogenic fungi are polyphages, while some of the species are monophages and can even colonize a specific part of plant e.i. *Septoria melissae* on lemon balm leaves [9, 11].

Due to the increase in contamination of raw material and the damage caused by pathogenic fungi, the main species of medicinal plants cultivated in Poland were subject of the investigation: lemon balm (*Melissa officinalis* L., *Lamiaceae*), peppermint (*Mentha* x *piperita, Lamiaceae*), St. John's wort (*Hypericum perforatum* L., *Hypericaceae*), lovage (*Levisticum officinale* Koch., *Apiaceae*), valerian (*Valeriana officinalis* L., *Valerianaceae*), caraway (*Carum carvi* L., *Apiaceae*), sweet basil (*Ocimum basilicum* L., *Lamiaceae*) and marjoram (*Origanum majorana* L., *Lamiaceae*). The identification of pathogens, evaluation of the risk of infection and above all else, the protection of the cultivated medicinal plants are of great importance as there are no papers and recommendations on protection programs.

MATERIAL AND METHODS

In 2012–2014, an experiment was conducted on eight medicinal plant species breeding nurseries grown in the field in Plewiska near Poznań (52°25'N, 16°58'E). The following species and cultivars of medicinal plants were investigated: lemon balm (*Melissa officinalis* L.), peppermint (*Mentha* x *piperita* L.), St. John's wort (*Hypericum perforatum* L.) cv. 'Topaz', lovage (*Levisticum officinale* Koch.) cv. 'Amor', valerian (*Valeriana officinalis* L.) cv. 'Polka', caraway (*Carum carvi* L.) cv. 'Kończewicki', sweet basil (*Ocimum basilicum* L.) cv. 'Wala', marjoram (*Origanum majorana* L.) cv. 'Miraż'. The analyzed plants of perennial species (lemon balm, peppermint, St. John's wort, lovage and valerian) were investigated in the first, second and third year of cultivation, respectively. The plants of sweet basil and marjoram were cultivated each year as annuals and caraway as a biennial. Eight fields, ca. 300 m^2 (6×50 m) each, were set up in the same type of soil (luvisol) in a complete block. Before the nurseries were established in 2012, the fields had been subjected to identical mineral fertilization, tillage and crop rotation scheme. A moderate intensity of management, in line with good agricultural practice was applied, meeting the high standard demands for medicinal plant raw material and seeds. Thus, no herbicide and fungicide inputs were incorporated and manual weeding methods were used in each case before and throughout the medicinal plant cultivation.

Each year, whole twenty plants of each species were randomly collected in the phase of full flowering. Fragments of leaf, stem, inflorescence and root tissues were randomly taken for identification of pathogenic fungi. Plant material for mycological analysis was rinsed in running water, then disinfected by soaking in 70% solution of ethanol for 1 min. and finally rinsed with distilled water. The disinfected leaves were cut up into pieces 3–5 mm long, the stems into 1 cm long fragments, then epidermal tissue samples were taken. The disinfected inflorescences were divided into single flowers. The disinfected tissue of root 1cm below the root crown was taken, then roots were fragmented into 3-5 mm pieces. Section of tissue were aseptically excised and placed into Petri dishes containing PDA medium within 7 days of incubation at 25°C. Small parts of colonies growing around inocula were transferred into glucose-potato medium. The obtained fungal colonies, after segregation, were identified to the species on selective or standard media (PDA, CLA, maltose medium, Czapek-Dox medium, OA, CA) according to available monographs [12-17].

Ethical approval: The conducted research is not related to either human or animal use.

RESUTS

Fourteen species of eleven genera of pathogenic fungi were isolated from the investigated medicinal plants: *Fusarium avenaceum, F. culmorum, F. equiseti, F. oxysporum, Botrytis cinerea, Alternaria alternata, Cladosporium* sp., *Rhizoctonia solani, Septoria* sp., *Boeremia exigua, Golovinomyces cichoracearum, Penicillium* sp., *Ramularia* sp., and *Sclerotinia sclerotiorum* (tab. 1-8). The most severe infection was caused by *Fusarium* sp. (6 species infested) and *Botrytis cinerea* (5 species). Also *Cladosporium* sp. (4 species), *A. alternata* and *Septoria* sp. (3 species) infected plant tissue. Leaves and stems were

Table 1.

Number of colonies on plant parts of lemon balm (Plewiska 2012–2014)

Pathogen	Plant parts			
	leaves	stem	inflorescence	roots
2012				
Septoria melissae	48	–	–	–
Alternaria alternanta	26	–	–	–
Cladosporium sp.	–	28	–	4
Rhizoctonia solani	10	18	–	–
Fusarium equiseti	–	10	–	–
Fusarium culmorum	–	8	–	–
Fusarium oxysporum	7	11	–	9
Boeremia exigua	10	8	–	–
2013				
Septoria melissae	23	–	–	–
Cladosporium sp.		–	–	11
Rhizoctonia solani	35	39	–	–
Fusarium culmorum	–	13	–	–
Fusarium oxysporum	10	12	–	22
2014				
Septoria melissae	33	–	–	–
Alternaria alternanta	38	–	–	–
Cladosporium sp.	–	15	–	8
Rhizoctonia solani	22	34	–	–

Table 2.

Number of colonies on plant parts of peppermint (Plewiska 2012–2014)

Pathogen	Plant parts			
	leaves	stem	inflorescence	roots
2012				
Septoria sp.	40	–	–	–
Botrytis cinerea	31	24	–	–
Alternaria alternara	22	26	–	–
Rhizoctonia solani	10	3	–	–
2013				
Septoria sp.	28	–	–	–
Rhizoctonia solani	–	6	–	–
2014				
Septoria sp.	22	–	–	–
Botrytis cinerea	31	–	–	–

Table 3.

Number of colonies on plant parts of St. John's wort (Plewiska 2012–2014)

Pathogen	Plant parts			
	leaves	stem	inflorescence	roots
2012				
Fusarium culmorum	–	9	29	4
Cladosporium sp.	–	3	24	–
Boeremia exigua	5	8	–	–
2013				
Fusarium culmorum	–	5	17	6
Cladosporium sp.	–	7	20	–
Boeremia exigua	10	17	–	–
2014				
Fusarium sp.	15	8	–	–
Cladosporium sp.	–	23	29	–
Boeremia exigua	7	26	–	–

Table 4.

Number of colonies on plant parts of lovage (Plewiska 2012–2014)

Pathogen	Plant parts			
	leaves	stem	inflorescence	roots
2012				
Alternaria alternata	–	8	–	–
Botrytis cinerea	–	6	34	–
Cladosporium sp.	20		–	–
Septoria sp.	18	9	–	–
Golovinomyces cichoracearum	–	7	–	–
2013				
Alternaria alternata	–	4	–	–
Cladosporium sp.	15		–	–
Septoria sp.	13	6	–	–
2014				
Alternaria alternata	–	17	–	–
Botrytis cinerea	–	–	27	–
Cladosporium sp.	14	–	–	–

Table 5.

Number of colonies on plant parts of valerian (Plewiska 2012–2014)

Pathogen	Plant parts			
	leaves	stem	inflorescence	roots
2012				
Botrytis cinerea	24	–	37	–
Fusarium oxysporum	12	–	–	–
Penicillium sp.	7	–	19	–
Ramularia sp.	9	6	–	–
Sclerotinia sclerotiorum	–	27	–	–

Pathogen	Plant parts			
	leaves	stem	inflorescence	roots
2013				
Fusarium oxysporum	11	–	–	–
Ramularia sp.	2	8	–	–
Sclerotinia sclerotiorum	–	12	–	–
2014				
Botrytis cinerea	34	–	33	–
Fusarium oxysporum	7	–	–	–

Table 6.

Number of colonies on plant parts of sweet basil (Plewiska 2012–2014)

Pathogen	Plant parts			
	leaves	stem	inflorescence	roots
2012				
Botrytis cinerea	33	12	–	–
Cladosporium sp.	16	10	–	–
Fusarium oxysporum	–	5	–	18
2013				
Cladosporium sp.	6	5	–	–
2014				
Botrytis cinerea	19	–	–	–
Fusarium oxysporum	–	11	–	–

Table 7.

Number of colonies on plant parts of caraway (Plewiska 2012–2014)

Pathogen	Plant parts			
	leaves	stem	inflorescence	roots
2012				
Botrytis cinerea	23	–	34	–
Fusarium oxysporum	–	10	–	–
2013				
Fusarium oxysporum	–	13	–	–
2014				
Botrytis cinerea	29	–	–	–
Fusarium oxysporum	–	6	–	–

Table 8.

Number of colonies on plant parts of marjoram (Plewiska 2012–2014)

Pathogen	Plant parts			
	leaves	stem	inflorescence	roots
2012				
Fusarium sp.	38	–	–	–
2013				
Fusarium sp.	21	–	–	–
Fusarium sp.	33	–	–	–

the most infected parts of the tested plants. Lemon balm, lovage and valerian were the most infected species, while marjoram was infected only with *Fusarium* sp.

In all years of evaluation, plants of lemon balm were infected with *Septoria melissae*, *Cladosporium* sp. and *Rhizoctonia solani* (tab. 1). The most infected parts of lemon balm plant were leaves attacked by *S. melissae*, *R. solani*, *A. alternata*, *P. exiqua* and *F. oxysporum* and stems infected with *Cladosporium* sp., *R. solani*, *F. equiseti*, *F. culmorum*, *F. oxysporum* and *B. exigua*, whereas lemon balm inflorescences were free of infection and roots were attacked only by *Cladosporium* sp., and *F. oxysporum*. In 2012, on lemon balm leaves the highest number of *S. melissae* colonies (48) occurred, while in 2013, the infection of *R. solani* was dominant (35), and, in 2014, the predominant species infecting leaves was *A. alternata* (38). In 2012, *Cladosporium* sp. colonies were the highest in number on lemon balm stems, then, in 2013 and 2014, *R. solani* occurred in the highest number (39 and 34, respectively). In 2013, lemon balm roots were infected mostly with *Fusarium oxysporum* (22 colonies).

Septoria sp. (each year), *R. solani* and *B. cinerea* (in two different years) were the most frequently occurring fungi on peppermint plants (tab. 2). Additionally, in 2012, the presence of *A. alternata* was noticed. The fungal diseases' symptoms appeared only on peppermint leaves and stems, while inflorescences and roots were free of infection. *Septoria* sp. occurred on leaves and *R. solani, B. cinerea* and *A. alternata* were isolated from both leaves and stems. In 2012 and 2013, on peppermint leaves occurred the highest number of *Septoria* sp. colonies (40 and 28, respectively), while in 2014, the infection with *Botrytis cinerea* was dominant (33). In 2012, the high number of *B. cinerea* colonies were also observed on peppermint leaves (31). In 2012, the low incidence of *A. alternata* was noticed on peppermint stems (26 colonies), and also colonies of *B. cinerea* occurred in high number (24) on peppermint stems.

F. culmorum, Cladosporium sp. and *B. exiqua* were identified in all years of the study on St. John's wort plants (tab. 3). *F. culmorum* was detected on all analyzed plant parts, while *Cladosporium* sp. occurred on stems and inflorescences and *B. exigua* on leaves and stems. In 2013, on St. John's wort leaves occurred the highest number of *B. exigua* colonies (10), while in 2014, the infection with *Fusarium* sp. was dominant (15). In 2013 and 2014, on St. John's wort stems the colonies of *B. exigua* were dominant (17 and 26, respectively) and, in 2014, the high number of *Cladosporium* sp. colonies were noticed (23). In all the investigated years, *Cladosporium* sp. occurred in the highest number on inflorescences (24, 20 and 29 colonies, respectively). Additionally, in 2012, the inflorescences were infected with *F. culmorum* (29 colonies).

In each year of the investigation, *A. alternata* and *Cladosporium* sp. were the predominant species among other fungi which occurred on lovage plants (tab. 4), while *Septoria* sp. and *B. cinerea* were isolated from leaves, stems and inflorescences in two different years. Only in 2012, *G. cichoracearum* was identified on stems. In all the analyzed years, on lovage leaves occurred the highest number of *Cladosporium* sp. colonies (20, 15 and 14, respectively). In 2012 and 2013, *Septoria* sp. frequently infected lovage stems (9 and 6 colonies respectively), while in 2014, *A. alternata* colonies occurred in the highest number (17). In 2012 and 2014, lovage inflorescences were infected with *B. cinerea* (34 and 27, respectively).

Leaves of valerian were invaded by *F. oxysporum* each year (tab. 5). In 2012 and 2013, *Ramularia* sp. was isolated from leaves and stems and *Sclerotinia sclerotiorum* was identified only on stems of valerian plants, while in 2012 and 2014, *Botrytis cinerea* occurred on leaves and inflorescences. *Penicillium* sp. was isolated sporadically from valerian leaves and inflorescences. No fungi were identified on the roots of valerian plants. In 2012 and 2014, on valerian leaves occurred the highest number of *B. cinerea* colonies (24 and 34, respectively), while, in 2013, the infection with *F. oxysporum* was dominant (11). In 2012 and 2013, *S. sclerotiorum* colonies were the highest in number on valerian stems (27 and 12, respectively). In 2012 and 2014, valerian inflorescences were infected mostly with *B. cinerea* (37 and 33, respectively).

B. cinerea, Cladosporium sp. and *F. oxysporum* occurred on sweet basil plants: in 2012 and 2014 *B. cinerea* and *F. oxysporum* and *Cladosporium* sp. in 2012 and 2013 (tab. 6). Sweet basil leaves were contaminated by *B. cinerea* and *Cladosporium* sp., while stems were infected with all the fungi. *F. oxysporum* was detected on sweet basil roots only in 2012. Inflorescences of sweet basil were free of pathogens. In 2012 and 2014, *B. cinerea* colonies occurred on sweet basil leaves in the highest number (33 and 19, respectively). In 2012, *B. cinerea* colonies were the highest in number on sweet basil stems (12), while, in 2014, *F. oxysporum* colonies occurred in the highest number (11). In 2012, sweet

basil roots were infected mostly with *F. oxysporum* (18 colonies).

The stems of caraway were invaded by *F. oxysporum* each year (tab. 7). The number of identified colonies of *F. oxysporum* on caraway stems was 10, 13 and 6 in three consecutive years. In 2012 and 2014, *B. cinerea* appeared on leaves (23 and 29 colonies, respectively) and inflorescences were infected only in 2012 (34 colonies), while roots were free of infection.

Each year, the leaves of marjoram were infected with *F. oxysporum* (tab. 8). The number of *F. oxysporum* colonies was 38, 21 and 33 in the consecutive years.

DISCUSSION

The similar results of pathogenic fungi occurrence on lemon balm plants were reported by Machowicz-Stefaniak *et al.* [18], who analyzed fungi colonized herb of lemon balm and thyme. They observed that lemon balm leaves were colonized by *A. alternata* and *S. melissae*, which caused many black spots. They also isolated fungi of *Fusarium* sp. (*F. avenaceum, F. culmorum, F. equiseti* and *F. oxysporum*) from lemon balm roots and the stem base in the second year of cultivation. Also Fróżyńska-Jóźwiak and Andrzejak [10] isolated *F. avenaceum* and *F. oxysporum* colonies on lemon balm roots and the stem base. Similar to our results, they also found lemon balm leaves to be infected with *Septoria melissae.* Fróżyńska-Jóźwiak and Andrzejak [10] isolated *B. cinerea* from lemon balm leaves and stems, while we did not.

Fróżyńska-Jóźwiak and Andrzejak [10] identified *F. oxysporum, F. culmorum* and *R. solani* on peppermint roots and the stem base, whereas no species of *Fusarium* were isolated from peppermint plants in our study, though peppermint leaves and stems were found to be infected with *R. solani*. Also Zimowska [3] isolated four species of *Fusarium*: *F. avenaceum, F. equiseti, F. culmorum* and *F. oxysporum* on peppermint roots, but also *Ph. strasseri*, which was found both on roots and stem base. She also isolated *A. alternata* from leaves showing symptoms of necrotic irregular spots.

Zimowska and Machowicz-Stefaniak [1], who examined one-year-old and two-year-old plantations of St. John's wort cv. 'Topaz' found disease symptoms caused by complex of pathogenic fungi as: *Fusarium* spp., *R. solani*, *B. cinerea* and *B. exigua*. While, in our study *Cladosporium* sp. was isolated from St. John's wort plants, except *F. culmorum* and *B. exigua*.

In our investigation, the analyzed parts of caraway plants were infected mainly with *B. cinerea* and *F. oxysporum*. Unfortunately, we did not examine caraway fruit, while Stojanovic *et al.* [6] isolated 24 fungi from caraway fruit. *A. alternata* and *A. solani* were the dominant species, which contaminated up to 75% of caraway fruit. The different species of *Fusarium* (among others F. *oxysporum*) were identified on 2-7% of caraway fruit. They also detected *B. cinerea*, *S. sclerotiorum*, *Coletotrichum* sp. and others. The authors analyzed only caraway fruit, excluding other parts of the plant. Fróżyńska-Jóźwiak and Andrzejak [10] identified *B. cinerea* on caraway leaves and stems, while in our research *B. cinerea* was the predominant pathogenic fungi on caraway leaves and inflorescences.

In our examination occurrences of *Fusarium* sp. were detected on different parts of the plants of lemon balm, St. John's wort, valerian, sweet basil, caraway and marjoram. Filoda *et al.* [11], who detected occurrences of *Fusarium* fungi on 12 medicinal plant species. The authors also found St. John's wort plants to be infected with *F. avenaceum* and *F. equseti*. In our detection, in 2012 and 2013, only *F. culmorum* was found on the St. John's wort stems, inflorescences and roots. *F. equseti* and *F. solani* were more frequent than other species and infected mainly roots and the base of stems. In our investigation, *F. oxysporum* was noticed on roots of lemon balm and sweet basil, while *F. culmorum* was observed on St. John's wort roots. Fróżyńska-Jóźwiak and Andrzejak [10] identified *F. culmorum* on St. John's wort roots and stem base, while in our experiment this species was found on stems, inflorescences and roots. Fróżyńska-Jóźwiak and Andrzejak [10] also found St. John's wort root to be infected with *Rizoctonia solani*.

Filoda *et al.* [11] found *F. equseti, F. oxysporum, F. redolens, F. sambucinum* and *F. solani* on marjoram plants, while Fróżyńska-Jóźwiak and Andrzejak [10] identified only *F. culmorum* on marjoram roots. In our study, the genus of *Fusarium* sp. was isolated from marjoram leaves.

CONCLUSION

1. Fourteen species of eleven genera of pathogenic fungi were isolated from different parts of eight species of medicinal plants.
2. *Fusarium* sp. and *Botrytis cinerea* caused the

most severe infection on most of the investigated medicinal plants.
3. Leaves and stems were the most infected parts of the tested plants.
4. Lemon balm, lovage and valerian were the most infected species.

Conflict of interest: Authors declare no conflict of interest.

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Grzyby patogeniczne zidentyfikowane na wybranych gatunkach roślin zielarskich

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Streszczenie

Wstęp: Grzyby patogeniczne porażające rośliny zielarskie w czasie uprawy powodują straty w plonie oraz obniżają jakość uzyskanego surowca.

Cel: Przedmiotem badań była identyfikacja grzybów chorobotwórczych występujących na uprawnych roślinach zielarskich i określenie stopnia ich porażenia.

Metody: W latach 2012–2014 badano występowanie grzybów patogenicznych na roślinach ośmiu gatunków roślin zielarskich. Materiał do badań pochodził ze szkółek hodowlanych założonych w Plewiskach. Badania obejmowały następujące gatunki i odmiany: melisa lekarska, mięta pieprzowa, dziurawiec zwyczajny odm. 'Topaz', lubczyk ogrodowy odm. 'Amor', kozłek lekarski odm. 'Polka', kminek zwyczajny odm. 'Kończewicki', bazylia pospolita odm. 'Wala' oraz majeranek ogrodowy odm. 'Miraż'.

Wyniki: Z pobranego materiału roślinnego wyizolowano czternaście gatunków należących do jedenastu rodzajów grzybów patogenicznych: *Fusarium avenaceum, F. culmorum, F. equiseti, F. oxysporum, Botrytis cinerea, Alternaria alternata, Cladosporium* sp., *Rhizoctonia solani, Septoria* sp., *Phoma exigua, G. cichoracearum, Penicillium* sp., *Ramularia* sp. oraz *Sclerotinia sclerotiorum.* Najczęściej występującymi grzybami były *Fusarium* sp. i *Botritis cinerea*, podczas gdy *Cladosporium* sp., *A. alternata* i *Septoria* sp. obserwowano w mniejszym stopniu. Najbardziej porażone przez grzyby były liście i łodygi badanych gatunków. Największa liczba grzybów patogenicznych występowała na roślinach melisy lekarskiej, lubczyku ogrodowego oraz kozłka lekarskiego.

Słowa kluczowe: ***rośliny zielarskie, grzyby patogeniczne, porażenie***