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FUNGI THREATENING THE CULTIVATION OF OREGANO (*Origanum vulgare* L.) IN SOUTH-EASTERN POLAND

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Abstract. Studies on diversity of fungi colonizing and damaging different plant parts of oregano (*Origanum vulgare* L.) were carried out in 2012–2014 on production plantations grouped in south-eastern Poland. Fungi were isolated from superficially disinfected roots, stems and leaves using mineral medium. Fungi from *Fusarium* spp., *Boeremia exigua* var. *exigua* and *Rhizoctonia solani* were obtained from the roots and stems showing necrosis and tissue disintegration. *Alternaria alternata* and *Stemphylium botryosum* were isolated from the leaves with symptoms of irregular, necrotic spots. *Colletotrichum fuscum* was commonly obtained from the leaves showing symptoms necrotic, concentrically zoned spots with lighter center and the slightly raised edge. This species had not been found in Poland earlier.

Key words: herbaceous plants, fungi, herb diseases, oregano, occurrence

INTRODUCTION

South-eastern Poland is the region where the climate and soil conditions, as well as the tradition passed down from generation to generation caused that different species of herbs are grown here, and in recent years *Origanum vulgare* L., commonly called oregano is an increasingly readily cultivated species [Machowicz-Stefaniak et al. 2002, Machowicz-Stefaniak et al. 2004, Zimowska 2008a b, Zimowska 2010a b]. A growing interest in oregano is due to the wide spread use of *Herba Origani*, which is commonly used in Poland and in the world in the food industry. Therefore, due to the antifungal and antibacterial activity of oregano oil, which is rich in phenolic compounds, ie. carvacrol and thymol, it is applicable in medicine. The concentration of herbaceous plantations in this region of Poland causes their frequent return to the same field, which con-

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tributes to the accumulation of pathogenic fungi in the environment, which decrease the quality and quantity of the yield [Zimowska 2007]. There is little information in the literature about the pathogens which cause diseases of oregano. In the United States, the fungi from genus *Pythium* causing the symptoms of roots rot were observed, while fungi of the genera: *Alternaria, Botrytis, Helminthosporium* and *Stemphylium* were noticed on the leaves showing such symptoms such as necrotic spots [Farr et al. 1995].

The present paper discusses the results of three-year-long studies on fungi colonizing and damaging the underground and aboveground parts of oregano and they are at continuations of a cycle of studies on mycosis of herbal plants from *Labiateae* family cultivated in south-eastern Poland.

MATERIAL AND METHODS

The studies were conducted in the years 2012–2014 on three two-year-old production plantations of oregano in the communes of Fajsławice and Suchodoły in the Lublin province. The forecrop on those plantations were usually other herbs, e.g. lemon balm, common thyme and sage. Each year the percentage of plants with disease symptoms was established twice during the vegetation period, directly on the plants. Plants with visible disease changes were taken for analysis in the laboratory. The fungi presence was determined on the basis of etiological signs occurring on the infected plant parts and on the basis of the mycological analysis. The fungi were isolated from the superficially disinfected roots, the stems up to the height of 10 cm from the base and from the leaves. A 10% solution of sodium hypochlorite was used for disinfection, while a mineral medium (0.7 g NH_4NO_3, 0.3 g KH_2PO_4, 0.3 g $MgSO_4 \times 7\ H_2O$, 0.01 g $FeCL_3 \times 6\ H_2O$, 0.01 g $ZnSO_4 \times 7\ H_2O$, 0.01 g $CuSO_4 \times 7\ H_2O$, 0.01 g $MnSO_4 \times 5\ H_2O$, 38 g saccharose, 20 g agar, 1000 ml H_2O) was used for isolation [Zimowska and Machowicz-Stefaniak 2004]. After the fungi were taken to one-spore cultures, they were taxonomically identified on malt agar medium (MA; Difco Laboratories, Detroit, USA) or on other media [Saccardo 1878, Rifai 1969, Ellis 1971, Ramirez 1982, Nelson et al. 1983, Boerema et al. 2004].

RESULTS

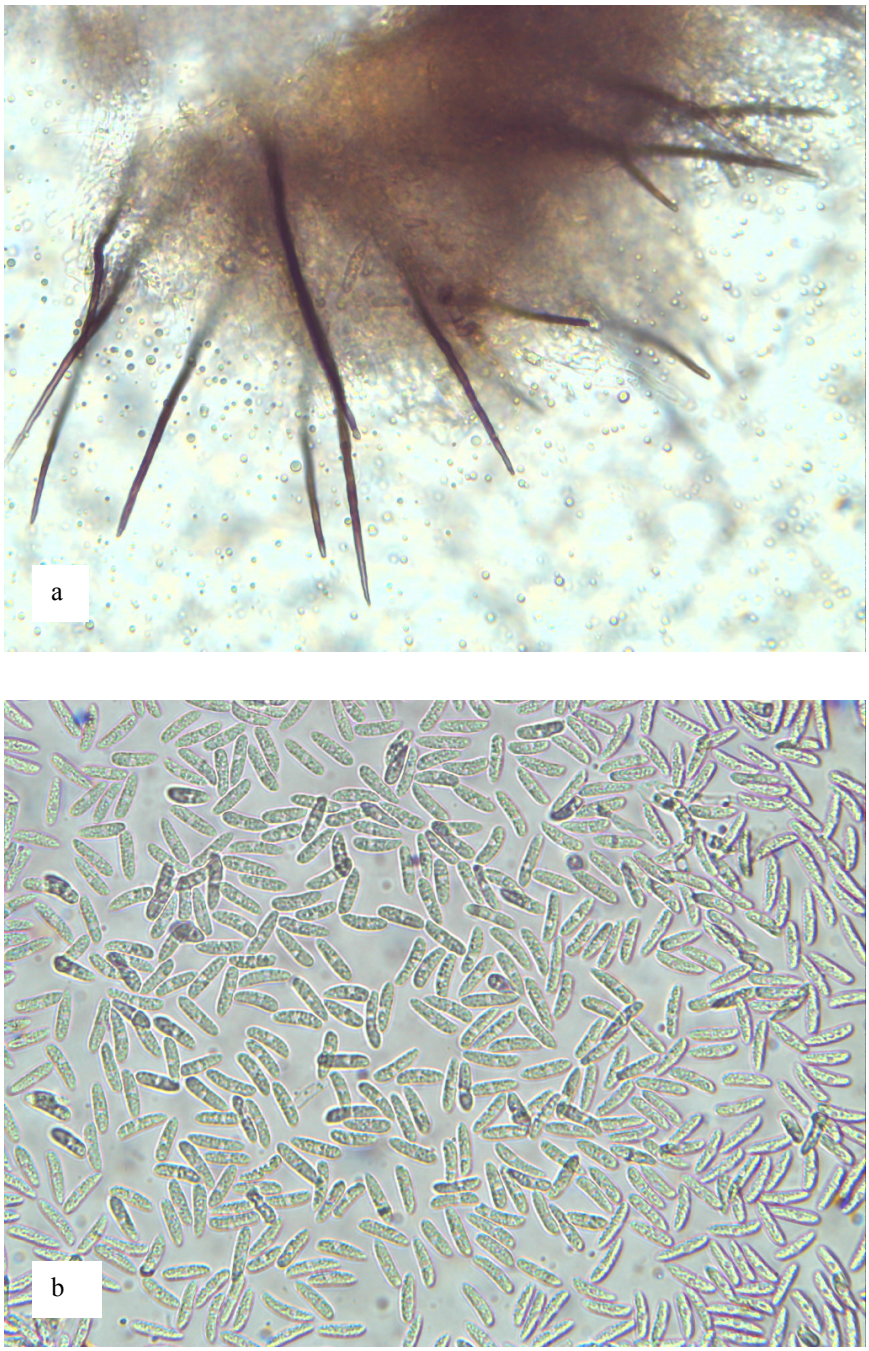
The studies pointed to the occurrence of different disease symptoms on the plants of oregano. Three types of disease symptoms were observed on the leaves. One of them were necrotic, irregular spots, often limited by leaves veins (phot. 1). Etiological signs were found in the place of such spots in the form of conidiophores and conidia typical of the species *Alternaria alternata.* The second type of disease symptoms observed on the leaves were oblong, necrotic spots that formed on the sides and tops of the leaf blade (phot. 2), where etiological signs in the form of conidiophores and conidia characteristic of species *Stemphylium botryosum* (phot. 3) were found. The third type of symptoms were necrotic, concentrically zoned spots with a lighter center and a slightly raised edge (phot. 4). On the surface of such spots acervuli including conidia typical of genus *Colle-*

Phot. 1. Necrotic, irregular spots from which *Alternaria alternata* was isolated. Photo B. Zimowska

Phot. 2. Oblong, necrotic spots on the shore and on the tops of the leaf blade from which *Stemphylium botryosum* was isolated. Photo B. Zimowska

Phot. 3. Conidia of *Stemphylium botryosum* × 500 Photo E. Zalewska

Phot. 4. Necrotic, concentrically zoned spots with lighter center and the slightly raised edge from which *Colletotrichum fuscum* was isolated. Photo B. Zimowska



Phot. 5. *Colletotrichum fuscum*; a – acervuli × 250; b – conidia × 450. Photo B. Zimowska

Phot. 6. Collar and rots with symptoms of necrosis and decay of tissues from witch *Fusarium* spp., *Rhizoctonia solani* and *Boeremia exigua* var. *exigua* were isolated. Photo B. Zimowska

totrichum were found (phots 5a b). All types of disease symptoms were observed both during the first analysis of plants, i.e. at the beginning of vegetation, and in full vegetation, with an increased number of spots on the leaves during the second observation. In addition, such spots merged together, which led to the drying out of the leaves and their premature defoliation. Necrosis and tissue decay were observed on the root collar and on the lower part of the stems (phot. 6). The roots of such plants showed similar disease symptoms. The percentage of plants with the above-mentioned disease symptoms ranged in the years 2009–2011 from 15 to 25% at the beginning of the vegetation to 20 to 47% at full vegetation. Particularly high severity of disease symptoms was observed in the second and third years of the study, when the average air temperature was higher than the long-term average and rainfall during the early growing season exceeded the standard long-term average (tab. 1).

Table 1. Comparison of average value of monthly temperature of air and rainfalls with average many years in vegetation periods in 2012–2014

Month	Means of the years 1963–1992		Difference of mean air temperature in comparison with means of the years			Percentage of the average annual rainfalls		
	air temperature (°C)	rainfalls (mm)	2012	2013	2014	2012	2013	2014
May	13.3	60.9	0.75	6.2	4.7	-28.3*	48.3	180.2
June	16.4	78.3	0.8	6.2	4.0	-36.7*	35.5	-2.1*
July	17.8	77.9	3.0	5.8	8.1	-36.1*	10.7	-1.8*
August	17.3	69.3	1.65	7.3	5.5	-25.0*	-52.5*	-12.7*
September	13.1	36.0	2.2	3.0	6.1	2.4	5.1	-9.5*

* – values with minus are low than the means of the years

Table 2. Fungi isolated from plants of oregano (*Origanum vulgare* L.) in 2012–2014

Fungus species	Number of isolates			Total number and (%)
	roots	stem	leaves	
Alternaria alternata (Fr.) Keissler	12	35	89	136 (13.56)
Boeremia exigua var. *exigua* (Desm.) Avescamp, Gruyter&Verkley, comb. nov.	21	9	5	35 (3.49)
Botrytis cienerea Pers.	4	15	21	40 (3.99)
Cladosporium cladosporioides (Fresen.) de Vries	–	7	10	17 (1.69)
Chaetomium globosum Kunze	–	2	13	15 (1.50)
Colletotrichum fuscum Laub.	–	–	118	118 (11.76)
Epicoccum nigrum Link	–	–	15	15 (1.50)
Fusarium avenaceum (Fr.) Sacc.	31	18	5	54 (5.38)
Fusarium culmorum (W.G.Smith) Sacc.	29	38	11	78 (7.78)
Fusarium equiseti (Corda) Sacc.	48	24	10	82 (8.18)
Fusarium oxysporum Schlecht.	28	10	–	38 (3.79)
Gliocladium fimbriatum Gilman & Abbott	9	8	10	27 (2.70)
Gliocladium roseum Bainier	13	12	12	37 (3.69)
Mucor hiemalis Wehmer	7	–	2	9 (0.89)
Penicillium chrysogenum Thom	7	2	2	11 (1.09)
Phoma leonuri Letendre	–	15	8	23 (2.30)
Phoma multirostrata var. *macrospora* Boerema	15	7	9	31 (3.09)
Phoma versabilis Boerem, Loer. & Hamers	–	10	6	16 (1.59)
Stemphylium botryosum Wallr.	3	15	27	45 (4.49)
Rhizoctonia solani Kühn	27	11	–	38 (3.79)
Talaromyces flavus (Klöcker) Stolk & Samson	11	5	–	16 (1.60)
Trichothecium roseum (Pers.) Link	–	5	7	12 (1.20)
Trichoderma harzianum Rifai	10	9	16	35 (3.49)
Trichoderma koningii Oud.	4	14	19	37 (3.69)
Trichoderma viride Pers.	1	10	27	38 (3.78)
Total	280	281	442	1003 100%

Totally, 1003 isolates of fungi represented by 25 species were obtained from the analyzed parts of oregano during the three years of studies (tab. 2). The biggest number of isolates was obtained from oregano leaves and their proportion was 44.07% of all fungi obtained during the studies (tab. 2). Among all the isolated species, *Alternaria alternata* was a commonly occurring species. The fungus was isolated both from the roots and stems but the biggest number of isolates was obtained from the leaves (tab. 2). Their proportion was, respectively, 4.29, 12.46 and 20.14% of all fungi isolated from those parts (fig. 1). Fungi from the genus *Fusarium* were isolated from all studied parts of oregano, while most isolates were obtained from the roots and stems, and their proportion was, respectively, 48.57 and 32.03% of all fungi obtained from these organs

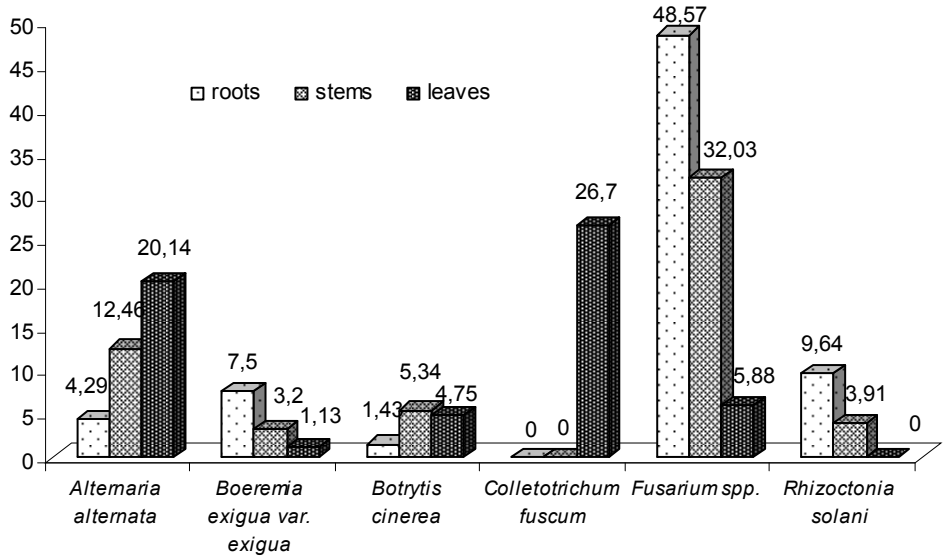


Fig. 1. Mean participation of some fungi isolated from examined organs of oregano in 2012–2014

(fig. 1). *Botrytis cinerea* and *Boeremia exigua* var. *exigua* were obtained from all plant parts (tab. 2). The biggest number of isolates of *B. cinerea* was obtained from the leaves and from the stems of oregano and their proportion was, respectively, 4.75 and 5.34% of all fungi isolated from those parts (fig. 1). *B. exigua* var. *exigua* was isolated mainly from the rots (tab. 2). Single isolates of this fungus were also obtained from the stems and the leaves of oregano and their proportion was, respectively, 7.5, 3.2 and 1.13% of all fungi obtained from those parts (fig. 1). *Stemphylium botryosum* was isolated from all studied parts of oregano, but the biggest number of cultures of this fungus was obtained from the leaves (tab. 2). *Rhizoctonia solani* was isolated from the roots and stems of oregano (tab. 2). Their proportion was, respectively, 9.64 and 3.91% (fig. 1). Species *Colletotrichum fuscum* was obtained only from the leaves of oregano. Isolates of the fungus constituted 26.7% of all fungi obtained from that part (tab. 2, fig. 1). *Phoma sensu lato* e.g. *P. leonuri*, *P. multirostrata* var. *macrospora* and *P. versabilis* were also isolated from the examinated parts of oregano (tab. 2). Cultures of *Trichoderma* spp. and *Gliocladium* spp. were obtained from all parts of plants (tab. 2).

DISSUSION

The present studies confirmed the occurrence of disease symptoms caused by fungi on the plants of oregano cultivated in the plantations in south-eastern Poland. The symptoms of necrosis and rot of the roots, the root collar and the lower part of the stem were most probably the consequence of the pathogenic effect of soil-borne fungi. This conclusion is justified by their frequent isolation from these plant parts of the species

from *Fusarium* genus and species *Boeremia exigua* var. *exigua* and *Rhizoctonia solani*, well-known plurivorous soil-borne pathogens of many crop species, including spices and medicinal plants [Machowicz-Stefaniak et al. 2002, Zimowska and Machowicz--Stefaniak 2004, Zimowska 2007, Zimowska 2008a b, Zimowska 2010a b, Zimowska 2013]. Species *F. avenaceum, F. culmorum* and *F. equiseti* isolated in the present study were found considered on the basis of pathogenicity tests to be the cause of dieback of germs and seedlings of lemon balm thyme [Machowicz-Stefaniak and Zalewska 2004, Zalewska and Machowicz-Stefaniak 2004]. Results of the mycological analysis indicated that the roots and stems were colonized by *F. oxysporum*. In the United States, in California and Oregon, this species was found to be the main cause of the dying out of sage as a result of root rot and wilt of plants [Subbiah et al. 1996]. In 2008, Saudi Arabia, this species caused heavy losses in the cultivation of lavender, causing dieback and vascular wilting of seedlings and plants [Perveen and Bokhari 2010]. Some special forms of *F. oxysporum* are known which are specialized in infecting specific plant species. *F. oxysporum* f.sp. *basilici* causes wilting of basil in Russia, Italy, Israel, France, Canada and Australia [Gamliel et al. 1996, Gamliel and Yarden 1998, Gullino et al. 1998]. *F. oxysporum* f. sp. *cumini* causes the wilting of cumin on the Greek island of Chios, in India, Egypt and Israel [Papas and Elena 1997], *F. oxysporum* f.sp. *corianderii* causes the wilting of coriander in India [Patel and Prasad 1963], while *F. oxysporum* f.sp. *zingiberi* the wilting of ginseng [Priya and Subramanian 2008]. Taking into the consideration the thermophilic character of the fungus and the thermal conditions that prevailed especially in the second and third years of the study, when the average air temperature was higher than the long-term average, it can be assumed that obtained *F. oxysporum* isolates could be involved in causing the symptoms on the roots and stems of oregano. For confirmation of this thesis, however, it is necessary to conduct pathogenicity tests in accordance with the requirements of Koch's postulates. *Rhizoctonia solani* belongs to the pathogens that cause great damage in herb crops. In India, this species has been recognized as one of the main causes of decline of the roots and rhizomes of peppermint, which was manifested as yellowing and dieback of the plants [Kalra et al. 2004]. In Italy the fungus caused massive dieback of basil seedlings [Minuto et al. 1997]. In Poland, the big harmfulness has been demonstrated for the roots and stems of ginseng [Berbeć and Pięta 1996]. In the process of pathogenesis isoenzymes are involved produced by the pathogen, mainly pectic metyloesterase which decompose pectin compounds of the central lamina [Banniza and Rutherford 2001]. *R. solani* isolates obtained from the roots and stems with necrosis and rot symptoms indicates the role of this pathogen in causing just such symptoms.

Basing on the studies and information from literature, *Boeremia exigua* var. *exigua* can be included within the complex of fungi responsible for the symptoms of necrosis and the rot of the roots and the lower part of the stems. The fungus is commonly found in the soil as a typical weakness pathogen. Besides, it often causes seedling blight of various plants, including herbs, as documented in the work by Machowicz-Stefaniak and Zalewska [2007]. In the process of pathogenesis α and β glycosidase are involved, the enzymes degrading cellulose by destroying the basic structure of the cell wall as well as toxins such as cytocholasin B, F, Z2 and Z3, deoxaphominea, ascosonchine and an antibiotic E, toxins of cytotoxic and phytotoxic properties [Rai et al. 2009]. In con-

nection with the occurrence of a complex of fungi discussed above in the cultivation environment of the studied oregano plants, the polyphagous character of parasitism of those fungi species should be taken into consideration while deciding upon the proper crop rotation. The root and pulse crops recommended as proper forecrop for oregano are very often the hosts of the fungi species described above [Łacicowa et al. 1974, Kurzawińska 2006]. Considering the above, introduction of phytosanitary plants, i.e., maize or a grass mixture, should be taken into account, with simultaneous one-year interval in the cultivation of oregano. Such a system of cultivation brought positive results on mint plantations in India, as far as the healthiness of plants is concerned [Kalra et al. 2004].

Isolation of *B. cinerea* in the present study mainly from the leaves and stems may indicate the role of this species in causing oregano diseases. It is the most dangerous pathogen of many herb species, e.g. basil cultivated in Italy as well as lemon balm, marjoram and sage cultivated in Israel [Garibaldi et al. 1997, Gamliel and Yarden 1998]. Since the fungus germ tube penetrate into the plants through its injuries, and the presence of a drop of water is necessary for an infection to occur, the collection of 2-year-old plants of *Herba Origani* performed two or three times during the growing season, should be carried out in dry weather. In addition, *B. cinerea* is transferred from the seeds of oregano as demonstrated by tests conducted by Zimowska [unpublished]. Seed colonization by *B. cinerea* contributes to reducing the viability of seed rot and damping-off [Łacicowa 1989]. Colonization of seeds by the pathogen depends mainly on the weather conditions during flowering, because a significant role in the transmission of the fungus conidia is played by pollinators [Coley-Smith et al. 1980].

The *Colletotrichum fuscum,* which had not been found on oregano in Poland or all in the whole world earlier, was considered as the main cause of characteristic symptoms on oregano leaves in the form of necrotic, rounded, concentrically zoned spots. Such a conclusion is justified by the fact of isolating the fungus cultures from the infected leaves, the presence of etiological signs in the form of the acervuli and conidia as well as by positive results of pathogenicity tests [personal communication]. The pathogen was previously recorded in Japan as the causal agent of anthracnose of nemesia (*Nemesia strumosa*), and in the United States as the pathogen of foxglove (*Digitalis purpurea*) (*Digitalis lanata*) [Goodman 1960, Tomioka et al. 2001]. The genus *Colletotrichum* includes a very important group of pathogens, taking into account the economic aspect they take the eighth place on the list of the most dangerous plant pathogens [Dean et al. 2012]. *C. gloeosporioides* causes anthracnose of John's wort in Germany, Switzerland and Poland [Debrunner and Rauber 2000] and basil cultivated in Italy [Garibaldi et al. 1997]. *C. dematium* is pathogenic for caraway [Machowicz-Stefaniak 2010]. It follows from studies on the effects of temperature on the growth and formation of infective material of *C. fuscum* [Zimowska unpublished.] that this species, like other species of *Colletotrichum* spp., has high thermal requirements and the optimum is 28°C. Severe occurrence of anthracnose of oregano caused by *C. fuscum* must be therefore taken into account during the vegetation periods when the temperature is high, and these conditions were observed especially in the last two years of the study when the average air temperature was higher than the long-term average.

Alternaria spp. and *Stemphylium botryosum* should be considered as the cause of the second and third types of disease symptoms on oregano leaves in the form of irregular,

necrotic spots. Common isolation of *A. alternata* and *S. botryosum* both from the seeds of oregano [Zimowska unpublished] and from the leaves as well as the presence of etiological signs instead of the above described symptoms indicate a potential risk of disease harmfulness for oregano plants posed by these species of fungi. Reports of fungi from the genus *Stemphylium* occurring on *Origanum vulgare* plants and causing spots symptoms come from the United States [Farr et al. 1995]. Moreover, the harmful effects of *A. alternata* does not only influence the *Herba Origani* yield, due to premature defoliation infected leaves, but the quality as well, because the fungus produces substances of toxic character that accumulate in plant tissues even during their growth [Tylkowska et al. 2003, Kalra et al. 2004].

Diversity of species *Phoma sensu lato* occurring in the cultivated environment of oregano documented in the present studies deserves attention. *Phoma leonuri* is widespread and common in Europe on dead stems of the perennial labiate herbs i.e. *Leonurus cardiaca*, *Ballota nigra* and others [Boerema et al. 2004]. *P. multirostrata* var. *macrospora* belongs to plurivorous soil-borne opportunistic plant pathogen, which may also cause blight and dieback symptoms. In India it is recorded as a leaf spots pathogen [Boerema et al. 2004]. *P. versabilis* has been recorded on dead stems of various herbaceous plants in Europe belonging to *Cruciferae* and *Coryophyllaceae* families [Boerema et al. 2004].

The occurrence of fungi from *Gliocladium* and *Trichoderma* genera, known for their antagonistic effect towards different pathogens of cultivated plants, including herbaceous ones, in the cultivation environment of the studied plants of oregano should be viewed as positive [Zimowska 2004, Sandoval et al. 2006].

CONCLUSIONS

1. The satisfactory and repeatable quality of raw materials of *Herba Origani* depends on the phytosanitary condition of plants during their cultivation. The present studies pointed to the diversity of fungi, including pathogenic species, which- as a result of injuring the plants- exerts a negative effect on the quality and quantity of *Herba Origani.*

2. Discovering the species *C. fuscum,* the causal agent of anthracnose of oregano leaves for the first time in Poland should be considered as particularly dangerous.

3. The negative aspect of the occurrence of *C. fuscum* on oregano leaves, in addition to reducing the amount of *Herba Origani* yield, is the ability to accumulate colleotine, toxic substance produced by the pathogen in the tissues.

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GRZYBY ZAGRAŻAJĄCE UPRAWIE LEBIODKI POSPOLITEJ (*Origanum vulgare* L.) W POŁUDNIOWO-WSCHODNIEJ POLSCE

Streszczenie. W latach 2012–2014 na plantacjach produkcyjnych zgrupowanych w południowo-wschodniej Polsce przeprowadzono badania nad grzybami zasiedlającymi i uszkadzającymi nadziemne i podziemne organy lebiodki pospolitej (*Origanum vulgare* L.). Grzyby izolowano przy użyciu pożywki mineralnej z powierzchniowo odkażonych korzeni, podstawy łodyg do 10 cm od podstawy oraz z liści. Z korzeni oraz z łodyg wykazujących objawy nekrozy oraz dezintegracji tkanek uzyskano gatunki *Boeremia exigua* var. *exigua* i *Rhizoctonia solani* oraz *Fusarium* spp. Z liści z objawami nekrotycznych, nieregularnych plam otrzymano kultury *Alternaria alternata* oraz *Stemphylium botryosum*. *Colletotrichum fuscum* powszechnie izolowano z liści z symptomami regularnych, nekrotycznych, koncentrycznie strefowanych plam z jaśniejszym środkiem i lekko wzniesionym brzegiem. Gatunek ten nie był notowany wcześniej w Polsce.

Słowa kluczowe: rośliny zielarskie, grzyby, choroby ziół, oregano, występowanie

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