

CHARACTERISTICS AND OCCURRENCE OF *Phoma* spp. ON HERBS FROM THE FAMILY *Lamiaceae*

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Abstract. Fungi belonging to *Phoma* spp. commonly occur on herbaceous plants, including herbal ones. In the years 2004–2006 isolates belonging to genus *Phoma* which were differentiated in morphological respects were obtained from herbs belonging to family *Lamiaceae*. Basing on constant morphological feature observed *in vitro* in the cultures developing on standard media, isolates of *Phoma* spp. were included within the proper section, and next the species were identified. *P. exigua* var. *exigua* was isolated from all parts of the examined plants. *P. nepeticola* cultures were obtained from the leaves and stems of motherwort, whereas isolates of *P. nemophilae* were from the roots. Apart from the above mentioned species, *P. capitulum*, *P. labilis*, *P. pereupyrena*, *P. subglomerata* and *P. septacidalis* were also isolated.

Key words: fungi, herbaceous plants, *Phoma* spp., motherwort, sage, savory

INTRODUCTION

Fungi of the genus *Phoma* are at present one of the more widespread ones in respect of their geography and they consist of a large number of species which one can find in varied ecological niches. From among 3000 taxa described so far, 110 are pathogenic species often infecting those plant parts that are important from the economic point of view [Aveskamp et al. 2008]. Some of them are included within a quarantine, because they cause serious problems for organizations creating legal settlements concerning plant quarantine [Miric et al. 1999, Mendes et al. 2006].

Herbs belong to plants that are frequently colonized by fungal species from the genus *Phoma*. *Phoma dictamnica*, *P. glaucii* and *P. multirostrata* var. *microspora* were isolated from the leaves and stems of thyme [Machowicz-Stefaniak et al. 2002]. Cultures of *P. exigua* var. *exigua*, *P. capitulum* and *P. glomerata* were obtained from the roots, stems and leaves of thyme, lemon balm and St. John's wort [Machowicz-Stefaniak et al. 2002, Zimowska and Machowicz-Stefaniak 2004, Machowicz-Stefaniak

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et al. 2004]. *P. strasseri* was isolated from the stems and rhizomes of peppermint with the symptoms of the black stem rot [Zimowska and Machowicz-Stefaniak 2005, Zimowska 2007a].

The author's own studies conducted in the years 2004–2009 on diseases of so far not studied species of herbs from the family *Lamiaceae* extended the collection of cultures of the Department of Plant Pathology and Mycology, University of Life Sciences in Lublin with the new isolates of *Phoma* spp. The present work relates to the occurrence and characteristics of *Phoma* spp., with special regard to the species described in the literature as pathogenic.

MATERIAL AND METHODS

The studies conducted in 2004–2006 concerned three plantations of motherwort (*Leonurus cardiaca* L.) and sage (*Salvia officinalis* L.) in the second year of cultivation, and in 2007–2009 three 1-year-old plantations of savory (*Satureja hortensis* L.). The plantations were situated in the communes of Fejsławice, Dziecinin and Suchodoły in the Lublin province. The procedure of taking plants for investigations and isolating the fungi was already described in detail by Zimowska [2008a]. The obtained isolates of *Phoma* spp. were identified after making single-spore cultures on standard media. The keys considering the current principles of the taxonomy of fungi from genus *Phoma* were used for identification [Boerema et al. 2004, De Gruyter and Noordeloos 1992]. Isolates: L 121 obtained from the roots of motherwort, S 458 from the stems of sage and Sh 6002 isolated from the roots of savory were chosen from the population of *P. exiqua* var. *exiqua* for further studies. Isolates L 284, L 291 and L 302 obtained from the leaves of motherwort represents the population of *P. nepeticola* and three isolates of *P. nemophilae* L 171, L 205 and L 248 from the roots of motherwort. The randomly chosen isolates were grown on the standard media MA (malt agar), OA (oatmeal agar) and CA (cheery agar) [De Gruyter and Noordeloos 1992]. The cultures were incubated during the first week in a thermostat without access of light at the temperature of 22°C, and for the next 13 hours in UV light and 11 hours in darkness. After this time measurements of the diameter of the colonies were done and also colour of the avers and the reverse, character of growth of the edge of the colony and character of growth of hyphae of the air mycelium were evaluated. After 14 days of keeping the cultures in the thermostat, the same features were described as after 7 days. Additionally on OA medium colour of the cultures after their reaction with 2–3 drops of 1N NaOH was studied. Moreover, the arrangement of the pycnidia was defined on OA medium. Shape of the pycnidia, character of the ostioles, colour of the conidial exudates and structure of the pycnidial wall surface were studied. After 2 weeks of the growth of the cultures on OA medium, the measurements of 300 conidia (3 isolates × 100 conidia) and 150 pycnidia (3 isolates × 50 pycnidia) were done for every species. Attention was also paid to presence of chlamydospores.

RESULTS

The present investigations showed that all organs of the analysed plant species were infected by *P. exigua* var. *exigua*, section *Phyllostictoides* (tab. 1). The cultures of this species were most frequently obtained from the roots showing the symptoms of necrosis and the set-back of the growth and from the bottom parts of stems with the symptoms of necrosis. Etiological signs in the form of pycnidia and conidia with the features typical for the above species were found on the infected organs. The isolated species from plants of motherwort, sage and savory was *P. glomerata* belonging to the section *Peyronellea* (tab. 1). The cultures of *P. nepeticola* from the section *Phyllostictoides* were received from the leaves and the stems of motherwort showing the symptoms in the form of regular, necrotic spots. Besides the characteristic symptoms, pycnidia were found on these organs, and in them conidia with the typical features of the *Phoma* genus. The isolates of *P. nemophilae*, species also belonging to the section *Phyllostictoides*, were obtained only from the roots of motherwort (tab. 1). Moreover, the isolates of *P. labilis* from the section *Phoma* were isolated from the roots and stems of motherwort and sage. Cultures of *P. septicialis* from the section *Paraphoma*, were obtained only from the stems and roots of motherwort, while *P. subglomerata*, species belonging to the section *Peyronellea*, was also isolated from sage plants (tab. 1).

Table 1. Occurrence of *Phoma* spp. on herbs from *Lamiaceae* family in 2004–2009
Tabela 1. Występowanie *Phoma* spp. na ziołach z rodziny *Lamiaceae* w latach 2004–2009

<i>Phoma</i> spp.	Section Sekcja	Plant species – Gatunki roślin								
		<i>Leonurus cardiaca</i> L.			<i>Salvia officinalis</i> L.			<i>Satureja hortensis</i>		
		a	b	c	a	b	c	a	b	c
<i>Phoma capitulum</i>					+				+	
<i>Phoma labilis</i>	<i>Phoma</i>	+	+		+	+				
<i>Phoma pereupyrena</i>					+	+				
<i>Phoma exigua</i> var. <i>exigua</i>		+	+	+	+	+	+	+	+	+
<i>Phoma nemophilae</i>	<i>Phyllostictoides</i>	+								
<i>Phoma nepeticola</i>			++	++						
<i>Phoma glomerata</i>	<i>Peyronellea</i>	+	+		+	+	+	+	+	
<i>Phoma subglomerata</i>		+	+		+	+	+			
<i>Phoma septicialis</i>	<i>Paraphoma</i>	+	+							

+ – frequency up to 5% – częstotliwość do 5%

++ – frequency from 5 to 10% – częstotliwość od 5 do 10%

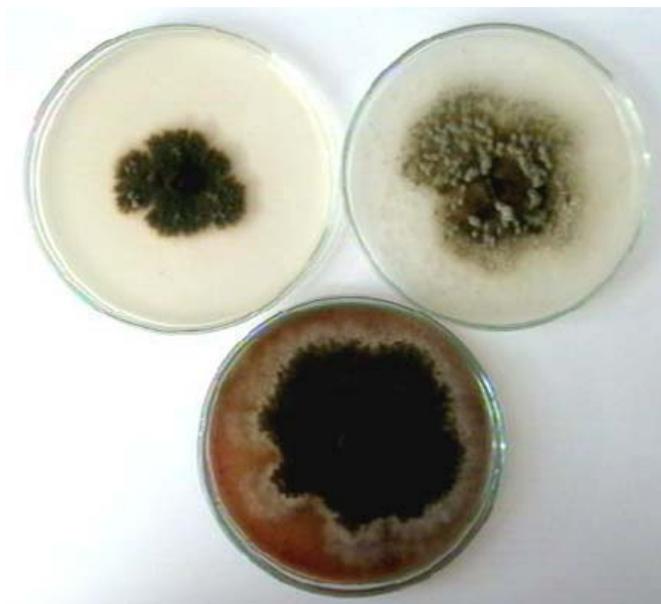
The investigations concern the growth of chosen isolates of *P. exigua* var. *exigua* on standard media showed that cultures of the fungus on MA medium after 7 days were characterized by brown-olive, flocky aerial hyphae of the air mycelium with a white edge (photo 1). The reverse of the colony was olive-grey. The character of the growth of

Table 2. Characteristics of pycnidia and conidia of *Phoma exigua* var. *exigua*, *Phoma nepiticola* and *Phoma nemophilae* (mean for 3 isolates)
 Tabela 2. Charakterystyka piknidiów i konidiów *Phoma exigua* var. *exigua*, *Phoma nepiticola* oraz *Phoma nemophilae* (średnia dla 3 izolatów)

Author Autor	<i>P. exigua</i> var. <i>exigua</i>			<i>P. nepiticola</i>			<i>P. nemophilae</i>		
	Pycnidia Piknidia	Conidia Konidia		Pycnidia Piknidia	Conidia Konidia		Pycnidia Piknidia	Conidia Konidia	
	a	b	c	a	b	c	a	b	c
Own data Badania własne	Globose with usually one ostiole, 74,2 × 195 Okrągłe, najczęściej z 1 ujściem, 74,2 × 195	On the agar, but partly submerged in the agar, glabrous but often with soft hyphal, outgrowths Na powierzchni podłoża lub częściowo zagłębione w podłożu, gładkie lub często z delikatnymi utworami strzępkowymi	Subglobose, ellipsoidal to oblong. Conidia aseptate 3,7–7,2 × 2,2–3,5, septate 6,9–9,5 × 2,1–3,2, with 1 ostiole Prawie okrągłe, elipsoidalne do podłużnych, Komidia bez przegród 3,7–7,2 × 2,2–3,5, Komidia z przegródą 6,9–9,5 × 2,1–3,2	Globose with 1 ostiole, 75 × 250 Okrągłe z 1 ujściem	On the agar or submerged, glabrous or sparsely covered by mycelial hairs Na powierzchni podłoża lub zagłębione, gładkie lub pokryte delikatnymi strzępkami	Conidia hyaline, oval to ellipsoidal. Conidia aseptate 6,2 × 2,9, septate 12,1 × 3,2, Komidia bezbarwne, owalne do elipsoidalnych. Komidia bez przegród 6,2 × 2,9, Komidia z przegródą 12,1 × 3,2	Globose to irregular with 1–3 sometimes elongated ostioles 63 × 257 Okrągłe do nieregularnych, ujęcia od 1 do 3, czasami wydłużone	Submerged in the agar, glabrous or covered by mycelial outgrowths Zagłębione w podłożu, gładkie lub pokryte strzępkami	Ellipsoidal to cylindrical mainly aseptate 6,3 × 1,5 septate 11,8 × 3 Elipsoidalne do cylindrycznych, głównie bez przegród 6,3 × 1,5, komidia z przegródą 11,8 × 3,2
Boerema et. al. 2004 Boerema i. in. 2004	Globose to subglobose with usually 1–2 ostioles, 75–200 Okrągłe do prawie okrągłych najczęściej z 1 do 2 ujść, 75–200	Variable in shape and dimension. Conidia aseptate 4–7(–12) × 2,5–3,5, septate 7–10(–13) × 2,5–3,5 Zróżnicowane w kształcie i rozmiarze. Konidia z 1 ujściem bez przegród 4–7(–12) × 2,5–3,5, z przegródą 7–10(–13) × 2,5–3,5	Subglobose to ellipsoidal, conidia aseptate 5–7 × 2,5 septate 9,5–14,5 × 2,5–5, Prawie okrągłe do elipsoidalnych, konidia bez przegród 5–7 × 2,5, z przegródą 9,5–14,5 × 2,5–5.	Globose with usually 1 ostiole 70 × 240 Okrągłe, najczęściej 70 × 240	Glabrous or sparsely covered by mycelial hairs Gładkie lub pokryte delikatnymi strzępkami	Globose to irregular with 1–5 usually ostioles later developing to an elongated neck, 60 × 260 Okrągłe do nieregularnych z 1–5 ujściami z czasem w kształcie wyciągniętej szyjki, 60 × 260	Glabrous or with some mycelial outgrowths Gładkie lub z nielicznymi wyrostkami strzępkowymi	Cylindrical allantoid, aseptate 4–9,5 × 1,5–2,5, septate conidia 12 × 3,5. Cylindryczne allantoidalne, bez przegród 4–9,5 × 1,5–2,5 z przegródą 12 × 3,5	

a – shape and dimension in μm – kształt i wymiary w μm ,
 b – arrangement and structure of wall surface – rozmieszczenie i struktura powierzchni ściany,
 c – shape and dimension in μm – kształt i wymiary w μm .

the colony and the colour of the averse and the reverse were similar on OA and CA media. The edge of the colony on all media was irregular, lightly sliced (photo 1). After 14 days the colour of the hyphae was more intensive. The diameter of the colony after 7 days ranged from 27 to 39 mm, and after 14 days from 60 to 78 mm on OA and CA media and from 40 to 45 on MA medium. The pycnidia formed on OA medium in great



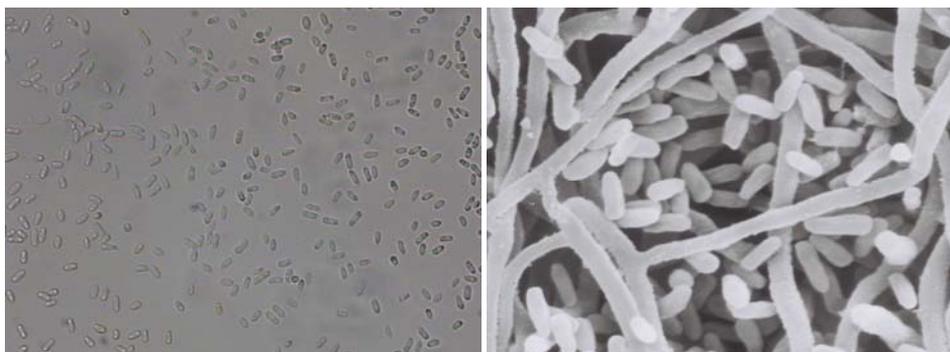
Phot. 1. 14-day old colonies of *P. exiqa* var. *exiqa* (L 121) on malt agar. Photo E. Zalewska

Fot. 1. 14-dniowe kolonie *P. exiqa* var. *exiqa* (L 121) na pożywce maltozowej. Fot. E. Zalewska



Phot. 2. Pycnidia of *P. exiqa* var. *exiqa* (L 121) – $\times 125$ (a) – photo E. Zalewska; SEM – $\times 790$ (b); Pycnidium and fragments of mycelium hyphae – photo M. Wróbel

Fot. 2. Piknidia *P. exiqa* var. *exiqa* (L 121) – $\times 125$ (a) – fot. E. Zalewska; SEM – $\times 790$ (b); Piknidium i fragmenty strzępek grzybni – fot. M. Wróbel



Phot. 3. Conidia of *P. exiqa* var. *exiqa* (L 121) – $\times 500$ (a) – photo E. Zalewska; SEM $\times 6500$ (b) – photo M. Wróbel

Fot. 3. Konidia *P. exiqa* var. *exiqa* (L 121) – $\times 500$ (a) – fot. E. Zalewska; SEM $\times 6500$ (b) – fot. M. Wróbel



Phot. 4. 14-day old colonies of *P. nepeticola* (L 284) on malt agar. Photo E. Zalewska

Fot. 4. 14-dniowe kolonie *P. nepeticola* (L 284) na pożywce maltozowej. Fot. E. Zalewska

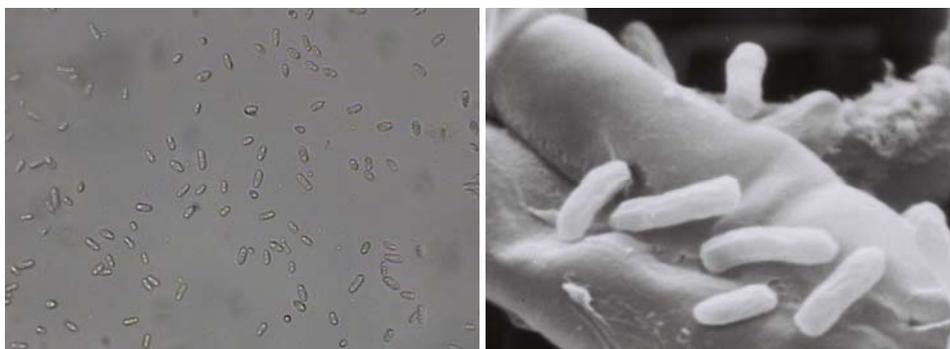
number were scattered over the whole surface of the colony in an irregular way, and they were partly sunken in agar. The pycnidia had a round shape and one ostiole from which creamy white drops of the conidial exudate flowed out (tab. 2). The walls of the pycnidia were generally smooth, with delicate hyphal outgrowths sometimes visible (photo 2). The size of the pycnidia ranged from 74.2 to 195.5 μm (tab. 2). The conidia

of the studied isolates of *P. exigua* var. *exigua* were differentiated in shape and size, most frequently being oval, elliptic or round, with single drops of fat on the poles, without secondary septum or rarely with one septum (photo 3). The dimensions of 1-cell spores – $3.5\text{--}7.2 \times 2.2\text{--}3.5 \mu\text{m}$, and 2-cell $6.9\text{--}9.5 \times 2.1\text{--}3.2 \mu\text{m}$ (tab. 2). The test with 1 N NaOH showed the positive reaction. The change of the tinge of agar was observed in all studied isolates around the colony from green to red. Chlamydospores were not observed.



Phot. 5. Pycnidia of *P. nepeticola* (L 284) – $\times 125$ (a) – photo E. Zalewska; SEM $\times 2400$ (b); Ostiole of pycnidium and fragments of mycelium hyphae – photo M. Wróbel

Fot. 5. Pliknidia *P. nepeticola* (L 284) – $\times 125$ (a) – fot. E. Zalewska; SEM $\times 2400$ (b); Ujście pliknidium i fragmenty strzępek – fot. M. Wróbel



Phot. 6. Conidia of *P. nepeticola* (L 284) – $\times 500$ (a) – photo E. Zalewska; SEM $\times 9900$ (b); Conidia and fragments of mycelium hyphae – photo M. Wróbel

Fot. 6. Konidia *P. nepeticola* (L 284) – $\times 500$ (a) – fot. E. Zalewska; SEM $\times 9900$ (b); Konidia i fragmenty strzępek grzybni – fot. M. Wróbel

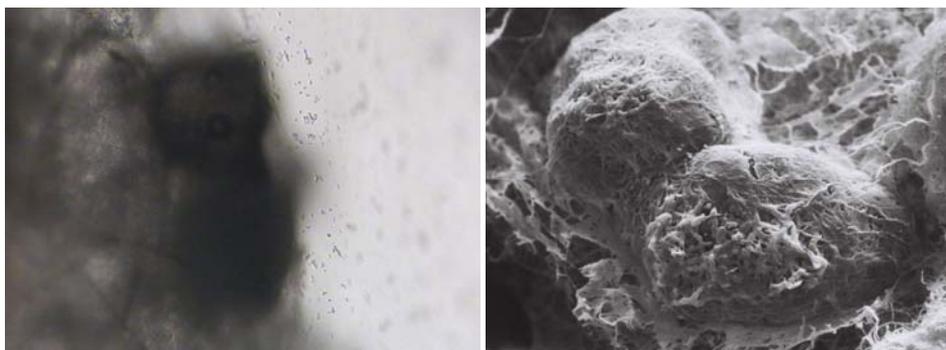
Isolates of *P. nepeticola* on MA medium after 7 days of the growth were characterized by quite compact hyphae of the air mycelium of the grey-olive colour. After 14 days on the same medium, the hyphae of the air mycelium produced a flocculent struc-



Phot. 7. 14-day old colonies of *P. nemophilae* (L 205) on malt agar. Photo E. Zalewska
 Fot. 7. 14-dniowe kolonie *P. nemophilae* (L 205) na pożywce maltozowej. Fot. E. Zalewska

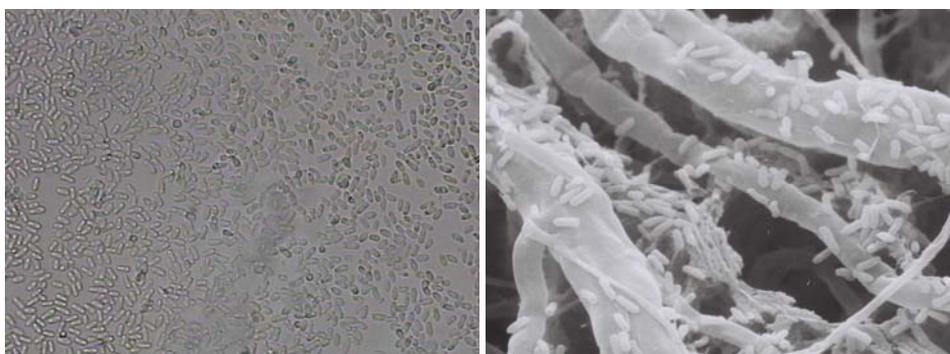
ture in the central part of the colony and they were of the white-cream colour (photo 4). The reverse was olive-green. Similar features were shown by the colonies growing on OA and CA media. The edge of the colony was slightly irregular. The isolates growing on OA medium reached the largest diameter and after 7 the days of growth it ranged from 40 to 43 mm. The diameter of the colonies was a little smaller on OA and CA media and it ranged from 35 to 38 mm. After 14 days on OA medium the diameter of the colony ranged from 74 to 76 mm while on CA from 45 to 49 mm. Numerous pycnidia formed on OA medium were round, smooth or covered with delicate hyphal outgrowths (photo 5). They were formed singly or in small aggregates. Drops of the conidial exudate of cream-salmon pink to pink colour went out from the ostioles. The size of the pycnidia ranged from 75 to 230 μm (tab. 2). The conidia were hyaline, oval to ellipsoidal, usually with several small drops of fat (photo 6). One-cell conidia with the mean dimensions of $6.2 \times 2.9 \mu\text{m}$ predominated, two-cell ones occurred rarely and their size was on an average $12.1 \times 3.2 \mu\text{m}$ (tab. 2). The reaction of the cultures of *P. nepeticola* with 1 N NaOH was negative; moreover, none of the isolates of the fungus formed chlamydo spores.

The isolates of *P. nemophilae* formed after 7 days of growth on standard media a dark olive air mycelium with a similar reverse. After 14 days the colour of the air mycelium became brown-olive tinge, and the hyphae of the air mycelium produced a flocculent structure. In the central part of the colony delicate, white hyphae of the air



Phot. 8. Pycnidia of *P. nemophilae* (L 205) – $\times 160$ (a) – photo E. Zalewska; SEM $\times 790$ (b) – photo M. Wróbel

Fot. 8. Pliknidia *P. nemophilae* (L 205) – $\times 160$ (a) – fot. E. Zalewska; SEM $\times 790$ (b) – fot. M. Wróbel



Phot. 9. Conidia of *P. nemophilae* (L 205) – $\times 500$ (a) – photo E. Zalewska; SEM $\times 6500$ (b); Conidia and fragments of mycelium hyphae – photo M. Wróbel

Fot. 9. Konidia *P. nemophilae* (L 205) – $\times 500$ (a) – fot. E. Zalewska; SEM $\times 6500$ (b) Konidia i fragmenty strzępek grzybnii – fot. M. Wróbel

mycelium were seen around the point of transfer. The edge of the colony was regular (photo 7). The diameter of the colony after 7 days of growth on all three media was similar and ranged from 65 to 73 mm, while after 14 days from 80 to 88 mm. The pycnidia formed in small aggregates were the most often round or slightly elongated. They were partly sunken in agar (photo 8). They had one to three elongated ostioles, which were surrounded by hyphal outgrowths. Drops of conidial exudate of creamy-white colour got out through the pycnidial ostioles. The conidia were hyaline, ellipsoidal to cylindrical with several small drops of fat on the poles (photo 9). One-cell conidia with the dimension $6.3 \times 1.5 \mu\text{m}$ predominated. Two-cell conidia occurred rarely, and their dimension was, on an average, $11.8 \times 3.2 \mu\text{m}$ (tab. 2). The reaction with 1 N NaOH on MA and OA media for all studied isolates of *P. nemophilae* was positive. The change of the tinge of agar was observed around the colony, initially into the green colour which changed in red. Chlamydospores were not observed.

DISCUSSION

The present studies showed that despite herbaceous plants produce substances of bacteriostatic and fungistatic properties [Kozłowska et al. 2010, Tajkarimi et al. 2010], they are commonly colonized and infected by fungi, including those belonging to *Phoma*.

The diversity of species of the genus *Phoma* occurring on the herbs from family *Lamiaceae* which was proved in the present paper confirms the information from the literature on their frequent occurrence, especially on herbal plants, including herbaceous ones [Aveskamp et al. 2008]. Saprobes commonly developing on different substrates include the species also isolated in the present studies: *P. capitulum*, *P. labilis*, *P. perepyrena*, *P. subglomerata* and *P. septicialis*, with the possibility that the latter species can cause necrosis of plants from family *Gramineae* [De Gruyter and Boerema 2002, Zimowska 2007b, 2008b]. The fact of obtaining isolates of *P. exiqua* var. *exiqua* from all the studied plants confirms the information in the literature that this species is one of the most frequently occurring of *Phoma* in the world [Aveskamp et al. 2008]. Isolates of this fungus have been so far obtained from over 200 genera of plants [Boerema et al. 2004], and results of these studies enrich the collection with successive hosts. *P. exiqua* var. *exiqua*, as a facultative pathogen can cause different disease symptoms, e.g. necrosis of the leaves and the stems, root rot or seedling blight [Van Der et al. 2000, Machowicz-Stefaniak et al. 2008]. Isolation of the fungus cultures from the studied plants showing symptoms of necrosis and growth inhibition of the root system, together with the presence of etiological signs on them can indicate that *P. exiqua* var. *exiqua* caused such symptoms. Infection of leaves and stems by the fungus should be assessed negatively. Due to the facultative character of the fungus parasitism, infection of these organs can occur through the injuries caused by cultivation, different weather conditions or interaction with other organisms.

P. nepeticola is described in the literature as a commonly occurring pathogen of catnip (*Nepeta cataria*) and other plants from family *Lamiaceae*. The list of host plants that can infect *P. nepeticola* includes *Leonurus cardiaca* and *Mentha* spp. [De Gruyter et al. 2002, Zimowska 2008b]. Isolation of this fungus cultures from the leaves and stems of motherwort showing symptoms of small necrotic spots, together with the presence of etiological signs on them may suggest that they were caused by the fungus species under discussion.

P. nemophilae is a pathogen known both in Europe and the United States to cause seedling blight and stem decay of plants from genus *Nemophila* [De Gruyter et al. 2002]. The fact of obtaining fungus isolates from motherwort roots can point to the role of this fungus species in causing disease symptoms on motherwort plants. However, in order to confirm the pathogenic abilities of the enumerated species of *Phoma* spp. it is necessary to conduct infection studies according to Koch's postulates.

Studies conducted on the morphology of the examined isolates confirmed the assumption put forward in the 1930's and 1940's by Wollenweber, Hochaphel and Dennis, and later continued by Boerema et al. They proved that establishing taxa within *Phoma* spp. is possible only on the basis of constant morphological features observed *in vitro* in cultures developing in standard conditions. It is only such a procedure that en-

abled to qualify differentiated isolates obtained from motherwort plants, sage and savory within the proper section, and next, within the section, to the species.

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CHARAKTERYSTYKA I WYSTĘPOWANIE *Phoma* spp. NA ZIOŁACH Z RODZINY *Lamiaceae*

Streszczenie. Gatunki należące do rodzaju *Phoma* powszechnie występują na roślinach zielnych, w tym również zielarskich. W latach 2004–2006 z ziół z rodziny *Lamiaceae* otrzymano zróżnicowane pod względem morfologicznym izolaty należące do rodzaju *Phoma*. Na podstawie obserwacji stałych cech morfologicznych, przeprowadzonych *in vitro* w kulturach rozwijających się na standardowych podłożach zakwalifikowano poszczególne izolaty *Phoma* spp. do odpowiedniej sekcji, a następnie do gatunku. Ze wszystkich organów badanych roślin izolowano gatunek *P. exiqua* var. *exiqua*. Kultury *P. nepeticola* otrzymano z liści i łodyg serdecznika pospolitego, a z korzeni uzyskano izolaty *P. nemophilae*. Oprócz wyżej wymienionych gatunków wyizolowano również *P. capitulum*, *P. labilis*, *P. pereupyrena*, *P. subglomerata* oraz *P. septicialis*.

Słowa kluczowe: grzyby, rośliny zielarskie, *Phoma* spp., serdecznik pospolity, szaflwia lekarska, cząber ogrodowy

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