

## OCCURRENCE AND CHARACTERIZATION OF *Colletotrichum fuscum*

Beata Zimowska, Ewa Dorota Zalewska, Ewa Dorota Król  
University of Life Sciences in Lublin

**Abstract.** During 2012–2014 in southeastern Poland the species *Colletotrichum fuscum* was isolated from the leaves of oregano (*Origanum vulgare* L.) showing the symptoms of necrotic, concentrically zoned spots with a lighter center and a slightly raised edge. Morphology of nine randomly chosen isolates from the fungus population and reference isolate CBS obtained from CBS-KNAW Fungal Biodiversity Centre were studied. Each isolate was cultured on PDA medium, at the temperatures 24°C for 14 days. The character of the cultures, the color of the averse and the reverse, the formation of morphological structures of the fungus, i.e. acervuli, conidia, appressoria and chlamydo-spores were studied. Ultrastructural observations of morphological structures were undertaken using scanning electron microscopy. The presence of setose acervuli, conidia, chlamydo-spores and appressoria was visible. Moreover, studies on the biotic effect between *C. fuscum* and other species of phyllosphere fungi of oregano showed that all tested fungi inhibited the growth of *C. fuscum*, but the size of the limiting effect was different. Fungi from genera *Trichoderma* and *Clonostachys* were found out to be the most effective and positive antagonists. *Alternaria alternata*, *Botrytis cinerea* and *Fusarium* spp. – despite the high values of IBE were considered negative antagonists.

**Key words:** *Origanum vulgare*, oregano, phyllosphere fungi, morphology, biotic activity

### INTRODUCTION

The genus *Colletotrichum* includes endophyte and saprophytic species, as well as plant pathogens from different groups of utility [Cannon et al. 2012]. They occur in all

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Corresponding author: Beata Zimowska, Department of Plant Pathology and Mycology, University of Life Sciences in Lublin, Leszczyńskiego 7, PL-20-069 Lublin, Poland, e-mail: beata.zimowska@up.lublin.pl

climatic zones and cause anthracnose disease [Lenné 2002]. *Colletotrichum* species cause devastating diseases of coffee berries, corn, sugarcane, sorghum in Africa. Moreover, they are the reason for anthracnose of tropical fruits, for example, mango, banana and avocado [Jeffries et al. 1990]. There are also the species which cause anthracnose of herbal plants. *Colletotrichum gloeosporioides* is one of the most important pathogens St. John's wort in Germany, Switzerland and Poland [Debrunner and Rauber 2000, Gärber and Schrenk 2002, Zimowska and Machowicz-Stefaniak 2004] and basil grown in Italy [Garibaldi et al. 1995]. *C. dematium* is a pathogen of caraway [Machowicz-Stefaniak 2010]. The genus *Colletotrichum* was recently voted the eighth most important group of plant pathogenic fungi in the world, based on perceived scientific and economic importance [Dean et al. 2012].

The author's own studies conducted in the years 2012–2014 on the healthiness of oregano (*Origanum vulgare* L.) grown in southeastern Poland pointed to common colonization of leaves with the symptoms of regular, necrotic spots by *Colletotrichum fuscum* Laub., the species not reported on oregano in Poland and in the world till now [Zimowska 2015]. The pathogenic character of the fungus was confirmed by means of pathogenicity tests according to Koch's postulates (personal communication). Furthermore, the analysis of regions ITS1 and ITS2 showed 99% nucleotide sequence similarity of three native isolates of *C. fuscum* to the reference isolate from CBS (personal communication).

Despite common isolation of *C. fuscum* cultures from the infected leaves, the occurrence of other fungi species was observed on those parts of oregano plants. Those fungi included antagonistic species from genera *Trichoderma*, *Clonostachys*, *Epicoccum*, *Cladosporium* as well as fungi from fast growing genera *Alternaria*, *Botrytis*, *Fusarium* and *Phoma sensu lato*, which are known for their competitive abilities [Zimowska 2015].

Because the literature lacks information on the occurrence of *C. fuscum* on oregano as well as on the biotic interactions of this species and other fungi colonizing the phyllosphere of the leaves of oregano, studies were undertaken to explain this problem. Besides, the work documented morphological features of the structures of the pathogen isolates tested *in vitro* which are important from the taxonomical point of view.

## MATERIAL AND METHODS

**Isolation and identification of *Colletotrichum fuscum*.** The research material consisted of isolates of *C. fuscum* obtained from the leaves of two-year-old plants of oregano grown on three plantations in the Lublin province in 2012–2014. The fore crops 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, i.e. in the third 10-days' period of May and the first 10 days of August, directly on the plantation. At the same time, sets of forty symptomatic oregano plants were collected each year from each plantation. For mycological analysis five symptomatic leaves were taken from each plants. Chosen leaf blades were surface-sterilized by soaking in a 10% bleach (0.525% sodium hypochlorite) solution for 3 min and then rinsing three times with sterile distilled water. Small (approximately 3 × 3 mm)

section of tissue were aseptically excised and placed into 90 mm diameter petri plates (10 pieces/plate) containing the mineral medium (0.7 g  $\text{NH}_4\text{NO}_3$ , 0.3 g  $\text{KH}_2\text{PO}_4$ , 0.3 g  $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ , 0.01 g  $\text{FeCl}_3 \times 6 \text{H}_2\text{O}$ , 0.01 g  $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$ , 0.01 g  $\text{CuSO}_4 \times 7 \text{H}_2\text{O}$ , 0.01 g  $\text{MnSO}_4 \times 5 \text{H}_2\text{O}$  + 38 g saccharose + 20 g agar + 1000 ml  $\text{H}_2\text{O}$ ). Within 4 days of incubation in the dark at 24°C, small parts of colonies growing around the inocula were transferred into PDA medium (Difco™ Potato Dextrose Agar, USA) slants. After 10 days, the obtained isolates were segregated and identified according to the description given by Sutton [1980] and Tomioka et al. [2001]. Nine isolates of *C. fuscum* 886/2012, 892/2012, 905/2012, 987/2012, 998/2013, 1001/2013, 1038/2013, 1108/2014, 1125/2014 and a reference isolate CBS 102 189 obtained from CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands, were randomly chosen from the collection of single – spore cultures for further studies. Each isolate was cultured in a thermostat, at the temperatures 24°C for 14 days [Machowicz-Stefaniak 2010]. The character of the cultures, the color of the averse and the reverse, the formation of morphological structures of the fungus, i.e. acervuli, conidia, appressoria and chlamydospores were studied after that time. To determine the structures mentioned above, the measurements of 100 acervuli (10 isolates per 10 acervuli) and 500 conidia (10 isolates per 50 spores) were made. Moreover, the size of appressoria, setoses and chlamydospores of the studied isolates was determined. Photographic documentation was made using a light and scanning electron microscopy Vega, Tescan.

**Biotic activity of *Colletotrichum fuscum*.** Because the literature lacks information on the biotic effect of *C. fuscum*, the studies considered different fungi species, regardless of the frequency of their isolation [Zimowska 2015]. The studies were conducted using the method of biotic series [Mańka 1974, Mańka and Mańka 1992, Mańka 1995] on PDA medium. This method was adopted for fungi colonizing the plants' phyllosphere [Zimowska 2004, Machowicz-Stefaniak et al. 2008]. Two discs of 3 mm in diameter from 14-day-old cultures, one of *C. fuscum* 987/2012 and one of the fungus representing the studied community, were taken. They were placed mycelium down, 2 cm apart in the center of the Petri dish, on the solidified medium. The control constituted of dishes with single fungi species. For each experimental combination, 4 dishes were considered which were treated as replications. They were kept in a thermostat at the temperature of 24°C. The biotic effect was estimated on the basis of an 8-degree scale after 10 days of common growth, and in the case of *Clonostachys* spp. the observation was extended to 34 days [Machowicz-Stefaniak 2010]. While evaluating the biotic effect, the overgrowth of the fungus colony by the accompanying fungus, the occurrence of the inhibition zone between two colonies and growth inhibition of the colony of one of the fungi were taken into consideration [Mańka 1995]. In the case of the overgrowth of *C. fuscum* by other fungi species, the studies examined changes in the appearance of morphological structures, i.e. the hyphae and conidia. The biotic effect of the accompanying fungi representing the phyllosphere of the leaves of oregano and *C. fuscum* was expressed as an individual biotic effect (IBE) [Mańka 1974]. Next, the general biotic effect (GBE) was estimated which was the product of the individual biotic effect and the multiplicity of the occurrence of particular fungi species. The algebraic sum of general biotic effects made it possible to determine the summary biotic effect (SBE), which reflected the effect of all studied species of phyllosphere fungi on

*C. fuscum* in the years 2012–2014. A positive value of IBE indicates the growth inhibition of the pathogen, whereas a negative value of IBE points to the lack of growth inhibition of the pathogen's colony. "0" value means a neutral effect of both fungi on each other [Mańka 1974, Mańka 1995].

## RESULTS

**Isolation and identification of *Colletotrichum fuscum*.** Isolates of *C. fuscum* were obtained from oregano leaves with necrotic, concentrically zoned spots with a lighter center and a slightly raised edge (figs 1A, 1B). On the surface of such spots acervuli including conidia typical of genus *Colletotrichum* were found (figs 1C, 1D). The percentage of plants with the above-mentioned disease symptoms ranged in the years of study from 15 to 25% at the beginning of the vegetation to 20 to 47% at full vegetation. The proportion of the fungus isolates in successive years of the studies was, respectively, 17.98, 29.01 and 38.23% (tab. 1). In total, 118 isolates of *C. fuscum* were obtained during the 3 years of studies, which constitutes 26.7% of all fungi obtained from the analyzed leaves of oregano (tab. 1).

Table 1. Participation of *Colletotrichum fuscum* isolates in fungal communities obtained from the diseased leaves of oregano in 2012–2014

Fungi	Number (and percent) of isolates in years			Total 2012–2014
	2012	2013	2014	
<i>Colletotrichum fuscum</i>	32 (17.98 %)	47 (29.01 %)	39 (38.23 %)	118 (26.70 %)
Other species of fungi	146 (82.02 %)	115 (70.98 %)	63 (61.76 %)	324 (73.30 %)
Total isolates	178	162	102	442

Table 2. Size ( $\mu\text{m}$ ) of morphological structures of *Colletotrichum fuscum* on PDA medium (mean for 10 isolates)

Author	Acervuli ( $\mu\text{m}$ )	Setoses ( $\mu\text{m}$ )	Conidia ( $\mu\text{m}$ )	Appressoria ( $\mu\text{m}$ )	Chlamydo­spores ( $\mu\text{m}$ )
Own data	350.68 $\times$ 251.45	17.81–85.59 $\times$ 4.20–5.85	14.07–21.98 $\times$ 3.58–4.89	8.49–17.68 $\times$ 5.22–7.83	7.23–10.97 $\times$ 7–11.32
Sutton [1980]	–	–	15–21 $\times$ 3.5–4	–	–
Sato [1996]	–	–	15–21 $\times$ 3.5–4	–	–
Tomioka et al. [2001]	120	24 $\times$ 80	13–22 $\times$ 4–5	8–18 $\times$ 5–7	8–12 $\times$ 8–10

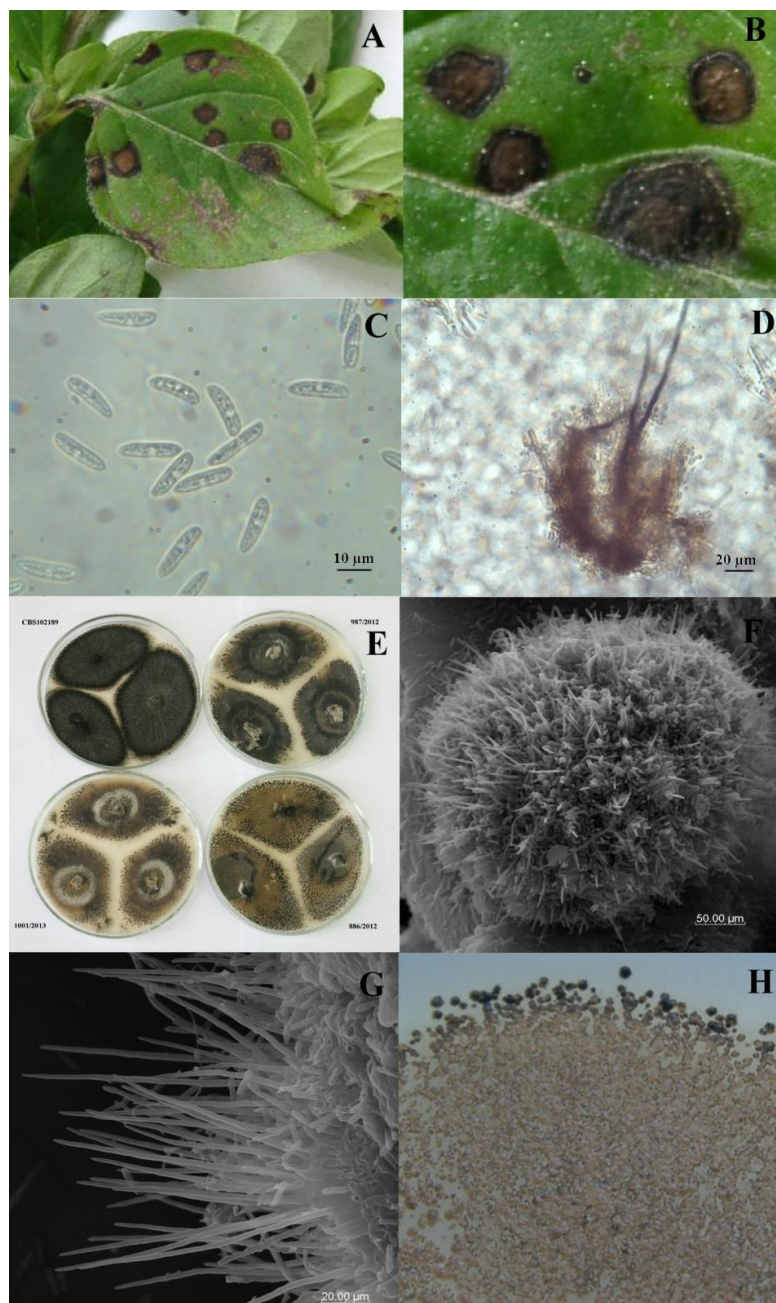


Fig. 1. *Colletotrichum fuscum*: A–B) Disease symptoms on oregano leaves; C) conidia under light microscope; D) acervulus under light microscope; E) 14-day-old colonies of four isolate on PDA medium; F) SEM micrographs showing acervulus with setoses; G) SEM micrographs showing sharp pointed setoses; H) salmon-cream drops of conidial exudate

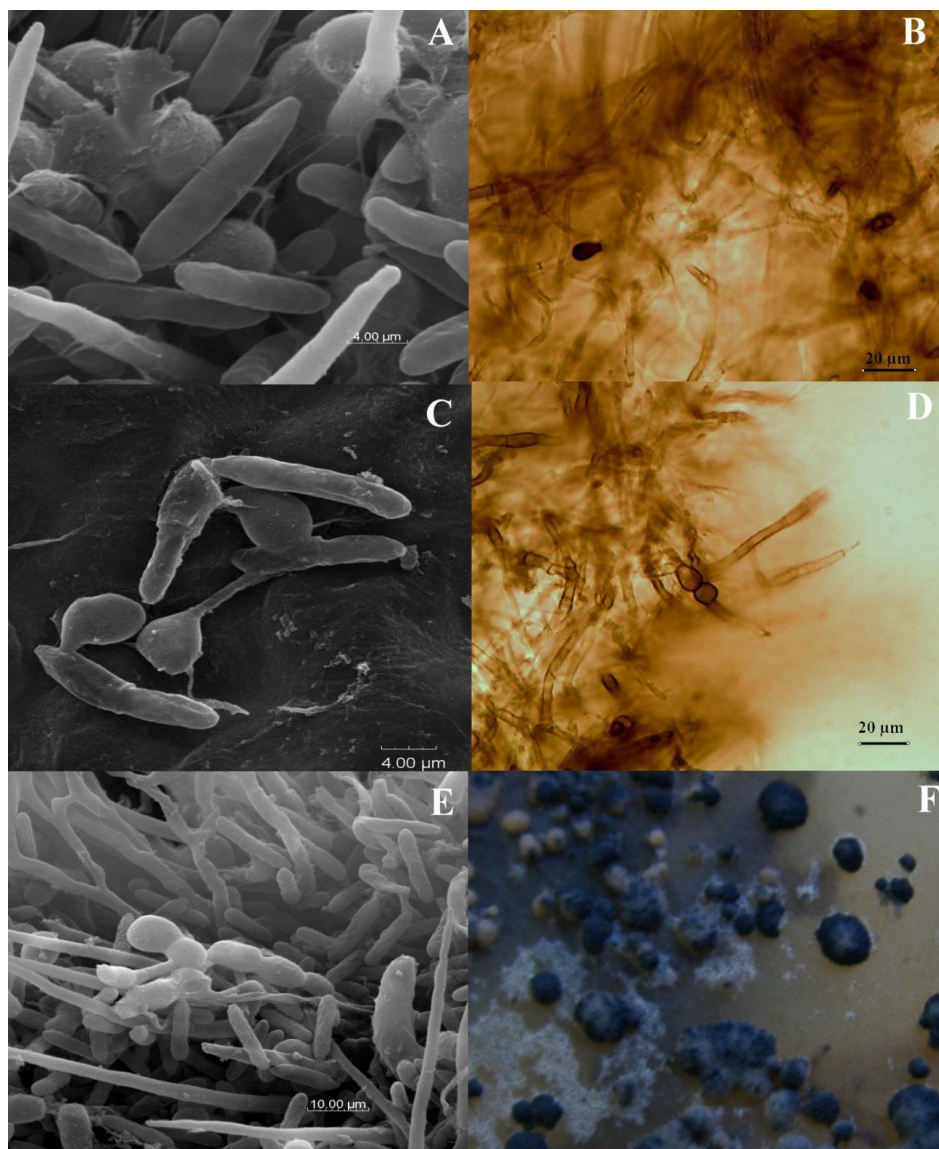


Fig. 2. *Colletotrichum fuscum*: A) SEM micrographs showing conidia; B) appressoria under light microscope; C) SEM micrographs showing germinating conidia with appressoria; D) chlamydospores under light microscope; E) SEM micrographs showing chlamydospores; F) sclerotia on PDA medium

After 14 days of culture, colonies of the fungus on PDA medium formed a dense dark-brown aerial mycelium, with sectors of a more loose, fluffy texture and gray colour (fig. 1E). The reverse of the colonies was black. Numerous acervuli (fig. 1F), were observed after this time, which were formed on the entire surface of the colony. They were black, oval or almost round and slightly immersed in the medium. The diameter of acervuli was  $350.68 \times 251.45 \mu\text{m}$  (tab. 2). On the surface of acervuli, numerous dark, sharp pointed setoses were formed, their size ranging from  $17.81 \mu\text{m}$  to  $85.59 \mu\text{m}$  in length and at the base  $4.20 \mu\text{m}$  to  $5.85 \mu\text{m}$  in width (tab. 2, fig. 1G). Salmon-cream drops of conidial exudate emerged from the acervuli (fig. 1H). *C. fuscum* formed hyaline, aseptate, smooth conidia. They are cylindrical to ellipsoid, straight or slightly bent. The top of the conidia is rounded, the base is slightly cut (fig. 2A). The size of the conidia was  $14.07\text{--}21.98 \times 3.58\text{--}4.89 \mu\text{m}$  (tab. 2). At the end or in the middle of hyphae abundant brown, ellipsoidal and lobe appressoria were formed (figs 2B, 2C). The size of appressoria was  $17.68 \times 5.22\text{--}7.83 \mu\text{m}$  (tab. 2). The tested isolates also formed round and smooth chlamydospores measuring  $7.23\text{--}10.97 \times 7\text{--}11.32 \mu\text{m}$  (tab. 2, figs 2D, 2E) and numerous conical, round or discoid black sclerotia (fig. 2F).

Table 3. Biotic effect of fungi isolated from leaves of oregano (*Origanum vulgare* L.) on *Colletotrichum fuscum*, after 10 days of dual growth

Species of fungus	IBE*	2012		2013		2014	
		frequency	GBE**	frequency	GBE**	frequency	GBE**
<i>Alternaria alternata</i> (Fr.) Keissler	+4	24	+96	31	+124	34	+136
<i>Epicoccum nigrum</i> Link	+5	4	+20	6	+30	5	+25
<i>Fusarium avenaceum</i> (Fr.) Sacc.	+4	2	+8	2	+8	1	+4
<i>Fusarium culmorum</i> (W.G.Sm.) Sacc.	+4	6	+24	3	+12	2	+8
<i>Fusarium equiseti</i> (Corda) Sacc.	+5	3	+15	5	+25	2	+10
<i>Clonostachys fimbriatum</i> Gilman et Abbott	+1	5	+5	2	+2	3	+3
<i>Clonostachys rosea</i> (Link) Schroers, Samuels, Seifert & W. Gams	+1	5	+5	2	+2	5	+5
<i>Phoma leonuri</i> Letendre	+3	2	+6	2	+6	4	+12
<i>Phoma multirostrata</i> var. <i>macrospora</i> Boerema	+5	3	+15	5	+25	1	+5
<i>Phoma versabilis</i> Boerem., Loer. & Hamers	+2	3	+6	1	+1	2	+4
<i>Stemphylium botryosum</i> Wallr.	+4	7	+28	11	+44	9	+36
<i>Talaromyces flavus</i> (Klöcker) Stolk & Samson	+1	1	+1	1	+1	3	+3
<i>Trichothecium roseum</i> (Pers.) Link	+4	2	+8	2	+8	3	+12
<i>Trichoderma harzianum</i> Rifai	+8	6	+48	5	+40	5	+40
<i>Trichoderma koningii</i> Oud.	+8	3	+24	14	+112	10	+80
Number of isolates	–	92	–	104	–	101	–
SBE***	–	–	+405	–	+515	–	+445

IBE\* – individual biotic effect; GBE\*\* – general biotic effect; SBE\*\*\* – summary biotic effect



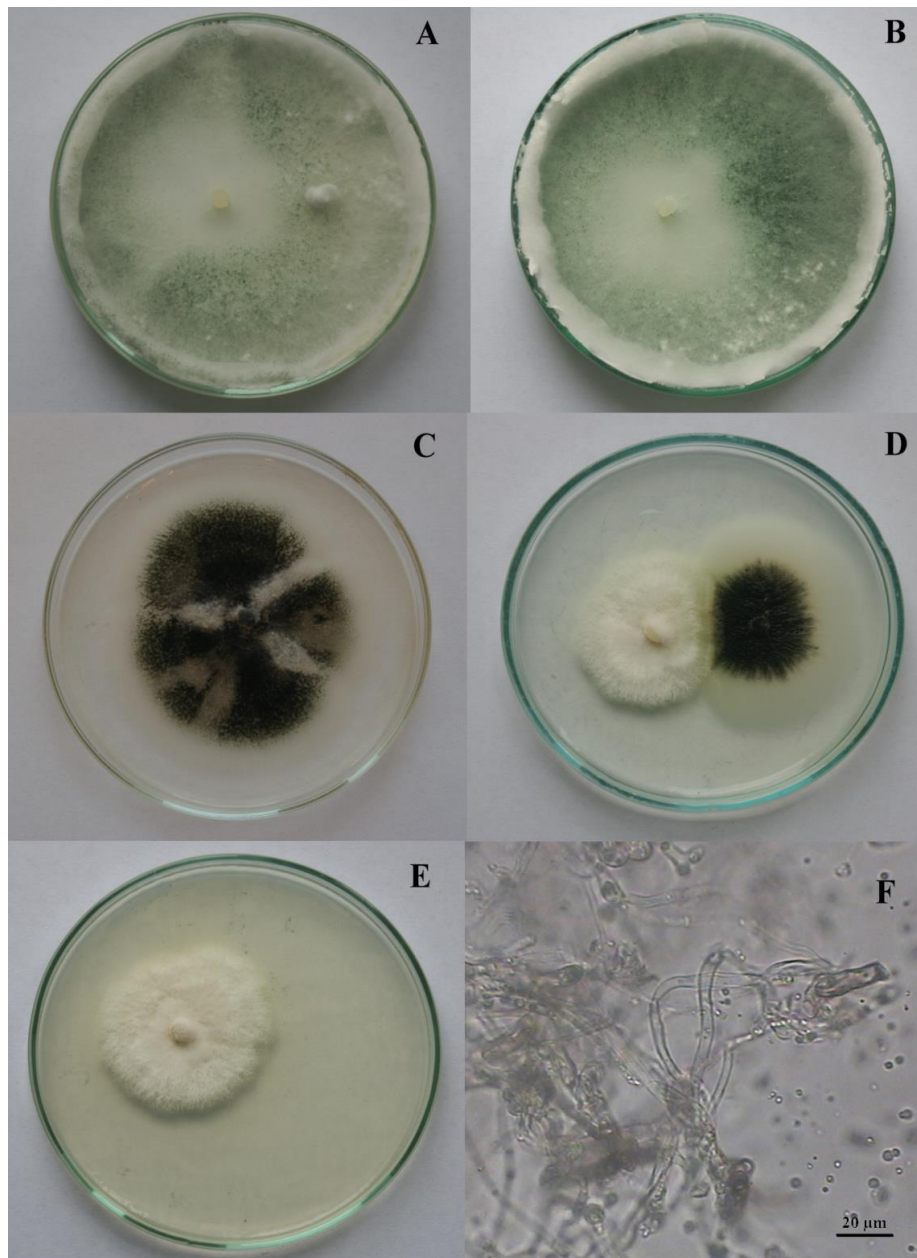


Fig. 3. Biotic activity of *Coletotrichum fuscum*: A) *Coletotrichum fuscum* (right) and *Trichoderma koningii* (left) after ten days of dual growth; B) individual growth of *T. koningii*; C) individual growth of *C. fuscum*; D) *C. fuscum* (right) and *Clonostachys rosea* (left) after ten days of dual growth; E) individual growth of *Clonostachys rosea*; F) degeneration of *C. fuscum* hyphae caused by *Clonostachys rosea*



**Biotic activity of *Colletotrichum fuscum*.** All the tested fungi inhibited the growth of *C. fuscum*, which is testified to by the positive values of IBE (tab. 3). The maximally positive values of +8 occurred in the case of *Botrytis cinerea*, *Trichoderma koningii* and *T. harzianum* and (tab. 3). *Trichoderma* spp. colonies overgrew the inoculum of *C. fuscum*, making the growth and sporulation of the pathogen impossible (figs 3A–3C). Besides, *Trichoderma* spp. caused degradation and dying out of hyphae and conidia of *C. fuscum*. The fungi species that considerably inhibited the growth of 10-days' pathogen colonies were *Epicoccum nigrum*, *Fusarium equiseti* and *Phoma multirostrata* var. *macrospora*, because the value of the individual biotic effect was +5 (tab. 3). Besides, with the common growth of *C. fuscum* with *P. multirostrata* var. *macrospora*, a 3 mm inhibition zone was observed. In the case of other tested species from the genus *Fusarium* and *Alternaria alternata*, *Chaetomium globosum* or *Stemphylium botryosum* the value of the individual biotic effect was +4 (tab. 3). The species that inhibited the growth of 10-days' colonies of *C. fuscum* only to a small extent were *Cladosporium cladosporioides*, *Clonostachys* spp. and *Talaromyces flavus* since the value of IBE was +1 (tab. 3). The colonies of *Clonostachys rosea* and *C. fimbriatum* met the colony of *C. fuscum* after 10 days of common growth (figs 3D, 3E), while after 14 days they overgrew  $\frac{1}{2}$ , of the surface of the pathogen colony. After 20 days of common growth, the whole colony of *C. fuscum* was covered with the mycelium of the micoparasite, which caused degradation and dying out of hyphae of pathogen (fig. 3F).

## DISCUSSION

Obtaining numerous isolates of *C. fuscum* from characteristic, regular, necrotic spots occurring on the leaves of oregano, as well as the positive results of pathogenicity tests carried out by the author (personal communication) make it possible to recognize *C. fuscum* as a new pathogen of oregano in Poland and propose the name for the disease, anthracnose of oregano leaves. The pathogen was previously recorded in Japan as the causal agent of anthracnose nemesia (*Nemesia strumosa* Benth.) [Tomioka et al. 2001], and the United States as a pathogen woolly foxglove (*Digitalis lanata* Ehrh.) and purple foxglove (*Digitalis purpurea* L.) [Goodman 1960]. The toxin produced by the pathogen called colleotine, composed mainly of a polysaccharide and peptides, plays an important role in the pathogenesis process [Goodman 1960]. Including the studied isolates in the species *C. fuscum* was possible through a careful analysis of macroscopic and microscopic features studied on PDA medium, which is recommended by many authors [Shen et al. 2001, Tomioka et al. 2001, Khan and Hsiang 2003, Machowicz-Stefaniak 2010] to the culture and identification of fungi from genus *Colletotrichum*. On PDA medium the tested isolates of *C. fuscum* formed all the characteristic morphological structures which are essential for the correctly identification. *Colletotrichum fuscum*, like most fungi of the genus *Colletotrichum*, for example, *C. coccodes*, *C. lindemuthianum*, *C. crassipes*, *C. caudatum*, *C. graminicola* or *C. dematium*, produces the acervuli covered with numerous sharp-pointed setoses, and despite the fact that the current system of taxonomy of ascomycetes anamorph gives the features of conidioma as a key feature, in the case of *C. fuscum* it appears to be insufficient to correctly identi-

fy the species. Very important morphological structures formed by *C. fuscum* are intercalary chlamydospores. Thanks to them, it is possible to distinguish the examined species from those which are very similar, i.e. *Colletotrichum acutatum*, *C. coccodes*, *C. gloeosporioides* and *C. higginsianum* [Sutton 1992, Tomioka et al. 2001]. The studied isolates of *C. fuscum* formed sclerotia and appressoria, the structures important from the taxonomical point of view and frequently reported in genus *Colletotrichum* [Sato 1996]. Especially important is the shape of appressoria. The features of the morphology and dimensions of appressoria produced by isolates of *C. fuscum* are in accordance with the description given by Tomioka et al. [2001].

The present studies showed that in case of all the tested fungi the IBE value was positive, which suggests that *C. fuscum* growth can be inhibited by those fungi in the phyllosphere of oregano leaves. A strong inhibiting effect of fungi from genus *Trichoderma* on *C. fuscum*, which is shown by complete overgrowth of the mycelium and degradation of the hyphae and conidia, should be positively estimated in the practical aspect of using the strains of *T. harzianum* and *T. koningii* in the biological control of *C. fuscum*. These fungi combine many of the features of a good antagonist, which include: rapid growth, abundant sporulation, prevalence in the rhizosphere as well as in the phyllosphere of plants, production a fungistatic substance (mainly antibiotics peptide), production of endo- and exoenzymes, the possibility of existence on many substrates, an easy use of organic and inorganic compounds and the ability for mycoparasitism [Fokkema 1993]. Strong inhibiting effects of fungi from genus *Trichoderma* were used, for example, in India, to control *Fusarium oxysporum* f. sp. *cumini* [Singh et al. 2007], in Canada to limit the occurrence of the wilt of purple coneflower caused by *Sclerotinia sclerotiorum* [Chang et al. 2006], as well as in Argentina to control coriander alternariosis caused by *Alternaria alternata* [Sandoval et al. 2006]. In Poland the ability of *Trichoderma koningii* and *T. harzianum* was shown to reduce the growth and cause the lysis of morphological structures of *Septoria carvi* and *Phomopsis diachenii*, pathogens of caraway [Machowicz-Stefaniak et al. 2008, Machowicz-Stefaniak 2009], *Colletotrichum dematium* [Machowicz-Stefaniak 2010], and *Boeremia strasseri* (*Phoma strasseri*) the pathogen of peppermint [Zimowska 2012]. A significantly slower antagonistic effect after 10 days of common growth was reported for the species of the genus *Clonostachys*. Those fungi are mainly known for their abilities for antibiosis and mycoparasitism; hence, the full antagonistic effect of those fungi is visible only after 30–40 days, which was observed in the present studies and earlier, for such pathogens as *Boeremia strasseri*, *Botrytis cinerea*, *Seimatosporium hypericinum* or *Septoria carvi* [Machowicz-Stefaniak 1998, Zimowska 2004, Machowicz-Stefaniak et al. 2008, Zimowska 2011]. Based on the undertaken studies, *Epicoccum nigrum* and *Chaetomium globosum* can be considered as positive antagonists. The first one is a well-known antagonist for many pathogens including *Colletotrichum gloeosporioides*, *B. cinerea* or *Monilinia laxa* [Pascual et al. 2002]. It was used in Egypt to reduce the impact of cotton damping-off caused by *Pythium ultimum*, *P. debaryanum* and *Rhizoctonia solani* [Hashem and Ali 2004]. In Argentina it effectively restricted the occurrence of sunflower root and stem rot caused by *Sclerotinia sclerotiorum* [Pieckenstain et al. 2001]. *Epicoccum nigrum* antagonistic properties rely primarily on competition and antibiosis thanks to the production of secondary metabolites, i.e. flavipine and epikorazine B [Piecken-

stain et al. 2001]. *Chaetomium globosum* restrict the development of foliar pathogens of wheat in India [Aggarwal et al. 2004] and protect the apple trees in front of their infection by *Venturia inequalis* [Cullen et al. 1984]. The limiting effect of *Phoma multirostrata* var. *macrospora* on *C. fuscum* is probably due to antibiosis, which may indicate the zone of inhibition produced by the first species of fungus. It is known that fungus *Phoma* sensu lato has abilities for producing secondary metabolites of various kinds, including fungistatic ones. *Boeremia exigua* var. *exigua* produce antibiotic E and cytochalasine B, metabolites of antibacterial and antifungal properties [Rai et al. 2009, Boerema and Höweler 1967]. *Plenodomus lingam* (*Phoma lingam*) phomenoic acid, phomenolactin and siredosmin PL, the metabolites of similar properties [Devys et al. 1984, 1986]. The fungi tested in this study and known for their antagonist effect on a variety of pathogens include *Cladosporium cladosporioides*, *Talaromyces flavus* and *Trichothecium roseum* [Huang and Kokko 1993, Jackson et al. 1997, Madi et al. 1997]. The first two species slightly limited the growth of *Colletotrichum fuscum* as evidenced by the low value of the IBE. The first species is known for reducing the growth of, among others, *Botrytis fabae* [Jackson et al. 1997]. It was also shown that the cultures of *C. cladosporioides* are the source of cladosporol inhibiting the synthesis of  $\beta$ -glucan and antibiotic izocumarine and thereby affecting the retardation hyphae of the fungus *Phytophthora capsici* [Sakagami et al. 1995]. Antagonistic interaction of *Talaromyces flavus* on *Sclerotium rolfsii*, and *Verticillium dahliae* was associated with antibiosis thanks to the fact that this species produces antifungal metabolites and with mycoparasitism, which is made possible by the fungus producing chitinolytic and cellulolytic enzymes [Madi et al. 1997]. Probably the full effect of the above-mentioned species of antagonistic fungi can be assessed after a prolonged period of growth.

The other species of the tested fungi, i.e. *Alternaria alternata*, *Botrytis cinerea* or *Fusarium* spp., cannot be regarded as positive antagonists since they belong to the species that are pathogenic towards a lot of herbal plants [Machowicz-Stefaniak and Zalewska 2004, Zimowska 2007, 2008a, b, 2010, 2013].

The study showed that *C. fuscum* is a weak competitor, and its development in the phyllosphere of oregano plants may be limited by numerous antagonists, which should be viewed positively in the context of plant health and quality of the crop of *Herba Origani*. In this study they did not reveal the capacity of *C. fuscum* for antibiosis despite the production of colleotine by the pathogen [Goodman 1960]. This toxin probably shows the phytotoxic and not fungistatic effect and therefore, an important role is attributed to it in the pathogenesis process of this fungus.

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## WYSTĘPOWANIE I CHARAKTERYSTYKA *Colletotrichum fuscum*

**Streszczenie.** W latach 2012–2014 w południowo-wschodniej Polsce z liści lebidki pospolitej (*Origanum vulgare* L.) z nekrotycznymi, koncentrycznie strefowanymi plamami z jaśniejszym środkiem i lekko wzniesionym brzegiem wyizolowano *Colletotrichum fuscum*. Przebadano morfologię wybranych losowo z własnej kolekcji kultur dziewięciu izolatów grzyba oraz izolatu referencyjnego otrzymanego z CBS-KNAW. Izolaty hodowano na pożywce PDA w temperaturze 24°C przez okres 14 dni. W przypadku każdego izolatu określano charakter wzrostu kolonii, kolor awersu i rewersu, tworzenie struktur morfologicznych, tj. acerwulusów, konidiów, apresoriów oraz chlamydospor. Ponadto przeprowadzono badania wyżej wymienionych struktur morfologicznych przy użyciu skaningowego mikroskopu elektronowego SEM. Przeprowadzone badania wskazały na tworzenie przez *C. fuscum* charakterystycznych struktur niezbędnych do prawidłowej identyfikacji patogena, tj. konidiów, acerwulusów ze szczecinami, chlamydospor oraz apresoriów. Ponadto wyniki badań nad biotycznym oddziaływaniem *C. fuscum* na gatunki grzybów występujące w fylosferze roślin lebidki wykazały, że wszystkie grzyby w różnym stopniu ograniczały wzrost *C. fuscum*. Grzyby z rodzajów *Trichoderma* i *Clonostachys* uznano za najbardziej efektywnych antagonistów *C. fuscum*. *Alternaria alternata*, *Botrytis cinerea* oraz *Fusarium* spp., pomimo wysokich wartości IBE, uznane zostały za negatywnych antagonistów.

**Słowa kluczowe:** *Origanum vulgare*, oregano, grzyby fylosferowe, morfologia, biotyczne oddziaływanie

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