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ПИКНИДИАЛНИ ГЪБИ ПО ЛАВАНДУЛАТА (*LAVANDULA OFFICINALIS* L.) PYCNIDIA FUNGI ON LAVENDER

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Abstract

In the period 2013 – 2014, a survey was carried out for determining the species of pycnidia fungi which can cause diseases in lavender. From leaves, skeletal branches and stems of lavender plants (*Lavandula officinalis* L.) with typical symptoms, the species *Phomopsis lavandulae* and *Phoma lavandulae* were isolated and identified. On the basis of symptoms and morphological characteristics the fungus *Septoria lavandulae* was defined. *Phomopsis lavandulae* attacks stems and leaves. On the leaves elliptical elongated spots were formed, starting from the lamina leaf. On the branches slightly elongated spots to irregular necrotic ones were detected. They developed cracks, the bark split and fell off. The pathogen formed dark colored pycnidia, rounded to slightly pear-shaped, with a diameter of 37.44 – 88.21 x 41.44–104.5 µm. The conidia were colorless, two cellular and elliptical in shape, slightly bent in the middle, with sizes of 6.16 to 8.75 x 2.77–4.55 µm. When *Phomopsis lavandulae* was associated with *Phoma lavandulae* and *Septoria lavandulae*, the damage on the plants increased. Small, yellow-brown spots appeared on the leaves of the plants infected with *Phoma lavandulae*. They subsequently merged and became necrotic. Yellowish spots formed on the stems, which progressively dried out and became gray-brown. They were filled with pycnidia of the pathogen, which were spherical, brown to black, with sizes of 125.80 x 110.50 µm. Unicellular, colorless, oval to cylindrical spores were formed with sizes of 6.12–5.10 x 1.70–1.36 µm. The symptoms occurring in the plants infected with *Septoria lavandulae* were well-formed rounded, reddish-brown dark-edged spots on the leaves. The pycnidia of the pathogen were often found on the upper surface. They were immersed in the leaf tissue, spherical, flattened, black, small with a diameter of 41.3–66.7 µm. The conidia were filamentous, straight or slightly curved, with pointed ends, multicellular, colorless, from 12.8 to 33.5 x 1.1–2.6 µm.

Key words: lavender (*Lavandula officinalis* L.), *Phoma lavandulae*, *Phomopsis lavandulae*, *Septoria lavandulae*.

INTRODUCTION

Lavender (*Lavandula officinalis* L.) is the main essential crop for Bulgaria. The essential oil, which is in the flowers, is used for the production of lavender oil, lavender concrete and lavender concrete alcohol (Yankulov, 2000; Terziev, 2006; Georgieva, 2005).

During the past years, the diseases attacking the culture have been increasing. According to scientific studies, reports can be found about appearance of phytoplasma (wet feet), Alfalfa mosaic virus, Cucumber mosaic virus, bacteriosis (*Xanthomonas campestris*); fungal diseases - *Phomopsis* (*Phomopsis lavandulae* Gabotto), *Phoma* (*Phoma lavandulae*), *Septoria* (*Septoria lavandulae* Dezm.), gray mold (*Botrytis cinerea*) and soil inhabiting pathogens from the genera *Fusarium*, *Verticillium*, *Sclerotium bataticola*, *Sclerotinia sclerotiorum* and *Phytophthora*.

In the foreign literature, data about damages caused by the fungus *Phomopsis lavandulae* are reported. The disease developed in an epidemic size during the second half of the XX century and it completely destroyed 3-4 years old the lavender plantations in France (Buczacki and Harris, 1998). The damages from *Phomopsis* increased when associated with other pycnidial pathogenic fungi from the genera *Septoria* and *Phoma* (Buczacki and Harris, 1998).

Another important and deleterious disease for lavender is caused by the fungus *Phoma lavandulae* Gabotto (1905).

Brierley, in 1916, identified a fungus found in the buds and leaves as *Phoma lavandulae*, the disease is known later under the name "Shab". The disease is spread through infected plants at the base of the branches. Similar symptoms were found in 1946 in the Botanical Garden of the University of Pavia. The plants were affected so seriously that the culture was almost destroyed. The cause was identified with a fungus of the genus *Phomopsis*. Up to now, the two genera have been combined into one *Phomopsis lavandulae* (Gabotto) Cif. et Vegni = *Phoma lavandulae* Gabotto (1905). The disease is well studied in France (Metcalf, 1931).

In Italy, *Septoria lavandulae* was isolated from *L. dentate* (Buonario et al., 1996). This species is also identified from *L. angustifolia* in Hungary (Nagy and Horvath, 2010) and Croatia (Vrandecic et al., 2014). In France, such symptoms of the disease are also described (Boudier, 1995).

In Bulgarian phytopathological literature, reports have been published about *Septoria lavandulae*, *Phoma lavandulae*, root rot and withering of lavender (Hristov, 1972, Margina, 2000; Bobev, 2009; Nakova, 2011). *Phomopsis* causes drying of separate branches or of whole plants. Stems become yellow, necrotic and filled with black pycnidia (Bobev, 2009). In 2008-2009 Nakova (2011) identified *Phomopsis lavandulae* (Gabotto) as the causal agent of the disease.

The aim of this study is to determine the types of pycnidial fungi that cause diseases in lavender.

MATERIALS AND METHODS

During the period 2013–2014, a survey was carried out to identify the pycnidial fungi that cause diseases of lavender (*Lavandula officinalis* L.). Plant materials for analysis were collected from the regions of Shumen, Stara Zagora

and Elhovo. Laboratory analyses were performed at the Department of Phytopathology, at the Agricultural University – Plovdiv.

Isolation of the pathogens

Observations under field conditions are conducted on symptomatic manifestations at different varieties of naturally infected lavender shrubs, leaves, skeletal branches. Small pieces of the infected tissues are cut from the border zone between the diseased and healthy part of the infected plants and then washed with running water. Isolations are made on PDA. From pure cultures, sporulation is examined and pathogenicity tests are carried out by inoculation of healthy branches of healthy lavender plants.

Pathogenicity tests

From a 12–14 day-old culture on PDA, a spore suspension is prepared and sprayed on the skeletal branches of the lavender (variety Seuthopolis). The control plants are sprayed with distilled water. The plants are placed in a growth chamber at 25°C, RH 70% and periodically sprayed with water to maintain the high humidity. If any symptoms appear, reisolation and microscopic analysis are carried out.

Determination of morphological characteristics

The determination of phytopathogens is performed by macroscopic signs such as symptoms (syndrome) and microscopic analysis on the morphological characteristics of the pycnidia and conidia (Tafradjiiski et al., 1973). For that purpose, fruiting bodies and conidia from a 12–14 day-old culture on PDA is measured.

RESULTS AND DISCUSSION

Within the period 2013–2014 samples of diseased lavender plants (*Lavandula officinalis* L.) in plantations located in the main production areas in the South Eastern, Central and Northern part of the country were analyzed.

From the leaves, skeletal branches and stems of the lavender plants (*Lavandula officinalis*) with typical symptoms the species *Phomopsis lavandulae* and *Phoma lavandulae* are isolated and identified. The pathogens are found in all lavender growing regions and for each isolate pathogenicity tests are carried out. On the basis of the symptom manifestations and morphological characteristics the species *Septoria lavandulae* is defined.

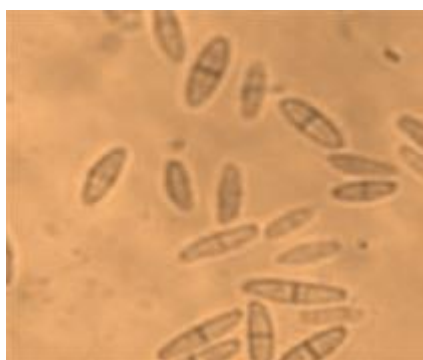
The disease with the causative agent *Phomopsis lavandulae* induces elliptically elongated leaf spots, starting from the periphery of the lamina. Subsequently, the spots become necrotic. There are elliptical, necrotic spots on the flowering stems. Often, flower withering appears. On the branches slightly elongated spots to irregular necrotic ones are found. They become shells, bark splits and falls off. The drying of individual skeletal branches is observed. Inside them, pycnidia of the pathogen are formed. The isolate used in the present study (Sh1/1.6) is from the region of Shumen, from a more than 8 years old lavender field, planted with Seuthopolis variety. On PDA, the pycnidia initially develop colorless mycelium, but later it becomes pale brown to gray-brown. While growing, the colonies become a zoned type. The base is colored dark brown. The pycnidia are partially submerged in the agar medium, and give concentric zoning of the

colonies. The fruiting bodies are also partially submerged into plant tissues. The pycnidia are rounded, dark colored with sizes – 104,5-88,21x41,55-35,61 μm (Fig. 1). The conidia are colorless, dual cellular and elliptical in shape, slightly bent in the middle with slightly pointed ends. They have the following size – 8,75-5,15 x 4,55-1,93 μm (Tabl. 1), (Fig. 1).

Таблица 1. Размери на пикнидии и конидиоспори на *Phomopsis lavandulae* (Sh1/1.6), μm

Table 1. Sizes of pycnidia and spores of *Phomopsis lavandulae*, μm

Номер на изолата Number of isolate		Среда: КДА Media: PDA				
		Размер – плодни тела			Размер - спори	
		Size – fruit bodies			Size - spores	
		Диаметър Diameter	Дължина Length	Ширина Width	Дължина Length	Ширина Width
Sh1/1.6	Средно Average	39,44	41,44	37,44	6,16	2,77
	Cv%	11,65	10,68	12,62	16,66	15,3
	S2	20,97	19,61	22,33	1,05	0,18
	S	4,58	4,43	4,73	1,03	0,42
	Max	98,45	104,5	41,55	8,75	4,55
	Min	37,78	88,21	35,61	5,15	1,93



Фиг. 1. Конидиоспори на *Phomopsis lavandulae*
Fig. 1. Conidiospores of *Phomopsis lavandulae*

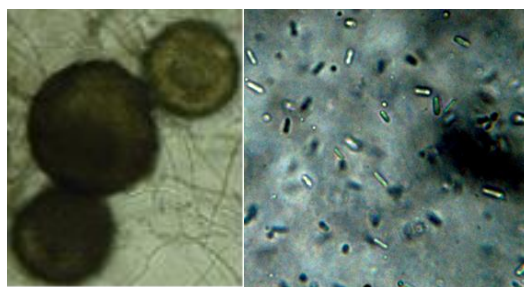
Symptom manifestations, caused by *Phoma lavandulae* on the crop, form specs with size 1–1,5 mm, and also fluidal necrotic spots – 1-1.5 x 3,5-4 mm. Yellowish spots are detected inside the stems, which progressively dry up and become gray-brown, often with 2-3 mm to 20-25 mm. The damage permeates deep into the stem, forming shells. The fungus pycnidia are found around them.

Spherical, brown to black – 119,00-108,80 x 142,80-108,80 μm (Fig. 2). The spores are ovate, unicellular, colorless - 6,12-5,10 x 1,70-1,36 μm (Tabl. 2). The damages reach the flower stems and often the flower dries during the mass flowering. The fungus mycelium is septate, colorless. On the PDA there is a radial mycelial growth, gray-brown with whitish periphery and a dark base in the central part. The pathogen used in current test was isolated from the region of Zimnitsa - Stara Zagora (3 years of age, Seuthopolis variety).

Таблица 2. Размери на пикнидии и конидиоспори на *Phoma lavandulae* (Zim3/1), μm

Table 2. Sizes of pycnidia and spores of *Phoma lavandulae*, μm

Номер на изолата Number of isolate		Среда: КДА Media: PDA				
		Размер – плодни тела			Размер – спори	
		Size – fruit bodies			Size – spores	
		Диаметър Diameter	Дължина Length	Ширина Width	Дължина Length	Ширина Width
Zim3/1	Средно Average	118	115.16	120.84	5.64	1.58
	Cv%	4.62	4.04	11.91	7.82	10.31
	S2	29.78	21.64	207.24	0.19	0.03
	S	5.46	4.65	14.4	0.44	0.16
	Max	125.8	119	142.8	6.12	1.7
	Min	110.5	108.8	108.8	5.1	1.36



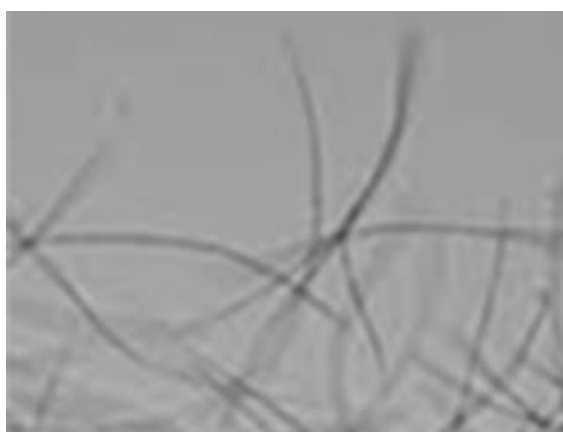
Фиг. 2. Пикнидии и конидиоспори на *Phoma lavandulae*
Fig. 2. Pycnidia and spores of *Phoma lavandulae*

On the basis of the symptom manifestations and morphological characteristics the fungus *Septoria lavandulae* is identified. The symptomatic manifestations on the leaves are in the form of rounded, reddish-brown spots with dark wreath. In the beginning of the vegetation the spots are small, subsequently they expand in size and can cover the entire lamina. Later the tissues in the spots

area becomes gray, and on the upper surface black pycnidia are observed. They are immersed, spherical, flattened, black, small (diameter 41,3-66,7 μm), with a round ostiole. The conidia are filamentous, straight or slightly wavy, with pointed ends, multicellular (1-3 septa without twist), colorless, 12.8 to 33.5 x 1,1-2,6 μm (Tabl. 3), (Fig. 3). The studied pathogen is from the region of Elhovo (Seuthopolis variety, 9 years old lavender).

Таблица 3. Размери на пикнидии и конидиоспори на *Septoria lavandulae*, μm
Table 3. Sizes of pycnidia and spores of *Septoria lavandulae*, μm

Номер на изолата Number of isolate		Растителни тъкани/Plant tissue				
		Размер – плодни тела			Размер – спори	
		Size – fruit bodies			Size – spores	
		Диаметър Diameter	Дължина Length	Ширина Width	Дължина Length	Ширина Width
<i>Septoria lavandulae</i>	Средно Average	58.52	50.82	66.21	25.69	1.88
	Cv%	9.39	21.85	4.2	39.3	39.87
	S2	30.22	123.35	7.73	101.97	0.56
	S	5.5	11.11	2.78	10.1	0.75
	Max	66.81	66.64	68	33.56	2.62
	Min	41.4	41.31	41.48	12.82	1.12



Фиг. 3. Конидиоспори на *Septoria lavandulae*
Fig. 3. Conidiospores of *Septoria lavandulae*

Quite frequently, association of the three pathogens is seen. It further complicates the plant health status. The situation worsens when there are frequent rains and high temperatures.

CONCLUSIONS

The research carried out on the morphological characteristics of pycnidia pathogens in the lavender agroecosystems across the country gives reason to make the following important conclusions:

1. Phytopathogenic fungi *Phomopsis lavandulae* (Gabotto) Cif. & Vegni, *Phoma lavandulae* Gabotto, *Septoria lavandulae* Dezm. are found and spread in some lavender plantation areas in Bulgaria.

2. *Phomopsis lavandulae* often develops in association with pathogens of genera, *Phoma* (*P. lavandulae*) and *Septoria* (*S. lavandulae*).

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