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PHYLLOSTICTA DECIDUA ELLIS ET KELLERMAN – A NEW PATHOGEN ON LEMON BALM (*MELISSA OFFICINALIS* L.) IN BULGARIA

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Abstract

A study on the identification and spread of harmful flora on lemon balm (*Melissa officinalis*) in the region of Dobroudzha was conducted during the period 2015 – 2016. The research established that the percentage of the diseased plants (in a plantation of 1 ha) was 9.42%, while the index of the infected leaves was 19.25%.

Small, irregular, light brown to necrotic spots with a darker edge were formed on the leaves. The spots were randomly scattered over the whole lamina. On the branches were found elongated light brown spots, resulting in the formation of shells as well as partial or complete drying of the plants.

A pycnidia fungus was isolated from the leaves and branches with typical symptoms. The pathogenicity of the isolates was proven. The morphological and cultural characteristics were tested. On this basis the pathogen *Phyllosticta decidua* Ellis et Kellermann was identified.

The fungicidal activity of 6 products with a different active base as well as of two biological extracts was tested *in vitro*.

Keywords: lemon balm (*Melissa officinalis* L.), spots (*Phyllosticta decidua*).

INTRODUCTION

The lemon balm (*Melissa officinalis*) has long been known and widely used as essential oils and medical plants. 0.33% of the essential oil is contained in the above-ground parts. The main ingredient is the citrate. Because of its strong lemon aroma, it is widely used in perfumery, cosmetics and food industry for flavoring (Yankulov, 2000).

In the literature on the lemon balm phytopathogens from different groups have been reported and described. The phytoplasmas are widely spread in the medical plants. It is estimated that they induce the formation of the phyllodia, the yellowing, and reddening of the plant, the atypical proliferation and the green color of the buds (Starovic et al., 2015). The Tulip Virus X is identified from the viral diseases affecting the culture in the United States, (Tzanetakis et al., 2005).

In 1940 the *Phyllosticta decidua* was established on the lemon balm in the USA (mycoportal.org). The pathogen was observed further in Canada and Indonesia (www.gbif.org).

In 2006 in Liguria, Italy the fungus *Botrytis cinerea* was identified on the crop. Symptoms of the disease, both on the leaves and stems are detected. At a later stage the plants wilt and dry (Pensa et al., 2007).

In 2009 in several gardens in Budapest, Hungary and Genci, Romania, symptoms of powdery mildew *Golovinomyces biocellatus* were observed (Kassai-Jager et al., 2010). In Hungary, the pathogenic fungus *Septoria melissae* was described, too (Nagy et al., 2010).

From the lemon balm in the Kraków Botanical Garden the fungal pathogens *Alternaria alternata*, *Epicoccum nigrum* and *Sordaria fimicola* are isolated (Kowalik, 2013).

During the period 1998 - 2001 in Poland from the roots and stem bases of *Melissa officinalis* L., the pathogens *Fusarium solani* and *Rhizoctonia solani* are isolated and identified. The fungus *Alternaria alternata*is observed from the infected leaves (Machowicz-Stefaniak et al., 2004).

In the Bulgarian literature only the species *Septoria melissae* Desmazieres is reported (Hristov, 1972; Margina, 2000; Bobev, 2009).

The species *Phyllosticta decidua* Ellis et Kellermann causes the formation of small spots with brown rounded edges. The pycnidia of the fungus are semi-submerged, spherical (about 75 µm), and black. The spores are oblong with rounded ends, unicellular and colorless, 5-10 x 3-3.5 µm (Hristov, 1972). The pathogen was not found in Bulgaria at that time.

During the period 1999-2001, the soilborn pathogens *Pythium* sp., *Rhizoctonia solani*, *Fusarium oxysporum* and *Pestalotia* sp. are isolated in Bulgaria (Mirkova et al., 2003).

In the recent years, the interest in the lemon balm (*Melissa officinalis*) as an essential-oil plant has grown. In the different regions of the country, the production crop areas are increasing, but a systematic research of the plant health regarding harmful flora has not been conducted yet.

From the performed monitoring through the country it is found a constantly growing infectious background from the fungal pathogens in the plantations of the lemon balm. That defines the purpose of the study.

MATERIALS AND METHODS

During the period 2015-2016, a survey was carried out to identify the health status of the lemon balm plantations. The plant materials for analysis were collected from the region of Dobrudzha. The laboratory analyses were performed at the Agricultural University, Plovdiv in the Department of Phytopathology.

The disease symptoms are described from the naturally infected plants and then compared with the artificially inoculated ones.

The spread of the diseases

The incidence rate was calculated as a percentage of the infected plants. The survey was done on the randomly selected plants taken from the diagonals of the lemon balm fields.

The calculations were performed according to the formula of Chumakov (1974):

$$P = a/A \times 100$$

P – Incidence of diseases [%];
a – Number of diseased plants;
A – Total number of reported plants.

The disease severity

The index of the disease severity is calculated by the formula of McKinney (Josifovich,1956):

$$I = \Sigma (n \times k)/N \times K \times 100,$$

I – Index of the disease [%];
n – Number of samples (leaves/branches) reported, at the respective scores/group;
k – Group scores;
N – Total number of recorded samples (leaves/branches);
K – The highest, score evaluated in the field.

To measure the spread of the spots on the leaves, 100 leaves taken from 5 different sites of the plantation are examined, using a six-point scale.

The degree of the attack on the leaves is determined according to the following scale:

0 - no signs of disease;
0.1 - small (about 1mm) spots;
1 - Spots on 25% of the leaf area;
2 - Spots on 50% of the leaf area;
3 - Spots on 75% of the leaf area;
4 - Spots on more than ≥ 75% of the leaf area.

The causal agent was isolated from the naturally infected lemon balm plants on a potato-dextrose agar (PDA) using standard phytopathology methods. Small pieces of the infected tissues were cut from the border zone between the diseased, and the healthy part of the infected plants then washed with running water and sterilized with ethanol. The Sporulation was examined from the pure cultures under the microscope. The pathogenicity tests were carried out by the inoculation of the healthy lemon balm plants.

Pathogenicity tests

From a 12-14-day-old culture on the PDA, a spore suspension is prepared and sprayed on the skeletal branches of the *Melissa officinalis*. The control plants are sprayed with distilled water. All plants are placed in a growth chamber at 25^{o}C, RH 70% and periodically sprayed with water to maintain the higher humidity. When symptoms appear, reisolation and microscopic analysis are carried out.

The pathogenicity is also proved using green apple fruits by the method of "Baiting bioassay" (Erwin and Ribeiro, 1996).

The identification of the phytopathogens causing the disease is performed both macroscopically based on the symptom (syndrome) characteristics and microscopically by morphological characters of the fruiting bodies and spores.

A hundred fruiting bodies and conidia are measured of the 12-14-day-old culture on the PDA for that purpose (Tafradjiiski et al., 1973). The data obtained were processed with programs Excel 2010 and SPSS 21.

A test of fungicidal activity

The effect of the different fungicides is evaluated by the method of Thornberry (modified). The following fungicides are tested:

Topsin M 70 ВДГ – 0.1% (а.в. tiofanat-methil),

Dithane M 45 – 0.2% (a.в. mankozeb),
Score 250 EK – 0.02% (a.в. difenkonazol),
Thiram – 0.3% (a.в. tiram),
Champion 50 BP – 0.15% (a.в. copper hydroxide),
Cuprocine Super M – 0.2% (a.в. mankozeb + copper oxychloride),
Biological extract 2,
Biological extract 2.1.

In every Petri plate, 9 ml of the PDA is poured together with the fungicide or the biological extract with a certain concentration. The Petri dishes are carefully homogenized by spinning. After that, the cooled dishes are infected by an 8-mm-micelia block from the 12-14-old–day culture of the pathogen. Each variant includes 3 repetitions.

The diameter of the colonies is measured in mm on the 3th, 6th, and 9th day. The results are calculated by the following formula:

(A-B)/A*100, %,

A – diameter of colony on fungus in the control, mm;
B – diameter of the colony on fungus, with fungicide, mm.

RESULTS AND DISCUSSION

From the observations carried out within the period of 2015-2016 in a 1 ha plantation of the lemon balm (*Melissa officinalis*) in the region of Dobrudzha, there are symptoms found on 9.42% of the plants. The index of attack on the leaves is 19.25%.

In dynamics, the disease development occurs in the following steps: initially, small (1.5 × 3 mm) and randomly scattered spots appear on the leaves. At later stages, the damaged tissues become brown and necrotic. Finally, a drying of the strongly infested plant is observed (fig. 1).

Fig. 1. *Symptoms of Phyllosticta decidua in natural infectious background*

Fig. 2. *Symptoms of Phyllosticta decidua on stems of lemon balm in artificial infectious background*

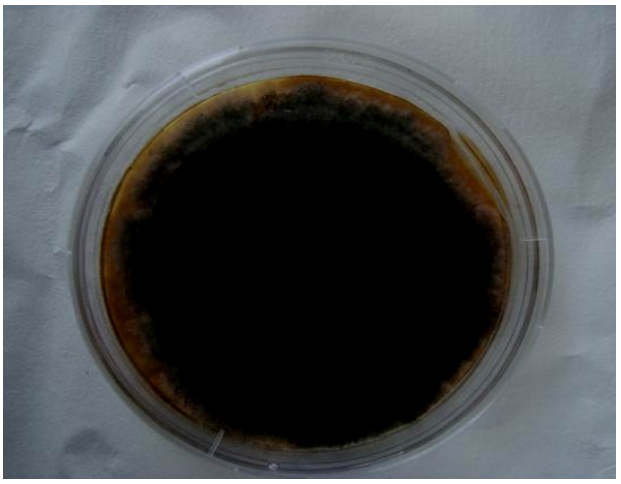

Fig. 3. *Culture characteristics of the colonies of isolate DR/01 on potato-dextrose agar*

Chlorotic spots are formed on the stems. They gradually dry up and become gray-brown. The spot size range is from 1.5-2 mm to 25-35 mm. The damage spreads into the stem and forms cracks. On the diseased leaf and shoot tissues, the fruiting bodies of the pathogen are formed as scattered small dots, semi-submerged in the tissues (fig. 2).

The morphological and cultural characteristics of all the received isolates (11) are similar. That is the reason for only the isolate DR 0/1 to be described below.

A colorless substrate mycelium is developed on the PDA which later transforms from light to dark gray (fig. 3). The fruiting bodies are immersed into the agar medium and are randomly scattered.

The pycnidia are rounded to slightly elongated, dark brown in color, 87.00-40.00 x 77.00-60.00 µm (fig. 4). The conidiospores are colorless, non-septate and oblong to ovoid with rounded ends, 10.00-4.60 x 3.00-1.60 µm. The data correspond to the ones described in the literature (Hristov, 1972).

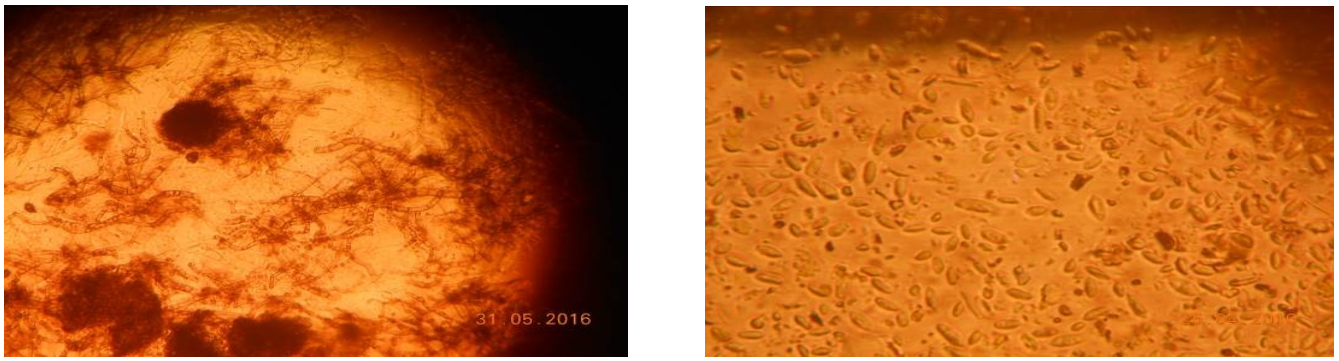

Fig. 4. *Pycnidia and conidiospores of isolate DR/01 from Phyllosticta decidua*

Table 1. The influence of temperature and relative humidity on the formation of pycnidia and conidiospores of *Phyllosticta decidua*, in a growth chamber

Temperature	Relative humidity, %	Formation of pycnidia, days	Formation of conidia, days
25^0C	80	7	11
20^0C		9	13
15^0C		11	16

Table 2. The efficacy [%] of some fungicides on the mycelial growth of the Isolate DR0/1

Isolate/ Fungicide		Control	Topsin M	Dithane	Score	Thiram	Champion	Cuprocine
		*M.G.	**ePPP	**ePPP	**ePPP	**ePPP	**ePPP	**ePPP
DR 0/1	Day 3	18.50	95.14	75.14	92.43	54.05	64.86	93.51
	Day 6	42.33	95.51	78.97	92.91	57.48	66.93	93.39
	Day 9	67.66	95.57	79.31	92.86	58.62	67.48	94.61

**M.G. – mycelial growth, mm; **ePPP – efficacy of plant protection product, %*

Table 3. The efficacy [%] of some biological extracts on the mycelial growth of the Isolate DR0/1

Isolate/Biological extract		Control	Biological extract 2.1	Biological extract 2
		*M.G.	**ePPP	**ePPP
DR 0/1	Day 3	18.50	87.02	84.86
	Day 6	42.33	87.71	85.87
	Day 9	67.66	88.17	86.45

**M.G. – mycelial growth, mm; **ePPP – efficacy of plant protection product, %*

The pathogenicity of the isolates is proven by the inoculation of the lemon balm plants and the green apple fruits. After the expiration of the incubation period, the typical symptoms of the disease caused by *Phyllosticta decidua* appear on the plant.

The observations on the effects of the temperature and relative humidity on the formation of the pycnidia and the conidia are presented in Table 1.

The data show that within the temperature range from 15^0C to 25^0C and at a relative humidity of 80% the formation of the fruiting bodies is carried out from 7 to 11 days, and the conidia are registered after 11 to 16 days.

The phytopathogenic fungus *Phyllosticta decidua* Ellis et Kellermann is determined as the causal agent of the leaf spots based on the analysis of the symptoms, the morphological characteristics of the fruiting bodies, the conidia and the cultural characteristics of the isolates. The data correspond to the publication of Hristov (1972).

The results of the *in vitro* experiments with the chemical and the biological means of control are presented in Tables 2 and 3.

The data are shown in Table 2 point out that the fungicides Topsin M, Cuprocine and Score inhibit the mycelium growth at the highest degree. Their efficiency on the third day is high and remains high until the ninth day - 92.43 - 95.57%. On a ninth day, the fungicidal effect of Dithane is 79.31%, of Champion - 67.48% and of Thiram - 58.62%.

Both biological products have a high efficacy on the mycelia development - Biological extract 2.1 - 88.17% and Biological extract 2 - 86.45% (Table 3).

CONCLUSIONS

The data from the studies on the phytosanitary situation in a compact lemon balm plantation in the region of Dobrudzha give the reason to make the following conclusions:

- The causal agent of the leaf spots on *Melissa officinalis* L. is *Phyllosticta decidua* Ellis et Kellermann. The spread of the pathogen in the observed plants is 9.42%, while the index of the attack on the leaves is 19.25%. That is an indication of an existing infectious background of the pathogen.
- In the *in vitro* testing the high fungicidal efficacy is shown by the fungicides Topsin M, Cuprocine and Score, and Biological extracts 2.1 and 2.

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