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Monitoring of fungal diseases of lavender

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Abstract. In the period between 2013 and 2014, phytosanitary monitoring was performed to establish the health status of lavender in relation to fungal diseases. During 2013, the diseases spread were investigated on 56,973 ha, and during 2014 – on 218,01 ha, respectively. The calculated disease incidence is 20.84% for the first, and 21.64% for the second year. The data analysis indicates that the phytosanitary situation is very complicated. As a whole, the pathogen infestation on the major varieties at different ages and in different regions was between 3.45% and 53.79%. The percentage of infected plants varies by regions: Karlovo (from 34.08 to 35.65%), Stara Zagora (16.04 – 30.27%), Kazanlak (20.01 – 23.43%), Elhovo (32.67 – 35.10%), Shumen (14.79 – 15.79%). Disease incidence on the major variety Seuthopolis, in 1 to 3-year-old lavender plantations differs: in Iganovo village, Karlovo region it was 36.18% and in Khan Krum village, Shumen region – 4.73%. This is an indication for the presence of high infection level in lavender fields from the early stages of plant development. It may be due to the usage of infected planting material or to the establishment of new plantations onto old lavender fields. Isolations have been made from different areas. In 2013, the diseases spread were investigated on 56.973 ha, and during 2014 – on 218.01 ha, respectively. The calculated disease incidence is between 3.45% and 53.79%. The percentage of infected plants varies by regions: in 2013 – 30.27%, in 2014 – 35.65%.

Keywords: lavender, diseases, Phoma, Phomopsis, Septoria, Phytophthora.

Introduction

Lavender (Lavandula officinalis) is mainly grown for the production of high quality essential oil, which is applied in cosmetics, in the perfumery and pharmaceutical industries, but also in medicine (Yankulov, 2000). Main lavender production regions are Stara Zagora and Plovdiv, and less lavender is grown in Pazardzhik, Blagoevgrad, Burgas, Varna, Shumen, Veliko Tarnovo and Vidin areas. In these regions lavender is planted on lands that are less productive and located at 800 to 1,000 m above sea level (Terziev, 2006; Dzhumariski et al., 2009).

The crop is considered as relatively resistant to the pathogenic microflora. In literature reports can be found about occurrence 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. Disease developed in epidemic size during the second half of the XX century, when it destroyed completely lavender plantations, 3-4 years old, in France (Buczacki and Harris, 1998). During the last decades the losses in lavender caused by Fusarium fungi are increasing. Pathogens have been reported in China (Ren et al., 2008), Saudi Arabia (Perveen and Bokhari, 2010), and in Croatia (Cosic et al., 2012). Damages from Phomopsis are increased when associated with other pycnidal pathogenic fungi from the genera Septoria and Phoma (Buczacki and Harris, 1998).

Information about the spread of Phytophthora fungi and losses caused are found in the publications of Putnam (1991), Minuto et al. (2001), Tsay (2002), Alvarez et al. (2007), Dervies et al. (2011), Nakova (2011). From diseased plants with symptoms most frequently isolated species of Phytophthora rot, is Phytophthora nicotianae var. parasitica Breda de Haan, syn. Phytophthora parasitica (Putman, 1991; Minuto et al., 1999; Minuto et al., 2001; Alvarez et al., 2007; Nakova, 2011; Faedda et al., 2013). Another species causing damages in lavender is Phytophthora cinnamomi (Orlikowski and Valjuskaite, 2007). In Spain, Phytophthora palmivora has also been reported (Paez et al., 1993). The hybrid Phytophthora x pelgrandis was described in the USA and Taiwan as a pathogen on ornamental plants. In lavender, it is found in the Netherlands (Bonnants et al., 2000), Hungary and Italy (Szijethy et al., 2012; Faeda et al., 2013). The species Phytophthora cryptogea is also reported on lavender and gerbera (Orlikowski, 1981; Orlikowski and Valjuskaite, 2007; Krober, 1991). In Italy, Septoria lavandulae was isolated from L. dentate (Buonario et al., 1996). This species is identified in Hungary on L. angustifolia, in 2010 (Nagy and Horvath, 2010) and in Croatia (Vrandecic et al., 2014). In France, the same symptoms of the disease are 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). The roots are dead and black branching rhizomorphs can be found – Armariella mellea, or white powdery mycelia and flat branched rhizomorphs - Rosellinia necaritix (Hristov, 1972, Margit, 2000). Phomopsis causes drying of the separate branches or whole plants. The stems become yellow, necrotic and filled with black picknidia (Bobev, 2009). In 2008 – 2009 Nakova (2011) identified Phomopsis lavandulae (Gabotto) as the causal agent of the disease. The plants carrying infection of the...
 Phytophthora spp. in the early stages have stunted growth, followed by a yellowing of the leaves and then wilting and defoliation (Nakova, 2011).

The aim of this study was to perform phytosanitary monitoring of the fungal diseases in lavender, calculate the percentage of diseased plants and diseases spread and severity, and determine the biodiversity of pathogens.

Material and methods

During the period 2013–2014, the diseases spread were determined in lavender plantations located in the main production areas of the Southern, Central and Northern part of Bulgaria. The laboratory analyses are carried out at the Department of Phytopathology, Agricultural University, Plovdiv.

Spread of the diseases

Incidence rate was calculated as a percentage of the infected leaves and skeletal branches, to total leaves and branches inspected. Tests were done walking on diagonals onto randomly selected shrubs of lavender fields. The calculations are performed according to the formula of Chumakov (1974):

\[ P = \frac{a}{A} \times 100 \]

where \( P \) is Incidence of diseases (%), \( a \) is Number of diseased plants, \( A \) is total number of reported plants.

Disease severity

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

\[ I = \sum \left( n \times k \right) / N \times K \times 100 \]

where \( I \) is Index of the disease (%), \( n \) is Number of samples (leaves/branches) reported, at the respective scores/group, \( k \) is Group scores, \( N \) is total number of recorded samples (leaves/branches), \( K \) is the highest, score evaluated in field.

The score is determined by counting the number of spots on the internodes of the skeletal branches of 50 plants of each variety/age using a five-point scale. To measure the spread of the spots on the leaves, 100 leaves of variety/age are examined using a six-point scale.

Disease severity on the branches is determined according to the following scale:

0 – healthy skeletal branches;
1 – skeletal branches with 1-2 spots at internode;
2 – skeletal branches with 3-5 spots at internode;
3 – skeletal branches with 6-10 spots at internode;
4 – skeletal branches with more than 10 spots at internode.

The degree of 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.

Identification of the phytopathogens that cause the diseases is performed macroscopically based on symptoms (syndrome) characteristics, and microscopically by morphological characters of the fruiting bodies and spores (Taftradjiski et al., 1973).

Isolation of the pathogens

Small pieces of infected tissues are cut from the border zone between the diseased and healthy part and then washed with running water. Isolations are made on PDA. From pure cultures, sporulation is examined and pathogenicity tests are carried by inoculation of healthy branches of healthy lavender plants.

Pathogenicity tests

From 12–14 days’ culture on PDA, a spore suspension of Phoma and Phomopsis is prepared and sprayed on skeletal branches of lavender. For isolates of Phytophthora, mycelial block is placed at the base of the branches. Control plants are sprayed with distilled water. Plants are placed in a growth chamber at 25°C, RH 70% and periodically sprayed with water to maintain high humidity. If symptoms appear, reisolation and microscopic analysis are carried out.

From lavender inoculated plants with pathogens of the genus Phytophthora, isolations on selective media PARP have been done after appearance of symptoms, and also the method of “baiting bioassay” is performed (Erwin and Ribeiro, 1996).

Results and discussion

In the foreign literature, data about percentage of losses are reported. In Tella region (Italy) in 2001, about 60% of the plants grown on loam soil exhibit symptoms of wilt and root rot (Dervies et al., 2011). During 2004, in Valencia, lavender plants are affected by stem and root rot. Infected plants are about 70% and have shown symptoms of wilting and lack of growth (Alvarez et al., 2007). During the period 2004–2005 in China, wilting has been observed resulting in high loss in yield, 15–20% (Ren et al., 2008). In 2010 in Turkey, symptoms of root rot were observed in 45% of the plants in the nursery (Dervies et al., 2011). During 2011 in plantations in the region of Slavonia, Croatia, symptoms of wilting have been observed on 20–30% of lavender plants (Cosic et al., 2012). In most cases the authors reported only the species which attack the lavender plants but not the percentage of losses.

The lack of current information about the health status of lavender crops in Bulgaria necessitates the conduction of phytosanitary monitoring in the main lavender producing areas of the country – Karlovo, Stara Zagora, Kazanlak, Elhovo, Shumen and others.

The study was performed in the period 2013 and 2014.

Percentage of infected plants was calculated and also the spread of the fungal phytopathogens. The total investigated area in 2013 was 57.963 ha, and the average percentage of diseased plants was 20.84%. In 2014, the investigated area was 218.01 ha, and the average percentage of diseased plants was 21.64%. Analysis of the data (Tables 1 and 2) shows that in some regions the spread of fungal diseases varies between different age groups: Karlovo from 6.47% to 46.91%, Plovdiv (35.85%–63.88%), Asenovgrad (5.15%–42.61%), Kazanlak (6.83%–47.76%), Stara Zagora (2.68%–45.12%), Shumen (4.73%–14.79%). In 2013, the highest percentage of diseases is observed in the region of Karlovo, village Iganovo on the one-year-old lavender – 48.95% and on 2–3-year-old...
Table 1. Spread of lavender fungal diseases in some regions of Bulgaria in 2013

<table>
<thead>
<tr>
<th>Region</th>
<th>Variety</th>
<th>Age of plantation, years</th>
<th>Area, ha</th>
<th>% of diseased plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burgas -Elhovo</td>
<td>Seuthopolis</td>
<td>8 - 9</td>
<td>1.5</td>
<td>32.67</td>
</tr>
<tr>
<td>Yambol-Zornitsa</td>
<td>Seuthopolis</td>
<td>1</td>
<td>3</td>
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</tr>
<tr>
<td>Zornitsa</td>
<td>Seuthopolis</td>
<td>2 - 3</td>
<td>9</td>
<td>13.04</td>
</tr>
<tr>
<td>Zornitsa</td>
<td>Druja</td>
<td>10</td>
<td>17</td>
<td>34.8</td>
</tr>
<tr>
<td>Zimnitsa</td>
<td>Seuthopolis</td>
<td>nursery</td>
<td>0.05</td>
<td>10.49</td>
</tr>
<tr>
<td>Plovdiv</td>
<td>Seuthopolis</td>
<td>4 - 5</td>
<td>0.006</td>
<td>10.45</td>
</tr>
<tr>
<td>Plovdiv</td>
<td>Raya</td>
<td>2 - 3</td>
<td>0.0042</td>
<td>4.35</td>
</tr>
<tr>
<td>Plovdiv</td>
<td>Hebar</td>
<td>2 - 3</td>
<td>0.0042</td>
<td>3.03</td>
</tr>
<tr>
<td>Plovdiv</td>
<td>Yubileyna</td>
<td>2 - 3</td>
<td>0.0042</td>
<td>3.45</td>
</tr>
<tr>
<td>Plovdiv</td>
<td>Druja</td>
<td>2 - 3</td>
<td>0.0042</td>
<td>12.5</td>
</tr>
<tr>
<td>Karlovo-Iganovo</td>
<td>Seuthopolis</td>
<td>1</td>
<td>0.8</td>
<td>48.95</td>
</tr>
<tr>
<td>Iganovo</td>
<td>Seuthopolis</td>
<td>3</td>
<td>1</td>
<td>26.25</td>
</tr>
<tr>
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<td>Seuthopolis</td>
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<td>1.5</td>
<td>25.64</td>
</tr>
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<td>Karnare</td>
<td>Seuthopolis</td>
<td>5 - 6</td>
<td>2.5</td>
<td>28.49</td>
</tr>
<tr>
<td>Rozino</td>
<td>Seuthopolis</td>
<td>2 - 3</td>
<td>5</td>
<td>41.06</td>
</tr>
<tr>
<td>Kazanlak-Manolovo</td>
<td>Seuthopolis</td>
<td>3 - 4</td>
<td>7.5</td>
<td>27.53</td>
</tr>
<tr>
<td>Kazanlak</td>
<td>Seuthopolis</td>
<td>nursery</td>
<td>0.6</td>
<td>16.48</td>
</tr>
</tbody>
</table>

The data reveal that there is a high infection level in the lavender censos, that is a factor for increased development of fungal diseases in the fields. Under favorable weather conditions pathogens can provoke an outbreak of epidemics. Plant health status was also complicated because of the association of fungal phytopathogens from different genera that parasitize lavender stems, roots, branches and leaves, leading to deep disorders in the growth and development of plants. The survey indicates complicated phytosanitary situation in young plantations aged from 1 to 3-4 years old. For example, in the village of Panicherevo in one-year-old plantations the diseases recorded on variety Hemus are 11.86% and in the village of Khan Krum – 4.73% (Seuthopolis variety). In 2-year-old plantations infestation is 36.18% in the Iganovo village (Seuthopolis), 3.73% in the village of Manolovo (Seuthopolis), 11.35% in Asenovgrad (Seuthopolis). In 3-4-year-old plantations, disease incidence is 45.19% in the village of Iganovo (Seuthopolis), 63.88% in the village of Dolna mahala, 35.55% in Manolovo, 46.91% in the village of Rozino.

The most widely grown variety in the country – Seuthopolis, is strongly attacked by phytopathogens, and the highest rate of disease incidence in 2013 was reported on one-year-old plants in the region of Iganovo 48.95%, while the lowest is in a 3-year-old plantation in the village of Zimnitsa – 1.60%. In 2014 the percentage of diseases is 63.88% – in 3-year-old plantations in the region of Dolna mahala. There are fields from that variety where the percentage of diseased plants is low – for example, 2-year-old lavender in the region of Manolovo (3.73%). In variety Hebar, 18-year-old plantation (Panicherevo), the percentage is 45.12%, and on variety Hemus (Panicherevo – 1-year-old lavender) – 11.86% are recorded.

The data received indicate the wide variation of the symptoms and the severity index on the leaves, skeletal branches and base of the plants (Figure 1). There is a trend of discovering more damages on the wood in the old plantations. But there are also cases registered with high index of diseases development on the leaves and wood in new fields aged 2-4 years, like Iganovo (Karlov) where disease severity is respectively 15.83% and 33.5%, Pavel Banya – 8.40% and 24.08%; Tarnichene – 21.0% and 35.0%; Manolovo – 3.6% and 30%. The analysis also point out that the main cultivated varieties in Bulgaria are sensitive to fungal pathogens. Diseases spread and severity in the different groups varies over a wide range on the leaves and the wood of the skeletal branches.

The macroscopic and microscopic diagnostics shows that plant health status of lavender is complicated. In the area of the town Elhovo, disease symptoms and sporulations (fruiting bodies and spores) are found from fungi of the genera: Phomopsis, Septoria, Phytophthora and Fusarium; in the region of Stara Zagora (Zimnica, Panicherevo) – Phoma, Phytophthora, Fusarium, Alternaria and Verticillium; near the town of Kazanlak (Kazanlak, Pavel banya, Tarnichene, Manolovo) – Phomopsis, Phoma, Septoria, Phytophthora, Fusarium, Verticillium and Alternaria; near the town of Karlovo (Iganovo, Karnare, Rozino, Vedrare) - Phoma, Phomopsis, Phytophthora, Alternaria, Verticillium; near the town of Plovdiv (Dolina Mahala, Asenovgrad, Novi izvor) – Fusarium, Alternaria, Phytophthora, Phomopsis, Phoma, Septoria. The town of Shumen (Khan Krum, Nikola Kozlevo) – Phytophthora, Phomopsis, Phoma. Infection levels, with more than 20% infested plants were reported in Karlovo, Yambol and Burgas in lavender field aged from 1 to 2 or 3 years old.

A prerequisite for the complex syndrome is the ability of pathogens to associate and parasite in various plant organs. In support of that are the results of the microscopic analysis and isolations of pathogens from diseased plants from different regions of the country (Table 3). In the second half of the vegetation of the culture, with the increase of temperature, the percentage of diseased plants is also rising. The reason for this is that during the...
Table 2. Spread of lavender fungal diseases in some regions of Bulgaria in 2014

<table>
<thead>
<tr>
<th>Region</th>
<th>Variety</th>
<th>Age of plantation, years</th>
<th>Area, ha</th>
<th>% of diseased plants</th>
</tr>
</thead>
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<tr>
<td>Iganovo</td>
<td>Seuthopolis</td>
<td>2</td>
<td>0.8</td>
<td>36.18</td>
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Figure 1. Disease severity on plants leaves and skeletal branches
period from July to September diseases caused by *Phoma lavandulae* and *Phomopsis lavandulae* are massively developing. During the warmer months, from July to August, peak moments occur for their development.

The severe attack of *Septoria lavandulae* causes leaf fall and weakening of the plants. In literature there are incidents of yield drops of the row material up to 24% and up to 13% of the fatness, as well as deterioration of the essential oil quality (Margina, 2000). The disease is also dangerous for the seedling production, reducing the percentage of rooting.

Over the past few years, root rot and wilting of plant also appears to be a serious problem in our country, especially in seedling production. *Phytophthora* rot is considered a major problem for some regions of the world where lavender is grown. The prerequisite for the massive spread of *Phytophthora* fungi are some biological characteristics of the pathogens, such as: the availability of a latent infection, in the culture media and plant tissue, general ecological plasticity and variability, high level of adaptation to the conditions of the environment, the presence of parasitic and saprophytic way of life, diversity of reproduction forms, and the presence of polycycle (Hansen et al., 1979, 1980).

When comparing the results with other authors, it is revealed that the percentage of diseased plants is high. This is the consequence of the inadequate care for crops during the growing season and the conditions stimulating the development of pathogens.

Data indicate that in addition to pathogens of the genera *Septoria*, *Phoma*, *Phomopsis* and *Phytophthora* and weaker parasitic fungi from the genera *Alternaria*, *Fusarium*, *Verticillium* are developed, that make the diagnosis of disease complicated and also take decisions control.

### Conclusion

The spread of phytopathogens on lavender in different regions of the country varies among different age groups from 3.24% to 53.29%. When the plants grow old the percentage of damage is increasing. The crop is vulnerable to attack by pathogens due to poor growing conditions that lead to deterioration of the quality and quantity of the production. A total of 156 isolates are made and identified from different regions of the country. The presence of the phytopathogens from the genera *Septoria* (S. lavandulae), *Phoma* (P. lavandulae), *Phomopsis* (Ph. lavandulae), *Phytophthora* hybrid, *Phytophthora parasitica* have been confirmed. The spread of the diseases can be reduced by using healthy planting material, removing diseased and wilting lavender bushes, and applying good agricultural practices.

### References


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Table 3. Derived groups of isolates of fungal pathogens from different regions of Bulgaria
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G. Dineva, V. Vlashev, L. Tsanov
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