



**SZENT ISTVÁN UNIVERSITY**

**THE EFFECT OF VARIETY AND NON-PESTICIDE METHODS AGAINST  
THE SEPTORIA LEAF SPOT DISEASE OF LEMON BALM**

**PHD THESIS**

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## 1. INTRODUCTION AND OBJECTIVES

The production of medicinal and aromatic plant products was a very successful sector of horticulture in the last decades of the XX<sup>th</sup> century in Hungary. However, nowadays the plant protection methods of several medicinal plants are not up-to-date except those which are considered major crops among them (BERNÁTH and ZÁMBORINÉ 2015). The development and authorisation of new plant protection products for use in medicinal and aromatic plants have obstacles, due to the decreasing number of plant protection products in the EU and the high costs of the development of new active substances. The number of new active substances only slightly increased in the last years, however, approval of many active substances is not renewed in the EU. This tendency will continue.

Lemon balm (*Melissa officinalis* L.) is a minor crop in Hungary. The most common and significant pathogen of lemon balm is *Septoria melissae* Desm. in Europe (NAGY 2002, ROLF *et al.* 2007). The fungus can cause serious yield losses and the septoria infection can also decrease the quality of the drug (D'AULERIO *et al.* 1995, NAGY and HORVÁTH 2010).

Only eight active substances (six only in premix) have got approval in lemon balm, which can be used for septoria control in Hungary (ANONYMUS 2020). The successful protection of the plant cannot always be achieved with the available chemical methods in a sometimes 16 weeks long growing season. On the other hand, the drugs of the plant have stricter requirements regarding the pesticide residues.

The use of disease tolerant or resistant varieties is a well-known and applied method in the growing of the major cereals and pome-fruits. The use of resistant cultivars can significantly reduce the disease incidence of the crop while higher yield can be expected and the cost of crop protection can be lower as well (GURURANI *et al.* 2012, LYNCH *et al.* 2017, KAISER *et al.* 2020). In addition, there are only a few descriptions considering the disease response of lemon balm varieties or accessions. However, less susceptible or resistant varieties could have important role in the lemon balm production (MEYERS *et al.* 2007).

Beyond the variety use, there are other non-pesticide agents e.g. essential oils, plant extracts, which are successfully used in several major crops (HOCHBAUM and NAGY 2013, ŽABKA *et al.* 2014). On the other hand, the antifungal effect of inorganic salts

and foliar fertilizers is reported by several studies (DELIOPOULOS *et al.* 2010). However, only a few field researches have been carried out in medicinal and aromatic plants.

In the light of the above mentioned factors, the main objectives of the present work are:

1. to evaluate the susceptibility of intraspecific lemon balm accessions to *Septoria melissae* Desm.
2. to evaluate the pathogen's effect on the yield and the quality of the drug
3. to evaluate natural substances and inorganic salts against *Septoria melissae* Desm. *in vitro*

## 2. MATERIALS AND METHODS

### 2.1 Evaluation of the accessions' susceptibility

In the present work the lemon balm susceptibility to *Septoria melissae* Desm. was evaluated in case of seven accessions and varieties<sup>1</sup> (**Altissima**, **'Ildikó'**, **'Lemona'**, **Lorelei**, **Quedlinburger Niederliegende**, **Soroksári**, **Wroclaw**). The plant material for the experiment was obtained from commercial trade and from the Gene Bank of the Medicinal and Aromatic Plants Department.

The three years long (2016-2018) field trial was carried out in the Experimental and Research Farm of the Szent István University in Budapest-Soroksár.

Evaluations were carried out weekly during the investigated years. The infection rate was defined for each plant by the following parameters: The **disease incidence** was calculated by the ratio of the infected leaves in each plant and expressed in percentage. The **disease severity index** was calculated by the formula of TOWNSEND and HEUBERGER (in GÄRTNER 1971) based on the area of the necrotized tissue compared to the total surface of the leaf. Leaves were classified into the following six infection categories<sup>2</sup>: 0 – healthy leaf, 1 – 1–5% necrotized leaf area, 2 – 6–25% necrotized leaf area, 3 – 26–50% necrotized leaf area, 4 – 51–75% necrotized leaf area,

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<sup>1</sup> Varieties which are in the national variety lists marked with apostrophe.

<sup>2</sup> Further mentioned as „i.c.”

5 – >75% necrotized leaf area. The comparison of the **count of leaves in different infection categories** was also investigated.

Statistical analyses MANOVA, Pearson Chi square method and z-test were used to evaluate the received data. All statistical analyses were carried out at 95% significance level.

## **2.2 Evaluation of the pathogen's effect on yield and quality**

Yield and the quality measurements were performed in 2017 and 2018. Harvest of the plants could be carried out once in 2017 and twice in 2018. Three plots were harvested in case of all tested accessions. The fresh weight of the shoots was measured by plots directly after harvest. Dry weight of the shoots was also investigated.

Quality of the drug was determined by the essential oil content (PH. HG. VII. 1986), the total polyphenol content (SINGLETON és ROSSI 1965) and the total hydroxycinnamic acid content (PH. HG. VIII. 2004) of the drug from shoots.

The effect of the septoria infection on the quality of the leaves was also studied. Healthy (i.c. 0), moderately (i.c. 2) and severely (i.c. 4) infected leaves were collected separately. The shoots' quality parameters mentioned were measured in case of the leaves as well in addition to the count of the healthy glandular hairs and the GC-MS analysis of the distilled essential oil.

Statistical analyses ANOVA, Pearson Chi square method completed with Fisher test were used to evaluate the data. All statistical analyses were carried out at 95% significance level.

### **2.2.1 The *in vitro* efficacy of natural substances and inorganic salts against *Septoria melissae* Desm.**

Isolates of the fungus were collected from naturally infected leaves of lemon balm in Budapest-Soroksár and Warsaw-Wilanów. Fungal cultures were developed in MEA and PDA media.

During the *in vitro* studies, the effect of **essential oils** (cinnamon, thyme, coriander), **water extracts** (clove, cinnamon, garlic, thyme, chamomile), copper based **foliar fertilizers** (Sergomil-L60, Damisol rézaminkomplex) and **inorganic salts** (sodium hydrogen carbonate, calcium-hydroxide, sodium silicate) on the mycelial

growth of the pathogen were investigated. Essential oil of cinnamon and water extracts were also tested against the conidia of the pathogen.

Statistical analyses ANOVA was used to evaluate the received data. All statistical analyses were carried out at 95% significance level.

### 3. RESULTS

#### 3.1 Susceptibility of the investigated accessions

Accession Soroksári was the most susceptible to *Septoria melissae* Desm. among the investigated accessions. During the investigated years the ratio of the infected leaves was 65% and the disease severity index was 27 in general on the plots of accession Soroksári. Before the first harvest of 2018 the disease incidence of the plants reached 93%. Moreover, the severely infected leaves (i.c. 4 and 5) have higher count in these plots than the other less susceptible accessions. Besides **Soroksári**, accessions **Lorelei** and **Quedlinburger** were **highly susceptible** to the pathogen as well.

According to the results obtained with cultivars 'Ildikó', 'Lemona' and accession *Wroclaw* are moderately susceptible to septoria leaf spot. The ratio of the infected leaves was around 35-51% in these plots. The disease severity indexes were under 20 in case of these cultivars and accession while on the plants of the susceptible ones the number was over 22 during the investigated years.

The investigated accession of the subspecies *altissima* (*Altissima*) was the least susceptible to the pathogen during the evaluations. The average rate of the infected leaves was 18% in the two investigated years which is significantly lower than other accessions. Besides this, the development of the symptoms was significantly slower than the susceptible accessions Soroksári or Lorelei.

#### 3.2 The pathogen's effect on the yield and quality

According to the statistical analyses, the observed differences in the yield of investigated accession were developed by the genotype. The septoria infection did not directly influence the fresh and dry weight of the shoots.

Among the tested quality parameters, the pathogen's influence on the essential oil content and hydroxycinnamic acid content could be confirmed by statistical analyses.

The quantity of glandular hairs and essential oil content of the separately collected leaves (infection categories: 0, 2, 4,) decreased in accordance with the increasing disease severity. The rate of citronellal compound was higher in the essential oil of the infected leaves compared to the healthy ones. The ratio of neral was decreased due to the higher leaf infection. Different tendency was observed in the investigated years in the development of the geranial.

The total polyphenol content and hydroxycinnamic acid content also significantly decreased due to the infection.

### **3.3 The *in vitro* effect of natural substances and inorganic salts on *Septoria melissae* Desm.**

Essential oil of *Cinnamomum zeylanicum* with 79% trans-cinnamaldehyde content was the most effective against the mycelia growth of the pathogen. This essential oil completely inhibited the growth of the pathogen and the germination of conidia in all applied concentrations (0.03%; 0.1%; 0.3%). The essential oil of thyme also reached this effect in the higher concentrations 0.1% and 0.3%.

Among the investigated water extracts, the clove extract gave approx. 100% inhibition in all investigated concentrations (12.5%; 25%; 50%). The extract of cinnamon and garlic also provided strong inhibition (84 and 99%, respectively) in the 25% and 50% concentrations. The thyme and chamomile extracts were less effective against the pathogen. Similar effect and differences could be observed in the tests which were carried out with the conidia of the pathogen. According to statistical analysis, the total polyphenol content of the extracts has particular correlation with the inhibitory effect.

The investigated fertilizers Sergomil-L60 and Damisol rézaminkomplex had different effect on the mycelial growth of the pathogen *in vitro*. Sergomil L-60 reached higher inhibitory effect in 0.3% and 0.5% concentrations than Damisol rézaminkomplex which had low efficacy in all tested concentrations.

Under field conditions, the effects of the foliar fertilizer treatments were similar both in 2017 and 2018. These treatments reduced the disease incidence by 24-27% in the first year of the trial. However, moderate effect could be obtained by the treatments in the second year.

Calcium-hydroxide had the strongest inhibitory effect among the investigated inorganic salts. This substance fully inhibited the growth of the pathogen in the applied 0.5% while sodium hydrogen carbonate and sodium silicate had good efficacy only in the higher 1% and 5% concentrations.

### **3.4 Summary of new scientific results**

1. According to the results of the field trials, the investigated intraspecific accessions of lemon balm have different susceptibility to *Septoria melissae* Desm.
2. The yield (fresh and dry shoot weight) of the accessions was not significantly influenced ( $F=4.519$ ;  $p>0.05$ ) by the pathogen. The effect of the genotype was stronger.
3. The pathogen has significant effect on the leaves' quality. The septoria infection significantly decreases the essential oil content by reducing the number of the healthy glandular hairs.
4. Applied in 0.1% and 0.3% concentrations, the essential of *Cinnamomum zeylanicum* with 79% trans-cinnamaldehyde content and the essential oil of *Thymus vulgaris* with 53% thymole can completely inhibit the growth of the pathogen in growing media. The essential oil of *Cinnamomum zeylanicum* can inhibit the germination of the pathogen's conidia in 0.03-0.3% concentrations as well.
5. The water extract of clove, cinnamon and garlic can effectively inhibit the mycelial growth of the pathogen and the germination of its conidia *in vitro*. Particular correlation between the inhibitory effect and the total polyphenol content was confirmed.
6. Foliar fertilizers with at least 30 g/l copper content can reduce the disease incidence of the treated plants under field conditions. Considering the results of the *in vitro* tests, the different copper compounds (copper sulphate pentahydrate and copper sulphate) have different effect against the mycelia growth of the pathogen.
7. The investigated inorganic salts – calcium-hydroxide, sodium hydrogen carbonate and sodium silicate – can effectively inhibit the mycelia growth of the pathogen in 1% concentration. The calcium-hydroxide has full inhibition in 0.5% concentrate as well.



## 4. DISCUSSION AND CONCLUSIONS

Based on the result of the studied years it can be concluded that there are significant differences between the investigated intraspecific lemon balm accessions in the susceptibility of *Septoria melissae* Desm..

Considering the literature data (GUEST and BROWN 1997, FINCHER *et al.* 2020) the observed differences between the accessions may be developed by the morphological and chemical properties of the plants. The high tolerance of accession Altissima also confirms this observation, because the morphological properties and the composition of the secondary metabolites of this accession are quite different compared to the other investigated accessions (DAWSON *et al.* 1988).

Besides the susceptibility of the accessions, the age of the crop can significantly influence the infection of the pathogen. The disease incidence reached a higher level much later in 2016 and 2017 in the new plantations than in the overwintered plants in 2018. The quantity of the infected fallen leaves was also higher in the last year of the trial.

Now, the main goals in lemon balm and medicinal plant breeding focus to the increase of the yield and quality of the drug. The research for resistant or tolerant accessions is not a main objective in this sector yet. However, the presented results show that the use of tolerant varieties can be an effective tool in the plant protection of lemon balm for which, nevertheless, future studies are required.

In the light of the data regarding the yield and drug quality, the investigated lemon balm accessions were different in this field, too.

There was no significant correlation between the fresh and dry weight of the shoots and the disease development. The effect of the genotype may be stronger on the yield than that of the pathogen.

The essential oil content of the plant is determined by the variety in accordance with the literature data (ZÁMBORINÉ-NÉMETH *et al.* 2019) and the obtained results. However, the count of the glandular hairs of the healthy and infected leaves showed significant difference. Therefore it can be concluded that the infection has significant effect on the essential oil content of the drug by decreasing the number of glandular hairs.

There were great differences between the accessions as regards the total polyphenol content and the total hydroxycinnamic acid content in accordance with the observations of ZÁMBORINÉ-NÉMETH *et al.* (2019). However, only the data considering the hydroxycinnamic acid content was in correlation with the infection development in case of the shoots. These two quality parameters were in negative correlation with the infection level of the healthy and infected leaves.

The investigated natural substances and inorganic salts could inhibit the mycelial growth and conidial germination of *Septoria melissae* Desm.

The effect of the investigated essential oils was in correlation with their chemical composition in accordance with the studies of ŽABKA *et al.* (2014) and FAYAERTS *et al.* (2018).

The effect of the tested water extracts had particular correlation with their total polyphenol content. The garlic extract is an exception, because it was effective against the pathogen, but this extract had the lower (4.6%) polyphenol content. Considering the descriptions of ANKIRI and MIRELMAN (1999) the antifungal effect of this extract may be developed by sulphur compounds.

The effect of the applied foliar fertilizers had different effect during the *in vitro* investigations. The results have correlations with the study of PHAM *et al.* (2019). Under field conditions, the application of fertilizer had similar efficacy. However, the effect of these agents depends on the time of the applications and the initial volume of the pathogen's inocula.

The effect of the investigated inorganic salts has strong inhibitory effect against the pathogen in 1% and 5% in accordance with other studies (BIGGS *et al.* 1997, ILHAN *et al.* 2006, BUCK *et al.* 2008). Among them, the calcium-hydroxide can provide the strongest growth inhibition, because in the laboratory test it fully inhibited the mycelial growth in 0.5%.

The sodium hydrogen carbonate and calcium-hydroxide are well known and applied basic substances in several fruit and vegetable crops. The presented results can give a basis to the authorisation of these substances for medicinal and aromatic plants.

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