ABSTRACT OF PhD THESIS

Research upon the biology and culture technology for *Melissa officinalis* L

Scientific coordinator
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INTRODUCTION

From ancient times man discovered the healing properties of the plants. Medicinal plants or cure plants represented one of the most important activities of mankind. Searching for the things necessary to live man observed that several plants used on wounds had a calming effect and rushed the healing process, and others if consumed they cured several diseases.

Knowledge of the medicinal plants had since the beginning of mankind, an important impact in its fighting for survival and development.

Once man reached in a development stage that allows to leave palpable samples, first unconsciously and then with the intention of leaving something behind, regarding the plant usage, began to have meaning a series of preoccupations in order to know the curative features of several plants. Medicinal plants have always represented the main cure method, although it is true that in the last decades their role was replaced with the synthesis medicine.

The synthesis medicine proved that they can have a negative effect in human body, through allergic reactions, addiction, intoxication and other unknown negative effects; in the last years it was stated a strong coming back for the naturist treatments, especially when it comes about plant therapy and also other traditional practices for treatment that entered that way, on the first place of the actual medical preoccupations.

Once with the coming back of the traditional therapeutic methods, it was noticed the need for a detailed research form the biological, ecological and agricol point of view, for the medicinal plants; the study of the chemical compounds characteristic for each specie, also the elaboration of the culture technologies specific to each medicinal plants species. Besides their curative role they have upon the living creatures, it was noticed that some species of plants may protect the soil of diseases and pests. Due to the fact that medicinal and aromatic plants were taken into culture, they become important in the context of the multiple valorification and also to protect the spontaneous flora.

The study made at USAMV Cluj-Napoca, within 2006-2008, presented in the PhD thesis contains research concerning Melissa officinalis L., specie referring to the biological material used to establish the experiences, aspects of biology and culture
technology used to enlarge the culture in our country and to achieve several high and constant production of vegetal row material.

The experiments took place in two locations, Cluj-Napoca and Pâglișa, the studies contained aspects upon the determination of the percent of clean seed, after using the plane sieves in the selection process; determination of the dynamic of seedlings formation for *Melissa officinalis* L; determination of the optimal nutrition space of *Melissa officinalis* L., there were established two experimental fields one near Cluj-Napoca that belong to Plants technology Department USAMV Cluj Napoca and one situated in a village Dăbâca- Pâglișa; The determination of the plant structure at *Melissa officinalis* L.; determination of the Melissa genotypes behavior, in the climatic conditions in our country aimed to establish the production and differences of production for 11 backgrounds (Germania, Germania O, Germania 1, Germania 2, Germania 3, Populația de Cluj, Timișoara, Polonia, Cehia, Franța Melissa 349) and two omologated cultivars in European Union (Lemona și Citronella); determination of the optimal period to sow/plant; identification of the chemical compounds and active principles at *Melissa officinalis* L.

I wish to deeply thank those who helped me to achieve the research presented in my PhD thesis. Scientific coordination of the research presented, results interpretation and thesis elaboration was made under the guidance of professor doctor Leon Sorin Muntean. I wish to thank him for all the professional and moral help he offered me on the entire period of my thesis.

Also I would like to thank the entire team from Plant technology Department for the help given to prepare my thesis.

Not at least I wish to thank my family for all the devotion they showed on the entire period of elaboration.
CHAPTER 1. GENERALITIES CONCERNING THE USE AND CULTIVATION OF MEDICINAL AND AROMATIC PLANTS

In the first chapter it is presented a short history upon the usage of medicinal plants in the world and also in our country. There are presented dates regarding the valorization of medicinal plants in the world and in Romania, active principles in the medicinal plants and factors determinant for agricol production.

CHAPTER 2. ASPECTS REGARDING THE BIOLOGY AND CROP TECHNOLOGY OF MELISSA OFFICINALIS L.

Chapter II presents aspects in the biology of Melissa officinalis L., and also the culture technology (crop rotation, fertilization, soil tillage, seeds and sowing, protection tillage, harvesting and conditioning the raw material.

CHAPTER 3. RESEARCH OBJECTIVES. PEDOCLIMATIC CONDITIONS IN WHICH THE RESEARCH TOOK PLACE. MATERIAL AND METHOD OF RESEARCH

3.1 RESEARCH OBJECTIVES
Research aimed the biology and culture technology of Melissa officinalis L. within 2005-2008 at University of Agricultural sciences and Veterinary Medicine, in two culture areas: at Cluj-Napoca, in the experimental fields of the Plant technologies department and in Pâglișa, Dâbâca and in laboratory conditions.

Achieving high Melissa production is possible only if its biological particularities are known. In our research we approached aspects regarding the biology and culture technology of this specie, with the following goals:

- Determination of the clean seed, using an equipment for separation with plane sieves at three amplitudes: 0.2 mm, 1 mm and 2.2 mm, depending on the
constructive parameters of the equipment, at three working time: 5, 10 and 15 minutes.

- Research upon the germination of seeds at Melissa (Populația De Cluj) depending on the age of seeds (from the previous harvest, 12 months and 18 months old) and temperature (20°, 20-30°- alternant temperature 8 hours 30ºC and 16 hours 20ºC and 10°).

- Determination for seedlings formation at *Melissa officinalis* L. specie, following its evolution from springing to planting from the point of size and number of leaves.

- The influence of the sowing/planting period at *Melissa officinalis* L. upon the production of vegetal row material and determination of the optimum nutrition space for *Melissa officinalisi* L., in two experimental fields, one in Cluj-Napoca (belonging to Plant Technologies Department USAMV Cluj Napoca) and one sitiated in Dâbâca, Pâglișa, Cluj County.

- Determination of the structure of plant at *Melissa officinalis* L. specie (two cultivates and eleven populations) regarding the size of the plant number of the stems and number of leaves.

- Determination of melissa genotypes production, in the condition of our country, 11 populations (Germania, Germania O, Germania 1, Germania 2, Germania 3, Populația de Cluj, Timișoara, Polonia, Cehia, Franța Melissa 349) and two cultivars homologated in European Union (Lemona and Citronella)

- Identification of the chemical compounds at cultivars and populations of *Melissa officinalis* L., cultivated in the condition from Pâglișa, Cluj county.

**3.2 PEDOCLINATIC CONDITIONS IN WHICH THE EXPERIMENTS TOOK PLACE**

In subchapters 3.2.1, 3.2.2 și 3.3.3 there are presented the conditions of clime and soil in which the experiments took place.

The soil on which the experiments took place belongs to the undeveloped soil type (protisoil). The soil is aluvial molic (low aluviosoil molic – after the Romanian System
for soil taxonomy in 2003), has at the horizonts at the surface (0-40 cm), a clay texture, and the following horizons (40-120 cm ) are characterized by an average clay class.

The soil the experiments were placed on, in Pâglișa, Dăbâca is a aluviosoil type, belongs to the protisoi class (OSPA, Cluj-Napoca). The experimental plot is situated on Lona stream, fact that determined in time the formation of deep layers.

In Cluj Napoca, during 2005 and 2006 the weather was warm, with an average temperature of that was with 1,4 respectively with 1,03°C less then the multiannual average temperature. During the vegetation period for Melissa, (April - September), in 2005, June was the hottest, with a difference towards multiannual temperature of 2,05 °C. August was the coldest month (with 1,39 °C less than the multiannual average temperature), due to the abundant precipitation amount in this period. In 2006, August was very hot, with 6,41 °C more compared to the multiannual average temperature.

The year 2005 in the vegetation period for Melissa (April- September) was a rich year in precipitations, all the months registering higher temperatures compared to the average temperature, in August being registered a difference of 123,6 mm. Compared to the year 2005, 2006 April had a lack of precipitations of 23,8 mm. May and June were richer in precipitations with 35,7 respectively with 80,9 mm. The highest lack of precipitations could be seen in July with 64,8 mm less than the average; in August with 47,2 mm less and in September less with 58,1 mm.

At Pâglișa the vegetation period for Melissa, April-September 2006-2008, was a warm period, the average monthly temperature being higher with 3,2°C than the multiannual average for this period in 2006, with 1,9°C in 2007 and in 2008 with 2,6°C.

The experimental years 2006-2008 are characterized by fewer precipitations compared to the multiannual average amount of precipitations in all the months in which the experiments took place. The sum of monthly precipitations on the vegetation period is less with 168,5 mm in 2006, with 153,5 mm in 2007 and with 157,6 mm in the last experimental year compared to the multiannual values.
3. 3 MATERIAL AND RESEARCH METHOD

3.3.1 Seed conditioning at Melissa officinalis L. specie using plane sieves

The experiment took place within the laboratory „Transfer phenomena and unitary operations in Food Industry“ at Department III Mechanization USAMV Cluj-Napoca.

The experimental factors taken into study were represented by:

- Factor A – separation time – with the graduations:
  - a₁ - 5 minutes
  - a₂ - 10 minutes
  - a₃ - 15 minutes

- Factor B – separation amplitude – with the graduations:
  - b₁ - 0.2 mm
  - b₂ - 1 mm
  - b₃ - 2.2 mm

Biologic material was represented by Melissa officinalis L., flowerings, “Populația De Cluj”, harvested in 2008, each sample had 40 g, and a humidity percent of 15%.

For conditioning, special equipment was used, with timer and variable amplitude (fig. 3.1).

Using RETSCH AS 300 equipment to separate, within the experiment it was tried to separate flowerings from seeds of Melissa officinalis L. specie. In order to do this there were taken measurements regarding the quantity of material on each sieve. The sieves used in the experimental determinations are presented in table 3.1.

![Fig. 3.1 RETSCH AS 300 separation equipment (original)](image)
Sieves for separation equipment AS 300 used to conditioning seeds of *Melissa officinalis* L. from flowerings

<table>
<thead>
<tr>
<th>Nr. crt</th>
<th>Number of sieve</th>
<th>Dimensions of the sieves (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sieve 1</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Sieve 2</td>
<td>2,5</td>
</tr>
<tr>
<td>3</td>
<td>Sieve 3</td>
<td>1,25</td>
</tr>
<tr>
<td>4</td>
<td>Sieve 4</td>
<td>0,63</td>
</tr>
<tr>
<td>5</td>
<td>Collector</td>
<td>-</td>
</tr>
</tbody>
</table>

The experimental data were statistically interpreted using variant analyze and Duncan test. The interpretation of the data was made only for the sieve number four. The diameter of the hole on this sieve is sufficient small (0,63 mm) to terrain seeds, this not reaching anymore in the collector.

3.3.2 Seed germination capacity and the dynamic of seedlings formation in the case of *Melissa officinalis* L.

The experiment was established in 2005 within the Teritorial Inspection for Control of Seeds Quality and planting material, Cluj-Napoca.

The variants taken into study were represented by:

- regarding the experiment for seeds age with the graduations:
  - $V_1$ - harvest
  - $V_2$ – 12 months from harvest (variant control)
  - $V_3$ - 18 months from harvest

At the experience concerning temperature with the following graduations:

- $V_1$ - alternant 20-30°C (8 hours 30°C and 16 hours 20°C)(variant control)
- $V_2$ - 20°C
- $V_3$ - 10°C

In order to establish the data regarding the biologic value of the seeds, the work methods are standardized regarding the environment, temperature, and also the light also
mentioned in STAS 1634/ 82 and STAS 72/ 92 for all laboratories for quality control of seeds and planting material in the country.

3.3.3 Dynamic for seedlings formation at Melissa officinalis L., Populația De Cluj

The experiment was placed in the greenhouse from USAMV Cluj-Napoca in 2005, in wood boxes. Seeds were placed in Fleurelle soil. This type of soil is rather acid, with a pH of 6.2-7. The biologic material was represented by Populația de Cluj.

For the determinations there were used 30 plants, they were measured regarding the dynamic for seedlings formation with determination at each 7 days; 14 days; 21 days; 41 days and 57 days.

Each plant was marked, in order to be identified much easier (fig. 3.5).

Statistical interpretation was made using the variation coefficient as follows:

- \( S\% < 10\% \) - small variability
- \( 10\% > S\% < 20\% \) - average variability
- \( S\% > 10\% \) - high variability

3.3.4 Research regarding production, plant structure and chemical composition for different cultivars and populations of Melissa officinalis L.

The experimental research regarding several cultivars and population of Melissa officinalis L., took place in the experimental field in Pâglișa, Dăbâca, Cluj County.

To determine production, the structure of plants and chemical composition there were established two experimental fields. (fig. 3.2 și 3.3)

At the experience regarding green production of different cultivars and populations the biological material used was:

- Populations of Melissa officinalis:
  - Populația De Cluj
  - Population Germania 2
  - Population De Timiș
  - Population Polonia

- Omologated cultivars in UE:
  - Citronella cultivar
  - Lemona cultivar
<table>
<thead>
<tr>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>Citronella</th>
<th>Germania 2</th>
<th>Cluj</th>
<th>Lemona</th>
<th>Timiș</th>
<th>Polonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>R&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Polonia</td>
<td>Lemona</td>
<td>Citronella</td>
<td>Germania 2</td>
<td>Cluj</td>
<td>Timiș</td>
</tr>
<tr>
<td>R&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Germania 2</td>
<td>Cluj</td>
<td>Timișoara</td>
<td>Polonia</td>
<td>Lemona</td>
<td>Citronella</td>
</tr>
</tbody>
</table>

Fig. 3.2 Placement of the experimental plots for the experience concerning populations and cultivars of *Melissa officinalis* L., Pâglișa 2006

<table>
<thead>
<tr>
<th>Citronella</th>
<th>Lemona</th>
<th>Fara</th>
<th>Germania O</th>
<th>Germania 349</th>
<th>Polonia</th>
<th>Timișoara</th>
<th>Lemona</th>
<th>Citronella</th>
<th>Populația De Cluj</th>
<th>Germania 3</th>
<th>Germania 2</th>
<th>Celia</th>
<th>Germania</th>
<th>Lemona</th>
<th>Citronella</th>
</tr>
</thead>
</table>

Fig. 3.3 Placement scheme for the experimental plots at the experience regarding the populations and cultivars of *Melissa officinalis* L., Pâglișa, 2006

### 3.3.5 Research concerning the optimal nutrition space at *Melissa officinalis* L.

#### 3.3.5.1 Research upon the optimal nutrition space at Melissa in the climatic conditions of Cluj-Napoca

In order to determine the influence of the nutrition space upon the production of herba at *Melissa officinalis* L., the variants taken into study were represented by:

- **V<sub>1</sub>** – planted at 50 cm between roes and 20 cm between plant on row, with a density of 83.333 plants/ha.

- **V<sub>2</sub>** – planted at 50 cm between rows and 30 cm between plants on row, with a density of 66.666 plants/ha.

- **V<sub>3</sub>** – planted at 60 cm between rows and 20 cm between plants on row, with a density of 83.333 plants/ha.
- \( V_4 \) – planted at 60 cm between roes and 30 cm between plants on row, with the density of 55.555 plants/ha.
- \( V_5 \) – planted at 70 cm between rows and 20 cm between plants on row, with the density of 71.429 plants/ha.
- \( V_6 \) – planted at 70 cm between rows and 30 cm between plants on row, with the density of 47.619 plants/ha.

Placement scheme of the experimental field is presented in figure 3.4.

<table>
<thead>
<tr>
<th>( V_4 R_3 )</th>
<th>( V_1 R_3 )</th>
<th>( V_5 R_3 )</th>
<th>( V_2 R_3 )</th>
<th>( V_6 R_3 )</th>
<th>( V_3 R_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_6 R_2 )</td>
<td>( V_5 R_2 )</td>
<td>( V_2 R_2 )</td>
<td>( V_1 R_2 )</td>
<td>( V_3 R_2 )</td>
<td>( V_4 R_2 )</td>
</tr>
<tr>
<td>( V_1 R_1 )</td>
<td>( V_2 R_1 )</td>
<td>( V_3 R_1 )</td>
<td>( V_4 R_1 )</td>
<td>( V_5 R_1 )</td>
<td>( V_6 R_1 )</td>
</tr>
</tbody>
</table>

Fig. 3.4 Placement scheme for *Melissa officinalis* L., culture used to determinate the optimal space, Populația De Cluj, Cluj-Napoca, 2006

### 3.3.5.2 Research upon the sowing period/planting and the optimal nutrition space at melisa in the climatic conditions in Pâglişa, Cluj County

The experiment regarding the optimal period of sowing/planting was placed in 2005 Pâglişa, Dâbâca.

The biologic material used was represented by Populația De Cluj, sowed and planted in different periods. The experimental plot had 319 m\(^2\), the size of a plot being of 10 m\(^2\). The experimental plots were randomized distributed on the entire experimental surface, in four repetitions.

Experimental variants for sowing and planting were:
- \( V_1 \) – sowed on 15.10.2005
- \( V_2 \) – sowed on 15.11.2005
- \( V_3 \) – sowed on 15.04.2006
- \( V_4 \) – planted on 15.04.2006
- \( V_5 \) – planted n 15.05.2006

Regarding the influence of the nutrition space upon the production of herba at *Melissa officinalis* L. specie, the experimental factors were:
Factor A – distance between plants on row – with the graduations:
- $a_1 = 20$ cm
- $a_2 = 30$ cm
- $a_3 = 40$ cm

Factor B – distance between rows – with graduations:
- $b_1 = 50$ cm
- $b_2 = 60$ cm
- $b_3 = 70$ cm

Experimental variants resulted from the factors interaction are presented in table 3.2.

### Table 3.2

<table>
<thead>
<tr>
<th>Distance between rows</th>
<th>Distance between plants on row</th>
<th>Plants density</th>
<th>Development space</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>20</td>
<td>100.000</td>
<td>0,1</td>
</tr>
<tr>
<td>50</td>
<td>30</td>
<td>66.666</td>
<td>0,15</td>
</tr>
<tr>
<td>50</td>
<td>40</td>
<td>50.000</td>
<td>0,2</td>
</tr>
<tr>
<td>60</td>
<td>20</td>
<td>83.333</td>
<td>0,12</td>
</tr>
<tr>
<td>60</td>
<td>30</td>
<td>55.555</td>
<td>0,18</td>
</tr>
<tr>
<td>60</td>
<td>40</td>
<td>41.666</td>
<td>0,24</td>
</tr>
<tr>
<td>70</td>
<td>20</td>
<td>71.429</td>
<td>0,14</td>
</tr>
<tr>
<td>70</td>
<td>30</td>
<td>47.419</td>
<td>0,21</td>
</tr>
<tr>
<td>70</td>
<td>40</td>
<td>35.714</td>
<td>0,28</td>
</tr>
</tbody>
</table>
CHAPTER 4. RESULTS OF RESEARCH UPON SEEDS CONDITIONING OF MELISSA OFFICINALIS L., POPULATIA DE CLUJ, USING THE SEPARATION METHOD WITH PLANE SIEVES

The results obtained upon the influence of the interaction between amplitude sieving factor and sieving time factor upon the seed quantity collected on the sieve number four is presented in figure 4.1.

![Fig. 4.1 Quantity of seed collected under the influence of interaction between time x sieving amplitude on the sieve with 0,63 mm diameter](image)

The graduation of the factor „sieving time” of 15 minutes, is higher than the variant control, the difference of 4,33 g being significant. At this time of sieving the quantity of the material collected on sieve number four is with 61,9% higher.

The differences, registered at the amplitude of 2,2 mm and sieving time of 10 and 15 minutes (-2,00g, respectively -0,67g) compared to the variant control 5 min x 2,2 mm are insignificant, so that it can be concluded that at this amplitude, the sieving time does not influence the quantity of the material collected on sieve number four.
Results obtained upon the influence of the interaction between the factor amplitude of sieving and time factor upon the quantity of seed collected on sieve are presented in figure 4.2.

![Graph showing seed quantity collected under the influence of the interaction between amplitude x time on sieve with the diameter of 0.63 mm](image)

Differences compared to the variant control can be highlighted at the amplitude of 2.2 mm (5 min and 10 min) and 1 mm (10 min).

**CHAPTER 5. RESEARCH RESULTS UPON THE GERMINATION AND DYNAMIC OF SEEDLINGS FORMATION OF MELISSA OFFICINALIS L., POPULATIA DE CLUJ**

The age of seeds influence in a positive way the germination, at this age at Melissa officinalis L. specie, the germination is significantly increased at 18 months from harvest compared to the variant control (V₂ – 12 months from harvest).

The temperature influences strongly the germination at this specie. So that at low temperatures (10°C) the seeds germination is very low, only 4% of the germinative faculty. Germination increases significant, from the statistical point of view, at 20°C,
reaching 92.7%. The germination of *Melissa officinalis* L., seeds, Populația De Cluj, in the conditions of alternant temperature was of 81.5%.

**Number of days from sowing and the size of plants are:**
- Sowing – plant emergence = 21 days; size of plants 0.2-0.6 cm
- Seed lobe leaves = 6 (27) days; size of plants 0.5-0.7 cm
- Formation of the first true leaves = 14 (41) days; size of plants 0.8-3.5 cm.
- Phase of 2-4 leaves = 16 (57) days; size of plants 2-4 cm
- Seedling completely formed = 21 (78) days; size of plants 7.81-13 cm

In figure 6.9 it is presented the seedling ready to be planted

**CHAPTER 6. RESULTS REGARDING THE GREEN MASS PRODUCTION, PLANT STRUCTURE AND CHEMICAL COMPOSITION OF SEVERAL SORTS AND POPULATIONS OF MELISSA OFFICINALIS L., IN THE CONDITION FROM PÂGLISA, CLUJ COUNTY**

Percentage differences of production between populations and cultivars are presented in figure 6.1.

**Fig. 6.1 Production differences e (%) for green production of cultivars / populations of Melissa officinalis L., in the three experimental years of culture (2006,2007,2008), Pâlși, Cluj County**

From figure 6.1 it can be observed that in the first year of vegetation the cultivars Lemona and Citronella had higher values compared to the variant control (Populația De
Cluj). In the case of Lemona cultivar, the difference was significantly different - 6.73 t/ha(166.4%); for Citronella cultivar, the difference registered - 6.39 t/ha(157.9%) was significant.

In the second year of vegetation, Polonia population registered significant differences in the negative way compared to Populația de Cluj. The rest of them were not different from the control variant, the differences were insignificant.

In the third year of vegetation all the populations had a high green production. Citronella cultivar had higher values compared to the control variant with 7.09 t/ha, the difference being distinct significant.

**Results upon the size** of the cultivars and populations of *Melissa officinalis* L. are presented in figure 6.2.

![Plant height comparison](image)

**Fig. 6.2** Size of the cultivars and populations of *Melissa officinalis* L., second year of culture (2007), second cycle of vegetation, in the climatic conditions from Pâglișa, Cluj County

Positive significant differences were registered at the populations Germania O, Franța and Lemona cultivar with 14 (15.9%), 12 (13.6%) respectively 13.4 cm (13.4%) higher than the variant control.

Citronella cultivar had a size of 105 cm, the difference compared to the variant control being distinctive significant (17 cm, 19.3%).
The very significant differences in a positive way were registered in the case of Melissa 349 population with a size of plant of 110.8 cm. Compared to the variant control it had the size with 22.8 cm (25.9 %) higher.

**Results upon the number of stems** at cultivars and populations of *Melissa officinalis L.* are presented in figure 6.3.

![Fig. 6.3 Number of stems on plant at the cultivars and populations of *Melissa officinalis L.*, the II of culture (2007), first cycle of vegetation, in the conditions from Pâglișa, Cluj County](image)

Significant differences were observed only in the case of Germania 1 population compared to the variant control (Populația De Cluj) with 35.2 (44.6%) stems and cultivar with a difference of 41.2 (52.2%) stems.

**Results regarding the number of leaves on stem** at cultivars and populations of *Melissa officinalis L.* are presented in figure 6.4.

The number of leaves on plant is different at the cultivars and species of *Melissa officinalis L.*. Citronella cultivar registers significant positive differences compared to the control variant (Populația De Cluj), with 42.4 (44%) more leaves on stem. Significant differences, but in the negative way are registered in the case of Timișoara population with 36.2 (37.6%) more leaves on stem. Significant positive differences are registered in the case of Germania O, Franța populations and Lemona cultivar compared to the variant control. These have with 48 (49.8%), 51.4(53.3%) and 59.8 (52.2%) more leaves on stems.
Results upon the volatile oil content are presented in figure 6.5.

Fig. 6.5 The content of volatile oil at cultivars and population of *Melissa officinalis L.*, Pâglişa, Cluj County, 2007

Cultivars and populations of *Melissa officinalis L.* had a different content of volatile oil.
The content of volatile oil was situated between 0,09 ml/100mg pv at Lemona cultivar and 0,32 ml/100 mg pv at Citronella cultivar. The rest of the population had a content in volatile oil between 0,11 ml/100mg pv and 0,2 ml/100 mg pv.

The samples analyzed contain volatile oil in the limits indicated by the scientific literature (0,1-0,4ml/100mg vegetal product).

In figure 6.6 it is presented the content of polyphenolic content in the case of cultivars and populations of Melissa officinalis L. taken into study.

![Fig. 6.6 Content of polyphenolic compounds (mg/g) in the case of cultivars and populations of Melissa officinalis L. taken into study](image)

The content of polyphenolic is different depending on the cultivar and population. So the smallest quantity for the polyphenolic compounds can be observed at Melissa 349 (22,08 mg/g) and the biggest quantity can be observed at Populația De Cluj (99,22 mg/g).

The content of rosmarinic acid differs. The richest populations are: Populația De Cluj (24,5 mg/g), Timișoara (27,34 mg/g) and Citronella cultivar (30,54 mg/g). Rosmarinic acid can be seen in all the samples analyzed. It represents on average 30% of the total phenol acids acizilor fenolici. At these populations there were made also analyses regarding te composition of phenolic compounds using extraction in ethanol 45%. The results obtained are presented in figure 6.7.
Fig 6.7 Identification of the phenolic acids in samples A- Populația De Cluj; B- Timișoara; C- Citronella
CHAPTER 7. RESULTS OF THE RESEARCH UPON THE OPTIMAL NUTRITION SPACE MELISSA OFFICINALIS L., SPECIE, POPULATIA DE CLUJ, IN CLIMATIC CONDITION FROM CLUJ-NAPOCA, 2006

The results obtained at Cluj-Napoca in the first year of culture, first and second cycle of vegetation, are presented in table 7.1.

Synthesis on the experimental data using Duncan test, first year, first and second cycle of vegetation, 2006

<table>
<thead>
<tr>
<th>Graduation of factors</th>
<th>Density at planting pl/ha</th>
<th>Production Kg/ha</th>
<th>Duncan test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vegetation cycle I</td>
<td>Vegetation cycle II</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16,33</td>
<td>7,83</td>
</tr>
<tr>
<td>50x20 cm</td>
<td>100000</td>
<td>14,92</td>
<td>6,83</td>
</tr>
<tr>
<td>50x30 cm</td>
<td>66666</td>
<td>15,32</td>
<td>7,32</td>
</tr>
<tr>
<td>60x20 cm</td>
<td>83333</td>
<td>18</td>
<td>9,10</td>
</tr>
<tr>
<td>60x30 cm</td>
<td>55555</td>
<td>14,83</td>
<td>6,18</td>
</tr>
<tr>
<td>70x20 cm</td>
<td>71429</td>
<td>13,17</td>
<td>5,92</td>
</tr>
<tr>
<td>70x30 cm</td>
<td>41666</td>
<td>5,92</td>
<td>1,07-1,16</td>
</tr>
</tbody>
</table>

First cycle of vegetation: DS$_{5\%}$ - 2,91-3,14
Second cycle of vegetation: DS$_{5\%}$ - 1,07-1,16

Production in the experimental field was between 13,17 t/ha at 41666 plants/ha and 18 t/ha at 55555 plants/ha. The variant that registered very significant differences from the green production point of view was with 55555 plants/ha.

In the second cycle of vegetation, the production in the experimental field was between 5,92 t/ha at 71429 plants/ha and 9,10 t/ha at 55555 plants/ha, being highlighted the same evolution of production as in the first cycle of vegetation.
CHAPTER 8. RESULTS OF RESEARCH REGARDING THE INFLUENCE OF SOWING/PLANTING PERIOD UPON THE PRODUCTION AND OPTIMAL NUTRITION SPACE AT MELISSA OFFICINALIS L., CLUJ COUNTY

Results of research regarding the influence of sowing/planting period are presented in table 8.1.

Table 8.1

Influence of sowing/planting period upon the production at Melissa officinalis L., Populația De Cluj, Păglișa, 2006

<table>
<thead>
<tr>
<th>sowing/planting period</th>
<th>Yield t/ha</th>
<th>Difference %</th>
<th>Difference t/ha</th>
<th>Meaning</th>
<th>Test Duncan</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₁</td>
<td>15,60</td>
<td>100,0</td>
<td>0,00</td>
<td>Mt</td>
<td>B</td>
</tr>
<tr>
<td>V₂</td>
<td>1,60</td>
<td>10,3</td>
<td>-14,00</td>
<td>Ooo</td>
<td>A</td>
</tr>
<tr>
<td>V₃</td>
<td>5,88</td>
<td>37,7</td>
<td>-9,72</td>
<td>Ooo</td>
<td>A</td>
</tr>
<tr>
<td>V₄</td>
<td>34,00</td>
<td>217,9</td>
<td>18,40</td>
<td>***</td>
<td>C</td>
</tr>
<tr>
<td>V₅</td>
<td>12,10</td>
<td>77,6</td>
<td>-3,50</td>
<td>-</td>
<td>B</td>
</tr>
</tbody>
</table>

DL 5% - 4.81  DL 1% - 6.75  DL 0.1% - 9.52

The production of the experimental field was between 1,60 t/ha at V₂ (sowed in November) and 15,60 t/ha at V₁ (sowed in October). The only variant that registered a plus of yield was V₄ (planted in April) with higher values than the variant control V₁ (sowed in October) with 18,4 t/ha, difference being from the statistic point of view very significant. The experimental variants V₂ and V₃ (sowed in November and April) had inferior production compared to the control variant, the differences being very significant in a negative way. Variant V₅ (planted in May) had an inferior production compared to the variant control, the difference is insignificant (4.81 t/ha).

Results of research regarding the optimal nutrition space at Păglișa, Cluj County in 2006 are represented in table 8.2.
Production in the experimental field had the value between 0,19 t/ha at $V_9$ (35714 plants/ha) and 1.35 t/ha at $V_5$ (55555 plants/ha). Variant 9 was insignificant as difference compared to the variants 8, 7 and 6 (47619, 71429 and 41666 plants/ha). The variant with the most significant difference regarding the green production was $V_5$ cu 55555 plants/ha. The variants with 100000 respectively 83333 plants/ha are significant superior to the variants with 66666 and 50000 plants/ha.

Table 8.2  

**Synthesis of the experimental data comparison using Duncan test, anul I, Pâglișa, Cluj County, 2006**

<table>
<thead>
<tr>
<th>Graduation of factors</th>
<th>Density of planting pl/ha</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>t/ha</td>
</tr>
<tr>
<td>50x20 cm</td>
<td>100000</td>
<td>0,96</td>
</tr>
<tr>
<td>50x30 cm</td>
<td>66666</td>
<td>0,94</td>
</tr>
<tr>
<td>50x40 cm</td>
<td>50000</td>
<td>0,61</td>
</tr>
<tr>
<td>60x20 cm</td>
<td>83333</td>
<td>0,93</td>
</tr>
<tr>
<td>60x30 cm</td>
<td>55555</td>
<td>1,35</td>
</tr>
<tr>
<td>60x40 cm</td>
<td>41666</td>
<td>0,66</td>
</tr>
<tr>
<td>70x20 cm</td>
<td>71429</td>
<td>0,72</td>
</tr>
<tr>
<td>70x30 cm</td>
<td>47419</td>
<td>0,44</td>
</tr>
<tr>
<td>70x40 cm</td>
<td>35714</td>
<td>0,19</td>
</tr>
</tbody>
</table>

Valori DS$_{5\%}$ - 0,30 – 0,35

Results obtained at Pâglişa, Cluj County, in the second year of culture, the first and second cycle of vegetation, are presented in table 8.3.

The yield from the experimental field in the first cycle of vegetation was between 7,91 t/ha at 47619 plants/ha and 21.73 t/ha at 71429 plants/ha. The variant that registered very significant differences from the yield point of view was the one with 71429 plants/ha. The variant with 83333 plants/ha is significant superior to all the variants taken into study.
In the second cycle of vegetation, the production in the experimental field was of 4,56 t/a (47419 plants/ha) and 12,6 t/ha la 71429 plants/ha.

The variant that registered the highest production was the one with the planting space of 70 x20 cm (71429 plants/ha).

**Synthesis of experimental data with the help of Duncan test, second year, first and second cycle of vegetation, 2007**

<table>
<thead>
<tr>
<th>Graduation of factors</th>
<th>Density of planting pl/ha</th>
<th>Vegetation cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I t/ha</td>
</tr>
<tr>
<td>50x20 cm</td>
<td>100000</td>
<td>15,99</td>
</tr>
<tr>
<td>50x30 cm</td>
<td>66666</td>
<td>15,19</td>
</tr>
<tr>
<td>50x40 cm</td>
<td>50000</td>
<td>15,36</td>
</tr>
<tr>
<td>60x20 cm</td>
<td>83333</td>
<td>18,17</td>
</tr>
<tr>
<td>60x30 cm</td>
<td>55555</td>
<td>15,51</td>
</tr>
<tr>
<td>60x40 cm</td>
<td>41666</td>
<td>8,89</td>
</tr>
<tr>
<td>70x20 cm</td>
<td>71429</td>
<td>21,73</td>
</tr>
<tr>
<td>70x30 cm</td>
<td>47419</td>
<td>7,91</td>
</tr>
<tr>
<td>70x40 cm</td>
<td>35714</td>
<td>8,06</td>
</tr>
</tbody>
</table>

First cycle of vegetation: DS₅% - 1.55 – 1.78
Second cycle: DS₅% - 0.90 – 1.03

The results obtained at Pâglișa, Cluj County, in the third year of culture, first and second year of vegetation, are presented in table 8.4

**Synthesis of experimental data using Duncan test, third year, first and second cycle of vegetation, 2008**

<table>
<thead>
<tr>
<th>Graduation factors</th>
<th>Density of planting pl/ha</th>
<th>Cycle of vegetation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I t/ha</td>
</tr>
<tr>
<td>50x20 cm</td>
<td>100000</td>
<td>27,7</td>
</tr>
<tr>
<td>50x30 cm</td>
<td>66666</td>
<td>25,12</td>
</tr>
<tr>
<td>50x40 cm</td>
<td>50000</td>
<td>20,69</td>
</tr>
<tr>
<td>60x20 cm</td>
<td>83333</td>
<td>29,63</td>
</tr>
<tr>
<td>60x30 cm</td>
<td>55555</td>
<td>27,37</td>
</tr>
<tr>
<td>60x40 cm</td>
<td>41666</td>
<td>23,89</td>
</tr>
<tr>
<td>70x20 cm</td>
<td>71429</td>
<td>22,97</td>
</tr>
<tr>
<td>70x30 cm</td>
<td>47419</td>
<td>11,12</td>
</tr>
<tr>
<td>70x40 cm</td>
<td>35714</td>
<td>10,67</td>
</tr>
</tbody>
</table>

First cycle of vegetation: DS₅% - 1.56 – 1.79
Second cycle of vegetation: DS₅% - 0.92 – 1.05
In the first cycle of vegetation, yield in the experimental field was of 10,67 t/ha at 35714 plants/ha and 29.63 t/ha at 71429 plants/ha. The variant that registered significant differences from the green yield point of view was the one with 100000 plants/ha.

In the second cycle of vegetation, the yield from the experimental field was of 6,07 t/ha at 35714 plants/ha and 17,01 t/ha at 71429 plants/ha. The variant that registered very significant differences from the green yield point of view was the planting variant with 71429 plants/ha.

CHAPTER 9 CONCLUSIONS AND RECOMMENDATIONS

Conclusion regarding the seed conditioning at *Melissa officinalis L.*, Populația de Cluj, using the separation method based on plane sieve
- Time necessary for sieving influences the quantity of seeds separated only at low amplitude.
- The amplitude influences the quantity of collected material, the more the amplitude is, the larger the separated seed quantity is, no matter the time of sieving.

Conclusions regarding the germination and the dynamic of seedlings formation at *Melissa officinalis L.*, Populația De Cluj
- Right after seeds harvest at *Melissa officinalis L.* had a low germination percent, this increases once with the seeds age, at 18 months from harvest the germination having a high value.
- Temperature strongly influences the germination of seeds at *Melissa officinalis L.* So, at low temperature seeds can not germinate or the germination value is very low, the optimal temperature is of 20ºC.
- The time for vegetation phases at *Melissa officinalis L.*, Populația De Cluj is:
  - Sowing – plant emergence = 21 days; size of plants 0.2-0.6 cm
  - Leaves seed lobe = 6 (27) days; size of plants 0.5-0.7 cm
  - Formation of true leaves = 14 (41) days; size of plants 0.8-3.5 cm.
  - Phase of 2-4 leaves = 16 (57) days; size of plants 2-4 cm
  - Seedlings completely formed = 21 (78) days; size of plant 7.81-13 cm

Conclusions regarding the green yield, structure of plant and chemical composition of several cultivars and populations of *Melissa officinalis L.*, in climatic conditions from Pâglișa, Cluj county
- The first year of vegetation is also the year the experience was established, has a production of 4.04 t/ha at Populația de Cluj (Mt) and 10.77 t/ha at Lemona cultivar.
- In the second year of culture, first cycle of vegetation none of the population or cultivar had a production higher than in the case of Populația De Cluj. Germania 2 population and Lemona and Citronella cultivars had inferior production compared to the control variant. In the second cycle of vegetation it is maintained the same direction, the control variant has the highest values.
In the third year of culture, the first cycle of vegetation, the production of population and cultivars was between 52.79 t/ha and 61.14 t/ha. Yield achieved by Populația De Cluj was exceeded by the yield of Citronella cultivar. In the second cycle of vegetation, the yield of population and cultivars was of 22.88 t/ha and 26.50 t/ha. The same as in the case of the first cycle of vegetation, Citronella cultivar had the highest yield.

**Conclusions regarding the structure of plants**
- Size of plants larger than Cluj population was noticed in the case of population Germania O, Franța and Lemona cultivar. Citronella cultivar had the largest size.
- Compared to populația De Cluj, the largest number of stems could be observed in the case of Lemona cultivar followed by populația Germania 1.
- Regarding the number of leaves on stems Citronella cultivar had the highest number of leaves compared to populația De Cluj.
- **There were identified four positive strong correlations** between the features size – number of stems at Populația De Cluj, Polonia, Franța and Melissa 349; 4 positive strong correlations between the number of stems-number of leaves at Germania 2, Franța, Melissa 349 and Citronella; 2 strongly positive correlations between the size – number of leaves on stems at Franța and Citronella.

**Conclusions regarding the chemical composition and production of active elements in the case of several cultivars and populations of Melissa officinalis L., in climatic conditions from Pâglișa, Cluj County**

**Content and volatile oil production**
- The highest content of volatile oil was registered in the case of Citronella cultivar. The samples analyzed contain volatile oil in the limits indicated in the scientific literature (0.1-0.4ml/100mg vegetal product). The lowest production of volatile oil was registered in the case of Lemona cultivar. The highest production of volatile oil was registered at Citronella cultivar. Populația de Cluj had a medium production of volatile oil.

**Content and production of polyphenol compounds**
- The lowest content in polyphenol compounds is different depending on cultivar and population, the smallest quantity of polyphenol compounds can be observed in the case of Melissa 349 population and a higher quantity was determined at Populația De Cluj.
- The content of rosmarinic acid also differs. The richest populations are: Populația De Cluj, Timișoara and Citronella cultivar. Rosmarinic acid is observed in all the samples analyzed. It represents on average 30% of the total of phenolic acids.
- Besides rosmarinic acid there were also identified other 4 phenolic acids (galic protocatecuic, cafeic and p-cumaric acid).
- Citronella cultivar is richer in p-Cumaric acid content, followed by Populația De Cluj.
- Populația De Cluj had the highest production of poliphenolic compounds. There were not noticed higher productions at neither the cultivars nor the populations taken into study.
Conclusions regarding the influence of sowing/planting period upon the production at *Melissa officinalis* L., Populația De Cluj, Pâglișa, 2006

- The experimental variant planted in April had the highest production the rest of the variant sowed in November and April had inferior production to the control variant, the differences being significant negative.

Conclusions regarding the optimal nutrition space at *Melissa officinalis* L. in climatic conditions from Cluj-Napoca, Populația De Cluj, anul 2006

- In 2006, first and second cycle of vegetation, the highest production were registered at 50 cm between rows and 20 cm between plants on row, except the planting distance of 60 x 30 cm where it was observed a superior production. Increasing the distance between plants on row and between rows determines a production decrease.

- At all the planting variants except the variant 60 x 20 cm, the correlation between the number of stems and the number of leaves on plants are significantly. Strong correlations between size and leaves on plant were determined at the planting variants 60 x 20 cm and 60 x 30 cm. L variant 60 x 20 cm there was determined a significant positive correlation between size and number of stems.

Conclusions upon the optimal nutrition space at *Melissa officinalis* L., Populația de Cluj, Pâglișa, Cluj County, first year of vegetation, 2006

- The same as in the case of the experience in Cluj, at Pâglișa in the first year of vegetation, the distance of 50 cm between rows and the distance of 20 cm between plants on row are the best distances for planting. It can be observed the distances of planting 60 x 30 cm that registers superior production.

Conclusions regarding the optimal nutrition space at *Melissa officinalis* L., specie Populația De Cluj, Pâglișa, Cluj County, the second year of culture, 2007

- In the second year of vegetation, the first and second cycle of vegetation the small distances between plants on row and large distances between rows have the highest productions.

Conclusions regarding the optimal nutrition space at *Melissa officinalis* L., species Populația De Cluj, Pâglișa, Cluj County, the third year of cultivation, 2008

- In the third year of vegetation, the first and second year of vegetation, the same as in the previous year, at small distances between plants on row and between rows, the green yield is high. The exception was observed in the case of variant 60 x 40 cm, where in both of the vegetation cycles there were registered higher production compared to the ones obtained in the case of control variant.

Conclusions regarding the evolution of production at *Melissa officinalis* L., populația De Cluj, in the conditions from Pâglișa, Cluj County (2007-2008)

- In the third year of vegetation, the production was high, all the experimental variants with significant differences of production compared to the second year, except the variants of planting at 70 cm between rows.

- In the second year of culture as in the third year of culture, the highest production of green yield was registered at the variants with the distance between plants on row of 20 cm.
- Production registered in the case of control variant (50 cm between rows and 30 or 40 cm between plants on row) were the highest, except the planting variant of 70 x 20 which registered a higher production, the differences being very significant.

**Recommendations**

- It is recommended to establish the culture of *Melissa officinalis* L. by planting, as soon as possible in early spring.
- It is recommended to use the seeds of at least 18 months from harvest in order to obtain a high percent of germination at a temperature of 20°C.
- Taking Populația De Cluj in culture and registering it in the Official Catalog of the cultivars, due to the high green yield and the high content in poliphenolic compounds.
- Planting the seedling of *Melissa officinalis* L., it is recommended to be achieved at small distances between rows (50 or 60 cm) and between plants on row (20 or 30 cm) in order to achieve a higher density of plants on hectare, fact that leads to significant yield.

**SELECTIVE BIBLIOGRAPHY**

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