PhD THESIS

Research on the influence of technological factors and abiotic stress on some varieties of lavender (*Lavandula angustifolia* Mill.)

(SUMMARY OF THE PhD THESIS)

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INTRODUCTION

Medicinal and aromatic plants, as well as lavender, have been known and used since ancient times.

Today there is a growing interest for *Lavandula angustifolia* Mill. in the industry, but it is also recognized in academia, health sciences, cosmetics and art.

The genus Lavandula includes over 45 species and about 400 varieties (KOULIVAND *et al*., 2013;). It is native to the Mediterranean Basin, from southern Europe to North and East Africa and the countries of the Middle East to southwest Asia and southeast India (MOKHTARZADEH *et al*., 2011; LIS-BALCHIN, 2017). It is considered an appreciated species, due to its cosmetic and pharmaceutical properties (MAHBOUBI and FEIZABADI, 2009; CANTOR *et al*., 2018). HERRAIZ-PEÑALVER *et al*., (2013),) states that lavender oils are most appreciated in the cosmetic industry because of its high content of *linalyl acetate*, *linalool* and low *camphor* content, while oils with a high concentration of *camphor* are more appreciated in aroma therapy.

Germination of the seeds from the *Lavandula* genus is a problem in the cultivation of these plants. The percentage of germination depends on the quality of the seeds, on the percentage of their dormancy (CHAVAGNAT, 1977). Germination biostimulators can influence the percentage of germination and can release dormancy, the dormant state of lavender seeds (YANG *et al*., 2020).

Relatively new concept in agriculture, the sustainability of agroecosystems, which in other words ensures modernity, evolution, ecosystem conservation and economic efficiency in the circumstances of promoting and boosting unconventional/organic, biological or ecological agriculture movements (GARY, 2004), this category also includes plant mulching with different ecological methods.

Nowadays, climate change is a growing problem for the population and the nature (LINDNER *et al*., 2010). According to NARESHKUMAR *et al*., (2020), plants are exposed to various stresses throughout the life phenophase that limits growth, development and affect production yield.

The harmful effects of climate change will soon be observed not only in arid and semi-arid regions, but also in areas with temperate climates, as well as the accumulation of dissolved salts in water used for irrigation will lead to salinization of many cultivated areas (FITA *et al*., 2015).

Plants can recognize abiotic stress, such as water deficiency and saline stress, and can activate appropriate physiological, biochemical, and molecular responses, with impaired metabolism, growth, and development (MBARKI *et al*., 2018). Environmental conditions such as salinity, temperature, water deficiency, heavy metals affect the quality and quantity of secondary metabolites of medicinal plants (SHARMA *et al*., 2016).

Based on the above mentioned, we consider it appropriate to approach technological and biochemical aspects of lavender to obtain and cultivate varieties which are tolerant to climate changes.

RESEARCH AIMS AND OBJECTIVES

Lavender cultivation being a profitable business, more and more farmers in Romania start cultivating it. Until now, as only a few researches have been made on the influence of germination biostimulators, mulching and the effect of abiotic stress on these plants.

The *aim* of the research of the doctoral thesis "Research on the influence of technological factors and abiotic stress on some varieties of lavender (*Lavandula angustifolia* Mill.)" was to study the influence of some technological factors of cultivation and water and salt stress of some varieties of lavender so they would adapt to the new pedoclimatic and cultivation conditions.

Given the purpose of the research, the following *objectives* were set:

1. Establishing the germination capacity of lavender seeds with and without biostimulators;
2. Determination of the influence of mulching methods on lavender plants;
3. The effects of water and salt stress on the growth of *Lavandula angustifolia* Mill plants;
4. Analysis of the content of photosynthetic pigments in lavender varieties under the influence of water and saline stress: chlorophyll a, chlorophyll b and total carotenoids;
5. Determination of ion content in lavender varieties under the influence of water and saline stress (calcium, sodium, potassium and chlorine);
6. Analysis of plant osmolites concentrations: proline and total soluble sugars in lavender leaves under the influence of stressors;
7. Analysis of malondialdehyde (MDA) concentrations under water and saline stress conditions;
8. Determination of non-enzymatic antioxidants under stress conditions (phenols and flavonoids);
9. Determination of enzymatic antioxidants under stress conditions (superoxide dismutase, catalase, ascorbate peroxidase and specific glutathione reductase activities);
10. Assessment of the lavender essential oil content of the two varieties of *Lavandula angustifolia* Mill. (‘Codreanca’ and ‘Sevtopolis’).

STRUCTURE OF THE THESIS

The paper entitled "Research on the influence of technological factors and abiotic stress on some varieties of lavender (*Lavandula angustifolia* Mill)", counts 148 pages and is written with according to university and national editing regulations. The doctoral thesis is structured in two parts, containing 11 chapters, 11 tables, 63 figures and graphs and 333 scientific literature citations.

The **first part** of the doctoral thesis, the literature review, is structured in 5 chapters and has 48 pages. **Chapter 1** summarizes information on the origin, distribution area, systematic classification, morphological description, ecological requirements and use of lavender. **Chapter 2** covers the propagation, establishment and maintenance of crops, the harvesting, drying and processing of lavender. **Chapters 3**, **4** and **5** present aspects on the influence of germination biostimulator, mulching and the effect of abiotic stress on lavender plants.

The **second part** of the doctoral thesis includes the personal contribution, is structured in 6 chapters and containing 99 pages. The illustration of the obtained results is highlighted by 9 tables and 50 figures. **Chapter 6** describes the purpose and objectives of the research. **Chapter 7** covers the geographical locations of the researches, the establishment of experiments and the related climatic conditions. **Chapter 8** presents the research materials and methods and also the organization of field experiments, Fitotron and the distillation of lavender essential oil. In **Chapter 9** we can find the results and discussions of the four research studies, followed by **Chapter 10** which includes the conclusions and recommendations based on the results. The originality and innovative contributions of this research are highlighted in **Chapter 11** of the doctoral thesis. The thesis ends with the bibliography followed by the abstract in Romanian and English language.

RESULTS AND DISCUSSIONS OF THE RESEARCH

Chapter 9 of the doctoral thesis consist of four experiments, according to the organization of experiments and the development of studies.

The *first experiment* of this thesis investigates the influence of germination biostimulator, gibberellic acid (GA_3) on the germination of *Lavandula angustifolia* var 'Codreanca' and var. 'Sevtopolis' seeds, at concentrations of 100, 200, 300 ppm, for 35 days. The first seeds germinated within 7 days in the GA_3 treated Petri dishes, but the germination of control seeds could be observed only after 11 days. As well a significant difference was observed in the percentage of germination as well, because the seeds treated with 300 ppm GA_3 registered a percentage of over 90%, followed by 200 ppm GA_3 with over 75%, 100 ppm GA_3 with 65% and the control seeds with only 30%.

The *second experiment* investigated the influence of mulching on weeds, as well as the effect on the lavender plant growth. We consider this experiment to be important, as weeds can pollute the content of the essential oil. The first experiment was to evaluate the density of weeds for the two varieties of lavender 'Codreanca' and 'Sevtopolis', mulched with straw/hay and mulching foil. The results obtained from the experiment showed that the mulching foil significantly reduced the density of weeds in the first year to an average of 20%, and in the second year to an average of 15%; followed by straw/hay, where weed density was reduced by an average of 43% (first year) and 33% (second year). The highest density of over 50% was recorded in the control (unmulched) variants (SZEKELY-VARGA *et al*., 2020a).

The second part of the experiment focused on the effect of mulch on the increases of the diameter of lavender plants compared to the control. In the first year of the research in all variants (control, straw/hay and mulching foil), the diameter of the lavender cuttings was similar. However, some changes were observed in the second year of growth. In the 'Codreanca' variety, the highest growth was recorded at the straw/hay variants (104.6 cm), being followed by the unmulched plants and mulching foil. However, at the *L. angustifolia* var. 'Sevtopolis', the largest increase in diameter was recorded in the straw/hay variant (48.38 cm), followed by the mulching foil and the control (SZEKELY-VARGA *et al*., 2021).

In the third year of the study both lavender varieties increase in diameter were almost identical to that of the second year, but what is important to mention is that the plants in the control variants exceeded the plants in the version with mulch foil. Also in this experiment, the increases in diameter for the two varieties were compared. At the beginning of the research, the diameter of all one-year-old lavender plants was almost similar for both varieties, about 24 cm. In the second year *L. angustifolia* var. 'Codreanca' registered an increase in diameter higher than var. 'Sevtopolis'. The measurements for the third year were similar to the second year, in which the 'Codreanca' variety showed an increase in diameter, up to 100 cm.

In the *third experiment*, the responses of lavender seedlings to water and salt stress were investigated. At the beginning of the study, the substrate in all pots did not show significant differences in humidity or electrical conductivity, with average values of 50% and 2 mS cm^{-1}, respectively. As expected, after 30 days of treatment, these two parameters showed significant differences from the initial values. Given the aerial parts of the plants, water deficit and salt treatments inhibited seedling growth compared to control plants, with a similar degree between the two varieties of *L. angustifolia*. However, the length of the root was significantly increased (2-3 cm) in water-stressed plants in both varieties. Regarding salt stress treatments, a slight increase in root length was detected in 'Codreanca' plants–not in the 'Sevtopolis' variety, but was statistically significant only at the highest salinity tested, 300 mM NaCl.

A sharp reduction in fresh root weight was observed in plants exposed to water stress, which was associated with dehydration of the roots. NaCl treatments, caused the inhibition of root growth in a concentration-dependent manner, but not significant water loss. As in the roots, the water deficit caused a substantial reduction in fresh weight in the stems and leaves, accompanied by a significant decrease in the water content. The salt stress affected the growth of the aerial parts of the plants more than that of the roots.

The fresh weight of stems and leaves decreased in parallel with the increase of external NaCl concentrations, reaching similar values as in the treatment of water stress in the presence of 300 mM NaCl. Contrary to the roots, salt stress caused a progressive reduction of the water content in the stems and leaves, although, in general, it was not as pronounced as in plants with water stress.

Chlorophyll a, decreased in both varieties under water stress, but under salt stress only at ‘Sevtopolis’. Chlorophyll b increased in both varieties at NaCl concentrations of 300 mM.

Na^+ and Cl^- concentrations increased in response to salt treatments. K^+ concentrations in the roots of plants subjected to water stress increased twice compared to the control. Regarding Ca^{2+} concentrations, similar qualitative patterns were observed in response to stress treatments.

The highest proline content was identified in seedlings under 200 mM NaCl stress. In the case of ‘Codreanca’ variety subjected to treatments, the total soluble sugars increased significantly compared to the control, but only a small fluctuation was detected at ‘Sevtopolis’ (SZEKELY-VARGA *et al*., 2020b).

Salt treatments induced an increase in MDA content, but in the case of water stress in ‘Codreanca’ variety the concentration decreased. In both varieties, a significant increase in the content of total phenolic compounds was detected in plants subjected to water stress; the total flavonoids content increased significantly in the plants subjected to stress. A significant increase in superoxide dismutase activity was observed in seedlings subjected to salt stress. Calatase activity increased significantly in response to salt treatments. The 'Codreanca' variety plants subjected to salt stress showed a strong decrease in the activity of ascorbate peroxide, in terms of glutathione reductase activity, both varieties showed a significant decrease at water stress and contrary in the presence of high salt concentrations (300 mM NaCl) activity has increased (SZEKELY-VARGA *et al*., 2020c).

In the *fourth experiment*, the compounds of the essential oil of the two varieties were investigated. The most important compounds of lavender essential oil are *linalool* and *linalyl acetate*; both varieties had a high content of these compounds.

CONCLUSIONS

Chapter 10 summarizes the conclusions regarding the research studies carried out in this thesis, as follow:

1. Lavender seeds of the variety *Lavandula angustifolia* var. 'Codreanca' and var. 'Sevtopolis' are influenced by the germination biostimulator (gibberellic acid) in a positive way, as follows: lavender seeds under the effect of GA_3 had a higher germination percentage ('Codreanca' 80%–95% și 'Sevtopolis' 65–90% în funcție de tratament) than the control ('Codreanca'–30% și 'Sevtopolis'–35%); seeds;
2. Weed control with mulching foil, reduces the average density of weeds to 20%, compared to straw/hay where the average density of weeds was 30-35% and control variant (unmulched) by 50%;
3. Although mulching may have an effect on plant growth due to its influences on environmental conditions, the data obtained indicated that the largest increase in plant diameter was observed at straw/hay mulching and the smallest at the mulching film;
4. Only small, insignificant differences were observed between the two varieties in response to abiotic stress, but there were some differences in some biochemical compounds;
5. The number of branches from control variant had the most branches compared to the other plants that were subjected to water and saline stress;
6. Diameter of lavender stems subjected to water stress decreased during treatment, also finding differences between the control and the variants subjected to abiotic stress;
7. The length roots, confirms the results obtained by other researchers, which showed that the roots of lavender plants penetrate into the soil up to 3-4 meters to absorb water. Our obtained results also confirmed, that the roots of lavender plants from the water stress treatments recorded the highest increases;
8. Results obtained on the determination of fresh material (aerial part, stem and root), reveal that abiotic stresses largely influence the two varieties of lavender, inhibiting the growth of these plants;
9. Concentration of photosynthetic pigments was influenced only by higher amounts of salt. The studied lavender varieties have accumulated remarkable amounts of ions (Na^+, Cl^-, K^+ and Ca^{2+}), these being important mechanisms of stress tolerance;
10. Proline and total soluble sugars are among the most important osmolites found in plants, in the experiment the concentration of osmolites increased, which led to the start of the mechanism of adaptation of lavender plants.

Oxidative stress has a small increase in lavender seedlings, subjected to stress, but the MDA concentration was significantly higher than the water stress;

11. The content of antioxidant compounds (TPC and TF) recorded an increase of the content in the seedlings exposed to water stress. Lavender plants subjected to stress treatments with salt or water deficiency have increased the specific activities of all enzymes tested, superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase, higher in the presence of NaCl than in the absence of water, these data may explain a relative tolerance of lavender to water and saline stress;
12. Analyze of the compounds of lavender essential oil, confirms that the two varieties of lavender had a high content of *linalool* and *linalyl acetate*.

RECOMMENDATIONS

We recommend a GA_3 concentration of 300 ppm, being the most effective for increasing the germination percentage of lavender seeds.

Mulch has a positive effect on weed control, therefore we recommend mulching with mulch foil, to obtain an uncontaminated lavender essential oil, without components from other plant species. However, we would like to indicate that this method of mulching influences in a small percentage the plant growth.

Although the two varieties selected for this thesis did not differ significantly in terms of stress tolerance, the results presented in this thesis open the possibility to use biochemical stress biomarkers, for the possible selection of lavender genotypes more resistant to abiotic stress factors and therefore better adapted to a climate change scenario.

The analysis of lavender essential oil compounds can be useful to cultivate a variety of lavender, which contains the most important components of the essential oil, *linalool* and *linalyl acetate*.

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