„ALEXANDRU IOAN CUZA" UNIVERSITY OF IAȘI
FACULTY OF BIOLOGY
DOCTORAL SCHOOL OF BIOLOGY

INTERDISCIPLINARY RESEARCH ON THE BIOLOGY OF SOME *LAVANDULA* L. TAXA CULTIVATED IN ROMANIA

SUMMARY OF THE DOCTORAL THESIS

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TABLE OF CONTENT

LIST OF ABBREVIATIONS

AACT – acetoacetyl-CoA thiolase;

DNA – Deoxyribonucleic acid;

RNA – Ribonucleic acid;

BDH – borneol dehydrogenase;

BERS – α-bergamotene synthase;

CADS – cadinol synthase;

CARS – β-caryophyllene synthase;

CINS – 1,8 - cineol synthase;

CoA – acetyl-coenzyme A;

CPS – 9-epi-caryophyllene synthase;

DMAPP – dimethylallyl pyrophosphate;

DXP – 1-deoxy-D-xylulose-5-phosphate;

DXS –1-deoxy-D-xylulose-5-phosphate synthase;

FPP – farnesyl pyrophosphate;

FPPS – FPP synthase;

G3P – glyceraldehyde 3-phosphate;

GC-MS – gas chromatography and mass spectrometry;

GERDS – germacrene D synthase;

GPP – geranyl pyrophosphate;

GPPS – GPP synthase;

HMBPP – 1-hydroxy-2-methyl-2-E-butene-4-pyrophosphate;

HMG-CoA – 3-hydroxy-3-methylglutaryl-CoA;

HMGR – 3-hydroxy-3-methylglutaryl-CoA reductase;

HMGS – 3-hydroxy-3-methylglutaryl-CoA synthase;

IPI – isopentenyl pyrophosphate isomerase;

IPP – isopentenyl pyrophosphate;

IR – retention index;

ISO – International Organization for Standardization;

K – potassium;

LIMS – limonen synthase;

LINS – linalool synthase;

LPP – lavandulyl pyrophosphate;

LPPS – LPP synthase;

MEP – 2-C-methyl-D-erythritol-4-phosphate;

MEV – mevalonate;

MVA – mevalonic acid;

MVD – mevalonate pyrophosphate decarboxylase;

MVPP – mevalonate-5-pyrophosphate;

NADPH – Nicotinamide adenine dinucleotide phosphate;

P – phosphorus;

pb – base pairs;

PCR – Polymerase chain reaction;

PHLS – β-phellandrene synthase;

RT – qPCR – Quantitative real-time polymerase chain reaction;

s. u. – dry substance;

SEM – scanning electron microscopy;

Work variants

M – control;

v1 – plants watered with H2O;

v2 – plants watered with standard Hoagland nutrient solution;

v3 – plants watered with Hoagland nutrient solution with the addition of K;

v4 – plants watered with Hoagland nutrient solution with the addition of P;

Cod – Codreanca variety;

PB – Provence Blue variety;

Sev – Sevtopolis variety;

Vera – Vera variety;

var. – variety;

PART I: THEORETICAL CONSIDERATIONS

INTRODUCTION

Since ancient times, man has taken from nature various species of spontaneous plants that were planted in areas specially designed for their cultivation. Thus, were born the crop plants that have been improved since prehistoric times in order to increase production, so that new varieties and even species have resulted.

A good knowledge of the biochemical constituents of plants provides important information in the effort to discover new complex phytochemical resources, which is the basis for the synthesis of valuable compounds for natural remedies and more.

The cultivation of aromatic and medicinal plants and their industrial transformation is an alternative that is currently of real interest for many regions of our country, activities that can help solve certain economic, social and environmental problems at zonal and regional level, given that we have witnessed in recent years both in Romania and in the rest of Europe, an accentuation of the interest for the study of aromatic and medicinal plants.

From an economic point of view, lavender species are suitable for cultivation in the climatic conditions of Romania, being introduced today on the list of plants of agricultural interest; at present, the granting of European-funded projects for the establishment and capitalization of lavender crops has increased the interest of farmers in this regard, which has materialized through an exponential increase in the number of lavender crops organized in the country.

In the context of the above, the topic addressed in this doctoral thesis is part, through the proposed objectives and methodology to achieve them in a field of fundamental applied research, research based on an interdisciplinary approach, which aimed to identify and investigate the interrelations that can be established between the cultivation conditions, the morpho-anatomical changes, the gene expression of certain genes involved in the biosynthesis of the volatile oil and its composition in some taxa of the genus *Lavandula* L.

CHAPTER 1. THE BIOLOGY OF THE GENUS *LAVANDULA* L.

Systematic classification

Species of the genus *Lavandula* have been used as medicinal and aromatic plants since antiquity; discussing the appearance of the popular name of lavender, the literature considers that the origin of the word "*lavandula*" comes from the Latin "*lavare*" = to wash, due to the fact that in ancient Greece, Persia and the Roman Empire people used this plant to perfume bathrooms (Lis-Balchin, 2002; Basch et al., 2004; Szekely-Varga and Cantor, 2019).

Recent studies on the taxonomy of the genus *Lavandula* divide the genus into 8 sections and 36 morphologically distinct species (Upson, 2002; Upson and Andrews, 2004; Passalacqua et al., 2017).

According to ITIS (Information System for Taxonomic Integration) (ITIS TSN 500370) and CABI (International Center for Agriculture and Bioscience), the genus *Lavandula* L. presented the following systematic positioning:

KINGDOM: *PLANTAE*

PHYLUM: *SPERMATOPHYTA*

SUBPHYLUM: *ANGIOSPERMAE*

CLASS: *DICOTYLEDONAE*

SUBCLASS: *ASTERIDAE*

ORDER: *LAMIALES*

FAMILY: *LAMIACEAE (LABIATAE)*

TRIBE: *LAVANDULEAE*

GENUS: *LAVANDULA*

Representatives of the genus *Lavandula* L.

The genus *Lavandula* is represented by four main species:

- *Lavandula x intermedia* Emeric: lavandin, Dutch lavender

It is a natural hybrid, which has sterile seeds, resulting from the crossing of *Lavandula angustifolia* Mill and *Lavandula latifolia* L. Like lavender, this hybrid has many varieties, one of the most popular in our country being Grosso (Lis-Balchin, 2002; Upson and Andrews, 2004).

- *Lavandula latifolia* Medik.: Portuguese lavender, broadleaf lavender, spike lavender

It is a woody shrub with a globular shape, reaching a height between 40 - 100 cm. The leaves are opposite, with shapes ranging from lanceolate to spatulate, with a revolute edge, gray-green to silver-gray, with dense indumentum. The inflorescences are peduncled (usually branched once, giving a 3-branched flower head) (Săvulescu, 1952; Zuzarte, 2013).

- *Lavandula stoechas* L.: French lavender, Spanish lavender

The lavender species belonging to this section are perennials that grow up to 1 m tall. They are mostly native to the Mediterranean region, while some grow in North Africa, Algeria, Asia and the Azores. The leaves are linear, lanceolate, oblanceolate or elliptical, with entire margins, and their color varies from green to gray-green. Sterile bracts are large and petal-like, variable in color (Mason, 2014).

- *Lavandula angustifolia* Mill. (Figure 1. 1.): lavender, English lavender

"It is a perennial shrub with lignified roots, up to 2-3 cm thick. In the first year after planting the plant develops the main root, which can reach up to 1.20 m. In the second year of vegetation it develops its secondary root system horizontally, as a projection of the aerial bush into the soil. The depth and richness of the root system give lavender drought resistance. The stem, strongly branched at the base, forms a globular bush, with a height between 30 - 70 cm or more. The branches that bear the inflorescences are 25-35 cm long and have leaves only at the bottom. The leaves are opposite, linear-lanceolate, sessile, acute, on ciliated edges; the lower ones gray, 1 - 2 cm long and 1.5 - 2 mm wide, on both sides with indumentum made of branched, stellate hairs; the upper ones are gray-green, 2 - 3.5 cm long and 3 - 6 mm wide, less hairy. Lavender leaves do not fall in autumn at the end of vegetation ".

According to the same authors, “The labiate-type flowers, with an aromatic scent due to the oleiferous glands, are grouped in a spiciform inflorescence, with a length of 3 - 8 cm, actually composed of 4 - 5 to 12 superimposed pseudoverticles. The fruits are represented by 4 nuts, located at the base of the persistent calyx, elongated ovate, with brown or gray surface, smooth and glossy. Lavender blooms in June - July ”(Săvulescu, 1952; Luncean et al., 2018; Goncearіuc et al., 2019).

Figure 1. 1. *Lavandula angustifolia* - (Köhler, 1887) **(**https://www.kalliergeia.com/en/english-lavender-lavandula-angustifolia-botanical-classification-and-varieties/**)**

CHAPTER 2. RESEARCH HISTORY

Currently, species of the genus *Lavandula* are intensively studied due to their content in volatile oils and socio-economic value for the medical industry (Shahdadi et al., 2017; Firoozeei et al., 2021), food, cosmetics, perfumery and aromatherapy (Cavanagh and Wilkinson, 2002; Zuzarte et al., 2010; Woronuk et al., 2011; Hassiotis et al., 2014; Prusinowska and Śmigielski, 2014; Lesage-Meessen et al., 2015; Salehi et al., 2018). The products (*Lavandulae flos*), represented by the lavender inflorescences, as well as the volatile oil (*Lavandulae atheroleum*), defined as the volatile oil obtained by steam hydrodistillation from the inflorescences (Verma et al., 2010; Saadatian and et al., 2013; European Pharmacopoeia 10.0., 2019).

Three species of the genus *Lavandula* are cultivated mainly for the commercial production of volatile oils, namely *Lavandula angustifolia* Mill. sin. *L. officinalis* Chaix (true lavender or English lavender), *Lavandula x intermedia* Emeric sin. *L hybrida* L. (lavandin) and *Lavandula latifolia* Medicus (Werker, 1993; Lis-Balchin, 2002; MacTavish and Harris, 2002; Prins et al., 2010; Nimet and Baydar, 2013; Lesage-Meessen et al., 2015; Aprotosoaie et al., 2017; el Hamdaoui et al., 2018; Mill et al., 2019).

Quality standards for the chemical profile of volatile oils have been established by the International Organization for Standardization (ISO), and for *Lavandula angustifolia* volatile oil this is ISO 3515: 2002; also the pharmaceutical quality of lavender volatile oil is strictly regulated by pharmacopeias (Table 2. 1.).

Table 2. 1. ISO and European Pharmacopoeia standards for volatile oils produced by *Lavandula angustifolia* (European Pharmacopoeia 10.0, 2019)

Compound (%)	ISO 3515:2002 *L. angustifolia*	European Pharmacopoeia *L. angustifolia*
Linalool	25 – 38	20 – 45
Linalyl acetate	25 – 45	25 – 47
1,8 - Cineol (eucalyptol)	1 – 2	< 2.5
Camphor	0.5 – 1	< 1.2
Limonene	< 1	< 1
β - ocimene	2.5 – 6	–
Terpinen-4-ol	2 – 6	0.1 – 8
Lavandulyl acetate	3.4 – 6.2	> 0.2
Lavandulol	> 0.1	> 0.1
α - Terpineol	< 2	< 2
3 - Octanone	–	0.1 - 5

The chemical composition of volatile oils

Of the 48 species of the genus *Lavandula*, only 17 have been studied in terms of the chemical composition of their volatile oils. Among them, the most known and studied species important for the pharmaceutical, medical and cosmetic industries and recognized for its volatile oil production is *Lavandula angustifolia* (Yukes and Balick, 2010; Dušková et al., 2016; Aprotosoaie et al., 2017; Sönmez et al., 2018).

Quantitatively, according to the European Pharmacopoeia, the main components that impart the basic odor of lavender oil are linalool (20 - 45%) and linalyl acetate (25 - 47%). Esters responsible for the oil's aroma, found in a smaller proportion, are eucalyptol (maximum 2.5%) and camphor (maximum 1.2%), the latter being considered to diminish the quality of the oil. In turn, lavandulol (minimum 0.1%) and lavandulyl acetate (minimum 0.2%) are the characteristic compounds of lavender oil (Ceaușescu et al., 1988; Lis-Balchin, 2002; Herraiz-Peñalver et al., 2013; Saadatian et al., 2013; Hancianu et al., 2014).

PART II: PERSONAL CONTRIBUTIONS

AIM AND OBJECTIVES OF THE RESEARCH

The research of this thesis aims to observe, highlight and discuss some histo-anatomical and micromorphological characteristics of the vegetative organs (leaves, stems, and roots), as well as some functional changes (extraction yield, composition spectrum, as well as the expression of genes coordinating

the biosynthesis process of volatile oils) of Romanian *Lavandula* L. volatile oil-producing taxa cultivated under specific experimental conditions.

In order to achieve the proposed goal, the following objectives have been set and achieved:

Aim 1. Comparative histo-anatomical and micromorphological evaluation of vegetative organs of some *Lavandula* L. genus taxa producing volatile oils cultivated in Romania.

Aim 2. To study the histo-anatomical and micromorphological changes induced by the application of Hoagland nutrient solution, as a cultivation supplement, to the vegetative organs (leaves and stems, depending on the type of investigation performed) in the lavender taxa under investigation.

Aim 3. To study the effects induced by the application of Hoagland nutrient solution as a cultivation supplement on the extraction yield and biochemical composition of volatile oils produced by vegetative and generative organs (floral stems) in the lavender taxa studied.

Aim 4. Quantification of gene expression involved in volatile oil synthesis in the lavender taxa studied.

Aim 5. The correlation of the gene expression involved in the volatile oils synthesis with the treatment variants applied to the lavender taxa analyzed.

CHAPTER 3. COMPARATIVE HISTO-ANATOMICAL AND MICROMORPHOLOGICAL ANALYSIS OF THE VEGETATIVE ORGANS OF SOME TAXA OF THE GENUS *LAVANDULA* L. CULTIVATED IN ROMANIA

Species belonging to the family Lamiaceae have different types of protective and secretory hairs, whose morphology, distribution, and density are important taxonomic features. Species of the genus *Lavandula* have been the subject of a significant number of studies based on various characteristics, such as the anatomy of vegetative and generative organs (Toma and Niță, 1982; Huang et al., 2005; Nikolakaki and Christodoulakis, 2006; Robu et al., 2011; do Rocio Duarte and Carvalho de Souza, 2014; Lungu et al, 2014; Riva et al., 2014; Brailko et al., 2017; Fakhriddinova et al., 2020; Tanase et al., 2020) and micromorphological characteristics of protective and secretory hairs (Huang et al., 2005, 2008; Zuzarte et al., 2010; Giuliani et al., 2020).

Biological material

Analyses were performed on varieties of *Lavandula x intermedia* Emeric and *Lavandula angustifolia* Mill. species (Table 3. 1.).

Table 3. 1. Lavender species and varieties studied

Species	Variety	Inventory number
***Lavandula angustifolia* Mill.**	Ellagance Pink	207011
	Ellagance Snow	207008
	Ellagance Purple	207007
	Munstead	207010
	Vicenza Blue	207006

Species	Variety	Inventory number
***Lavandula intermedia* x Emeric.**	Grosso	207005

Histo-anatomical investigations

Method and working techniques for making cross-sections through vegetative organs of plants

The preparation of histological sections was carried out according to a protocol comprising the following steps:

- Fixation and preservation of biological material;
- Sample sectioning;
- Staining of cross-sections;
- Photographing the cross-sections.

Results and discussions

Stem

The outline of the cross-section is square, modified by four relatively prominent wings (characteristic outline of plants of the Lamiaceae family). The epidermis is covered with numerous, massive, stellate-branched, multi-cellular protective hairs, dead at maturity, which give the stem and leaves a whitish appearance.

- *Lavandula angustifolia* Ellagance Pink (Figure 3. 1.)

The outline of the cross-section is square, with slightly rounded edges at the corners. The epidermis is composed exclusively of cells transformed into branched single- and multicellular protective hairs and single- and multicellular secretory hairs. The bark has islands of angular collenchyma at the corners of the stem, between which are two to three layers of parenchymal assimilatory cells.

- *Lavandula angustifolia* Ellagance Purple (Figure 3. 2.)

The structure of the stem is similar to that of Ellagance Pink and Ellagance Snow, in that the section has a square outline, with rounded edges at the corners, the pith is rhomboidal, persistent, made up of parenchyma cells, slightly rounded, leaving small gaps between them.

The epidermis disorganizes and exfoliates and with it much of the bark and secondary free is disorganized.

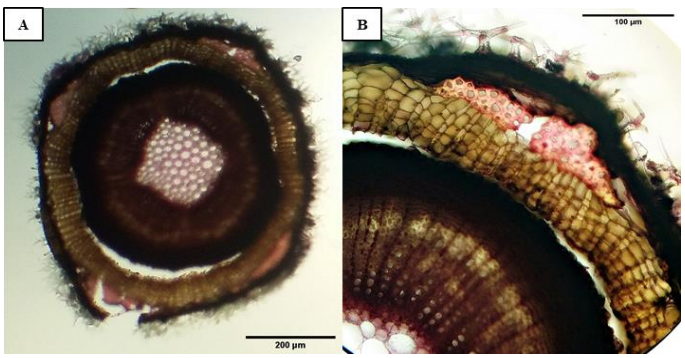

Figure 3. 1. Cross-sections through the stem of *Lavandula angustifolia* Ellagance Pink (A - general view; B - exfoliation of the suber, angular collenchyma and epidermis) (original)

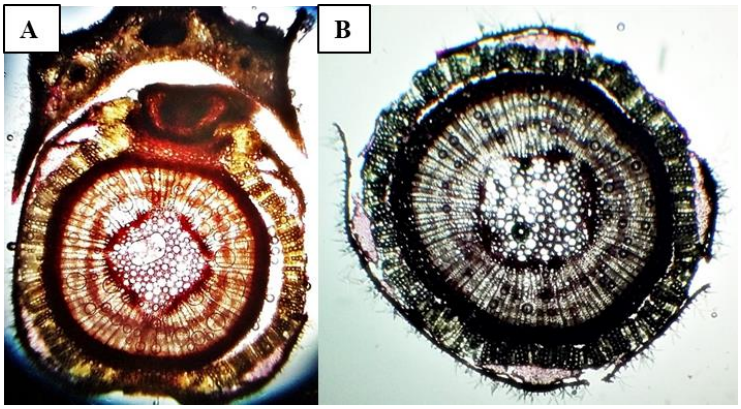

Figure 3. 2. Cross-sections through the stem of *Lavandula angustifolia* Ellagance Purple (A, B - overview, skin and bark exfoliation can be seen) (original)

Varieties of *Lavandula angustifolia* Munstead (Figure 3. 3.) and Vicenza Blue (Figure 3. 4.) show a similar characteristic not found in varieties of the Ellagance group: angular collenchyma almost absent in the stem ribs, instead present in the form of well defined islands (7 - 10 layers) at the periphery of the free (primary and secondary).

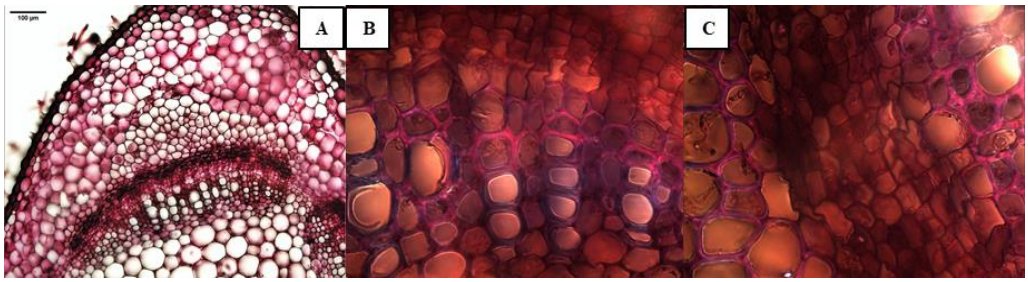

Figure 3. 3. Cross-section through the stem of *Lavandula angustifolia* Munstead (A - detail; angular collenchyma in ribs; B - detail with woody conducting tissue; C - detail with phloemic conducting vessels) (original)

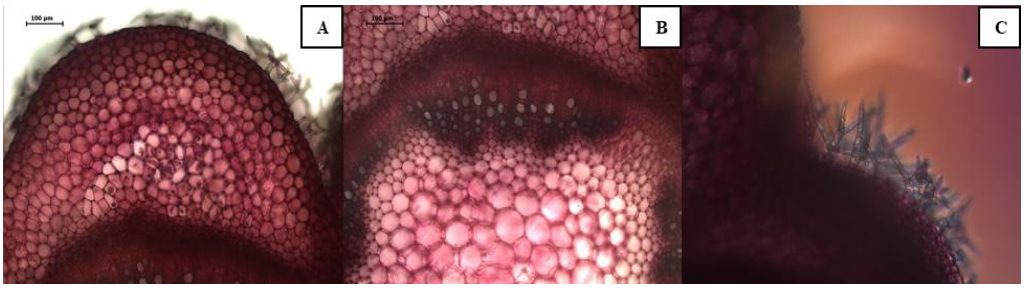

Figure 3. 4. Cross-section through the stem of *Lavandula angustifolia* Vicenza Blue (A - rib with protective hairs and angular collenchyma; B - vascular bundle; C - protective hairs) (original)

- *Lavandula x intermedia* Grosso (Figure 3. 5.)

The outline of the cross-section is square, with the four ribs slightly projecting as described in the classical pattern for species of the family Lamiaceae.

In the central cylinder, open collateral vascular bundles of different sizes can be distinguished. The marrow retains some rhomboidal outline and is persistent.

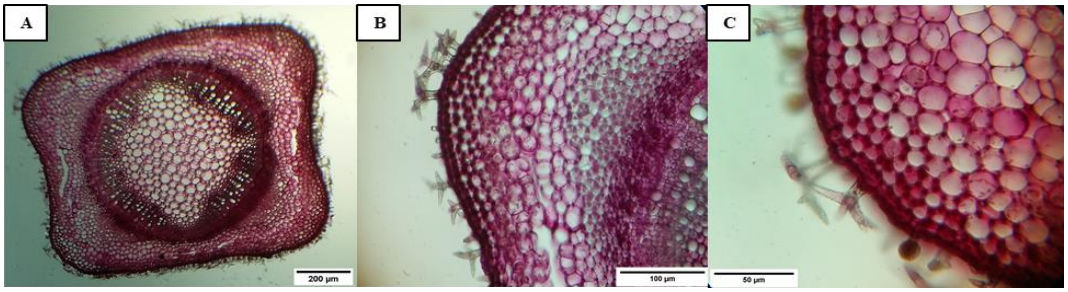

Figure 3. 5. Cross-section through the stem of *Lavandula x intermedia* Grosso (A - overview; B - rib with angular collenchyma; C - epidermis covered with secretory and protective hairs) (original)

Secretory and protective hairs

On the surface of the stem, there are more or less long secretory hairs with bi-, tetra-, or octocellular glands (Figure 3. 6. - A, B, C, D).

On the surface of both epidermis of the leaf blade, there are secretory hairs with short pedicel and uni, bi-, tetra-, or octocellular glands. Compared to the protective hairs, which are more abundant in the upper epidermis, the secretory hairs are more abundant in the lower epidermis (Figure 3. 6. - E, F).

On the surface of the stem there are numerous protective hairs, much more frequent in the vallecula and of two categories: uni- or bicellular, but simple, short and pointed at the tip and pluricellular, longer, always branched (Figure 3. 7.), results supported by experimental data reported in the literature (do Rocio Duarte and Carvalho de Souza, 2014; Giuliani et al., 2020).

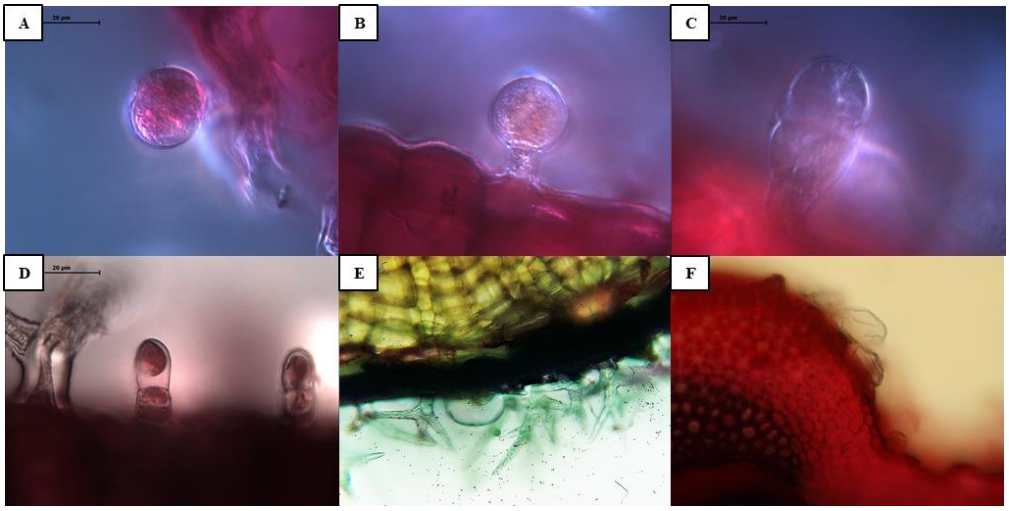

Figure 3. 6. Optical micrographs of types of glandular trichomes (A, B - capitate secretory hairs with unicellular secretory gland; C, D - capitate secretory hairs with bicellular secretory gland; E - peltate secretory hairs; F - cuticle of peltate secretory hairs) (original)

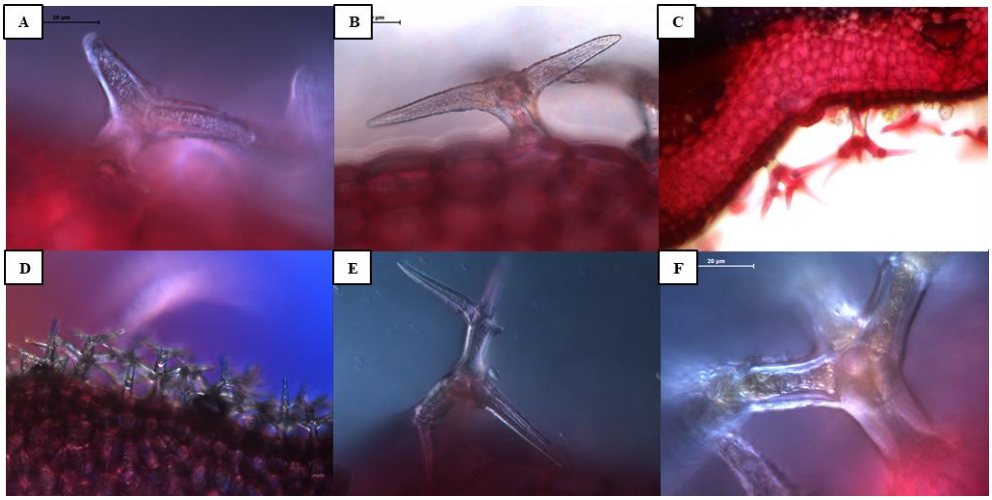

Figure 3. 7. Optical micrographs of types of protective trichomes (A, B - bicellular protective hairs; C, D, E, F - multicellular branched hairs) (original)

Micromorphological aspects

Working method and technique for scanning electron microscopy (SEM)

The plant material (previously described in subchapter **Biological material**) used for this study consisted of fresh leaves, collected from the *Lavandula* genus taxa studied, prepared according to SEM techniques adapted from standard techniques described by Bozzola et al., 1999.

The samples were observed and photographed under the Tescan VEGA II SBH scanning electron microscope.

Leaf area measurements and the counting of secretory and protective formations were performed on digital images and analyzed with ImageJ software using the Cell Counter plugin (Abràmoff et al., 2004).

Results and discussions

The analysis of leaf blade surfaces by scanning electron microscopy (SEM) revealed aspects of the density and structure of protective and secretory hairs.

Capitate and peltate secretory hairs (Figure 3. 8.) identified on the leaf surfaces of the studied varieties showed similar characteristics to secretory hairs reported for other species of the genus *Lavandula* (Martínez-Natarén et al., 2011; do Rocio Duarte and Carvalho de Souza, 2014; Brailko et al., 2017).

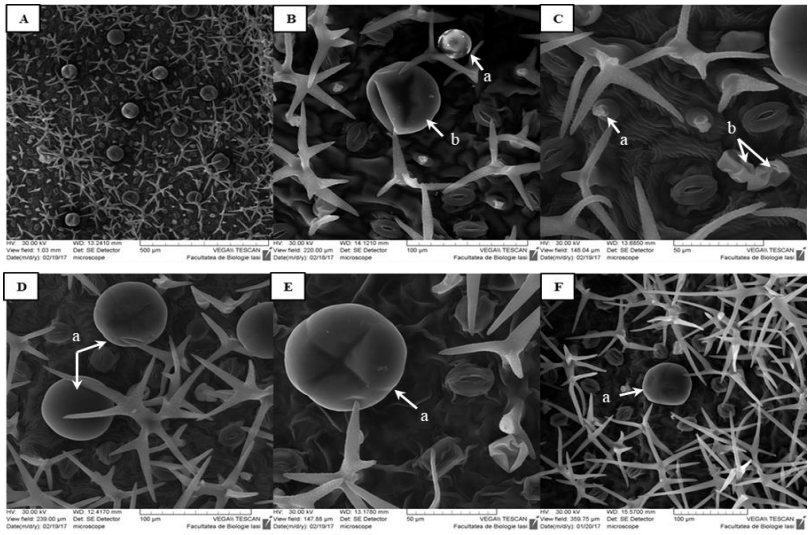

Figure 3. 8. Scanning electron micrographs of secretory trichomes on the leaf surface (A - *L. angustifolia* Munstead, lower epidermis; B - *Lavandula x intermedia* Grosso, a - bicellular capitate secretory hair; b - peltate trichome with tetracellular secretory gland; C - *L. angustifolia* Munstead, a, b - unicellular and bicellular glandular hairs in the post-secretory phase; D - *L. angustifolia* Ellagance Purple, a - peltate secretory hair with tetracellular gland; E - *L. angustifolia* Munstead, a - peltate secretory hair with tetracellular gland; F - *L. angustifolia* Vicenza Blue, a - peltate trichome with eight secretory cells) (original)

The highest values of protective hairs density were found in the Vicenza Blue variety of *L. angustifolia* on both epidermis surfaces, followed in descending order by *Lavandula x intermedia* Grosso, *L. angustifolia* Ellagance Snow, Munstead (with close values), Ellagance Purple and Ellagance Pink, in which the number of protective hairs was higher on the upper than on the lower epidermis.

Based on morphology and cell number, protective hairs have been divided into two types: single or multicellular, branched or multicellular (Figure 3. 9.).

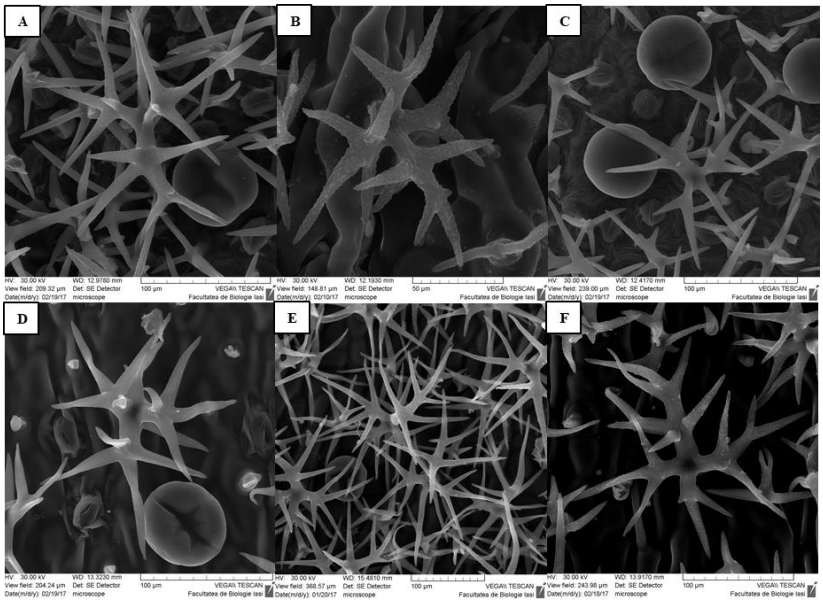

Figure 3. 9. Scanning electron micrographs of protective trichomes on the leaf surface (A – *L. angustifolia* Ellagance Snow; B – *L. angustifolia* Ellagance Pink; C – *L. angustifolia* Ellagance Purple; D – *L. angustifolia* Munstead; E – *L. angustifolia* Vicenza Blue; F – *L. x intermedia* Grosso) (original)

The highest density of secretory pear was observed for the Munstead variety on both leaf surfaces (Table 3. 2.), this characteristic being correlated with the economic importance of this cultivar, known for its high production capacity of volatile oil, often used in various industries (Adaszyńska-Skwirzyńska et al, 2013). Also, in this cultivar, numerous uni-, bi- or multicellular protective hairs were observed on the surface of both epidermises, which were scarcely branched and smaller in size than those identified in the other taxa analyzed.

Table 3. 2. Density/mm^2 of secretory and protective hairs in taxa of the genus *Lavandula: L. angustifolia* Ellagance Pink, *L. angustifolia*`Ellagance Purple, *L. angustifolia*`Ellagance Snow, *L. angustifolia* Munstead, *L. angustifolia* Vicenza Blue, *Lavandula x intermedia* Grosso

Species/variety		Upper epidermis				Lower epidermis			
		Secretory hairs			Protective hairs	Secretory hairs			Protective hairs
		Capitate	Peltate	Total		Capitate	Peltate	Total	
***Lavandula angustifolia* Ellagance Pink**	Average	7.20	3.81	11.01	75.38	22.49	11.16	33.65	50.56
	Standard deviation	2.98	0.61	2.37	3.75	3.24	3.26	0.03	4.84
***Lavandula angustifolia* Ellagance Purple**	Average	7.25	6.04	13.30	85.02	24.77	30.59	55.36	104.36
	Standard deviation	1.14	0.57	1.71	8.57	3.57	3.60	7.17	2.85
***Lavandula angustifolia* Ellagance Snow**	Average	13.18	3.07	16.25	113.76	35.28	13.75	49.03	127.29
	Standard deviation	3.75	0.62	3.13	10.74	7.52	3.56	3.96	8.12
***Lavandula angustifolia* Munstead**	Average	64.34	7.84	72.18	95.80	73.60	15.26	88.86	127.66
	Standard deviation	9.57	5.05	11.58	13.67	2.66	3.13	0.47	15.81
***Lavandula angustifolia* Vicenza Blue**	Average	36.38	10.91	47.30	185.52	60.37	11.30	71.67	200.21
	Standard deviation	10.31	5.15	15.47	5.27	9.79	5.17	14.96	2.36
***Lavandula x intermedia* Grosso**	Average	13.99	0.00	13.99	98.00	45.04	8.68	53.72	143.74
	Standard deviation	0.08	0.00	0.08	1.78	7.18	1.02	6.16	8.24

Preliminary conclusions

Following histo-anatomical analysis of the vegetative organs, consultation of the literature and microscopic observations, the following can be stated:

- there are no significant structural differences at the root level, the differences that do occur are of a quantitative nature and concern the parenchyma cells in the central wooden body;
- at the stem level, in the varieties of the Ellagance group, the epidermis is disorganized and exfoliated, the outline of the section becoming circular, with the disappearance of the specific diagnostic element of Lamiaceae - the quadrate outline; in the other varieties the quadrate outline is preserved, with four prominent ribs; a quantitative difference is found in the varieties Munstead and Vicenza Blue, in which the angular collenchyma is present in the form of well-defined islands;
- all varieties have numerous protective, branched arborescent hairs and numerous secretory hairs with bi-, tetra- or octocellular glands on the stem surface, characters specific to the genus *Lavandula.*

The epidermal leaf surfaces of the species and varieties studied show an indumentum consisting of secretory and protective hairs. The density of hairs varies interspecifically as well as intraspecifically, with two types of secretory hairs observed: capitate and peltate. The peltate secretory hairs consist of a massive head of four to eight secretory cells; these are absent in the upper epidermis of *Lavandula x intermedia* Grosso. Capitate secretory hairs are the dominant type of glandular hairs, much more abundant on the lower epidermis of all taxa analyzed.

Protective hairs are the dominant formations on the surface of both epidermises for all species and varieties analyzed, much more abundant on the surface of the lower epidermis. They were divided into two types: simple unicellular or multicellular, branched unicellular or multicellular, with a higher density than the secretory hairs for all taxa analyzed.

CHAPTER 4. COMPARATIVE HISTO-ANATOMICAL AND MICROMORPHOLOGICAL CHANGES OF VEGETATIVE ORGANS IN FOUR VARIETIES OF *LAVANDULA ANGUSTIFOLIA* MILL. SUPPLEMENTED WITH DIFFERENT TYPES OF HOAGLAND NUTRIENT SOLUTION

Species of the genus *Lavandula* have been the subject of several experiments on cultivation (Economakis et al., 2002; Chrysargyris et al., 2016) and on the use of the products resulting from the processing of their vegetative and generative organs (Zhao et al., 2015; Salehi et al., 2018; Ciocarlan et al., 2021; Mushtaq et al., 2021), as well as on the establishment of methods to improve production and the chemical composition of the volatile oil obtained from them (Mulder-Krieger et al, 1988; Lis-Balchin, 2002; Urwin and Mailer, 2008; Prins et al., 2010; Zuzarte et al., 2010; Hassanpouraghdam et al., 2011; Woronuk et al., 2011; Herraiz-Peñalver et al., 2013; Lesage-Meessen et al., 2015; Tomescu et al., 2015; Erland and Mahmoud, 2016; el Hamdaoui et al., 2018; Segura et al., 2019; Ciocarlan et al., 2021).

In the context of the above, the results of the research included in this chapter aim at the comparative histo-anatomical and micromorphological evaluation of vegetative organs belonging to four varieties of *Lavandula angustifolia* Mill. cultivated in our country in the counties Botoșani, Vrancea and Cluj, supplemented during the vegetative cycle with different types of Hoagland nutrient solutions.

Biological material and experimental design

The plant material used was supplied by local producers from Vorona, Botoșani County (47°34′32″N 26°37′39″E), Satu Nou, Vrancea County (45°53′22″N 27°5′53″E) and Sânpaul, Cluj County (46°52′15″N 23°25′09″E). It consisted of vegetative organs (stems and leaves) belonging to mature plants, in flowering period, grown in specially organized crops for oil production, as shown in Table 4. 1.

Table 4. 1. List of varieties belonging to the species *Lavandula angustifolia* Mill. analyzed

Species	Variety	Morphological description	Inventory number
***Lavandula angustifolia* Mill.**	Codreanca	Romanian variety of lavender from the English Hidcote variety, acclimatized by INCDA Fundulea, approved in 1992. Medium-sized plants with dark purple inflorescences and high volatile oil content. It is considered one of the best varieties for growing in	207002

		Romania's climatic conditions, being resistant to low temperatures and frost.	
	Provence Blue	An early to late summer flowering variety, with inflorescences of purple flowers and beautiful, intensely fragrant blue hues.	207009
	Vera	A variety of English lavender with dark blue-purple inflorescences and compact bushes, grown commercially for the production of sweet-scented volatile oil.	207001
	Sevtopolis	A variety native to Bulgaria, of great commercial interest due to its high volatile oil content, with a bush height of 40 - 60 cm and a width of 50 - 60 cm, the individuals have long green-grey leaves and blue flowers.	207004

The experiment was carried out in a protected (greenhouse) and unprotected (field) space in order to estimate with a high degree of accuracy the influence of the addition of nutrients to the growing medium on the production of volatile oils, a parameter analyzed both quantitatively and qualitatively.

To achieve the aims proposed in this experiment, four varieties of *Lavandula angustifolia* Mill were used and, respectively, four experimental variants (Figure 4. 1.).

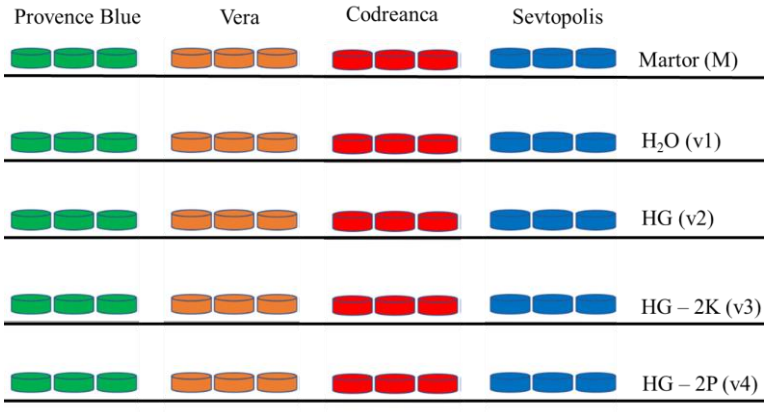

Figure 4. 1. Experimental cultivation scheme (different types of Hoagland solution supply) of the 4 lavender varieties analyzed

In addition to the four working variants, the control (M) plants were grown in an unprotected space (field) and irrigated with water.

Experimental cultivation protocol

In the autumn of 2017, about 100 lavender cuttings belonging to several varieties of *L. angustifolia* were purchased from specially organized agricultural cultures in Romania.

In the spring of 2018, at the end of April, the cuttings were dug up and transplanted into production pots with a volume of 3 L. Their planting was done in a 3:1 mixture of peat and perlite.

After planting, the pots were transferred to a greenhouse located in Vânători, Vrancea county (45°43′22″N 27°15′16″E), for the start of the experiments.

Thus, an experimental model was developed comprising four experimental variants:

- watered with H_2O (v1);
- watered with standard Hoagland nutrient solution (HG) (v2);

- watered with Hoagland nutrient solution containing twice the amount of K (HG – 2K) (v3);
- watered with Hoagland nutrient solution containing twice the amount of P (HG – 2P) (v4).

Each experimental variant consisted of 3 replicates.

Chemical composition of the nutrient solution

By preparing this solution, an attempt was made to obtain a universal nutrient solution that would provide the necessary nutrients for plants grown in greenhouses or hydroponic systems.

The chemical composition of the stock solutions and the quantity required to prepare one litre of nutrient solution are given in Tables 4. 2. - 4.4.

Table 4. 2. The standard composition of the Hoagland nutrient solution (Hoagland and Arnon, 1950)

Components	Stock solution [g/L]	Stock solution [ml/L]
KNO_3	202	2.5
$Ca(NO_3)_2 \times 4H_2O$	236	2.5
FeEDTA	15	1.5
$MgSO_4 \times 7H_2O$	493	1
NH_4NO_3	80	1
KH_2PO_4	136	0.5
Microelements		
H_3BO_3	2.86	0.5
$MnCl_2 \times 4H_2O$	1.81	0.5
$ZnSO_4 \times 7H_2O$	0.22	0.5
$CuSO_4 \times 5 H_2O$	0.051	0.5
$H_3MoO_4 \times H_2O$	0.09	0.5

Table 4. 3. The composition of the Hoagland nutrient solution supplemented with K

Components	Stock solution [g/L]	Stock solution [ml/L]
KNO_3	202	5
$Ca(NO_3)_2 \times 4H_2O$	236	2.5
FeEDTA	15	1.5
$MgSO_4 \times 7H_2O$	493	1
NH_4NO_3	80	1
KH_2PO_4	136	0.5
Microelements		
H_3BO_3	2.86	0.5
$MnCl_2 \times 4H_2O$	1.81	0.5
$ZnSO_4 \times 7H_2O$	0.22	0.5
$CuSO_4 \times 5 H_2O$	0.051	0.5
$H_3MoO_4 \times H_2O$	0.09	0.5

Table 4. 4. The composition of the Hoagland nutrient solution supplemented with P

Components	Stock solution [g/L]	Stock solution [ml/L]
KNO_3	202	2.5
$Ca(NO_3)_2 \times 4H_2O$	236	2.5
FeEDTA	15	1.5
$MgSO_4 \times 7H_2O$	493	1
NH_4NO_3	80	1
KH_2PO_4	136	1
Microelements		
H_3BO_3	2.86	0.5
$MnCl_2 \times 4H_2O$	1.81	0.5
$ZnSO_4 \times 7H_2O$	0.22	0.5
$CuSO_4 \times 5 H_2O$	0.051	0.5
$H_3MoO_4 \times H_2O$	0.09	0.5

Plants belonging to variants v1 and M were watered rhythmically with water according to functional needs throughout the growing interval, and plants belonging to variants v2, v3 and v4 were watered twice a month with dilutions of nutrient stock solutions, depending on the experimental variant, while during the rest of the interval they were also watered as needed with water.

Histo-anatomical investigations

Biological material and working method

Plant material was collected from mature, flowering specimens in June 2019 and vegetative organs (stems and leaves) were fixed in 70% ethanol for histo-anatomy studies.

Leaf and stem cross-sections for plants watered with H2O (v1) and for those watered with standard Hoagland nutrient solution (v2) were made by the method described in Chapter 3 (see section **Method and working technique for making cross-sections through vegetative organs of plants**).

Results and discussions

Stem

All the layers and structures described in other anatomical studies on *Lavandula angustifolia* species (Toma and Niță, 1982; do Rocio Duarte and Carvalho de Souza, 2014; Fakhriddinova et al., 2020; Ștefan et al., 2021) were identified at the stem level.

In its upper third, the stem has a square contour in cross-section, with four very prominent ribs (Figure 4. 1. - 4. 2.). Towards the base of the stem, the ribs attenuate, the epidermal cells in the ribs become much elongated tangentially, the elements of the collenchyma cords flatten much, the last layer of the bark dedifferentiates and gives a phellogen, which produces a thick suber and a few layers of small phelloderm cells; the cambium becomes inelar, so that from its activity results a thin ring of secondary phloem (with sieved tubes, attachment cells and parenchymal cells) and another sinuous ring composed of secondary xylem, thicker along the ribs (consisting of vessels, few parenchymal cells and a lot of libriform); the medullary rays at the level of the wood are sclerified and lignified, forming with it a continuous ring (Toma and Niță, 1982; Fakhriddinova et al. , 2020; Ștefan et al., 2021).

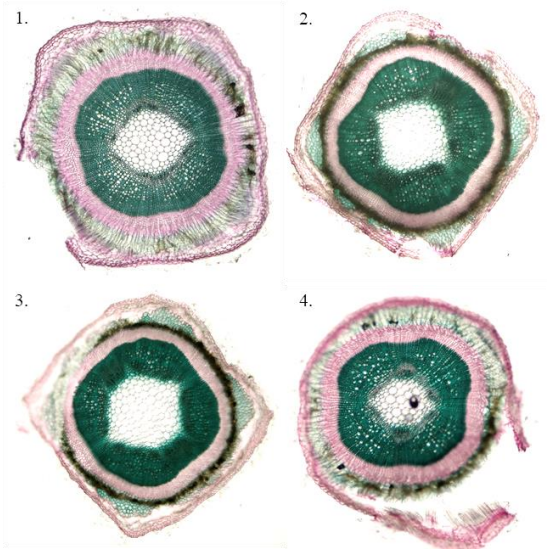

Figure 4. 1. Cross sections through the stem of lavender plants, varieties Codreanca (1), Provence Blue (2), Vera (3) and Sevtopolis (4) watered with H_2O (variant v1) (x40), (original)

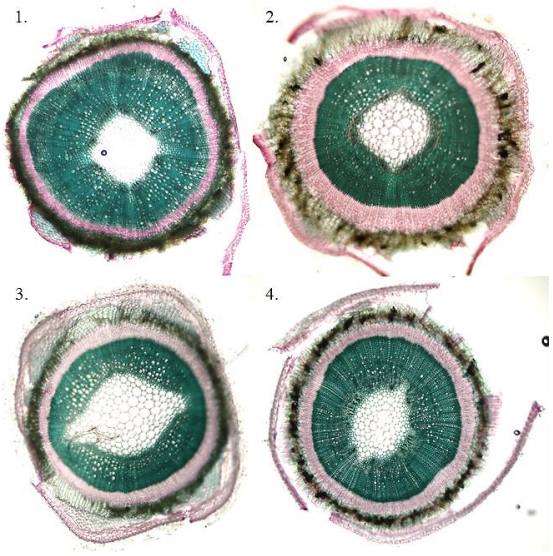

Figure 4. 2. Cross sections through the stem of lavender plants, varieties Codreanca (1), Provence Blue (2), Vera (3) and Sevtopolis (4) watered with standard Hoagland nutrient solution (variant v2) (x40 (original)

The dedifferentiation of the secondary meristem, called phellogen, located in a deep position near the central cylinder, on account of an inner layer of the bark, causes a pluristratified suber to form outwards, with dead elements at maturity. For this reason, everything on the outside (the rest of the bark and epidermis) will exfoliate and, therefore, at some point the stem will have only suber, phellogen, xylem (primary and secondary), phloem (primary and secondary) and the pith, a fact confirmed by other scientific studies (Toma and Niță, 1982; Fakhriddinova et al., 2020; Ștefan et al., 2021).

As the plant grows and develops, the stem undergoes a process of lignification, typical of subshrubs such as lavender, and the stem loses its epidermis and bark, as well as the secretory and protective hairs along with them, these structures remaining visible only on the green portions of the stems, grown in each new year of cultivation.

Leaf blade

The histo-anatomical characteristics of the leaf can be seen in Figures 4. 3. - 4. 4.

At the level of the cross sections through the leaf blade, a single layered epidermis with rounded, elliptical or rectangular cells (slightly tangentially elongated), with the outer wall slightly thicker than the others and covered by a thin cuticle, was identified on both sides of the leaf for all varieties presented, a fact also confirmed by the relevant literature (Toma and Niță, 1982; Brailko et al., 2017; Fakhriddinova et al., 2020).

Numerous protective hairs (uni-, bi- or multicellular, uniserial or branched) and glandular secretory hairs (with short pedicel and uni-, bi-, tetra- or octocellular secretory gland) specific to this species are found on the surface of both epidermises (Iriti et al., 2006; Nikolakaki et al., 2006; Huang et al., 2008; Zuzarte et al., 2010; Lungu et al., 2014; Giuliani et al., 2020).

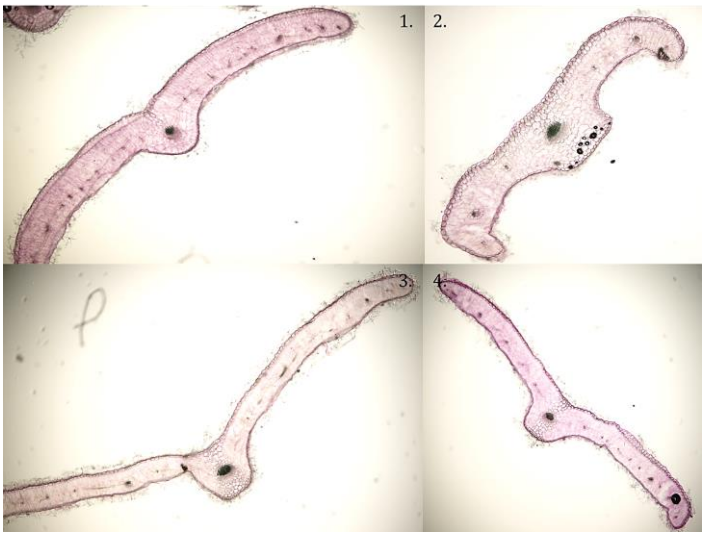

Figure 4. 3. Cross sections through the leaf blade of lavender plants, varieties Codreanca (1), Provence Blue (2), Vera (3) and Sevtopolis (4) watered with H_2O (variant v1) (x40) (original)

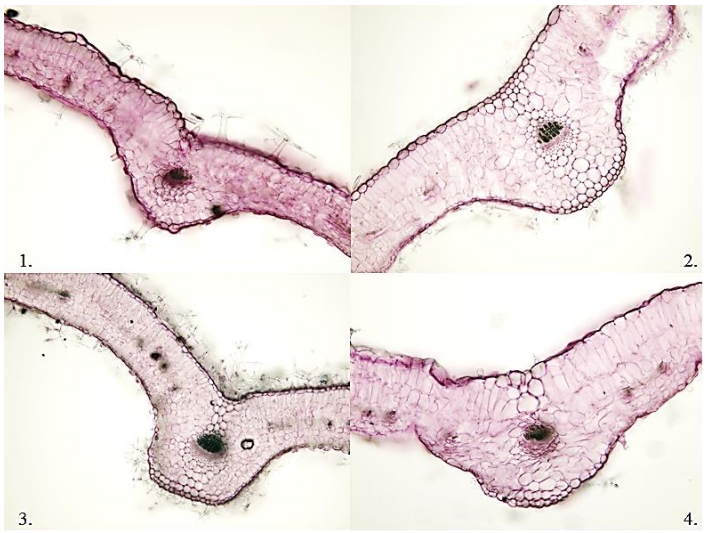

Figure 4. 4. Cross sections through the midrib of lavender plants, varieties Codreanca (1), Provence Blue (2), Vera (3) and Sevtopolis (4) watered with Hoagland nutrient solution (v2) (x100) (original)

Leaf blade micromorphology

Biological material and working technique

The plant material represented by fresh leaves belonging to varieties of *Lavandula angustifolia* species grown under controlled environmental conditions (see section **Biological material and experimental design**) was collected from mature, flowering specimens in June 2019.

The microscopic specimens for all varieties and experimental variants were prepared using the method described previously in Chapter 3 (see section **Method and working technique for scanning electron microscopy (SEM)**).

Results

It is well known that plants of the genus *Lavandula* have numerous secretory and protective hairs on both the upper and lower surfaces of the leaf blade (Huang et al., 2008; Zuzarte et al., 2010).

The secretory hairs are further divided into glandular and peltate hairs, depending on the structure of the secretory gland. In both cases the secreted substances are accumulated in a subcuticular space. Peltate hairs produce most of the volatile oil, with terpenes as main components (Turner et al., 2000; Boz et al., 2009; Zamfirache et al., 2009, 2010; Zuzarte et al., 2010; Burzo and Toma, 2013; Zuzarte, 2013).

Lavandula angustifolia – Codreanca variety

On both epidermises of the leaf blade of lavender plants belonging to the Codreanca variety, two categories of epidermal formations were morphologically identified: simple, branched protective hairs and secretory hairs with unicellular and multicellular glands, with a higher abundance on the abaxial surface of the leaf for all experimental variants (Table 4. 5.).

After analysis of the micrographs for this variety, differences between the control and experimental variants were observed, showing lower densities, especially for individuals watered with H_2O (v1) and those supplemented with Hoagland solution (v2).

Table 4. 5. Density of secretory and protective hairs in *Lavandula angustifolia*, variety Codreanca, in all treatment variants with Hoagland nutrient solution (no. hairs/mm^2) (n=3):

Species/ variety/ variant		Upper epidermis				Lower epidermis			
		Secretory hairs			Protective hairs	Secretory hairs			Protective hairs
		Capitate	Peltate	Total		Capitate	Peltate	Total	
***L. angustifolia* var. Codreanca, variant v1**	Average	13.41	3.72	17.14	42.01	29.71	10.20	39.91	45.94
	Standard deviation	4.63	1.56	6.19	3.53	11.26	1.55	12.75	8.21
***L. angustifolia* var. Codreanca, variant v2**	Average	36.27	4.62	40.89	52.41	44.49	19.06	63.56	69.70
	Standard deviation	9.46	1.96	7.72	6.71	5.38	2.36	6.33	10.53
***L. angustifolia* var. Codreanca, variant v3**	Average	25.63	4.87	30.50	33.76	91.80	20.68	112.48	85.67
	Standard deviation	1.81	1.01	1.26	2.99	6.20	1.38	4.92	9.95
***L. angustifolia* var. Codreanca, variant v4**	Average	31.54	4.70	36.24	138.92	47.01	17.07	64.08	195.11
	Standard deviation	3.81	1.55	5.25	11.01	8.34	3.44	10.31	11.67
***L. angustifolia* var. Codreanca, variant M**	Average	25.73	6.93	32.66	128.98	42.44	12.93	55.37	204.95
	Standard deviation	4.33	1.72	3.99	10.15	3.90	0.98	4.55	11.08

Lavandula angustifolia - Provence Blue variety

Two categories of epidermal formations were identified on the surface of the upper and lower epidermis of the leaf blade in lavender plants belonging to the Provence Blue variety: simple branched arborescent protective hairs and capitate and peltate secretory hairs.

Analyzing the density of protective hairs, it can be seen that in all experimental variants a higher number of protective hairs are present on the lower side of the leaf (Table 4. 6.).

Table 4. 6. Density of secretory and protective hairs in *Lavandula angustifolia*, variety Provence Blue, in all Hoagland nutrient solution treatments (no. hairs/mm^2) (n = 3):

Species/ variety/ variant		Upper epidermis				Lower epidermis			
		Secretory hairs			Protective hairs	Secretory hairs			Protective hairs
		Capitate	Peltate	Total		Capitate	Peltate	Total	
***L. angustifolia* var. Provence Blue, variant v1**	Average	29.08	18.27	47.36	148.77	16.70	25.51	42.22	212.60
	Standard deviation	7.76	5.08	11.27	19.18	9.04	2.39	10.79	14.54
***L. angustifolia* var. Provence Blue, variant v2**	Average	23.55	6.89	30.44	80.54	24.53	14.87	39.40	143.17
	Standard deviation	8.47	2.01	10.10	12.13	2.26	3.12	5.33	15.13
***L. angustifolia* var. Provence Blue, variant v3**	Average	27.78	2.26	30.04	84.62	59.61	13.91	73.51	115.26
	Standard deviation	3.92	1.48	2.58	5.77	3.46	1.69	5.10	7.42
***L. angustifolia* var. Provence Blue, variant v4**	Average	58.36	5.57	63.93	80.00	67.71	15.12	82.83	114.97
	Standard deviation	3.13	0.56	3.07	8.19	6.86	2.11	8.89	11.59
***L. angustifolia* var. Provence Blue, variant M**	Average	47.42	8.18	55.60	91.58	37.77	18.71	56.48	217.00
	Standard deviation	4.07	2.28	4.10	2.46	3.50	1.94	3.87	11.99

Following the applied treatments, in the case of experimental variants v3 and v4, the development of protective hairs was inhibited and the frequency of capitate secretory hairs was stimulated on the surface of the lower epidermis for plants watered with the nutrient solution with K supplementation (v3) and on the face of both epidermis for plants watered with the nutrient solution with P supplementation (v4).

Lavandula angustifolia – Vera variety

In the analyzed epidermis of the aerial vegetative organs (leaves) of Lavandula angustifolia - variety Vera, two types of hairs are observed: secretory and protective. The number of hairs per area unit varies on the two sides of the leaf for all experimental variants (Table 4. 7.).

A comparative assessment of the density of secretory hairs shows higher values of their number on the lower side of the leaf blade compared to the upper side in all experimental variants of this lavender variety, the dominant type of hairs being the capitate type.

Table 4. 7. Density of secretory and protective hairs in *Lavandula angustifolia*, variety Vera, in all Hoagland nutrient solution treatments (no. hairs/mm^2) (n = 3):

Species/ variety/ variant		Upper epidermis				Lower epidermis			
		Secretory hairs			Protective hairs	Secretory hairs			Protective hairs
		Capitate	Peltate	Total		Capitate	Peltate	Total	
***L. angustifolia* var. Vera, variant v1**	Average	32.78	3.95	36.72	110.19	38.13	28.10	66.24	228.26
	Standard deviation	13.03	0.92	13.21	4.05	11.67	10.21	17.79	17.38
***L. angustifolia* var. Vera, variant v2**	Average	34.75	9.43	44.19	96.47	35.05	16.64	51.68	166.55
	Standard deviation	3.25	1.02	3.90	0.51	6.46	1.82	8.18	6.65
	Average	43.11	5.83	48.94	162.35	56.97	14.84	71.80	278.58

***L. angustifolia* var. Vera, variant v3**	Standard deviation	2.31	1.16	3.05	4.64	6.24	2.28	5.03	5.10
***L. angustifolia* var. Vera, variant v4**	Average	20.10	1.62	21.72	140.73	48.14	9.89	58.02	281.10
	Standard deviation	3.39	0.56	2.94	4.18	4.01	2.60	6.32	5.69
***L. angustifolia* var. Vera, variant M**	Average	25.83	8.28	34.11	150.32	40.33	22.35	62.68	250.10
	Standard deviation	2.64	1.57	3.15	5.13	7.98	2.93	9.62	9.12

Comparing the densities of both types of hairs in the plants analyzed, their numerical difference is observed in the control and in the plants supplemented with nutrient solution with phosphorus addition (v4).

Lavandula angustifolia – Sevtopolis variety

On the surface of both leaf epidermis of lavender plants belonging to the Sevtopolis variety, as in the case of the other varieties analyzed, two categories of morphologically and functionally different epidermal formations were identified: simple, branched protective hairs and secretory hairs with unicellular and multicellular glands, more abundant on the abaxial surface of the leaf in most of the experimental variants of this lavender variety (Table 4. 8.).

Table 4. 8. Density of secretory and protective hairs in *Lavandula angustifolia*, variety Sevtopolis, in all Hoagland nutrient solution treatments (no. hairs/mm^2) (n = 3):

Species/ variety/ variant		**Upper epidermis**				**Lower epidermis**			
		Secretory hairs			**Protective hairs**	**Secretory hairs**			**Protective hairs**
		Capitate	**Peltate**	**Total**		**Capitate**	**Peltate**	**Total**	
***L. angustifolia* var. Sevtopolis, variant v1**	Average	28.32	7.97	36.29	76.20	23.38	12.17	35.55	143.67
	Standard deviation	6.96	2.63	9.05	14.92	1.70	2.55	1.08	10.53
***L. angustifolia* var. Sevtopolis, variant v2**	Average	44.58	5.95	50.54	76.84	55.75	12.40	68.15	167.70
	Standard deviation	7.63	1.59	8.95	2.42	5.40	0.18	5.22	21.62
***L. angustifolia* var. Sevtopolis, variant v3**	Average	31.28	5.54	36.82	192.07	18.61	25.19	43.80	196.21
	Standard deviation	3.22	1.53	4.08	5.13	1.42	6.54	6.66	1.81
***L. angustifolia* var. Sevtopolis, variant v4**	Average	44.58	7.05	51.63	177.30	48.48	19.65	68.12	263.30
	Standard deviation	3.60	1.47	4.74	8.02	1.00	2.01	2.49	8.94
***L. angustifolia* var. Sevtopolis, variant M**	Average	17.98	3.21	21.19	94.39	46.05	25.12	71.17	142.01
	Standard deviation	2.40	0.56	1.90	5.97	0.60	2.90	3.40	6.10

Discussions

All the varieties and experimental variants examined showed numerous uni-, bi- or multicellular, uniseriate or branched protective hairs and many glandular secretory hairs of different types, most commonly with short pedicel and uni-, bi-, tetra- or octocellular secretory gland on the leaf surface, specific to the genus *Lavandula* (Huang et al., 2005; Nikolakaki and Christodoulakis, 2006; Lungu et al., 2014; Ștefan et al., 2021).

In the material analyzed (lavender varieties and experimental cultivars) the highest density of protective hairs was recorded on the lower leaf surface of the Vera variety, in the case of plants watered with

Hoagland nutrient solution with the addition of P and K (v4 -281.1 and v3 - 278.58), and on the upper epidermis of the Sevtopolis variety in plants belonging to the variants watered with nutrient solution with the addition of K and P (v3 - 192.07 and v4 - 177.3 respectively).

The highest density of secretory hairs on the lower surface of the lavender leaves was found in plants watered with Hoagland nutrient solution supplemented with K (v3 - 112.48) of the Codreanca variety, followed by those watered with the solution supplemented with P (v4 - 82.83) of the Provence Blue variety. For the upper surface of the leaf blade, the highest values were found in plants watered with Hoagland solution with the addition of P (v4 - 63.93), followed by plants from the control group (M - 55.6) of the Provence Blue variety.

Preliminary conclusions

Data obtained by histo-anatomical and micromorphological analysis of vegetative organs (stems and leaves) in the four varieties of the genus *Lavandula*, with four experimental variants, discussed in comparison with the information presented in the specialized literature in this domain (Robu et al, 2011; do Rocio Duarte et al., 2014; Lungu et al., 2014; Riva et al., 2014; Brailko et al., 2017; Tanase et al., 2020) allows us to consider that all the histological structures identified in the taxa analyzed are generally circumscribed to the typical structural plan of the genus.

The comparative analysis of the four varieties of lavender under examination reveals several qualitative differences:

- <u>at the stem level</u>:

- variations in the contour of the cross-section are observed and the epidermis and bark tend to exfoliate due to the formation of a suber in the depth of the bark;

- the pith presents different shapes, which can be square, hexagonal or rhomboid;

- <u>at the leaf blade level</u>:

- variations in anatomical characters are noted regarding the contour of the section, the thickness of the mesophyll, the number of protective formations (protective and secretory hairs) and the number of conducting vascular bundles corresponding to the lateral ribs;

- an indumentum consisting of glandular and protective hairs is present on the surface of both leaf blade epidermises for the varieties studied as well as for the experimental variants;

- the density of secretory and protective hairs is improved in plants watered with Hoagland nutrient solution with the addition of K and P for all varieties studied.

The comparative numerical analysis of the protective and secretory hairs in the four varieties of lavender, depending on the treatment applied, revealed the following aspects:

At the Codreanca variety:

- The highest density of protective hairs was found in control plants (M) and those watered with Hoagland nutrient solution with addition of P (v4).
- Stimulation of the development of the number of secretory hairs was observed in the experimental variant with the addition of K (v3) on the lower surface of the leaf blade.

For the Provence Blue variety:

- The highest density of protective hairs was found in the control (M) and H_2O-watered plants (v1).

- Plants watered with Hoagland nutrient solution with double addition of P (v4) showed the highest frequency of secretory hairs.
- The lowest values were recorded for the protective hairs in plants treated with nutrient solution supplemented with K and P (v3 and v4, respectively), which showed close values.

Vera variety:

- Hoagland solution treatment with double amounts of K and P increased the density of protective hairs on the surface of the lower epidermis.
- The highest values of secretory hairs were observed in plants watered with Hoagland nutrient solution with addition of K (v3).
- Plants watered with standard Hoagland nutrient solution (v2) had the lowest frequency of secretory hairs, and those watered with the solution with addition of P (v4) had the lowest density of secretory hairs on the face of both leaf epidermis.

At the Sevtopolis variety:

- Following treatment with Hoagland nutrient solution, there was an increase in the density of protective hairs in plants watered with nutrient solution with double amounts of K and P (v3 and v4 respectively) on the surface of both leaf blade surfaces.
- The secretory hairs showed a high frequency in the control lot (M) on the lower surface of the leaf, in plants watered with standard Hoagland nutrient solution (v2) and in those with P addition (v4), which showed similar values.
- The lowest density of secretory hairs was found in plants watered with H_2O (v1) for the lower epidermis and in field-grown - control plants (M) for the upper epidermis.

CHAPTER 5. COMPARATIVE QUANTITATIVE AND QUALITATIVE ANALYSIS OF THE VOLATILE OIL PRODUCED BY FOUR VARIETIES OF *LAVANDULA ANGUSTIFOLIA* MILL. SUPPLEMENTED WITH DIFFERENT TYPES OF HOAGLAND NUTRIENT SOLUTION

Volatile oil extraction

Plant material and working method

The plant material for analysis was represented by hand-harvested flower stems from three lavender plants belonging to the species *Lavandula angustifolia* Mill., varieties Codreanca, Provence Blue, Vera and Sevtopolis, cultivated in experimental variants according to the protocol presented in **Chapter 4** under point **Biological material and experimental design**.

Volatile oil extraction was carried out by steam distillation with a Clevenger hydrodistillation device heated under direct fire under strict supervision.

GC-MS analysis and characterisation of volatile oil

Compound separation was performed using the Agilent 6890N GC gas chromatograph equipped with a flame ionization deprotector (FID) and coupled to the MSD 5975 mass spectrometer (Agilent Technology, USA), operating at an electron ionization energy of 70 eV.

The capillary column used was Teknokroma TBR-5MS (length 30m, internal diameter 0.25mm, film thickness 0.25 μm), inlet: 230°C, split ratio 200:1 (MS), 100:1 (FID), He flow rate 0.5 ml/min (MS), 1.8 ml/min (FID), GC-MSD transfer line: 280°C, temperature program 60°C, 4°C/min -> 220°C, 20°C/min -> 320°C.

Extraction efficiency

The amount of volatile oil obtained from plant extraction varied both between the plant varieties analyzed and between the experimental variants (watered with different types of Hoagland nutrient solutions) (Table 5. 1.). Thus, *Lavandula angustifolia* var. Provence Blue grown in the field (M) is the richest in volatile oil, reaching a maximum yield of 5.11% in dried flower stems, followed by plants watered with standard Hoagland nutrient solution (v2) belonging to the Sevtopolis variety (3.64%).

Table 5. 1. Extraction efficiency of volatile oils obtained by hydrodistillation from four varieties of lavender - *Lavandula angustifolia* Mill., supplemented with different types of Hoagland nutrient solution

Species/variety	g d. s.	ml oil	%
***L. angustifolia* – var. Codreanca (v1)**	**4.76**	**0.08**	**1.68**
***L. angustifolia* – var. Codreanca (v2)**	**7.14**	**0.14**	**1.96**
***L. angustifolia* – var. Codreanca (v3)**	**5.40**	**0.07**	**1.30**
***L. angustifolia* – var. Codreanca (v4)**	**1.97**	**0.03**	**1.52**
***L. angustifolia* – var. Codreanca M**	**24.86**	**0.53**	**2.13**
***L. angustifolia* – var. Provence Blue (v1)**	**18.42**	**0.45**	**2.44**
***L. angustifolia* – var. Provence Blue (v2)**	**11.85**	**0.3**	**2.53**
***L. angustifolia* – var. Provence Blue (v3)**	**8.74**	**0.06**	**0.69**
***L. angustifolia* – var. Provence Blue (v4)**	**5.79**	**0.08**	**1.38**
***L. angustifolia* – var. Provence Blue M**	**24.45**	**1.25**	**5.11**
***L. angustifolia* – var. Vera (v1)**	**8.20**	**0.16**	**1.95**
***L. angustifolia* – var. Vera (v2)**	**12.93**	**0.28**	**2.17**
***L. angustifolia* – var. Vera (v3)**	**8.78**	**0.14**	**1.59**
***L. angustifolia* – var. Vera (v4)**	**7.01**	**0.12**	**1.71**
***L. angustifolia* – var. Vera M**	**7.39**	**0.13**	**1.76**
***L. angustifolia* – var. Sevtopolis (v1)**	**10.12**	**0.28**	**2.77**
***L. angustifolia* – var. Sevtopolis (v2)**	**11.55**	**0.42**	**3.64**
***L. angustifolia* – var. Sevtopolis (v3)**	**15.42**	**0.23**	**1.49**
***L. angustifolia* – var. Sevtopolis (v4)**	**10.42**	**0.22**	**2.11**
***L. angustifolia* – var. Sevtopolis M**	**20.70**	**0.43**	**2.08**

Chemical characterisation of volatile oils

***Lavandula angustifolia* – Codreanca variety**

From the qualitative and quantitative analyses carried out (Table 5. 2.) it can be deduced that the volatile oil produced by this variety of lavender, in the experimental variants of supplementation with Hoagland nutrient solution, contains more than 60 compounds, among which, in high concentrations linalool, linalyl acetate, eucalyptol, borneol, terpinen-4-ol, camphor, lavandulyl acetate and caryophyllene oxide.

Table 5. 2. Biochemical profile of volatile oils produced by lavender plants, *Lavandula angustifolia* Mill. - variety Codreanca, supplemented with different types of Hoagland nutrient solution

RI	Compound	Composition (%)			
		Variant M	Variant (v1)	Variant (v2)	Variant (v3)
811	butylacetate	0.007	-	-	-
823	hexyl methyl ether	0.013	-	-	-
906	propyl isobutyrate	0.475	-	-	-
926	tricyclene	0.006	-	-	-
929	a-thujene	0.007	-	-	-
937	a-pinene	0.211	0.051	0.039	0.021
952	camphene	0.006	0.195	0.148	0.08
981	b-pinene	0.169	-	0.082	0.341
984	**3-octanone**	**0.727**	**0.147**	**0.589**	**0.652**
991	b-myrcene	1.1	0.293	0.623	0.926
1011	hexyl acetate	0.234	0.097	0.146	0.212
1014	3-carene	0.054	-	-	0.055
1019	terpinolene	0.03	-	0.218	0.33
1026	o-cymene	0.09	0.128	0.066	0.161
1031	D-limonene	0.288	0.137	0.19	0.382
1035	**eucalyptol**	**6.754**	**2.919**	**2.892**	**4.991**
1047	**trans-b-ocimene**	**3.009**	**0.402**	**3.058**	**2.619**
1060	g-terpinene	0.011	-	0.412	-
1069	sabinene hydrate	0.215	0.011	0.234	0.018
1074	cis-linalool oxide	0.525	0.018	0.144	0.026
1090	trans-linalool oxide	0.292	-	-	-
1104	**linalool**	**22.919**	**19.549**	**17.437**	**17.75**
1111	**1-octenyl-3-yl acetate**	**0.974**	**0.678**	**3.712**	**1.064**
1122	3-octanol acetate	0.109	0.213	0.447	0.366
1149	camphor	0.601	0.659	0.461	0.782
1156	nerol oxide	0.064	-	-	-
1186	p-cymen-8-ol	-	0.166	-	0.04
1160	sabine ketone	0.032	-	-	-
1162	myrtenol	-	0.215	-	-
1170	**borneol**	**2.312**	**3.295**	**2.076**	**2.562**
1182	**terpinen-4-ol**	**3.104**	**1.575**	**2.203**	**1.506**
1189	cryptone	0.166	0.527	0.878	0.197
1195	**a-terpineol**	**4.011**	**2.863**	**2.746**	**2.751**

RI	Compound	Composition (%)			
		Variant M	Variant (v1)	Variant (v2)	Variant (v3)
1208	2,6-dimethyl-3,5,7-octatriene-2-ol, E,E	0.249	0.199	0.314	0.072
1212	chrysanthenone	0.052	-	-	0.261
1221	cis-carveol	0.159	0.118	0.132	-
1230	nerol	0.919	0.945	0.546	0.747
1232	bornyl formate	-	0.156	0.296	0.227
1243	cumaldehyde	0.392	0.311	0.135	0.282
1247	D-carvone	0.19	0.146	0.052	0.137
1257	**linalyl acetate**	**27.134**	**34.497**	**24.839**	**32.212**
1291	**lavandulyl acetate**	**3.64**	**6.1**	**2.811**	**5.637**
1301	carvacrol	-	-	1.8	-
1330	hexyl tiglate	0.012	-	0.029	-
1364	**neryl acetate**	**1.111**	**1.252**	**1.171**	**1.14**
1383	**geranyl acetate**	**2.091**	**2.515**	**2.394**	**2.252**
1426	a-santalene	0.375	0.506	0.269	0.452
1430	**trans-b-caryophyllene**	**2.661**	**2.707**	**2.501**	**1.983**
1441	trans-a-bergamotene	0.199	0.176	0.244	0.159
1451	b-sesquiphellandrene	0.016	-	-	-
1459	E-b-farnesene	0.245	0.239	0.153	0.156
1463	humulene	-	-	-	0.009
1469	1,4,7-cycloundecatriene 1,5,9,9-tetramethyl, Z,Z,Z	0.015	0.024	-	-
1490	germacrene D	0.353	0.655	0.421	0.372
1513	b-bisabolene	0.02	-	-	-
1522	g-muurolene	0.201	0.389	0.212	0.35
1525	benzenemethanol, 4-methyl-a-(1-methyl-2-propenyl)	-	-	0.377	1.525
1558	cis-sequisabinene hydrate	0.025	-	-	-
1570	humulene-1,2-epoxide	0.026	-	-	-
1594	**caryophyllene oxide**	**2.394**	**2.63**	**2.895**	**3.662**
1633	epicubenol	0.094	0.247	-	-
1668	**t-cadinol**	**0.875**	**2.270**	**1.253**	**1.545**
1721	muurol-5-ene-4-one	0.015	0.106	0.252	0.074
1777	benzyl benzoate	0.02	-	-	-
1812	5-hydroxy-calamenene	-	-	0.065	-
	Total	91.982	90.326	81.962	91.086

For the volatile oils obtained from the Codreanca variety, the major groups of compounds were monoterpenesters (31.66 - 44.58 %) and monoterpenols (25.01 - 33.27 %) for all experimental variants analysed. The highest abundance of esters was identified in plants grown in medium watered with H_2O (v1), and for alcohols the highest values were found in the control group (M), represented by plants grown in the field.

None of the oils analyzed met the pharmaceutical standard requirements and were considered unsuitable for use for this purpose.

According to the standards presented in the European Pharmacopoeia, the compounds in the oils analyzed for this cultivar, which do not fall within the required values, are represented by linalool, which was below the limit for all experimental variants (v1, v2, v3), α-terpineol and eucalyptol, in which the determined amounts exceeded the requirements for all four types of oil analyzed, and lavandulol, which was not identified in any of the samples.

***Lavandula angustifolia* - Provence Blue variety**

The results (Table 5. 3.) indicate that more than 70 different organic compounds are found in the volatile oil produced by this lavender variety, in this case the major components being linalool (12 - 40 %), identified both in the free state and partially esterified as linalyl acetate (15 - 30 %), lavandulyl acetate (2,3 - 5,1 %), borneol (0,8 - 5,1 %), terpinene-4-ol and α-terpineol (0,4 - 5,9 %, respectively 1,1 - 3,7 %).

Table 5. 3. Biochemical profile of volatile oils produced by lavender plants, *Lavandula angustifolia* - variety Provence Blue, supplemented with different types of Hoagland nutrient solution

RI	Compound	Composition (%)				
		Variant M	Variant (v1)	Variant (v2)	Variant (v3)	Variant (v4)
811	n-butyl acetate	0.026	-	0.008	-	-
823	hexyl methyl ether	0.171	-	0.009	-	-
906	propyl isobutyrate	-	-	0.008	-	-
926	tricyclene	-	-	-	-	0.045
929	a-thujene	-	-	0.13	-	-
937	a-pinene	0.113	0.13	0.196	0.156	0.04
949	butyl isobutyrate	-	-	-	0.066	-
952	camphene	0.17	0.295	0.049	0.652	0.639
973	1,3,5-cycloheptatriene,3,7,7-trimetyl	-	-	-	0.155	-
978	1-octen-3-ol	-	-	-	0.501	-
981	b-pinene	-	0.099	0.147	0.398	0.233
984	**3-octanone**	**2.892**	**0.758**	**0.326**	**0.666**	**0.809**
991	b-myrcene	0.973	0.212	0.094	0.035	0.686
994	butyl butyrate	0.683	-	-	-	-
1008	a-phellandrene	0.087	-	-	-	-
1011	hexyl acetate	1.081	0.496	0.298	1.508	0.652
1014	3-carene	0.007	0.069	0.047	-	-
1019	terpinolene	0.243	-	-	-	-

RI	Compound	Composition (%)				
		Variant M	Variant (v1)	Variant (v2)	Variant (v3)	Variant (v4)
1026	o-cymene	0.153	0.078	0.118	0.501	0.077
1031	D-limonene	1.515	0.261	0.371	0.398	0.578
1035	**eucalyptol**	**1.652**	**0.548**	**0.436**	**0.838**	**3.154**
1047	**trans-b-ocimene**	**3.62**	**5.351**	**5.952**	**0.302**	**0.626**
1060	g-terpinene	0.087	-	0.056	0.143	-
1069	sabinene hydrate	0.175	0.134	0.258	-	0.009
1074	cis-linalool oxide	-	-	0.314	-	-
1090	trans-linalool oxide	0.068	0.798	0.307	0.277	-
1104	**linalool**	**40.744**	**30.769**	**23.311**	**12.106**	**25.33**
1129	a-campholenal	-	-	-	0.459	-
1131	(Z)-b-ocimene epoxide	-	-	-	0.239	0.033
1111	**1-octen-1-ol, acetate**	**0.863**	**0.895**	**1.144**	**4.14**	**1.07**
1122	3-octanol, acetate	0.31	0.103	-	0.656	-
1128	pinocarvone	-	-	-	0.181	-
1147	n-hexyl isobutyrate	0.078	-	-	-	-
1149	camphor	0.167	0.386	0.317	0.44	1.393
1168	**lavandulol**	**1.523**	**1.073**	**-**	**-**	**-**
1170	**L-borneol**	**0.869**	**1.578**	**1.218**	**2.018**	**5.162**
1174	(3Z, 5E)-1,3,5-undecatriene	0.121	-	0.115	-	-
1182	**terpinen-4-ol**	**2.523**	**2.336**	**5.974**	**0.482**	**2.845**
1195	**a-terpineol**	**3.777**	**2.858**	**2.525**	**1.149**	**2.628**
1210	cis-piperitol	0.077	-	-	-	-
1221	cis-carveol	0.046	-	0.019	-	-
1232	bornyl formate	0.061	-	-	-	-
1189	cryptone	0.079	1.162	-	-	-
1190	n-hexyl butyrate	2.007	-	0.432	-	1.207
1162	myrtenol	0.04	-	-	-	-
1230	nerol	0.685	0.575	0.527	0.39	0.731
1236	hexyl 2-methyl butyrate	0.048	0.329	-	12.01	-
1237	hexyl acetoacetate	-	-	0.059	-	-
1243	cumaldehyde	0.435	0.097	0.051	0.25	0.055
1247	D-carvone	0.132	0.128	0.015	0.099	0.227
1257	**linalyl acetate**	**16.344**	**28.084**	**27.712**	**15.364**	**18.473**
1301	carvacrol	-	-	-	0.046	0.266
1279	fragranyl acetate	-	-	-	0.756	-
1291	**lavandulyl acetate**	**5.151**	**4.325**	**3.926**	**2.315**	**4.256**

RI	Compound	Composition (%)				
		Variant M	Variant (v1)	Variant (v2)	Variant (v3)	Variant (v4)
1330	hexyl tiglate	0.065	0.074	0.025	0.1	-
1352	a-terpinyl acetate	0.035	-	-	-	-
1364	neryl acetate	0.894	0.822	0.766	0.537	1.023
1383	**geranyl acetate**	**1.594**	**1.586**	**1.708**	**1.229**	**2.071**
1394	sequithujene	-	-	0.018	-	-
1385	n-hexyl hexanoate	0.18	-	-	-	-
1426	a-santalene	-	0.357	-	0.174	0.383
1394	zingiberene	0.05	-	-	-	-
1430	**trans-b-caryophyllene**	**1.973**	**2.476**	**4.792**	**-**	**-**
1378	a-cedrene	-	-	0.014	-	-
1441	trans-a-bergamotene	0.062	0.122	0.364	0.513	-
1459	(E)-b-farnesene	2.377	1.271	1.209	0.174	0.144
1469	1,4,7,-cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	-	0.127	-	-	-
1490	germacrene D	0.484	0.421	0.31	-	0.37
1513	a-selinene	-	-	0.031	-	
1522	g-muurolene	0.058	0.384	0.069	0.152	-
1565	nerolidol	0.041	-	-	-	-
1530	cis-calamenene	-	0.155	0.139	-	-
1570	humulene-1,2-epoxide	-	-	-	0.15	-
1594	**caryophyllene oxide**	**0.587**	**1.593**	**2.694**	**14.83**	**8.208**
1633	epicubenol	-	0.133	0.225	-	-
1647	trans-geranyl geraniol	0.049	-	-	-	-
1668	**t-cadinol**	**0.427**	**1.407**	**2.277**	**1.055**	**0.914**
1721	muurol-5-en-4-one cis-14-nor	-	0.204	0.031	0.686	0.227
1723	farnesol	0.123	-	-	-	-
1777	benzyl benzoate	-	0.18	-	0.108	-
1797	2-pentadecanone, 6,10,14-trimethyl	-	-	-	0.041	-
	Total	98.795	95.239	91.123	84.564	79.561

In the case of the volatile oils obtained from the dried inflorescences of this variety, the major groups of compounds were esters (20.10 - 34.92 %) and alcohols (16.15 - 50.12 %) for all samples analyzed. The highest values of monoterpenesters were found in plants watered with H_2O (v1) and in plants watered with

standard Hoagland nutrient solution, which showed similar values, and monoterpenols were found in high concentrations in the control lot (M), represented by field-grown plants.

None of the oils tested met, by their composition, the pharmaceutical standard requirements set out in the Pharmacopoeia for use for medicinal purposes and were therefore considered unsuitable for such use.

***Lavandula angustifolia* – Vera variety**

According to the results obtained (Table 5. 4.), 64 different compounds can be found in the volatile oil extracted from the experimental variants of this lavender variety.

Table 5. 4. Biochemical profile of volatile oil produced by lavender plants, *Lavandula angustifolia* - variety Vera, supplemented with different types of Hoagland nutrient solution

RI	Compound	Composition (%)				
		Variant M	Variant (v1)	Variant (v2)	Variant (v3)	Variant (v4)
811	n-butyl acetate	-	-	-	0.015	-
823	hexyl methyl ether	-	0.021	-	-	0.011
926	tricyclene	0.029	-	-	-	-
937	a-pinene	0.182	0.1	0.133	0.074	1.047
949	butyl isobutyrate	-	-	-	0.201	-
952	camphene	0.44	0.261	0.457	0.126	0.314
978	1-octen-3-ol	0.527	-	-	0.375	0.196
981	b-pinene	-	0.652	0.16	0.081	0.235
984	**3-octanone**	**1.067**	**0.832**	**1.013**	**0.34**	**0.251**
991	b-myrcene	0.381	0.789	1.232	0.32	0.187
994	butyl butyrate	-	0.285	-	-	-
1008	a-phellandrene	0.106	0.036	-	-	-
1011	hexyl acetate	0.237	0.668	0.257	0.362	0.302
1013	p-mentha-1(7),-8-diene	-	0.078	-	0.065	-
1014	3-carene	-	-	0.026	-	-
1026	o-cymene	0.092	0.166	0.795	0.086	0.343
1031	D-limonene	0.021	0.553	-	0.363	0.112
1035	**eucalyptol**	**2.737**	**2.95**	**6.302**	**5.615**	**0.337**
1047	**trans-b-ocimene**	**1.047**	**1.344**	**2.043**	**0.198**	**0.175**
1060	g-terpinene	0.11	-	-	-	-
1069	sabinene hydrate	0.464	0.251	0.158	0.174	-
1074	cis-linalool oxide	0.631	0.289	-	-	0.239
1090	trans-linalool oxide	0.625	-	0.346	0.766	0.181
1104	**linalool**	**37.816**	**36.613**	**26.981**	**35.719**	**28.131**
1111	**1-octen-1-ol, acetate**	**0.652**	**3.048**	**0.904**	**2.075**	**9.485**
1122	3-octanol, acetate	-	-	0.306	-	-
1149	camphor	1.083	0.651	0.681	0.501	0.866

RI	Compound	Composition (%)				
		Variant M	Variant (v1)	Variant (v2)	Variant (v3)	Variant (v4)
1168	**lavandulol**	**7.251**	**0.595**	**-**	**0.752**	**-**
1170	**L-borneol**	**2.336**	**2.294**	**2.384**	**1.865**	**2.312**
1182	**terpinen-4-ol**	**3.753**	**3.404**	**2.583**	**2.173**	**1.913**
1195	**a-terpineol**	**1.108**	**1.694**	**5.315**	**1.904**	**1.239**
1221	cis-carveol	-	-	0.199	0.219	-
1232	bornyl formate	-	0.141	-	0.172	0.156
1189	cryptone	-	-	0.674	0.196	
1190	n-hexyl butyrate	1.15	1.079	-	-	1.055
1208	2,6-dimethyl-3,5,7-octatriene-2-ol, E, E	0.171	-	-	0.175	-
1212	chrysantenone	-	-	-	0.087	-
1230	nerol		0.253	1.139	0.379	0.2
1236	hexyl 2-methyl butyrate	-	0.061	-	0.067	-
1243	cumaldehyde	0.071	0.215	0.1	0.604	-
1247	D-carvone	0.123	0.11	0.051	0.251	-
1257	**linalyl acetate**	**10.659**	**19.861**	**23.513**	**16.593**	**12.18**
1301	carvacrol	-	-	-	0.164	-
1291	**lavandulyl acetate**	**3.009**	**4.308**	**3.995**	**2.685**	**8.064**
1300	o-cymen-5-ol	0.154	-	-	-	-
1330	hexyl tiglate	0.092	0.064	0.036	-	0.04
1364	neryl acetate	0.387	0.449	1.541	0.589	0.319
1383	**geranyl acetate**	**0.161**	**0.762**	**3.05**	**1.206**	**0.847**
1385	n-hexyl hexanoate	0.717	-	-	-	-
1426	a-santalene	-	-	-	0.288	0.334
1430	**trans-b-caryophyllene**	**4.726**	**2.119**	**1.697**	**1.258**	**2.588**
1391	b-cubebene	0.297	-	-	-	-
1441	trans-a-bergamotene	-	-	0.106	0.161	-
1459	(E)-b-farnesene	0.103	0.639	0.131	0.435	1.19
1463	humulene	0.216	-	-	-	-
1490	germacrene D	-	0.093	0.273	0.107	0.152
1522	g-muurolene	-	0.231	0.214	0.366	-
1594	**caryophyllene oxide**	**5.965**	**1.693**	**2.182**	**4.769**	**6.62**
1633	epicubenol	-	-	0.102	0.223	-
1668	**t-cadinol**	**0.324**	**0.727**	**0.939**	**1.904**	**1.621**
1721	muurol-5-en-4-one cis-14-nor	-	0.121	0.212	0.377	-
	Total	91.02	90.5	92.23	83.242	87.425

For the volatile oils obtained from the inflorescences of Vera cultivar, the major groups of compounds identified were monoterpenesters (14.22 - 32.41 %) and monoterpenols (33.8 - 52.26 %) for all experimental variants analysed. The highest abundance of esters was identified in plants watered with standard Hoagland nutrient solution (v2), and for alcohols the highest values were found in the control lot (M), represented by field-grown plants.

According to international quality standards, the compounds in the volatile oil samples analyzed for this cultivar do not fall within the values required by the Pharmacopoeia. Values exceeding the requirements were found for α-terpineol in plants watered with standard Hoagland solution (v2), for lavandulyl acetate in the experimental variant watered with Hoagland solution supplemented with P (v4), and eucalyptol was identified in increased concentration in all experimental variants except the one also watered with Hoagland nutrient solution with P addition (v4).

***Lavandula angustifolia* – Sevtopolis variety**

From the GC - MS results, more than 90 organic compounds were detected for the Sevtopolis variety, the majority of which are linalool (24 - 33.6%) and linalyl acetate (13 - 24%).

Other important compounds in the composition of the oil are lavandulyl acetate (2.6 - 4.3%), eucalyptol (1.5 - 4.9%), terpinen-4-ol (1.8 - 2.4%), α-terpineol (2.6 - 3.8%), borneol (1.3 - 3.9%), as shown in Table 5. 5.

Table 5. 5. Biochemical profile of volatile oil produced by lavender plants, *Lavandula angustifolia* - variety Sevtopolis, supplemented with different types of Hoagland nutrient solution

RI	Compound	Composition (%)				
		Variant M	Variant (v1)	Variant (v2)	Variant (v3)	Variant (v4)
782	2-butenal, 3-methyl	0.036	-	-	-	-
811	butylacetate	-	-	0.026	-	0.025
823	hexyl methyl ether	0.006	-	0.075	-	0.07
862	1-hexanol	0.022	-	-	-	-
893	5-hexanal, 4-methylene	0.009	-	-	-	-
906	propyl isobutyrate	-	-	-	-	0.021
929	a-thujene	0.038	-	0.023	0.022	0.007
937	a-pinene	0.173	-	0.109	0.093	0.184
949	butylisobutyrate	-	-	0.018	0.093	0.038
952	camphene	0.339	-	1.277	0.405	0.657
973	1,3,5-cycloheptatriene, 3,7,7-trimethyl	0.016	-	-	-	-
981	b-pinene	0.007	-	0.225	0.225	0.37
984	**3-octanone**	**1.095**	**1.508**	**1.784**	**1.665**	**1.912**
991	b-myrcene	0.766	-	0.79	0.679	0.759
1009	Dehydroxyhydro linalool oxide	0.317	-	-	-	-

RI	Compound	Composition (%)				
		Variant M	Variant (v1)	Variant (v2)	Variant (v3)	Variant (v4)
1011	hexyl acetate	0.552	0.824	0.77	0.874	0.996
1014	3-carene	0.032	-	0.097	-	-
1026	o-cymene	0.705	0.322	0.331	0.187	0.458
1031	D-limonene	0.592	0.602	0.871	0.442	0.74
1035	**eucalyptol**	**3.49**	**3.172**	**2.661**	**1.523**	**4.992**
1047	**trans-b-ocimene**	**1.461**	**0.686**	**3.249**	**1.383**	**0.752**
1060	g-terpinene	0.094	-	0.007	-	0.011
1069	sabinene hydrate	0.358	-	0.134	0.13	0.293
1074	cis-linalool oxide	2.646	0.59	0.263	0.688	0.669
1090	trans-linalool oxide	1.953	-	0.316	0.492	0.79
1104	**linalool**	**25.441**	**33.66**	**33.17**	**24.998**	**30.148**
1111	**1-octenyl-3-yl acetate**	**1.631**	**0.816**	**0.715**	**2.865**	**1.155**
1122	3-octanol acetate	-	-	0.373	0.514	0.4
1128	pinocarvone	-	-	-	0.458	-
1129	a-campholenal	0.035	-	-	-	-
1131	(Z)-b-ocimene epoxide	0.13	-	-	0.103	0.211
1133	n-butyl tiglate	-	-	0.023	-	0.127
1137	cis-p-mentha-2,8-dien-1-ol	-	-	0.023	-	-
1149	camphor	0.123	0.378	0.383	0.61	1.008
1156	nerol oxide	0.075	-	-	0.068	-
1186	p-cymen-8-ol	0.232	-	-	0.278	-
1162	myrtenol	-	-	-	0.816	-
1168	lavandulol	0.364	0.529	-	-	-
1170	**borneol**	**1.415**	**1.331**	**1.485**	**2.275**	**3.957**
1182	**terpinen-4-ol**	**2.406**	**2.237**	**1.978**	**1.833**	**2.192**
1189	cryptone	1.211	-	0.148	1.383	0.158
1190	n-hexyl butyrate	-	1.28	-	-	1.729
1191	butanoic acid, octyl ester	-	-	1.493	-	-
1195	**a-terpineol**	**3.198**	**3.85**	**3.253**	**3.08**	**2.656**
1199	butyl 2-methyl butanoate	-	-	-	0.244	-
1200	E-ocimenol	1.017	-	-	-	-
1207	propanal, 2-methyl butanoate	-	-	-	0.28	-
1208	2,6-dimethyl-3,5,7-octatriene-2-ol, E,E	-	-	0.022	0.326	0.215
1210	cis-piperitol	-	-	0.074	-	-
1221	cis-carveol	0.044	-	-	0.153	0.073

RI	Compound	Composition (%)				
		Variant M	Variant (v1)	Variant (v2)	Variant (v3)	Variant (v4)
1230	nerol	0.732	0.82	0.631	0.613	0.62
1232	bornyl formate	0.14	-	0.099	0.244	0.257
1236	hexyl 2-methyl butyrate	-	-	0.041	-	0.118
1243	cumaldehyde	0.376	-	0.407	-	0.531
1247	D-carvone	0.176	-	0.026	0.143	0.242
1257	**linalyl acetate**	**19.318**	**21.009**	**24.009**	**13.602**	**15.75**
1262	phenol,2,3,5,6-tetramethyl	-	-	-	0.252	-
1271	citral	0.061	-	-		-
1291	**lavandulyl acetate**	**3.783**	**4.334**	**2.671**	**3.59**	**4.021**
1300	o-cymen-5-ol	-	-	-	-	0.177
1301	carvacrol	-	-	-	0.797	
1330	hexyl tiglate	-	-	0.061	0.069	-
1345	hexyl caproate				0.494	-
1364	**neryl acetate**	**0.893**	**1.155**	**0.902**	**0.993**	**0.856**
1378	a-cedrene	-	-	-	0.219	-
1383	**geranyl acetate**	**1.746**	**2.218**	**1.688**	**1.739**	**1.644**
1384	isocaryophyllene	-	-	-	1.03	-
1385	hexyl hexanoate	-	-	0.102	-	0.163
1394	sesquithujene	-	-	0.012	-	-
1422	2-epi-a-funebrene	0.132	-	-	-	-
1426	a-santalene	0.273	-	0.267	-	0.344
1430	**trans-b-caryophyllene**	**0.457**	**-**	**1.891**	**-**	**0.84**
1441	trans-a-bergamotene	-	-	0.147	0.117	0.012
1459	E-b-farnesene	0.153	1.631	1.89	0.973	0.762
1490	germacrene D	-	-	0.376	0.173	0.214
1513	b-bisabolene	-	-	0.108	-	
1522	g-muurolene	0.342	-	0.257	0.348	0.268
1525	benzenemethanol, 4-methyl-a-(1-methyl-2-propenyl)	-	-	-	1.479	-
1530	cis-calamenene	0.061	-	0.04	0.082	-
1549	naphthalene, 1,2,3,4-tetrahydro-2, 5,8-trimethyl-	0.021	-	-	-	-
1558	cis-sequisabinene hydrate	-	-	-	-	0.02
1565	nerolidol	-	-	0.099	-	-
1570	humulene-1,2-epoxide	0.197	-	-	0.342	-

RI	Compound	Composition (%)				
		Variant M	Variant (v1)	Variant (v2)	Variant (v3)	Variant (v4)
1594	**caryophyllene oxide**	**7.435**	**-**	**1.359**	**8.447**	**5.262**
1633	epicubenol	-	-	0.096	0.376	-
1667	cis-muurola-4(14),5-diene	-	1.015	-	-	-
1668	**t-cadinol**	**1.332**	**-**	**0.982**	**2.108**	**1.045**
1702	a-calacorene	-	-	0.137	-	-
1721	muurol-5-ene-4-one	0.258	-	0.158	0.144	0.047
1740	muurola-4,10(14)-dien-1b-ol	-	-	0.023	-	-
1777	benzyl benzoate	0.045	-	-	0.261	-
1770	(4aS,8S,8aR)-8-isopropyl-5-methyl-3,4,4a,7,8,8a-hexahydronaphthalen-2-yl)methanol	0.034	-	-	-	0.032
	Total	89.994	83.967	94.645	91.184	87.82

For the volatile oils obtained from plants belonging to this lavender variety, the major groups of compounds identified were monoterpene esters (20.44 - 29.64 %) and alcohols (32.8 - 42.43 %) for all the experimental variants analyzed, followed by sesquiterpenes (1.63 - 9.59 %). The highest amount of esters was identified in plants watered with standard Hoagland nutrient solution (v2), and for monoterpenols the highest values were recorded in plants watered with H_2O (v1).

None of the oils analyzed were within the international standard quality requirements, the compounds in the samples analyzed for this cultivar that did not fall within the ranges required by the Pharmacopoeia were linalyl acetate, which was below the limit for all variants analyzed, α-terpineol and eucalyptol, which recorded amounts exceeding the standard requirements for most of the samples investigated, and lavandulol, which was not identified for any of the experimental variants watered with Hoagland nutrient solutions (v2, v3 and v4).

Discussions

Quantitative and qualitative analysis of the volatile oils obtained by hydrodistillation of dried inflorescences belonging to four varieties of Lavandula angustifolia watered with different types of Hoagland nutrient solution revealed the following: the concentration of the components of all varieties and experimental variants fluctuated intraspecifically and interspecifically, depending on the variety analysed and the cultivation conditions provided, practically confirming the statements in the literature in this regard (Tucker et al, 1984; Koç, 2000; Baranauskienė et al., 2003; Arabaci and Bayram, 2004; Biesiada et al., 2008; Camen et al., 2016; Chrysargyris et al., 2016, 2017; Silva et al., 2017; Skoufogianni et al., 2017).

Analysis of the 19 samples of volatile oil extracted from the variants tested showed a wide numerical range of identified components - between 22 and 59. In this respect we highlight the Sevtopolis variety, with the highest number of components of the extracted volatile oils in all the experimental variants - between 56

and 59 biochemical compounds - with the exception of plants belonging to variant v1 (watered with H_2O), in whose oil only 22 compounds were identified.

The major components detected in all the samples of volatile oils extracted from the inflorescences of the four *L. angustifolia* variants were linalool (12.1 - 40.7%), linalyl acetate (10.7 - 34.5%), camphor (0.12 - 1.39%) and eucalyptol (0.34 - 6.75%). The results obtained confirm data from similar studies, which reported linalool, linalyl acetate, eucalyptol, β-ocimene, terpinen-4-ol and camphor as major constituents of the volatile oil of the *L. angustifolia* species (Lis-Balchin, 2002a; Zamfirache et al, 2010; Robu et al., 2011a; Jianu et al., 2013; Saadatian et al., 2013; Tomescu et al., 2015; Aprotosoaie et al., 2017; Blažeković et al., 2018).

In the experiments carried out, none of the samples of volatile oil extracted from lavender varieties grown in variants treated with different types of Hoagland nutrient solutions met all the quality parameters required by international standards, with the greatest differences being observed in the concentration of linalool and linalyl acetate, compounds which varied in quantity both within the varieties tested and within some of the treatment variants applied.

Preliminary conclusions

The biochemical composition of the 19 samples of volatile oil extracted from the floral stems of the 4 varieties of *Lavandula angustifolia* Mill. grown in 4 experimental variants was found to be similar in terms of the number of main biochemical compounds identified, the amount of which varied considerably within both the varieties and the experimental variants.

The common major components of the lavender oils tested are: linalool, linalyl acetate, trans β - ocimene, D - limonene, terpinen-4-ol, lavandulyl acetate, β - caryophyllene, caryophyllene oxide, 1,8 - cineol (eucalyptol), camphor, borneol and α -terpineol.

None of the volatile oil samples extracted from the plant material in question, grown under the given cultivation conditions, fell entirely within the quality parameters required by the current edition of the European Pharmacopoeia (10. 0., 2019).

The results obtained from the experiments carried out complete the data presented in the literature on the composition of lavender volatile oils and are practically significant in the effort to evaluate these compounds extracted from the floral stems belonging to the four varieties of *Lavandula angustifolia* Mill. cultivated in our country. In this respect, the research aimed at cultivating these varieties in a controlled environment, experimentally supplemented with different types of Hoagland nutrient solutions, as a possible basis for establishing special techniques for supplying plants with nutrients that can support a quantitative and qualitative biosynthesis of volatile oils suitable for their efficient industrial processing for different purposes.

CHAPTER 6. EVALUATION OF THE EXPRESSION OF SOME GENES INVOLVED IN THE SYNTHESIS OF VOLATILE OILS IN VARIETIES OF THE *LAVANDULA ANGUSTIFOLIA* MILL. SPECIES

Research on terpene biosynthesis in species belonging to the genus Lavandula has led to the characterization of four monoterpene synthases (limonene, linalool, 1,8-cineole and β-panandrene) (Landmann et al, 2007; Demissie et al., 2011, 2012) and four sesquiterpene synthases (bergamotene, germacrene D, β-

caryophyllene and τ-cadinol) (Landmann et al., 2007; Demissie et al., 2011, 2012; Sarker et al., 2012, 2013; Jullien et al., 2014).

Plant material and RNA isolation

Plant tissue (represented by leaves and flowers) used in the analysis was harvested in June 2019 at 50% flowering stage from Codreanca, Provence Blue, Vera and Sevtopolis varieties belonging to the species *L. angustifolia* Mill. and all experimental variants (see section **Biological material and experimental design**) in RNA Save (Biological Industries, Beit Haemek Ltd, Israel) and stored at -80 °C for further isolation.

The SV Total RNA Isolation System kit (Promega Corporation, Madison, Wisconsin, USA) was used for RNA isolation.

Genes of interest

Volatile oils are important secondary metabolites produced in many plant species by two complex natural pathways (MVA and MEP) involving different enzymatic reactions.

Terpene synthesis (Figure 6. 1.) takes place in the mevalonate cycle (MVA), carried out by terpene synthases (TPS) and has as its initial product acetyl-coenzyme-A (acetyl CoA). In this cycle monoterpenes, sesquiterpenes, diterpenes, triterpenes and tetraterpenes are synthesized. In the first step of terpene biosynthesis, 3-hydroxy-3-methylglutaryl CoA is formed from three molecules of acetyl CoA. In the second step, catalyzed by hydroxymethyl-glutaryl CoA reductase, mevalonate is formed. By decarboxylation and phosphorylation, from mevalonate results isopentyl pyrophosphate (IPP), from which geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) are formed. These substances are precursors for the various terpenes. Thus, from geranyl pyrophosphate the monoterpenes linalool and limonene are formed; from farnesyl pyrophosphate the sesquiterpenes and squalene are formed (Chizzola, 2013; Lange and Ahkami, 2013; Aprotosoaie et al., 2017).

In the context of the above, the research that is the subject of this chapter of the PhD thesis aimed to characterize five genes involved in the synthesis of lavender volatile oil, namely 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and 1-deoxy-D-xylulose-5-phosphate synthase (DXS) that catalyze the first step of the MEP pathway for the production of volatile oil constituents: borneol dehydrogenase (BDH), an enzyme that generates camphor through the oxidation of borneol, limonene synthase (LIMS), directly responsible for limonene biosynthesis, and linalool synthase (LINS), an enzyme that coordinates the production of linalool, as genes involved in terpene biosynthesis in four different varieties of lavender, *Lavandula angustifolia* Mill. Provence Blue, Sevtopolis, Vera and Codreanca.

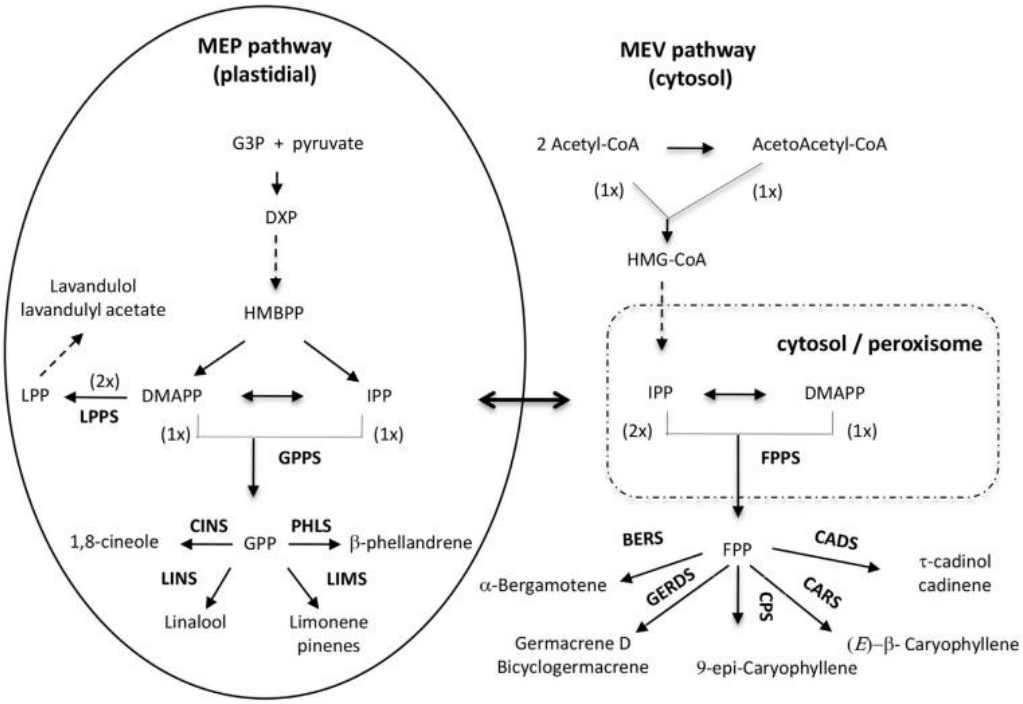

Figure 6. 1. Biosynthesis pathways of mono- and sesquiterpenes in *Lavandula angustifolia* Mill. BERS, α-bergamoten synthase; CADS, τ-cadinol synthase; CARS, β-caryophyllene synthase; CINS, 1,8-cineole synthase; CPS, 9-epi-caryophyllene synthase; DMAPP, dimethylallyl pyrophosphate; DXP, 1-deoxy-D-xylulază-5-phosphat; FPP, farnesyl pyrophosphate; FPPS, FPP synthase; GERDS, germacrene D synthase; G3P, glyceraldehydă-3-phosphat; GPP, geranyl pyrophosphate; GPPS, GPP synthase; HMBPP, 1-hydroxy-2-methyl-2 (E)-butenyl 4-pyrophosphat; IPP, isopentenyl pyrophosphate; LIMS, limonen synthase; LINS, linalool synthase; LPP, lavandulyl pyrophosphate; LPPS, LPP synthase; MEP, 2-C-methyl-D-erythritol-4-phosphate; MEV, mevalonate; (Despinasse et al., 2017)

Real-Time PCR (RT-qPCR) technique and analysis

The Real-Time Quantitative PCR technique is a variant of the PCR technique in which amplicon production can be visualized in real time using fluorochromes. Real-time Quantitative PCR allows accurate quantification of PCR products during the exponential phase of the PCR amplification process (Wang et al., 1989).

Quantitative quantification of RNA

After RNA isolation, quantitative and qualitative analysis was performed by spectrophotometric reading using the Nanodrop 2000 spectrophotometer (Nanodrop Technologies).

Standard preparation

Reverse-transcription reaction

To prepare the standards that were used for absolute quantification of the expression of the genes of interest, previously isolated total RNA was used from which cDNA was obtained by reverse transcription using the GoScript™ Reverse Transcription Mix + Oligo (dT) kit (Promega Corporation, Madison, Wisconsin, USA).

PCR amplification of genes of interest

The cDNA obtained from the reverse transcription reaction was used for PCR amplification of the genes of interest for standard preparation.

Amplicon validation by agarose gel electrophoresis

At the end of the PCR program, the products obtained were checked by agarose gel electrophoresis.

PCR product purification

The PCR products obtained were purified in columns using the Wizard SV Gel and PCR Clean-Up System kit (Promega Corporation, Madison, Wisconsin, USA).

Evaluation of gene expression by RT - qPCR method

Absolute quantification of the expression of the genes of interest was performed by RT- qPCR technique, using 6 standards and normalized dilutions of RNA. The absolute expression level of each gene was calculated for each experimental variant as an average concentration (copies/reaction) of 6 expressed values (three plant tissue samples/experimental variant and 2 RT-qPCR replicates).

Both reverse transcription and RT-qPCR amplification were performed in a single step using the GoTaq®1-Step RT-qPCR kit (Promega Corporation, Madison, Wisconsin, USA). The HRM Rotor-Gene 6000 Analysis System (Corbett Life Science, California, USA) was used for amplification and analysis.

Data interpretation and analysis

Results were read and interpreted as absolute values of target gene expression (expressed as number of copies) using Rotor-Gene Q-Pure v2.2.3 detection and analysis software (Qiagen, Redwood City, CA, USA).

The experimental data obtained were centralized in tables using Microsoft Office Excel 2019 (Microsoft Corporation, USA) and statistically analyzed by applying a one-way analysis of variance (ANOVA) followed by Tukey post-hoc test for detailed analysis.

Box plots were generated using R software version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria) and the R analysis package - ggplot2. All data were processed using custom R scripts (Kassambara, 2013; Wickham, 2016).

Results

The present research aimed to investigate if the administration of different Hoagland nutrient solution treatments to the varieties Codreanca, Provence Blue, Vera and Sevtopolis of *Lavandula angustifolia* Mill., grown under controlled conditions, alters the expression of genes (LIMS, LINS, BDH, DXS, HMGR) involved in terpene biosynthesis in the tissues analyzed (flower and leaf).

Quantification and normalization of RNA samples

Following the sample processing of flowers and leaves to obtain purified RNA solutions, concentration values were found to range from 10.9 - 139.0 ng/µl for flowers and 19-181.8 ng/µl for leaves.

Since the samples showed variable concentrations, the lowest value being 9.8ng/µl, all samples were normalized to the value of 9ng/µl to eliminate errors in the evaluation of gene expression caused by fluctuations in the concentration of RNA introduced in the amplification reactions..

PCR product analysis following amplification

PCR amplification of the cDNA sample obtained from the reverse transcription reaction using the primer pairs described in Table 6.1. was performed to produce the standards and the amplification products were validated by electrophoresis.

Table 6. 1. Molecular markers used to determine gene expression

Primer	Primer sequence	Fragment length (bp)	Literature
LINS	F-5' -ACACGCACGACAATTTGCCA-3'	124	(Lane et al., 2010)
	R-5' -AGCCCTCCAATGAAGTGGGAT-3'		
DXS	F-5'-CCAACTCCGTGAAGCAGCAAA-3'	102	(Lane et al., 2010)
	R-5'-TTGCCCGCGAATCCTTTCAGA-3'		
LIM	F: 5'-GCGCCACACAACTAGAAATTAAGT-3'	152	(Guitton et al., 2010)
	R: 5'-TTGCACAGTCAGCTCAGCG-3'		
BDH	F-5' -AATCGGAGCGGCAGCATAATCT-3'	167	(Sarker et al., 2012)
	R-5' -TAATACGGCGAGACGCAGTTCA-3'		
HMGR	F-5'-TTAACGCCGAGTTCCCAGACA-3'	104	(Lane et al., 2010)
	R-5'-TGATTTGCCACGGCCTTCGAT-3'		
β - ACT	F-5'-TGTGGATTGCCAAGGCAGAGT-3'	118	(Sarker et al., 2012)
	R-5'-AATGAGCAGGCAGCAACAGCA-3'		

Discussions

Numerous recent studies have focused on the biosynthesis and biological activities of lavender volatile oil (Landmann et al., 2007; Zuzarte et al., 2009; Lane et al., 2010; Demissie et al., 2011, 2012; Woronuk et al., 2011; Sarker et al., 2012; Lesage-Meessen et al., 2015; Wells et al., 2020).

The terpene biosynthesis pathway in volatile oils is derived from two 5-carbon compounds, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) generated by both the 2-C-methyl-D-erythritol-4-phosphate (MEP) and mevalonate (MVA) pathways (Li et al., 2021).

Following a literature review, five genes active in the volatile oil biosynthesis pathway were selected, two of them precursors of the MVA (HMGR gene in cytosol) and MEP (DXS gene in plastids) biosynthesis pathways and three genes involved in monoterpene biosynthesis, namely genes responsible for the biosynthesis of linalool (LINS), limonene (LIMS) and camphor (BDH), essential compounds of lavender volatile oil (Guitton et al, 2010; Lane et al., 2010; Sarker et al., 2012).

HMGR gene (3-hydroxy-3-methylglutaryl-CoA reductase)

The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), is a well-known key enzyme responsible for terpene biosynthesis in plants. It catalyzes the first step of isoprenoid biosynthesis in the MVA pathway (Diarra et al., 2013) in the cytosol.

It has also long been assumed that terpene biosynthesis via the MEP and MVA pathways are independent. However, recent studies suggest that metabolite exchanges exist between the two pathways (Bick and Lange, 2003; Hemmerlin et al., 2003; Laule et al., 2003; Schuhr et al., 2003; Dudareva et al., 2005; Lane et al., 2010; Mendoza-Poudereux et al., 2015).

The results obtained from the practical research show that the highest gene expression values for the HMGR gene (Figure 6. 2.) were observed in the flowers of Sevtopolis plants watered with Hoagland nutrient

solution with the addition of K (v3) (3.43E+06), followed by the control lot (M), belonging to the Vera variety (3. 13E+06); the lowest expression values of this gene were observed in plants belonging to variant v2 of the same Vera variety, watered with standard Hoagland nutrient solution (8.53E+04), while at the same time improvements in the expression of this gene were recorded in most plants belonging to variants v3 and v4, watered with Hoagland nutrient solution with the addition of K and P respectively.

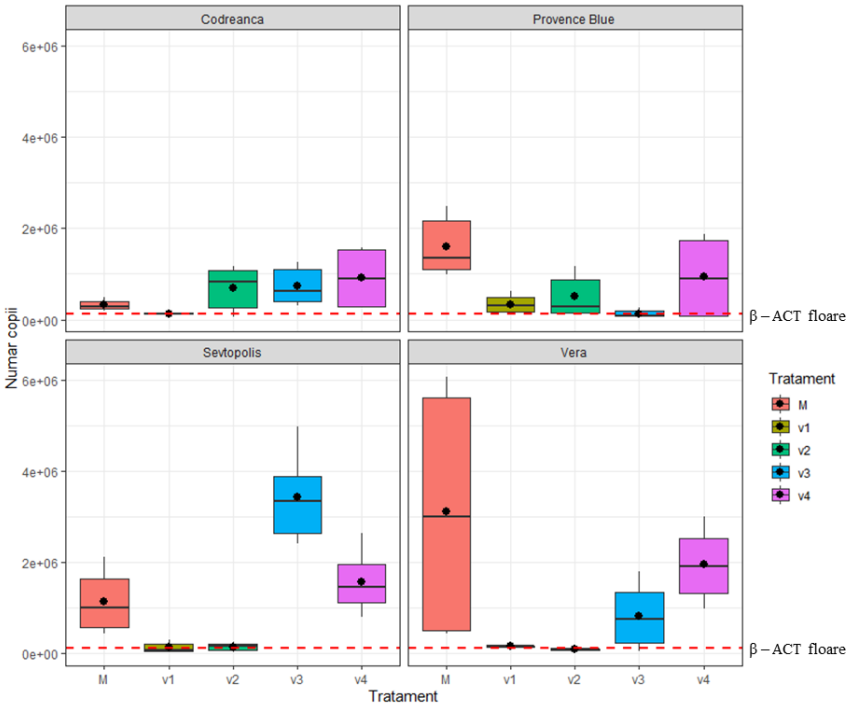

Figure 6. 2. Boxplot diagram of HMGR gene expression in flowers of all experimental varieties and variants of lavender, *Lavandula angustifolia* Mill., reported to the reference gene β-actin (M - control; v1 - plants watered with H_2O; v2 - plants watered with standard Hoagland nutrient solution; v3 - plants watered with Hoagland nutrient solution with K addition; v4 - plants watered with Hoagland nutrient solution with P addition). Values are means ± S.E.M. (n=6) For Tukey posthoc test: **M** - Vera vs Codreanca: p = 0.0225 (* p<0.05); **v1** – Provence Blue vs Codreanca: p = 0.0317 (* p<0.05); Sevtopolis vs Provence Blue: p = 0.0340 (* p<0.05); **v2** – Vera vs Codreanca: p = 0.0365 (* p<0.05); **v3** – Sevtopolis vs Codreanca: p = 0.0000033 (*** p<0.001); Sevtopolis vs Provence Blue: p = 0.0000002 (*** p<0.001); Vera vs Sevtopolis; p = 0.0000056 (*** p<0.001); **v4** – F = 1.597; p = 0.235 (p >0.05)

In the leaves, the highest number of RNA copies was observed for the HMGR gene (Figure 6. 3.) in plants watered with Hoagland nutrient solution supplemented with K (v3) of the Sevtopolis variety (1.92E+06), followed by plants watered with Hoagland nutrient solution supplemented with P (v4) of the Provence Blue variety (1. 63E+06), and the lowest gene expression values for this gene were recorded in plants watered with standard Hoagland nutrient solution (v2) of the variety Vera (1.17E+05).

Comparing the results obtained for all varieties and experimental variants in relation to the expression for this gene, an increase in values was observed in leaves of plants supplemented with the nutrient solution with added K and P (v3 and v4, respectively).

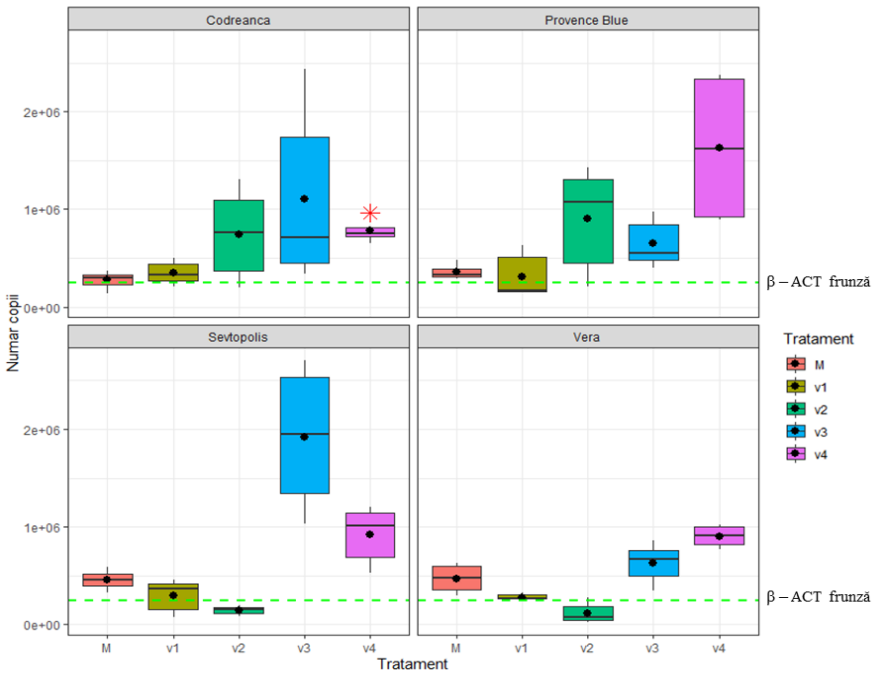

Figure 6. 3. Boxplot diagram of HMGR gene expression in leaves of all experimental varieties and variants of lavender, *Lavandula angustifolia* Mill., reported to the reference gene β-actin (M - control; v1 - plants watered with H_2O; v2 - plants watered with standard Hoagland nutrient solution; v3 - plants watered with Hoagland nutrient solution with K addition; v4 - plants watered with Hoagland nutrient solution with P addition). Values are means ± S.E.M. (n=6) For Tukey posthoc test: **M** – Sevtopolis vs Codreanca: p = 0.0296 (* p<0.05); Vera vs Codreanca: p = 0.0393 (* p<0.05); **v1** – F = 0.193; p = 0.9 (p >0.05); **v2** - Sevtopolis vs Codreanca: p = 0.0398 (* p<0.05); Vera vs Codreanca: p = 0.0307 (* p<0.05); Sevtopolis vs Provence Blue: p = 0.0078 (** p<0.01); Vera vs Provence Blue: p = 0.0059 (** p<0.01); **v3** - Sevtopolis vs Provence Blue: p = 0.0079 (** p<0.01); Vera vs Sevtopolis: p = 0.0070 (** p<0.01); **v4** – F = 3.284; p = 0.0525 (p >0.05)

DXS gene (1-deoxy-D-xylulose-5-phosphate synthase)

Volatile oil components are synthesized by plants from the universal isoprenoid precursor isopentenyl pyrophosphate (IPP) and its double bond isomer dimethylallyl pyrophosphate (DMAPP) via the mevalonate (MVA) and 2-C-methyl-D-erythritol-4-phosphate (MEP) pathways (Hemmerlin et al, 2003; Dudareva et al., 2005; Guitton et al., 2010; Lane et al., 2010; Chizzola, 2013; Mendoza-Poudereux et al., 2015; Li et al., 2021).

At the flower level (Figure 6. 4.), no significant changes in gene expression were observed for the DXS gene in the statistical analysis, but the highest values were observed for the H_2O (v1) watered plants of the Vera variety (7. 39E+05), followed by plants watered with Hoagland nutrient solution with addition of K (v3) of the variety Sevtopolis (6.42E+05), and the lowest number of copies was observed in plants watered with standard Hoagland nutrient solution (v2) of the variety Vera (1.07E+05).

No significant influence of plant treatments to increase gene expression levels was observed for the DXS gene at the level analyzed.

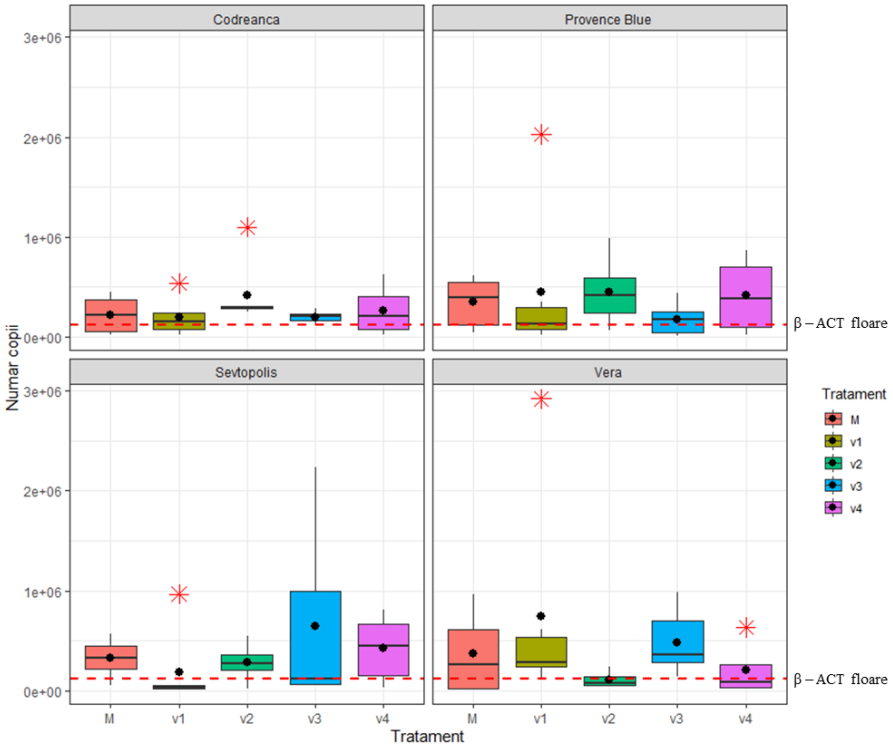

Figure 6. 4. Boxplot diagram of DXS gene expression in flowers of all experimental varieties and variants of lavender, *Lavandula angustifolia* Mill., reported to the reference gene β-actin (M - control; v1 - plants watered with H_2O; v2 - plants watered with standard Hoagland nutrient solution; v3 - plants watered with nutrient solution with K addition; v4 - plants watered with nutrient solution with P addition). Values are means ± S.E.M. (n=6). For ANOVA test: **M** – F = 0.331; p = 0.803 (p >0.05); **v1** – F = 0.832; p = 0.492 (p >0.05); **v2** – F = 2.329; p = 0.105 (p >0.05); **v3** – F = 1.235; p = 0.323 (p >0.05); **v4** – F = 0.477; p = 0.703 (p >0.05)

In the leaves, however, significant changes were observed for the DXS gene (Figure 6. 5.) when irrigated with Hoagland nutrient solution with the addition of P (v4) and K (v3), respectively.

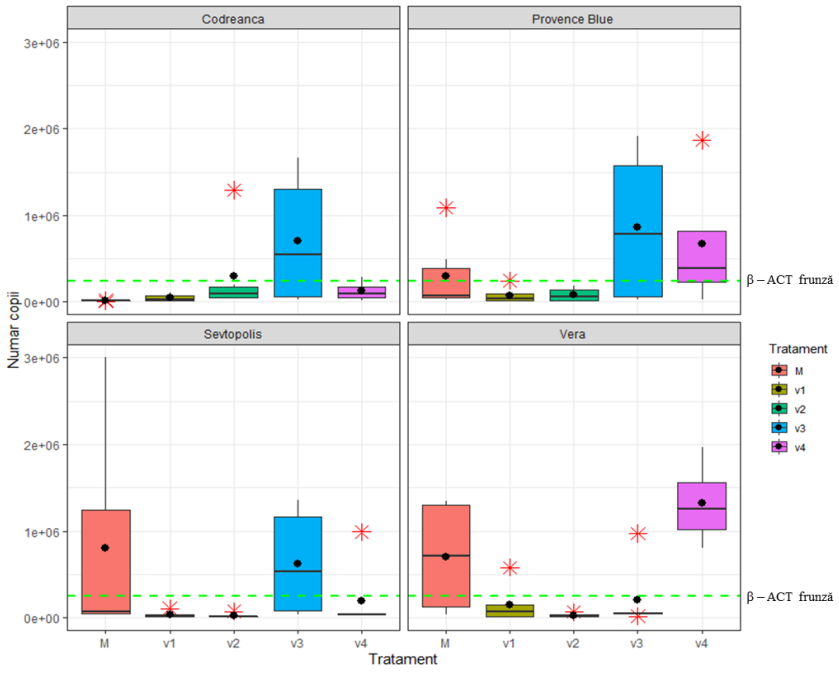

Figure 6. 5. Boxplot diagram of DXS gene expression in leaves of all experimental varieties and variants of lavender, *Lavandula angustifolia* Mill., reported to the reference gene β-actin (M - control; v1 - plants watered with H_2O; v2 - plants watered with standard Hoagland nutrient solution; v3 - plants watered with nutrient solution with added K; v4 - plants watered with nutrient solution with added P). Values are means ± S.E.M. (n=6) For Tukey posthoc test: **M** – F = 1.356; p = 0.288 (p >0.05); **v1** – F = 1.065; p = 0.386 (p >0.05); **v2** – F = 1.582; p = 0.225 (p >0.05); **v3** – F = 1.034; p = 0.399 (p >0.05); **v4** – Vera vs Codreanca: p = 0.0219 (* p<0.05); Vera vs Sevtopolis: p = 0.0179 (* p<0.05)

The DXS gene showed lower expression compared to the HMGR gene in all variants and experimental variants, but the determined values were within the range presented by similar studies (Landmann et al., 2007; Lane et al., 2010; Segura et al., 2019).

LIMS gene (limonene synthase)

Numerous monoterpene synthases in the leaves and flowers of *L. angustifolia*, such as linalool, limonene and bergamot synthase (Landmann et al., 2007), have been functionally characterized until now, of which limonene synthase (LIMS) catalyzes the conversion of geranyl pyrophosphate to limonene, a minor constituent of lavender oil (Muñoz-Bertomeu et al., 2008; Tsuro and Asada, 2014).

Following the analysis of the results obtained practically for the gene involved in limonene synthesis (LIMS), the highest gene expression values were observed in flowers (Figure 6. 6.) in plants watered with H_2O (v1 - 6.47E+04) and in those watered with standard Hoagland nutrient solution (v2 - 3.14E+04) belonging to the Codreanca variety.

For the rest of the varieties, also at the flower level, the highest expression values were found in plants watered with H2O (v1 - 2.39E+04 in the Provence Blue variety, 7.33E+03 in the Vera variety and 1.70E+04 in the Sevtopolis variety).

Apart from the Codreanca variety, watering with standard Hoagland nutrient solutions as well as with the addition of K and P respectively (variants v2, v3 and v4) did not bring improvements in LIMS gene expression in flowers.

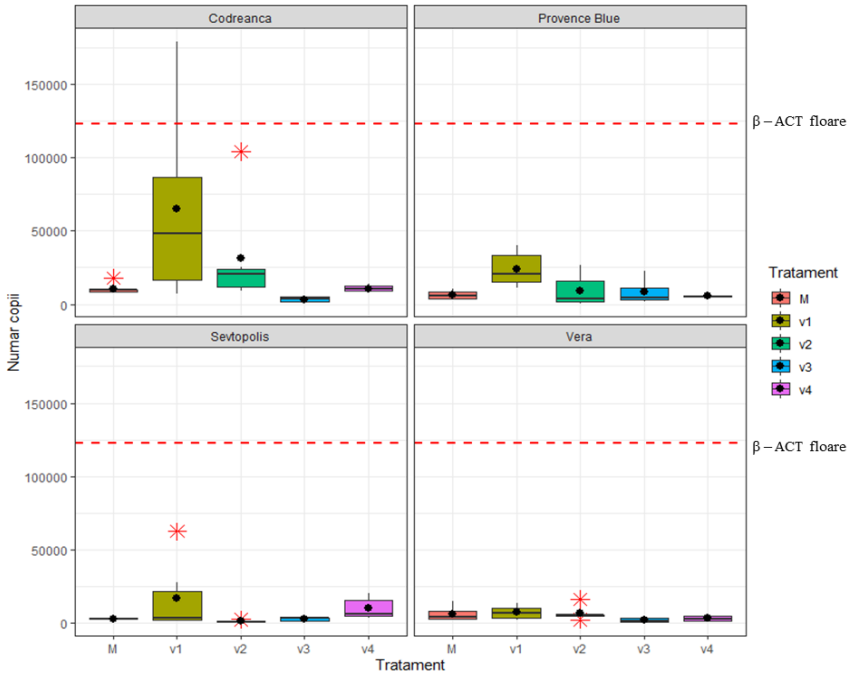

Figure 6. 6. Boxplot diagram of LIMS gene expression in flowers of all varieties and experimental variants of lavender, *Lavandula angustifolia* Mill., reported to the reference gene β-actin (M - control; v1 - plants watered with H_2O; v2 - plants watered with standard Hoagland nutrient solution; v3 - plants watered with nutrient solution with added K; v4 - plants watered with nutrient solution with added P). Values are means ± S.E.M. (n=6) For Tukey posthoc test: **M** – Sevtopolis vs Codreanca: p = 0.0123 (* p<0.05); **v1** – F = 3.03; p = 0.0533 (p >0.05); **v2** - F = 2.894; p = 0.0607 (p >0.05); **v3** - F = 2.412; p = 0.0969 (p >0.05); **v4** – F = 2.388; p = 0.113 (p >0.05)

For leaves (Figure 6. 7.), the results obtained from LIMS gene expression analysis showed the highest expression values in plants watered with standard Hoagland nutrient solution (v2 - 1.58E+04), followed by field-grown control plants (M - 8.78E+03) of the Provence Blue variety, and the lowest expression values of this gene were observed in plants watered with H_2O (v1 - 3.06E+02) of the Codreanca variety.

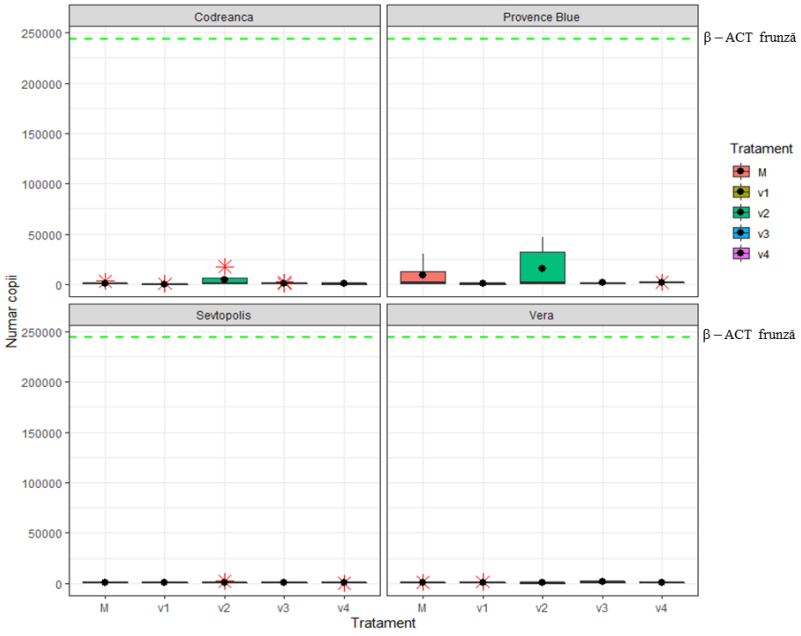

Figure 6. 7. Boxplot diagram of LIMS gene expression in leaves of all experimental varieties and variants of lavender, *Lavandula angustifolia* Mill., reported to the reference gene β-actin (M - control; v1 - plants watered with H_2O; v2 - plants watered with standard Hoagland nutrient solution; v3 - plants watered with nutrient solution with added K; v4 - plants watered with nutrient solution with added P). Values are means ± S.E.M. (n=6). For Tukey posthoc test: **M** – F = 2.226; p = 0.12 (p >0.05); **v1** – F = 1.689; p = 0.201 (p >0.05); **v2** - F = 2.166; p = 0.124 (p >0.05); **v3** - F = 2.97; p = 0.0565 (p >0.05); **v4** – Sevtopolis vs Provence Blue: p = 0.0406 (* p<0.05)

Comparative analysis of LIMS gene expression in the four varieties showed lower expression of the gene in the Vera and Sevtopolis varieties compared to Codreanca and Provence Blue, both in flowers and leaves. Gene overexpression was also observed in flowers compared to leaves.

Compared to the reference gene, the LIMS gene was found to be underexpressed in both tissues analysed, in all varieties and for all treatment variants.

The results obtained in the present research are consistent with the results reported in similar research carried out on different species of the genus *Lavandula* (Landmann et al., 2007; Muñoz-Bertomeu et al., 2008; Tsuro and Asada, 2014).

LINS gene (linalool synthase)

Linalool is synthesized in plants, like other monoterpenes, by the enzyme linalool synthase (LINS) from geranyl pyrophosphate (GPP), a compound generated by the condensation of IPP and DMAPP by geranyl pyrophosphate (GPP) synthase (Segura et al., 2019).

Following analysis of the data obtained for the LINS gene, higher gene expression values were observed in flowers (Figure 6. 8.) compared to leaves (Figure 6. 9.) for all experimental variants and varieties of *Lavandula angustifolia* Mill.

The highest values of LINS gene expression in flowers were observed in plants watered with Hoagland nutrient solution with the addition of K (v3 - 4.31E+04) of the Sevtopolis variety, which showed at least 3 times the number of copies, compared to the rest of the samples analyzed, in which the values recorded ranged from 6. 07E+02 to 1.44E+04. At the same time, the lowest expression values of this gene were recorded in H_2O-watered plants (v1 - 6.07E+02), grown under controlled conditions, of the Codreanca variety.

Increases in gene expression following irrigation with Hoagland nutrient solution compared to field-grown control (M) plants were observed in the variety Codreanca in plants watered with standard Hoagland nutrient solution (v2), in the Vera variety in the plants watered with Hoagland nutrient solution with added K and respectively P (v3 and v4), and in the Sevtopolis variety in the plants watered with Hoagland nutrient solution with double the amount of K (v3).

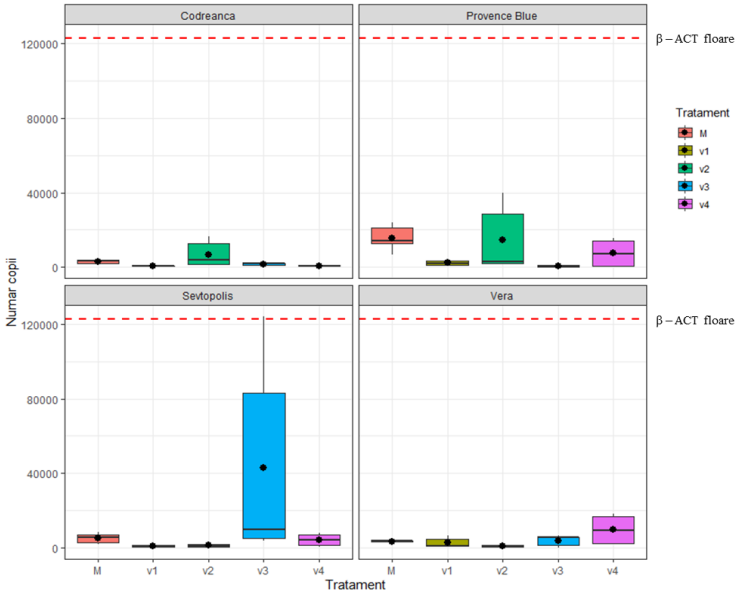

Figure 6. 8. Boxplot diagram of LINS gene expression in flowers of all varieties and experimental variants of lavender, *Lavandula angustifolia* Mill., reported to the reference gene β-actin (M - control; v1 - plants watered with H_2O; v2 - plants watered with standard Hoagland nutrient solution; v3 - plants watered with nutrient solution with added K; v4 - plants watered with nutrient solution with added P). Values are means ± S.E.M (n=6). For Tukey posthoc test: **M** - Provence Blue vs Codreanca: p = 0.000105 (*** p<0.001); Sevtopolis vs Provence Blue: p = 0.000826 (*** p<0.001); Vera vs Provence Blue: p = 0.000576 (*** p<0.001); **v1** – F = 2.466; p = 0.0918 (p >0.05); **v2** – F = 2.405; p = 0.0975 (p >0.05); **v3** – F = 3.185; p = 0.0461 (* p<0.05); **v4** – F = 1.82; p = 0.19 (p >0.05)

At the leaf level (Figure 6. 9.), following RT - qPCR amplification of the gene of interest, overexpression of the gene involved in linalool synthesis (LINS) was found in all varieties in plants watered

with Hoagland nutrient solution with the addition of K and P respectively (v3 and v4) compared to control plants (M).

The highest values were observed in plants watered with Hoagland nutrient solution with the addition of K (v3 - 3.06E+03) of the Provence Blue variety and the lowest in plants watered with standard Hoagland nutrient solution (v2 - 3.20E+02) of the Sevtopolis variety.

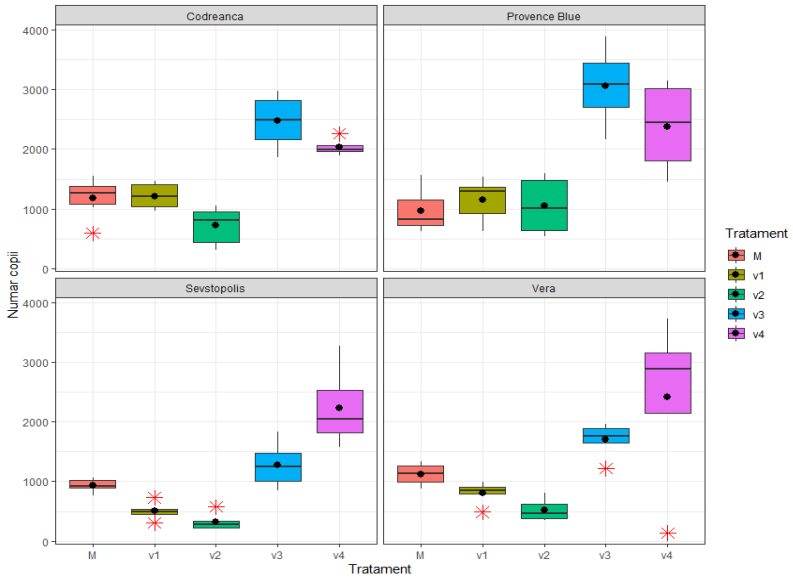

Figure 6. 9. Boxplot diagram of LINS gene expression at leaf level in all varieties and experimental variants of lavender, *Lavandula angustifolia* Mill.: (M - control; v1 - plants watered with H_2O; v2 - plants watered with standard Hoagland nutrient solution; v3 - plants watered with nutrient solution with added K; v4 - plants watered with nutrient solution with added P). Values are means ± S.E.M (n=6). For Tukey posthoc test: **M** - F = 1.067; p = 0.388 (p >0.05); **v1** – Sevtopolis vs Codreanca: p = 0.000252 (*** p<0.001); Vera vs Codreanca; p = 0.0384 (* p<0.05); Sevtopolis vs Provence Blue: p = 0.000672 (*** p<0.001); **v2** – Sevtopolis vs Provence Blue: p = 0.0028 (** p<0.01); Vera vs Provence Blue: p = 0.0354 (* p<0.05); **v3** – Sevtopolis vs Codreanca: p = 0.00090 (*** p<0.001); Vera vs Codreanca; p = 0.0364 (* p<0.05); Sevtopolis vs Provence Blue: p = 0.0000062 (*** p<0.001); Vera vs Provence Blue: p = 0.000224 (*** p<0.001); **v4** – F = 0.146; p = 0.931 (p >0.05)

Compared to the reference gene, the LINS gene was underexpressed in both flowers and leaves in all varieties and experimental variants.

BDH gene (borneol dehydrogenase)

As with other terpene synthases in these plants, BDH gene expression levels are higher in flowers compared to leaves for all experimental variants and varieties of *Lavandula angustifolia* species investigated.

In flowers (Figure 6. 10.), the highest expression values of this gene were determined in plants watered with Hoagland nutrient solution with additional P (v4 - 2.94E+03) of the Codreanca variety, followed by plants watered with Hoagland nutrient solution with additional K (v3 - 2. 29E+03) of the Provence Blue variety, and the lowest values were obtained in plants watered with standard Hoalgand nutrient solution (v2 - 2.62E+02) of the Sevtopolis variety. It was also observed that for Provence Blue and Vera varieties the lowest values in the experimental variants were also recorded in plants watered with standard Hoagland nutrient solution (v2).

Following the administration of Hoagland nutrient solutions, increases in gene expression for the BDH gene were recorded for most varieties in plants watered with nutrient solution with the addition of K and respectively P (v3 and v4).

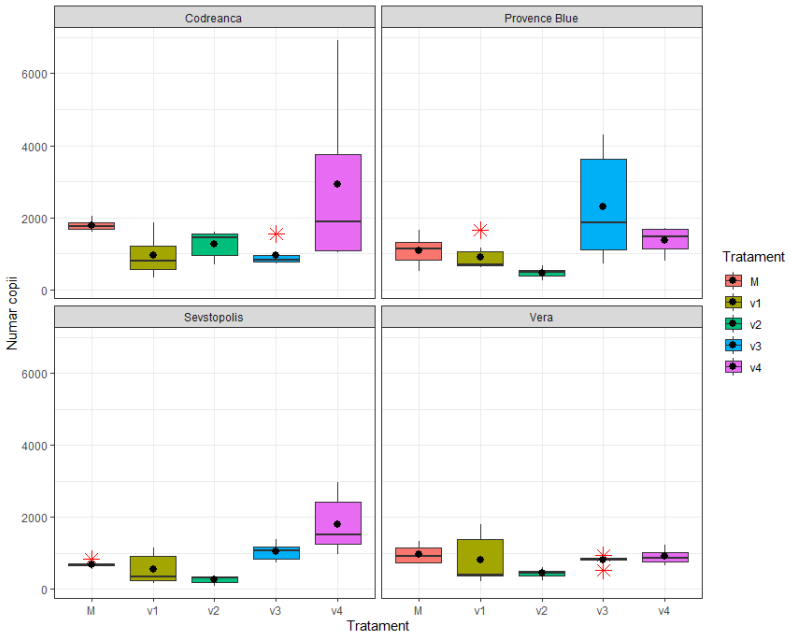

Figure 6. 10. Boxplot diagram of BDH gene expression in flowers of all varieties and experimental variants of lavender, *Lavandula angustifolia* Mill.: (M - control; v1 - plants watered with H_2O; v2 - plants watered with standard Hoagland nutrient solution; v3 - plants watered with nutrient solution with added K; v4 - plants watered with nutrient solution with added P). Values are means ± S.E.M (n=6). For Tukey posthoc test: **M** – Provence Blue vs Codreanca: p = 0.00146 (** p<0.01); Sevtopolis vs Codreanca: p = 0.0000071 (*** p<0.001); Vera vs Codreanca: p = 0.00101 (** p<0.01); **v1** – F = 0.6; p = 0.622 (p >0.05); **v2** – Provence Blue vs Codreanca: p = 0.0000502 (*** p<0.001); Sevtopolis vs Codreanca: p = 0.0000022 (*** p<0.001); Vera vs Codreanca: p = 0.0000332 (*** p<0.001); **v3** – Provence Blue vs Codreanca: p = 0.0434 (* p<0.05); Vera vs Provence Blue: p = 0.0231 (* p<0.05); **v4** – F = 1.569; p = 0.241 (p >0.05)

In leaves (Figure 6. 11.) the highest expression values were observed in plants watered with standard Hoagland nutrient solution (v2 - 5.13E+02) belonging to the Codreanca variety, and the lowest values were observed in control plants (M - 7.70E+01) of the Sevtopolis variety.

In the case of Provence Blue, Vera and Sevtopolis varieties, low levels of BDH gene expression were recorded in field-grown control plants (M), while for the variety Codreanca, the lowest values were observed in plants watered with H_2O (v1).

Also, following treatments with standard Hoagland nutrient solution and with the addition of K and respectively P (v2, v3 and v4), an increase in BDH gene expression was observed in all varieties tested compared to the control (M).

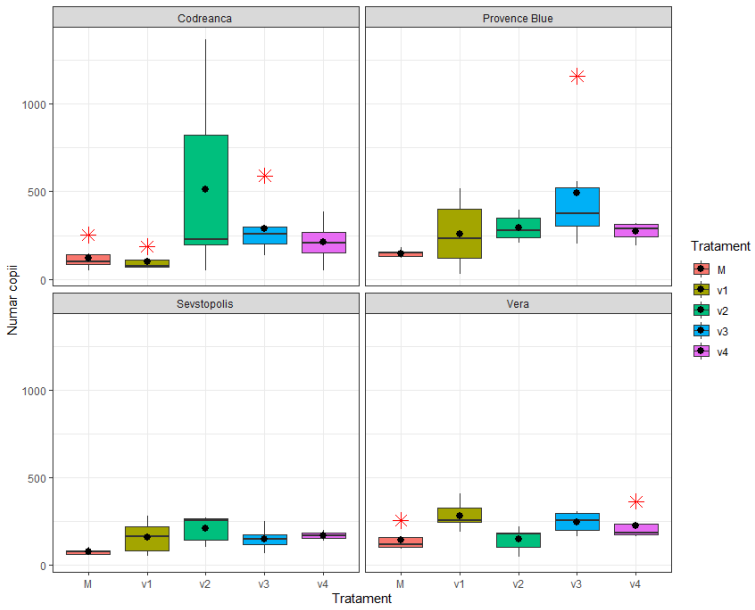

Figure 6. 11. Boxplot diagram of BDH gene expression in leaves of all varieties and experimental variants of lavender, *Lavandula angustifolia* Mill.: (M - control; v1 - plants watered with H_2O; v2 - plants watered with standard Hoagland nutrient solution; v3 - plants watered with nutrient solution with added K; v4 - plants watered with nutrient solution with added P). Values are means ± S.E.M. (n=6) For Tukey posthoc test: **M** – F = 2.336; p = 0.108 (p >0.05); **v1** – F = 3.228; p = 0.0443 (* p<0.05); **v2** – F = 1.935; p = 0.156 (p >0.05); **v3** – Sevtopolis vs Provence Blue: p = 0.0319 (* p<0.05); **v4** – F = 1.205; p = 0.344 (p >0.05)

Compared to the reference gene, the BDH gene is found to be underexpressed in all tissues and for all treatment options.

In flowers, linalool synthase (LINS) and limonene synthase (LIMS) were expressed at higher levels, while expression of the borneol dehydrogenase (BDH) gene was reduced. All genes tested were poorly expressed in leaves, suggesting that these tissues have a minor contribution to the biochemical composition of volatile oil, data supported by other similar research (Landmann et al., 2007; Ashour et al., 2010; Chen et al., 2011; Sarker et al., 2012).

Preliminary conclusions

All five tested genes involved in terpene biosynthesis were expressed in both types of plant tissue analysed (flower and leaf).

For the HMGR gene, involved in the cytosolic MVA synthesis pathway, increases in leaf expression were observed following the watering of plants with Hoagland nutrient solutions with the addition of K and P (v3 and v4) in all varieties studied.

In the case of the DXS gene, the first enzyme involved in the MEP pathway in plastids, an increase in the expression in leaves was found in plants watered with Hoagland nutrient solution with the addition of K (v3) of the varieties Codreanca and Provence Blue and in plants supplemented with Hoagland nutrient solution with the addition of P (v4) of the varieties Provence Blue and Vera.

The LIMS gene, involved in the limonene biosynthesis pathway, was strongly expressed in flowers of H_2O-watered plants (v1) for all varieties studied.

For the gene involved in lianlool synthesis (LINS) it was found that application of Hoalgand nutrient solution with addition of K (v3) and P (v4) positively influenced gene expression in leaves for all varieties of *Lavandula angustifolia* species analyzed.

The BDH gene, which upon borneol oxygenation synthesizes camphor, had low expression in both tissue types, but the lowest values were recorded in leaves.

CHAPTER 7. GENERAL DISCUSSION

For species of the genus Lavandula, flowers and leaves are the key sources of terpene biosynthesis, among which monoterpenes are the major compounds, together with sesquiterpenes, which are synthesized in much smaller amounts, depending on the developmental stages of the plants (Boeckelmann, 2008).

The research covered by this thesis carried out on biological material represented by four varieties of *Lavandula angustifolia* Mill. cultivated in a protected area (glasshouse) and in the open air, aimed at:

(1) to determine whether the cultivation regime of the test plants (specific growing conditions, watering regime with Hoagland nutrient solutions of different compositions) obviously influences their volatile oil production, expressed by specific quantitative and qualitative parameters and (2) to highlight the existence of a possible correlation between the biochemical composition of volatile oil and the expression of certain genes involved in terpene biosynthesis.

The volatile oils in lavender are produced by specialized glandular secretory hairs covering the leaves, stems and are found abundantly on the surface of the flower calyx. Depending on the number of cells of which the secretory gland is composed, glandular secretory hairs identified in lavender taxa belong to two types: capitate and peltate (Huang et al., 2008; Zuzarte et al., 2010; Stefan et al., 2021).

Analysis of micrographs of vegetative organs (leaves) obtained by scanning electron microscopy (SEM) revealed an increase in the density of secretory hairs in plants watered with Hoagland nutrient solution with the addition of K and P (v3 and v4) for all *Lavandula angustifolia* varieties studied.

According to existing data in the literature, the quality of the volatile oil can be correlated with the proportion of its constituents, which varies according to the variety, the treatment applied to the plants and the environmental conditions in which they were grown (Angioni et al. 2006; Woronuk et al. 2011; Dudareva et al. 2013; Carvalho et al. 2016). In this context, analyzing the specific conditions of Vânători locality in Vrancea county, the growing area of the test plants analyzed in this paper, we perceive that this is a rather new region for lavender cultivation, characterized by a temperate climate with hot, dry summers and cold winters. In view of these environmental conditions, we aimed to identify through the research carried out the possible presence of individual characteristics of the volatile oils produced by the four lavender varieties tested in the treatment variants under consideration. In this respect, their biochemical analysis revealed that the percentage of some essential volatile oil compounds (linalool, linalyl acetate, lavandulol) in the plants grown in the locality of Vânători differs slightly from the optimal concentration range established by ISO (*L. angustifolia* ISO 3515) and the European Pharmacopoeia.

In the investigations, linalool and linalyl acetate, the two major compounds detected in all samples of volatile oils extracted from the inflorescences of the four *L. angustifolia* varieties analyzed, showed concentrations ranging from 12.1 - 40.7% and 10.7 - 34.5% respectively. These were followed by minor

compounds such as α-terpineol (1.1 - 5.3%), terpinen-4-ol (0.5 - 6%), β-ocimene (0.2 - 6%), camphor (0.1 - 1, 4%), borneol (0.8 - 5.1%), eucalyptol (0.3 - 6.7%), lavandulyl acetate (2.3 - 8.1%), limonene (0 - 1.5%) and geranyl acetate (0.2 - 3.1%).

Terpene production and, as a consequence, the composition of volatile oils is also affected at the transcriptional level of genes involved in terpene biosynthesis and vary during different stages of plant development (Lane et al., 2010). To study the effect of gene expression on lavender oil composition, the expression of two genes that initiate synthesis pathways was explored: MVA (3-Hydroxy-3-methylglutaryl-CoA reductase - HMGR) and MEP (1-deoxy-D-xylulose-5-phosphate synthase - DXS) from cytosol and plastids, respectively, and three genes involved in monoterpene biosynthesis in different lavender tissues, in particular the gene expression of lilanool synthase (LINS), limonene synthase (LIMS) and borneol dehydrogenase (BDH) in leaves and inflorescences was monitored using quantitative real-time PCR.

The HMGR gene, was the most highly expressed gene, with high values in both flowers and leaves, followed in tissue abundance by the DXS gene, which showed higher values in flowers compared to leaves.

The practical results obtained during the present thesis showed that for the three tested genes involved in monoterpene biosynthesis (LINS - lilanool synthase, LIMS - limonene synthase and BDH - borneol dehydrogenase), linalool synthase and limonene synthase were expressed in the plant material tested (selected lavender variants, in the applied treatment variants) at high levels especially in the flowers, while the expression of the borneol dehydrogenase gene was reduced.

At the same time, the experiments carried out aimed to test whether the expression of the linalool synthase gene was correlated with the linalool content of lavender oil, and it was observed that it was expressed in tissues belonging to both organ types analysed (flowers and leaves). It was also observed that LINS gene expression was detected in higher abundance in flowers and in much lower quantity in leaves, suggesting that only floral stems should be harvested to obtain high-quality lavender essential oil.

The results of the research thus conducted are in agreement with data presented in the scientific literature, that LINS gene expression predominated in flowers (Demissie et al., 2011) and that linalool accumulation in *L. angustifolia* was directly correlated with transcript levels for it (Lane et al., 2010).

In species of the genus *Lavandula*, the biosynthesis of camphor, as well as other volatile oil compounds extracted from these plants, takes place in the glandular secretory hairs through a series of biochemical reactions. Like other monoterpenes, camphor is derived from isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), the universal precursors of all terpenes, and camphor will be generated through the oxidation of borneol due to the catalytic activity of borneol dehydrogenase (BDH) (Sarker et al., 2012; Dudareva et al., 2013; Despinasse et al., 2017; Li et al., 2021).

Practical investigations carried out on the plant material under consideration led to the observation that borneol and camphor had a different abundance both in the leaves and in the flowers of the experimental varieties and variants of *L. angustifolia* under consideration. The flowers showed this compound in higher concentrations compared to the leaves, but overall, it was the most poorly expressed gene of those considered.

GENERAL CONCLUSIONS

The results obtained in the present PhD thesis are based on original research, whose main objectives were to highlight and discuss some histo-anatomical and micromorphological characteristics of vegetative

organs (roots, stems and leaves) in taxa of the genus *Lavandula* L. cultivated in Romania, as well as to determine the composition of volatile oils produced by vegetative and generative organs (floral stems) and the gene expression of five genes involved in the process of terpene biosynthesis in four varieties of *Lavandula angustifolia* Mill. supplemented with Hoagland nutrient solutions of different compositions and cultivated under specific experimental conditions.

The conclusions of the research can be summarised as follows:

- **From a histo-anatomical point of view:**

Comparison of the structure of vegetative organs in six taxa of the genus *Lavandula* (*L. angustifolia* Ellagance Pink, *L. angustifolia* Ellagance Purple, *L. angustifolia* Ellagance Snow, *L. angustifolia* Munstead, *L. angustifolia* Vicenza Blue, *Lavandula x intermedia* Grosso) revealed the following aspects:

- there are no structurally significant differences between the taxa analysed;
- in plants belonging to taxa of the Ellagance group, the stem tends to exfoliate the epidermis, the organ losing the square outline specific to the genus;
- all taxa have on the stem surface numerous branched arborescent protective hairs and numerous secretory hairs with bi-, tetra- or octocellular glands, characters specific to the genus *Lavandula;*

The analysis of the anatomical results of the experimental variants of the four varieties of *Lavandula angustifolia* Mill. under consideration (Codreanca, Provence Blue, Vera and Sevtopolis) watered during cultivation with H_2O (v1) and supplemented with standard Hoagland nutrient solution (v2) revealed the following structural aspects:

- the histological structures identified are circumscribed to the typical structural plane of the genus;
- at the stem level there are structural differences (variation in the outline of the section, absence of pericycle in plants watered with H_2O - variant v1 belonging to varieties Codreanca and Sevtopolis) and differences in the outline of the central area occupied by the pith (square, hexagonal or rhomboid);
- at the level of the leaf blade, variations are observed in the contour of the section, the number of vascular bundles in the lateral veins and the number of epidermal formations (protective and secretory hairs).

- **From a micromorphological point of view:**

Comparative analysis of the density of protective and secretory hairs in the six *Lavandula* genus taxa analyzed (*L. angustifolia* Ellagance Pink, *L. angustifolia* Ellagance Purple, *L. angustifolia* Ellagance Snow, *L. angustifolia* Munstead, *L. angustifolia* Vicenza Blue, *Lavandula x intermedia* Grosso) revealed the following:

- manifestation of intra- and interspecific variation in the frequency of the epidermal formations;
- absence of secretory peltate hairs on the upper surface of the epidermis of the leaf blade in *Lavandula x intermedia* Grosso;
- capitate secretory hairs are the dominant type of hairs, much more abundant on the lower epidermis of the leaf blade of all taxa analyzed;

Protective hairs are the dominant formations on the surface of both leaf blade surfaces in all species and varieties analyzed, being more abundant on the lower blade surface.

Comparative numerical analysis of protective and secretory hairs in the four varieties of *Lavandula angustifolia* Mill. (Codreanca, Provence Blue, Vera and Sevtopolis), depending on the Hoagland nutrient watering option applied, revealed the following:

- the appearance of the hairs varies in the taxa analyzed, with the identification of two types of secretory hairs: capitate and peltate and two types of protective hairs: branched and unbranched;
- the density of secretory and protective hairs increased in plants watered with Hoagland nutrient solution with the addition of K and respectively P (v3 and v4) for all lavender varieties tested;
- protective hairs show a higher frequency, compared to secretory hairs, on both leaf blade epidermises in all taxa analyzed.

➢ **On the production and biochemical analysis of volatile oils:**

Quantitative and qualitative analysis of the volatile oils obtained by hydrodistillation of dried flowering stems belonging to four varieties of *Lavandula angustifolia* Mill. (Codreanca, Provence Blue, Vera and Sevtopolis) watered with different types of Hoagland nutrient solution revealed the following:

- the amount of volatile oil obtained from the biological material subjected to hydrodistillation showed quantitative and qualitative variations depending on the lavender variants and the experimental varieties analyzed;
- the highest extraction yield was obtained for the field-grown variety Provence Blue (M), which showed an extraction yield of 5,11 %;
- *Lavandula angustifolia* Mill. volatile oil extracted showed qualitative variations both within varieties and according to the Hoalgand nutrient solution treatment applied (working variants);
- The common major components of the lavender oils extracted are: linalool (12.1 - 40.7%), linalyl acetate (10.7 - 34.5%), β - ocimene (0.2 - 6%), D - limonene (0 - 1.5%), terpinen-4-ol (0.5 - 6%), lavandulyl acetate (2.3 - 8, 1%), α -terpineol (1.1 - 5.3%), β -caryophyllene (0 - 4.8%), caryophyllene oxide (0 - 14.8%), eucalyptol (0.3 - 6.7%), camphor (0.1 - 1.4%) and borneol (0.8 - 5.1%);
- none of the samples of volatile oil extracted from processed lavender flower stems fully met the quality parameters required by the current edition of the European Pharmacopoeia and ISO standards.

➢ **Concerning the expression of some genes involved in the synthesis of volatile oils:**

Expression analysis of five genes (LIMS, LINS, BDH, DXS, HMGR) involved in terpene biosynthesis in flowers and leaves of four varieties of *Lavandula angustifolia* Mill. (Codreanca, Provence Blue, Vera and Sevtopolis) grown under experimental conditions and supplemented with Hoagland nutrient solutions of different compositions revealed the following:

- all five genes tested were expressed in both types of plant tissue analyzed (flowers and leaves), showing stronger expression in flowers compared to leaves for most of the experimental varieties and variants studied;
- the comparison of the results obtained from the gene expression analysis to the reference gene (β-actin) showed an overexpression of HMGR and DXS genes and a reduced expression of LIMS, LINS and BDH genes in both types of tissues analyzed (flowers and leaves); HMGR was the most highly expressed gene, the highest values being observed in flowers;

- following treatments applied to *Lavandula angustifolia* Mill. plants, the expression of HMGR, DXS and LINS genes increased in plants watered with Hoagland nutrient solution with the addition of K and respectively P (v3 and v4) for all lavender varieties analyzed;
- LIMS gene was highly expressed in flowers of H_2O-watered plants (v1) of all varieties tested;
- BDH gene, responsible for camphor synthesis, was the most poorly expressed gene in both tissue types analysed, the lowest values being recorded in leaves.

The present work, by its subject matter, belongs to a field of fundamental applied research, which aimed to identify and investigate the interrelationships that can be established between cultivation conditions, morpho-anatomical changes, gene expression of certain genes involved in the biosynthesis of volatile oils and their composition in taxa of the genus *Lavandula* L. cultivated in Romania.

Based on an interdisciplinary, integrative approach of three specific fields of plant biology (anatomy, biochemistry and plant genetics), the paper provides additional arguments on the importance of growing lavender plants in protected and unprotected conditions, highlighting the effect of supplementing them with Hoagland nutrient solutions of different compositions on the quantity and biochemical profile of volatile oils, the practical approach to the subject and the correlation of research results being authentic and strictly novel for the field.

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