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# HISTO-ANATOMICAL AND MICROMORPHOLOGICAL INVESTIGATIONS ON SIX *LAVANDULA* L. TAXA

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**Abstract:** Species of the genus *Lavandula*, belonging to the Lamiaceae family, popularly known as lavenders are widely used in various fields and industries as an aromatic plant and also in ornamental gardening. This paper presents the histo-anatomical and micromorphological characteristics of the root, stem and leaves of two *Lavandula* taxa (*Lavandula x intermedia* Emeric `Grosso` and five cultivars of *Lavandula angustifolia* Mill.: `Ellagance Pink`, `Ellagance Purple`, `Ellagance Snow`, `Munstead` and `Vicenza Blue`). Since there is little knowledge on the structural differences between cultivars of the *Lavandula* genus, this study was elaborated using color photographs acquired from optical microscopy and scanning electron microscopy (SEM) observations, in order to assist in the identification. The root investigations revealed for the analyzed samples three main structures common for all plants: a layered periderm, which brakes and dies, a secondary cortex and the central cylinder, with many secondary wood vessels. At stem level, the images outline the square shape of the cross-section, with numerous protective and secretory trichomes. The SEM micrographs on the surface of the leaf blade, showed differential densities and types on the protective and secretory hairs. These results contribute to the taxonomic knowledge on lavender and could be used as taxonomic criteria for species and cultivar differentiation for the *Lavandula* genus.
**Keywords:** *Lavandula* taxa, histo-anatomy, optical microscopy, scanning electronic microscopy (SEM), trichome density.

## Introduction

Throughout its history, man has taken from nature various species of spontaneous plants, which he has planted in special, anthropized areas, specially intended for them. Thus, were born the crop plants, which, in order to increase production, have been improved since prehistoric times, so that new varieties and even species have resulted.

The taxonomy of species of the genus *Lavandula* has been quite controversial, the number of species ranging from 4 to 32 depending on the author and the morphological characteristics taken into account. Recent taxonomic studies divide the genus *Lavandula* into 8 sections and 36 morphologically distinct species (Upson, 2002; Upson and Andrews, 2004; Passalacqua et al., 2017).

Three species of the genus *Lavandula* are grown mainly for the commercial production of their volatile oils, namely: *Lavandula angustifolia* Mill. (syn. *L. officinalis* Chaix) - true lavender or English lavender, *Lavandula x intermedia* Emeric (syn. *L hybrida* L.) - lavandin and *Lavandula latifolia* Medicus (Lesage-Meessen et al., 2015). More than 50 varieties of *L. angustifolia* are known, and some of the most popular cultivars include Lady, Munstead, Hidcote, Vera (Erland and Mahmoud, 2016).

Currently the species of the genus *Lavandula* are intensely studied due to their content in essential oils and socio-economic value for the medical (Shahdadi et al., 2017; Firoozeei et al., 2021), food, cosmetics, perfumery and aromatherapy industries (Cavanagh and

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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 scent of these plants is primarily due to the presence of monoterpenoids that are synthesized and accumulate in the aerial parts, mainly in flowers (Hassanpouraghdam et al., 2011). The most popular lavender oils for the perfume and cosmetics industry are those with high linalyl acetate and linalool content and low in camphor, while the richest in camphor are used mainly in aromatherapy and phytotherapy (Herraiz-Peñalver et al., 2013).

The species belonging to the Lamiaceae family have different types, distribution, morphology and density of protective and secretory hairs, being important taxonomic features. Species of the *Lavandula* genus have been subject to a number of studies based on various data, such as its anatomy (Toma and Niță, 1982; 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 the trichomes (Huang et al., 2005, 2008; Zuzarte et al., 2010; Giuliani et al., 2020).

On the surface of the stem and leaves there are numerous glandular and protective trichomes, specific for the Lamiaceae genera, which gives the particular aspect and aromatic attributes for the species in this family.

Secretory trichomes are epidemic formations located on leaves, stems, sometimes on the bracts of inflorescences or on the scales of buds. Secretion products, especially volatile oils, accumulate between the wall of the secretory cell (or cells) and the cuticle, which it bulges. When the cuticle is broken, the volatile oil diffuses outwards (Burzo and Toma, 2013).

The leaf blade has protective hairs in both epidermises, uni-, bi- or multicellular, uniserated or branched, more common on the upper face of the limb (Robu et al., 2011).

The aim of the study is to asses by comparison the histo-anatomical differences between *Lavandula x intermedia* and five cultivars of *Lavandula angustifolia* by optical microscope analysis of the root and stem and by electronic microscope scan analysis on the leaves surface.

## Materials and methods

The plant material consists of root, stem and leaf parts of plants of the *Lavandula* genus (*Lavandula x intermedia* `Grosso` and five cultivars of *Lavandula angustifolia* Mill.: `Ellagance Pink`, `Ellagance Purple`, `Ellagance Snow`, `Munstead` and `Vicenza Blue`), as described in table 1.

Table 1. Species and cultivars used in the study

| Species | Morphological description |
|---|---|
| ***Lavandula angustifolia* `Ellagance Pink`** | This cultivar produces grey-green leaves with spikes of delicate pink flowers and reaches a height between 30 – 35 cm. |
| ***Lavandula angustifolia* `Ellagance Snow`** | This plant has grey-green leaves with spikes of pure white flowers and reaches the height of 30 – 40 cm. |
| ***Lavandula angustifolia* `Ellagance Purple`** | This cultivar has grey-green leaves with spikes of deep violet-blue flowers and has a height of 25 – 30 cm. |

| | |
|---|---|
| ***Lavandula angustifolia* `Munstead`** | This lavender cultivar has a compact bushy shrub with aromatic, narrow grey leaves and spikes of purple-blue flowers that reaches 40 – 60 cm. |
| ***Lavandula angustifolia* `Vicenza Blue`** | This compact, bushy strain has grey-green foliage and spikes of deep lavender-blue flowers that reach 25 – 30 cm. |
| ***Lavandula x intermedia* `Grosso`** | This hybrid forms a bushy mound of grey-green foliage, with long spikes of blue-purple flowers and has a height of 60 – 75 cm. |

**Optical microscopy**

Plant material was collected from specimens grown in cultures from Romania, Vrancea (coordinates 45°31′01.4″N 27°23′48.1″E). The samples were identified and the vegetative organs (leaf and stem) were fixed in 70% ethanol for anatomical studies. Cross sections of the material used for the study were carried out manually using a microtome and a botanical razor and double stained with iodine green and ruthenium red.

The sections were fixed in glycerol-gelatin and photographed using a NOVEX Holland photonic microscope using a digital camera and a Leica DFC 290 camera attached to a Confocal Laser Scanning Microscope CLSM—Leica TCS SPE DM 5500Q.

**Scanning Electron Microscopy**

The material used for this study consisted of fresh leaves prepared according to standard SEM techniques (Bozzola and Russell, 1999). The leaves were first washed with distilled water to remove dust particles and then dried to eliminate excess water. Small fragments were fixed on a carbon band and the fragments were then covered with a thin layer of gold particles. Samples were observed and photographed on a Tescan VEGA II SBH Scanning Electron Microscope, at the Electron Microscopy Laboratory of the Faculty of Biology, Alexandru Ioan Cuza University of Iași.

**Trichome density**

In this investigation, the type and frequency of glandular and non-glandular trichomes were examined on both leaf sides. The areas of leaves were measured from digital images and were analyzed by ImageJ software using Cell Counter plugin (Abràmoff et al., 2004). Trichome density was calculated by dividing the hair number by the leaf area (Gonzáles et al., 2008).

## Results and discussions

***Histo-Anatomical Aspects of the Lavender Root***

At the root level, the contour of the cross section is circular. The root structure is already secondary generated by the activity of the two lateral meristems: the cambium and the phellogen. The cambium, first formed, generated inwards a central wood cylinder with relatively numerous vessels, arranged radially and separated by libriform. Outwardly, the cambium produced a thin secondary phloem ring consisting of sieved tubes, attachment cells, and phloem parenchymal cells (Fig. 1).

The second secondary meristem, the phellogen differentiated outwards, producing multilayered suber; the outer layers are in the process of exfoliation (Fig. 1A). The secondary wood consists of vessels, relatively numerous, arranged in radial rows and surrounded by libriform. In some places, single- or biseriate medullary rays can be observed, with radially

elongated cells with uniformly thickened walls. The cambium works inwards relatively balanced in the sense that it produces secondary wood and libriform in approximately equal quantities. Such histological behavior generally characterizes plants in crops.

- *Lavandula angustifolia* `Munstead` (Fig. 1)

The central wood cylinder that remains after the disorganization and exfoliation of the tissues from its exterior consists of xylem vessels, more numerous than *Lavandula x intermedia* `Grosso`, also arranged in radial rows, and dominated by libriform, but also by wood parenchyma cells quantitatively reduced than those noticed in *Lavandula x intermedia* `Grosso` (Fig. 1B).

- *Lavandula x intermedia* `Grosso` (Fig. 2)

By the appearance in the depth of the root bark of a differentiated phellogen and by its operation, especially to the outside, results a multilayered suber. This suber with dead cells at maturity will cause forced exfoliation of the rhizodermis, exodermis, cortical parenchyma, the root structure being represented only by the central wood cylinder (Fig. 2A). The central cylinder comprises xylem vessels arranged in radial rows, quantitatively dominated by libriform (sclerenchyma fibers with uniformly thickened but non-lignified walls) and woody parenchyma cells with thin walls, cellulose – pectic (Fig. 2B).

***Histo-Anatomical Aspects of the Lavender Stem***

The contour of the cross section through the stem is square, modified by four relatively prominent wings (contour characteristic of plants of the Lamiaceae family). At the level of the epidermis, the presence of numerous multicellular, massive, branched, dead protective trichomes is observed, which gives the stems and leaves a whitish appearance. At the sectioned level, the pith is persistent, parenchymal-meatic. At the rib, the epidermis is unilayered, with small cells, slightly elongated radially. In the subdermal position there are 4-6 layers of angular collenchyma, with polygonal cells with thickened cellulose walls only at the corners.

On the surface of the stem there are more or less short secretory hairs, with a singular (Figs. 3A, B), bi- (Figs. 3C, D), tetra- or octocellular gland (Fig. 3E), with a massive secretory head and an intact cuticle. The secreted product accumulates between the secretory cell, its wall and the strongly convex cuticle (Fig. 3F).

Among the secretory trichomes there are numerous protective trichomes, much more frequent in valleculas and of two categories: uni- or bicellular (Figs. 4A, B), but simple, short and sharp at the tip and multicellular, longer, always branched (Figs. 4C, D, E, F).

- *Lavandula angustifolia* `Ellagance Snow` (Fig. 5)

The pith disorganizes leaving a large air cavity in the center of the stem. Correlated with the disorganization of the pith, at the periphery of the section is observed the exfoliation and disappearance of the epidermis, the angular collenchyma (diagnostic character for the Lamiaceae family), the parenchymal cortex, the contour of the cross section becoming circular (Fig. 5A, B). The epidermis and bark disorganize very quickly, from the early stages when the pith still persists.

- *Lavandula angustifolia* `Ellagance Pink` (Fig. 6)

The contour of the cross section is square, with the edges slightly rounded at the corners (Fig. 6A). The epidermis is represented exclusively by cells transformed into branched uni- and multicellular protective hairs and uni- and multicellular secretory

trichomes. The bark presents at the corners of the stem islands of angular collenchyma, between which there are two, maximum three layers of assimilating parenchymal cells.

The central cylinder comprises a secondary phloem ring and remnants from the primary phloem to the outside, cambium and a secondary wood ring to the inside. The elements of the primary wood are very difficult to distinguish. The pith has a rhomboidal shape, is persistent, consists of parenchymal cells, slightly rounded, leaving small ducts between them. By dedifferentiating some layers from the parenchymal cortex, a phellogen appears that works mainly to the outside giving a multilayered suber (6-8 layers). As the elements of the suber are dead to maturity, the connection between the living phelogen and the last layer of suber in its vicinity will be broken, which will lead to a massive exfoliation of the suber, angular collenchyma and epidermis (Fig. 6B).

- *Lavandula angustifolia* `Ellagance Purple` (Fig. 7)

The structure of the stem is similar to that of the varieties `Ellagance Pink` and `Ellagance Snow`, in that the contour of the section is square, with rounded edges at the corners, the pith has a rhomboidal shape, is persistent, consisting of parenchymal cells, slightly rounded leaving small meatus between them (Fig. 7A).

The epidermis disorganizes and exfoliates and with it most of the bark and the secondary phloem is disorganized (Fig. 7B).

The *Lavandula angustifolia* varieties `Munstead` (Fig. 8) and `Vicenza Blue` (Fig. 9) have a similar characteristic not found in the varieties `Ellagance`: angular colenchyma almost absent in the ribs of the stem, instead present in the form of well-defined islands (7-10 layers) at the periphery of the phloem (primary and secondary).

- *Lavandula x intermedia* `Grosso` (Fig. 10)

The square contour of the steam can be observed in fig. 10A. Four slightly protruding ribs can be observed, those features being described also in the literature for the Lamiaceae family.

The bark has small islands of angular colenchyma at the corners, alternating with very well-developed assimilative parenchyma (7-9 layers) (Figs. 10B, C). In some places there are air spaces resulting from the local disorganization of the parenchymal cortex. In the central cylinder can be distinguished collateral vascular bundles of very different sizes.

***Micromorphological aspects of the leaf surfaces***

Following the analysis of the leaf surfaces under the scanning electron microscope (SEM), aspects regarding the density and the structure of the protective and secretory hairs were observed.

All varieties have numerous protective trichomes, with arborescent branches and many secretory hairs with a bi-, tetra- or octocellular gland (Fig. 11) on the surface of the stem and leaf, specific to the genus *Lavandula* (Nikolakaki and Christodoulakis, 2006; Lungu et al., 2014).

Peltate and capitate trichomes found on the surface of the leaves of the studied cultivars showed similar characteristics to glandular trichomes reported for other species of the *Lavandula* genus (Martínez-Natarén et al., 2011; do Rocio Duarte and Carvalho de Souza, 2014; Brailko et al., 2017).

After analyzing the density of secretory and protective trichomes among the studied taxons, non-glandular hairs were widely observed on both surfaces of the leaves and their number was higher than glandular ones. Based on the morphology and cell number, the non-

glandular trichomes were divided into four types: simple unicellular or multicellular, branched unicellular or multicellular (Fig. 12). The highest density of secretory trichomes was seen for the `Munstead` cultivar, on both leaf surface (Table 2).

Table 2. Mean density per mm$^2$ of different trichomes on leaves of *Lavandula* taxons

| Species | Adaxial | | Abaxial | |
|---|---|---|---|---|
| | **Secretory** | **Protective** | **Secretory** | **Protective** |
| ***Lavandula angustifolia* `Ellagance Pink`** | 11.010 | 75.376 | 33.641 | 51.622 |
| ***Lavandula angustifolia* `Ellagance Purple`** | 13.296 | 85.019 | 55.339 | 104.368 |
| ***Lavandula angustifolia* `Ellagance Snow`** | 15.810 | 118.138 | 49.012 | 125.541 |
| ***Lavandula angustifolia* `Munstead`** | 63.931 | 93.144 | 88.860 | 127.736 |
| ***Lavandula angustifolia* `Vicenza Blue`** | 23.300 | 102.692 | 47.660 | 136.112 |
| ***Lavandula x intermedia* `Grosso`** | 13.987 | 97.915 | 53.687 | 149.218 |

A particularity feature was identified in *Lavandula x intermedia* `Grosso`, in which the adaxial surface of the leaf blade didn't have any peltate secretory trichomes (Table 3).

Table 3. Density per mm$^2$ of glandular and protective trichomes in *Lavandula angustifolia* cultivars and *Lavandula x intermedia*

| Species | | Adaxial | | | | Abaxial | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Capitate** | **Peltate** | **Secretory** | **Protective** | **Capitate** | **Peltate** | **Secretory** | **Protective** |
| ***Lavandula angustifolia* `Ellagance Pink`** | Mean | 8.5 | 4.5 | 13 | 89 | 20 | 9 | 29 | 44.5 |
| | Std. dev. | 3.535 | 0.707 | 2.828 | 4.242 | 11.313 | 1.414 | 12.727 | 23.334 |
| ***Lavandula angustifolia* `Ellagance Purple`** | Mean | 9 | 7.5 | 16.5 | 105.5 | 25.5 | 31.5 | 57 | 107.5 |
| | Std. dev. | 1.414 | 0.707 | 2.121 | 10.606 | 3.535 | 3.535 | 7.071 | 3.535 |
| ***Lavandula angustifolia* `Ellagance Snow`** | Mean | 15 | 3 | 18 | 134.5 | 41 | 16 | 57 | 146 |
| | Std. dev. | 4.242 | 0 | 4.242 | 19.091 | 8.485 | 4.242 | 4.242 | 11.313 |
| ***Lavandula angustifolia* `Munstead`** | Mean | 71.5 | 4 | 75.5 | 110 | 79.5 | 16.5 | 96 | 138 |
| | Std. dev. | 20.506 | 2.828 | 17.677 | 16.970 | 2.121 | 3.535 | 1.414 | 18.384 |
| ***Lavandula angustifolia* `Vicenza Blue`** | Mean | 20 | 7 | 27 | 119 | 43 | 12.5 | 55.5 | 158.5 |
| | Std. dev. | 2.828 | 0 | 2.828 | 2.828 | 5.656 | 0.707 | 6.363 | 10.606 |
| ***Lavandula x intermedia* `Grosso`** | Mean | 16 | 0 | 16 | 112 | 57 | 11 | 68 | 189 |
| | Std. dev. | 1.414 | 0 | 1.414 | 8.485 | 8.485 | 1.414 | 7.071 | 26.870 |

## Conclusions

Lavender species are important medicinal, aromatic and industrial plants due to their active principles, as demonstrated by long anatomical, biochemical and molecular research on them.

This study was based on the histo-anatomical and micromorphological analysis of six taxa of the genus *Lavandula*: *Lavandula x intermedia* Emeric and *Lavandula angustifolia* Mill.

In comparison with other works on the species of the genus *Lavandula* (Robu et al., 2011; do Rocio Duarte and Carvalho de Souza, 2014; Lungu et al., 2014; Riva et al., 2014; Brailko et al., 2017; Tanase et al., 2020), all histological structures in investigated taxa are generally circumscribed in the typical structural plan of the genus and Lamiaceae family.

Following the histo-anatomical analysis on the vegetative organs, the consultation of the specialized literature and the personal microscopic observations, the following were determined: at the root level there are no significant differences from a structural point of view, the differences that appear are of a quantitative nature, and refer to the parenchyma cells in the central wood cylinder; at the level of the stem, in the varieties from the `Ellagance` group the epidermis is disorganized and exfoliates, the contour of the section becoming circular, disappearing the diagnostic element specific to Lamiaceae, the square contour; the rest of the varieties keeping the square contour, with four prominent ribs; a difference is found in the `Munstead` and `Vicenza Blue` varieties, in which the angular colenchyma is present in the form of well-defined islands.

The leaf epidermal surfaces of the studied species and cultivars have indumentum consisted of glandular and non-glandular hairs. The density of trichomes varied between these taxa and two types of secretory trichomes were observed: capitate and peltate. Peltate trichomes were constituted by a massive head formed of four to eight secretory cells, absent for the superior epidermis of *Lavandula x intermedia* `Grosso`. The capitate trichomes are the dominant type of glandular trichomes.

The protective hairs were divided into branched and unbranched, with higher density than the secretory trichomes for all analyzed taxa.

Trichome density, the presence or absence of some types and their typology can be used as taxonomic characters for intragenic classification of the genera.

Our research had in prospection the creation of a histo-anatomical and micromorphological diagnosis sheet for each taxon studied, a sheet that would allow their correct identification when initiating or on existing cultures with economic impact. This is the reason why in the Laboratory of Plant Morphology and Anatomy the plant material studied by us is preserved and has the value of a voucher and can be made available at any time to any researcher who has similar pursuits as us.

Given the economic importance of the genus *Lavandula* and the possibilities of entrepreneurial activities related to the initiation and feasible exploitation of crops in the local economy, we consider it very important that these histo-anatomical and micromorphological diagnosis sheets must accompany each cultivated taxon as benchmarks for establishing the purity of a culture and the authenticity of cultivated taxa, thus achieving a solid link between the economic and research environment.

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**EXPLANATION OF THE PLATES**

PLATE I

Fig. 1. Cross-sections of the root of *Lavandula angustifolia* `Munstead`
- A - general view; the periderm, the rest of the primary bark and even the phloem are exfoliating
- B - fragment of the central wooden body

Fig. 2. Cross-sections of the root of *Lavandula x intermedia* `Grosso`
- A - general view
- B - fragment of the central wooden body

PLATE II

Fig. 3. Optical micrographs of types of glandular trichomes
- A, B - single celled head
- C, D - bicellular secretory head
- E - peltate trichomes
- F - cuticle of peltate trichomes

Fig. 4. Optical micrographs of types of protective trichomes
- A, B - bicellular trichomes
- C, D, E, F - multicellular branched trichomes

PLATE III

Fig. 5. Cross-section through stem of *Lavandula angustifolia* `Ellagance Snow`
A - general view
B - fragment of the stem

Fig. 6. Transversal sections from the stem of *Lavandula angustifolia* `Ellagance Pink`
A - general view
B - the deep periderm exfoliates with the rest of the primary bark and the hairy epidermis

Fig. 7. Transversal sections from the stem of *Lavandula angustifolia* `Ellagance Purple`
A, B - general view; the rhomboid contour of the pith is observed

PLATE IV

Fig. 8. Cross-section through stem of *Lavandula angustifolia* `Munstead`
A - detail of the angular collenchyma of a rib
B - detail of the woody conductive tissue
C - detail of the phloemic conducting tissue

Fig. 9. Cross-section through stem of *Lavandula angustifolia* `Vicenza Blue`
A - detail from a rib with epidermal hairs and angular collenchyma
B - detail of the vascular bundles
C - epidermal hairs

Fig. 10. Cross-section through stem of *Lavandula x intermedia* `Grosso`
A - general view
B, C - details from a rib with epidermal hairs and angular collenchyma

PLATE V

Fig. 11. Scanning electron micrographs of secretory trichomes on the leaf surface
A - *Lavandula angustifolia* `Munstead`, abaxial epidermis view
B - *Lavandula x intermedia* `Grosso`, bicellular secretory trichome and peltate trichome with tetracellular secretory gland
C - *Lavandula angustifolia* `Munstead`, post-secretory uni- and two-celled glandular trichomes
D - *Lavandula angustifolia* `Ellagance Purple`, peltate trichomes with tetracellular head
E - *Lavandula angustifolia* `Munstead`, peltate trichome with tetracellular head
F - *Lavandula angustifolia* `Vicenza Blue`, peltate trichome with eight secretory cells

Fig. 12. Scanning electron micrographs of protective trichomes on the leaf surface
A - *Lavandula angustifolia* `Ellagance Snow`
B - *Lavandula angustifolia* `Ellagance Pink`
C - *Lavandula angustifolia* `Ellagance Purple`
D - *Lavandula angustifolia* `Munstead`
E - *Lavandula angustifolia* `Vicenza Blue`
F - *Lavandula x intermedia* `Grosso`

**PLATE I**

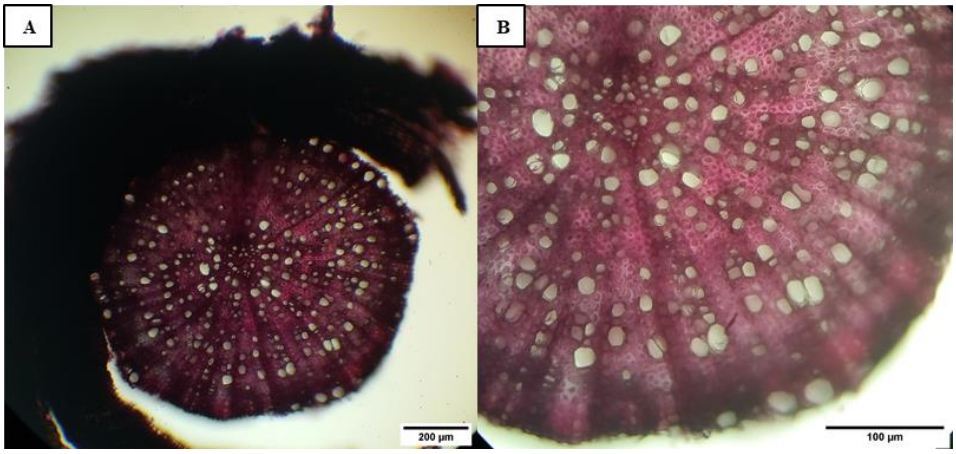


Figure 1

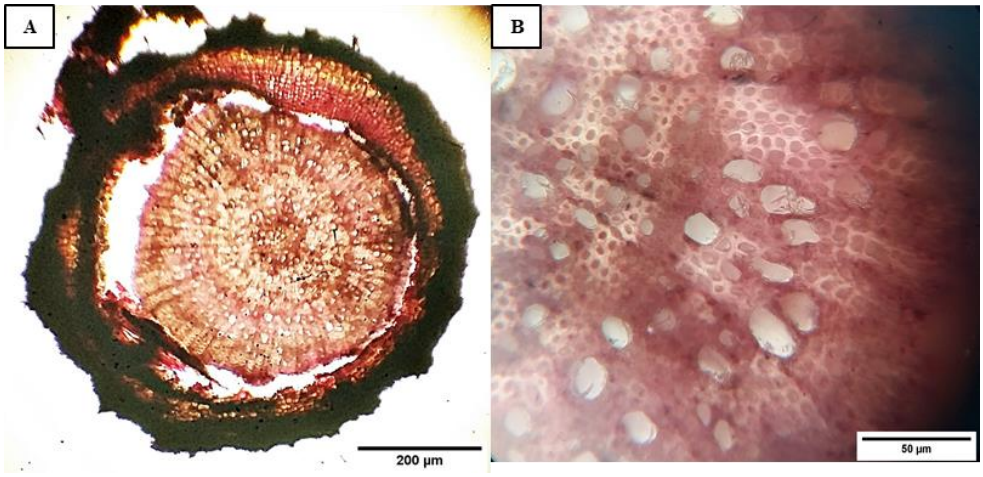


Figure 2

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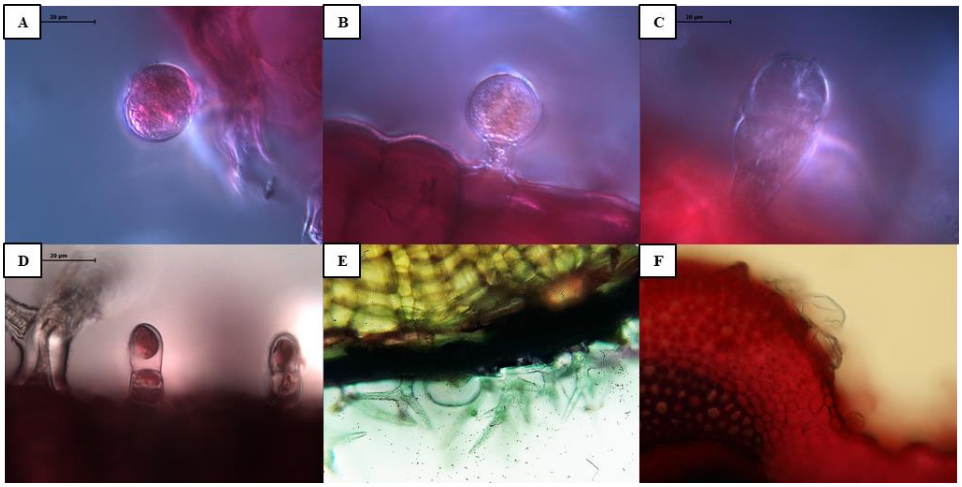


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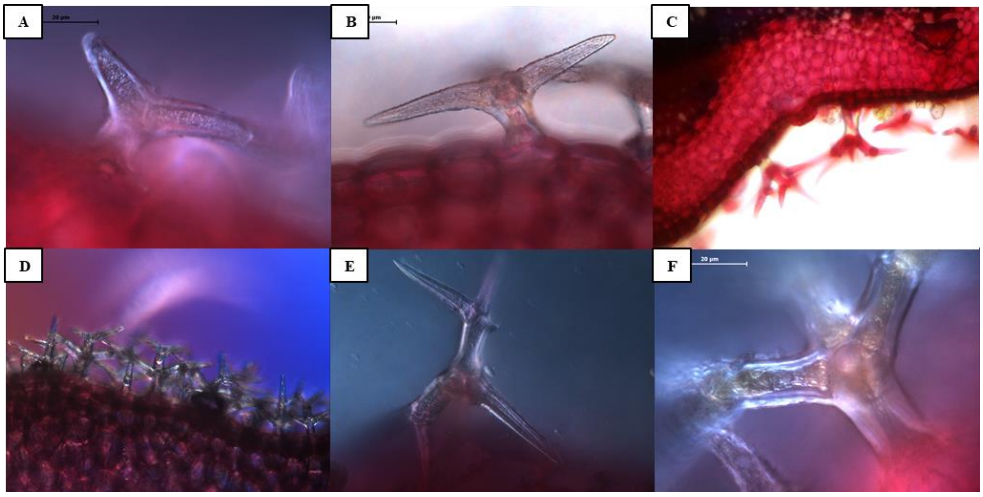
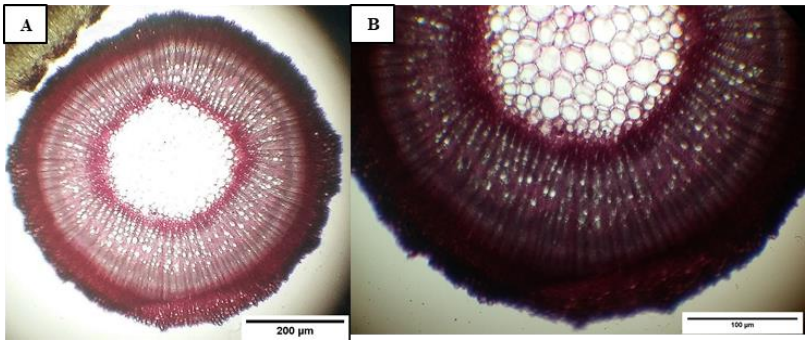



Figure 4

**PLATE III**

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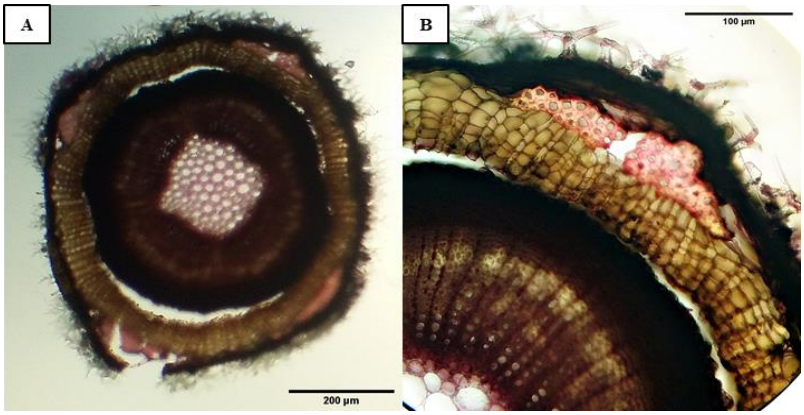


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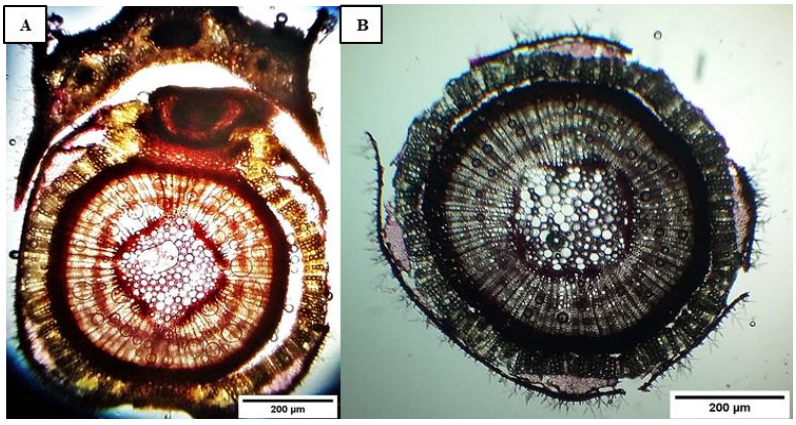


Figure 7

**PLATE IV**

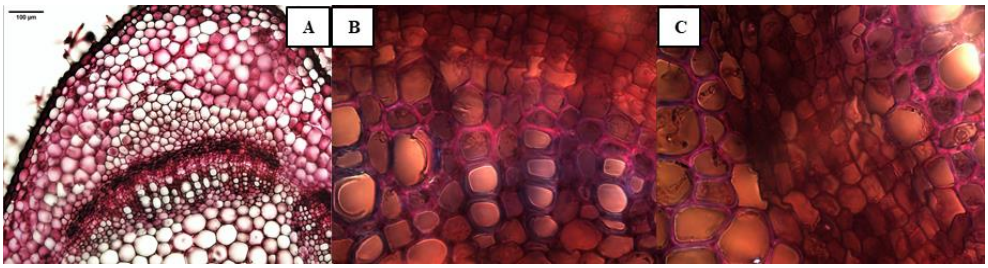


Figure 8

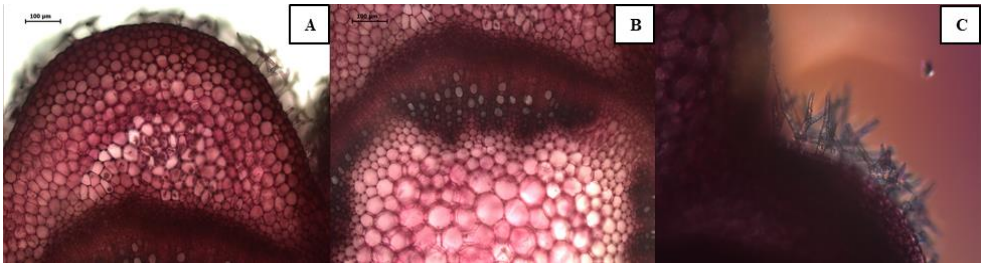


Figure 9

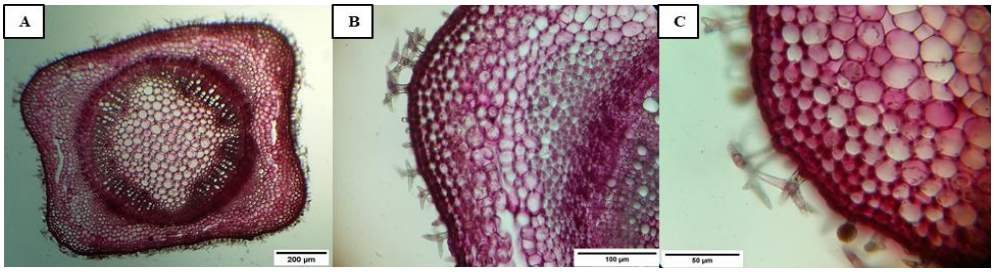


Figure 10

**PLATE V**

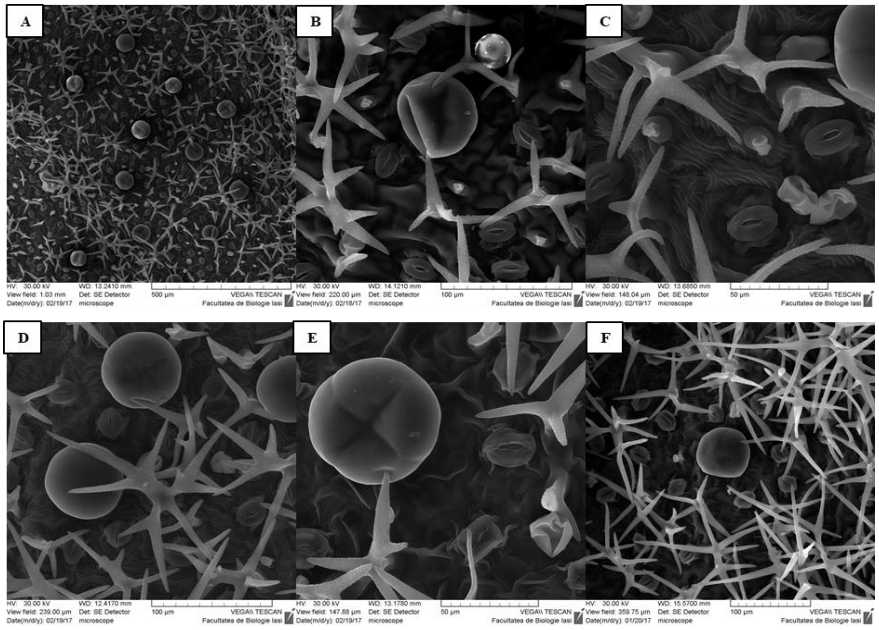


Figure 11

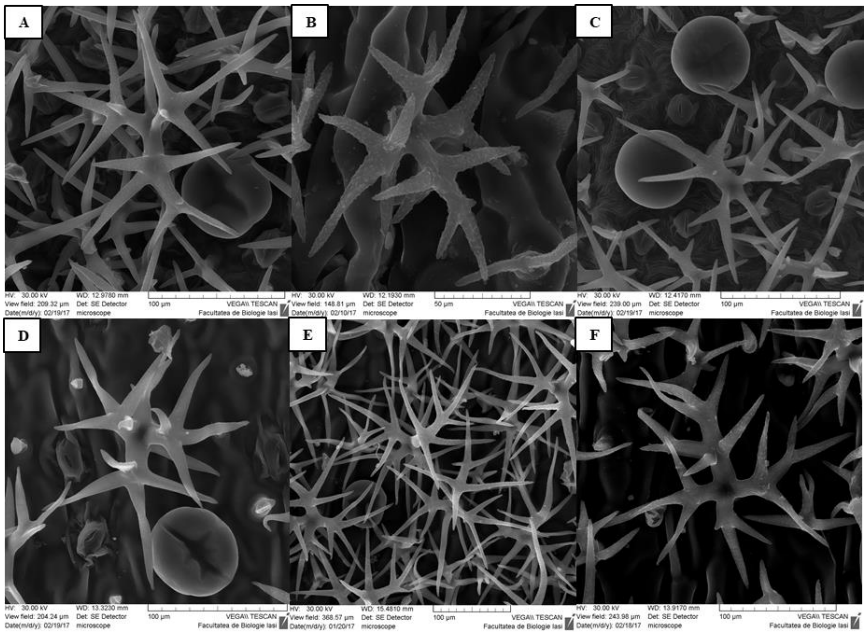


Figure 12