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MORPHO-ANATOMICAL STUDY ON *MELISSA OFFICINALIS* L.

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Key words: *lemon balm, histo – anatomical study*

INTRODUCTION

There are many species of Lamiaceae (lavender, mint, thyme, basil, lemon balm, etc.) in the spontaneous flora of Romania, mainly used for medicinal, pharmacological, or culinary purposes. Due to their essential oils, they are grown as spices, aromatic, and ornamental herbs. Despite the curative properties of its volatile oil, the species is not extremely popular and valued in our country [1-5].

Mellisa offícinalis L. is a herbaceous, perennial plant, known as lemon balm. Its active principles are spasmolitic and sedative, recommended for gastro - intestinal spasms and cardiac condition. The volatile oil is antiseptic, sedative, carminative, choleretic, laxative, stomachic, cicatrisant, galactagogue, and insecticide action.The lemon balm is a precious melliferous species, therefore often cultivated in many gardens [7-9].

Our previously published scientific studies on lemon balm included biotechnological, biometrical and biochemical research results. As the inner structure of lemon balm is not entirely known, our goal was to observe each and every aspect in this species' development, as well as its organs'histo-anatomy [10-13].

MATERIAL AND METHODS

The vegetal material used in the histo – anatomical research was represented by axial and lateral vegetative organs of lemon balm plants originating from Greece, acclimatised and cultivated on a plot in Racova village, Bacău county (fig 1). The tested plants were in various developmental stages. The histo - anatomical sections were accomplished by means of botanic scalpels and microtomes. The laboratory methods were in accordance to the standards. There were some steps in the laboratory tests in order to provide the preparates: the various age plants were fixed and preserved in ethanol (70% concentration). Many cross sections were made, in the study of every vegetative organ.

The cell content was subsequently removed from all vegetative tissues (the sections were immerged into sodium hypochlorite reagent, and maintained for several minutes, depending on the plant organ where they were harvested from (underground organs – about 20 minutes; aerial stems–approximately 15 minutes; leaves - 10 minutes).

The sections were rinsed in acetic water, and in tap water (twice) afterwards. Superficial sections through leaves and aerial stemswere not emptied. The next stage was staining in Carr reagent, and acetic orcein for a few dozens of seconds, subsequently rinsed twice in tap water. The colored preparates were mounted on glass slides into a waterdrop, and covered with coverglass. The preparates were observed using an optical microscope (Olympus CX31) at the objectives 4X, 10X, 40X, and 100X, then photographed by means of a digital camera (Olympus C 5060).

RESULTS AND DISCUSSIONS

*Morphological aspects of the vegetative parts.*The analyzed plants were approximately35-45 cm in height, dispalyed 2-3 secondary stems of various diameters. Fibrous, very thin roots.

Structure of the vegetative organs

The root

The root contour was circular in cross sections, with a thin periderm (made of 2-3 layers of cork flat, thin wall cells). The cortex was thin, compact, unclearly divised in exodermis, cortical parenchyma, and endodermis. It contained 3-4 layers of oval cellulose thin-walled cells (fig. 3, 4).

There were evinced many Casparian strips in the endodermis. They contain suberin and are deposited on radial and cell walls in the root endodermis of vascular plants. They prevent passive water flow together with minerals to and from the central cylinder. The latter one is compact, its vessels' walls are slightly lignified. The floem is arranged in small isles of few sieve elements and ajoined cells (fig. 3, 4).

The underground stem

Histo-anatomical observations on the underground organs displayed that the main root presents some structural features for a typical underground stem (rhizome). The emerging roots are adventitious, provided by the cambial activity. The rhizome maintains the same square shape as the aerial stem (fig. 5), including the four bulges of

collenchyma nearby the main vascular tissues. The epidermis contains even, aggregated cells with a thicker outer cell wall. Below the epidermis, in the four corners of the cross section, there are small isles of angular collenchyma. The cortical parenchyma is rather thin in our analyzed plants, contains 6-9 layers of elongated cells (very small air spaces inbetween cells)(fig. 5-7).

The central cylinder is well represented, made of numerous cell layers, most of which is pith parenchyma,that dissolves in many cases, favouring a central large air space. The vascular tissue is proeminent: four main fascicles facing the corner colenchyma, and a few smaller fascicles (containing xylem).

They join in a ring of vascular tissue as plants develop. The large vascular bundles include ray arrangemnt in xylem vessels with thickended lignified walls, and the phloem comprises sieve tubes and theirassociated elements (fig. 5-7).

The parenchymatic-cellulosic pith is well represented in this organ (a third ofthe entire surface). The pith cells are large, poligonal shape in the outer side, then elongated. The cells have thin walls. There are aerial spaces of various sizes in between cells, some very large. The cells sometimes tend to be disorganised, becoming an aerial space.

The aerial stem. Cross sections of aerial stems are square -shaped(fig. 8).

The epidermis – comprises isodiametriccells with bulging outer wall covered in a thin cuticle. The cells lack chlorophyll and nutrients (fig. 8, 9, 12).There may scarcely be seen multicellular trichomes and tetracellular secreting glandular trichomes (fig. 8-11).

The cortex (cortical parenchyma) contains 6-7layers of thin wall, round cells, small air spaces in between cells (fig. 9, 12). The first layer displays thicker walls than the innermost layers (fig. 8, 9, 12).

The central cylinder maintains the square contour in cross section. One may observe the four xylem-phloem open bundles.In between the main bundles, there are 2-3 secondary bundles observed in the cross sections through the upper stem; they can join in the inferior stem third in a tissue ring of xylem and phloem.

The xylem is disposed in rays of large conductive vessels inside the main bundles, among which there are xylemic fibres with thick hard walls. The phloem, much thinner, comprises smaller elements than the xylem. The peripheral regions of the phloem contain groups of sclerenchyma fibres (1-2) with thickened walls. Therefore, the secondary structure developed in large vascular bundles. The vessels arranged in rays are separated by xylemic parenchyma cells (fig. 8, 12-15).

The pith is very well represented, made of large cellulosic cells, small air spaces in between, that scarcely tend to desorganise (fig. 8, 15).

The leaf. Cross sections through the petiole and lamina were made in view of histo-anatomical observations.

a. The petiole is crescent - shapedin cross section (fig. 16). The epidermis contains isodiametrical cells with slightly bulged and thickened outer wall. Pluricellular trichomes emerge among the epidermis, together with the secreting tetracellular glandular trichomes, similar to the formations described in the aerial stems.

The cortical parenchyma comprises large cells with a polygonal or roundish shape, air spaces in between cells. At the corners, under the epidermis, there are 2 or 3 layers of angular collenchyma, although there are more numerous in mature plants (5-6).

There is a large central xylemic-phloemic bundle (open collateral type) and two secondary ones, nearby. (fig. 16).

The xylem is formed by large vessels, ray arranged cells (4-5), with lignified thick walls. The parenchyma cells (xylemic, cellulosic) are scarce among the vessels. The phloem is thinner and may provide a multicellular sheath of parenchyma cells.

The lateral bundles contain xylemic vessels (4 - 6) with thickened cell walls, yet lignified, and are separated by cellulosic xylemic parenchyma (very thin cell walls). The phloem is smaller than the xylem, surrounded by an outer parenchyma sheath (fig. 16).

b. The lamina. The cross sections throughout the leaf lamina show a normal, typical histo-anatomical structure for the herbs in *Lamiaceae*family.Therefore, one may observe a bifacial hetero facial structure of the lamina (fig. 17, 18).

*Front view of the epidermis.*The upper epidermis contains sinuous epidermal cells (fig. 19). Bi- or three-cellular very long trichomes may be observed on the main lamina bundle or nearby (fig. 21). Secreting glandular trichomes are scarce. The lower epidermis contains smaller cells, with very sinuous lateral walls.

Both the upper and the lower epidermis comprise cells with bulging thick outer walls (fig. 18).

There is a layer of palisade collenchyma below the outer epidermis. The palisade contains very many chloroplasts. A lacunar multilayeredtissue may be observed below the palisade. Furthermore, the lacunar cells contain chloroplasts, as well. Many long trichomes were depicted among lower epidermic cells (fig. 18, 21).

The vascular system (tissue, and vascular bundles) are crescent shaped, the xylem is disposed to the outer side, 6-7 rays of vessels in the plants observed by us, separated by cellulosic xylemic parenchyma cells.

The xylemic vessels displayed thick lignified walls (17, 20).The phloem is crescent shaped as well,

lies below the xylemic tissue, and comprises sieve elements and parenchyma, as the schlerenchyma is present only in secondary structure (fig. 20).

CONCLUSIONS

- *Melissa officinalis* L. is a herbaceous perennial species of the *Lamiaceae* family, valuable for its active principles of its essential oils, that are: sedative, spasmolitic, antiseptic, or cure gastro-intestinal disorders, etc.
- The underground stem, the root the aerial stem and the leaves were thoroughly analyzed from the histo-anatomical viewpoint.
- The main root diplays typical structure and feature as the rhizome.
- The collenchyma in the four stem corners is underdeveloped. The cortical parenchyma in the analyzed plants is very well delimitted from the central cylinder, contains slightly elongated cells, with thin walls. At the corners, under the epidermis, there are 2 or 3 layers of angular collenchyma, more numerous in mature plants.
- Cross sections through the petiole and lamina were made in view of histo-anatomical observations.
- One may observe a bifacial hetero facial structure of the lamina.
- There were evinced pluricellular trichomes and tetracellular secretory glandular trichomes both in stem, and in leaves.

ABSTRACT

Mellisa officinalis L. is a herbaceous, perennial plant, known as lemon balm. Our previously published scientific studies on lemon balm included biotechnological, biometrical and biochemical research results.

As the inner structure of lemon balm is not entirely known, the purpose of this scientific study was to observe each and every aspect in this species' development, as well as its organs' histo-anatomy.

The vegetal material used in the histo – anatomical research was represented by axial and lateral vegetative organs of lemon balm plants originating from Greece, acclimatised and cultivated in Racova village, Bacău county. The tested plants were in various developmental stages.

Fig. 1. *Melissa officinalis* – the plants in an experimental field in Racova (Bacău county), subsequently harvested for lab experiments

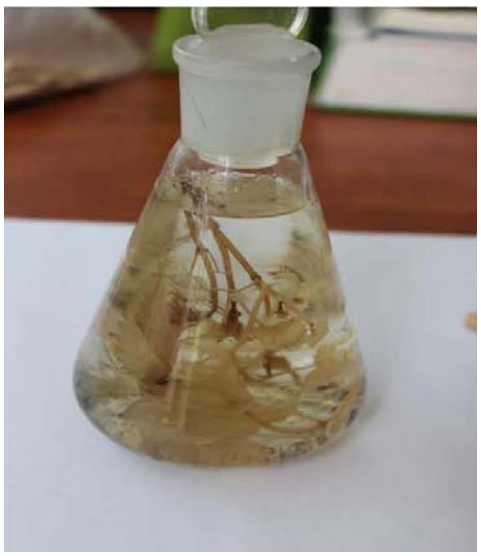

Fig. 2. Lemon balm (preserved in 70% ethanol)

Fig. 3. Cross section through the root of *Melissa officinalis* L. –detail on the central cylinder

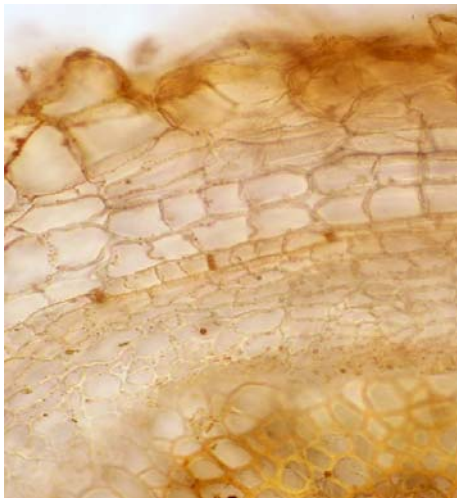

Fig. 4. Cross section through the root of *Melissa officinalis* L. –detail on the endodermisand the Casparian stripes

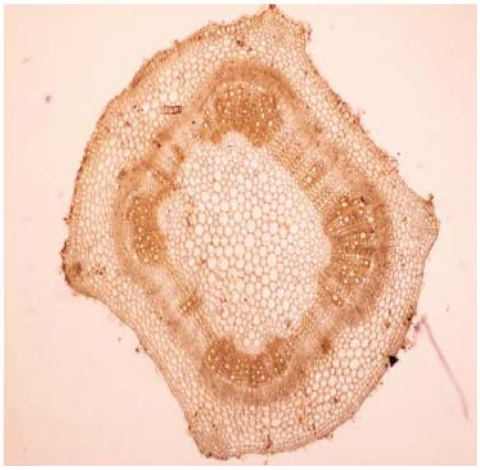

Fig. 5. Cross section through the underground stem of *Melissa officinalis* L.

Fig. 6. Cross section through the underground stem of *Melissa officinalis* L. –detail

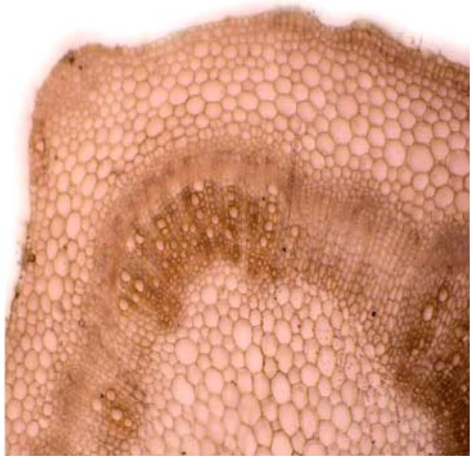

Fig. 7. Cross section through the underground stem of *Melissa officinalis* L. –detail (0-10 µm)

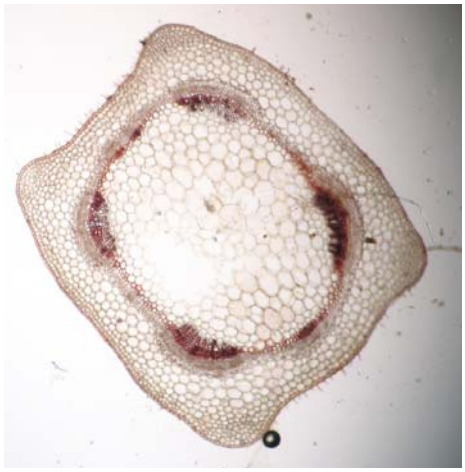

Fig. 8. Cross section through the aerial stem of *Melissa officinalis* L.

Fig. 9. Cross section through the aerial stem of *Melissa officinalis* L. – detail of the epidermis and cortical parenchyma

Fig. 10. Epidermis (front view) with tetracellular secreting glandular trichomes in stem epidermis

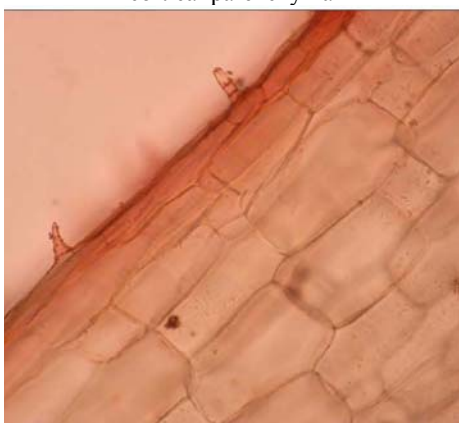

Fig. 11. Longitudinal section through the aerial stem of *Melissa officinalis* L.- detail of epidermis, trichomes and cortical parenchyma tissue

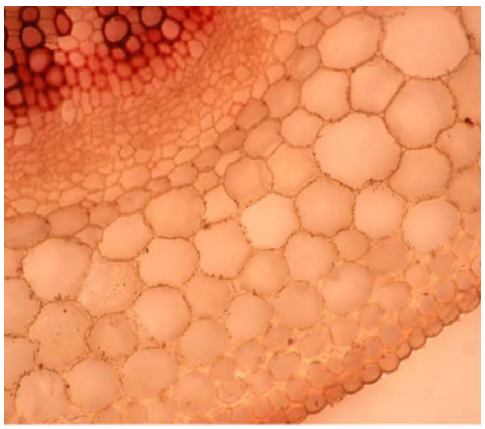

Fig. 12. Cross section through the aerial stem of *Melissa officinalis* L. –detail of epidermis and cortex

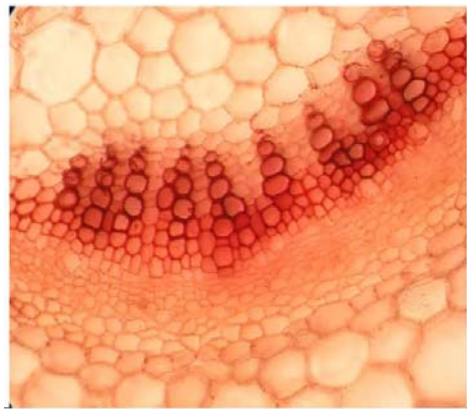

Fig. 13. Cross section through the aerial stem of *Melissa officinalis* L. –detail of main xylemic-phloemic bundle

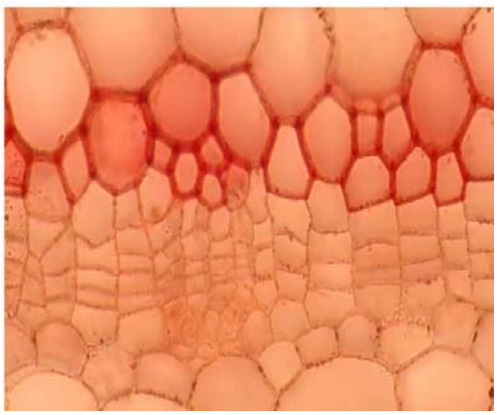

Fig. 14. Cross section through the aerial stem of *Melissa officinalis* L. –detail of secondary bundle

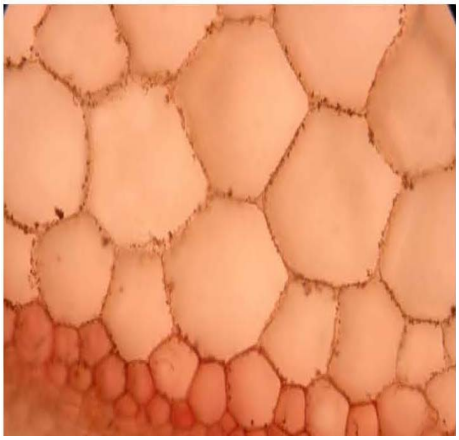

Fig. 15. Cross section through the aerial stem of *Melissa officinalis* L. –detail of pith

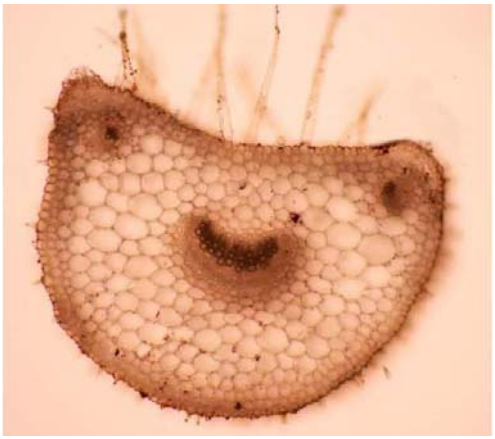

Fig. 16. Cross section through the petiole of *Melissa officinalis* L. – general view

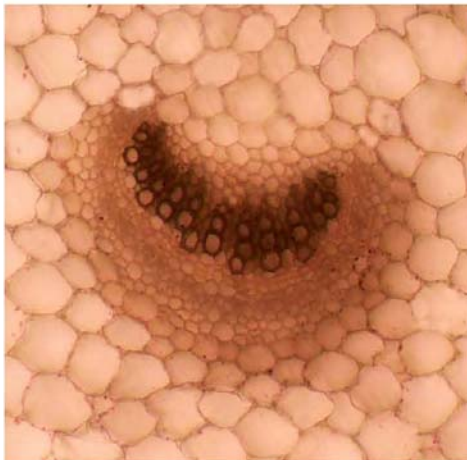

Fig. 17. Cross section throughout the lamina

Fig. 18. Lamina cross section – bifacial and hetero facial typical structure

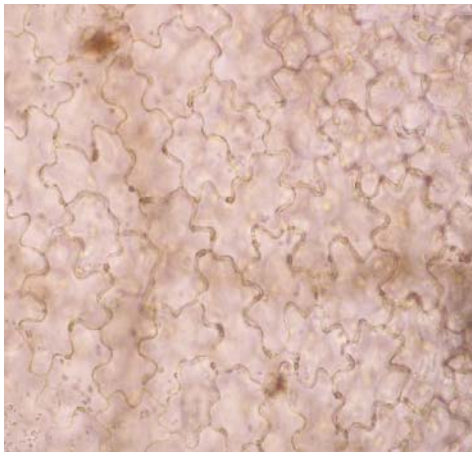

Fig. 19. Outer epidermis

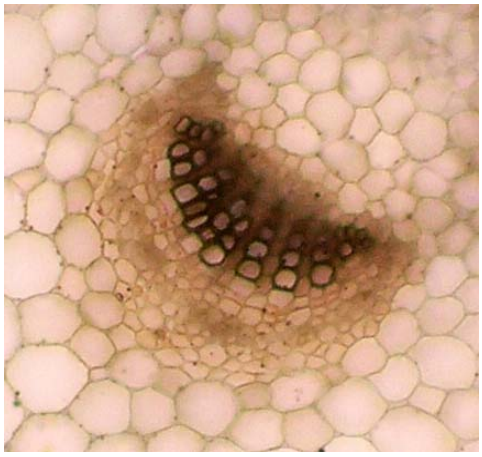

Fig. 20. Cross section – leaf lamina – vasculary bundle detail

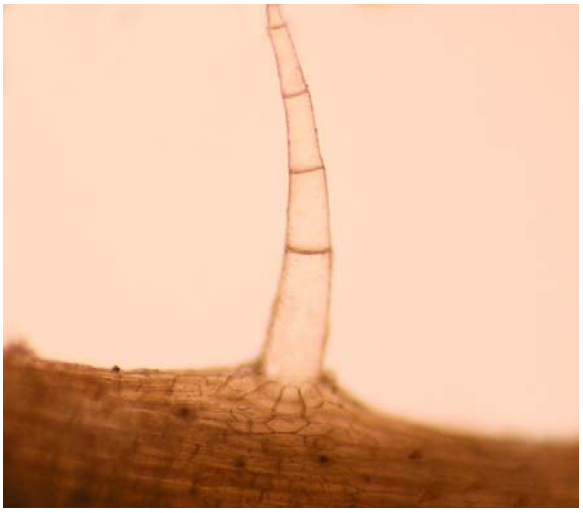

Fig. 21. Trichome – lower epidermis

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