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ANATOMICAL AND BIOCHEMICAL RESEARCH ON THE SPECIES *OCIMUM BASILICUM* L. (LAMIACEAE) CULTIVATED IN THE NUTRIENT FILM TECHNIQUE SYSTEM

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Abstract:

The article presents aspects regarding the anatomy and composition of volatile oil in the species Ocimum basilicum L. *(Lamiaceae), cultivar 'Aromat de Buzău'. Anatomical observations were made on cross-sections in the stems and lamina of the leaf. The epidermis of the leaf is unilayered, with the outer walls covered by a thin cuticle. Glandular and nonglandular hairs are present on both epidermis, especially on the lower epidermis. There are short glandular hairs, capitate or peltate and the non-glandular ones are unicellular or multicellular, sharp or with a curved tip. The stomata are present in both epidermis but more numerous in the lower epidermis, being of the diacytic type. The leaf mesophylle is bifacial with palisade tissue under the upper epidermis consisting of a single row of elongated cells, rich in chloroplasts and spongy tissue, below the lower epidermis, consisting of 3-4 rows of ovoid cells and with intercellular spaces. The oil has been extracted during the flowering period, by hydro-distillation and analyzed by gas chromatography – mass spectrometry (GC-MS). The main chemical compounds present in the volatile oil were: methyl chavicol (63.56 %), linalool (17.10 %), 1,8-cineole (4.01%) and α-epi-cadinol (2.64 %).*

Key words*: Ocimum, anatomy, secretory hairs, volatile oil, linalool.*

INTRODUCTION

The species *Ocimum basilicum* belongs to the Lamiaceae family, it is a plant with strong aromatic and medicinal properties being used for pharmaceutical, food and cosmetic purposes. (Nurzyńska-Wierdak et al., 2012). Due to its unique aroma, it is a plant often used in the fragrance industry, since ancient times. The interest in cultivating herbs and spices has increased recently, including for the species *Ocimum basilicum.* A number of new cultivars were introduced and gradually expanded into culture, differing in terms of morphology (colour, leaf shape, height, so on), production, and chemical composition.

All these features give the plant uses in various industries. Basil leaves and young shoots can be used in flavouring wines as well as for making teas and infusions (Vînătoru et al., 2019). The *Ocimum basilicum* 'Aromat de Buzău' is used as an aromatic, food, medicinal, ornamental, and melliferous plant. The flavours identified in this cultivar are varied depending on the presence of chemical compounds, such as: lemon flavour given by citral, anise and clove flavour provided by eugenol, camphor flavour imprinted by the high content of camphor and camphene, anise flavour given by the substance called anethole (Jailawi et al., 2021).

The production of volatile oil is carried out by secretory structures, in the species of the Lamiaceae family being frequently found pelted and capitate secretory hairs (Fahn, 1988; Berciu (Boz), 2009). The predominant chemical compounds in the volatile oil extracted from *Ocimum basilicum* plants were: methyl chavicol (estragole), 1,8-cineole, methyl eugenol, linalool (Elsherbiny et al., 2016), linalool, eugenol, methyl eugenol (Akgül, 1989), 1,8-cineole, linalool, geraniol, α-trans-bergamotene, epi-α-cadinol, methyl chavicol, nerol (Sajjadi, 2006), linalool, 1, 8-

cineole, eugenol, α-cubebene, methyl cinnamate, caryophyllene, β-ocimene, and α-farnesene (El-Soud et al., 2015), 1,8-cineole, linalool, camphor, α-terpineol, methyl chavicol and eugenol (Barcelos et al., 2013).

The composition of volatile oil can be influenced by environmental conditions, genetics, different chemotypes (Özcan & Chalchat, 2002; Nassar et al., 2014) as well as the application of treatments and fertilizers to plants.

The results obtained by Burducea et al. (2018) have shown that conventional fertilizers can increase fresh yield, while organic fertilizers positively change the composition of volatile oil, leading to increased crop quality.

Growing basil plants in the greenhouse with hydroponic system allows high yields and commercial quality both for the fresh market and for the essential oils industry (Guerrero-Lagunes et al., 2020). Previous studies have shown that the volatile oil of the *Ocimum basilicum* species is rich in substances that possess antibacterial, anti-insecticidal and antifungal activities.

The purpose of this study is to analyze anatomically and biochemically the basil plants grown in the hydroponic system.

MATERIALS AND METHODS

Material

The biological material used was represented by plants of *Ocimum basilicum* cultivar 'Aromat de Buzău', grown in hydroponic system in the greenhouse within the Research Center for Studies of the Food Quality and Agricultural Products, U.S.A.M.V. Bucharest. The analyses were performed during the flowering period, in May, 2021.

Anatomical and micromorphological characterization

Anatomical and micromorphological analyses were performed on the stems, leaves and petals of the *Ocimum basilicum* 'Aromat de Buzău' (Figures 1-3). In the stems, cross sections made at at the level of the internodes were made, and in the leaf in the middle part of the lamina of the leaf.

The sections were clarified with chloral hydrate and stained with carmine-alum and iodine green, according to the classical method (Morlova et al., 1966).

Observations and photographs were taken with the LEICA DM 1000 LED optical microscope, LEICA DFC 295 video camera and LEICA S 8 APO stereomicroscope, using 10x lenses, and micromorphological observations using by the Inspect S50 scanning electron microscope (SEM), existing in the endowment of the Research Center for Studies of Food Quality and Agricultural Products.

Volatile oil analysis

The extraction of volatile oil was made from the whole plant (stems, leaves, flowers).

The fresh harvested plants (300 g) were hydro-distilled for 3 h in a Clevenger-type apparatus. Analysis of the essential oils was performed on an Agilent 6890 GC coupled with a 5973 Network single quadruple mass spectrophoto-meter detector in Electron Ionization (EI) mode and 7673 injector on a HP-5MS capillary column (30 m × 0.25 mm id, 0.25 μm film thicknesses). The following column oven operating conditions were employed: 50°C for 8 min, then a 4°C/min ramp to 280°C. Helium was used as carrier gas with a constant flow of 1.0 mL/min, injection volume 3 μL with a split ratio 50: 1. The temperatures for inlet, MS transfer line and ion source was 250°C, 250°C and 230°C, respectively. The GC column was coupled directly to the spectrometer in EI mode at 70 eV with the mass range of 50-550 amu at 2 scan/s (Ion et al., 2020).

RESULTS AND DISCUSSIONS

Stem anatomy

The anatomy of the stem is similarly to the species in the *Lamiaceae* family (Metcalfe & Chalk, 1983; Toma & Rugină, 1985; Garner & Catherine, 2017). The outline of the cross section is rectangular, with four rounded ribs. In the cross section, the epidermis, the cortex and the central cylinder can be seen (Figure 1a). The epidermis consists of a single row of cells, with the outer walls covered by a thin cuticle. In the epidermis, from place-to-place stomata and uniseriate nongladular hairs (with 3-5 cells) curved, very rarely capitate glandular hairs, with one or two cells were observed (Figure 1b).

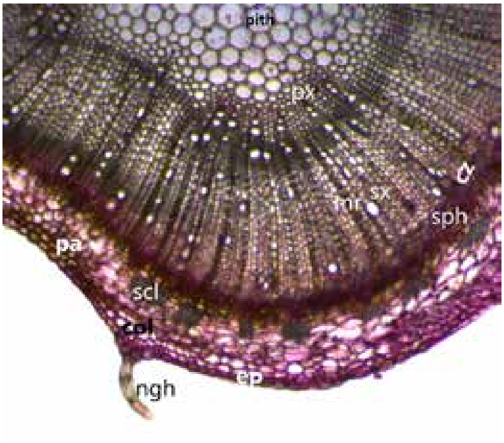

Figure 1a. Transversal section of the *Ocimum basilicum* 'Aromat de Buzău' stem: ep – epidermis; col – collenchyma; pa – parenchyma; scl – sclerenchyma; sph – secondary phloem; sx – secondary xylem; cz - cambium zone; mr – medullar race, ob. 10 x

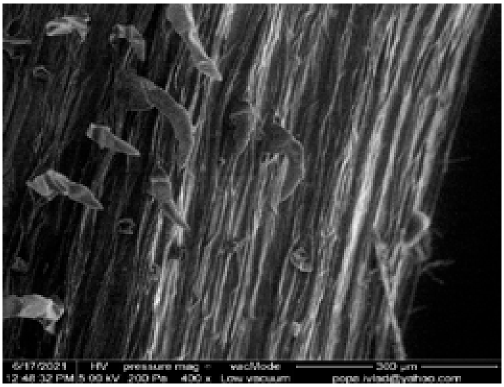

Figure 1b. Scanning electron microscopy (SEM) of the *Ocimum basilicum* 'Aromat de Buzău' stem,400 x

The bark consists of cords of angular colenchyma in the 4 ribs (3-4 rows of cells), and in the rest bands of parenchyma (chlorenchyma) 3-4 rows of cells. Along the ribs, at the level of the parenchymal tissue, there are also large air cavities. The last layer of the bark is primary type endoderm. In the central cylinder, the conducting bundles are collateral, of secondary origin, forming two concentric rings. On the outside is the thin secondary phloem ring, consisting of sieved vessels, attachment cells and cellulosic parenchyma. The phloem has thin segments of sclerenchymal fibers at the periphery. Inside there is the thick secondary xylem ring, made up of wooden vessels, wooden parenchyma and wood fibers. The xylem vessels are separated by sclerified medullary rays. The conducting bundles are larger at the corners. The wood of primary origin is reduced, and the wooden vessels are separated by parenchymal medullary rays. The marrow is parenchymal, cellulose in nature, multi-layered, 10-14 rows of cells.

Leaf anatomy

The epidermis of the leaf seen from the front is made up of cells with strongly wavy walls.

The stomata are present in both epidermis, but more numerous in the lower epidermis, the leaf being amphistomatic with diacytic stomata (Figure 2a, 2b).

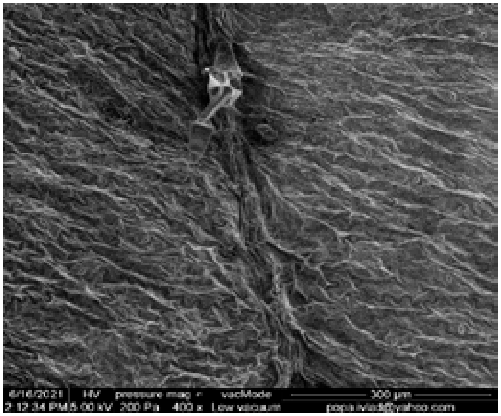

Figure 2a. Scanning electron microscopy (SEM) image of the upper leaf epidermis of the *Ocimum basilicum* 'Aromat de Buzău' 400x

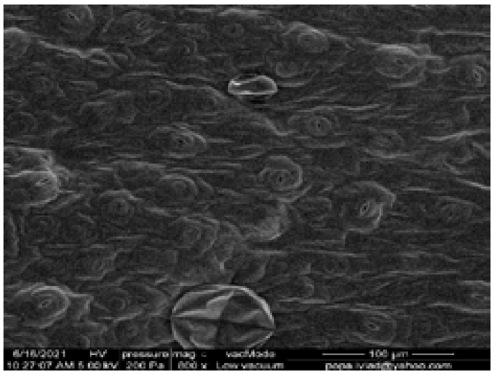

Figure 2b. Scanning electron microscopy (SEM) image of the lower leaf epidermis of the *Ocimum basilicum* 'Aromat de Buzău', 800 x

In some of the published articles it was noted that the stomatas are anomocytic type on the leaves of basil (Nassar et al., 2014). The median rib is strongly prominent on the lower face, with a hypodermic colenchyma and a free woody conducting bundle, located centrally, with xylem on the outside and phloem on the inside. On the adaxial side, numerous bent uniseriate non-glandular hairs are present, consisting of 3-5 cells, rarely hairs capitate or peltate secretory. In cross-section, both epidermis are unistratified, consisting of tabular cells tightly joined together, with the outer walls covered by a thin cuticle. Some regions of the upper and lower epidermis are made up of papillary cells, consisting of much larger oval cells.
In both epidermis there are nongladular and glandular hairs, peltate or capitate and scaly specific to the basil leaf (Zamfirache et al., 2008; Trettel et al., 2019; Poursaeid et al., 2020).

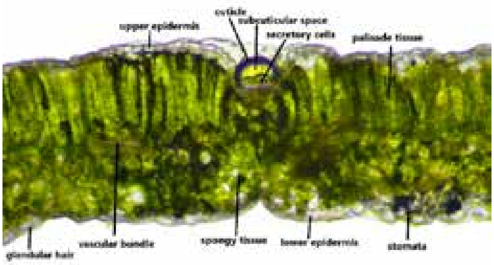

Figure 2c. Transversal section in the lamina leaf of the *Ocimum basilicum* de 'Aromat Buzău', ob. 10 x

The capitate hairs are composed of a base cell, a short stem cell, and a terminal cell consisting of either an elongated cell or two broad cells, seen in both epidermis. The peltate hairs consist of four terminal cells, seen in the lower epidermis. The scaly hairs were observed in the upper epidermis, being made up of a basal cell, on which secretory cells arranged in the rosette are present. The leaf mesophyll is bifacial, with palisade tissue under the upper epidermis, consisting of a single row of elongated cells, rich in chloroplasts and spongy tissue, below the lower epidermis, consisting of 3-4 rows of ovoid cells and with intercellular spaces.

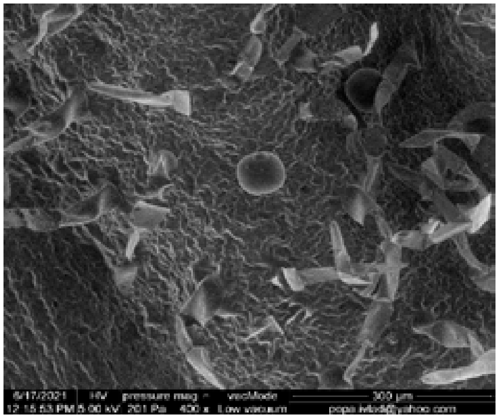

Figure 3. Scanning electron microscopy (SEM) image of the petal flower of the *Ocimum basilicum* 'Aromat de Buzău', 400 x

The composition of the volatile oil
Ocimum basilicum species has numerous local cultivars and populations, which have a very varied chemical composition of volatile oil (Burzo, 2015). Characteristic of the volatile oil are as follows: high content of methyl chavicol (63.56%), linalool (17.10%), followed smaller amounts of 1,8-cineole (4.01%) and α-epi cadinol (2.64%) were found, as showed in Figure 4.

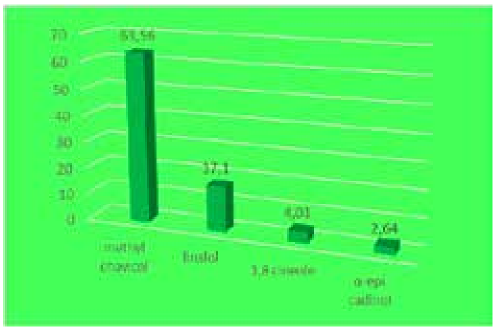

Figure 4. Major chemical compounds in the *Ocimum basilicum* 'Aromat de Buzău'

In addition, some compounds were detected in quantities of 1.06 % to 1.73% such as: δ-guaien (1.06%), β-ocimene (1.27%) and germacrene D (1.73%). Other constituents (the remainder which comprised 48 components) had a value below 1% (from 0.02% α-phellandrene to 0.95% bicyclogermacrene).

Other studies found that the chemical compound methyl chavicol (estragole) was also noted in the volatile oil of the species *Ocimum ciliatum* having the highest share (87.6%) followed by methyl eugenol (2.6%) and 1,8 - cineole (1.7%). Studies conducted by Moghaddam et al. (2014) showed that the oil extracted from this species had antimicrobial activity on all bacteria tested. The chemical compound linalool is a high weight monoterpenoid, commonly found in the composition of *Ocimum basilicum* volatile oil.

It has a role as a plant metabolite, a antimicrobial agent and, a fragrance (source:https://pubchem.ncbi.nlm.nih.gov/compound/Linalool).

The research conducted by Jailawi (2021) shows that the main chemical compounds present in the volatile oil extracted from the 'Aromat de Buzău' grown in hydroponic system during the flowering period were: linalool 27.52%, methyleugenol 17.48%, 1,8-cineole 6.69%, α-epi-cadinol 5.78% and α-bergamotene 4.69%. From the analyzes performed by Al Abbasy et al. (2015) it is showed that linalool has highest amount (69.87%) compared to other bioactive compounds considered major such as: geraniol (9.75%), p-allylanisole (6.02%), 1,8-cineole (4.90%), trans-α-bergamotene (2.36%) and neryl acetate (1.24%). Also, the chemical compound, linalool, predominated in the volatile oil analyzed by Guerrero-Lagunes et al. (2020) in plants grown in a greenhouse in a hydroponic system. *Ocimum basilicum* is characterized by a high linalool content, which makes it useful in the food, pharmaceutical and perfumery industries (Al Abbasy et al., 2015).

The species *Ocimum basilicum* is characterized by a high linalool content, which makes it useful in the food, pharmaceutical and perfumery industries (Al Abbasy et al., 2015).

The category of the main chemical compounds present in the volatile oil of *Ocimum basilicum* also includes 1.8 cineole (eucalyptol).

Following the studies performed by Omer et al., (2008) of three species of basil and four varieties grown in saline soil, it was showed that the main chemical compounds were: linalool (19.93%-40.41%), 1,8 - cineole (19.1%-10.52%), methyl chavicol (28.92%-46.48%), eugenol (4.08 %), γ-elemene (4.18%-3.22%) α-trans-bergamotene (2.52%- 4.57%), germacrene D (4.04%), t-cadinol (4.17%-10.9%). Eucalyptol is a terpenoid oxide with anti-inflammatory and antioxidant effects in various conditions such as pancreatitis, respiratory, cardiovascular and neurodegenerative diseases (Seol & Kim, 2016). The results obtained are in accordance with those of the literature.

CONCLUSIONS

The species *Ocimum basilicum* 'Aromat de Buzău' showed secretory hairs on the epidermis.

Curved uniseriate nonglandular hairs, are present in the epidermis of the basil stem.

The leaf blade is amphistomatic, with diacytic type stomata.

The nongladular and glandular hairs, peltate or capitate and scaly are present in both epidermis of the leaf.

The mesophile of the lamina is bifacial, with palisade tissue under the upper epidermis, consisting of a single row of elongated cells and spongy tissue, below the lower epidermis, consisting of 3-4 rows of ovoid cells and with intercellular spaces.

Regarding the composition of the volatile oil, the study shows that the *Ocimum basilicum* plants, the 'Aromat de Buzău', is an important source of chemical compounds that can be used in different industries.

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