Volume 26(1), 23 - 28, 2022
JOURNAL of Horticulture, Forestry and Biotechnology
www.journal-hfb.usab-tm.ro

Aspects of secretory tissues and the composition of volatile oil at basil cultivated in the hydroponic system

Govoreanu Elena Alina[1], Ion Violeta Alexandra[2], Badea Monica Luminița[1], Săvulescu Elena[1], Popa V.[2], Drăghici Elena Maria[1*]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Research Center for Studies of Food Quality and Agricultural Products, 59 Mărăști Blvd, District 1, Bucharest, România

*Corresponding author. Email: draghiciem@yahoo.com

Abstract The species *Ocimum basilicum* L. it is an aromatic and medicinal plant with uses in various industries. In the 'Macedon' and 'Crispum' basil cultivars were analyzed the secretory hairs and the composition of the volatile oil from the leaves and flowers during the flowering period. At the level of the leaves, glandular hairs were found in both epidermis (upper and lower) identified by the Inspect S50 scanning electron microscope. The oil has been extracted by hydro distillation and analyzed by gas chromatography – mass spectrometry (GC-MS). The majority of the chemical compounds to the 'Crispum' were 1,8-cineole 3.32%, terpinen-4-ol, 4.56%, α-epi-cadinol 4.68%, trans-α-bergamotene 5.53%, eugenol, 17.30%, methyl chavicol 18.42% and linalool 26.94%. In the composition of the volatile oil from 'Macedon' the chemical compounds were represented by cis-verbenol 2.01%, α-bisabolene 2.52%, 6-methyl-5-heptene-2-one 2.45%, isocaryophylene 3.17%, geraniol 3.67%, nerol 5.83%, neral 30.71% and geranial 36.25%..

Key words

leaf, secretory hairs, linalool, methyl chavicol

Ocimum basilicum L. (*Lamiaceae*) is an annual plant, native to Africa and Asia [33]. The genus *Ocimum* comprising of more than 150 species throughout the world [21].

New cultivars were created in Romania, which were gradually expanded in culture [33]. Basil leaves are widely used for flavoring culinary preparations, due to the variation of chemical compounds secreted by the secretory hairs present in the leaves, sporadically and in the petals. It is known that in plants of the *Lamiaceae* family monoterpenes predominate, the volatile oil being produced by secretory hairs located in the aerial parts [11].

Basil leaves have two types of glandular trichomes, pelted and capitate, characteristic of species of the *Lamiaceae* family. Secretory glands consist of several cells providing different functions [6].

The pharmaceutical properties and aroma of *Lamiaceae* plants are due to the presence of monoterpenes and sesquiterpenes [28; 29]. The volatile oil extracted from leaves, flowers and young stems contains chemical compounds such as linalool, methyl cavicol, camphor, cineole, α-pinene, methyl cinnamate, eugenol, oleanolic acid, β-sitosterol, anethole, triterpene saponosides, tanoids etc. [20].

Essential oil from *Ocimum basilicum* has multiple properties: antifungal [24; 4; 14], repellent insecticide [4], anti-inflammatory [17; 23], antioxidants [19] and antimicrobials [30; 21; 22]. Many species of the *Lamiaceae* family, such as *Tymus*, *Salvia*, *Mentha* and *Origanum*, are cultivated as sources of essential oils [13; 25] and are used to treat various ailments such as diabetes, colds, migraines, fever, cardiovascular disease etc. [22]. The purpose of this work is to analyze the composition of the volatile oil in *Ocimum basilicum* 'Crispum' and 'Macedon' and also the micromorphological of the secretory hairs.

Material and Method

The biological material used was represented by plants of *Ocimum basilicum* 'Crispum' and 'Macedon' grown in hydroponic system in the greenhouse within the Research Center for Studies of Food Quality and Agricultural Products, U.S.A.M.V. Bucharest (Figure 1). The analyses were performed during the flowering period, in May, 2021.

Micromorphological observations were made on basil leaves and flowers using the Inspect S50 scanning electron microscope (SEM).

Figure 1. Basil culture in the greenhouse

Volatile oil analysis

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

The fresh harvested plants (300 g) were hydrodistillated for 3 h in a Clevenger-type apparatus. Analysis of the essential oils was performed on an Agilent 6890N GC coupled with a 5973 Network single quadruple mass spectrophotometer detector in Electron Ionization (EI) mode and a ALS 7683 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 [9].

Results and Discussions

Secretory tissues

Ocimum species have glandular and non-glandular hairs on both the adaxial and abaxial surfaces of the leaves, on the sepals and sometimes on the petals [31]. In the species *Ocimum basilicum* 'Crispum' and 'Macedon', peltate and capitate glandular hairs were observed, as well as non-glandular hairs (Figure 2-7). 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. Peltate hairs generally have a large subcuticular space, with a rigid cuticle that becomes thin during the maturation of the gland, thus facilitating its rupture and removal of secreted substances.

Peltate glandular hairs are also common in other species of the *Lamiaceae* family, such as *Salvia verticillata* [12], *Salvia officinalis* [7], *Dracocephalum moldavica* [3], *Mentha x piperita* [32], and *Rosmarinus officinalis* [14]. In basil plants, peltate hairs show a variability in the number of secretory cells. In the species *Ocimum gratissimum*, large glands with four cells were found, as well as glands with 8 secretory cells [6].

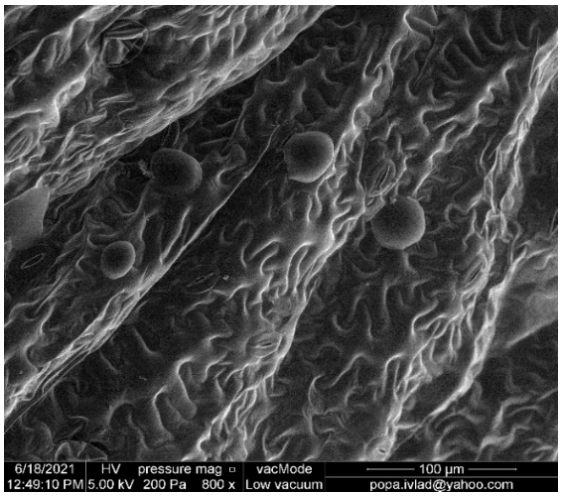

Figure 2. Scanning electron microscopy (SEM) image of the lower leaf epidermis of the *Ocimum basilicum* 'Macedon', 800 x

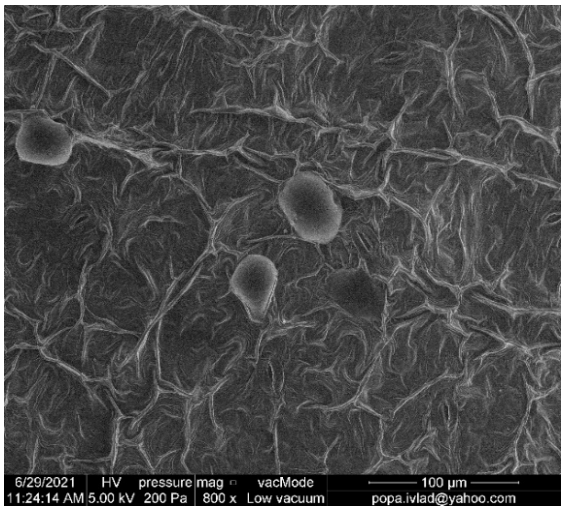

Figure 3. Scanning electron microscopy (SEM) image of the upper leaf epidermis of the *Ocimum basilicum* 'Macedon', 800 x

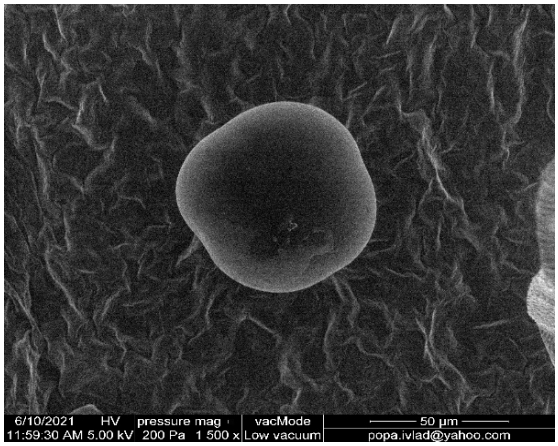

Figure 4. Scanning electron microscopy (SEM) image of the petal flower of the *Ocimum basilicum* 'Crispum', 1500 x

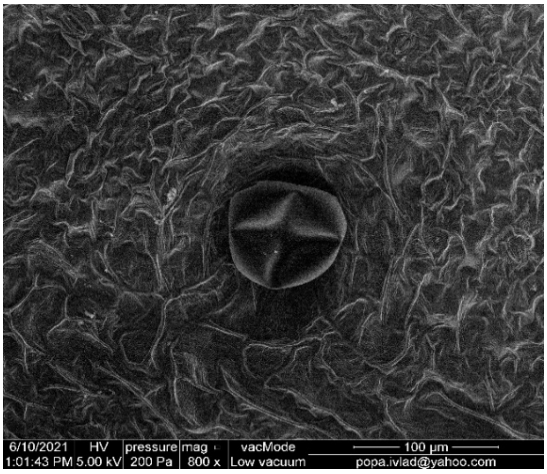

Figure 5. Scanning electron microscopy (SEM) image of the lower leaf epidermis of the *Ocimum basilicum* 'Crispum', 800 x

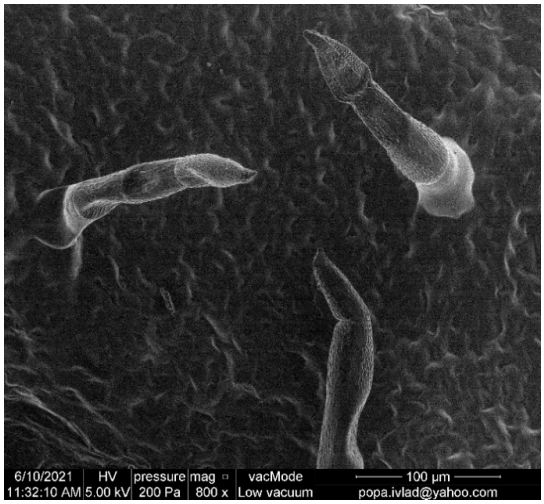

Figure 6. Scanning electron microscopy (SEM) image of the petal flower of the *Ocimum basilicum* 'Crispum', 800 x

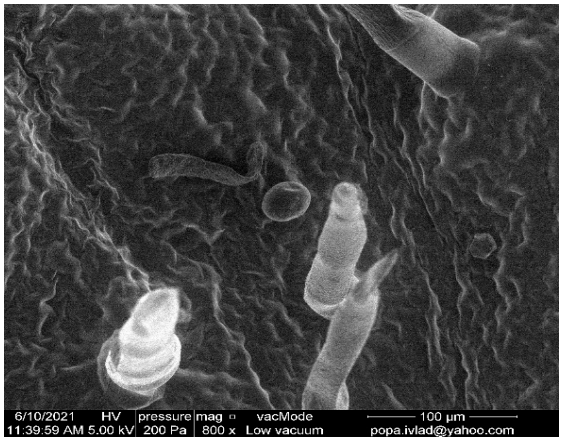

Figure 7. Scanning electron microscopy (SEM) image of the petal flower of the *Ocimum basilicum* 'Crispum', 800 x

The composition of the volatile oil

The composition of the volatile oil in *Ocimum basilicum* varied depending on the cultivars. The major chemical compounds for the 'Crispum' were: 1,8-cineole 3.32%, terpinenen-4-ol, 4.56%, α-epi-cadinol 4.68%, trans-α-bergamotene 5.53%, eugenol 17.30%, methyl chavicol 18.42%, and linalool 26.94% (Figure 8). The presence of minor chemical compounds specific to the 'Crispum' was also observed, such as α-phellandrene, α-terpinene, o-cymene, γ-terpinene, cis-sabinene hydrate, camphor, isoborneol, L-α-terpineol, terpinen-4-ol, L-α-bornyl acetate, methyl cinnamate, β-elemene, α-cubebene, methyleugenol, cis-α-bergamotene, α-guaiene, cis-muurola-3,5-diene, γ-muurolene, δ-guaiene, γ-cadinene, cubenol, and α-cadinol. Minor chemical compounds specific to the 'Macedon' were represented by: n-octanal, 2-hexen-1-ol, acetate, exo-fenchol, β-citral, trans-chrysanthemal, citronellal, limonene oxide, cis-verbenol, geranyl acetate, nerol acetate, geranyl acetate, cis-β-farnesene, and α-bisabolene. The majority of chemical compounds in the species *Ocimum basilicum* found in other research studies were: methyl chavicol, linalool (Sastry et al., 2012), methyl chavicol, linalool, epi-α-cadinol, trans-α-bergamotene (Sajjadi, 2006), methyl eugenol and methyl chavicol (Joshi, 2014), 1,8-cineole, linalool, camphor, α-terpineol, methyl chavicol, eugenol (Barcelos, 2013), methyl chavicol, linalool, geranial, eucalyptol, methyl cinnamate, α-bergamotene, and eugenol (Nour et al., 2012). In the composition of the volatile oil analyzed by Oliveira et al. (2013), from dry leaves and fresh basil leaves prevailed linalool (29.50%-32.26%) methyl chavicolul (36.81%-41.62%) și eucalyptolul (9.99%-7.68%). The chemical compounds linalool (13.1% and 21.1%) and methyl chavicol (56.7% and 37.2%) also prevailed in the volatile oil from the leaves and inflorescences of basil plants grown in hydroponic system [5]. The analysis of the studies showed that methyl chavicol and linalool predominated in the composition of the volatile oil in

the species *Ocimum basilicum*. The composition of the volatile oil varied depending on the growing conditions, ecotype, the organ analyzed and the condition of the plant material (dry or fresh). The results of the study conducted by Hossain et al., 2010 suggest that organic oils and extracts of *Ocimum basilicum* could be a source of natural antimicrobial agents for use in the food and / or pharmaceutical industries (Figure 8 and 9).

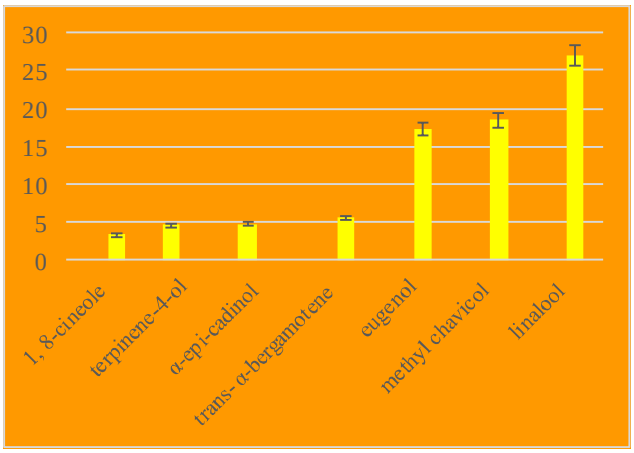

Figure 8. Major compounds chemical in 'Crispum' cultivar

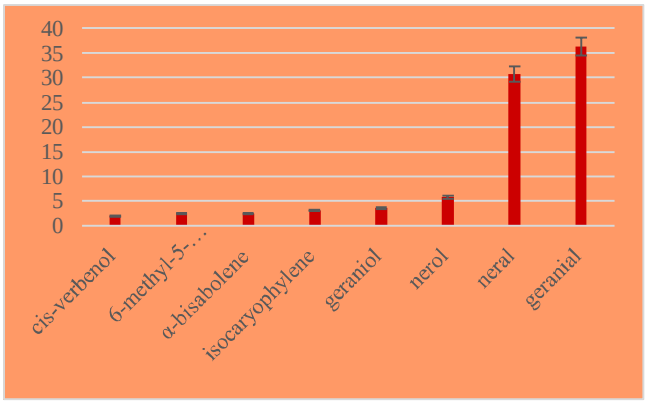

Figure 9. Major compounds chemical in 'Macedon' cultivar

Acknowledgements

The data presented in this paper have been obtained in for the doctoral thesis *Morpho-anatomical, biochemical and antimicrobial research on some species of aromatic and medicinal plants from the Lamiaceae family* from the Doctoral School of Engineering and Management of Vegetal and Animal Resources from the Faculty of Veterinary Medicine. University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Conclusions

Secretory hairs, pelted, and capitate were present in both basil cultivars, more frequently in the lower epidermis of the leaf and very rarely in flowers.

The major chemical compounds present in the volatile oil in the species *Ocimum basilicum* 'Crispum' were: 1,8-cineole, terpinene 4-ol, α-epi-cadinol, trans-α-bergamotene, eugenol, methyl chavicol, and linalool.

In 'Macedon' the majority of the chemical compounds were cis-verbenol, α-bisabolene, 6-methyl-5-heptene-2-one, isocaryophylene, geraniol, nerol, neral, and geranial.

The variation in the chemical composition of the volatile oil gives the plants a specific flavour having a wide use both in gastronomy and in the pharmaceutical and cosmetic industry.

References

[1] Asmaa A. J., Jerca I. O., Drăghici E. M. (2021). Study on the cultivation of 'Genovese' and 'Aromat de Buzău' basil varieties in the NFT

system., Journal of Horticulture, Forestry and Biotechnology, 25 (2), 27-31, www.journal-hfb.usab-tm.ro

[2] Barcelos R. C., Jham G. N., Dhingra O. D, Mendonça F.A., Valente V. M. M. (2013). Identification and quantification of the major fungi toxic components of the brazilian basil (Ocimum basilicum L.) essential oil. J Food Res., vol. 2, no. 5.

[3] Dmitruk M., Sulborska A., Żuraw B., Stawiarz E., Weryszko-Chmielewska, E. (2019). Sites of secretion of bioactive compounds in leaves of Dracocephalum moldavica L.: anatomical, histochemical, and essential oil study, Brazilian Journal of Botany, 42:701–715

[4] Dube S., Upadhyay P.D.,Tripathi S.C. (1989). Antifungal, physicochemical, and insect repelling activity of the essential oil of Ocimum basilicum, Can. J. Bot. 67, 2085–2087.

[5] Hassanpouraghdam M.B., Gohari G. R., Tabatabaei S. J. and Dadpour M. R. (2010). Inflorescence and leaves essential oil composition of hydroponically grown Ocimum basilicum L. J. Serb. Chem. Soc. 75 (10) 1361–1368.

[6] Hazzoumi Z., Moustakime Y., Joutei K.A. (2017), Efect of arbuscular mycorrhizal fungi and water stress on ultrastructural change of glandular hairs and essential oil compositions in *Ocimum gratissimum*, Chem. Biol. Technol. Agric., 4:20.

[7] Hazzoumi Z., Moustakime Y., Joutei K.A. (2019), Essential oi land glandular hairs: Diversity and Roles, Essential Oil-Oils of Nature, 1-16.

[8] Hossain M.A., Kabir M.J., Salehuddin S.M., Mizanur Rahman S.M., Das A.K., Singha S.K., Alam Md.K., Rahman A. (2010). Antibacterial properties of essential oils and methanol extracts of sweet basil *Ocimum basilicum* occurring in Bangladesh, Pharmaceutical Biology, 48(5): 504–511.

[9] Ion A.V., Nicolau F., Petre A., Bujor O.C., Bădulescu L. (2020). Variation of bioactive compounds in organic *Ocimum basilicum* during freeze-drying processing. Scientific Papers, Series B, Horticulture, vol. LXIV, No 1, 397-404, ISSN-L 2285-5653.

[10] Joshi R.K. (2014). Chemical composition and antimicrobial activity of the essential oil of *Ocimum basilicum* L. (sweet basil) from Western Ghats of North West Karnataka, India. Anc Sci Life, 33:151–6.

[11] Kowalski R., Kowalska G., Jankowska M., Nawrocka A., Kałwa K., Pankiewicz U., Włodarczyk-Stasiak M. (2019). Secretory structures and essential oil composition of selected industrial species of Lamiaceae, Acta Sci. Pol. Hortorum Cultus, 18(2), 53–69, ISSN 1644-0692.

[12] Krstic L., Malencic D., Anackov, G. (2006). Structural investigations of trichomes and essential oil composition of *Salvia verticillata*, Botanica Helvetica, 116: 159-168.

[13] Lewinsohn E., Ziv-Raz I., Dudai N., Tadmor Y., Lastochkin E., Larkov O., Chaimovitsh D., Ravid U., Putievsky, E., Pichersky E., Shoham Y., (2000). Biosynthesis of estragole and methyl-eugenol in sweet basil (*Ocimum basilicum* L). Developmental and chemotypic association of allylphenol O-methyltransferase activities. Plant Science 160, 27–35.

[14] Marin M., Koko Y., Duletić-Laušević S., Marin P.D., Rančić D., Dajic-Stevanovic Z. (2006). Glandular trichomes on the leaves of *Rosmarinus officinalis*: Morphology, stereology and histochemistry. Volume 72, Issue 3, 378-382.

[15] Nour A.H., Nour A.H, Yusoff M.M., Jessinta S. (2012). Bioactive compounds from basil (*Ocimum basilicum*) essential oils with larvicidal activity against aedes aegypti larvae 3rd International Conference on Biology, Environment and Chemistry IPCBEE, vol.46, 21-24.

[16] Oxenham S.K., Svoboda K.P., Walters D.R. (2005). Antifungal activity of the essential oil of basil *(Ocimum basilicum*). Journal of Phytopathology, 153: 174–80.

[17] Okoye F.B.C, Obonga W.O, Onyegbule F.A., Ndu O.O.,Ihekwereme C.P. (2014). Chemical composition and anti-inflammatory activity of essential oils from the leaves of Ocimum basilicum L. and *Ocimum gratissimum* L. (Lamiaceae). Int J Pharm Sci Res; 5:2174-80.

[18] Oliveira R.A., de Moreira I.S., Oliveira F.F. (2013). Linalool and methyl chavicol present basil (Ocimum sp.) cultivated in Brazil, Rev. Bras. Pl. Med., Campinas, v.15, n.2, 309-311.

[19] Pandey V., Patel A., Patra D.D. (2016). Integrated nutrient regimes ameliorate crop productivity, nutritive value, antioxidant activity and volatiles in basil (*Ocimum basilicum* L.). Industrial Crops and Products. 87: 124–31.

[20] Pârvu C. (2006). The universe of plants. Ed. ASAB, Bucharest, 97.

[21] Poonkodi K. (2016). Chemical composition of essential oil of *Ocimum basilicum* L. (basil) and its biological activities–an overview, Innovare Academic Sciences Pvt Ltd., Vol 3, Issue 3, ISSN- 2394-5125, 56-62.

[22] Purushothaman B., Prasanna Srinivasan R., Suganthi P., Ranganathan B., Gimbun J., Shanmugam K. (2018). A Comprehensive Review on *Ocimum basilicum*, Journal of Natural Remedies, ISSN: 2320-3358, Vol 18 (3), 71-85.

[23] Raina P., Deepak M., Chandrasekaran C.V., Agarwal A., Wagh N., Kaul-Ghanekar R. (2016). Comparative analysis of anti-inflammatory activity of aqueous and methanolic extracts of *Ocimum basilicumin* RAW 264.7, SW1353 and human primary chondrocytes. Journal of Herbal Medicine, 6(1): 28–36.

[24] Reuveni R., Fleischer A., Putievsky E. (1984). Fungistatic activity of essential oils from *Ocimum basilicum* chemotypes, Phytopath. Z. 110, 20–22.
[25] Sangwan N.S., Farooqi A.H.A., Shabih F., Sangwan R.S. (2001). Regulation of essential oil production in plants. Plant Growth Regul. 34: 3-21.
[26] Sajjadi S.E. (2006). Analysis of the essential oils of two cultivated basil (*Ocimum basilicum* L.) from Iran. Daru, vol. 14, no.3, 128-130.
[27] Sastry K.P, Ramesh Kumar R., Niranjan Kumar A., Sneha G., Elizabeth M. (2012). Morpho-chemical description and antimicrobial activity of different *Ocimum* species. J Plant Dev, 19:53-64.
[28] Skoula M., Harborne J.B. (2002). The taxonomy and chemistry of Origanum. In: Oregano: the genera *Origanum* and Lippia, Kintzios S.E. (ed.), 1st ed., Vol. 25. Taylor and Francis, London, 67–108.
[29] Stahl-Biskup E. (2002). Essential oil chemistry of the genus *Thymus* – a global view. In: Thyme: The genus *Thymus* L., Stahl-Biskup, E., Saez, F. (eds.), 1st ed., Vol. 24. Taylor, Francis, London–New York, 75–124.
[30] Suppakul P., Miltz J., Sonneveld K., Bigger S.W. (2003). Antimicrobial properties of basil and its possible application in food packaging. Journal of Agricultural and Food Chemistry. 51: 3197–207.
[31] Ștefan M., Zamfirache M, Pădurariu C., Trută E., Gostin I. (2013). The composition and antibacterial activity of essential oils in three Ocimum species growing in Romania. Cent. Eur. J. Biol., 8(6):600-608.
[32] Turner G.W., Gershenzon J., Croteau R.B. (2000). *Distribution of Peltate Glandular Trichomes on Developing Leaves of Peppermint*, Plant Physiol. 2000 Oct; 124(2): 655–664.
[33] Vînătoru C., Mușat Bianca, Bratu Camelia (2019). Tratat de legumicultură specială. Editura ALPHA MDN, Buzău.