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RESEARCHES CONCERNING THE VOLATILE OILS PRODUCED BY SOME SPECIES OF AROMATIC PLANTS CULTIVATED IN THE NORTHERN AREA OF ROMANIA AND THEIR POSSIBLE PHYTOTHERAPEUTIC EFFECTS

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SYNOPSIS

Were investigated 2 populations of *Ocimum basilicum* L. and to 3 taxa of *Pelargonium* genus - *P. radens*, *P. fragans*, and *P. zonale*. The analysis of secretor hairs was performed by a light microscopy method. The volatile oils extraction was conducted using a Clevenger hydro distillation system; the component separation was made by gas chromatography combined with the mass spectrometry. Essential oils were tested upon strains of *Escherichia coli* and *Staphylococcus aureus,* using the antibiogramme method. In all studied taxa, the secretor hairs from vegetative organs are multi cellular structures, with specific conformation, depending on taxon and its age. The number of volatile compounds identified in the oil samples is variable, according to the analyzed taxon, the harvesting moment of the samples, as well as the analyzed organ. The investigated oil groups have a variable inhibitory effect, depending on their composition and concentration on *S. aureus* strain and a mild influence on *E. coli* strain. The response of tested fungi varies by the specific growth speed, which was evaluated at analysis intervals.

INTRODUCTION

The *Ocimum* genus, belonging to the *Lamiaceae* family, includes annual and perennial herbal plants, as well as shrubs, from tropical and subtropical zones of Asia, Africa and South America, plants that are widely spread in the world. The complex taxonomy of the genus, determined by interspecific hybridizations and polyploidy, includes 150 species, according to some authors (Labra et al., 2004; Pushpangadan and Bradu, 1995, cf. Telci et al., 2006), while Paton et al., 1999 (cf. Telci et al., 2006) proposed 65 species; other researchers accept only the existence of 35 species to be included in this genus. The classification is difficult to be realized because of great intra- and inter specific morphological variability of the genus and because of man intervention in cultivation process, by selection and hybridizing.

The most important species of *Ocimum* genus are *O. sanctum* L**.** and *O. basilicum* L.; this latter species, usually named common basil or sweet basil, is a herbal, annual, allogamous plant, with extraordinary culinary and medicinal properties, and it is characterized by a considerable morphological and biochemical variability (Telci et al., 2006); in Romania it grows in Baragan, Oltenia and Timis plains.

There are numerous chemo types for both species. Their representatives, very similar by morphology, can be differentiated by their chemical composition (Kothari et al., 2004). The leaf volatile oils contain, as shown by gas-chromatography, eugenol, eugenal, carvacrol, methyl-chavicol (estragol), limatrol, cariophyllin, while the seed volatile oils have fatty acids and sitosterol; in addition, the seed mucilage contains some levels of sugars and the anthocyans are present in green leaves.

In *Ocimum basilicum* L. (*common basil)* a remarkable infraspecific variation exists in plant morphology and in essential oil composition (Pascual-Villalobos and Ballesta-Acosta, 2003). The leaves of this taxon contain numerous active principles, but the most important component is the volatile oil (0.20-1.00%). The quantity and quality of these volatile oils are influenced by a lot of factors (soil nature and properties, climate, harvesting moment, subspecies, chemo variability etc). In addition, its volatile oils contain nematicidal, fungi static and anti fungal substances (against *Candida albicans, Penicillium notatum*, and *Microsporeum gyseum)* or antimicrobial ones (against *Staphylococcus aureus, Salmonella enteritidis* and *Escherichia coli)*. The insecticidal effect of the volatile essential oils from *Ocimum basilicum* L. on *Callosobruchus maculates* coleopteran, showed that the highly toxic oils, those with a 40-100% insect mortality rate, were the oils that contained eugenol or methyl chavicol as a main compound (Pascual-Villalobos and Ballesta-Acosta, 2003).

In literature there are few references regarding the toxicity of the chemical components from the species belonging to the *Ocimum* genum. However it is known that common basil contains these kinds of dangerous substances - safrol, cafeic acid, triptophan, quercetin. Triptophan conducts to a respiratory disease for cows, the cafeic acid (a phenolic acid) can inhibit the digestion for ruminant animals and quercetinul leads to cancer secondary to a bovine papilomavirus.

We can state that for *Ocimum sanctum* and *Ocimum basilicum*, as well as other species from the *Ocimum* genum, there is a great site-specific variance, fact that opens up the favorable perspectives on improvement and selection.

The *Geranium*, specie of the *Pelargonium* genus – *Geraniaceae* family, belongs to the group of rustic species used at interior decorations, balconies, windows and gardens. Because of its abundant and beautiful foliage its continuous flowering, the representatives of the kind are very popular flowers, loved and widely cultivated. At present, 250-300 cultivated taxa belonging to *Pelargonium* genus are known all over the world (Serbanescu, 1958; Gostin et al., 2000). Nowadays we encounter the mass production of certain kinds in such a manner that limits the selection and production to some botanical gardens (Clifton, 1945).

The species from the *Pelargonium* genus impose themselves by their decorative and odorant qualities, especially considering the leaves, (Serbanescu, 1958), as well as by their potential usage in aromatherapy. We already know the composition of the volatile oil extracted from the *P. citronellum* leaves (Demarne and van der Walt, 1993), and the one obtained from the *P. graveolens* ones (Fang et al., 1989) (cf. Burzo et al., 2005).

In the context presented above, our research try to analyze by comparison the volatile oil produced by two population belonging to the *Ocimum basilicum* L. specie, and by three taxa from the *Pelargonium* genus, cultivated in the Northern part of Moldavia (Romania), found in the flowering moment of vegetation, moment when the production of these products is considered maximal, their components giving the volatile oils obvious phytotherapeutic and aromatic properties.

MATERIAL AND METHODS OF RESEARCH

The biological material used for the present study consists of two populations of *Ocimum basilicum* L. and three species of *Pelargonium* genus: *P. zonale* (L.) L'Hér., *P. radens* (H.E. Moore) and *P. fragans* (Willd.), cultivated in unprotected (free) areas (the populations of *Ocimum basilicum* species), and in protected spaces (green houses) in "Anastasie Fatu" Botanical Garden of Iasi (the species of *Pelargonium* genus).

The experimental group of plants consisted of 40 individuals belonging to each of the studied taxa, and the tests were done in the period starting before the flowering until the senescence stage.

For the morph-anatomic researches, material processing was conducted in the Vegetal Morphology and Anatomy Laboratory, belonging to the Biology Faculty in the “Alexandru Ioan Cuza” University, Iasi, following classic material processing protocol, for vegetal material destined for optic microscopy studies. Pointing out the secretor structures was done by using the photographs of the surface of fresh foliar limb with Novex microscope at x 200. The microscopic images of the secretor structures which produce volatile oils were analyzed and graphically processed by a Minolta analogical photographic camera.

For the investigations regarding volatile oils composition, the biological material consisted of leaves and stems freshly harvested from plants in the flowering stage. The analyses were performed in *„Hortical" Laboratory* - centre for the study of fruits and vegetable quality, belonging to the Faculty of Horticulture from University for Agriculture Sciences and Veterinary Medicine, Bucharest. The work protocol included: volatile oils extraction using a Clevenger hydro distillation process, with a plant material / water ratio being of approximately 1:3, in a 3-4 hours extraction time; separation of volatile oil components by a gas chromatographic method combined with the mass spectrometry one, by using a GC-MS Agilant 6890 N with a spectrometric mass detector 5973 and an auto sampler; the DB5 chromatographic column has a length of 25 m and an interior diameter of 0.25 m. The separated compounds were identified by means of the Nist spectra database, and the peaks' position was confirmed by the Kovats indices.

The antimicrobial and antifungal effects of the volatile oils were accomplished on collection microorganisms:

- *for the antimicrobial effect, Staphylococcus aureus* ATCC-6538 strain (Gram positive bacteria) and *Escherichia coli* ATCC-10536 strain (Gram negative bacteria), from collections of the Microbiology Laboratory of Faculty of Biology, "Alexandru Ioan Cuza" University of Iasi, have been used. The testing was also carried out in this laboratory, by using the Kirby-Bauer disk diffusion method (Nimitan et al., 1995). Gelose was the culture medium, and the incubation was performed at 37^0C, for 16-18 hours; the bacterial cultures were used both for variants and control seeding (oil was diluted with D.M.S.O. - dimethyl sulfoxide - as solvent and was placed upon paper discs and inside glass cylinders); the results were established by measuring the inhibition area diameter (mm), for 2-3 times in different directions, using a marked rule. Result expression consisted of direct transcription of inhibition area diameter in categories of sensitive, resistant or intermediary strains.
- *for the fungistatic effect,* were used *Penicillium chrysogenum* and *Aspergillus niger* strains, also present in the collections of previously cited laboratory; the tests were conducted in Laboratory of Microbiology from Biological Research Institute of Iasi, by using the method described by Schadler et al., 2006, consisting in fungi cultivation on Sabouraud media; the colonies were measured at 5 and 10 days, moment when the colonies of control cultures reached the edges of the Petri dish; for the oval colonies, more diameters were measured, finally the average value of the measurements being considered; the comparative inhibition of growth was calculated using a specific formula by which the growth inhibition is determined, in % face to control.

RESULTS WITH DISCUSSIONS

Morph-anatomic results: The morph-anatomic investigations conducted on the airy vegetative organs on *Ocimum basilicum* confirm a data series presented in the specific literature regarding the structural idiosyncrasies of the *lamiacees* in general, but also bring new information: the presence of secretor hairs in a great number in the upper and middle third of the stem (Fig. 1 - images a and b) and their absence in the lower third; the hair presence in both epidermis of the foliar limb (Fig. 1 – image c); most numerous are the secretor hairs with a unicellular base, unicellular pedicel and a bi-cellular secretor part (present especially on the stem), as well as the ones with tetra- (rarely octo-) cellular secretor part (present mostly on the leaves); the secretor hairs with bi-cellular pedicel and unicellular secretor part are fewer (present mostly on the stem); the presence, on the stem, of some stomata located well beyond the epidermis cellular level, aspect that can be correlated with ecologic conditions that disposed the plants through the vegetation stage (enhanced environment relative humidity).

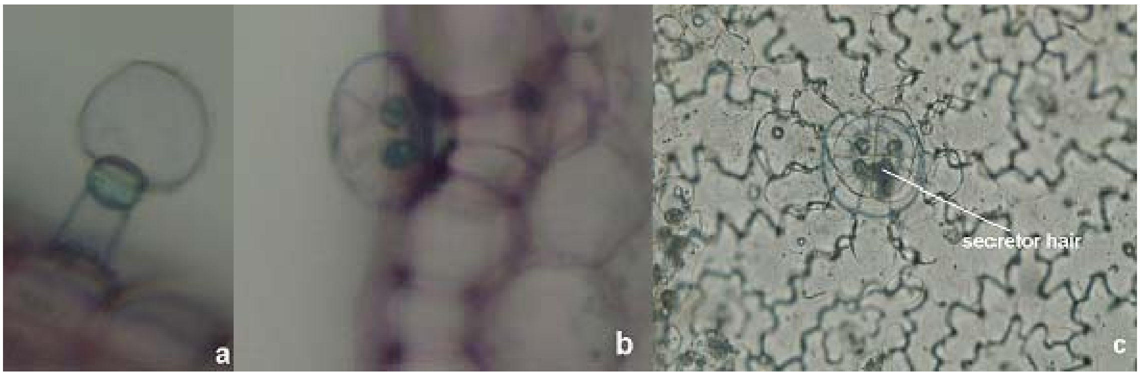

Fig. 1 – Types of secretor hairs for *Ocimum basilicum* L. placed on the upper third of the stem – a and b and on the foliar limb (inferior epidermis) - c (x400)

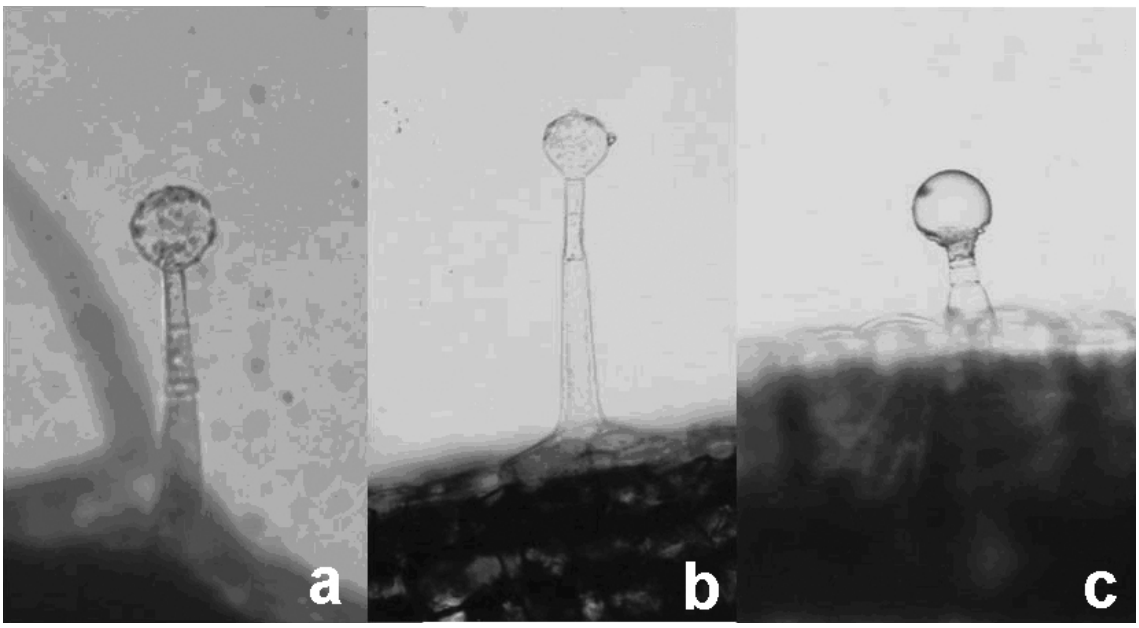

Fig. 2 - Secretive hairs (multi cellular hairs) in *P. zonale* (image a) and *P. radens* longer hair foot (image b); shorter hair foot (image c) (×200).

In *P. zonale* the analysis of the foliar surface points out the existence of multi cellular secretor hairs on the two sides of the limb; these formations have big dimensions compared to the component cells of the multi cellular leg at *Pelargonium zonale* (Fig. 2 – image a), and small ones at *Pelargonium odoratissimum*. The basis of the hair is situated between the epidermic cells, unicellular and polygonal. The aspect that wasn't pointed out during the ontogeny of the analyzed species was the morphological differences of the secretor hairs inducted by the age of the leaves. The secretor structures of the *P. radens*' essential oil were found to be multiple celled hairs located both in upper and in lower epidermis. The length of the hairs is variable, in accordance with the hair foot component cells (Fig. 2 – images b and c).

Volatile oil composition: For the available biologic material, the volatile oil analyses from the two *Ocimum basilicum* populations lead to the following interpretations: (fig. 3 – 4):

The number of identified compounds among the two populations is greater for sample I (28 compounds) compared to sample II (25 compounds). From total, 17 compounds are common for both samples, and 11 from these reach the maximum percentage in both groups, fact that confers the aromatic feature to the respective oils. In the first 5 positions, considering the registered concentration, we note: Germacren D (24.103, respectively 10.267%); β - Elemen (14.67, respectively 10.010%); r - Cadiden (5.632, respectively 9.095/); α - Guaien (5.095, respectively 3.487); γ- Elemen (5.915, respectively 1.504%).

The volatile oil composition analysis, conducted on the available plant material, did not show the presence of methyl cinamate and linalool, compounds found in the chemo types presented in literature (Burzo et al., 2005). This fact is probably related to the analysis moment, possibly not strictly corresponding to the biosynthesis stage of these compounds, as well as to the oil producing organ (Fig. 3 and Fig. 4)

At taxa from the *Pelargonium* genus the comparison analysis of the composition of the oils extracted from leaves of the analyzed species show a qualitative difference in relation to the chosen analysis moment (according to a specific ontogenetic stage). In *Pelargonium* taxa, before flowering moment, 11 compounds were identified for *P. fragans* and 64 for *P. radens*, while in the flowering stage 44 volatile compounds were noted for *P. radens* and 161 for *P. zonale*.

The component fractions of the volatile oils separated by the two analysis moments have an obvious valor variance, the preponderance of one or other component inducing specific aromatic properties for each analyzed sample. (Fig. 5 and Fig. 6).

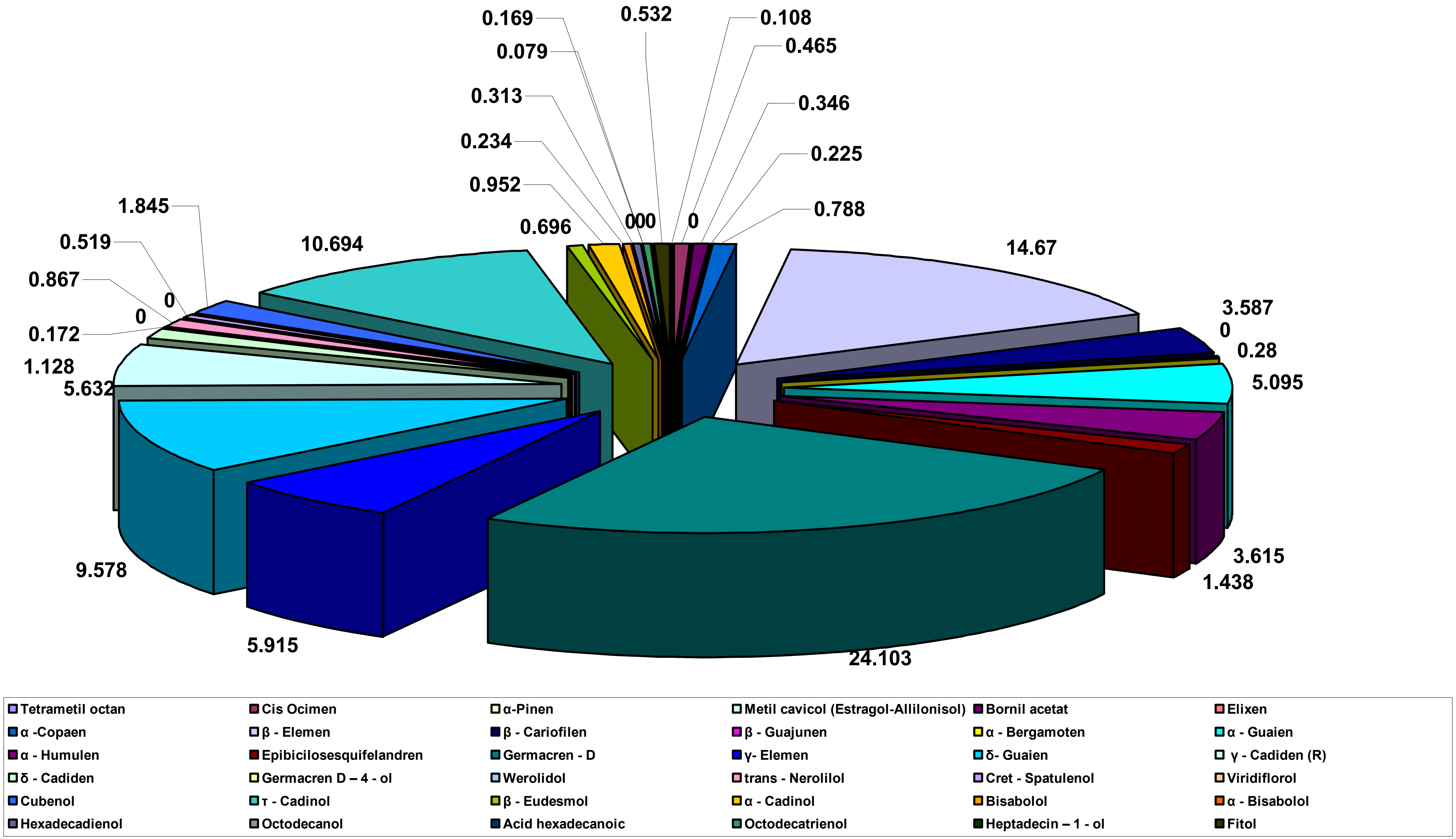

Fig. 3 - The composition of the volatile oil production at *Ocimum basilicum* L at flowering moment (sample I).

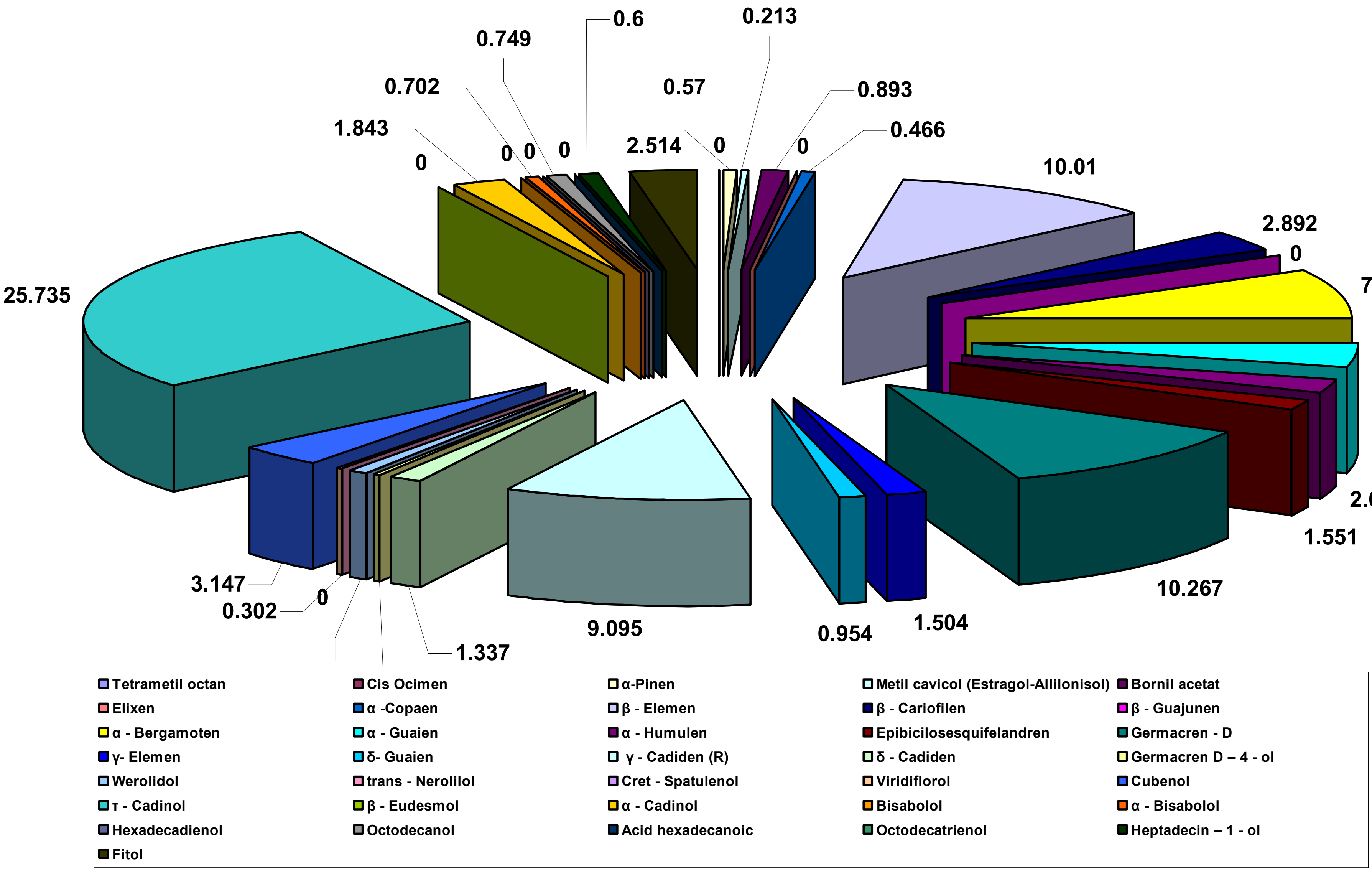

Fig. 4 - The composition of the volatile oil production at *Ocimum basilicum* L at flowering moment (sample II).

The testing of the antibacterial activity of the two analyzed oil samples from *Ocimum basilicum* plants, expressed by the diameter of the inhibition areas, shows that the inhibition is present only on the Gram positive strain for both 500 and 1000 ppm concentrations. So, for the Gram negative bacteria *Escherichia coli*, none of the two oil samples inhibits the growth and development of the microorganism, fact confirmed by the absence of inhibition areas. But for the Gram positive bacteria *Staphylococcus aureus* a different action pattern was evidenced. A weaker or a stronger inhibition of microorganism development was registered, correlated to the applied oil concentration. Among the two tested oil samples, probe I has a greater inhibitory effect at 1000 ppm concentration. The data presented by us is similar with the ones presented by the specific literature (Pascual-Villalobos et al., 2003; Kothari et al., 2004). For control, represented by a DMSO solvent for volatile oils, a slight inhibiting effect for the *Staphylococcus aureus* strain was observed. In the case of *Escherichia coli*, any effect was registered.

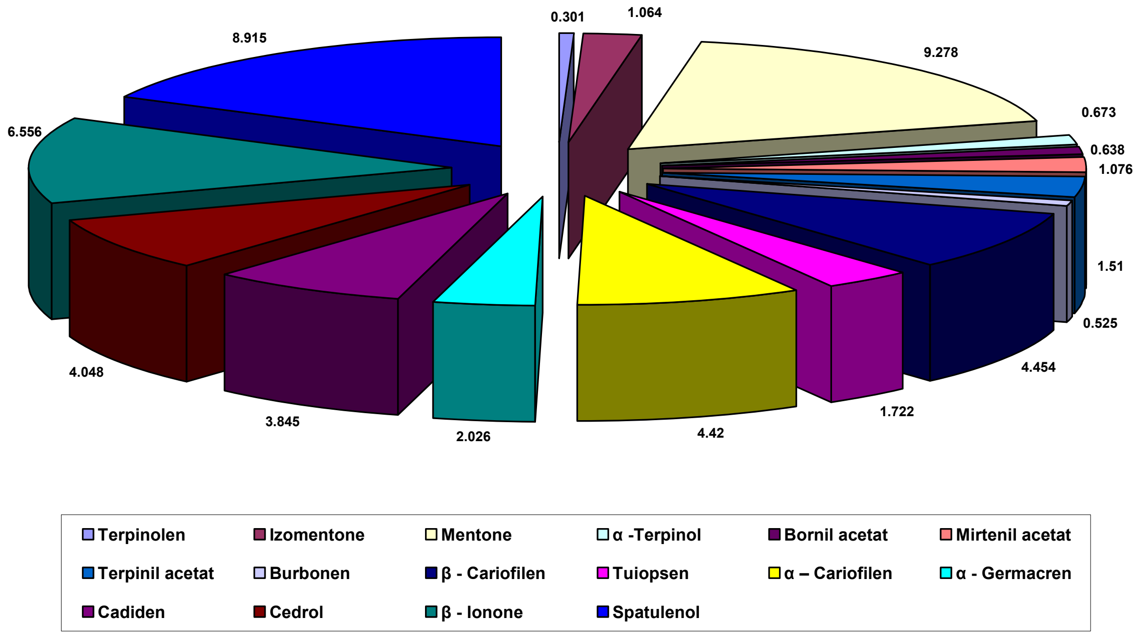

Fig. 5 - The composition of the volatile oil production at *Pelargonium zonale* L. before flowering moment.

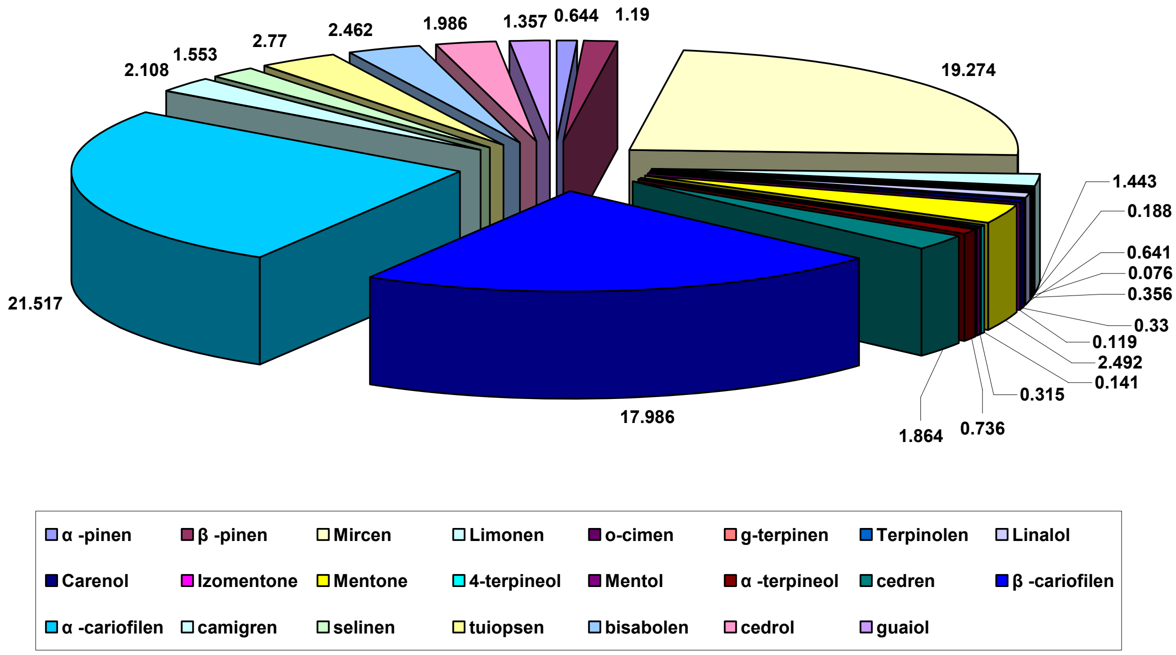

Fig. 6 - The composition of the volatile oil production at *Pelargonium zonale* L. at flowering moment

The fungi static activity testing of the volatile oil groups of *Ocimum basilicum* showed a different behavior for the two fungi species. In this case, the solvent induces an inhibition of the *Penicllium* cultures, but it does not act on the *Aspergillus* cultures sooner than the end of the cultivation period. The different fungus inhibition patterns suggest that the two groups of oils have different properties. The variants with 500 ppm and 1000 ppm concentration from first oil sample have different influences on culture growth compared to variants with 500 ppm and 1000 ppm concentration from second oil sample. At the same time, the two species have a different response to volatile oil action: the *Penicillium* cultures are more inhibited by oils from first oil investigated sample, and the influence being more significant after 5 days of cultivation. For *Aspergillus,* the influence is greater at the end of the cultivation period, and the effect being significant for oils belonging to second investigated sample.

For oil produced by *Pelargonium* taxa, the effect of D.M.S.O. upon the **microbial cultures** was null, for both *Escherichia coli* and *Staphylococcus aureus*. The solvent used for the essential oil (D.M.S.O) has no influence upon the microbial cultures, which means that only the effect upon bacteria is due to the presence of the essential oil. The effect of *P. radens* essential oil is null upon *Escherichia coli* strains using both paper discs and glass cylinders, but the effect of this kind of essential oil can be noticed upon *Staphylococcus aureus* **fungus strains** using paper discs or glass cylinders.

CONCLUSION

• The morph-anatomic investigations conducted on the available vegetal material confirm that data presented in the specific literature and also completes the structural picture of the airy vegetative organs for the investigated taxa, with specific references to the type of volatile oils secretor hairs present on leaves and stems, as well as the stomata location on these organs, as a adaptation to the environment conditions in witch the investigated plant populations developed.

• The efficiency of extraction of essential oils has different values depending on the taxon and on the ontogenetic stage the producing plant is in. The composition of volatile oils in the two stages of the ontogenetic cycle differs in quantity and quality and develops specific aromatic characteristics.

• The number of components of the essential oils extracted is larger during the flowering period for the essential oils extracted from *Pelargonium zonale* and *Pelargonium fragans*, and lower for *Pelargonium radens*.

• The composition analysis of the volatile oils extracted from the two populations of *Ocimum basilicum* did not show the presence of methyl-cinamat and linalool, compounds specific in some proportions, to the presented chemo types as reported by the specific literature; this reality can be motivated, we assume, by the analysis moment (which probably did not strictly correspond to the biosynthesis stage of these compounds) or by the investigated oil producing organ.

• The preliminary test for the *Pelargonium radens* essential oil was encouraging for further tests, revealing inhibitory effects upon the *Staphylococcus aureus* bacterial strain. Therefore, the rest of the essential oils extracted, other bacterial strains as well as the minimum inhibitory concentration are to be tested.

• The antibacterial effect of *Ocimum basilicum* volatile oils confirms the results presented in specific literature, according to which, for the pure extracts, the effect is obvious for Gram positive bacteria, and partially positive for the Gram negative ones (*Escherichia, Salmonella, Shigella)*.

• The preliminary testing of the fungi static effect of the two oils of different concentrations shows that fungal species react differently, they having different growth speeds, evaluated at analysis intervals.

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