RESEARCH REGARDING THE VOLATILE OILS COMPOSITION FOR *OCIMUM BASILICUM* L. AND THEIR POSSIBLE PHYTOTHERAPEUTIC EFFECTS

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Abstract: This paper presents the volatile oil composition for two *Ocimum basilicum* L. populations (common basil or sweet basil), as well as preliminary results regarding their tested antimicrobial effects. The analyzed material (stems and leaves) was harvested in the blossoming stage, when the secreted compounds have obvious phytotherapeutic and aromatic effects. The number of identified compounds among the two populations (generically noted sample I and II) is greater for sample I (28 compounds) compared to sample II (25 compounds). The volatile oil analysis not evidenced the presence of methyl cinnamate and linalool, compounds specific – in certain proportions - to the chemotypes presented in literature. This fact is probably related to the analysis moment, which possibly not strictly corresponds to the biosynthesis stage of these compounds, as well as to the oil producing organ. The investigated oil groups have a variable inhibitory effect, depending on concentration, on *Staphylococcus aureus* strain (Gram positive bacteria) and a mild influence on the growth and development of *Escherichia coli* strain (Gram negative bacteria). 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 interspecific 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 chemotypes 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 *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 species 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, chemovariability etc.), so that a lot of chemotypes are established: with linalool or with methyl chavicol, or a mixture of linalool and methyl chavicol, or a mixture of linalool and eugenol, or a mixture of methyl chavicol and methyl eugenol etc. In addition, its volatile oils contain nematicidal, fungistatic and antifungical 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).

POLITEO et al., 2007 recently confirmed the antioxidant capacity of the basil’s free volatile aglycons and of its volatile oils. So, by its antioxidant properties, basil helps the organism fight against free radicals, radicals that are related to aging and illness processes. It also has an adaptive role, with antistress effect, it helps nervous and emotional processes, and it has an immunostimulant effect. It also ensures, together with vitamin C, caroten, calcium and phosphorus, the skin health and the tonicity of nervous system, consolidating the memory processes.

In the context of the presented data, our research wishes to achieve a comparative analysis of the volatile oils produced by two basil populations cultivated in the northern part of Moldavia. The populations are found at the anthesys period, moment when the production of these compounds is at its peak, and these components give the oil its obvious phyto therapeutic and aromatic properties.

MATERIAL AND METHOD

►For the investigations regarding volatile oils composition, the biological material consisted of leaves and stems freshly harvested from plants in the anthesis stage, belonging to 2 local populations, generically noted as sample I and sample II. The analyses were performed in *„HORTICAL"* - center 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 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; identification of volatile oils possible by using the NIST spectra bank and Kovats indexes.

►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, "Al. I. Cuza" University of Iasi, have been used. The testings were also carried out in this laboratory, by using the Kirby-Bauer disk diffusion method (NIMIŢAN 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 (DMSO - dimethyl sulfoxide - as solvent for volatile oils); 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,* we 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, 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 AND DISCUSSIONS

For the available biologic material, **the volatile oil analyses** from the two basil populations lead to the following interpretations: (fig. 1 – 2):

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%).

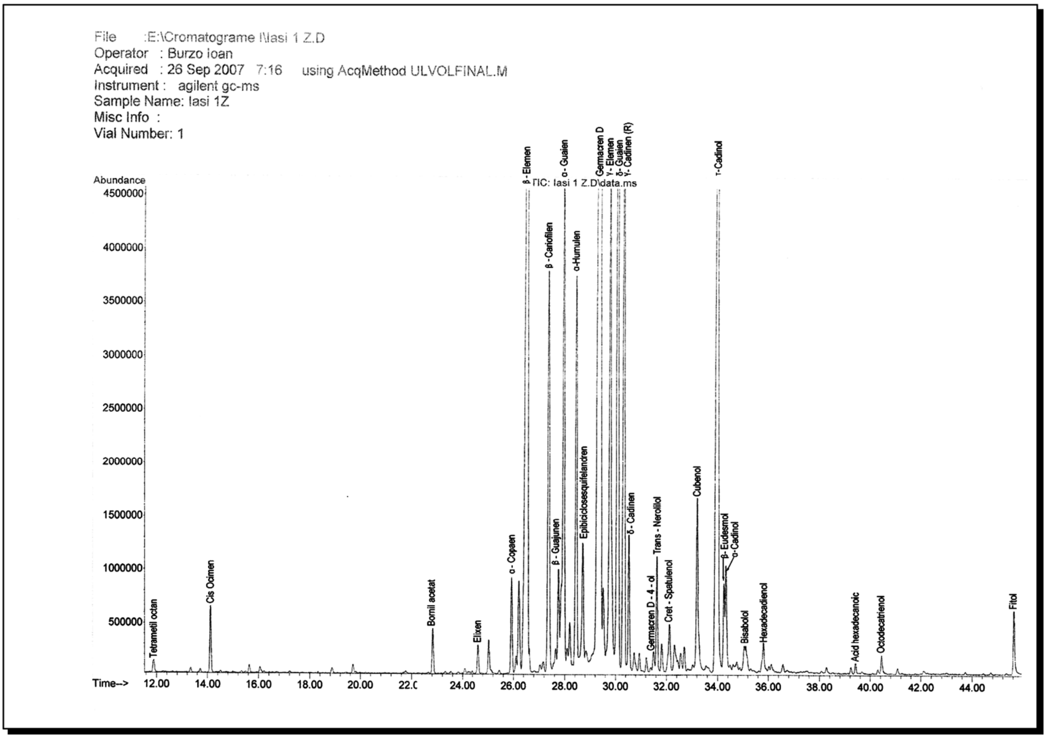

Figure 1. Volatile oil composition for *Ocimum basilicum* (sample I)

Among the characteristic compounds giving the specific properties of each analyzed sample, we mention the following compounds, depending on their percentage:

In sample I: trans – Nerolidol (0.867%); Spatulenol (0.519%); cis - Ocimen (0.465%); Hexadecadienol (0.313%);

In sample II: α – Bergamoten (7.189%); Nerolidol (0.816%); Octodecanol (0.749%); α – Bisabolol (0.702%); Heptadecan – 1 – ol (0.600%).

According to the specific literature, the common basil, which contains a lot of camphor, has numerous chemo types, with a certain predominant component. This fact leads to different kind of volatile oils. GULATI and SINHA (1989), (cf. BURZO and MIHĂIESCU, 2005) have identified three basil chemo types; the volatile oil extracted from the first chemo type had mostly: 42,23 % methyl cinnamate and 38,23 % linalool, the second one contained 41,80 % methyl cinnamate, 42,60 % linalool, and the third one presented 51,34 % methyl cinnamate and 19,68 % linalool.

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 chemotypes 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.

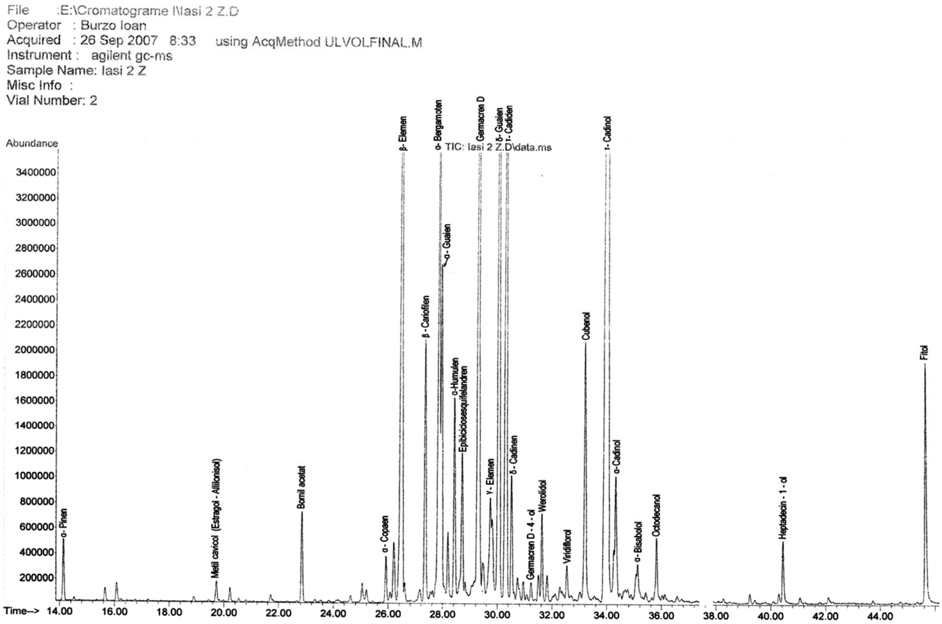

Figure 2. Volatile oil composition for *Ocimum basilicum* (sample II)

The testing of the antibacterial activity of the two analyzed oil samples, 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 (Table 1).

Table 1. Inhibitory effect induced by the oils extracted from *Ocimum basilicum* L. on some test bacteria strains

Sample	Volatile oil concentration (ppm)	Inhibition area diameter (mm)	
		Staphylococcus aureus	*Escherichia coli*
Sample I	500	18	0
	1000	20	0
Sample II	500	11	0
	1000	14	0

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.

The fungistatic activity testing of the volatile oil groups showed a different behaviour for the two fungi species (Table 2).

Table 2. The inhibitory effect induced by *Ocimum basilicum* L. volatile oils on some test fungal colonies

Variant	*Penicillium chrysogenum*				*Aspergillus niger*			
	5 days		10 days		5 days		10 days	
	Ø (mm)	I (%)	Ø (mm)	I (%)	Ø (mm)	I (%)	Ø (mm)	I (%)
Control M	14		18		39		70	
M_{DMSO}	10	*28*	14	*22*	48	*0*	45	*35*
C_1 (500 ppm)*	6	*57*	19	*0*	37	*5*	50	*28*
C_2 (1000 ppm)*	8	*42*	17	*5*	38	*2*	40	*42*
C_3(500 ppm)**	12	*14*	12	*33*	36	*7*	27	*61*
C_4(1000 ppm)**	10	*28*	15	*16*	26	*33*	45	*35*

* - sample I volatile oil; ** - sample II volatile oil

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. C_1 and C_2 variants have different influences on culture growth compared to C_3 and C_4. At the same time, the two species have a different response to volatile oil action: the *Penicillium* cultures are more inhibited by oils from C_1, respectively C_2 variants, the influence being more significant after 5 days of cultivation.

For *Aspergillus,* the influence is greater at the end of the cultivation period, the effect being significant for oils belonging to C_3 and C_4 variants.

CONCLUSIONS

The investigated basil populations are biochemical different, their composition being strictly influenced by moment of oil determination.

The aromatic and therapeutic efficiency can be settled for the same plant by the respective stage from ontogenetic cycle, depending on proper metabolic transformations in strict relationship with environmental conditions.

The antibacterial effect of *Ocimum* 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 fungistatic 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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