J. Plant Develop.
18(2011): 81-86

COMPOSITION OF THE VOLATILE OIL EXTRACTED FROM *ABIES ALBA* MILLER LEAVES PARASITIZED BY *MELAMPSORELLA CARYOPHYLLACEARUM* (DC.) J. SCHRÖT.

MANOLIU ALEXANDRU[1], IRIMIA ROMEO[1],
MIRCEA CORNELIA[2], ŞPAC ADRIAN[2]

Abstract: Researches results highlights both qualitative and quantitative influences exercised by the parasitic species *Melampsorella caryophyllacearum* on the composition of the volatile oil extracted from Abies alba leaves, prelevated in 2010 from Oituz river basin (Bacău county). The isolation of volatile oils has been realized by hydrodistillation in Neo-Clavenger installation, followed by gas-chromatography coupled with mass spectrometry analysis. The increase of the monoterpenes concentration in the parasitized sample could be explained by the degradative action of the enzymes produced by the pathogenic species *Melampsorella caryophyllacearum* or by the incapacity of syntheses from these monoterpenes of some compounds presenting a more complex structure in the parasitized plant case.

Keywords: volatile oil, monoterpenes, sescviterpenes, *Abies alba*, *Melampsorella caryophyllacearum*

Introduction

The decline of *Abies alba* Mill. has been the subject of great concern in Central Europe and North America since the early 1970s [SKELLY & INNES, 1994]. Among the main proposed causes of fir decline were air pollutants, climatic and biotic factors. The use of dendro-ecological techniques has enabled researchers to date with annual resolution, and to quantify precisely the effects of fungal pathogens on radial growth [CHERUBINI & al. 2002].

The fungus *Melampsorella caryophyllacearum* (DC.) J. Schröt. (Fungi, Basidiomycota) also called fir broom rust, has been reported to cause serious damage on *Abies* species [NICOLOTTI & al. 1995; MANOLIU & al. 2009]. The fungus causes the production by the tree of witches' brooms, and hypertrophied ring growths on the trunk or branches resulting in spherical swellings [SOLLA & al. 2006]. Of greater concern, *M. caryophyllacearum* may contribute to a tree's death by weakening it such that wind breaks the tree at the site of the swelling.

The disease is common wherever firs grow, being present in North America [MERRILL Z & al. 1993], Europe [FRIGIMELICA & al. 2001], and Asia [ALEKSEEV & al. 1999].

[1] "Alexandru Ioan Cuza" University, Biology of Faculty, bd. Carol I, 20A, 700505, Iaşi - Romania
[2] "Gr. T. Popa" Medecine and Pharmacy University, Pharmacy Faculty, st. University, 16, 700115, Iaşi - Romania

Material and methods

Identification and quantification of volatile oil [ŞTEFĂNESCU, 1988] have been realized using healthy and parasitized leaves samples from *Abies alba* (fir). Because the pathogenic fungus *Melampsorella caryophyllacearum* can not be cultivated on nutritive media in laboratory, the analyzed samples have been collected from trees growing in Oituz river basin (46°08,091' N; 26°30,985' E, 651 m alt.) and transported in freezers in the laboratory. The vegetal material vegetal has been dried and crumbled. The two samples have been encoded in this way: *Fr.S.* – healthy leaves sample and *Fr.B.* – parasitized leaves sample. Separated volatile oil has been analyzed by gas-chromatography coupled with mass spectrometry, using a Neo-Clavenger installation, (GC) Agilent Technologies gas chromatograph - type 6890N.

Method: 50 g of dry vegetal material crumbled in II sieve (*Farmacopeea Română*, Xth edition) have been treated with 500 ml distilled water and 30 ml glycerin. The glycerin added on the vegetal product has the role to favor hydratation and volatile oil extraction. After the introduction of the water in graduated tube of the device and in the separator, the samples have been distilled for 3 hours. After distillation, the separation of the volatile oil has been favored by adding of 1 ml xylene; this quantity will be dropped from the final volume of the volatile oil. The separated volatile oil has been inserted in a graduated tube where its volume has been identified and reported to 100 g vegetal product.

$$\text{ml volatile oil (\%)} = 100\ V/a$$

where:

V – extracted volume of volatile oil, expressed in ml;

a – the mass of the used dry vegetal material, expressed in g.

Results and discussions

The achieved extraction capacity, expressed in ml volatile oil in 100g vegetal material, highlights a content of 2.76 in this type of compounds for *Fr.S.* sample and 0.37 for *Fr.B.* sample, where a content by approximate 7.5 times smaller is observed. The analyzed volatile oil is predominantly constituted by monoterpenes and sescviterpenes (**Table 2**, **Fig. 1 and 2**).

Tab. 1. The main compounds identified in volatile oil samples

t_R (min.)	Compound	Aria %	
		Fr.S.	***Fr.B.***
4.788	santene	3.74	1.94
5.429	tricyclene	1.64	0.78
5.602	α–pinene	6.46	13.97
5.887	camphene	6.94	5.62
6.329	β–pinene	10.18	15.51
6.407	myrcene	0.69	0.84
6.718	α–phellandrene	0.12	0.11
6.891	α–terpinene	0.08	0.06
7.013	p-cymene	0.07	0.10
7.116	limonene	9.48	10.06
7.160	sabinene	2.18	4.29
7.532	γ-terpinene	0.09	0.08

7.965	α-terpinolene	1.50	0.57
8.034	p-cymenil	0.04	0.08
8.138	L-linalool	0.40	-
8.198	t-allocymene	0.26	-
8.389	mentha-1,4,8-triene	0.02	-
8.519	fenchol	0.09	0.05
8.623	α-campholenic aldehyde	0.42	0.26
8.978	camphor	0.08	0.11
9.107	exo-methyl-camphenilol	0.14	0.06
9.194	pinocarvone	0.02	0.14
9.229	isoborneol	0.03	-
9.298	α-phellandrene-8-ol	-	0.04
9.358	endoborneol	1.66	-
9.419	isopinocamphone	0.05	0.05
9.454	terpinen-4-ol	0.12	0.07
9.557	cis-m-menth-8-ene	0.08	
9.670	α-terpineol	1.37	0.80
10.371	t-β-ocymene	0.20	-
10.544	piperitone	0.01	-
10.596	(E)-2-decenal	0.02	0.14
10.873	lavandulyl acetate	0.05	-
10.916	felandral	0.03	0.09
11.003	(-)-bornyl acetate	12.14	7.68
11.419	t.t-2,4-decadienal	-	0.06
11.548	1,3,5-tris(methylene)cycloheptane	0.08	-
11.812	(+-)-m-mentha-1,8-diene	-	0.15
11.825	α-cubebene	0.14	0.08
11.981	α-longipinene	1.87	0.92
12.301	α-copaene	0.20	0.14
12.206	δ-3-carene	0.80	-
12.345	longicyclene	-	0.19
12.466	β-elemene	0.27	0.33
12.552	sativene	0.06	-
12.596	α-ylangene	0.03	0.24
12.691	(-)-isoledene	0.49	-
12.700	aromadendrene	-	0.17
12.838	isolongipholen	1.21	-
12.959	(−)-β-caryophyllene	6.05	8.35
13.003	α-cedrene	2.43	1.03
13.063	(-)-sinularene	-	0.08
13.106	α-guaiene	0.04	-
13.202	cis-β-bisabolen	0.03	-
13.271	t-β-farrnesene	0.37	0.33
13.375	α-himachalene	0.64	0.25
13.444	α-humulene	2.69	4.59
13.609	cis-cariophyllene	1.31	0.50
13.660	γ-muurolene	-	0.31

13.756	γ-himachalene	0.65	-
13.816	widdrene	1.34	-
13.877	β-selinene	3.13	-
13.989	α-selinene	-	1.12
14.050	β-himachalene	1.91	1.16
14.206	α-amorphene	1.24	0.69
14.266	δ–cadinene	2.54	1.81
14.396	allo-aromadendrene	-	0.18
14.509	aromadendrene VI	-	0.24
15.063	valencene	0.03	-
15.184	cariophyillen oxide	0.29	1.22
15.513	longiborneol	0.54	-
15.833	α-gurjunene	2.10	3.64
15.842	(-)-longipholene	-	2.22
15.963	β-paciulen	0.55	-
15.980	δ-cadinene	-	0.37
16.127	α-cadinol	0.85	-
16.327	t-muurolol	-	1.32
20.507	β-bisabolene	0.14	-

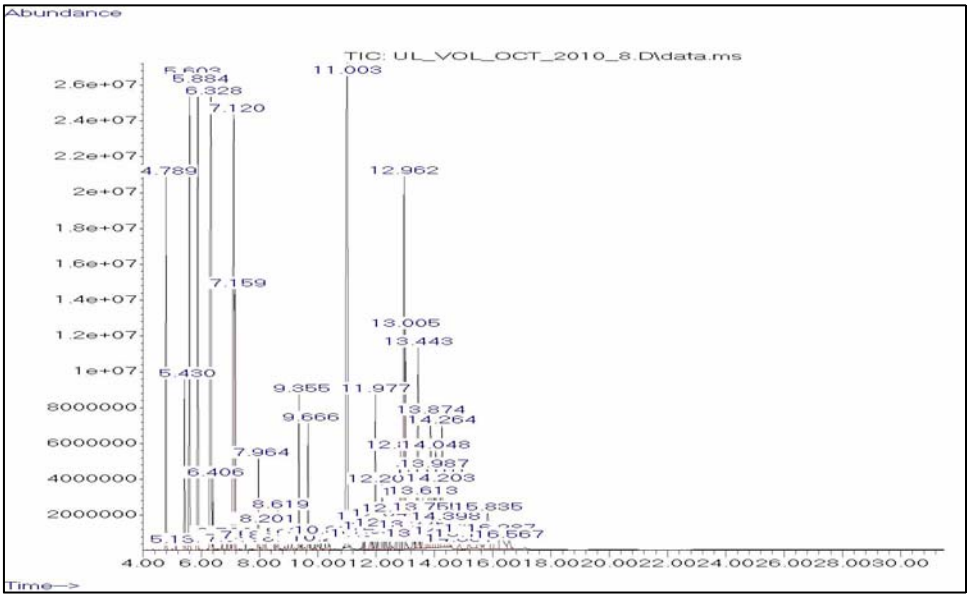

Fig. 1. Gas-chromatogram of the volatile oil – healthy leaves sample

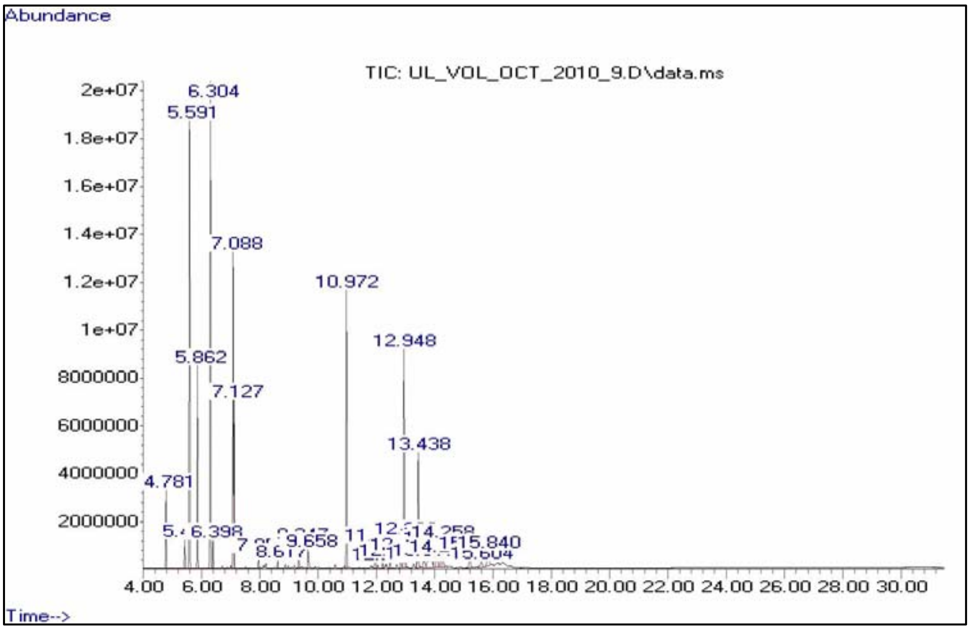

Fig. 2. Gas-chromatogram of the volatile oil – parasitized leaves sample

By comparison with *Fr.S.* sample, the *Fr.B.* sample is characterized by an increased level of monoterpenes (61.39% *Fr.S.*, 56.61% *Fr.B.*). The major monoterpenic structures are hydrocarbons, the concentration of the oxygenated derivates being increased in *Fr.S.* sample (16.61%) comparative with *Fr.B.* sample (9.35%), so the increasing of the monoterpenes content is realized through a increasing of the concentration in hydrocarbons structures. The sescviterpenes concentration is the same in both two samples, a slightly increased value being registered yet in *Fr.S.* sample (33.94% comparative to 31.48%). Instead, the oxygenated sescviterpenes presents an increased concentration in the parasitized sample (2.54% in *Fr.B.* sample comparing to 1.68% in *Fr.S.* sample). The reduction of the concentration in oxygenated compounds will determine a reduction of the therapeutic properties of the fir volatile oil de brad or the limitation of its use in aromatherapy.

Conclusions

The increase of the monoterpenes concentration in the parasitized sample could be explained by the degradative action of the enzymes produced by the pathogenic species *Melampsorella caryophyllacearum* or by the incapacity of syntheses from these monoterpenes of some compounds presenting a more complex structure in the parasitized plant case.

The major compounds characteristic and common in both samples are the next monoterpenes: santene, α- and β-pinene, camfene, limonene, sabinene, bornilacetate, and also some sescviterpenic derivates: β-cariophyllene, α-cedrene, α-humulene, β-himachalene, Δ-cadinene and α-gurjunene.

Modification of the content in volatile oil and of the quality of the volatile oil samples represents the consequence of the pathogenic process, which determines the impossibility of parasitized vegetal material use in order to obtain the volatile oil necessary in pharmacy, perfumes industry and aromatherapy.

References

1. ALEKSEEV V. A., ASTAPENKO V. V., BASOVA G., BONDAREV A. I., LUZANOV V. G., OTNYUKOVA T. N. & YANOVSKII V. M. 1999. The condition of the Kuznetsk-Alatau fir forests. *Lesnoe Khozyaistvo*, **4**: 51-52.
2. CHERUBINI P., FONTANA G., RIGLING D., DOBBERTIN M., BRANG P. & INNES J. L. 2002. Tree-life history prior to death: two fungal root pathogens affect tree-ring growth differently. *J. Ecol*., **90**: 839-850.
3. FRIGIMELICA A., CARPANELLI F., STERGULC M., KNIZEK B. & GRODZKI W. 2001. Monitoring of widespread forest diseases in Friuli-Venezia Giulia (north-eastern Italy). *J. For. Sci*., **47**: 81-84.
4. MANOLIU A., IRIMIA R., GRĂDINARIU P. & UNGUREANU E. 2009. The influence of the attack of the fungus *Melampsorella caryophyllacearum* (DC.) J. Schrot. (“witch brooms” on fir) on the peroxidase and catalase activity in host plant. *Anale Şt. Univ. Iaşi a. Genetică şi biol. molec*., **10**(3): 25-28.
5. MERRILL W., WENNER N. G. & PEPLINSKI J. D. 1993. New host distribution records from Pennsylvania conifers. *Plant Dis*., **77**: 430-432.
6. NICOLOTTI G., CELLERINO G. P. & ANSELMI N. 1995. Distribution and damage caused by *Melampsorella cariophyllacearum* in Italy. *Shoot and Foliage Diseases in Forest Trees*, pp. 289-29.
7. SKELLY J. M. & INNES J. L. 1994. Waldsterben in the forests of Central Europe and Eastern North America: Fantasy or reality?. *Plant Dis*., **78**: 1021-1032.
8. SOLLA A., SÁNCHEZ-MIRANDA Á. & CAMARERO J. J. 2006. Radial-growth and wood anatomical changes in *Abies alba* infected by *Melampsorella caryophyllacearum*: a dendroecological assessment of fungal damage. *Ann. For. Sci*., **63**: 293-300.
9. ŞTEFĂNESCU E. 1988. Uleiuri eterice din cetina principalelor specii de răşinoase din România. *Revista Pădurilor*. Bucureşti, **3**.