Chemical Composition and Protective Antifugal Activity of *Mentha Piperita L.* and *Salvia Officinalis L.* Essential Oils Against *Fusarium Graminearum Spp.*

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In present paper we studied the chemical composition of two essential oils (EOs), respectively Mentha piperita (peppermint) oil and Salvia officinalis L (sage) oil and the antifungal effect against Fusarium graminearum spp. in lab condition on culture media. The antifungal bioassay objectives were to determine the minimum concentration of essential oils for mycelium growth (MCMG) and to establish the EOs concentration with fungicidal effect (CFE). We tested different concentration of essential oils (1-20 mg·L^{-1}). As control it was used the treatment with thiophanat methil a pesticide recommended against Fusarium graminearum spp. development on wheat. The main components identified by gas-chromatography coupled with mass spectrometry (GC/MS) in Mentha piperita oil were menthol (42.35%) and menthone (29.10%), respectively camphor (20.64%), camphene (11.59%) and eucalyptol (11.75%) in Salvia officinalis oil. The antifungal assay show that MCMG was 5 mg·L^{-1} for Mentha piperita EOs and for sage 15 mg·L^{-1} but CFE are not remaining in time, relative to thiophanat methyl treatment neither for sage or peppermint EOs.

Keywords: Essential oils, Mentha piperita, Salvia officinalis L., Fusarium graminearum

In Romania, peppermint represents one of the most important aromatic and medicinal plants. Harvested during flowering strains contain 0.3-0.5% EO and leaves 1-2% EO with different chemical composition depending on provenance [14]. Along with menthol, a substance which gives aroma, and representing up to 70% of volatile oil, peppermint oil contain substances such as menthon, isomenthone, methofuran, a-pinene, limonene, carvone, cineole, piperitone, etc. [3]. Peppermint has several pharmaceutical uses and is recommended especially in digestive, respiratory and circulatory diseases. Menthol has antispasmodic effect, antiseptic, anti-inflammatory and vasodilators effect on sinus nasal mucosa [23].

The garden sage (*Salvia officinalis L.*) is native to Southern Europe, currently being successfully cultivated as a medicinal plant in Europe. In Romania, sage finds best culture conditions, especially on land, rocky and limestone, exposed to the sun, which maintains very well the colours in leaves and the fragrance. The medicinal effect is due by the antispasmodic, antiseptic, and astringent properties [15]. Monoterpenoids of the sage EO induces antimicrobial, anti-inflammatory and antioxidant properties [2,4].

Due to their bioactivity in the vapour phase, their non-toxicity and antifungal properties, EOs have found applications in recent years as fumigants for cereals protection. During storage, under favorable conditions of temperature and humidity, plant products are susceptible to fungi contamination with toxic effects on the human and animal body. The application of certain EOs to control plant pathogens in cereals has been highlighted in literature [5; 12; 17; 22; 24].

This paper studies the chemical composition of EOs obtained from *Menta piperita L.* and *Salvia officinalis* grown in Romania and establish their antifungal and fungicidal effect against *Fusarium graminearum* in vitro conditions.

Experimental part

Materials and methods

Obtaining of essential oils

The plant material was cultivated at Didactic Station (21^{0}13'E longitude, 45^{0}45' N latitude) of Banat's University of Agricultural Sciences and Veterinary Medicine *"King Mihai I of Romania"* from Timisoara, Romania. Inflorescences harvesting was done in 2014 at the time of flowering when accumulation of volatile oils is maximum. Identification of the species was confirmed by department of Aromatic plants from USAMVB Timisoara and a voucher specimen was preserved. Fresh herbs were conditioned and allowed to dry in a cool place, away from sunlight. The oils were obtaining during Hydrodistillation for 3 h using Clevenger equipment. The EOs was collected and stored at 4°C until used.

Determination of chemical composition

The chemical composition was determined using Gas-Chromatograph Agilent Technology 7820A (Agilent Scientific, USA) coupled with mass spectrometer MSD 5975. A capillary column DB 5: (30 m X 250 μm X 0.25μm, Agilent, USA) was used. The carrier gas was helium; with a mass flow of 1 mL/min. GC gradient was used: 40 °C for 1 min, rate of 5 °C/min to 210°C. The injector and ion source temperatures were 250 and 150 ^{0}C, respectively. The injection volume was 1 μL with a split rate 1:20. The NIST

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No.	RT (min)	Compounds	% from total
1	7.893	a-pinene	0.88
2	9.033	b-pinene	0.63
3	9.120	a-phellandrene	1.00
4	10.668	d-limonene	2.28
5	**10.746**	**Eucalyptol**	**5.65**
6	10.941	z-ocimene	0.40
7	11.817	cis-Beta-Terpineol	1.64
8	**14.435**	**Menthone**	**29.10**
9	14.691	Isopulegol	3.72
10	**15.064**	**Menthol**	**42.35**
11	16.846	Pulegone	0.73
12	17.257	Piperitone	0.24
13	18.328	Isomenthol acetate	0.75
14	21.692	Cedrene	3.70
15	22.533	Farnesene	0.34
16	23.231	beta-Cubebene	4.98
17	23.604	alpha-valencene	0.45
Total (%)			**98.89**

Table 1
CHEMICAL COMPOSITION OF *MENTHA PIPERITA L.* EO

spectra library has been used to identify the volatile compounds.

Bioassay for antifungal activity

Our preliminary activity was to obtain new mycelium of *Fusarium graminearum* on CYA medium (Sigma manufacturing). The *Fusarium graminearum* isolate was obtained from wheat by harvesting from diseased seeds and putting on Potato Dextrose Agar (PDA) [10]. The new fungal mycelium was cutting in small pieces, around 8 mm in diameter, and was transferred on CYGA culture media with different concentration of oils (V1-1mg·L^{-1}, V2-5mg·L^{-1} V3-10mg·L^{-1}, V4-15mg·L^{-1}, V5-20mg·L^{-1}). The control sample was CYGA without any addition. The Petri dishes were incubated in the dark at 22 ±2°C. Daily was check the growth rate of mycelium and the dates of 6th day were recorder and used in statistical approach for establishment of MCMG. The nature of fungitoxicity (fungistatic or fungicidal) was assessed by Thompsom method [19] after which the fungal-discs fully inhibited were transferred into CYGA media without oils. The revival of *Fusarium graminearum* growth on fresh medium was checked in the 5-th day. In one samples of medium was added thiophanate methyl, a commercial fungicide which is recommended in agricultural practices. The samples VoT was used as negative control. The experiments were conducted twice. The inhibition percent of mycelial growth (MIC) was determined by calculation [13] with formula:

$$MIC = [C-V/C] \times 100,$$

where:

C – mycelia growth diameter (mm) on control, CYA with no addition,

V – mycelia growth diameter (mm) on variants (V1 to V5)

Results and discussions

Chemical composition

In tables 1 and 2 are presented chemical compositions of *Mentha piperita*, respectively *Salvia officinallis* EOs, taking into consideration the chemical compounds which was found in higher percentage of 0.20% of the total compounds. The EOs are a mixture of different major and minor components which act together for their biological activities [12].

No.	RT(min)	Compounds	% from total
1	5.765	3,4-Octadiene, 7-methyl-	0.22
2	7.555	Tricyclene	0.49
3	7.707	alpha-Thujene	0.21
4	**7.893**	**alpha-Pinene**	**9.59**
5	**8.309**	**Camphene**	**11.58**
6	**9.124**	**beta-Pinene**	**5.04**
7	9.558	beta-Myrcene	0.83
8	10.299	alpha-Terpinen	0.23
9	10.538	para-Cymene	0.25
10	10.655	d-limonene	1.44
11	**10.733**	**1,8 cineole (eucalyptol)**	**11.75**
12	11.561	gama-Terpinene	0.45
13	12.450	Terpinolene	0.51
14	12.779	Linalool	0.60
15	**12.961**	**alpha-Thujone**	**8.64**
16	13.278	trans-Thujone	1.98
17	**14.106**	**Camphor**	**20.64**
18	**14.730**	**Borneol**	**8.80**
19	15.064	4-Terpineol	0.24
20	18.112	L-alpha-bornyl acetate	5.09
21	21.684	trans-Caryophyllene	3.41
22	22.542	alpha-Humulene	4.99
23	25.885	Viridiflorol	1.04
24	35.457	Epimanool	0.39
Total (%)			**98.51**

Table 2
CHEMICAL COMPOSITION OF *SALVIA OFFICINALIS L.* EO

Variants	Mint e- oil		Sage e- oil	
	MG (mm)	MIC%	MG (mm)	MIC%
Control	17.3±2.3	0	17.3±2.3	0
V_1-1mg·L^{-1}	5.5±1.3	69	15±1	13.2
V2-5mg·L^{-1}	0	100	9.3±1.3	46
V3-10mg·L^{-1}	0	100	1.6±0.3	90.7
V4-15mg·L^{-1}	0	100	0	100
V5-20mg·L^{-1}	0	100	0	100
VoT	0	100	0	100

Table 3
MYCELIUM GROWTH (MG) AND THE INHIBITION PERCENT OF MYCELIAL GROWTH (MIC) OF *FUSARIUM GRAMINEARUM* RECORDED IN PRESENCE OF MINT AND SAGE ESSENTIAL OILS WITH DIFFERENT CONCENTRATION

In *Mentha piperita* spp. were identified 33 volatile compounds, of which 17 in percentage of over 0.2%, totaling of 98.89% of the total components. EO obtained from *Mentha piperita spp.* cultivated in west Romania was comparable with previous reports from other countries. In *Mentha piperita spp.* oil main chemotype identified is menthol (42.35%), followed by menthone (29.10%) and eucalyptol (5.65%). The results are in compliance with those reported by Znini, 2011; Sokovic, 2009; Scavroni, 2007; Iscan, 2002 [22;17; 16; 8]. Opposite, pulegone (44.45%) were the major components of *Mentha piperita spp.* EO [9].

In *Salvia officinalis L.* were identified in total 38 components of which 24 in a proportion of 0.2% (98.516%). The chemical composition shown that the oxygenated monoterpenes (camphor, 1,8-cineole, α-thujone and borneol) are the most abundant compounds. Camphor (20.64%) is the dominant component followed by eucalyptol (11.75%), borneol (8.80%) and α-thujone (8.64%). The monoterpene hydrocarbon fractions are also recovered in important quantities: camphene (11.58%), alfa pinene (9.59%) and beta pinene (5.04%). In contrast, the sesquiterpene fraction represented a lower percentage in the oil. Previously studies reported that alfa thujone (40.9%) and camphor (26.12%) are the main components in sage oil cultivated in Brasil (Porte) [11], while manole and cineole were the main compounds identified in Lithuania [4]. Cineole was also detected in high concentration (39.5-50.3%) in different sage varieties from Jordan [1]. In Mediterranean region camphor was 24.59% [21] in the range of our obtained data.

Bioassay for antifungal activity

The lowest concentration of essential oils that did not permit any visible fungal growth was taken as MCMG. In this case the mint oil had a significant inhibitory effect on the *Fusarium graminearum* mycelia growth that was observed compared to sage oil. Mint oil has MCMG 5mg·L^{-1} since the MCMG for sage is corresponding to 15mg · L^{-1}. Previously studies shown that limonene and menthol are responsible for *F. verticillioides* inhibition at low concentration (75 ppm respectively 200 ppm), while thymol prevent mold grow at higher concentration (500 ppm) [6; 7].

CFE is the lowest concentration of EOs at which was recorded no revival of *Fusarium* mycelial growth. Our results shown that in V2 (5mg·L^{-1}) mint oil has an fungistatic effect, after 5 days has been observed the revival of *Fusarium graminearum* micelial growth. In variants with addition of sage essential oil the fungicidal effect on *Fusarium graminearum* growth is obtained only in V5 (20 mg·L^{-1} sage oil). In the case of 15 mg·L^{-1} sage oil (V4) the antifungal effect is temporarily for *Fusarium graminearum*.The variant VoT (with thiophanate) has a fungicidal effect, the rate of inhibition of mycelial growth is 100%. The same data were recorded for V3, V4 and V5, in case of mint essential oils addition. In variants with addition of sage essential oil the fungicidal effect on Fusarium growth is obtained only in V5, with 20mg·L^{-1} sage oil. For 15mg·L^{-1} sage oil (V4) the effect is fungistatic for *Fusarium graminearum.*

Conclusions

The chemical composition of *Mentha piperita* EO shown that menthol (42.35%) and menthone (29.10%) are the main compounds, while camphor (20.64%), camphene (11.59%) and eucalyptol (11.75%) represents the major components in *Salvia officinalis* EO.

The antifungal effect of *Mentha piperita* EO against *Fusarium graminearum* expressed as MCMG was 5mg·L^{-1} , respectively 15mg·L^{-1}for *Salvia officinalis L* EO, but the fungicidal effect expressed as was lower relative to thiophanat methyl treatment.

Taking into account the antifungal effects, correlated with the absence of toxicity, the essential oils would be recommended as a natural preservative for stored food commodities.

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