ANTIFUNGAL ACTION OF THE ESSENTIAL OIL OF MONARDA DIDYMA

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Abstract. *This study refers to the establishment of minimum inhibitory concentrations of mycelial growth (MIC) in vitro conditions on fungi: Botrytis cinerea and Aspergillus flavus and the correlation of this index with the chemical composition of Monarda didyma oil. We notice that the volatile oil of Monarda didyma inhibits the development of the mycelium of the two fungi, at a minimum inhibitory concentration (MIC) of 0.3%, having a high antifungal potential, due to the favorable ratio between linalool and thymol compounds. Cultura de Monarda was located in the experimental field of the Experimental Didactic Resort of the University of Agricultural Sciences and Veterinary Medicine of Banat "King Mihai I of Romania" from Timisoara. From 282 g total amount of dried grass was obtained 10 ml of essential oil, and from 300 grams of fresh grass (inflorescences and leaves) was obtained 2 ml of essential oil, by Soxleth extraction method. The analysis of the volatile oil of Monarda didyma cultivated in the years 2018-2019 highlighted up to 29 compounds, highlighting a high content of linalool 34.84-56.29%, thymol 12.29-19.90%, o-cimol 10.77- 18.31%, terpinen range 5.14-15.46% which varied depending on the condition of the material from which the fresh (green) or dry volatile oil was extracted, the time of harvest, the climatic conditions and the different ratio between inflorescences and leaves of the samples from which the oil was extracted. Medicinal plants have been used since ancient times in the human body's struggle with disease. Modern science has, now, demonstrated the subtle effect they have on the body. In search of what was necessary for life, man noticed that some plants, placed on wounds, relieved the pain, favouring their scarring, and that others, when consumed, cured some diseases. The cultivation of medicinal and aromatic plants is favoured by the pedoclimatic conditions in Romania. The variety of natural conditions favours a variety of species, which are sources of plant material for medicine and nutrition. Medicinal and aromatic plants are known from Antiquity, the ancient civilizations contributing to the knowledge and use of as many species as possible. Parallel to the development of chemistry, it was also possible to know the active substances (active principles) of plants. In the production of medicinal plants and herbs, the quality of the products is given by the content of active principles. The quantity of active principles in the plant is subject to ecological factors, species zoning, cultivation technology, biological value of the cultivar (population, variety, hybrid, etc.) and, last but not least, primary and secondary processing arrangements. When choosing a species for a given crop area, the complexity of the interaction of different vegetation factors shall be taken into account to ensure an optimal ratio between the pedoclimatic conditions and the biological requirements of the plants. This avoids situations where natural conditions may increase the production of plant biomass to the detriment of the content in active principles. The cultivation of aromatic and medicinal plants is favoured by the pedoclimatic conditions in Romania. Their variety favours a variety of species, which are a source of plant material for medicine, perfumery and nutrition.*

Keywords*: Monarda Didyma, antifungal, esential oil, Botritys, Aspergilius*

INTRODUCTION

In Romania a series of traditional herbs are grown – sage, mint, lavender, etc. for which agrotechnical processes have been developed. At the same time, research is being carried out to broaden the variety of herbs. [1] Monarda is among herbaceous plants whose healing properties were known in Antiquity. The flower is often used as a medicine, as well as added to foods as spices. Monarda was brought to Romania in the 19th century and, for a long time, it had a decorative use. Today, the herb is widely used to treat various conditions, strengthen the immune system, and solve cosmetic problems. The name of this genus was given by the 18th-century Swedish physicist and physician, Karl Linné, in honour of the Spanish researcher and physician Nicholas Monardes of the 16th century, who published, in 1574, a paper on the new plants of America. Among the varieties of the genus Monarda there are annual and perennial plants. (RUS C.F. – 2015)

The plant contains biologically active compounds and auxiliary components. Bioactive elements are terpenoids – substances in the composition of essential oil. Also included in Monarda's composition are: Amino acids, Bitter substances (bitterness), Flavonoids, Tannins, Cellulose, Pectins, Timol, Carvacrol.

Some varieties of Monarda contain characteristic substances that mimic the taste and smell of mint and lemon. (POP G., NIȚĂ S., IMBREA F., MOCIOI I- 2001)

It has been scientifically proven that prolonged use of Monarda-based medicinal products is not addictive. Comprehensive treatment of a number of diseases with this plant and antibiotics accelerates recovery.

The plant contains large amounts of vitamins, organic compounds and essential oils useful for the human body. Monarda has the following effects: Bactericide, Immunomodulator; Anti-virus, Antisclerotic, Antioxidant, Radioprotective, Antidepressant, Antispasmodic;, Anticarcinogen.

Essential oils derived from this flower are widely used in cosmetology. It turns out that they accelerate the process of regeneration and rejuvenation at cellular level. During massage, herbal oil is applied directly to the body.

Monarda-based decorations and infusions effectively eliminate the effects of radiation exposure. The plant helps the body recover after chemotherapy. (RICHARD G. HAWKE – 1998)

Pregnant women and children under 12 years of age take Monarda with caution.

Modern pharmacology widely uses the herb in the manufacture of medicines.

MATERIAL AND METHODS

Essential oils were obtained by continuous hydro-distillation (Soxleth extraction) in the laboratory of the Interdisciplinary Research Platform "Organic, Sustainable and Food Safety Agriculture". The water boiling balloon and the plant material storage balloon have a capacity of 2 l. To obtain the essential oil, Hyssopus dried herba was distilled using a quantity of vegetable material of 280-300 g/sample. At the time of boiling, the water vapour penetrates the herba, destroys the shell of the oleiferous glands, volatilizes the oil, and then mixes with it. The boiling time of a sample was about 30 minutes after the first steam passed through the dry herba mass. The extracted essential oil was stored in the refrigerator at 4°C in a brown bottle until use. Analysis of Hyssopus oil was performed by gas chromatography coupled with mass spectrometry (GC-MS): 50 microliters essential oil were taken in vial with 1 ml hexane from which 1 microlitre at 220oC was injected into the gas chromatograph gas Shimadzu GCMS-QP2010 Plus. The column used to separate compounds from ATWAX volatile oil L = 30 m, diameter 0.32 mm, granulation 1.0 micrometre is polar, and the analysis was carried out in a temperature gradient. Temperature programme for the separation of compounds in GC

The initial temperature Ti = 40oC was maintained for 1 min, then increased at a speed of 5°/minute to 250oC, where it was maintained for 7 min. Total analysis time was 50 min.

The carrier gas used He, with a flow rate of 1.81 ml/min.

Detection of separate compounds was achieved using the mass spectrometer as a detector, in scan acquisition mode, in the range 40-450 m/z, ionization energy EI = 0.9 kV, T ionic source = 220oC, T interface 255°C. The identification of the compounds separated from the essential oil was carried out using the NIST 5 database and on the basis of the retention indices calculated on the basis of the retention times obtained for each constituent and retention times of the linear alkanes C5-C25.

RESULTS AND DISCUSSIONS

Table 1.

Compounds of volatile oil of *Monarda didyma* Herba in the 2018-2019

Nr. Crt.	Compus		RI	Monarda (h. uscată) 2018	Monarda (h.uscată) 2019	Monarda (h.uscată) 2019	Monarda (h. verde) 2019	Monarda (h. verde) 2019
1.	**alfa Thujene**	MH	1022	**2.02608**	**3.5812**	**3.03956**	**4.44606**	**5.19258**
2.	Camphene	MH	1059	0.24878	0.18433	0.35233	0.20226	0.20088
3.	beta Pinene	MH	1096	0.6656	-	-	-	-
4.	Sabinene	MH	1113	0.30649	0.20451	1.12238	0.2419	0.26036
5.	**(+)-4-Carene**	MH	1148	**1.32356**	**4.72333**	**0.1033**	**4.85156**	**6.47001**
6.	Alfa-Phellandrene	MH	1158	-	0.08767	0.11039	0.16253	0.18401
7	**Beta Myrcene**	MH	1164	-	**1.87995**	**0.08185**	**4.10846**	**5.40417**
8.	beta.-Phellandrene	MH	1195	0.52745	0.20117	0.25859	0.19453	0.34982
9.	D-Limonene	MH	1206	0.65699	0.83426	0.84809	0.96833	1.19695
10.	**Gamma Terpinene**	**MH**	**1241**	**-**	**5.1393**	**-**	**9.64655**	**15.46044**
11.	3-Octanone	HO	1251	0.15171	-	-	-	-
12.	**o-Cymol**	**MH**	**1266**	**13.36495**	**17.76471**	**18.31698**	**12.69662**	**10.77229**
13.	1,3,8-p-Menthatriene	MH	1409	0.11434	-	-	-	-
14.	cis-.beta.-Terpineol	MO	1442	0.78859	-	-	-	-
15.	3-Pinocamphone	MO	1536	1.61402	-	-	-	-
16.	**Linalool**	**MO**	**1554**	**48.27249**	**45.31203**	**56.29301**	**38.09843**	**34.84518**
17.	Bornyl acetate	MO	1564	0.1812	-	-	-	-
18.	**Thymyl methyl eter**	**MO**	**1582**	**3.56064**	**1.33771**	**2.81667**	**0.76397**	**0.65698**
19.	Beta Caryophyllene	SH	1598	1.11263				
20.	Alloaromadendrene	SH	1670	0.17061	-	-	-	-
21.	.alpha.-Caryophyllene	SH	1694	0.08568	-	-	-	-
22.	Germacrene D	SH	1710	1.40388	-	-	-	-
23.	Alfa Farnesene	SH	1748	-	0.28437	0.7714	0.50604	0.2888
24.	Germacrene B	SH	1854	0.56148	0.47941	0.57093	1.44628	0.91732
25.	gamma Muurolene	SH	1934	0.1655	-	-	-	-
26.	Anethol	MO	1960	0.17452	-	-	-	-
27.	Caryophyllene oxide	SO	1998	0.42125	-	-	-	-
28.	Elemol	SO	2066	0.87145	-	-	-	-
29.	Viridiflorol	SO	2103	0.02332	-	-	-	-
30.	**Thymol**	**MO**	**2162**	**18.71025**	**12.29597**	**13.69153**	**19.90425**	**16.5665**
31.	Carvacrol	MO	2189	2.105	5.3861	1.13411	1.45201	0.88476
32.	beta Eudesmol	SO	2205	0.21633	-	-	-	-
	Total, din care			**99.82479**	**100**	**99.511**	**99.68978**	**99.65103**
		MH		**19.23424**	**34.60325**	**24.23347**	**37.5188**	**45.49151**
		MO		**75.55842**	**64.33**	**73.93532**	**60.21866**	**52.9534**
		SH		**3.49978**	**1.24311**	**1.34233**	**1.95232**	**1.20612**
		SO		**1.53235**	**-**	**-**	**-**	

Analysis of volatile oil extracted from *Monarda didyma* herba cultivated in 2018-2019 has highlighted up to 29 compounds, including a high content of linalool 34.84-56.29%, timol 12.29-19.90%, o-cimol 10.77-18.31%, terpinen range 5.14-15.46%, which varied depending on the condition of the material from which the volatile green-dry oil was extracted, the harvest period, climate conditions, part of the plant, and the different ratio between inflorescences and leaves of the samples from which they were extracted.

A similar profile has been reported for the volatile oil of *Monarda scorpio*, a hybrid obtained from *Monarda didyma* and *Monarda fistulosa* by Katarzyna Wróblewskaa et al. 2019, a hybrid that was cultivated in the Psary region of Wrocław (long 17.00 E; lat. 51.05 N), linalool 49.1% and timol 17.7%. Variants of *Monarda didyma* grown in Italy, France, and

Canada had as a main component either linalool or timol, excluding the presence of both compounds. Donata Ricci 2017, Oyeboade Adebayo 2013.

The content in linalool varies depending on the vegetation phase being maximum during the flowering period, July-August and predominating in inflorescences.

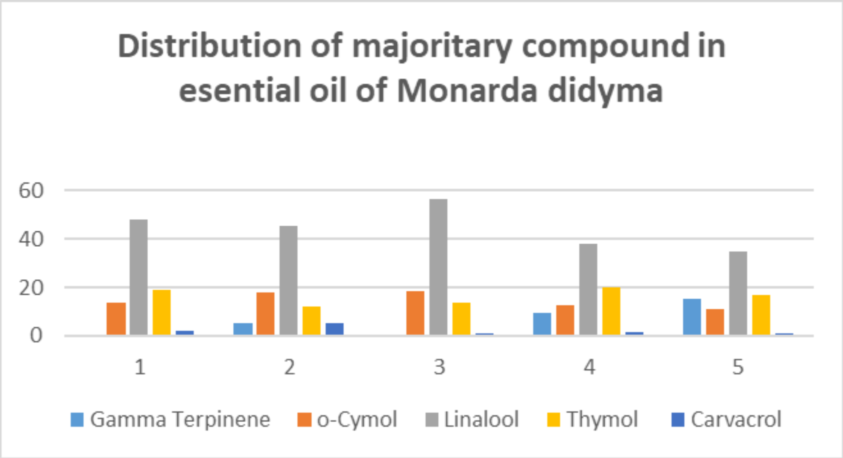

Fig 1. Distribution of majoritary compound in esential oil of Monarda didyma

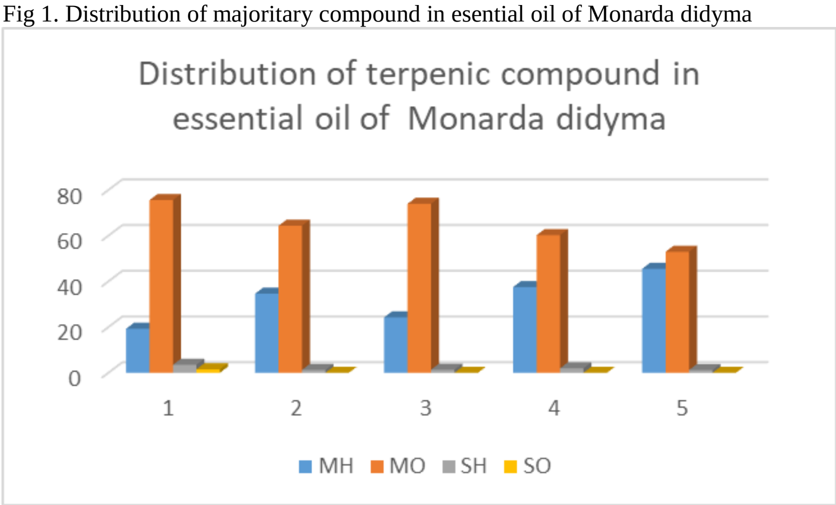

Fig 2. Distribution of terpenic compound in essential oil of *Monarda didyma*

The linalool content of essential oil has maximum values in dry samples; in the samples of oil extracted from green material, it decreases while the percentage of timol and isotimol (carvacrol) increases. In the drying process, part of the timol undergoes a reduction process with the opening of the aromatic cycle, turning into linalool. Differences in the content of linalool between oil samples extracted from the dry material grown in 2019 are due to the different ratio between inflorescences and leaves (the flowers have a higher content of linalool) with preservation of the percentage of o-cimol, and the terpinen range, hydrogenated monoterpene, present in the green material in percentage 9.64% and 15.46% respectively, undergoes an oxidation process: its content decreases to 5.11%, having a common track of biosynthesis with linalool.

Oxygenated monoterpene compounds are the majority in all samples analysed, but their percentage is higher in dry samples, so that some of the dry hydrogenated monoterpenes are oxidized, the MO/MH ratio being inversely proportional.

The profile of the volatile oil of Monarda didyma containing the two main components linalool and timol, known for their antifungal effect, led to the study of its antifungal effect on two fungal species: Botrytis cinerea and Aspergillus flavus.

The results of the EO test of Monarda didyma on Botrytis cinerea are presented in Table 2.

Table 2.

The results of the EO test of *Monarda didyma* on *Botrytis cinerea*

Botrytis cir	diametru cresteremiceliu (mm)								
		10µL	20µL	30µL	50µL	100µL	150µL	200µL/15 ml mediu	
Monarda 2	control	0.06%	0.12%	0.20%	0.30%	0.60%	1%	1.30%	Control (-)
r1	18	13.5	12	8	8	8	8.00	8.00	8
r2	18.5	13	12	9	8	8	8.00	8.00	8
r3	18.6	15	12.5	8.6	8	8	8.00	8.00	8
media	18.37	13.83	12.17	8.53	8.00	8.00	8.00	8.00	8.00
ab st	0.32	1.04	0.29	0.50	0.00	0.00	0.00	0.00	0.00
Isop albastru									
R1	18	18	18	18	18	16.2	8	8	8
R2	18.5	18	18.5	18	18	16	8	8	8
R3	18.6	18.5	18	18.5	18.3	16	8.2	8	8
media	18.37	18.17	18.17	18.17	18.10	16.07	8.07	8.00	8.00
ab st	0.32	0.29	0.29	0.29	0.17	0.12	0.12	0.00	0.00
Isop mov									
R1	18	18	17	16	14	9	8	8	8
R2	18.5	18.5	17.3	16.5	15	8.5	8	8	8
R3	18.6	18.5	17	16.8	15.3	9	8	8	8
media	18.37	18.33	17.10	16.43	14.77	8.83	8.00	8.00	8.00
ab st	0.32	0.29	0.17	0.40	0.68	0.29	0.00	0.00	0.00
Rozmarin									
R1	18	18.3	18	18	18	14	8	8	8
R2	18.5	18.5	18.5	18	18	13	8.6	8	8
R3	18.6	18.6	18	18	17.5	13.5	9	8	8
media	18.37	18.47	18.17	18.00	17.83	13.50	8.53	8.00	8.00
ab st	0.32	0.15	0.29	0.00	0.29	0.50	0.50	0.00	0.00

Volatile oil of Monarda didyma has been observed to inhibit the development of Botrytis mycelium at a concentration of 0.3% while, in hyssop and rosemary oils, it is 1% and 1.3%, respectively. The results obtained correlate with data from literature, the oil tested having higher antifungal potential than Botrytis, due to a ratio linalool/timol = 2.5, the minimum inhibitory concentration (MIC) obtained being lower than that found in literature and then that of linalool and timol individually. Studies show that, in the process of oil vapour fumigation, the required concentration is lower and spore production is inhibited. Shin et al. (2014) showed that the antifungal activity of timol was 4 times higher than that of linalool in the control of mycelium ash mould on table grapes because 30 µg / ml of timol controlled the development of fungal mycelium just like 120 µg / ml of linalool.

The results of the EO test of Monarda didyma on Aspergillus flavus are presented in Table 3.

Table 3.

The results of the EO test of Monarda didyma on Aspergillus flavus

aspergillus flavus									
Monarda 2018		10µL	20µL	30µL	50µL	100µL	150µL	200µL/15 ml mediu	
control	Control	0.06%	0.12%	0.20%	0.30%	0.60%	1%	1.30%	Control(-)
R1	18.5	17	11	10.8	8	8	8	8.00	8
R2	18	17.5	11	10.3	8	8	8	8.00	8
R3	18	17	12	10.2	8	8	8	8.00	8
media	18.17	17.17	11.33	10.43	8.00	8.00	8.00	8.00	8.00
abatere st	0.29	0.29	0.58	0.32	0.00	0.00	0.00	0.00	0.00
Isop albastru									
R1	18.5	18.5	18	18	17	9.2	9	8.00	8
R2	18	18	18	17	18	9.2	9	8.00	8
R3	18	18	18.5	18	17	9	9.2	8.00	8
media	18.17	18.17	18.17	17.67	17.33	9.13	9.07	8.00	8.00
ab st	0.29	0.29	0.29	0.58	0.58	0.12	0.12	0.00	0.00
Isop mov									
R1	18.5	18	15	14	11	8	8	8.00	8
R2	18	17	17	13	10	8	8	8.00	8
R3	18	17	17	14.00	11	8.5	8	8.00	8
media	18.17	17.33	16.33	13.67	10.67	8.17	8.00	8.00	8.00
ab st	0.29	0.58	1.15	0.58	0.58	0.29	0.00	0.00	0.00
Rozmarin									
R1	18.5	18.5	18	16	14	12	9	8	8
R2	18	18	18	**17**	14	11	9.2	8	8
R3	18	18	18	15.5	14	11	9.1	8	8
media	18.2	18.2	18.0	16.2	14.0	11.3	9.1	8.0	8.0
ab st	0.3	0.3	0.0	0.8	0.0	0.6	0.1	0.0	0.0

Volatile oil of Monarda didyma inhibits the development of Aspergillus flavus mycelium at a concentration of 0.3%, while in hyssop and rosemary oils, it is 1% and 1.3%, respectively.

CONCLUSIONS

1. Monarda Didyma is a species less known as a cultivated plant in Romania.
2. Under the pedoclimatic conditions of the BUASVMT Didactic Station, cultivated as an aromatic plant, Monarda finds a high degree of favourability expressed both by the yield of the oil extracted and the chemical composition of the essential oil of Monarda, which differs from the data in literature.
3. Analysis of Monarda oil was carried out by gas chromatography coupled with mass spectrometry (GC-MS): 50 microlitres of essential oil were taken in vial with 1 ml hexane from which 1microlitre at 220oC was injected into a chromatograph gas Shimadzu GCMS-QP2010 Plus.
4. The analysis of the volatile oils of Monarda didyma grown in 2018-2019 highlighted up to 29 compounds, with a high content of linalool 34.84-56.29%, timol 12.29-19.90%, o-cimol 10.77-18.31%, and terpinen range 5.14-15.46% which varied depending on the condition of the material from which the volatile green-dry oil was extracted, harvest period, climate conditions and the different ratio between inflorescences and leaves of the samples from which they were extracted.

5. The content in linalool varies depending on the vegetation phase, being maximum during the flowering period, July-August and predominating in inflorescences.

6. The linalool content of essential oil has maximum values for dry samples: in the oil samples extracted from green material, it decreases while the percentage of timol and isotimol (carvacrol) increase.

7. Oxygenated monoterpene compounds are the majority in all the samples analysed, but their percentage is higher in dry samples: some of the hydrogenated monoterpenes by drying are oxidized, the MO/MH ratio being inversely proportional.

8. The profile of the volatile oil of Monarda didyma containing the two main constituent linalool and timol, known for their antifungal effect, led to the study of its antifungal effect on two fungal species: Botrytis cinerea and Aspergillus flavus.

9. The antifungal activity of the essential oil of Monarda didyma is the result of the synergistic action of the components and not only of the main ones, but the composition is variable depending on the harvest period, climate conditions, and variety and, thus, one cannot establish a standard antibacterial or antifungal concentration.

10. Essential oils and their components have a variety of targets, especially the membrane and cytoplasm and, in certain situations, they completely alter the morphology of cells.

11. Essential oils are classified as "Generally recognized as safe" (GRAS) by the FDA, so they are not harmful and, due to their natural origin, are much more widely accepted by consumers than "synthetic" agents. The antimicrobial or antifungal activity of essential oils is determined by the properties of terpenes/terpenoids which, due to their lipophilic nature and low molecular weight, are able to disturb the cell membrane, causing death or inhibition of spores and germination of fungi that alter food.)

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