CHEMICAL CONSTITUENTS, ANTIOXIDANT AND ANTIFUNGAL POTENTIAL OF MELISSA OFFICINALIS FORMULATIONS

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Abstract

The purpose of this paper is to chemically characterize lemon balm (Mellissa officinalis) cultivated in the western part of Romania, as well as to obtain essential oil, aromatic water and alcoholic extract, which were characterized in terms of chemical composition and of active principles content with possible role in antifungal action against Fusarium graminearum.

Characterization of the plant matrices was carried out in the laboratories of the Interdisciplinary Research Platform of BUASVM from Timisoara by determining the proximal composition (moisture, protein content, lipid, mineral substances, macro and microelements content) using the official AOAC methods. Alcoholic extracts were obtained by macerating the plant in 70% ethyl alcohol, and volatile oils were obtained by water vapor entrainment. The chemical composition of the oil was determined by GS-MS and the alcoholic extracts by LC-MS.

Melissa Officinalis proximal composition results were: 15.3% moisture, 5.7% total mineral substances, 7.37% protein content and 0.94% lipid content, Na 83.34 ppm, Mn 16.41 ppm, Fe 119 , 4 ppm, Zn 29, 163 ppm, Ni 1.067 ppm, Cu 6.589 ppm

Key words: *lemon balm, chemical composition, alcoholic extracts, volatile oil*

1. INTRODUCTION

Medicinal plants were the main source of natural remedies from ancient times until today, being used in the treatment of several human diseases for their pharmacological and therapeutic properties. The medicinal plants have a great importance due to chemical constituents with antioxidant activity that can be found in very large concentrations and can be responsible for the effects in various diseases, such as cancer and cardiovascular problems (Jafer et al 2009).

Lemon balm (*Melissa officinalis L.*) is a perennial aromatic plant, has curative properties and belongs to *Lamiaceae* family (Craciun et al., 1992). Lemon balm comes from southern Europe, Asia and south of North America, the lemon balm populations being distributed in all Mediterranean countries (Jafer et al., 2009). Currently is used as a food additive, in cosmetics, ornamental plant, in medicine and pharmacology, phyto-pathology and food preservation as an antimicrobial and antioxidant compound (Sar and Ceylan 2002), also is used as antibacterial and stimulator agent for the immune system (Sadraei et al. 2003). The tea made from lemon balm leaves is often used as antispasmodic, analgesic, anti-inflammatory, antipyretic, diuretic and sedative (Carlini et al., 1986).

Mellissa officinalis essential oil is recommended for its antimicrobial activity and the water extracts is recommended for antiviral and antioxidative properties, anti-inflammatory and anti-diabetic effects (Abdellatif F., et al., 2014). Lemon balm oil might be exploited as natural antibiotic for the treatment of different infectious diseases (Bachir R.G., Benali M, 2012).

This paper aim was to characterize lemon balm (Mellissa officinalis) in terms of physical-chemical properties, samples cultivated in the western part of Romania and to obtain essential oil, aromatic water and alcoholic extract, which were characterized in terms of active principles content with possible role in antifungal action against *Fusarium graminearum.*

2. MATERIALS AND METHODS

2.1. Plant material

Plants were purchased from private producers from Western Romania. The characterization of the plant matrices was carried out in the laboratories of the Interdisciplinary Research Platform of *Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara,* by determining the proximal composition (moisture, protein content, lipid, mineral substances, macro and microelements content) using the official AOAC methods (AOAC 1997): protein (954.01) Lipids (920.39); Ash (923.03) and humidity (925.09) and atomic absorption spectrometry (AAS).

2.2. Alcoholic plant extracts

Alcoholic herbal extracts were obtained by macerating matrices, dried and ground in 70% ethyl alcohol in a ratio of 1:10 (m/v) and then the extracts were filtered and stored at 2-40 ° C until antioxidant and antifungal activity was tested.

2.2.1. Total polyphenols (TP) determination of extracts

Alcoholic extracts have been characterized in terms of total polyphenols (TP) content using Folin-Ciocalteu method (Singleton, et al., 1999).

0.5 ml extract alcoholic is treated with 1.25 ml Folin-Ciocalteu reactive diluted 1:10 with water. The sample was incubated for 5 minutes at room temperature and 1 ml of Na2 CO3 60g/l was added. After 30 min of incubation at 50 ° C the absorbance of the samples was measured at 750 nm using a UV-VIS spectrophotometer (Analytic Jena Specord 205). The calibration curve was obtained using gallic acid GA as standard (concentration range 5-250 μg / ml). The results were expressed in μg / ml of extract.

2.2.2. Determination of individual polyphenols by LC-MS

The main compounds responsible for the biologically active action of alcoholic extracts were determined by LC-MS. The major polyphenols characteristic of the vegetable matrices were determined by LC-MS using the Shimadzu Chromatograph System equipped with a detector SPD-10A UV and LC-MS 2010, column EC 150/2 NUCLEODUR C18 Gravity SB 150 x 2mm x 5 um.

The chromatographic conditions were as follows: mobile phases A: water acidified with formic acid at pH-3, B: acetonitrile acidified with formic acid at pH-3, gradient program: 0.01-20 min. 5% B, 20.01-50 min 5-40% B, 5-55 min, 40-95% B, 55-60 min 95% B. The solvent flow rate 0.2 ml / min, temperature 20 ° C, the monitoring wavelength was 280 nm. The calibration curves were performed in the range of 20-50 μg / ml.

2.3. EO extraction

Essential oils were obtained by water steam distillation using the Clevenger plant extraction installation according to the *Pharmacopeia method* (1996). The obtained oils were packaged, labeled and stored at 2-40C until used for in vitro and in vivo tests.

2.3.1. GC-MS

The chemical composition of the essential oils was determined using a gas-chromatograph with a mass spectrometer (GS/MS) Agilent Technology 7820A (Agilent Scientific, USA) coupled with MSD 5975 mass spectrometer and equipped with a capillary column with the following characteristics: DB WAX 30 m X 250 μm X 0.25 μm). The carrier gas was helium at a flow rate of 1 mL / min. For the separation of compounds, the following program was used: 40° C for 1 min, 5° C min $^{-1}$ to 210°C for 5 min. Injector and ion source temperatures were 250° C and 150° C respectively. The injection volume was 1 μL at a ratio of 1:20. The NIST database was used to identify volatile compounds.

2.4. Antifungal activity

The lemon balm essential oil was introduced into the CYA (chloramphenicol-yeast-agar) culture medium in 3 different amounts to achieve concentrations of 0.02%, 0.04 and 0.06% (poison environment technique). The inoculation of the added medium was done with the young mycelium (5 days). Three Petri dishes / box were used, each one representing a determination. The boxes were incubated in the dark at 23-25ºC. In parallel, CYA medium supplemented with methyl thiophanate was also used – a fungicide used in plant protection - as a negative control and CYA medium without any addition as positive control.

On day 6, mycelial growth was measured on two perpendicular diameters around each mycelium, the average serving for estimating mycelial growth.

3. RESULTS AND DISCUSSIONS

3.1. Proximal chemical composition of Melissa off

The proximal composition (moisture, protein content, lipid, mineral substances, macro and microelements content) determined for lemon balm plant samples, are presented in figures 1 and 2, as mean of 3 determination and standard deviation.

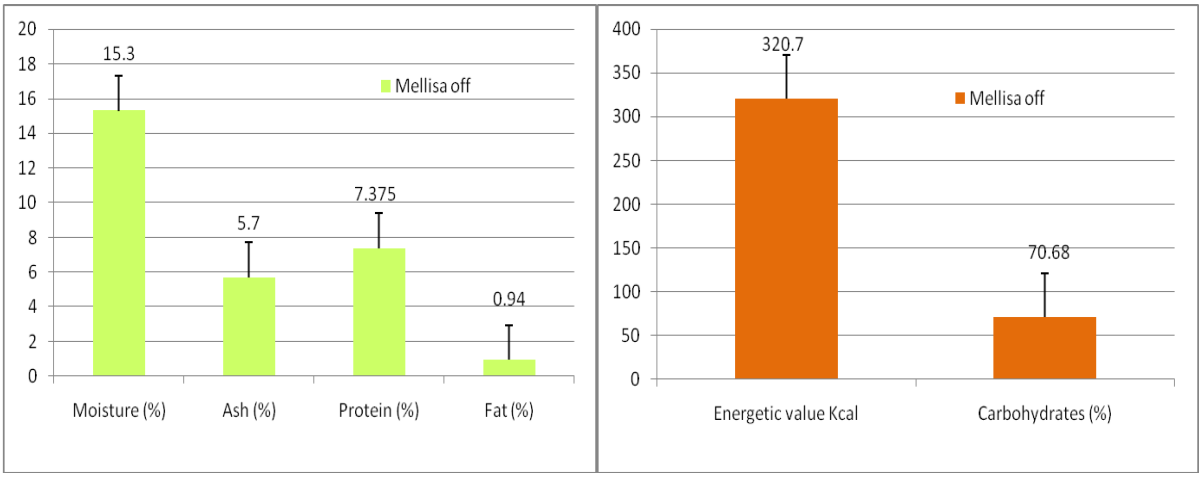
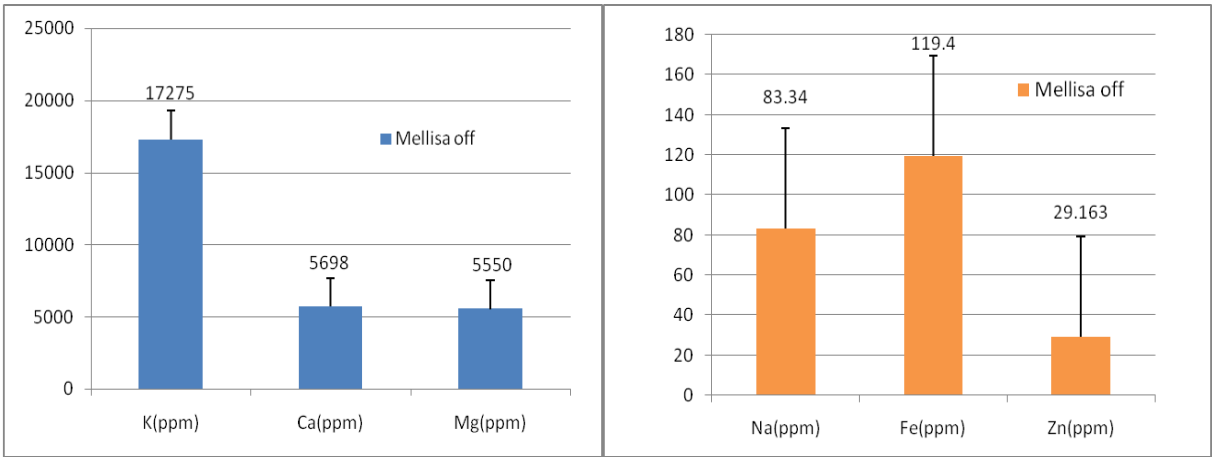


Figure 1. Proximate chemical composition in *Melissa officinalis* plant samples



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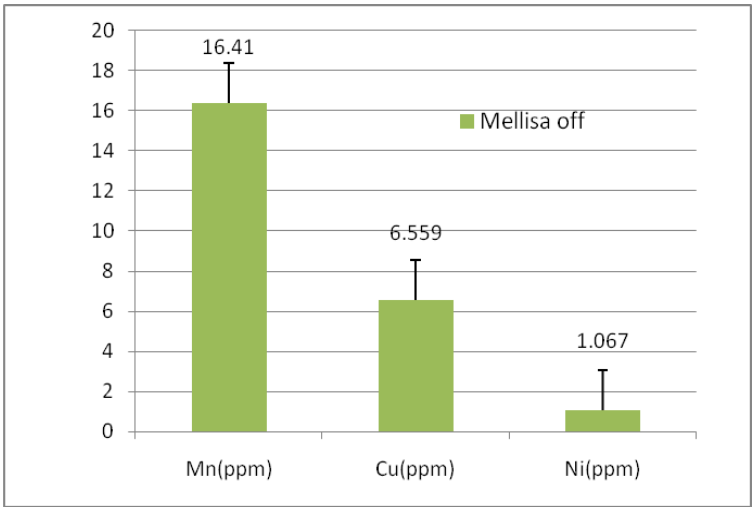

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Figure 2. Macro and micro elements in plant matrices (A - Mg, Ca and K content in lemon balm); (B – Zn, Fe and Na content in lemon balm); (C- Ni, Cu and Mn content in lemon balm)

The results shown in figure 1 show that the moisture content of *Melissa officinalis* samples, previously free-dried, was 15.3%, the ash value which reflects the mineral content of the samples, was 5.7%, the protein content of the studied samples was 7.375% and the lipid content had a value of 0.94%. The energy value of *Melissa officinalis* samples was 320.7 Kcal and the carbohydrate level was 70.68%.

The proximate composition of the studied vegetable matrix is in accordance with the data previously reported in the literature. Ullah et al., 2013 showed a moisture level of 8.71%, 9.16% ash, 6.82% protein, 6.89% lipids and 61.37% carbohydrates in lemon balm samples.

Experimental results on the macro and microelements composition reveal that potassium was the most abundant macro element present in the analyzed lemon balm samples (17275 ppm) followed by calcium (5698 ppm), magnesium (5550 ppm), iron (119.4 ppm), Sodium (83.34 ppm), Zn (29.163 ppm). Lower values were recorded for Mn 16.41 ppm, Cu 6.559 and Ni 1.067 (figure 2).

The level of macroelements in plants depends on preferential plant uptake, soil element content, its characteristics and culture technology (Yagi et al., 2013). The values registered correspond to those reported by other authors in literature data (Gjorgieva, et al., 2011).

3.2. Total polyphenols (TP) in alcoholic extracts

The obtained results indicated a high content of total polyphenols (TP) in the Melissa officinalis alcohol extract of 1625.89 μg / ml.

Literature studies have indicated that in medicinal plants belonging to the Lamiaceae family, the TP content ranges between 61-137 mg GAE·g^{-1} (Velickovic si colab., 2011), 40-60 mg GAE g^{-1}d.m. (Spiridon, 2011) si 14.53-33.22 mg GAE·g^{-1} (Derakhshani si colab. 2012).

3.3. Individual polyphenols by LC-MS

The main polyphenols characteristic of lemon balm plant matrices were determined by LC-MS. The results were expressed in mg · g^{-1}d.m. 11 compounds were identified, the retention times, the samples chromatogram and the results obtained are shown in figures 3 and 4.

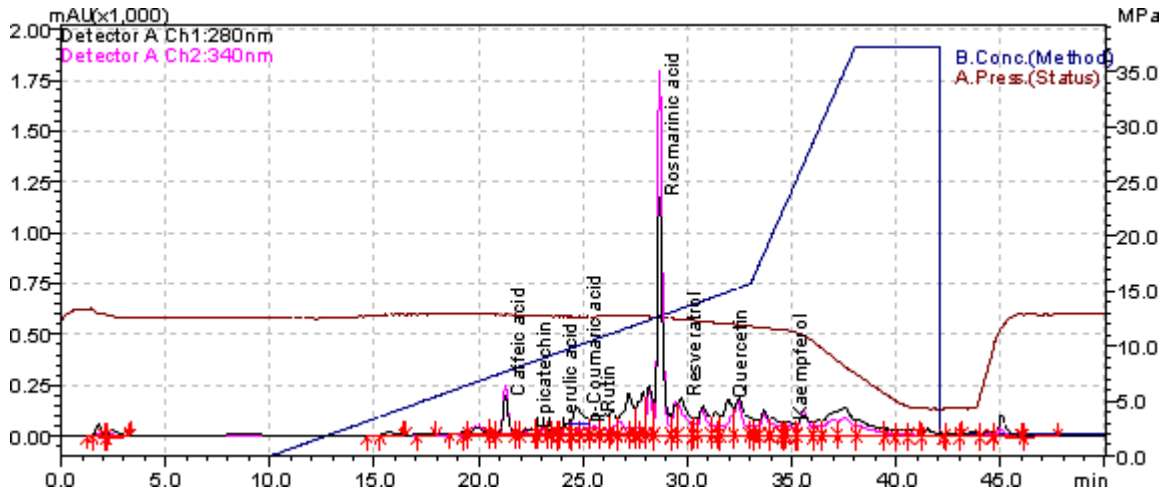

Figure 3. Chromatographic profile of individual polyphenols separated by LC-MS

In figures 3 and 4 are presented experimental results obtained of the chromatographic profile of individual polyphenols separated by LC-MS. Of the polyphenols one third is hydroxycinnamic acids (HCAs), such as caffeic acid (CA) with a level of 27.42 mg · g^{-1}d.m, epicatechin (EC) with a level of 39 mg · g^{-1}d.m, and rozmarinic acid (RO) with a value of 84.3 mg · g^{-1}d.m., which are phenolic compounds with a very high abundance in fruits, vegetables and cereals. Lower values were determined in the case of resveratrol (RS) with a content 7.75 mg · g^{-1}d.m., followed by kaempherol (KE) with a level of 4,3 mg · g^{-1}d.m., rutin (RU) with 3,93 mg · g^{-1}d.m. and the lowest level was found in the case of cumaric acid (CU) 0,34 mg · g^{-1}d.m. (figure 4).

The values determined in the lemon balm alcoholic extract studied samples are in accordance with those reported by other authors (Miron et al., .2013).

Acids such as CA, FE, CU were detected in lower levels comparing with RO (figure 4). CA level was 27.42 ug/ml in lemon balm extracts, value comparable to those reported by Proestos et al. 2013.

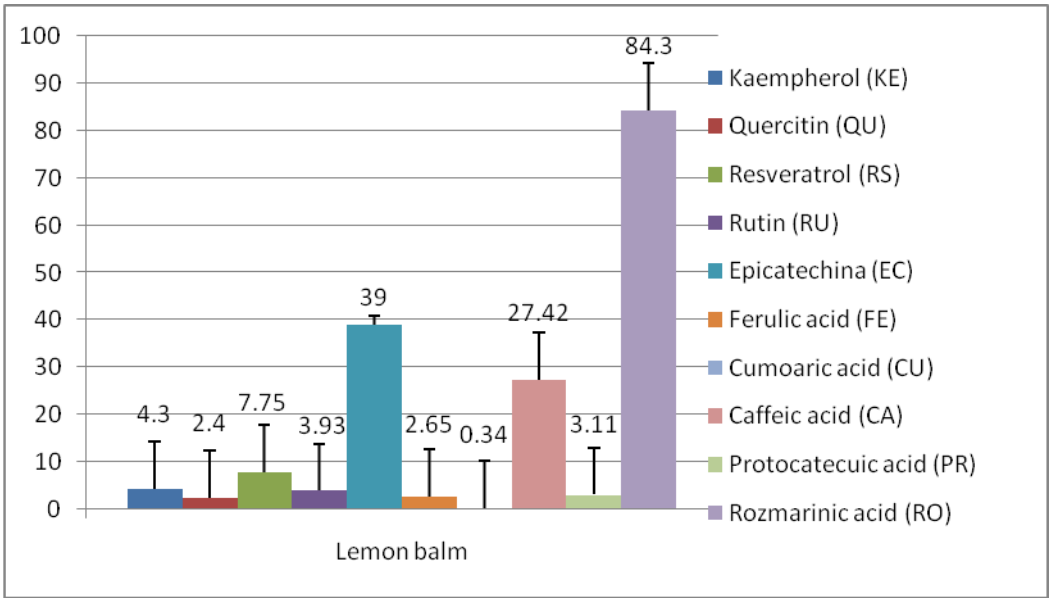

Figure 4. Individual polyphenols content (ug/ml) determined by LC/MS in plant extracts

3.4. EO yield

The yield of EO in studied lemon balm was low (0.3%), but in accordance with other studies that reported EO yield in lemon balm between 0.09 to 0.45%, in samples from 17 European regions (Patora et al. 2003).

3.5. EO composition

The chromatographic profile of lemon balm oil shows that m-menthane is a major compound with a percentage of 39.264%. The other compounds determined were in the following order 3-carene 17,013 %, limonene 14,548 %, gamma-terpinene 10.373 %, alpha –phinene 6.405 % (figure 5). Lowest values were recorded to cyclopenthane 0.376 % and acetaldehyde 0.302%. The experimental results obtained are in agreement with the literature data (Rus et al., 2015, Ruangamnart A. et al., 2015).

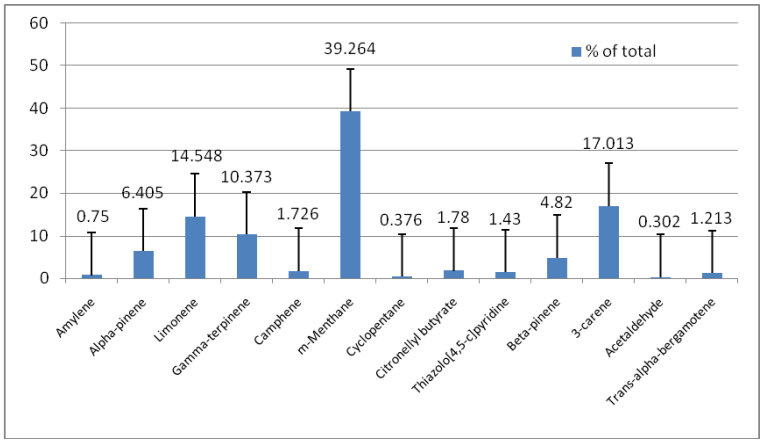

Figure 5. Chemical composition of *Mellissa officinalis* EO

3.6. Antifungal activity of Melissa officinalis formulation

Antifungal activity of *Mellissa officinalis* essential oil and water obtained after hydro-distillation and alcoholic extract, were studied against *Fusarium graminearum* mycelial strains.

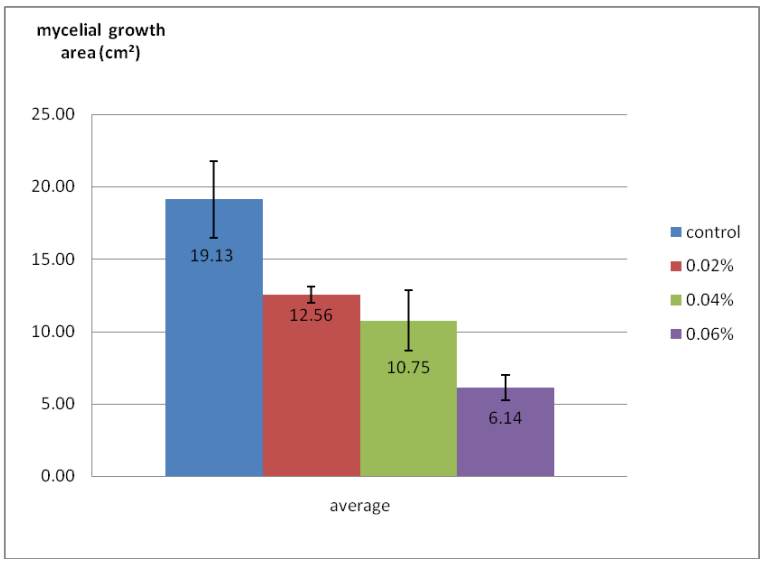

Figure 6. Effect of *Melissa off* EO at different concentrations on *Fusarium graminearum* mycelial growth

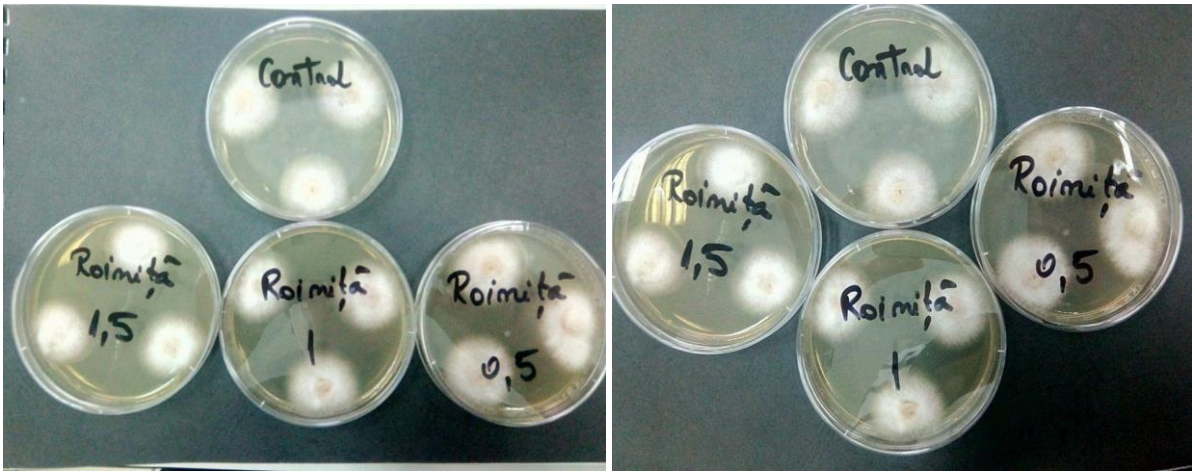

Figure 7. *Fusarium graminearum* mycelial growth under the effect of lemon balm EO

The results obtained and shown in figure 6 and 7 show that Melissa officinalis essential oil have an inhibitory effect on the *Fusarium graminearum* mycelium growth and therefore can be used as a natural antifungal agent. The effect is observable even at 0.02% concentration in culture medium. The increase of the oil concentration in the medium is in the inverse correlation with Fusarium's surface area, thus the highest inhibitory effect is observed in the case of the 0.06% lemon balm EO concentration. In this variant, the value of the mycelial growth surface is only 6.14 cm² respectively 32% reported to the control sample.

As have been demonstrated by other studies, the antifungal activity of Melissa off essential oil is not limited to the inhibition of dermatophyte pathogens (Menezes et. al, 2015) but also to filamentous fungi such as *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* (El Quadi, et al., 2017) .

The results obtained regarding antifungal concentration of *Melissa officinalis* EO are in agreement with results obtained of other researchers, showing that the total antifungal effect is above 1 µl / ml (Abdellatif, 2014). In our case, at 0.6 µl / ml concentration, a significant decrease in *Fusarium* mycelium was achieved compared to the control sample. Given the significant losses on cereals, due to the *Fusarium sp* attack during the growing season and during storage, *Melissa officinalis* EO represents a viable alternative in postharvest period for the storage of cereal products in order to limit the propagation of seed pathogen agents.

Regarding water obtained after hydrodistillation and alcoholic extracts, no antifungal activity against *Fusarium graminaerum* was observed. Our research strengthens the idea that the EO majority compounds are responsible for the antifungal activity and not polyphenolic compounds of the methanol extract. Future studies are needed to elucidate the fungal growth inhibition mechanism.

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CONCLUSIONS

The chemical analysis of *Melissa officinalis* plant highlights the high intake of macro and microelements, but also a high content of total polyphenols (TP). The experimental results regarding individual polyphenols showed that acids such as CA, FE, CU were detected in lower levels

comparing with R0 acids. The yield of EO in studied Mellissa officinalis samples was low, the chromatographic profile of lemon balm oil showing that m-menthane is the major compound.

The results of antifungal activity revealed that the essential oil of *Melissa officinalis* inhibited the mycelia growth of *Fusarium graminearum* and therefore can be used as a natural antifungal agent. The increase of the oil concentration in the medium is in the inverse correlation with Fusarium's surface area, thus the highest inhibitory effect is observed in the case of the 0.06% lemon balm EO concentration.

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