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Organic and mineral fertilization and chemical composition of lemon balm (*Melissa officinalis*) essential oil

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Abstract: *Melissa officinalis* L., Lamiaceae, is an herb with great growth prospects in the cosmetic industry due to its essential oil. In order to improve its production, it is necessary to study related agricultural practices. This study evaluated the effect of organic and mineral fertilization on the chemical composition of lemon balm (*Melissa officinalis* L.) essential oil. The assay was conducted at the "Fazenda Experimental do Glória" of the Federal University of Uberlândia, and essential oil extraction and GC/MS analyses were completed by the Centre for Research and Development on Plant Genetic Resources of the Campinas Agronomic Institute. The assay was conducted in a randomized complete block design with three replications. The tested treatments were six types of fertilization (0, 1, 2, 4, 8 kg.m^{-2} of cattle manure and mineral fertilizing with 60 g.m^{-2} of NPK 4-14-8 + 4 g.m^{-2} of boric acid) with four replications. The essential oil was extracted by hydrodistillation in a modified Clevenger apparatus. The chemical composition was analyzed by GC/MS. The essential oil presented the same compounds for all treatments; however, the relative proportion of some chemical constituents was altered according to the treatment. Neral, geranial, and citronellal were the major constituents.

Introduction

Lemon balm (*Melissa officinalis* L., Lamiaceae) is a medicinal and aromatic plant from Asia and Europe, adapted to subtropical and moderate weather and found throughout Brazil (Corrêa Junior et al., 1994). The leaves are used to make tea, which serves as a digestive aid and as a tranquilizer. Lemon balm is used to combat headaches, including migraine headaches, gas pains and cramps, and viral infections (colds, herpes, mumps, chickenpox); it facilitates menstruation and encourages the formation of bile. Its essential oil, due to its citrus fragrance and its power to induce relaxation, has great possibilities for use in the cosmetic industry and perfumery (Rigueiro, 1992).

Chemical constitution analyses of aerial part extracts show evidence of the presence of essential oils (citral, citronellal, geraniol, camphor), mucilage, tannins, saponins, and resins. Some of the constituents are active principles for cosmetic and medicine production (Sarer & Kökdil, 1991; Van Den Berg et al., 1997; Carnat et al., 1998; Sorensen, 2000; Blank et al., 2005).

There are many studies that show the medicinal effect of lemon balm's essential oil. Edris (2007) describes ways that lemon balm's essential oil can help treat some diseases, such as cancer and cardiovascular diseases, and describes its bactericidal, antiviral, antioxidant, and therapeutic effects.

Factors that influence essential oil production in herb and aromatic plants can be intrinsic and extrinsic. Extraction methods, drying temperature, geographical differences, temperature, harvest time, seasonality, day length, light intensity and quality, water quantity, nutrients available in the soil, and altitude are considered extrinsic. Everything involving genetic control is intrinsic and can be influenced by extrinsic factors (Sorensen, 2000).

It is known that organic fertilization is recommended for herb cultivation. Organic fertilization is the main source of nutrients and energy for soil microorganisms, providing macronutrients and mostly micronutrients for the plants. Organic fertilizers also benefit agriculture by keeping costs low and returns high

(Alvarenga, 2004).

As suggested by Mapeli et al. (2005), plant feeds should be compared and contrasted because nutrient excess or deficiency can intervene in the production of biomass and the quantity of active principles. When herbs, aromatic plants, and spice plants are cultivated, the influence that the nutrients have on plant development and its active principle production must be observed. Thus, the purpose of this study was to evaluate the *Melissa officinalis* essential oil composition, as a function of mineral and organic fertilization.

Materials and Methods

The study was conducted at Glória Research Farm (18°57' S e 48°12' W), part of the Universidade Federal de Uberlândia (Federal University of Uberlândia), located in Uberlândia-MG. The minimum medium temperature during the period of the assay was 19 °C and maximum was 27 °C; the medium precipitation during the three months of assay conduction was 95.33 mm.

The seedlings of *Melissa officinalis* L., Lamiaceae, were each produced in a gerbox maintained in BOD. Four days after germination, they were transplanted into polystyrene trays with 200 cells, using Plantmax commercial substrate. The seedlings in the polystyrene trays were maintained in a greenhouse. After sixty days, the seedlings were transplanted to the field.

The experiment was arranged in a complete random block design with six treatments and four replications. The organic fertilizer used was cattle manure at the following doses: 0, 1, 2, 4, 8 kg m^{-2} (T0, T1, T2, T3, T4), and the mineral fertilizer (T5) was 30 g of NPK 4-14-8 and 2 g of boric acid per meter. The spacing used was 50 cm between rows and 40 cm between plants. Each plot was composed of four lines with seven plants. The plants were irrigated daily by an overhead sprinkler system. The plants were harvested 83 days after their transplantation into a field; plants were cut at soil level. The cut leaves and stalks were transported to the phytotechnology laboratory and weighted in a precision balance. Fresh weight of leaves and stalks was obtained from each plot. From the fresh leaves of each plot a sample of 100 g was separated for essential oil extraction and chemical analysis. The rest of the leaves were dried at 40 °C for seven days in a forced air oven.

The essential oil chemical composition analysis and extraction was accomplished at the Laboratory of Natural Products from IAC Centre for Research and Development on Plant Genetic Resources. Dry and frozen 100 g samples of essential oil extraction leaves were used. The material was put into gallons of 3,52 pts and immersed in distilled water. The essential oil was obtained by hydrodistillation in a modified Clevenger apparatus. The process officially started when the first drops of essential oil condensed; the remaining extraction took two hours. The essential oil was stored in amber bottles and maintained in a freezer until the chemical composition analysis productivity calculation.

The qualitative analysis of the essential oil compounds was performed on a gas chromatography coupled to a mass spectrometer (GC-MS Shimadzu, QP5000), operating at an MS ionization voltage of 70 eV. The chromatography was equipped with a fused silica capillary column OV-5 (30 m x 0.25 mm x 0.25 µm), and helium was used as the carrier gas. The following chromatography conditions were used: injector at 240 °C, detector at 230 °C, gas flow 1.0 mL/min., split: 1/20, initial column temperature of 60 to 135 °C at rate of 3 °C/min then to 135 to 165 °C at rate of 8 °C/min then to 165 to 240 °C at rate of 5 °C/min, ending with a 5 min isothermal at 240 °C, and injecting 1 µL of solution (1 mg of essential oil and 1 mL of ethyl acetate).

The compounds were identified by the comparative analysis of the acquired mass spectra with those stored in the GC/MS database of the system (Nist 62.Lib), literature (McLafferty & Stauffer, 1989), and Kovats retention indices obtained by the co-injection of the samples and a mixture of *n*-alcanes (C_9H_{20}-$C_{25}H_{52}$, Sigma Aldrich, 99%) employing a column temperature program as follows: 60 to 240 °C at a rate of 3 °C/min (Adams, 2007).

The samples were compared by the Tukey test ($p<0.05$) using the software Sisvar® (Ferreira, 2000). Contrasts between control and mineral fertilizer, as well as between the medium of treatments T1, T2, T3, and T4 and mineral fertilizer, were accomplished for essential oil extracted from fresh and dried leaves, using the F test.

Results and Discussion

The content of essential oil extracted from fresh (OF) and dry (OS) leaves did not present significative variation for cattle manure doses (Tables 1 and 2). This calibrates or contradicts findings in earlier studies. Ming (1994), studying *Lippia alba*, verified that gradually increased doses of cattle manure promoted higher biomass production, but resulted in decrease of essential oil content. Morais (2006) tested different doses of poultry manure as organic fertilizer for *Ocimum basilicum* and did not observe significative differences in biomass production; however, for linalool content, the poultry manure doses presented a significative influence.

Significative differences for cattle and poultry manure, both organic fertilizers, in *Hyptis suaveolens* plants have been observed, but the poultry manure (6 kg m^{-2}) resulted in higher essential oil yield when compared with the cattle manure (12 kg m^{-2}) (Maia, 2006). Ming (1992) explained that fertilization cannot be dissociated from other components that interfere in plant development and in essential oil yield and content. The author suggested that, besides generic factors, there are environmental factors, such as soil microorganism and stresses suffered by plants, interfering in the biosystolic route.

The treatments 8 kg m^{-2} of cattle manure and mineral fertilizer did not result in higher essential oil content and yield (Tables 1 and 2). Different results were noted in *Hyptis marrubioides* plants, in which higher essential oil content and yield were correlated to higher doses of organic fertilizers (Sales, 2006). In *Mentha x villosa* plants the essential oil content was reduced progressively when the manure doses were increased (Chaves et al., 1998). The mineral and organic fertilizer did not present this tendency in the present study.

Neral, geranial, and citronellal were major constituents of the essential oils of all treatments (Tables 1 and 2). Increasing doses of cattle manure resulted in a decrease in citronellal in essential oil extracted from dried leaves (Table 2). The quality of essential oil is very important for the import/export market, and chemical analysis is required (Blank et al., 2005). In the case of *Melissa officinalis*, commercial essential oil must present the major chemical compounds neral, geranial, and citronellal, and preferably not present nerol and geraniol (Blank et al., 2005). Geraniol content progressively increased with higher cattle manure doses (Tables 1 and 2) when compared with the control.

Citronellal content of the essential oil extracted from dried leaves of *M. officinalis* decreased from 6.37 to 3.77% when 8 kg m^{-2} of cattle manure was used. Increase of linalool, citronellol, and geraniol content was observed using the highest cattle manure doses (Table 1). Neral content of the essential oil extracted from fresh leaves of *M. officinalis* increased when 6 kg m^{-2} of cattle manure was used (Table 2). Analyzing these results, we can conclude that nutrient management in the soil can promote different concentrations of chemical compounds in the essential oil of *M. officinalis*.

Contrasts between the control and mineral fertilizer data were observed as were contrasts between the medium of treatments T1, T2, T3, and T4 and mineral fertilizer for essential oil extracted from fresh and dried leaves (Table 3). In the contrasts between control and mineral fertilizer, no differences were observed for the compounds of essential oil extracted from dried leaves (Table 3). Significative differences were observed in the contrasts between control and mineral fertilizer for the compounds citronellol and methyl geranate, and the mineral fertilizer resulted in higher content of the compounds. The contrasts among the medium of the treatments T1, T2, T3, and T4 and mineral fertilizer resulted in no significative differences for the essential oil compounds extracted from fresh and dried leaves.

Table 1. Constituents of lemon balm (*Melissa officinalis*) essential oil distilled from fresh leaves harvested in plants cultivated with organic and mineral fertilizers.

Compounds	Percentage						KI
	T0	T1	T2	T3	T4	T5	
pentyl propanoate	0.295	0.275	0.265	0.237	0.262	0.307	973
3-octanone	0.445	0.475	0.485	0.522	0.585	0.442	979
mircene	0.135	0.1500	0.1475	0.1475	0.1375	0.1175	988
cis-β-ocimene	0.267	0.4050	0.3425	0.2950	0.4450	0.3200	1044
linalool	-	-	-	-	-	-	1096
perylene	0.170	0.172	0.165	0.115	0.192	0.167	1094
citronellal	7.392	5.767	5.565	6.275	5.817	7.522	1147
isomentol	1.440	1.610	1.520	1.557	1.612	1.490	1176
citronellol	-	0,220	0,137	0,202	0,247	0.280	1227
neral	31.970	33.395	33.777	33.330	33.570	32.512	1236
geraniol	0.227	0.770	0.447	0.485	0.660	0.557	1251
methyl citronellate	0.695	0.405	0.405	0.535	0.350	0.552	1257
geranial	50.455	49.310	49.510	49.372	49.897	48.822	1267
methyl geranate	0.265	0.250	0.265	0.297	0.245	0.272	1319
geranyl acetate	1.802	2.165	1.772	1.650	1.862	1.960	1379
β-caryophyllene	0.515	0.385	0.637	0.567	0.302	0.577	1413
Essential oil content (%)	0.075	0,084	0.072	0.100	0.087	0.071	
Essential oil yield (mL plant^{-1})	0.298	0.437	0.289	0.403	0.391	0.326	

T0, T1, T2, T3, T4 (0, 1, 2, 4, 8 kg m^{-2} of cattle manure, respectively) and T5 (60 g m^{-2} of NPK 4-14-8 + 4 g m^{-2} of boric acid). KI=Kovats Index.

Table 2. Constituents of lemon balm (*Melissa officinalis*) essential oil distilled from dried leaves harvested in plants cultivated with organic and mineral fertilizers.

Compounds	Percentage						KI
	T0	T1	T2	T3	T4	T5	
pentyl propanoate	0.332	0.367	0.347	0.290	0.362	0.387	913
3-octanone	0.935	2.092	1.510	1.090	1.872	1.330	979
mircene	0.275	0.315	0.270	0.217	0.392	0.260	988
cis-β-ocimene	0.725	0.712	0.827	0.605	0.795	0.760	1044
linalool	0.312	0.397	0.302	0.382	0.495	0.290	1096
perylene	0.502	0.657	0.582	0.575	0.985	0.567	1094
citronellal	6.372	5.785	5.560	5.062	3.770	5.670	1147
isomentol	-	-	-	-	-	-	1176
citronellol	0,772	1,107	1,002	0,850	1,722	0.787	1227
neral	31.030	30.217	30.952	38.895	28.890	31.855	1236
geraniol	0.817	1.367	1.337	1.125	2.520	0.925	1251
methyl citronellate	1.200	1.092	0.890	0.950	0.675	0.867	1257
geranial	39.955	38.577	40.057	39.695	37.392	40.835	1267
methyl geranate	0.552	0.610	0.512	0.572	0.670	0.495	1319
geranyl acetate	4.490	4.837	4.602	5.892	6.415	4.360	1379
β-caryophyllene	2.710	2.860	2.365	4.087	4.012	2.642	1413
Essential oil content (%)	0.127	0.141	0.115	0.100	0.112	0.115	
Essential oil yield (mL plant^{-1})	0.570	0.660	0.460	0.479	0.449	0.458	

T0, T1, T2, T3, T4 (0, 1, 2, 4, 8 kg m^{-2} of cattle manure, respectively) and T5 (60 g m^{-2} of NPK 4-14-8 + 4 g m^{-2} of boric acid). KI=Kovats Index.

Table 3. Estimations of contrasts for control *versus* mineral fertilization for compounds of essential oil extracted from dried and fresh leaves of *Melissa officinalis*.

Compounds	Control vs mineral fertilization	
	EO from dried leaves	EO from fresh leaves
pentyl propanoate	-0.0550^{ns}	-0.0125^{ns}
3-octanone	-0.3950^{ns}	0.0025^{ns}
mircene	0.0150^{ns}	0.0175^{ns}
cis-β-ocimene	-0.0350^{ns}	-0.0558^{ns}
linalool	0.0225^{ns}	---
perylene	-0.0650^{ns}	0.0025^{ns}
citronellal	0.7025^{ns}	-0.1300^{ns}
isomentol	-0.1100^{ns}	-0.0500^{ns}
citronellol	-0.0150^{ns}	-0.2825**
neral	-0.8250^{ns}	-0.5425^{ns}
geraniol	-0.1075^{ns}	-0.3300^{ns}
methyl citronellate	0.3325^{ns}	0.1425^{ns}
geranial	-0.8800^{ns}	1.6325^{ns}
methyl geranate	0.0575^{ns}	-1.9838**
geranyl acetate	0.1300^{ns}	0.2000^{ns}
β-caryophyllene	0.0675^{ns}	-0.1575^{ns}

**, *, ns - Significative at 1, 5%, and not significative, respectively (F-test).

Fertilization could not be dissociated from other components that interfere in plant development, and its essential oil yield and composition, and environmental factors, such as soil microorganisms and plant-suffered stress, interfere in the biosystolic route (Ming, 1992).

Essential oil distilled from fresh and dried leaves grown with different doses of cattle manure and mineral fertilization presented the same compounds, but different percentages of some compounds were observed.

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