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Organic and mineral fertilization influence on biomass and essential oil production, composition and antioxidant activity of *Lippia origanoides* H.B.K.

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ABSTRACT

In the present work, the effect of organic and mineral fertilization were evaluated on biomass production, essential oil content, essential oil composition, and antioxidant activity in comparison with plants cultivated without fertilization.

Field evaluations reported that fertilization does not affect biomass and essential oil production of *L. origanoides* plants. However some variations were observed in the essential oils composition. Carvacrol was the most abundant component followed by *p*-cymene, γ-terpinene, β-caryophyllene, and thymol. Changes observed in the antioxidant potential of the different essential oils may be related to essential oils (EO's) compositions. Essential oils from organic and mineral fertilization reported, respectively, higher and lower antiradical activity, while no differences were observed in the reducing power of the different treatments. Nevertheless, it was possible to partially discriminate the different fertilization treatments applied thanks to the EO's chemical composition and the bioactive potential.

We conclude that for biomass and essential oil production, fertilization plans are not needed in *L. origanoides* plants at the applied conditions, however if it is necessary to enhance the bioactivity of the essential oils from *L. origanoides*, organic fertilization is encouraged.

1. Introduction

Lippia origanoides H.B.K. (Verbenaceae), is an aromatic plant native from Central and northern South America (Vicuña et al., 2010). At domestic level, this aromatic plant is used for food seasoning but is also widely used in folk medicine. *Lippia origanoides* leaves are used as infusions to deal with stomach pain, flatulence, and indigestions. This plant is also believed to have effects as expectorant in lung and bronchial infections, is recommended for asthma treatments, tuberculosis, and is an antispasmodic agent (García-Barriaga, 1992; Stashenko et al., 2010). Part of the health benefits of this plant is attributed to the essential oil. *Lippia origanoides* is a species rich in essential oil (EO), however, its content vary considerably from 1% (Oliveira et al., 2007) and 4.6%. Variations are also observed in the EO composition of *L. origanoides*. These species present three different chemotypes according to the EO main components: carvacrol and thymol, α- and β-phellandrene, and ρ-cimene and limonene (Stashenko et al., 2010). The chemotypes characterized by high amounts of carvacrol and thymol exhibit antimicrobial activity against fungi and bacteria, reporting also considerable antioxidant potential. (Hyldgaard et al., 2012). Studies conducted with Verbenaceae species revealed that *L. origanoides* is promising due to its low toxicity level, as well as, its high capability to scavenge free radicals.

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Table 1
Soil characteristics of the experimental site used in the present study.

Soil characteristics	Experimental field characteristics
Organic matter (%)	0.29
pH	4.9
P ($mg\,dm^{-3}$)	6
K	6
Ca + Mg	2.4
Ca	1.7
Mg	0.7
Al	0.1
H + Al	2.64
Na	0.24
S	2.91
CTC	5.55

Table 2
Characterization of the organic fertilizer used in the present study.

	Organic fertilizer characterization
pH	6.8
Density ($g\,cm^{-3}$)	0.78
Moisture (%)	26.7
C/N ratio	11
Organic matter (%)	14.4
C	8.01
N	0.73
P	0.19
K	0.11
Ca	1.08
Mg	5.55
S	0.08

Free radicals and reactive oxygen species are fundamental in cellular metabolism. However, when present in excess they can cause oxidative stress, causing cellular changes that ultimately are responsible for the appearance of diversified diseases as cardiovascular, neurological, cancer, diabetes, among others (Aruoma, 1998). The majority of the compounds present in EO's are recognized as natural antioxidant agents, being capable to inhibit the formation of free radicals and scavenge them. Therefore, in the last decades the demand for safer and natural sources of antioxidants has increased, in order to substitute synthetic antioxidants (Mimica-Dukic et al., 2004; Ćavar et al., 2008). Due to such importance and promising potential, more recently, the food industry become the second most consuming industrial sector in EO's (Bakkali et al., 2008).

To include new and natural sources of antioxidants, free of synthetic molecules medicinal and aromatic plants can be a promising alternative for the medicinal, pharmaceutical and food industrial sectors. Several studies point out the potential of aromatic species (Alcântara et al., 2010; Bozin et al., 2006; Cansian et al., 2010; Mensor et al., 2001; Mimica-Dukic et al., 2004; Vicentino and Menezes, 2007).

Due to the great potential observed in *L. origanoides* in folk medicine as well as in several scientific reports, new studies are necessary to enable the commercialization of EO's. One of the great problems is related to the variation of EO chemical composition. Essential oil chemical composition is affected by a diversified array of factors: plant cultivation practices, harvest moment, climatic factors, moisture, luminosity, part of the plant extracted, transportation, storage, drying, and extraction processes. All these factors can affect considerably the composition of EO's, affecting their safety and active principles efficiency.

Therefore, the objective of this research was to study the production and composition of *L. origanoides* EO's in plants cultivated under different fertilization programs, and observe the effect of such practices in their antioxidant capacity.

2. Experimental

2.1. Samples cultivation and fertilization

The experiments were held in the experimental field of the State University of Feira de Santana (location: 12°16′00″ S, 38°58′00″ W; altitude – 234 m; precipitation – 750 mm annual average; temperature – 26 °C annual average) (soil characteristics are presented in Table 1).

Lippia origanoides plants were propagated by cuttings and grew in a greenhouse during 100 days before transplantation to the experimental field. On August 2011, *L. origanoides* plants were transplanted, previously tilled, in a compass of 1 × 0.50 m. 48 independent plots of 6 plants each were constituted with a total surface of $6.0\,m^2\,plot^{-1}$. According to the recommendation of Raij et al. (1996) and based on soil analysis, mineral fertilizer composed by 80-200-80 $kg\,ha^{-1}$ (N, P_2O_5, and K_2O) was applied. Half of the nutrients were applied at transplanting and the other half 30 days after transplanting.

In the organic fertilization it was used an organic fertilizer made from vegetable residues of the experimental site, applied to an equivalent dose of $10\,t\,ha^{-1}$. The chemical composition of the organic fertilizer is reported in Table 2.

After 150 days of growth in the field (January 2012), plants were harvested. From each plot four sub-samples were made. In each plant, biometric parameters were taken: plant height, stalk diameter, stalk dry weight, flowers and inflorescences dry weight, and total plant dry weight. Relative growth rates were calculated for plant height, stalk diameter, and plant dry weight.

2.2. Essential oils extraction

Leaves and inflorescences of *L. origanoides* plants cultivated under organic and mineral fertilization, as well as in plant material without fertilization (control samples), were dried at 40 °C until constant weight. The essential oils extraction was carried out in triplicate by hydro-distillation as performed by Teles et al. (2012, 2013) with some modifications. Briefly, 100 g of dried and milled plant material were extracted with sufficient water amount during 3 h in a Clevenger-type apparatus. The oils extracted were dried with anhydrous sodium sulphate. The extraction yield was calculated in mL of oil per 100 g of dried material. The collected oil was then stored in sealed vials at −20 °C and protected by the light with aluminum foil after any analysis.

2.3. Determination of essential oils composition

The determination of the essential oils of *L. origanoides* was analyzed by GC/FID and by GC/MS. Before injection, 20 mg of essential oil were diluted in 1 mL of ethyl acetate, and 1 μL of the volume was injected.

2.3.1. Gas chromatography with flame ionization detection (GC/FID) analysis

The GC used was a Shimadzu GC-2010 model, equipped with an autosampler AOC-20i, and a FID detector. The analysis were performed with an Rtx 5 capillary column, 30 m × 0.25 mm i.d., DF = 0.25 μm. The temperatures of the FID and of the injector were 240 °C and 220 °C respectively. The oven temperature was programmed at 60 °C with an increase of 3 °C/min until 240 °C, and maintained for 20 min. The carrier gas was helium, at a flow rate of 1.2 mL/min. The analysis was performed in split mode with a ratio of 1:20.