Lettuce and lemon balm intercropping: crops productivity, yield and phytochemical quality of lemon balm’s essential oil

Consórcio entre alface e melissa: produtividade das culturas, teor e qualidade fitoquímica do óleo essencial de melissa

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**ABSTRACT**

Intercropping is a recommended to conserve the agroecosystem balance, but identifying which species in the intercropping benefit each other is a challenge. The aim of this study was to evaluate the viability and effect of spacing between plants in the intercropping of lettuce with lemon balm on the yield and phytochemical quality of lemon balm essential oil. Lettuce was harvested at 41 days after transplanting (DAT) and fresh and lemon balm was harvested at 69 DAT. Equivalent Area Index (EAI) was used to evaluate the intercropping between lemon balm and lettuce, which has proven feasible, since the EAI were higher than 1.0. The yield of the two species intercropped was not significantly different from that obtained in the monocropping and there was no reduction in the essential oil content. There was also an increase in the major constituents of the lemon balm essential oil.

**Keywords:** crop association, volatile compounds, land equivalent ratio, Lactuca sativa L, Melissa officinalis L, medicinal plant

**RESUMO**

O consórcio entre culturas contribui para o equilíbrio do agroecossistema, entretanto identificar quais espécies se beneficiam no consórcio é um desafio. O objetivo deste trabalho foi avaliar a viabilidade do consórcio entre alface e melissa e o efeito do espaçamento entre plantas sobre a produtividade das culturas, o teor e a qualidade fitoquímica do óleo essencial de melissa. A alface foi colhida 41 dias após o transplante (DAT) e a melissa foi colhida aos 69 DAT. O Índice de Equivalência de Área (IEA) foi utilizado para avaliar o consórcio entre melissa e alface, que se mostrou viável, uma vez que o IEA foi maior que 1,0. A produtividade das duas espécies consorciadas não diferiu significativamente daquela obtida no monocultivo, e não houve redução no teor de óleo essencial de melissa. Verificou-se incremento nos constituintes principais do óleo essencial de melissa.

**Palavras-chave:** associação de culturas, compostos voláteis, índice de equivalência de área, Lactuca sativa L, Melissa officinalis L, planta medicinal.

**1 INTRODUÇÃO**

Intercropping between vegetables and medicinal plants can promote beneficial interactions between the crops, resulting in food supply diversification to different markets and ensure a safer and more continuous source of income for farmers. It is an applicable and accessible technology with advantages such as synergistic or compensatory effect of one crop on another and less environmental impact compared to monocropping (MOTA et al. 2010).

Efficient land use, assessed by the Equivalent Area Index (EAI), is often used to compare intercropping with monocropping systems and allows the biological efficiency of intercropping systems to be evaluated. Intercropping is considered efficient when the EAI is above 1.0 and
detrimental to production when below 1.0; any value greater than 1.0 indicates yield advantage for intercropping (GLIESSMAN, 2000).

The intercropping system is mainly used by small farmers to maximize the utilization of land available for crop production, inputs and labor use in weeding, fertilization, input applications and other cultural treatments. The system also allows increased yield and diversification per unit of cultivated area (CAETANO et al. 1999; MONTEZANO & PEIL 2006; CARVALHO et al. 2009; and FONSECA et al. 2016).

The valuation of vegetables resulting from the increase in population's awareness of a nutritious and healthier diet and the use of medicinal plants has created a need for studies on the generation of agro-technologies and information to enhance the production processes of vegetables and medicinal species of good quality.

Lettuce (*Lactuca sativa* L.) is one of the most consumed leafy vegetables in the country and in the world, with great importance from the economic point of view; it is, therefore, cultivated in practically all regions of the country (CARVALHO FILHO et al., 2009). Its high consumption is due to the ease of acquisition, low cost, and high contents of vitamins of B complex, Vitamin C, Vitamin E, and minerals, as well as its low caloric content (OHSE et al., 2009).

Lemon balm (*Melissa officinalis* L) is important for its therapeutic properties, which is associated with the essential oil extracted from its leaves. Among the properties of this species, we highlight sleep induction, an action related to citral, a major constituent of the essential oil (PASSOS et al., 2009; SHAKERIET al., 2016). In addition, the essential oil extracted from the leaves is widely used by the pharmaceutical industry for its antioxidant, antmycotic, sedative, and antiviral activities, mainly against Herpes Simplex Virus. It also has antibiotic, antibacterial, antifungal, analgesic, relaxing, expectorant, antiallergic, astringent, antiseptic, anti-inflammatory, anti-diarrheal, diuretic, and antispasmodic activities, and even as an invigorating tonic to the skin (HARDER et al., 2005; BLANK et al., 2005, SHAKERI et al., 2016).

This species was also selected in the State Program of Medicinal Plants of Minas Gerais (Green Component of the Pharmacy Network), which aims to integrate phytotherapy into the State Health System, expand the therapeutic options of users of the National Health System (SUS), and encourage the development of the productive chain of medicinal plants. It is noteworthy that both the leaves and the essential oil of lemon balm have high market value, thus its cultivation is an alternative for job and income generation for farmers.
However, genetic, edaphoclimatic and management factors may interfere with the concentration and quality of the active principles of therapeutic interest of medicinal plants (LUZ et al., 2014). The intercropping between species is a traditional practice among family farmers in the country, but although the intercropping of vegetables is widespread, it is still under-researched.

Plant spacing is one of the variables that needs adjustment to increase yield per unit area. Nevertheless, there is a limit to it, considering that increased plant density may result in increased competition, with consequent damage to plant development and decreased yield and/or quality. Planting density favors yield of several plant species, as long as it does not affect the production and partition of photoassimilates (MUNARIN et al., 2010).

One of the great challenges in the development of research and recommendation on intercropping of vegetables and medicinal plants to farmers is the selection of the species. This study aimed to evaluate the yield of lettuce and lemon balm in monocropping and intercropping systems, as well as their effect on the content and chemical composition of the essential oil extracted from lemon balm leaves.

2 MATERIAL AND METHODS
2.1 PLANT MATERIAL

The experiment was carried out at the field experimental station, in the municipality of Oratórios-MG, latitude 20° 30' S, and 43° 00' W, 500 m altitude. The predominant climate at the site is the type Cwa, tropical humid, Aw, semi-humid with hot summers, as classified by Köppen, and the original vegetation is a semideciduous tropical forest (CUNHA et al., 2000).

The experiment was arranged in a randomized block design with six treatments and five replications. The treatments consisted of the intercropping of lemon balm and lettuce and monocropping of each species at the spacings 0.25 x 0.25 m and 0.30 x 0.30 m. The plots of each treatment contained sixteen plants of each species and four plants were evaluated per plot. Lemon balm (ISLA) seeds were soaked in water at 25 °C for 48 hours to break dormancy and keep the germination uniform. The seeds of lemon balm (Melissa officinalis) and lettuce (Lactuca sativa) (cv. Veronica) were sown in polystyrene trays (200 cells) containing commercial substrate (Plantmax®) in a protected environment. Seedlings were transplanted at 28 days after sowing. The soil site for planting was prepared by one disc harrowing to raise ridges, and fertilization with cattle manure compost according to soil analysis and to meet the needs of the species studied. The experiment was irrigated using a micro sprinkler system, and manual control of invasive species was carried out when necessary. The
chemical characteristics of the mature cattle manure used for organic fertilization were as follows (%): N (1.15), P (0.31), K (0.88), Ca (0.59), Mg (0.23), S (0.41), CO (7.48); C/N (6.74).

2.2 HARVESTED AND DRYING

Fully developed lettuce plants were harvested at 41 days after transplanting (DAT) and head height and diameter (cm) were measured in the field with a ruler. After harvest, shoot fresh and dry masses were weighed in an analytical scale, and the number of leaves per plant was counted. Dry mass was determined in a representative sample of plant material from each plot incubated in an oven with forced air circulation at 65 °C to constant weight.

Lemon balm was harvested at 69 DAT, and shoot fresh mass, length and volume of the root system were evaluated. The fresh mass yields of lettuce and lemon balm were calculated based on the production of the four plants sampled from each plot and converted into yield per hectare, according to the spacing used.

The essential oil from lemon balm leaves was extracted and its chemical constituents were identified. To this purpose, a representative sample of plants was collected from each treatment, weighed, placed in paper bags, and incubated in an oven with forced air circulation (40° C) to constant weight.

2.3 ESSENTIAL OIL: EXTRACTION, QUALITATIVE AND QUANTITATIVE ANALYSIS

The essential oil was extracted by hydrodistillation, in a Clevenger type apparatus, adapted to a 2 L round bottom flask, during 150 min, using 50 g of dried leaves and 1 L of distilled water. After extraction was complete, the essential oil obtained was removed from Clevenger, and was transferred to an amber vial.

Identification of the chemical constituents of essential oils was performed on Shimadzu GC–MS QP5050A equipment, equipped with SE54 chromatographic column (30m x0.25mm id x0.25 μm pore thickness), using helium as the carrier gas, at a flow of 1.8 mL min−1, split ratio of 1:5 and the solvent cut-off time of 4 min. The injector and detector temperatures were maintained at 220 °C and 240 °C, respectively. The initial temperature of the column was maintained at 40 °C for 2 min, being programmed to have increases of 3 °C min−1 until reaching the maximum temperature of 240 °C, in which it was maintained for another 5 min. In the mass spectrometer, only ions with mass/charge ratio (m/z) between 40 and 500 were detected. The volume of the sample injected was 1.0 μL, at a concentration of 10,000 ppm, using dichloromethane as the solvent (brand SYNT, Hexis Scientific
(Hexis, Brazil). For the identification of the compounds, the obtained spectra were compared with those recorded in the equipment database and evaluation of calculated Kovats Indices (KI), compared with those described in the literature (Adams, 2007). A mixture of hydrocarbons (C7 through C30) was analyzed under the same chromatographic conditions, and the respective retention times served as the basis for calculating the KI of the components of the essential oils. Quantification of essential oil constituents was performed on a Shimadzu GC-17A gas chromatography - flame ionization (GC-FID), with fused silica capillary column with stationary phase SPB5 (30m x0.25mm id x0.25 μm pore thickness), nitrogen as carrier gas, at a flow of 1.8 mL min–1, temperature at the injector of 220 °C and temperature at the detector of 240 °C. The initial temperature of the column was maintained at 40 °C for 4 min, programmed to have increments of 3 °C every minute, until reaching the maximum temperature of 240 °C, which was maintained during 3 min. The volume of the sample injected was 1.0 μL, at a concentration of 10,000 ppm, using dichloromethane as the solvent. The normalization method was used, where the sum of the peak areas for all identified constituents was 1.0 %, and the percentage of each signal calculated by its area.

2.4 INTERCROPPING VALIDATION

The intercropping validation was performed by the Equivalent Area Index (EAI), according to Willey (1979), using the formula: \( IEA = \frac{Mc}{Ms} + \frac{Ac}{As} \), where: \( Mc \) is the yield of lemon balm intercropped; \( Ac \) is the yield of lettuce intercropped; \( Ms \) is the yield of lemon balm monocropped; and \( as \) is the yield of lettuce monocropped.

2.5 STATISTICAL ANALYSIS

Data were submitted to analysis of variance (ANOVA) and subjected to Tukey's test, at 5% probability, using the statistical analysis program SAEG® version 9.1 (SAEG, 2007).

3 RESULTS AND DISCUSSION

3.1 AGRONOMIC EVALUATION

The Equivalent Area Indices (EAI) of lemon balm and lettuce intercropping were 2.94 (0.25 x 0.25 m) and 3.02 (0.30 x 0.30 m), respectively, and indicate the viability of the intercropping, regardless of plant spacing. Equivalent Area Indices greater than 1.0 indicate yield advantage in intercroppings (GLIESSMAN, 2000).
In relation to spacing, the highest fresh mass yields of lettuce and lemon balm were recorded in the more densely packed intercropping, with the 0.25 x 0.25 m spacing (Table 1). These results corroborate with other studies on intercropping between lettuce and medicinal species. Brandão et al. (2014) found good yields of biomass in the intercropping of yarrow, lettuce, and lemon balm. Vieira et al. (2012) reported the viability of intercropping lettuce and basil, and Fonseca et al. (2016) found increasing the yield of the medicinal species in the intercropping between lettuce and marigold.

|                | Yield (kg ha⁻¹) |
|----------------|-----------------|
| Lemon balm intercropped (0.25 x 0.25 m) | 32.320.00 |
| Lemon balm monocropped (0.25 x 0.25 m)   | 22.040.00 |
| Lemon balm intercropped (0.30 x 0.30 m)   | 20.531.00 |
| Lemon balm monocropped (0.30 x 0.30 m)   | 11.836.66 |
| Lettuce intercropped (0.25 x 0.25 m)      | 60.432.00 |
| Lettuce monocropped (0.25 x 0.25 m)       | 40.816.00 |
| Lettuce intercropped (0.30 x 0.30 m)      | 42.272.22 |
| Lettuce monocropped (0.30 x 0.30 m)       | 32.744.44 |

The spacing showed no effect on height, diameter, and volume of roots when the lemon balm was cultivated in the monocropping system, (Table 2). Reduction in lemon balm root length (RL) was found only in the intercropping with lettuce at denser spacing (Table 2). Root length determines the plant's ability to absorb water and nutrients (BOUMA et al., 2000). This characteristic is directly related to shoot growth and plant yield, which were distinct for the two species. However, we found no effect of the spacing on height, diameter, number of leaves, fresh and dry mass of lettuce leaves in the intercropping system, with means of 6.00 cm, 37.60 cm, 42; 118.60 g/plant, and 6.22 g/plant, respectively. This result indicates that intercropping lettuce with lemon balm is not detrimental to the development of the vegetable. Likewise, the spacing and the intercropping of lemon balm with lettuce had no effect on fresh and dry masses of shoot and root of lemon balm, with means of 142.01 g/plant, 27.81 g/plant, 38.20 g/plant and 22.61 g/plant, respectively.
Table 2. Root length (RL), root volume (RV), height (H) and diameter (DIAM) of the lemon balm (*Melissa officinalis* L.) in monocropping and intercropping with lettuce, in two spacings.

| Treatments                        | RV (cm³) | RL (cm)  | H (cm)  | DIAM (cm) |
|-----------------------------------|----------|----------|---------|-----------|
| Lemon balm 0.30 x 0.30 m          | 43.50    | 19.23 AB | 35.44   | 56.37     |
| Lemon balm x Lettuce 0.25 x0.25 m| 50.51    | 16.38 B* | 31.33   | 54.00     |
| Lemon balm x Lettuce 0.30 x 0.30 m| 54.83    | 20.96 A  | 34.76   | 47.80     |
| Lemon balm 0.25 x 0.25 m          | 55.26    | 20.34    | 35.19   | 55.15     |

Analysis of variance:

- Mean: 51.02
- p-value (Treatments): 0.031
- C.V (%): 29.54

Means followed by the same letter are not significantly different by the Tukey test at 5% probability. * Different from control (lemon balm at the spacing 0.25 x 0.25 m).

### 3.2 EFFECT OF INTERCROPPING ON THE YIELD AND CHEMICAL COMPOSITION OF LEMON BALM ESSENTIAL OIL

The intercropping had no influence on the lemon balm essential oil yield, with mean of 0.92%. However, there was influence of the spacing on the concentration of some chemical constituents in the essential oil of lemon balm leaves: neral, geranial, geranyl acetate, caryophyllene oxide, and citral (Table 3 and 4).

The majority constituents of lemon balm essential oil increased in the denser intercropping (0.25 x 0.25 m). The concentration of neral increased from 11% to 34%, that is, 23%, and the concentration of geranial increased from 14% to 47%, that is, an increase of 33% (Table 3). The results of this study reinforce the possibility of increasing the biomass of medicinal plants and the yield of their essential oils by intercropping them with vegetables (Santos et al., 2009; Brandão et al. 2014).

Table 3. Mean concentration of the chemical constituents of lemon balm essential oil grown in intercropping system with lettuce, in two spacing

| Treatments                        | Neral  | Geranial | Thymol | Geranyl acetate | Caryophyllene oxide | Constituent not identified | Citral  |
|-----------------------------------|--------|----------|--------|-----------------|---------------------|---------------------------|---------|
| Lemon balm 30 x 30 cm             | 15.22  B| 10.51    B| 6.10   | 8.04            | 9.35 AB             | 6.245                     | 25.73   |
| Lemon balm x Lettuce 25 x 25 cm   | 30.89 A*| 50.40 A* | 1.97   | 1.34 *          | 4.77 B*             | 3.45                      | 81.29A  |
| Lemon balm x Lettuce 30 x 30 cm   | 18.26 B| 28.89 AB | 2.60   | 2.47            | 13.88 A             | 13.49                     | 47.15   |
| Lemon balm 25 x 25 cm             | 9.30   | 19.31 AB | 3.35   | 13.64           | 10.83               | 9.66                      | 28.61   |

Analysis of variance:

- Mean: 18.41
- p-value (Treatments): 0.003
- C.V (%): 22.15

Means followed by the same letter are not significantly different by the Tukey test. * Different from control (lemon balm at the spacing 0.25 x 0.25 m).
Table 4. Essential oil compounds from *Melissa officinalis* grown in intercropping system with lettuce, in two spacing (AxM1= 25 x 25 cm e AxM2= 30 x 30 cm) and monocropped (M1= 25 x 25 cm e M2= 30 x 30 cm)

| tR  | RI* | RI** | Compounds          | Concentration (%) |
|-----|-----|------|---------------------|-------------------|
|     |     |      |                     | AxM1             |
| 24.321 | 1240 | 1238,573 | geranial            | 30,89            |
| 25.825 | 1270 | 1273,650 | geranial            | 30,89            |
| 26.683 | 1290 | 1296,907 | thymol              | 1,97             |
| 27.987 | 1323 | 1326,899 | methyl geranate     | *                |
| 30.711 | 1383 | 1386,619 | geranyl acetate     | 1,34             |
| 32.160 | 1418 | 1419,785 | trans-caryophyllene | 1,38             |
| 39.078 | 1581 | 1585,511 | caryophyllene oxide | 4,77             |
| 41.403 | 1645 | 1645,501 | murolol             | *                |
| 41.871 | 1653 | 1657,628 | alpha-cadinol       | *                |
| 49.052 | 1871,096 | Ni |                   | 1,28             |
| 49.700 | 1871,855 | Ni |                   | *                |
| Total | 94.4 | 81.88 |                     | 72.31            |

tR - retention time, RI*- Literature retention indices; RI** - Calculated retention indices, Ni- not identified compound

4 CONCLUSIONS

The intercropping between lettuce and lemon balm allows better utilization of land available for crop production and, consequently, higher production volume per unit area. It is a viable and advantageous alternative considering the substantial increase in yield of the two species, without changing the lemon balm essential oil yield. In addition, the use of this agrotechnology allows the production of higher concentrations of some chemical constituents of therapeutic interest of lemon balm essential oil.

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