Cultivation of Mint (Mentha x gracilis) in Intercropping with Fruit Trees in an Agroforestry System: Production and Quality of Essential Oil

Dalva Paulus¹*, Dislaine Becker², Gilmar Antônio Nava¹, Daiane Luckmann¹ and Cláudia de Andrade Moura¹

¹Department of Agronomy, Federal University of Technology – Paraná, Campus Dois Vizinhos, 85660-000, Dois Vizinhos, Paraná, Brazil.
²Postgraduate Program in Agroecosistemas, Federal University of Technology – Paraná, Campus Dois Vizinhos, Brazil.

Authors’ contributions

This work was carried out in collaboration among all authors. Authors DP and DB designed the study and wrote the first draft of the manuscript. Authors DP, DB and GAN performed the experiments. Authors DP, DB, GAN and DL participated in fieldwork and laboratory analysis. Authors DP, DL and CAM managed the analyses of the study. Author CAM and DL managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The cultivation of medicinal plants in intercropping with other species of agricultural use has been an alternative to make production sustainable in family farming. The objective of this work was to evaluate the growth, biomass production, and chemical composition of the essential oil of mint (Mentha x gracilis Sole) in intercropping with fruit species in an agroforestry system.

Study Design: The experimental design was randomized blocks with four treatments, mint interplanted with citrus (Citrus sinensis L. Osbeck), bananas (Musa spp.), blackberries (Morus nigra), or Barbados cherries (Malpighia glabra).

*Corresponding author: E-mail: dalvapaulus@utfpr.edu.br;
Place and Duration of Study: The experiment was conducted in the agroforestry located in the sector of Olericultura of the Federal University of Technology – Paraná (UTFPR), Brazil, in the period between November 2015 to February 2017.

Methodology: We analyzed light intensity, relative chlorophyll index, height, leaf area, biomass accumulation, essential oil content, oil production and chemical composition of mint grown in agroforestry.

Results: The highest production of biomass and essential oil were obtained in the intercropping of mint with citrus and Barbados cherries, possibly due to the edaphic climatic conditions, such as greater light intensity, that favored the growth, production, and chemical composition of the mint essential oil. Bananas and blackberries intercropped with mint were not beneficial for the growth and production of essential oils.

Conclusion: The intercropping of mint with citrus and Barbados cherries resulted in higher growth, biomass accumulation, and essential oil content and production. The major components of the essential oils were linalool and carvone, with higher percentages in the intercropping of mint with citrus and Barbados cherries. The cultivation of mint by intercropping with fruit species such as citrus and Barbados cherries is an option to diversify the production of medicinal plants, making it sustainable.

Keywords: Medicinal plants; essential oil; linalool; intercropping; sustainable.

1. INTRODUCTION

The cultivation of medicinal plants as an income alternative for small farmers grows as the market for herbal medicines evolves, which is stimulated by the increasing appreciation of the quality of life in society [1].

There is a global demand for food production systems with less dependence on external energy inputs. Agroforestry contributes through the intentional incorporation of woody vegetation with agricultural crops, grasses, or livestock in the same land unit, to produce ecological, financial and social benefits [2].

The inclusion of medicinal plants in agroforestry systems has become an alternative for the integration of forest species with species of agricultural use, since they allow sustainable production, avoiding contamination of the soil with agrochemicals that can alter the composition of the active components of medicinal plants [3]. The objective of including medicinal plants on family farms is the creation of sustainable use and management technologies that reduce environmental impacts and that can make this production system socially and economically feasible.

Intercropping is one of the ways by which small farmers can increase productivity and economic efficiency [4], as well as maximize the utilization of a given area and the environmental resources available, improving pest control, and reducing diseases, weeds, and the use of fertilizers and agrochemicals [5]. The efficiency and benefits of intercropping systems are mainly based on the complementarity between the crops involved, which increases the possibility of minimizing the negative effects of establishing a single crop.

In the choice of medicinal species for cultivation in agroforestry systems, it is important that it presents adequate development and adaptation to the edaphoclimatic conditions of the agroforestry system. The genus Mentha (Lamiaceae) comprises plants such as mint, which are highly cultivated due to the production of essential oils produced by glandular trichomes present in the leaves and stems of plants. These essential oils are used commercially as food flavorings, flavoring agents, cosmetics, perfumes, and medications [6]. Mentha x gracilis Sole is a hybrid that originated from M. arvensis L. and M. spicata L. [7] that produces essential oils rich in monoterpene, such as linalool. This component is of great economic interest because it is widely used to fix fragrances. Approximately 70 % of the cosmetics produced in the perfume industry have linalool in its formula. The most refined cosmetics and perfumes prioritize the use of natural linalool [8].

Faced with the scarcity of studies on the insertion of medicinal plants in agroforestry, the need and feasibility of implementing them to improve environmental, social, and economic conditions, justifies the present study. The objective of this work was to evaluate the growth, biomass production, and composition of the essential oil of mint (Mentha x gracilis Sole) in intercropping with fruit species in agroforestry systems.
2. MATERIALS AND METHODS

2.1 Plant Material and Growing Conditions

The experiment was conducted in the agroforestry located in the sector of Olericultura of the Federal University of Technology – Paraná (UTFPR), Brazil, in the ecoclimatic region of Southwest Paraná, Brazil (latitude, 25242′S; longitude, 53206′W; average altitude, 520 m) [9]. The local soil is of the Red Nitrous Distroferric type [10]. Between the depths of 0 and 10 cm, the soil had chemical characteristics during the pre-planting stage according to Table 1.

Table 1. Chemical characteristics of experimental soil in the 0 to 20 cm depth layer

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH CaCl₂</td>
<td>5.70</td>
</tr>
<tr>
<td>Organic matter (g·dm⁻³)</td>
<td>56.29</td>
</tr>
<tr>
<td>P (mg·dm⁻³)</td>
<td>83.30</td>
</tr>
<tr>
<td>K⁺ (cmolc·dm⁻³)</td>
<td>1.00</td>
</tr>
<tr>
<td>Ca²⁺ (cmolc·dm⁻³)</td>
<td>8.70</td>
</tr>
<tr>
<td>Mg²⁺ (cmolc·dm⁻³)</td>
<td>4.80</td>
</tr>
<tr>
<td>Cu (mg·dm⁻³)</td>
<td>5.71</td>
</tr>
<tr>
<td>Fe (mg·dm⁻³)</td>
<td>47.53</td>
</tr>
<tr>
<td>Zn (mg·dm⁻³)</td>
<td>14.29</td>
</tr>
<tr>
<td>Mn (mg·dm⁻³)</td>
<td>198.54</td>
</tr>
<tr>
<td>Sum of bases (mg·dm⁻³)</td>
<td>14.50</td>
</tr>
<tr>
<td>H⁺ + Al³⁺ (mg·dm⁻³)</td>
<td>3.52</td>
</tr>
<tr>
<td>Base saturation (%)</td>
<td>80.47</td>
</tr>
</tbody>
</table>

Notes: Methodology according to Teixeira et al. [11]

Mint seedlings were produced from 10 cm apical cuttings taken from the apex of the mother plant. The cuttings were taken at the University’s Medicinal Garden and placed in a protected environment, in rows of 128-cell trays containing commercial Carolina Soil® substrate. Seedlings with 5 to 6 leaves were transplanted into pits at a spacing of 0.50 x 0.30 m, between rows of four-year old fruit species. The fruit species were planted according to the preference of the farmers of the region who work with agroforests. No control method was applied for pests and diseases; only weeding was done in the plots for the control of spontaneous plants.

According to data from the agrometeorological station (16 UTC), the average temperature during the experiment was 23°C, with a maximum of 33.5°C, minimum of 15.9°C, average rainfall of 275.3 mm, and mean relative humidity of 82.6%. The station located at UTFPR - Dois Vizinhos is 500 m from the agroforest [12].

2.2 Treatments and Experimental Design

The experimental design was randomized blocks with four treatments and five replicates. Each plot consisted of 20 plants, where the treatments were different intercropping combinations of mint with citrus (Citrus sinensis L. Osbeck) (T1), bananas (Musa spp.) (T2), blackberries (Morus nigra) (T3), and Barbados cherries (Malpighia glabra) (T4). The plots were 2.0 x 6.0 m long, with four plant lines in the intercropping system.

2.3 Evaluated Parameters

The luminous intensity was measured in six plants per replicate, in the median region and at the apex of the leaves, with an Instrutemp® digital luxmeter model ITLD-300. The measurements were performed at three times, 10:00 (morning), 12:00, and 15:00 (evening), and on three dates 40, 50, and 70 days after planting in the agroforest.

At 40 days after transplantation, the relative index of total chlorophyll in the abaxial and adaxial parts of the last two expanded leaves of each plant were determined at 11:00 using a Clorofilog Falker® chlorophyll meter.

Harvesting was performed when the plants were in full bloom, 70 days after transplanting. Leaf area was determined using a Cl-202® leaf area meter, plant height was measured with a ruler from the apex to the base, and fresh mass of the aerial parts was determined using a precision scale. For all the analyzed characteristics, eight plants of each experimental unit were collected.

The extraction of the essential oil was achieved by using the hydrodistillation method and a modified Clevenger device for 01.20 h on one plant sample from each plot [13]. Samples for the essential oil extraction were 60 g of leaves, inflorescences, and stems dried in a forced air circulation oven at a temperature of 40°C to a constant mass. The mass of the essential oil was obtained on a precision scale (0.01 g).

The essential oil content (%) of extracted vegetable biomass (inflorescences, leaves and stems) was calculated based on moisture free dry matter [14]. The oil production (PO) in liters per hectare was calculated considering the density of the essential oil as of 0.9 g cm⁻³ [15].

A sample of essential oil from each plot was diluted in chloroform (1%) and then 1 μL of each solution was injected in to split-mode gas...
chromatography (1:20). High resolution gas chromatography was performed using GC Agilent Technologies 7820 A apparatus, equipped with the split-splitless injector attached to a Supelcowax-10 column (30 m × 0.2 mm), with a film thickness of 0.2 μm (Supelco), and fitted to a flame-ionization detector (FID). The carrier gas was H2 (6 mL min\(^{-1}\)). Temperatures were set as follows: injector at 200°C (split: 1/30), FID detector at 220°C, while the column temperature was linearly programmed starting from 50°C at 0 min and reaching 200°C at an increasing rate of 3°C min\(^{-1}\). Data acquisition software used was the Open lab (Agilent).

The GC–MS was performed on GCMS-QP2010 ULTRA (Shimadzu) analytical system equipped with an Supelcowax-10 column (30 m × 0.2 mm, film thickness 0.2 μm). Carrier gas was He (4 mL min\(^{-1}\)). Other chromatographic conditions were the same as those for GC-FID. The transfer line was heated at 240°C. All mass spectra were acquired in electron impact (EI) mode with an ionization voltage of 70eV, in a range of m/z 40–450. Data acquisition software: GCMS Solution (Shimadzu).

The compounds were identified by comparing the mass spectra fragmentation patterns with those of a computer library [16,17] and the linear retention indices (RI), based on a homologous series of C\(_8\)–C\(_{32}\) n-alkanes, compared with those of authentic products included in the laboratory database, and/or literature data [16]. Relative amounts of individual components were calculated based on the GC peak areas without FID response factor correction.

2.4 Statistical Analysis

The data of the collected variables were submitted to an analysis of variance paired with the F test (\(P <0.01\)). The variances of the treatments were tested for homogeneity by the Bartlett test and the means were compared by the Tukey test at 5% probability with the aid of the SAS Studio* [18].

3. RESULTS AND DISCUSSION

The climatic conditions (average temperature of 23°C, maximum of 33.5°C, and minimum of 15.9°C) were favorable to the growth of mint in the different intercropping combinations in the agroforest. The optimum temperatures for mint growth are 30°C and 18°C during the day and night, respectively, while diurnal temperatures exert a greater influence on plant growth and essential oil yield [19].

Regarding the luminous intensity, at 10:00, 12:00, and 15:00, the highest values were obtained in the intercropping of mint with citrus and Barbados cherries. The lowest luminous intensity was found in the mint and bananas or blackberry intercropping combinations (Table 2). This can be explained by the fact that most solar radiation in an agroforest is intercepted by canopy components such as branches and leaves. Bananas and blackberries presented a denser canopy with larger leaves; therefore, more radiation was intercepted than that in other intercropping combinations. The luminous intensity affects the growth of the species by its direct effects on photosynthesis, chlorophyll synthesis, and stomatal opening [20].

The most important factor that influenced the development of mint plants in fruit growing systems was possibly the light intensity. Thereby, the satisfactory growth of some species in environments with different light availability can be attributed to their ability to adjust their physiological behavior to maximize the acquisition of resources in the environment [21].

<table>
<thead>
<tr>
<th>Intercropping</th>
<th>Light intensity (Lux)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10:00 AM</td>
</tr>
<tr>
<td>Mint with citrus</td>
<td>2273.29 a*</td>
</tr>
<tr>
<td>Mint with bananas</td>
<td>1021.48 b</td>
</tr>
<tr>
<td>Mint with blackberries</td>
<td>1057.85 b</td>
</tr>
<tr>
<td>Mint with Barbados cherries</td>
<td>2190.45 a</td>
</tr>
<tr>
<td>Mean</td>
<td>1635.79</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>18.5</td>
</tr>
</tbody>
</table>

Note. *Means followed by the same letter in the column do not differ significantly by Tukey test, at \(P=0.05\);

C.V.: Coefficient of variance
According to Willey and Reddy [22], shading is more important than ground competition in an intercropping combination of pearl millet and groundnut in India.

The relative chlorophyll a and total chlorophyll index were influenced by the intercropping of mint with citrus and Barbados cherries, respectively, which responded positively to the ability of the leaf to absorb light (Table 3). Determination of the relative indexes of leaf chlorophyll is important because the photosynthetic activity of the plant depends partly on the ability of the leaf to absorb light [23]. The higher relative index of chlorophyll in response to the higher uptake of solar radiation than during the photosynthesis process resulted in chemical energy gains in the form of ATP and NADPH in mint plants [24].

There were significant differences in the height and leaf area of mint plants between the different intercropping combinations (Table 4). Mint combined with citrus or Barbados cherries had higher leaf height and area than that of mint combined with blackberries or bananas. The leaf area results can be explained by the light intensity, that is, the intercropping combination that received the highest luminous intensity resulted in a larger leaf area, which is related to the photosynthetic activity of the plant [24].

The results obtained here show that there were differences in the ability of the plants to self-regulate in relation to the equilibrium of the interference relations. This is because source-sink relations can be altered by cropping conditions, especially in agroforestry, usually due to a combination of different species, where roots explore the soil at different depths, or where the leaves can respond differently to competition for light [25].

The mint fresh mass was significantly influenced by the intercropping combination, with the highest biomass increment in mint with citrus or Barbados cherries (Table 5). The mint and citrus intercropping showed a 23.97% gain in biomass compared to that of the mint and banana intercropping combination, which presented the lowest accumulation of biomass. The higher biomass gain in the mint with citrus or Barbados cherries can be explained by the higher luminous intensity, relative chlorophyll index, and leaf area, which contributed to increased photosynthesis. Increased leaf area provides an increase in the ability of the plant to harness solar energy for photosynthesis and can thus be used as a parameter related to fresh mass yield and the production of compounds such as essential oils [26].

It is important to note that the values of fresh aerial mass of mint in the agroforestry system decreased with increased shading, indicating that this species prefers environments with greater luminous intensity. This information is of great importance in the definition of the species that best adapt to the microclimate of agroforestry.

The results of essential oil content, yield, and production show that the highest averages were obtained in the mint combined with citrus or Barbados cherries (Table 5). This might be due to more favorable growth conditions, such as greater light intensity and less competition for water and nutrients. Results of biomass production and yield of essential oil obtained in the mint with citrus or Barbados cherries can be explained by several factors, including size and less dense architecture of the fruit species, which allowed higher light penetration. As the banana and blackberry trees presented more dense leaf architecture, they provided greater shading, which interfered in the accumulation of biomass and the secondary metabolism of mint.

Table 3. Relative index of chlorophyll a, chlorophyll b, and total chlorophyll in mint plants grown in intercropping combinations with citrus, bananas, blackberries or Barbados cherries

<table>
<thead>
<tr>
<th>Intercropping</th>
<th>Chlorophyll a</th>
<th>Chlorophyll b</th>
<th>Total chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mint with citrus</td>
<td>32.30 a</td>
<td>4.67 a</td>
<td>36.97 a</td>
</tr>
<tr>
<td>Mint with bananas</td>
<td>25.10 b</td>
<td>5.00 a</td>
<td>30.10 b</td>
</tr>
<tr>
<td>Mint with blackberries</td>
<td>26.70 b</td>
<td>4.33 a</td>
<td>31.03 b</td>
</tr>
<tr>
<td>Mint with Barbados cherries</td>
<td>30.50 a</td>
<td>5.63 a</td>
<td>36.13 a</td>
</tr>
<tr>
<td>Mean</td>
<td>28.65</td>
<td>4.91</td>
<td>33.56</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>3.27</td>
<td>17.91</td>
<td>4.57</td>
</tr>
</tbody>
</table>

*Note.* Means followed by the same letters in the columns do not differ significantly by Tukey test, at P = 0.05; C.V.: Coefficient of variance
Table 4. Height, leaf area and aerial fresh mass in mint plants grown in intercropping combinations with citrus, bananas, blackberries or Barbados cherries

<table>
<thead>
<tr>
<th>Intercropping</th>
<th>Height (cm)</th>
<th>Leaf area (cm² planta⁻¹)</th>
<th>Aerial fresh mass (g planta⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mint with citrus</td>
<td>33.52 a</td>
<td>1618.97 a*</td>
<td>252.50 a</td>
</tr>
<tr>
<td>Mint with bananas</td>
<td>29.16 b</td>
<td>1229.82 c</td>
<td>191.98 c</td>
</tr>
<tr>
<td>Mint with blackberries</td>
<td>28.30 b</td>
<td>1453.88 b</td>
<td>215.83 b</td>
</tr>
<tr>
<td>Mint with Barbados cherries</td>
<td>34.59 a</td>
<td>1604.15 a</td>
<td>249.31 a</td>
</tr>
<tr>
<td>Mean</td>
<td>31.39</td>
<td>1476.70</td>
<td>227.36</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>21.3</td>
<td>20.8</td>
<td>20.2</td>
</tr>
</tbody>
</table>

Note.*Means followed by the same letters in the columns do not differ significantly by Tukey test, at P= 0.05; C.V.: Coefficient of variance

Table 5. Essential oil content (E.O.C.), essential oil yield (E.O.Y.) and essential oil productivity (E.O.P.) in mint plants grown in intercropping combinations with citrus, bananas, blackberries or Barbados cherries

<table>
<thead>
<tr>
<th>Intercropping</th>
<th>E.O.C. (%)</th>
<th>E.O.Y. (g planta⁻¹)</th>
<th>E.O.P. (L ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mint with citrus</td>
<td>0.97 a</td>
<td>2.29 a</td>
<td>135.42 a</td>
</tr>
<tr>
<td>Mint with bananas</td>
<td>0.66 c</td>
<td>1.65 c</td>
<td>79.78 c</td>
</tr>
<tr>
<td>Mint with blackberries</td>
<td>0.85 b</td>
<td>1.97 b</td>
<td>92.15 b</td>
</tr>
<tr>
<td>Mint with Barbados cherries</td>
<td>1.08 a</td>
<td>2.24 a</td>
<td>141.63 a</td>
</tr>
<tr>
<td>Mean</td>
<td>0.89</td>
<td>2.04</td>
<td>112.24</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>15.29</td>
<td>20.05</td>
<td>19.30</td>
</tr>
</tbody>
</table>

Note.*Means followed by the same letters in the columns do not differ significantly by Tukey test, at P= 0.05; C.V.: Coefficient of variance

There are reports of increased essential oil content in aromatic plants with increased luminosity rate [27]. Solar intensity influences concentration as well as the composition of essential oils. The development of glandular trichomes, plant structures that biosynthesize and store essential oils, is a light-dependent process [28]. Low light intensity and low temperatures cause significant variations in the amount of essential oil constituents and total yield [29].

According to Costa et al. [30] the ratio of light quality and composition of peppermint oil (Mentha piperita) may be associated with the biosynthetic route of the compounds, as the conversion of menthone to menthol is influenced by low light and short photoperiod conditions. These conditions are favorable for the synthesis of linalool, where the precursor is geranyl diphosphate [31].

The cultivation of mint with bananas showed the lowest production of biomass and essential oil, possibly due to excessive shading, because the bananas had large leaves that provided greater shading than the other evaluated fruit species. [32] obtained similar results with black mesh (shading of 75, 50, and 25%) for lemon balm (Melissa officinalis L.). The plants cultivated with 75 % shading presented less fresh and dry weight development and lower essential oil content.

The desired characteristics in therapeutic plants are the phytomass and the active component, and if intercropping increases the productivity of the agroecosystem and maintains local biodiversity, favoring the natural control of pests and diseases, as well as the recycling of nutrients [33], the cultivation of medicinal plants associated with fruit, becomes a viable alternative.

The demand of the pharmaceutical and cosmetics industry for essential oils and their derivatives has encouraged research in the agronomic area of Mentha species. Telci et al. [34] obtained an average content of 1.66% essential oil in a study with M. spicata conducted in the field. According to the authors, the oil content was influenced by favorable climatic conditions for essential oil production, such as temperatures between 21.1°C and 22.3°C and sufficient light intensity. In relation to the chemical composition of the essential oils, it was possible to confirm variation in the constituents between the intercropping combinations (Table 6). Twelve constituents were identified in the mint essential oils. Mint combined with citrus and Barbados cherries resulted in higher percentages of α pinene, 1,8 cineole, linalool, and carvone.
The major components of the essential oils were linalool (49.87 to 41.57%) and carvone (15.41 to 18.30%), which are secondary compounds (terpenes). Linalool is a monoterpane, which is of great importance, as it is used in cosmetic products, such as face creams, body lotions, cream fragrances, deodorants, perfumes, and soaps [35]. Carvone is an important antimicrobial agent against pathogenic bacteria and fungi, used in food and antiseptic products. It also has insecticidal activity, acting against fruit flies and insect larvae, including Aedes aegypti, the vector of hemorrhagic dengue [36].

Interestingly, in the cultivation of mint in intercropping systems, although resulting in lower biomass production and oil content than those previously reported [34], the chemical composition of the essential oils correlated with that mentioned in the Brazilian Pharmacopoeia [37] and the results obtained by Garlet et al. [29] in hydroponic cultivation, with the same species.

The cultivation of mint intercropped with fruit species presented significant and important results for farmers to efficiently take advantage of the agroforestry area, with the option of commercializing the leaves for tea and essential oils for the pharmaceutical industry. Agroforestry systems produce wood, firewood, fruits, and other products that will make a profit and reduce the release of carbon dioxide into the atmosphere [38].

4. CONCLUSION

The intercropping of mint with citrus and Barbados cherries resulted in higher growth, biomass accumulation, and essential oil content and production. The mint combined with bananas or blackberries resulted in lower growth and production of essential oils, due to the excessive shading provided by these species.

Mint intercropped with citrus or Barbados cherries resulted in higher percentages of α-pinene, 1,8 cineole, linalool, and carvone. Linalool was the major component of mint essential oil grown in agroforestry combinations.

The cultivation of medicinal plants, such as mint, in agroforestry contribute to the use of the soil in a more efficient way, enabling farmers to develop more than one plant in the same area, thus having sustainable and more profitable production, becoming a source of income, while the fruit species are in the initial phase.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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