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Agronomic Practices in Lemon Balm Production under Temperate Climate Conditions: Raw Material Yield and Active Substances Content

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Abstract: The cultivation of lemon balm (*Melissa officinalis* L.) is necessary for pharmaceutical and cosmetic production. The aim of our work was to investigate the effect of the plant density as well as the harvesting method on the yield and quality characteristics of M. officinalis, which is used as a source of polyphenols and essential oil in the pharmaceutical, food, and cosmetic industries. The field experiment was carried out in two growing seasons (2019 and 2020). The experiment was set up as a 2-factor experiment; the factors studied were the plant spacing (30 × 30 cm or 40 × 40 cm) and the harvesting method (single harvesting or double harvesting). The lemon balm herb was cut twice (in mid-July and early September) or once (in early September) depending on the combination. The air-dried leaves were subjected to laboratory tests to determine the essential oil, total tannin, and total flavonoid contents. The yield of lemon balm in the individual years in the study depended more on the harvesting method than on the plant density. The essential oil content was 0.25–0.38% depending on the plant density. Considering the yields of the fresh and air-dried herb and leaves, essential oil yield and oil, and tannin and flavonoid contents, a higher plant density (40 × 40 cm) is a better agronomic option than a lower density (30 × 30 cm). Harvesting the herb twice proved to be more efficient in terms of quantity and quality than a single harvest.

Keywords: aromatic plants; *Melissa officinalis* L.; field cultivation; biomass yield; essential oil; flavonoids; tannins

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1. Introduction

Aromatic and medicinal plants (MAPs) are widely used in the pharmaceutical, food, cosmetic, alcohol, and chemical industries. The raw materials of MAPs (leaves, flowers, herbs, and roots) are obtained from cultivation and natural sites. Crop management is essential to balance the basic parameters of MAPs, such as biomass, and to produce high-quality essential oils and extracts with medicinal properties [1]. It has been emphasized that the integrated and sustainable cultivation of MAPs can contribute to the conservation and enhancement of biodiversity in agroecosystems and the restoration of degraded land [2]. One of the essential plants included in MAPs is lemon balm (*Melissa officinalis* L., Lamiaceae), which contains a variety of bioactive compounds, including essential oil, flavonoids, phenolic acids, tannins, and triterpenes, which are responsible for many beneficial medicinal effects [3,4]. The raw material of lemon balm (*Melissae folium*/*Melissae herba*) has antioxidant, antimicrobial, neuroprotective, anti-anxiety, spasmolytic, and other effects [4–6]. Due to its antioxidant activity, lemon balm protects against the development of degenerative diseases such as Alzheimer's disease, cardiovascular disease, cancer, and dermatological disorders [7].

Lemon balm is grown in various agrotechnical options (fields, hydroponic systems, and cover crops). The most productive countries are considered to be the United States, Iran, the United Kingdom, Brazil, Italy, Poland, and Germany, as well as Turkey, Serbia, and Spain [8]. The commercial raw material of lemon balm is not uniform; it is characterized by chemical composition and biological activity variability. Genetic [9,10], ontogenetic [11,12], and environmental [13–15] factors are mentioned as the main reasons for this variability. Avci et al. [16] showed that the harvesting stage of lemon balm (before, during, and after flowering) influences its plant height, fresh and dry herb yield, and essential oil content. Significant effects of specific agronomic treatments, such as the planting method and timing or number of harvests, among others, on the medicinal value of the lemon balm raw material have also been observed [16–19]. Agrotechnical treatments have the beneficial effect of increasing the raw material yield, active substance content, or both depending on the environmental conditions of the crop. Damavandi and Sayfzadeh [20] obtained in northern Iran (Takestan) the highest number of leaves to obtain essential oil from a lemon balm plant under the following cultivation combinations: a 40 × 40 cm density, mineral fertilizer, a square planting arrangement × mineral fertilizer, a rhomboid planting arrangement × a 40 × 40 cm density, and mineral fertilizer × a 30 × 30 cm density. Nahed [21] reports that spring planting lemon balm was beneficial for plant growth under Egyptian conditions, and harvesting in the second half of October gave better results for most parameters than in mid-July. Based on a study conducted in India, the highest yield of fresh and dry lemon balm herb and essential oil was obtained 160 days after planting and the lowest when it was harvested 120 days after planting [22].

Chrysargyris et al. [1] note the need to study the requirements of individual MAP species at each stage, including the growth, development, and metabolism stages. There is no doubt that these studies should be conducted under different environmental conditions and also on different plant genotypes. Our study aimed to determine the effects of the lemon balm plant density as well as one- and two-stage harvesting on the yield and quality characteristics of the raw material, which is a potential source of polyphenols and essential oil for pharmaceutical, food, and cosmetic production.

2. Materials and Methods

2.1. Plant Material, Location of Cultivation, and Growth Conditions

The object of this study was an aromatic medicinal plant, lemon balm (*Melissa officinalis* L.). The studies were conducted on annual plants. The seeds of lemon balm were obtained from the Breeding and Seed Company W. Legutko (Jutrosin, Poland). The experiments were conducted in 2019–2020 at the Experimental Farm of the University of Life Sciences in Lublin, located in south-eastern Poland (51.23° N; 22.56° E). The lemon balm was grown on loess soil developed from loess formations on chalky marls, containing 1.6% organic matter. The soil reaction was neutral (pH_{H2O} 6.5–7.4), and the following nutrient concentrations were in mg dm^{-3}: 29.8 for $N\text{-}NO_3$, 774 for Ca, 164 for K, 74 for $P\text{-}PO_4$, and 87 for Mg. The weather conditions prevailing during the study period were presented based on measurements taken in a standard manner at the Research Station of the Department of Agrometeorology of the Lublin University of Life Sciences located at the Experimental Farm Lublin-Felin (51°15′00″ N; 22°34′00″ E; 183 m a.s.l.) (Figure 1). A study on agroclimatic changes in Poland [23] identified 2019 and 2020 as the two warmest years in the 1971–2020 multi-year period. The highest temperature (over 23 °C) was recorded in the 2nd decade of June (2019) and the 2nd and 3rd decades of this month (2020). The highest rainfall (more than 70 mm) occurred in the 2nd decade of May (2019) and the 3rd decade of June (2020). Better solar conditions prevailed in 2019 than in 2020, with the highest sunshine (96 h) found at the beginning of June, followed by over 86 h at the end of June, July, and August in 2019 and at the end of July and beginning of August (over 70 h) in 2020.

Figure 1. Weather conditions during the research period.

2.2. Research Hypotheses—Experimental Field Design and Management Practices

The experiment was set up as a 2-factor experiment; the factors studied were the plant spacing (30 × 30 cm or 40 × 40 cm) and harvesting method (single harvesting or double harvesting). Optimal plant spacing facilitates plant growth, effectively utilizing solar radiation and soil nutrients. It was assumed that an optimal plant density would produce a high yield of raw lemon balm material with a high content of bioactive components. Under temperate climate conditions, lemon balm does not always flower in the first year of vegetation (which was the case in this study). Harvesting twice, in July and September, was assumed to stimulate plant abscission and the formation of young shoots to increase the herb yield. The hypothesis of the beneficial effect of this harvesting technique on the biological value of lemon balm was also verified. The single harvest was set for early September for the plants to form maximum biomass and develop and strengthen before winter, which is vital for a perennial crop, especially in colder climates.

The field experiment was established with seedlings produced in a greenhouse. The seeds were sown in mid-March into sowing boxes with a peat substrate and covered with a 2 mm thick layer of sand. Emergence occurred after two weeks, and three weeks after sowing, the plants were quilted into cone-shaped multi-tubes. The hardened seedlings were planted in a field on 20 May. The plant spacing was 30 × 30 cm or 40 × 40 cm. The plants were planted in four replications. The plot size with the 30 × 30 cm spacing was 2.16 m^2, and it was 3.2 m^2 for plants planted with the 40 × 40 cm spacing. Mineral fertilization was applied at 90 kg $N \cdot ha^{-1}$, 60 kg $P_2O_5 \cdot ha^{-1}$, and 80 kg $K_2O \cdot ha^{-1}$. Before planting the seedlings, phosphorus and potassium fertilizers were fully introduced during the field preparation. Nitrogen fertilizer was applied in divided doses: half of the dose was applied during the field preparation, and the remaining dose was applied as a single feeding to the plants after the seedlings had taken root. The plants were weeded twice by hand during the growing season, loosening the inter-rows. Subsequent weeding was not necessary due to the density of the plants, which shaded the soil and kept weeds out.

The lemon balm herb was cut depending on the combination: twice, both in mid-July and early September, or once in early September. The herb was cut by hand with secateurs at a height of 5 cm above the soil surface. After harvesting, the fresh herb yield was determined (kg·100 m^{-2}). The harvested herb was dried in a thermal dryer at 35 °C. After drying, the stems were separated from the leaves by hand. When assessing the yield, the following were also determined: the dry herb yield (kg·100 m^{-2}), the dry leaf yield (kg·100 m^{-2}), and the proportion of rubbed herb in the dry herb (kg·100 m^{-2}). If harvested twice, the herb and leaf yields from both dates were summed. The plant material (dry leaves) was subjected to laboratory tests to determine the contents of essential oil, total tannins, and flavonoids. The contents of the stated active substances for the double harvest were given as the average of both dates. The oil yield (kg·100 m^{-2}) was determined as the oil content × the air-dried herb yield.

2.3. Chemical Analyses of Plant Material

2.3.1. Essential Oil

Analyses were performed with a Clevenger apparatus [24] from Szymglass Lodz Poland. For the determination, a sample of 20 g of the air-dried herb was taken, placed in a 1000 mL round-bottomed flask, and 400 mL of distilled water was added. The liquid in the flask was heated to boiling. The distillation rate was then regulated. Distillation was carried out for 3 h. After this time, the heating was turned off, and after 10 min, the volume of oil that was collected in the calibrated tube was read. The oil content was calculated according to the following formula:

$$X(\%) = (a \times 100)/b$$

where

a—the oil volume (cm^3);
b—the sample weight (g).

2.3.2. Tannins

Tannins were determined spectrophotometrically (Hitachi U-2900 UV-Vis model, Tokyo, Japan) by extracting them from the dried herb according to the method described in Polish Pharmacopoeia IX [24]. In a 250 mL volumetric flask, 1 g of finely powdered (with a 0.16 mm sieve) crude was weighed out, and 150 mL of water was added and kept in a boiling water bath for 30 min. The mixture was cooled with a stream of water and quantitatively transferred to a 250 mL volumetric flask. The flask was rinsed by collecting the washings in the volumetric flask, and was supplemented to 250 mL with water. The precipitate was allowed to settle, and the liquid was filtered through a 125 mm diameter tissue paper filter. The first 50 mL of the filtrate was discarded; the remaining filtrate was used for the determination. To determine total polyphenols, 5.0 mL of the filtrate was supplemented to 25.0 mL with water. Then, 1.0 mL of phosphomolybdate reagent (VWR Chemicals, Gdansk, Poland) and 10.0 mL of water were added to 2 mL of this solution and supplemented with sodium carbonate solution (290 g·L^{-1}; Merck, Poznan, Poland) to 25.0 mL. After 30 min, the absorbance at 760 nm was measured using water as a standard (A1). To determine if the polyphenols were not bound to the leather powder, 0.10 g of leather powder was added to 10.0 mL of the filtrate, shaken vigorously for 1 h, and filtered. An amount of 5.0 mL of the filtrate was supplemented to 25.0 mL with water, and then 2.0 mL of this solution was added to 1.0 mL of phosphomolybdate reagent and 10.0 mL of water and supplemented to 25.0 mL with sodium carbonate solution (290 g·L^{-1}). After 30 min, the absorbance at 760 nm was measured using water as a standard (A2). The standard was prepared just before the determination by dissolving 50.0 mg of pyrogallol (Merck, Poznan, Poland) in water and supplementing this to 100.0 mL with water. An amount of 5 mL of the resulting solution was supplemented to 100.0 mL with water. An amount of 2.0 mL of this solution was mixed with 1.0 mL of phosphomolybdate reagent and 10.0 mL of water and supplemented to 25.0 mL with sodium carbonate solution (290 g·L^{-1}). After 30 min, the absorbance at 760 nm was measured using water as a reference (A_3). The tannin content (%) was calculated from pyrogallol according to the following formula:

$$\frac{62.5(A_1 - A_2)m_2}{A_3 m_1}$$

where

A_1—the absorbance of polyphenols in the test solution;
A_2—the uptake of polyphenols not related to the powdery skin in the test solution;
A_3—the absorbance of a pyrogallol comparison solution;
m_1—the initial mass of the raw material;
m_2—the sample with pyrogallol in g.

2.3.3. Flavonoids

Flavonoids were determined spectrophotometrically after extracting them from the raw material [25]. The stock solution was prepared as follows: 0.5 g of crude powder (with a 0.315 mm sieve) was weighed into a round-bottomed flask, and 20 mL of acetone, 2 mL of HCl (250 g·L^{-1}), and 1 mL of an aqueous solution of urotropine (methenamine) (5 g·L^{-1}) were added and kept for 30 min in a water bath in a reflux condenser. The hydrolysate was filtered through cotton wool into a 100 mL volumetric flask, the precipitate with the cotton wool was placed in a round-bottomed flask, and 20 mL of acetone was added and heated again to boiling in a reflux condenser for 10 min. The digestion in this way was repeated once more, and the resulting extracts were filtered into a volumetric flask and supplemented to 100 mL with acetone. Into a separatory funnel, 20 mL of the solution and 40 mL of water were poured and shaken with 15 mL of ethyl acetate, followed by 3 more additions of 10 mL of ethyl acetate each time and shaking. The combined organic phases were washed twice with 40 mL of water, filtered into a 50 mL volumetric flask, and supplemented with ethyl acetate. The test solutions were then prepared. To 10 mL of the stock solution, 2 mL of aluminum chloride solution (20 g·L^{-1}) was added and supplemented to 25 mL with a mixture (1:19) of acetic acid (1.02 kg·L^{-1}) and methanol. To prepare the reference solution, 10 mL of the stock solution was supplemented with a mixture (1:19) of acetic acid (1.02 kg·L^{-1}) and methanol to 25 mL. The performance of the assay was as follows: after 45 min, the absorbance of the solutions was measured at 425 nm using the reference solution as a reference. The total flavonoid content (%) was expressed as quercetin, according to the following formula:

$$X = (A \cdot k)/m$$

where

A—the absorbance of the test solution;
k—the conversion factor for quercetin, where k = 0.875;
m—the mass of the raw material.

2.4. Statistical Analysis

Statistical analyses were performed using Statistica 13.3.721.0 (http://statistica.io (accessed on 5 April 2023); TIBCO Software Inc., Tulsa, OK, USA, 2017). The determination of the significance of differences between the group means with the analysis of variance system was performed using Tukey's HSD test at a 0.05 significance level. In order to test the significance of differences between the means, an analysis of variance (ANOVA) was performed for the multivariate designs. The spacing (30 × 30 or 40 × 40) and the harvesting method (OTH—1-time harvest; TH—twice harvest) were the analyzed factors. The statistical analysis did not reveal any significant interactions for the tested factors.

3. Results

The yield of lemon balm in the individual years of this study depended more on the harvesting method (single or double harvesting) than on the planting density (Table 1 and Figure S1). In both years of this study, double harvesting was more favorable than single harvesting for obtaining the highest yields of fresh and air-dried herb and air-dried leaf weights. Plants grown at a lower density (40 × 40 cm) yielded a significantly higher fresh herb yield (2020) and air-dried leaf yield (2019) compared with the others.

Table 1. Yield of lemon balm (kg·100 m^{-2}) in different agrotechnical variants.

Density	Fresh Herb			Air-Dried Herb			Air-Dried Leaves		
	OTH	TH	Mean	OTH	TH	Mean	OTH	TH	Mean
					2019				
30 × 30	108.7	184.3	146.5 [a]	29.3	40.5	34.9 [ab]	14.5	30.0	22.2 [ab]
40 × 40	122.6	196.1	159.4 [a]	34.5	40.8	37.7 [a]	21.2	30.2	25.7 [a]
Mean	115.6 C	190.2 A		31.9 BC	40.7 A		17.8 B	30.1 A	
					2020				
30 × 30	92.7	132.2	112.4 [b]	22.8	34.6	28.7 [b]	12.4	26.0	19.2 [b]
40 × 40	117.3	158.2	137.8 [a]	32.0	36.2	34.1 [ab]	21.4	26.7	24.0 [ab]
Mean	105.0 C	145.2 B		27.4 C	35.4 AB		16.9 B	26.3 A	

OTH—one-time harvest; TH—twice harvest. Means marked with the same lowercase and uppercase letters in the columns/rows do not differ significantly from each other.

Like yield, the yield structure was also more dependent on the harvesting method than on the plant density (Table 2). Regarding the share of the air-dried herb weight in the fresh herb weight, single harvesting was more favorable (2019), while the share of air-dried leaves in the air-dried herb was significantly higher with double harvesting than with single harvesting in both years of this study. Lower plant densities yielded a significantly higher proportion of air-dried leaves in the air-dried herb in 2019 than higher densities did.

Table 2. Yield structure (%) of lemon balm in different agrotechnical variants.

Density	Air-Dried Herb/Fresh Herb			Air-Dried Leaves/Air Dry Herb			Air-Dried Leaves/Fresh Herb		
	OTH	TH	Mean	OTH	TH	Mean	OTH	TH	Mean
					2019				
30 × 30	0.27	0.22	0.25 [a]	0.49	0.74	0.62 [b]	0.13	0.17	0.15 [a]
40 × 40	0.28	0.21	0.25 [a]	0.62	0.74	0.68 [a]	0.17	0.15	0.16 [a]
Mean	0.28 A	0.22 B		0.55 B	0.74 A		0.15 A	0.16 A	
					2020				
30 × 30	0.25	0.26	0.26 [a]	0.55	0.75	0.65 [a]	0.14	0.20	0.17 [a]
40 × 40	0.27	0.23	0.25 [a]	0.67	0.74	0.70 [a]	0.18	0.17	0.18 [a]
Mean	0.26 AB	0.24 AB		0.61 B	0.74 A		0.16 A	0.18 A	

OTH—one-time harvest; TH—twice harvest. Means marked with the same lowercase and uppercase letters in the columns/rows do not differ significantly from each other.

In the first year of this study, lemon balm plants grown at higher densities accumulated significantly more essential oil than the others (Table 3). In contrast, plants grown at lower densities had significantly more tannins than others (in both growing seasons). Harvesting lemon balm twice resulted in a higher oil yield than a single harvest. The contents of essential oil, tannins, and flavonoids depended on the harvest date (Figures S2 and S3).

Table 3. Active substances in lemon balm raw material.

Density	Essential Oil Content (%)			Essential Oil Yield (g·100 m^{-2})			Tannins (%)			Flavonoids (%)		
	OTH	TH	Mean	OTH	TH	Mean	OTH	TH	Mean	OTH	TH	Mean
						2019						
30 × 30	0.20	0.30	0.25 ^{ab}	30.2	89.5	59.8 ^{ab}	4.23	4.50	4.29 ^{b}	0.50	0.45	0.48 ^{b}
40 × 40	0.13	0.20	0.16 ^{b}	26.3	61.6	43.9 ^{b}	5.20	5.33	5.26 ^{a}	0.58	0.43	0.50 ^{b}
Mean	0.16 B	0.25 AB		28.3 C	75.5 AB		4.71 A	4.84 A		0.54 AB	0.44 B	
						2020						
30 × 30	0.20	0.30	0.25 ^{ab}	25.3	81.5	53.4 ^{ab}	4.18	4.45	4.31 ^{b}	0.65	0.53	0.59 ^{ab}
40 × 40	0.35	0.40	0.38 ^{a}	75.0	104.8	89.9 ^{a}	5.20	5.30	5.25 ^{a}	0.58	0.70	0.64 ^{a}
Mean	0.28 AB	0.35 A		50.1 BC	93.2 A		4.69 A	4.88 A		0.61 A	0.61 A	

OTH—one-time harvest; TH—twice harvest. Means marked with the same lowercase and uppercase letters in the columns/rows do not differ significantly from each other.

By averaging the results obtained in each growing season, a comprehensive evaluation of the influence of the studied factors on the yield and quality of lemon balm, irrespective of weather conditions in 2019–2020, was developed (Figure 2). Lemon balm grown at lower densities was characterized by significantly higher fresh and air-dried herb yields, air-dried leaf yield, and tannin and essential oil contents than plants grown at higher densities. Harvesting the herb twice resulted in significantly higher fresh and air-dried herb and leaf yields, the proportion of air-dried leaves in the herb, and tannin content and oil yield compared with a single harvest.

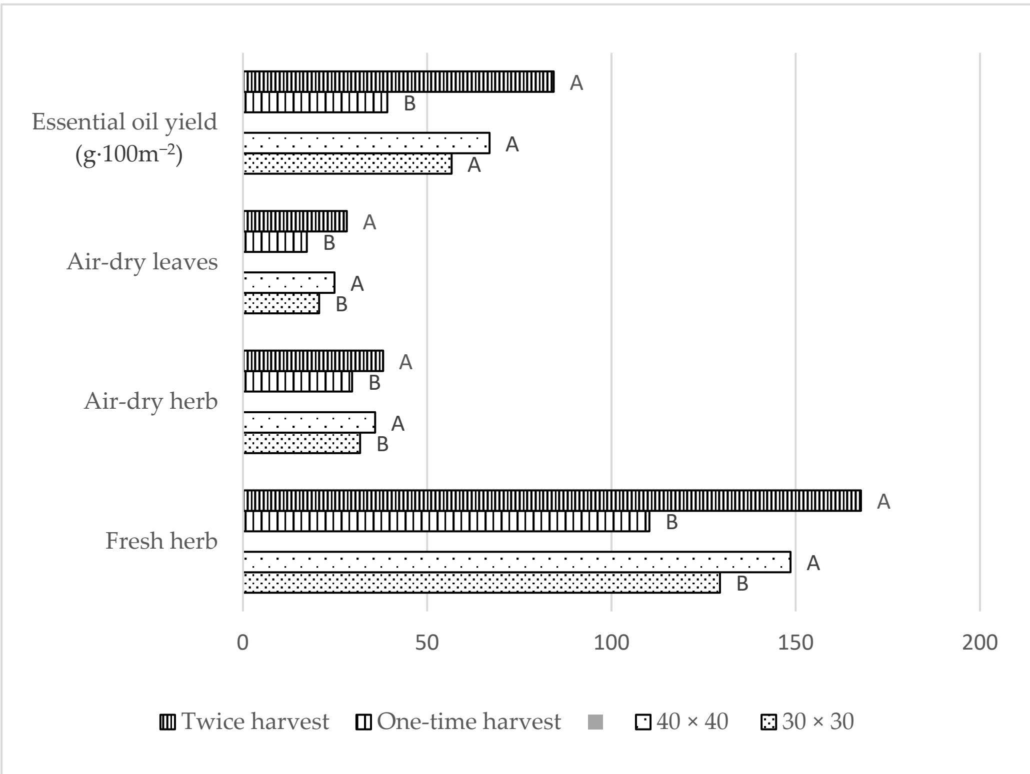

Figure 2. *Cont.*

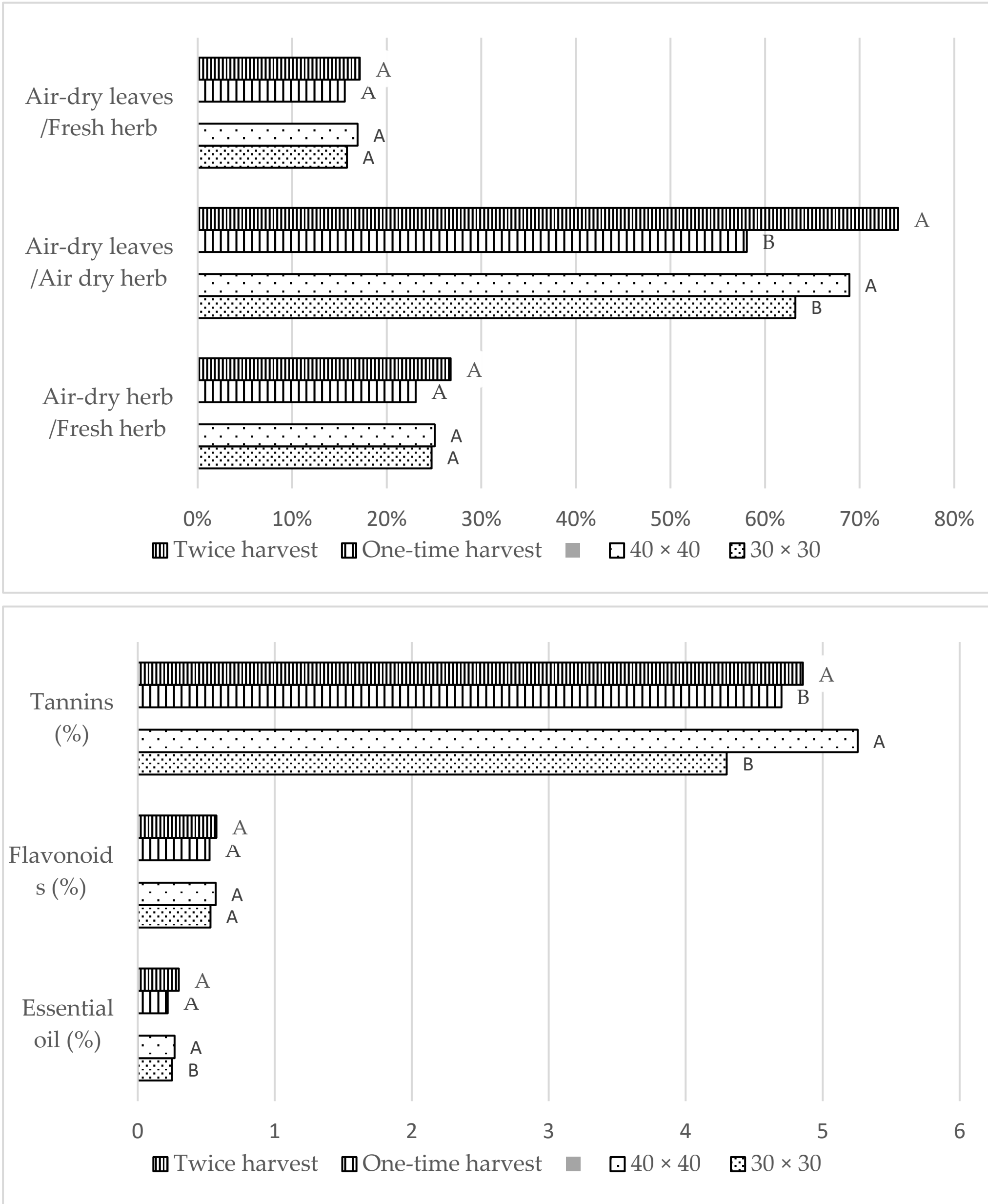

Figure 2. Lemon balm yield and active substances content in the tested agrotechnical variants (2019–2020) (Bar chart marked with the same letters do not differ significantly from each other).

4. Discussion

The growth, development, and yield of aromatic plants are strongly influenced by ontogenetic and environmental factors [26]. The yield of lemon balm herb we achieved was significantly lower than that in western Turkey [16], which weather factors should mainly explain. On the Turkish coast, under the conditions of a subtropical Mediterranean climate, regions with favorable environmental conditions for lemon balm cultivation have been designated [27]. In our study, the highest fresh herb yield came from the double harvest in 2019, when very good thermal and solar conditions prevailed. The double harvesting of the herb proved to be more efficient mainly because it allowed us to harvest young shoots (July), stimulate plant abscission and further growth, and perform an additional harvest of regrowing young shoots (September). The single harvest carried out in September involved older shoots, which were generally poorer in active ingredients, and proved to be less effective in terms of quantity and quality, as has also been shown in the cultivation of other aromatic plants [16,28].

The main active constituents of lemon balm are volatile compounds, triterpenes, phenolic acids, and flavonoids. The factors affecting the content of metabolites are the time of harvest and the methods used to determine the contents of the biosubstances [4]. Knowing the environmental requirements of lemon balm (a species of Mediterranean origin), a lower yield and, above all, a lower essential oil content can be expected under temperate climate conditions. Meanwhile, the essential oil contents we achieved (0.16–0.38%) did not differ from those shown in a study conducted in Turkey (0.27–0.36%) [16]. Virchea et al. [7] showed that the essential oil content in lemon balm herb grown under a temperate continental climate (in central Romania) is 0.17 mL·100 g^{-1} of the plant material, the flavonoid content is 0.965 g rutoside·100 g^{-1}, and the antioxidant activity is 90.40%. In our study, the essential oil content was higher (0.26% on average), and the flavonoid content per quercetin was 0.55% on average. The differences in the levels of volatile substances and polyphenols in the raw material of lemon balm may be due to various factors. Radásci et al. [13] report that water deficiency significantly affects the height and diameter of lemon balm plants, as well as their biomass production, flavonoid and phenolic compound contents, and antioxidant capacity. The most pronounced differences were found in plants grown under continuous, long-term drought conditions. Chrysargyris et al. [1] report that deficit irrigation is an environmentally friendly practice that can be applied in conventional and organic lemon balm growing systems, aiming to reduce water use and compensate for a reduced herb yield with an increased essential oil yield and polyphenol content. A study by Németh-Zámboriné et al. [19] showed that the content of phenolic compounds was highest during the vegetative phase, and in some cases, similar values were measured up to the flower bud formation phase. After a sharp decline during flowering, a second peak was detected at the end of the growing season in several cases. Similarly, the highest levels (0.239–1.152%) for flavonoids were found in the first half of the growing season, but with characteristic differences between sites. It should also be added that the level of polyphenols depends on the extraction method and solvent used, and the extract's different components strongly influence the biological activity [4].

The process of essential oil accumulation is highly dependent on ontogenetic and environmental factors [11,19]. Therefore, harvest time is one of the most critical agrotechnical factors in the cultivation of aromatic plants. Mansoori [28] obtained more essential oil and dry matter on the first harvest date of mint (25 May) than on the second harvest date (10 September). Chizzola et al. [12] report that lemon balm leaves from the first harvest (June) had more oil than those from the second harvest (August). Said-Al Ahl and Hussien [29] showed that lemon balm plants harvested in the 1st swath (August) and dried for 5 days had the most essential oil (0.31–0.32% in the herb and 0.39–0.40% in the leaves); these plants also yielded the highest oil yield (10.2–10.8 L·fed^{-1}). A study by Németh-Zámboriné et al. [19] shows that the maximum essential oil accumulation in lemon balm occurs during the flower bud formation phase, while earlier, during the vegetative phase, the plants accumulate the most polyphenols. The described dynamics of metabolite accumulation depend more on the habitat than the cultivar; oil accumulation reached maximum values in the flower bud stage (Budapest, Hungary) and flowering stage (Poznań, Poland). Similar relationships occurred in our study, although not all differences have been statistically confirmed. Lemon balm accumulates the most essential oil in its leaves (0.01–0.47%) [3,5,10,11]; however, lemon balm stems have less oil [12]. The oil from *M. officinalis* leaves isolated via hydrodistillation was a pale yellow and had a lemony odor [3]. In our study, the color and odor of the oil were similar, and its content (0.13–0.40%), depending on the plant density, harvesting method, and growing season weather conditions, was similar to that achieved under Turkish coastal conditions [16]. Therefore, it can be assumed that in lemon balm, weather conditions determine the yield to a greater extent than the essential oil biosynthesis. On the other hand, León-Fernández et al. [17] report that lemon balm from a typical plantation in Cuba accumulates more oil when grown in full sunlight than in the shade, while the time of day has no significant effect on oil levels.

Plant density differentially affects the growth and yield of oil plants and the content of essential oil and other bioactive substances [30,31]. Row spacing determines plant habits, the influencing light, water and nutrient use, and plant characteristics in mechanical harvesting [32]. EL-Leithy et al. [33] showed that the highest level of nitrogen fertilizer combined with the smallest plant spacing for *Satureja hortensis* L. (15 × 50 cm) influenced the increase in the essential oil content. Similarly, in the cultivation of *S. mutica*, Fisch C.A. Mey found the highest fresh and dry matter yields and essential oil content at a high plant density (80,000 ha^{-1}) [31]. In contrast, our results indicate that the lemon balm grown at a lower density (40 × 40 cm) showed higher fresh and air-dried herb and leaf yields than those grown at a higher density (30 × 30 cm). Damavandi and Sayfzadeh [20] obtained the highest plant height and dry leaf weight for lemon balm at a 30 × 30 cm density and the strongest branching, number of leaves per plant, and stem dry weight at a density of 40 × 40 cm. Our previous research [34] indicates that tarragon plants growing at a higher density (40 × 40 cm) have a lower oil content but higher fresh and air-dried herb yield and a higher oil yield than plants growing at a lower density (50 × 50 cm). Similarly, Askari et al. [35] report that as the density of yarrow plants increases, the essential oil content also increases, which is also influenced by the age of the plant. Conversely, with lemon verbena, the plants were found to produce less essential oil at higher densities [30]. Similarly, in winter savory (*Satureja montana* L.), the herb yield and essential oil content increase with a decreasing plant density, with the essential oil content and yield not depending on the number of harvests and harvest date [36]. In contrast, for another savory species (*S. sahendica* Bornm.), the highest number of shoots and essential oil content were obtained at the lowest density (80 × 80 cm), and the highest number of branches, flower shoot yield, and essential oil yield were obtained at the highest planting density (20 × 20 cm) [37]. The biosynthesis of active substances is dependent on light regimes and plant respiration. Light is the energy source that triggers the light phase reactions in photosynthesis in plants but also regulates the synthesis and accumulation of many metabolites, including polyphenolic compounds [38]. Appropriate cultivation techniques for Lamiaceae plants, such as shading, have positive effects, especially in the case of *M. officinalis* L. Shading allows a higher content of essential oil and its main constituents, as well as greater antioxidant and antimicrobial power [39]. Malek Maleki et al. [40] showed that the leaves of *Thymbra spicata* L. contain the most essential oil, phenols, flavonoids, and anthocyanins when grown at the highest density of 20 plants per m^2. It seems that the above differences should be explained not only at the genetic level (in terms of species and variety) but also taking into account environmental variability (in temperature, light, and humidity).

5. Conclusions

In summary, based on two growing seasons that are climatically favorable for the growth and development of lemon balm, we determined that taking into account the yields of fresh herb, air-dried herb, air-dried leaf, and oil, as well as the contents of essential oil, tannins, and flavonoids, a lower plant density (40 × 40 cm) is a better agronomic option than a higher density (30 × 30 cm). In practice, the production objective can guide the optimal harvesting date. Our research has shown that in the first year of lemon balm cultivation, a combined herb harvest in July and again in September produces better results than a single harvest in September.

The cultivation methods (in terms of the plant density and way of harvesting) and environmental conditions (light, temperature, and precipitation) determine the yield and quality of raw lemon balm. Considering the contents of essential oil and other bioactive substances, it can be concluded that cultivating lemon balm under temperate climate conditions, with a favorable set of climatic factors, can be as effective as in warmer regions. The agronomic practices described herein to obtain a high yield of lemon balm raw material and high levels of essential oil and polyphenols could also improve the production process; therefore, further investigations on the effect of the growing season, other agricultural practices, and the drying and storage conditions on other genotypes are still necessary.

Further research should focus on new agrotechnical methods in the cultivation of herbal plants to increase the yield of raw material and improve its quality.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13051433/s1: Figure S1: Lemon balm yield in different agrotechnical variants; Figure S2: Essential oil content and yield of lemon balm; Figure S3: Active substances in lemon balm raw material.

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