

EFFECTS OF MINERAL FERTILIZATION AND PLANT SPACING ON *Melissa officinalis* L. YIELD AND PREDICTIVE RELATIONSHIPS UNDER WESTERN ROMANIAN PEDOCLIMATIC CONDITIONS

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Abstract

Melissa officinalis L., commonly known as lemon balm, is a melliferous species from the Lamiaceae family, known and used since antiquity for its medicinal properties. The study investigated the impact of mineral fertilization and planting distance on yield and associated technological relationships in lemon balm cultivation. The bifactorial experiment included three levels of nitrogen fertilization (N0, N30, N60) and two planting densities (50/30 and 60/30), arranged in a randomized block design with four replications. Fresh and dry biomass yields were determined, along with the contribution of each factor to total production. Statistical analysis focused on assessing the significance of differences using ANOVA, as well as on linear correlations among factors and regression coefficients, with the aim of identifying predictive relationships between technological inputs and plant response. Preliminary results highlight a significant influence of mineral fertilization on yield, enhanced by optimal planting densities, with relevant interactions between factors. Calculated correlations indicate direct relationships between fertilization level and biomass performance, while regression models outline predictive trends useful for developing sustainable cultivation technologies.

Key words: lemon balm, mineral fertilization, plant spacing, biomass yield, regression analysis.

INTRODUCTION

The *Lamiaceae* family represents a key taxonomic group in medicinal plant research, as many of its genera are characterized by a high biosynthetic capacity for pharmacologically relevant compounds (Sârbu et al., 2013; Nieto, 2016; Gurita et al., 2019). *Melissa officinalis* L., commonly known as lemon balm, is a perennial herbaceous plant, widely appreciated for its aromatic, medicinal, and melliferous properties, that has long been cultivated across Europe, particularly in regions with temperate climates (Miraj et al., 2016; Beicu et al., 2021; Balasoiu et al., 2024). In recent years, the demand for lemon balm has increased, driven by its extensive use in the pharmaceutical, cosmetic, and food industries, particularly due to its essential oils, flavonoids, and antioxidant compounds (Shakeri et al., 2016; Petrisor et al., 2022; Ciobotaru et al., 2025; Pop et al., 2025). To meet this growing demand and ensure sustainable, high-yield cultivation, it is essential to optimize agronomic practices, notably fertilization and spatial arrangement,

according to specific environmental conditions (Chizzola et al., 2018; Banjaw & Gebre, 2024). Mineral fertilization remains one of the most effective strategies for enhancing plant growth, biomass production, and phytochemical content in medicinal and aromatic species. (Tepecik et al., 2016; Abdellatif et al., 2021; Banjaw & Gebre, 2024; Fallah et al., 2024; Gordanić et al., 2025). Among macroelements, nitrogen is particularly influential in promoting vegetative growth and biomass accumulation, but its optimal dosage can vary significantly depending on species, soil fertility, and climatic conditions (Imbrea et al., 2016; Gurita et al., 2019; Nurzyńska-Wierdak et al., 2023). Excessive or insufficient application can lead not only to reduced yields but also to environmental risks such as nutrient leaching and soil degradation (Sodré et al., 2012; Pinc et al., 2022; Fallah et al., 2024). In addition, the interaction between fertilization and planting, distance is another key agronomic factor that can significantly impact plant morphology, intra-specific competition, and resource availability (light, nutrients, and water).

Planting distance plays an important role in the development of the root system and the branching of the bush, while influencing air circulation and plant access to light. By determining the competition between plants for environmental resources, this technological factor can affect the growth rate and biomass accumulation of lemon balm. Identifying the optimal balance between planting distance and nutrient supply is crucial for achieving both high productivity and plant health (Rahmati, 2024; Gordanić et al., 2025). Given the specific pedoclimatic conditions of western Romania, characterized by cernoziom cambic soils with moderate fertility, a temperate continental climate, and variable rainfall patterns, it is necessary to adapt technological inputs accordingly. This region offers favorable conditions for cultivating *Melissa officinalis*, but scientific data on the combined effects of mineral fertilization and planting density under these local conditions remains limited. Also, the analysis of linear relationships between these factors and production allows for obtaining predictive information, in order to establish precise and sustainable cultivation strategies.

MATERIALS AND METHODS

The research was conducted during the 2023-2024 period at the Medicinal Plants Experimental Platform of the Faculty of Agriculture, within the University of Life Sciences ‘King Mihai I’ from Timișoara, located in western Romania, an area characterized by pedoclimatic conditions specific to the Banat Plain. The biological material used in the experiment was cultivated under open-field conditions following conventional agronomic practices. The experiment was conducted as a bifactorial trial with three replications, arranged in a randomized complete block design. Factor A was planting distance, with two levels (50/30 cm and 60/30 cm), and Factor B was mineral fertilization level, with three treatments: unfertilized control (N₀), N₃₀, and N₆₀. Mineral fertilization was applied according to the experimental protocol, in accordance with the standard cultivation technology for lemon balm.

Harvesting was performed at technological maturity, and fresh biomass yield was determined and expressed in kg/ha. The experimental data were statistically processed using analysis of variance (ANOVA). The statistical significance of the effects of planting distance and fertilization level, as well as the differences among treatment means, was evaluated using the Student’s t test and Duncan’s multiple range test, with the N₀ variant considered as the control. The contribution of each experimental factor to total yield variation was determined based on the analysis of variance, and linear correlation and regression analyses were used to statistically describe the relationships between the studied factors and biomass yield. The production data analyzed refers to fresh material.

RESULTS AND DISCUSSIONS

The results of the influence of planting distance on the production of lemon balm, are presented in Table 1. The analysis of variance showed that planting distance (Factor A) significantly influenced yield in 2023 and in the first harvest of 2024 ($p < 0.001$), while its effect was not statistically significant during the second harvest of 2024. Wider spacing (60/30 cm) consistently resulted in higher mean yields compared to the 50/30 cm distance, indicating improved plant development and reduced intra-specific competition.

Table 1. Effect of planting distance on yield of *Melissa officinalis* L.

Year / Harvest	Planting distance (cm)	Yield (kg/ha)	Standard error
2023	50/30	3917.27	428.52
	60/30	4345.03	403.17
2024 – Harvest I	50/30	5055.36	438.95
	60/30	5430.51	418.13
2024 – Harvest II	50/30	2912.55	432.03
	60/30	3154.10	280.02
2024 – Total	50/30	7967.92	867.87
	60/30	8584.61	692.26

In 2023, mean yield increased from 3917 kg/ha at 50/30 cm to 4345 kg/ha at 60/30 cm. A similar trend was observed in the first harvest of 2024, where yields reached 5431 kg/ha at the wider spacing. However, during the second

harvest, yield differences between planting distances were small and statistically non-significant, suggesting that spatial arrangement becomes less important during regrowth phases, when plants rely more on established root systems.

Overall, planting distance contributed modestly to yield formation, explaining between 1.3% and 3.2% of total variance, depending on year and harvest.

Yield differences were highly significant in 2023 and the first harvest of 2024, but non-significant in the second harvest, indicating that planting distance becomes less influential during regrowth.

The results regarding the influence of mineral fertilization on the production are shown in Table 2.

Table 2. Effect of fertilization level on yield of *Melissa officinalis* L.

Year/Harvest	Fertilization level	Yield (kg/ha)	Standard Error
2023	N ₀	2718.22	174.50
	N ₃₀	4111.29	19.87
	N ₆₀	5563.94	132.32
2024 – Harvest I	N ₀	3793.64	149.55
	N ₃₀	5196.49	10.48
	N ₆₀	6738.68	112.58
2024 – Harvest II	N ₀	1685.45	197.95
	N ₃₀	3319.86	18.72
	N ₆₀	4094.68	17.20
2024 – Total	N ₀	5479.08	347.51
	N ₃₀	8516.36	29.20
	N ₆₀	10833.36	95.39

Mineral fertilization (Factor B) exerted a very highly significant effect on yield in all years and harvests ($p < 0.001$).

Yield increased progressively with nitrogen level, confirming that nutrient availability is the main driver of biomass production in lemon balm under the studied conditions.

In 2023, fertilization accounted for 94.4% of yield variability. Compared to the unfertilized control (N₀), yield increased by 51.2% at N₃₀ and by 104.7% at N₆₀. Similar responses were recorded in 2024, with yield increases ranging from 37–55% at N₃₀ and from 77–98% at N₆₀, depending on harvest.

Duncan and Student tests consistently separated the three fertilization levels into distinct homogeneity groups, indicating that each nitrogen increment resulted in statistically

assured yield gains. These results emphasize the central role of mineral fertilization in optimizing lemon balm productivity in western Romania.

Duncan’s multiple range test separated all fertilization levels into distinct homogeneity groups in all experimental variants, confirming statistically assured differences among N₀, N₃₀, and N₆₀ (Table 3).

Table 3. Effect of fertilization level on yield of *Melissa officinalis* L. (Duncan’s test, $p \leq 0.05$)

Fertilization level	Yield (kg/ha)	Significance group
N ₆₀ (b3)	10830	a
N ₃₀ (b2)	8516	b
N ₀ (b1)	5479	c

The interaction between planting distance and fertilization (A×B) showed a differentiated response depending on year and harvest (Table 4 and Figure 1). While fertilization effects were consistent across planting distances, the magnitude of yield increase varied slightly between spatial arrangements.

Table 4. Interaction effect (A×B) on yield of *Melissa officinalis* L. – mean values (kg/ha)

Planting distance	N ₀	N ₃₀	N ₆₀
50/30 cm	lower	moderate	high
60/30 cm	higher	higher	highest

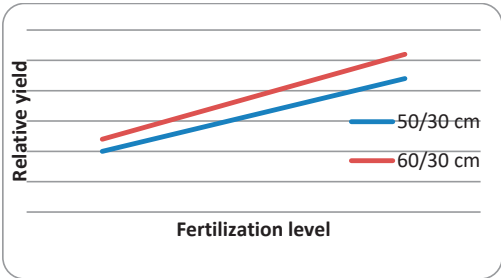


Figure 1. Interaction plot between planting distance and fertilization level for yield of *Melissa officinalis* L.

In years and harvests where planting distance was significant (2023 and first harvest of 2024), nitrogen-induced yield increases were more pronounced at the wider spacing (60/30 cm). This suggests that reduced plant competition enhanced nutrient uptake efficiency and biomass accumulation. In contrast, during the second harvest of 2024, the

interaction effect was weak, reflecting the diminished role of spatial arrangement during regrowth.

Although fertilization remained the dominant factor, the A×B interaction highlights that optimal yield expression occurs when adequate nutrient supply is combined with appropriate plant spacing, particularly during the main growth phase.

The contribution of experimental factors to the production of lemon balm are presented in Table 5 and Figure 2.

Table 5. Contribution of experimental factors to yield variability (%)

Year/Harvest	Planting distance (%)	Fertilization (%)
2023	3.20	94.40
2024 - Harvest I	2.34	96.11
2024 - Harvest II	1.36	93.80
2024 - Total	1.90	95.70

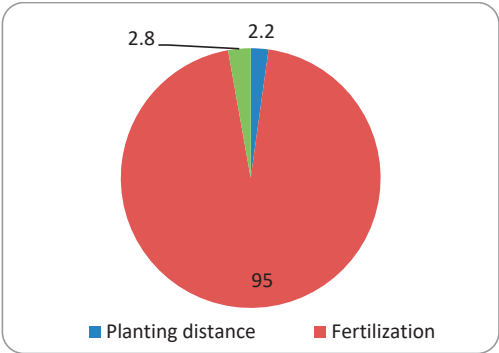


Figure 2. Relative contribution of planting distance and fertilization to yield variation

The contribution analysis clearly demonstrated the predominance of mineral fertilization in yield formation. Across all experimental variants, fertilization explained between 93.8% and 96.1% of total yield variability, whereas planting distance contributed less than 4%.

This disproportionate contribution confirms that, under the pedoclimatic conditions of western Romania, nutrient availability represents the primary limiting factor for *Melissa officinalis* productivity. Planting distance acts mainly as a secondary optimization factor, fine-tuning yield expression rather than driving it.

The variability analysis indicated a low coefficient of variation for planting distance (CV = 9.4%), reflecting the controlled experimental design, while fertilization dose and yield parameters showed high variability (CV > 20%), highlighting their strong influence on production levels (Table 6). Descriptive statistical parameters presented were calculated based on 18 observations.

Yield variability was higher in the second harvest of 2024, reflecting increased sensitivity of regrowth to environmental and nutritional factors.

Simple linear correlation analysis revealed a very strong and statistically significant positive relationship between fertilization level and yield across all years and harvests ($r = 0.95-0.98$, $p < 0.001$). These relationships are illustrated in Figure 3 (a, b), which shows the linear correlation between fertilization level and yield for: (a) first harvest in 2023 and 2024, and (b) second harvest and cumulative yield in 2024.

Table 6. Descriptive statistics of yield by harvest period

Experimental condition	Mean	Minimum	Maximum	Variance	Standard deviation	Standard error	Coefficient of variation
Harvest 2023-I	4131.151	2327.942	5860.13	1514614	1230.697	290.078	29.8
Harvest 2024-I	5242.937	3458.752	6990.63	1593753	1262.439	297.5598	24.1
Harvest 2024-II	3033.328	1242.393	4133.46	113806	1066.799	251.4469	35.2
Total Harvest 2024	8276.265	4701.144	11046.75	532029	2306.575	543.665	27.9
Planting distance	55.000	50.000	60.00	26	5.145	1.2127	9.4
Fertilization level	30.000	0.000	60.00	635	25.205	5.9409	84.0

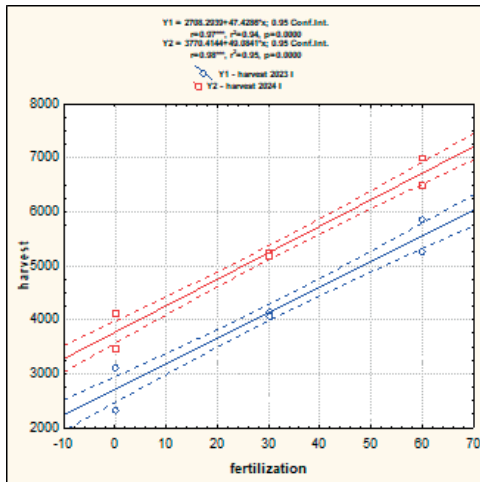


Figure 3a. Correlation between the level of fertilization

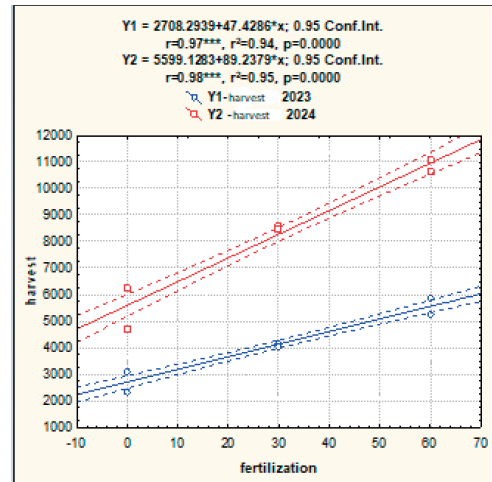


Figure 3b. Correlation between the level of fertilization

Figures 3a and 3b illustrate the correlation between fertilization level and yield, based on a comparative analysis of the first harvest obtained in the two experimental years, as well as on the comparison of total yield recorded in 2023 and 2024.

The three-dimensional response surface illustrates the combined effect of planting distance and fertilization level on the yield of *Melissa officinalis* L. in 2023 (Figure 4).

The relationship between the two experimental factors and yield was described using multiple regression analysis, indicating a positive contribution of both planting distance and fertilization level to yield variation.

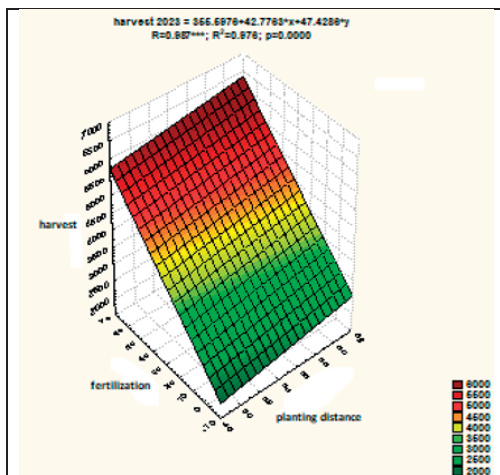


Figure 4. Relationship between planting distance, fertilization and yield in 2023

The high values of the correlation coefficient ($R = 0.987$) and coefficient of determination ($R^2 = 0.97$) indicate an excellent model fit, demonstrating that 97% of yield variability is explained by the combined action of the two experimental factors.

The slope of the response surface shows a much steeper gradient along the fertilization axis compared to the planting distance axis, highlighting fertilization as the dominant factor influencing yield.

An increase in fertilization level leads to substantial yield gains across the entire range of planting distances, confirming the strong positive effect of nitrogen supply on biomass accumulation.

This finding is consistent with the ANOVA and correlation analyses, which identified fertilization as the main determinant of yield. In contrast, planting distance exerts a moderate but positive effect

The three-dimensional response surface illustrates the combined effects of planting distance and fertilization level on the cumulative yield of *Melissa officinalis* L. in 2024 (sum of two harvests) (Figure 5).

The relationship between planting distance, fertilization level, and total yield was described using multiple regression analysis, indicating a positive and additive effect of both experimental factors on total biomass production.

The very high values of the correlation coefficient ($R = 0.98$) and the coefficient of

determination ($R^2 = 0.97$) indicate an excellent model performance, demonstrating that 97% of the variability in total yield is explained by the combined influence of planting distance and fertilization.

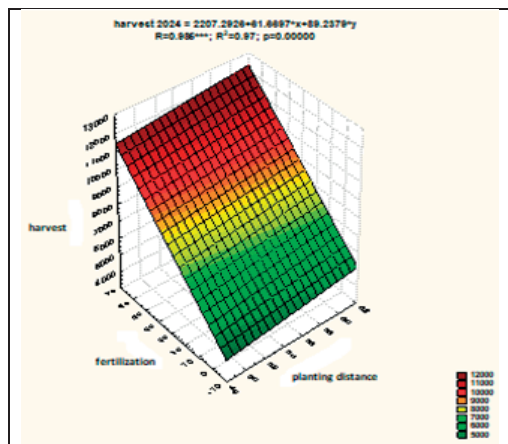


Figure 5. Relationship between planting distance, fertilization level and total yield in 2024

The response surface shows a pronounced and steep gradient along the fertilization axis, confirming fertilization as the dominant factor controlling cumulative biomass production. Increasing fertilization levels resulted in substantial yield gains across the entire range of planting distances, emphasizing the central role of nutrient availability in sustaining regrowth and multiple harvests.

Compared to 2023, the regression coefficient associated with fertilization is even higher, indicating that fertilization becomes increasingly important when total seasonal yield is considered. This reflects the cumulative effect of nitrogen supply on both primary growth and subsequent regrowth after the first harvest.

Planting distance exhibited a positive but secondary effect on total yield, as indicated by the smaller slope of the surface along the distance axis. Wider spacing enhanced yield mainly by improving the efficiency of fertilization, likely through reduced intra-specific competition and improved resource capture during successive harvest cycles.

The near-planar nature of the response surface indicates a weak interaction between planting distance and fertilization, suggesting that

fertilization consistently increased yield regardless of planting distance. This confirms that nutrient supply acts independently and decisively, while planting distance fine-tunes yield expression rather than altering the response pattern.

Overall, the response surface demonstrates that maximum cumulative yields in 2024 were achieved at higher fertilization levels combined with wider planting distances, providing strong quantitative and graphical support for fertilization centred management strategies in lemon balm cultivation under the pedoclimatic conditions of western Romania.

The fresh biomass yields obtained in our study are comparable, in terms of response pattern, with those reported under temperate climate conditions in Poland by Nurzyńska-Wierdak et al. (2023). In their study, fresh herb yield ranged approximately between 11 and 16 t·ha⁻¹, depending on plant spacing and harvest management, with higher values associated with wider spacing and multiple harvests. Although the absolute yield levels recorded in western Romania were lower, the results consistently confirm the dominant role of cultivation technology, particularly fertilization and harvest regime, over planting density in determining lemon balm biomass production (Nurzyńska-Wierdak et al., 2023).

In line with our findings, Gordanic et al. (2025) reported that planting density and cultivation practices significantly influenced agronomic performance and fresh biomass yield of lemon balm under temperate conditions in Serbia, which supports the observed role of planting density and technical management in yield variation (Gordanic et al., 2025).

Comparable agronomic research in Bulgaria by Zorovski et al. (2020) showed that fertilization treatments increased fresh biomass yield of lemon balm up to approximately 9.9 t·ha⁻¹, further emphasizing the positive effect of fertilization on yield performance in similar temperate European environments (Zorovski et al., 2020).

Research results demonstrate the importance of fertilization in optimizing fresh biomass yield in *Melissa officinalis* L., highlighting its dominant role compared to planting distance under field conditions.

CONCLUSIONS

The research results confirm that mineral fertilization is the main technological factor influencing yield in lemon balm under the pedoclimatic conditions of western Romania. Fertilization accounted for more than 95% of yield variability across experimental years and harvests, highlighting its dominant role in biomass accumulation and yield stability.

From an agronomic perspective, the results indicate that the application of moderate to higher nitrogen doses (N₃₀–N₆₀) leads to significant and statistically assured yield increases, irrespective of planting distance. The consistent yield response to fertilization demonstrates that nitrogen management represents a priority component of lemon balm cultivation technology, particularly in systems with multiple harvests per growing season.

Planting distance had a lower influence on yield; however, wider spacing (60/30 cm) proved advantageous by improving the efficiency of fertilization, especially during periods of active vegetative growth. Therefore, wider planting distances can be recommended as a complementary practice to enhance resource use efficiency, reduce intra-specific competition, and promote uniform crop development.

The weak interaction between planting distance and fertilization suggests that fertilization strategies may be applied uniformly across different planting densities, thereby simplifying technological decisions for growers.

The combined application of optimized fertilization regimes and appropriate planting distance represents a sustainable approach for increasing lemon balm productivity, supporting both economic efficiency and rational resource use in medicinal plant cultivation.

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