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EFFECTS OF SOWING DENSITY ON GROWTH AND YIELD OF LEMON BALM (*Melissa officinalis* L.) MICROGREENS

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Abstract: Lemon balm (*Melissa officinalis* L.) is a perennial medicinal and aromatic plant traditionally used in the form of herbal teas, extracts, and fresh biomass. Although it has been extensively studied under conventional cultivation systems, information regarding its agronomic performance when grown as microgreens remains limited. The present study aimed to evaluate the effect of sowing density on germination dynamics, early growth, and fresh yield of lemon balm cultivated as microgreens under controlled environmental conditions. The experiment was conducted in 2025 under laboratory conditions using three sowing densities commonly applied in microgreen production: 3 g m^{-2} (control, C), 6 g m^{-2} (T1), and 9 g m^{-2} (T2). Seeds were sown in plastic trays filled with a commercial peat-based substrate and grown under controlled temperature, relative humidity, and artificial lighting. Based on the thousand-seed weight (0.45 g) and seed use value determined according to ISTA rules (72%), the applied sowing densities corresponded to approximately 4.800, 9.600, and 14.400 plants m^{-2}, respectively. Germination, plant height, relative height increase, relative leaf area expansion, and fresh yield were monitored until harvest, which was performed 22 days after sowing at the stage of fully developed cotyledons and the onset of true leaf formation. Germination began on the 12th day after sowing and was completed by the 15th day in all treatments. Lower sowing density promoted earlier germination and faster early seedling development, whereas higher sowing density resulted in a greater number of established plants per unit area. Differences in plant height and relative leaf area were pronounced during the early growth stages but diminished by the time of harvest. Sowing density had a pronounced effect on fresh biomass yield, with the highest density (T2) achieving the greatest yield per unit area (approximately 90 g m^{-2}**)** due to increased plant density, despite reduced individual plant weight. In contrast, the control treatment (C) produced heavier individual plants but resulted in the lowest total fresh yield (approximately 56 g m^{-2}**).** The results indicate a clear density–yield trade-off in lemon balm microgreen production, suggesting that higher sowing density is more suitable for maximizing fresh biomass yield. This study provides new agronomic insights into the cultivation of *Melissa officinalis* as microgreens and contributes to the limited body of literature on medicinal and aromatic plants grown in this production form.

Keywords: Density, Germination dynamics, Early growth, Fresh yield

INTRODUCTION

Lemon balm (*Melissa officinalis* L.) is a perennial aromatic and medicinal plant belonging to the family Lamiaceae, traditionally used in the form of herbal teas, infusions, aqueous and alcoholic extracts, as well as in the food, pharmaceutical, and cosmetic industries. The dried aerial parts of the plant, primarily the leaves, are most commonly utilized due to their characteristic lemon aroma and the presence of numerous bioactive compounds. In modern production systems, lemon balm is cultivated as an open-field or protected crop aimed at producing herbal raw materials or fresh biomass (Waheed et al., 2020). In addition to conventional cultivation systems, recent studies have highlighted the potential of lemon balm for cultivation under controlled conditions, including indoor and closed-environment systems, where its responses to light and other environmental factors have been investigated (Appolloni et al., 2021). In this context, *Melissa officinalis* L. has recently been considered a suitable species for microgreen production, opening new possibilities for its utilization at early growth stages, characterized by a short production cycle and a cultivation approach distinct from conventional systems (Newman et al., 2023). Microgreens represent a relatively new category of horticultural products, comprising young plants of vegetable, aromatic, and medicinal species grown from emergence until the stage of fully developed cotyledons and the appearance of the first true leaves. Owing to their short production cycle, limited space requirements, and suitability for controlled-environment cultivation, microgreens are increasingly recognized as an appropriate production system for urban and intensive agriculture, as well as a model system for studying the effects of agronomic factors on plant growth and yield (Partap et al., 2023). Previous research on microgreens has primarily focused on vegetable species, particularly those belonging to the family Brassicaceae, whereas aromatic and medicinal plants remain underrepresented in the scientific literature. Nevertheless, several studies have reported that species from the family Lamiaceae, including basil, mint, and lemon balm (*Melissa officinalis* L.), exhibit good adaptive potential for microgreen production, with pronounced differences in growth and yield depending on genotype and cultivation conditions (Partap et al., 2023; Di Gioia et al., 2023; Newman et al., 2023). Fresh yield is a key production parameter in microgreen cultivation, as it directly determines the economic viability of this production system. Previous studies have shown that microgreen yield varies considerably among species and is strongly influenced by sowing density, seed size, and the number of shoots per unit area. An optimal sowing density enables efficient space utilization while preventing excessive intra-specific competition, which may otherwise result in reduced individual plant biomass and overall yield (Gordanić et al., 2025). In aromatic plant species, sowing density plays an additional role by influencing plant physiology, growth intensity, and biomass accumulation efficiency within the very short growth period characteristic of microgreens (Xu et al., 2023). Although *Melissa officinalis* L. has been extensively studied as a perennial medicinal plant under conventional cultivation systems, information regarding its growth behavior and production characteristics at the microgreen stage remains limited. Existing literature has largely focused on the biochemical properties of mature plants or the effects of light on metabolism, whereas basic agronomic parameters, such as sowing density and its impact on microgreen yield, have not been sufficiently investigated. Therefore, there is a clear need for systematic research on the production aspects of lemon balm grown as microgreens, with particular emphasis on the effect of sowing density on yield performance. Understanding these relationships is essential for defining optimal production technologies and may contribute to the wider adoption of lemon balm in commercial microgreen production. The objective of this study was to evaluate the effect of three different sowing densities on the fresh yield of lemon balm (*Melissa officinalis* L.) microgreens in order to identify the optimal density that ensures maximum yield per unit area under controlled growing conditions.

MATERIAL AND METHODS

Seeds

The experiment was conducted using reproductive material (seeds) of lemon balm (*Melissa officinalis* L.) originating from the collection of the Institute for Medicinal Plant Research "Dr Josif Pančić", Pančevo, Serbia (44°52′20.0″ N; 20°42′04.7″ E). The thousand-seed weight was 0.45 g, while the seed use value, determined in accordance with the ISTA rules (International Rules for Seed Testing), was 72%.

Experimental Design

The experiment was conducted in late March 2025 in the laboratory of the Department for Agricultural Research and Development, Belgrade, Serbia (44°49′ N; 20°28′ E), under controlled environmental conditions, to assess the effects of different sowing densities on germination dynamics and early growth of lemon balm (*Melissa officinalis* L.) cultivated as microgreens, up to the stage of fully developed cotyledons and the onset of the first true leaves. Three sowing density treatments commonly applied in microgreen production were evaluated: 6 g m^{-2} (T1) and 9 g m^{-2} (T2), while a standard sowing density of 3 g m^{-2}, according to the methodology of Stepanović et al. (2009), was used as the control (C). All treatments were arranged in four replicates. Based on the thousand-seed weight (0.45 g) and seed use value determined in accordance with the ISTA rules (72%), the applied sowing densities corresponded to approximately 4.800 plants m^{-2} (C), 9.600 plants m^{-2} (T1), and 14.400 plants m^{-2} (T2). Plant density was not considered an experimental factor but was calculated as a derived parameter reflecting germination success and early seedling establishment under microgreen cultivation conditions. Sowing was performed in plastic trays ("Dno-Flex") measuring 31×51 cm (surface area 0.15 m^2), previously filled with a commercial peat-based substrate ("Plagron Grow Mix"). The substrate consisted of a mixture of white and black peat enriched with fibers and perlite, with a neutral reaction (pH H_2O 6-7), electrical conductivity (EC) ranging from 1.0 to 1.5 mS cm^{-1}, and a nutrient composition of NPK 12:14:24, including a potassium oxide (K_2O) content of 4.6%.

Growing Conditions

After sowing, the trays were placed under controlled environmental conditions to ensure optimal parameters for seed germination and seedling growth. Artificial lighting was provided using cool fluorescent tubes with a 12 h photoperiod. Relative air humidity in the growth chamber ranged between 60 and 70%. Air temperature was maintained between 20 and 24 °C, while substrate temperature was kept stable at 21 ± 2°C. Air temperature and relative humidity were monitored using a HAXO-8 data logger, whereas substrate temperature was measured using a Testo 110 thermometer (Gordanić et al., 2025).

Growth Measurements

During the germination and early growth phase of lemon balm, seed germination was evaluated daily during the first seven days following emergence, in accordance with the ISTA standard, using the following equation:

Germination rate = (total number of seeds) / (number of germinated seeds) × 100

Subsequently, relative plant height increase was monitored following the method described by Radford (1967):

Relative height increase = (Hfinal - Hinitial) / Hinitial × 100

Where:
Hfinal - plant height at the end of the measurement period (e.g., at the end of the experiment),
Hinitial - initial plant height (e.g., at the start of the experiment or measurement).

In addition, relative leaf area increase was quantified using the Canopeo software, following the methodology developed by Patrignani et al. (2015).

Harvest and Yield Determination

Harvest was performed 22 days after sowing, at the stage characterized by fully developed and turgid cotyledons and the onset of true leaf formation. The number of plants per tray was recorded for each treatment. Seedlings were harvested by cutting at the substrate level using sterile cutting tools, and the collected biomass was immediately weighed. Fresh yield was determined using an analytical balance (KERN) and expressed on an area basis as g m^{-2}.

Statistical Analysis

Collected data were organized and preliminarily processed using Microsoft Excel. Statistical analysis was performed using one-way analysis of variance (ANOVA), and differences among treatment means were evaluated using Duncan's multiple range test at a significance level of $p < 0.05$. All statistical analyses were conducted using SPSS software.

RESULTS AND DISCUSSION

Germination Dynamics of lemon Balm Microgreens

Seed germination and emergence of lemon balm (*Melissa officinalis* L.) microgreens exhibited a delayed onset compared with many commonly cultivated microgreen species. Germination in all sowing density treatments began on the 12th day after sowing and was completed by the 15th day. This delayed germination behavior is characteristic of *Melissa officinalis* and has been associated with small seed size, lower initial metabolic activity, and specific physiological requirements for successful germination (Stepanović et al., 2009; Di Gioia et al., 2023).

The control treatment (3 g m^{-2}) exhibited the earliest and most uniform germination response, with significantly higher germination values recorded during the initial germination phase (days 12 and 13) compared with higher sowing density treatments. In contrast, the highest sowing density (9 g m^{-2}) showed a slightly slower initial response but reached the highest cumulative germination by day 15.

Table 1. Average seed germination per treatment (%)

Days after sowing	T1	T2	C
1-11	/	/	/
12	8.5 ± 1.2b	6.2 ± 0.9c	12.4 ± 1.5a
13	24.6 ± 3.1b	21.3 ± 2.7b	38.7 ± 4.2a
14	52.8 ± 4.9b	60.4 ± 5.6a	65.2 ± 5.1a
15	68.3 ± 5.7b	82.6 ± 6.4a	74.5 ± 6.0ab

* Values followed by the same lowercase letter within a row are not significantly different ($p < 0.05$)

These results indicate that lower seed density provided more favorable microenvironmental conditions for early germination, whereas higher density ensured a greater number of established seedlings per unit area, consistent with previous reports on microgreen establishment patterns (Di Gioia et al., 2023).

Plant Height Development

Average plant height measurements revealed distinct growth patterns during early development, followed by convergence among treatments at harvest. At 15 and 18 days after sowing, plants grown at the lowest sowing density exhibited significantly greater individual height compared with those grown at higher densities, reflecting reduced intra-specific competition for light, water, and nutrients.

Table 2. Average plant height per treatment (cm)

Days after sowing	T1	T2	C
15	0.82 ± 0.09b	0.65 ± 0.07c	1.10 ± 0.11a
18	1.95 ± 0.17b	1.82 ± 0.15b	2.24 ± 0.19a
20	2.95 ± 0.21a	3.02 ± 0.23a	3.05 ± 0.22a
22 (harvest)	3.80 ± 0.24a	3.88 ± 0.26a	3.75 ± 0.25a

*Values followed by the same lowercase letter within a row are not significantly different ($p < 0.05$)

By harvest (22 days after sowing), no statistically significant differences in plant height were observed, indicating that sowing density primarily influenced early growth dynamics rather than final plant height. Similar growth convergence has been reported for other microgreen species (Radford, 1967; Kyriacou et al., 2016).

Relative Increase in Plant Height

Relative plant height increases followed trends similar to absolute height values. During early growth, the control treatment exhibited the highest relative increase, while higher sowing densities showed slower relative growth.

Table 3. Average relative height increases per treatment (%)

Days after sowing	T1	T2	C
15	14.2b	11.6c	19.8a
18	38.4b	34.9b	46.7a
20	71.5a	73.2a	74.8a
22	100.0a	100.0a	100.0a

*Values followed by the same lowercase letter within a row are not significantly different ($p < 0.05$)

By harvest, relative height increases values converged across all treatments, confirming the compensatory growth capacity of lemon balm microgreens (Radford, 1967; Lee et al., 2017).

Relative Leaf Area Development

Relative leaf area expansion was significantly influenced by sowing density during early growth stages. Plants grown at lower density exhibited higher relative leaf area values, likely due to reduced competition and greater lateral expansion.

Table 4. Average relative increase in leaf area per treatment (%)

Days after sowing	T1	T2	C
15	18.4b	14.9c	29.6a
18	41.2b	38.5b	55.8a
20	62.9b	65.4ab	68.1a
22	100.0a	100.0a	100.0a

* Values followed by the same lowercase letter within a row are not significantly different ($p < 0.05$)

As growth progressed, differences diminished, and by harvest all treatments reached comparable relative leaf area values, consistent with canopy closure effects reported in previous studies (Patrignani et al., 2015; Wang et al., 2019).

Fresh Yield and Yield Components

Fresh yield parameters clearly demonstrated the dominant role of sowing density in determining lemon balm microgreen productivity.

Table 5. Fresh yield parameters per treatment (harvest at 22 DAS)

Treatment	Number of plants (m^{-2})	Plant weight (mg $plant^{-1}$)	Fresh yield (g m^{-2})
T1	118.4 ± 8.6b	0.043 ± 0.003b	72.4 ± 4.8b
T2	154.6 ± 11.9a	0.038 ± 0.002c	89.6 ± 6.3a
C	74.2 ± 6.1c	0.052 ± 0.004a	56.1 ± 3.9c

* Values followed by the same lowercase letter within a column are not significantly different ($p < 0.05$)

The control treatment produced the heaviest individual plants, whereas the highest sowing density achieved the greatest fresh yield due to a higher number of plants per unit area. This density yield trade-off confirms that total biomass production in microgreens is more strongly driven by plant density than by individual plant size (Di Gioia et al., 2023; Zhang et al., 2021).

CONCLUSION

This study showed that sowing density significantly affects germination dynamics and fresh yield of lemon balm (*Melissa officinalis* L.) grown as microgreens. Germination began on the 12th day after sowing and was completed by the 15th day in all treatments, with lower sowing density promoting earlier germination and faster early development.

However, differences in plant height and leaf area among treatments diminished by harvest 22 days after sowing.

Fresh yield was primarily determined by plant density, ranging from approximately 56 g m^{-2} in the control treatment to 72 g m^{-2} at the intermediate sowing density and reaching nearly 90 g m^{-2} at the highest sowing density. The highest sowing density produced the greatest biomass per unit area despite reduced individual plant weight, while the control treatment yielded heavier individual plants but the lowest total yield. These results indicate a clear density-yield trade-off and suggest that higher sowing density is more suitable for maximizing fresh yield of lemon balm microgreens under controlled conditions.

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REFERENCES

Appolloni, E., Pennisi, G., Zauli, I., Carotti, L., Paucek, I., Quaini, S., Gianquinto, G. (2022). Beyond vegetables: Effects of indoor LED light on specialized metabolite biosynthesis in medicinal and aromatic plants, edible flowers, and microgreens. Journal of the Science of Food and Agriculture, 102(2), 472-487.

Di Gioia, F., Renna, M., Santamaria, P. (2023). Yield performance, mineral profile and nitrate content of microgreens. Frontiers in Plant Science, 14, 1220691.

Gordanić, S., Dragumilo, A., Mrđan, S., Golijan Pantović, J., Mikić, S., Prijić, Ž., Marković, T. (2025). The impact of sowing density on the productivity and growth dynamics of *Ocimum basilicum* grown as microgreens. Proceedings: XIV International Symposium on Agricultural Sciences AgroReS *2025*, Trebinje, Bosnia and Herzegovina, University of Banja Luka, Faculty of Agriculture, 12-18.

Gordanić, S.V., Radanović, D., Rajković, M., Lukić, M., Dragumilo, A., Mrđan, S., Marković, T. (2025). Influence of mulching and planting density on agronomic and economic traits of Melissa officinalis L. Horticulturae, 11(8), 866.

Kyriacou, M.C., El-Nakhel, C., Pannico, A., Graziani, G., Soteriou, G.A., Giordano, M., Ritieni, A., De Pascale, S. (2016). Microgreens as a component of space life support systems: A cornucopia of functional food. Frontiers in Plant Science, 7, 1117.

Lee, J.S., Lee, H.J., Kim, S.Y. (2017). Growth dynamics and density effects in leafy vegetables. Scientia Horticulturae, 225, 22-29.

Newman, R.G., Moon, Y., Tou, J.C., McManus, T., Waterland, N.L. (2023). Harvest stage and brewing conditions impact mineral content, phenolic compounds, and antioxidant capacity of lemon balm (*Melissa officinalis* L.) herbal tea. Plant Foods for Human Nutrition, 78(2), 336-341.

Partap, M., Sharma, D., Thakur, M., Verma, V., Bhargava, B. (2023). Microgreen: A tiny plant with superfood potential. Journal of Functional Foods, 107, 105697.

Patrignani, A., Ochsner, T.E. (2015). Canopeo: A powerful new tool for measuring fractional green canopy covers. Agronomy Journal, 107, 2312-2320.

Radford, P.J. (1967). Growth analysis formulae. Their use and abuse. Crop Science, 7(3), 171-175.

Stepanović, B., Radanović, D., Turšić, I., Nemčević, N., Ivanec, J. (2009). Uzgoj ljekovitog i aromatičnog bilja. Jan Spider, Pitomača.

Waheed, K., Nawaz, H., Hanif, M.A., Rehman, R., Ogunwande, I.A. (2020). Lemon balm. *Medicinal* Plants of South Asia, 465-478.

Wang, Q., Zhang, T., Wang, W. (2019). Effects of plant density on early growth and leaf area development in leafy vegetables. Journal of Plant Growth Regulation, 38, 987-996.

Xu, A., Zhu, Y., Wang, W. (2023). Micro green technology innovation affects green finance pilot policy from the perspectives of action points and green value. Journal of Business Research, 159, 113724.

Zhang, Y., Xiao, Z., Li, X., Wang, F. (2021). Optimization of sowing density in microgreen production systems. Horticulturae, 7, 356.