

The impact of sowing density on the productivity and growth dynamics of *Ocimum basilicum* grown as microgreens

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Abstract

The increasing interest in fresh, functional, and nutraceutical products has driven the demand for healthy and longevity-promoting foods, with microgreens gaining popularity due to their vibrant colors, delicate texture, and rich phytonutrient content. Among various microgreens, *Ocimum basilicum* is widely recognized for its aromatic, bioactive properties, and nutritional composition, making it a promising functional food. This study aimed to investigate the impact of sowing density on the productivity, growth dynamics, and quality of *O. basilicum* cultivated as microgreens, focusing on improving production efficiency. The experiment was conducted in November 2024 in a controlled environment with three sowing densities: 2.5 g/m² (control, C), 3.5 g/m² (T1), and 4.5 g/m² (T2). Key parameters such as seed germination, plant height, leaf area, and fresh yield were evaluated throughout the growth cycle. Results showed that higher sowing densities (T2) positively affected seed germination and plant height during the initial growth stages. Although initial growth was more vigorous in the control treatment (C), the differences between treatments became less pronounced over time. The study concluded that optimizing sowing density is crucial for maximizing microgreens yield and improving the overall sustainability of basil production, providing valuable insights for commercial growers.

Key words: basil, germination, growth dynamics, vegetative development, fresh yield

INTRODUCTION

The interest in fresh, functional, and nutraceutical products has increased due to the growing societal focus on healthy diets and the preservation of health (Rizzo et al., 2018). Consumers are seeking products that support health and longevity while maintaining gastronomic qualities. Microgreens, a new category of specialized crops, consist of immature plant shoots (Xiao et al., 2012). They gained popularity in the late 1980s due to their vibrant colors, delicate texture, ability to enhance flavor, and rich phytochemical content (Sun et al., 2013; Xiao et al., 2015). Demand is growing due to emerging culinary trends, with species selection dependent on collaborations with chefs and consumer sensory preferences (Renna, 2017). Microgreens are distributed either as fresh products or in their growing phase (Choe et al., 2018), with commonly used species from the Brassicaceae, Asteraceae, and Lamiaceae families, among others (Xiao et al., 2015). Microgreens differ from sprouts, which are typically grown under moist, dark conditions and are associated with foodborne outbreaks (Rizzo et al., 2018; Xiao et al., 2015). In contrast, microgreens have superior flavor-enhancing properties and are rich in phytochemicals such as vitamins A, C, and E, as well as minerals (Pinto et al., 2015; Xiao et al., 2012). While microgreens are attractive due to their high market prices and short production cycles, they are limited by factors such as low productivity and short shelf life (Chandra et al., 2012; Kou et al., 2013). Microgreens are still in the early stages of development, and research on their potential as a superfood is revealing new insights. The focus is on pre-harvest and post-harvest factors that influence quality and resilience (Pinto et al., 2015).

Basil (*Ocimum basilicum*) is one of the most well-known microgreens, valued for its aromatic properties, bioactive compounds, and nutritional content. It is rich in phytochemicals, including flavonoids and essential oils with antimicrobial, anti-inflammatory, and antioxidant properties (Zhang et al. 2021). Basil also contains vitamins A, C, E, and minerals such as calcium, magnesium, and iron (Fayezizadeh et al., 2023). Its distinct flavor enhances the sensory properties of dishes, making it a unique addition to culinary preparations. As such, basil has significant potential as a functional food that contributes to health, particularly in the prevention of chronic diseases (Gupta et al., 2023).

The objective of this study is to investigate the impact of different sowing density on the growth, development, and quality of *O. basilicum* grown as microgreens, with a particular focus on improving production efficiency. The study will analyze the physiological and post-harvest characteristics of plants at varying sowing density, including plant height, leaf mass, and development dynamics. Additionally, the aim is to identify optimal sowing density that will maximize yield, which is critical for the commercial production of microgreens. This study also seeks to contribute to a better understanding of the factors influencing the efficiency and sustainability of microgreens production, with the goal of providing precise recommendations for growers.

MATERIAL AND METHODS

Reproductive Material

For the experiment, reproductive material (seeds) of basil (*Ocimum basilicum*) was used, sourced 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 1000-seed mass was 2.5 grams, with a germination rate of 63%.

Experimental Design

The experiment was conducted during the last decade of November 2024 in the laboratory of the Department of Research and Development in Agriculture, Belgrade, Serbia (geographical coordinates: 44°49' N, 20°28' E). The study was designed to assess the effects of different basil sowing density during germination and early growth stages (until the appearance and development of the first true leaves). The treatments included two sowing density variations: in the first treatment (T1), seeds were sown at a density of 3.5 g of seeds per m², while in the second treatment (T2), a density of 4.5 g of seeds per m² was used. The control treatment (C) involved a standard sowing density of 2.5 g of seeds per m², according to Stepanović et al. (2010). All treatments were conducted in four replicates. Seeds were sown in plastic trays (brand "Dno-Flex"), measuring 31 x 51 cm (area 0.15 m²), previously filled with a commercial substrate "Plagron Grow Mix." This substrate is a blend of white and black peat enriched with fibers and perlite, with a neutral pH (H₂O) ranging from 6-7, and an EC (mS/cm) of 1.0-1.5. The nutrient composition was NPK 12:14:24, with potassium oxide (K₂O) content of 4.6%.

After sowing, the trays were placed in controlled conditions to ensure optimal parameters for seed germination and plant growth. Artificial lighting was provided using cool fluorescent tubes, with a photoperiod of 12 hours. The relative humidity in the room ranged from 60-70%. Air temperature was maintained between 20 °C and 24 °C, while the substrate temperature was kept constant at 21 ± 2 °C. Air temperature and relative humidity were monitored using the HAXO-8 data logger, and substrate temperature was controlled using the Testo 110 thermometer.

During the germination and early growth phases of basil, which lasted from 1 to 3 weeks (Ramin et al., 2006), seed germination was assessed daily during the first 7 days according to the ISTA standard:

$$\text{Germination rate} = (\text{total number of seeds}) / (\text{number of germinated seeds}) \times 100$$

Afterward, the relative plant height increase was monitored using the method of Radford (1967):

$$\text{Relative height increase} = (\text{H}_{\text{final}} - \text{H}_{\text{initial}}) / \text{H}_{\text{initial}} \times 100$$

Where:

H_{final} - plant height at the end of the measurement period (e.g., at the end of the experiment),
H_{initial} - initial plant height (e.g., at the start of the experiment or measurement).

Additionally, the relative leaf area increase was quantified using the software program Canopeo, following the method developed by Patrignani et al. (2015).

Upon reaching the commercial harvest stage, defined by the presence of fully expanded, colored, and turgid cotyledons, as well as the initial growth of true leaves (16 days after sowing), plant counting was performed for each treatment. The microgreens were then harvested by cutting the seedlings just above the surface of the growing medium, using sterile blades. After harvesting, the microgreens were weighed to determine the fresh yield. Fresh yield per treatment was measured using an analytical balance (KERN), expressed in g/m².

Data Evaluation

The obtained data were processed using Excel, and statistical analysis was performed using one-way analysis of variance (ANOVA). The mean values were compared using Duncan's test ($p < 0.05$) with the SPSS software package.

RESULTS AND DISCUSSION

In this section of the paper, we will provide a detailed analysis of the research results related to seed germination, plant height, leaf area, and plant yield under different treatments. The data collected over a 16-day period from the beginning of the experiments were analysed, with a particular focus on changes during the critical stages of plant growth.

Table 1. Average seed germination per treatment (%)

Parameters	Days	Treatments		
		T1	T2	C
Germination of seeds from 1 to 10 days	1.	/	/	/
	2.	/	/	/
	3.	7±1,58b	7,25±1,5b	8±0,7a
	4.	12,8±1,87a	13,5±1,7a	13,5±1,7a
	5.	41,5±4,61b	61±4,2a	33,75±4,0c
	6.	74,25±6,76b	95±6,3a	51,5±2,5c
	7.	103,25±7,56b	145,75±11,7a	63±7,6c

*/-no results were recorded; values between treatments marked with the same lowercase letter are not statistically significantly different ($p < 0.05$).

Table 1 shows the average seed germination across different days of the experiment. Based on the analysis of the results, treatment T2 yielded the most favourable results in terms of seed germination. Specifically, as early as day three, the seed germination rate for treatment T2 was significantly higher (7.25%) compared to T1 (7%) and the control K (8%), with these differences becoming even more pronounced as the experiment progressed. On day seven, treatment T2 reached a germination rate of 145.75%, while treatments T1 and C recorded values of 103.25% and 63%, respectively. Statistical analysis indicates that treatment T2 was highly effective in stimulating germination, which aligns with studies confirming that certain treatments can significantly accelerate seed germination (Adams et al., 2016). This result suggests that treatment T2 promotes accelerated seed development, possibly due to specific stimulating factors such as temperature or nutrient treatments. On the other hand, the C treatment, which did not include any additional stimulation, demonstrated a significantly lower germination rate, implying that basic conditions for germination without additional treatments are insufficient for optimal results. This is significant as it corroborates the findings of previous studies that suggest treatments involving seed stimulation can offer long-term benefits in terms of both germination speed and quality (Smith et al., 2018).

Table 2. Average height of plants per treatment

Parameters	Days	Treatments		
		T1	T2	C
Average plant height per treatment (cm)	1.	/	/	/
	2.	/	/	/
	3.	0,31±0,05b	0,26±0,04b	0,43±0,04a
	4.	0,45±0,05b	0,43±0,08b	0,55±0,05a
	5.	1,35±0,23b	0,85±0,11b	1,18±0,11a
	6.	2,78±0,23b	3,20±0,19b	4,28±0,39a
	7.	3,78±0,23b	4,20±0,19b	5,30±0,39a
	8.	4,80±0,19b	5,10±0,12b	5,48±0,27a
	9.	5,23±0,22a	5,45±0,11a	5,65±0,30a
	10.	5,68±0,24a	5,85±0,39a	5,88±0,22a
	11.	5,95±0,15a	6,03±0,31a	5,98±0,18a
	12.	6,05±0,11a	6,18±0,23a	6,03±0,27a
	13.	6,03±0,13a	6,15±0,21a	5,98±0,25a
	14.	6,03±0,13a	6,15±0,21a	5,98±0,25a
	15.	6,05±0,11a	6,20±0,23a	6,10±0,21a
	16.	6,10±0,21a	6,23±0,26a	6,05±0,15a

*/-no results were recorded; values between treatments marked with the same lowercase letter are not statistically significantly different ($p < 0.05$).

Table 2 presents the results regarding plant height throughout the experiment. At the beginning of the experiment, the control treatment (C) exhibited a statistically significant greater plant height (0.43 cm) compared to T1 (0.31 cm) and T2 (0.26 cm). This result suggests that plants under treatment C had more favourable initial conditions for growth, potentially due to a higher initial root count or better conditions for water and nutrient absorption. However, as the experiment progressed, the differences in plant height among the treatments diminished. By the end of the experiment, all groups achieved similar plant heights: 6.1 cm (T1), 6.23 cm (T2), and 6.05 cm (C). These findings indicate that, over a longer period, all treatments have comparable effects on plant growth. This observation aligns with previous research demonstrating that long-term advantages in plant height are not necessarily determined by initial treatment differences (Johnson et al., 2020). This result could be particularly relevant for agronomic practices aimed at optimizing resources and outcomes based on various treatment applications.

Table 3 presents data on the relative increase in plant height throughout the experiment. In the initial phases (days 3 and 5), treatment C exhibited the highest relative increase in plant height (7.02% on day 3), suggesting that treatment C stimulated more rapid growth during the early stages. In contrast, T1 and T2 demonstrated slower growth during this phase but achieved comparable growth rates in the later days. By days 9 to 16, all three treatments reached nearly identical values for relative height increase, approximately 100%. This trend indicates that, although the treatments began with differing growth rates, all treatments facilitated similar plant growth dynamics by the end of the experimental period. Similar changes in relative growth rates have been reported in previous studies, which suggest that initial advantages in plant height are not sustained in the long term (Lee et al., 2017).

Table 3. Average relative height increase of plants per treatment

Parameters	Days	Treatments		
		T1	T2	C
Relative increase in plant height per treatment (%)	1.	/	/	/
	2.	/	/	/
	3.	5,12b	4,22b	7,02a
	4.	7,38b	6,83b	9,09a
	5.	22,13a	13,65b	19,42a
	6.	45,49b	51,41b	70,66a
	7.	61,89b	67,47b	87,60a
	8.	78,69b	81,93b	90,50a
	9.	85,66a	87,55a	93,39a
	10.	93,03a	93,98a	97,11a
	11.	97,54a	96,79a	98,76a
	12.	99,18a	99,20a	99,59a
	13.	98,77a	98,80a	98,76a
	14.	98,77a	98,80a	98,76a
	15.	99,18a	99,60a	100,83a
	16.	100,00a	100,00a	100,00a

*/-no results were recorded; values between treatments marked with the same lowercase letter are not statistically significantly different ($p < 0.05$).

Table 4 presents the results regarding the relative increase in leaf area. In the early days of the experiment, treatment C once again demonstrated the highest relative increase in leaf area, with a value of 33.2% on day 5. However, as the experiment progressed, differences between the treatments diminished, and by the end of the experiment, all three treatments reached a relative increase of 100%.

Table 4. Average relative increase in leaf area per treatment (from leaf emergence to harvest)

Parameters	Days from sowing	Treatments		
		T1	T2	C
Relative increase in leaf area per plant (%)	1.	/	/	/
	2.	/	/	/
	3.	/	/	/
	4.	/	/	14,2a
	5.	12,21b	11,3b	33,2a
	6.	36,41b	31,43b	50,99a
	7.	36,41b	31,43b	50,99a
	8.	37,93b	34,57b	50,99a
	9.	42,48b	40,86b	53,61a
	10.	53,11b	45,58b	58,84a
	11.	53,11b	50,29b	58,84a
	12.	54,62bb	50,29b	58,84a
	13.	65,24b	65,44a	66,68a
	14.	83,45a	89,58a	83,68a
	15.	91,04a	92,72a	87,61a
	16.	100,00a	100,00a	100,00a

*/-no results were recorded; values between treatments marked with the same lowercase letter are not statistically significantly different ($p < 0.05$).

These results suggest that the initial acceleration of leaf growth observed in treatment C was effective but did not result in long-term advantages regarding leaf area. In most cases, the later growth stages show that all treatments achieve similar outcomes in leaf development, which aligns with studies investigating the effects of various treatments on plant growth (Wang et al., 2019).

The results presented in Table 5 demonstrate significant differences in fresh yield parameters across treatments T1, T2, and C, where treatment T2 achieved the highest fresh yield ($83.14 \pm 11.67 \text{ g/m}^2$), followed by T1 ($62.58 \pm 1.98 \text{ g/m}^2$) and the control group C ($45.56 \pm 2.12 \text{ g/m}^2$). This outcome is primarily attributed to the significantly higher plant density in T2 ($145.75 \pm 11.69 \text{ plants/m}^2$) compared to T1 (103.25 ± 7.56) and C (63.00 ± 7.58), which enabled better cumulative resource utilization and increased productivity despite similar individual plant weights in T1 and T2 ($0.04 \pm 0.002 \text{ mg/plant}$). In contrast, treatment C exhibited the highest individual plant weight ($0.05 \pm 0.004 \text{ mg/plant}$), likely due to reduced competition for resources; however, this advantage was insufficient to offset the lower plant count, leading to significantly lower overall yield. These results align with previous studies that emphasize the critical role of plant density in maximizing yields by ensuring optimal resource use (Zhang et al., 2021; Smith et al., 2018). The findings also suggest that treatments enhancing germination and early seedling establishment, as seen in T2, are key to achieving higher productivity, while untreated control conditions, such as in C, provide suboptimal results. Overall, the results highlight the importance of targeted agronomic practices, including the use of fertilizers, growth stimulants, or seed priming, to improve plant density, resource efficiency, and fresh yield outcomes, consistent with findings from Adams et al. (2016) and Wang et al. (2019).

Table 5. Fresh yield parameters per treatment

Treatments	Parameters		
	Number of plants	Plant weight per treatment (mg/plant)	Yield (g/m^2)
T1	$103,25 \pm 7,56b$	$0,04 \pm 0,002b$	$62,58 \pm 1,98b$
T2	$145,75 \pm 11,69a$	$0,04 \pm 0,002b$	$83,14 \pm 11,67a$
C	$63,00 \pm 7,58c$	$0,05 \pm 0,004a$	$45,56 \pm 2,12c$

*/-no results were recorded; values between treatments marked with the same lowercase letter are not statistically significantly different ($p < 0.05$).

CONCLUSION

Based on the obtained results, it can be concluded that treatment T2 is the most effective in stimulating seed germination, plant growth, and achieving high yields. While treatment C demonstrated initial advantages in plant height and leaf area, it did not result in long-term benefits regarding yield. Treatment T1, although not producing the best results in any single parameter, exhibited moderate growth and yield, which may be useful under specific agronomic conditions. These findings are valuable for future studies and agronomic practices focused on optimizing seed treatments to achieve higher yields and efficient plant growth.

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