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Original article

The Effects of Sowing Density Applications on Yield and Some Quality Parameters in Different Vegetable Microgreens

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Abstract

Microgreens are plants consumed before reaching maturity. Vegetable seeds are generally used in their production. Microgreens are used in salads and various dishes in terms of their aromatic aspects. It is known that microgreens are an important source of antioxidants. Although microgreens are easy and fast to produce, producers' lack of knowledge limits the production of microgreens. Seed spacing has also been not correctly verified in previous studies, as well. Within the scope of the study, okra (*Abelmoschus esculentus* L. cv. 'Sultani'), carrot (*Daucus carota* L. cv. 'Nantes'), leek (*Allium porrum* L. cv. 'Hotanlı'), spinach (*Spin*acia *oleracea* L. cv. 'Matador'), cress (*Lepidium sativum* L. cv. 'Tere') vegetables were sown at two different sowing densities (the average amount of seeds that can fit on a 1 cm² surface area-dense sowing and 1/3 of this amount-sparse sowing). Plant weight, yield, width of root collar, plant height, water soluble dry matter, ascorbic acid were evaluated. Results indicated that ocra microgreens'weight was higher than the other microgreens' and had higher values in terms of yield. Ocra microgreens also demonstrated the highest total soluble solids value. The yield of okra, spinach and cress microgreens increased with dense planting. However, it was found that the increase in planting density and the increase in yield were not at the same rate. Cress microgreens had the highest value in terms of ascorbic acid value.

Keywords: Microgreen, Sowing Density, Yield, Ascorbic Acid.

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INTRODUCTION

Many vegetables, medicinal plants and herbaceous plants have thin stems and cotyledon leaves in their early stages. These plants are called microgreens. Microgreens can be in different colors, shapes and flavors (Bhatt and Sharma, 2018). Microgreens can be harvested at the stage when the cotyledon leaves are growing or when they have the first two true leaves (Li et al., 2021; Gerovac et al., 2016; Waterland et al., 2017). Gioia et al. (2023) indicated the commercial harvesting stage of microgreens as fully grown cotyledons and the initial growth of true leaves. Microgreens can be harvested by cutting the microgreens together at the root collar with a pair of scissors. Some of the important advantages of microgreens are that they can be grown in a short time and can be produced in a relatively small area.Microgreens had delicate tissues. Microgreens can be included in salads, soups, and sandwiches (Choe et al., 2018).

Crops that have the potential to be grown as microgreens include amaranth flower, fennel, arugula, spinach, beet, mustard, buckwheat, celery, radish, coriander, red cabbage, broccoli, lettuce, and carrots (Bhatt and Sharma, 2018). Recently, okra microgreens have also started to be used in garnishes or meals (Anonymous, 2020b)However, vegetables of the nightshade (*Solanaceae*) family carry harmful toxins in their shoots. The microgreens of cucumber, watermelon and melon vegetables are bitter. It is known that for the reasons listed, these vegetables are not used as microgreens (Anonymous, 2020a).

Microgreens can be easily cultured and require minimal resources to be cultured. Microgreens contain nutrients such as minerals, vitamins and antioxidants, and they also have a protective potential against many diseases when consumed in small amounts (Bhatt and Sharma, 2018). In the study (Wojdylo et al., 2020), 9 essential (Anonymous, 2020c) amino acids were detected in some different sprouts and micrgreens, including leek (*Allium porrum* L.) sprouts.

Microgreen cultivation is a lucrative business line if a market opportunity can be found. Microgreen cultivation is not sufficiently known in Çanakkale and there is very limited production. However, as a result of the recognition of microgreens in Çanakkale region, there is a potential for marketing in various restaurants.

Microgreen production is carried out with dense sowing technique. In the case of dense sowing and planting, there is increased competition for external resources and space for growth. At the same time, more seeds are needed. Considering that various vegetable seeds used in microgreen production are of different sizes, the number of seeds planted per unit area should vary according to the varieties. In addition, excessively dense or sparse sowing of seeds of the same varieties in microgreens will reveal negative results. For this reason, producers who want to use their seeds in microgreen production need a preliminary study on different vegetables and seed amounts.

The aim of the study was to determine the yield and some quality parameters of microgreens produced using different vegetable varieties at different sowing densities (in a growing medium usually used as a standard in seedling production - 2/3 peat, 1/3 perlite). Another objective of the study was to provide an easy way for the producers to utilize the surplus seeds of standard varieties with decreasing germination rate.

MATERIALS and METHODS

The study was carried out in the greenhouse located on the Dardanos campus of Çanakkale Onsekiz Mart University, in the Çanakkale province of Turkey.

Seeds of okra (*Abelmoschus esculentus* L. cv. Sultani), spinach (*Spinacia oleracea* L. cv. Matador), carrot (*Daucus carota* L. cv. Nantes), cress (*Lepidium sativum* L. cv. Tere) and leek (*Allium Porrum* L. cv. Hotanlı) vegetables were used as plant material in the experiment. All varieties in the trial are standard varieties.

In the study, a uniformly mixed 2/1 ratio of peat and perlite mixture was used as the planting medium.

Properties of the peat material used:

Organic carbon in dry matter of biological origin (C): 70 %, Organic Nitrogen (N): 0.8 %, Organic Matter: 95 %, pH ($CaCl_2$): 5.0-6.0, Electrical Conductivity (mS/m): 40, Water Holding Capacity (%): 70, Fertilizer Amount: NPK 17 10 14 1 kg/m^3.

Seeds were planted in 27×36×7 cm^3 growing containers (0.0972 m^2 surface area). An equal number of holes were drilled in the bottom of the growing containers to drain excess water. 5994 cm^3 of peat and perlite mixture (2/1) was used in each container. During the planting process, each container was filled with medium at a height of 5 cm. After the seeds were planted uniformly by hand, the seeds were covered with 1 cm of medium and lightly compressed. In order to increase uniform distribution, the container surface and seed amount were divided into 8 and the seeds were spread separately in each region. The containers were watered once a day until the first emergence appeared. The containers were watered in the greenhouse twice a day, at the same time in the morning and evening, until water came from the holes at the bottom of the growing containers. The containers were irrigated by spraying water in small particles. Thus, the humidity in the environment was increased and uniform irrigation of the growing surface was ensured.

The thousand grain weights of the seeds were calculated as 56.64 g for okra seeds, 12.3 g for spinach seeds, 2.61 g for leek seeds, 1.16 g for carrot seeds and 1.82 g for cress seeds.

In the experiment, planting was done at 2 planting densities (the amount of seeds that can fit into 1 cm² surface area for each variety - Dense Planting, one third of the amount of seeds that can fit into 1 cm² surface area - Sparse Planting). Accordingly, in the dense planting application, 4 okra, 9 spinach, 17 leek, 30 carrot and 30 cress seeds were planted on 1 cm² surface area.

First of all, the number of seeds to be planted on the surface area (0.0972 m^2) of the growing containers was calculated. Then, the amount of seeds (g) sowed in each container was calculated using the thousand grain weights (g) of the seeds.

Germination power of vegetable seeds was determined according to Ellis and Roberts, (1980). Germination rate was determined as 98% for okra and spinach seeds, 92% for cress and carrot seeds and 90% for leek seeds.

The amount of non-emerging seeds (g) was added as the percentage of non-emerging seeds. For example, the amount of extra seeds was obtained from same seeds had 4% low germination.

X=Percentage of seeds that added calculated with using the formulas (if germination percentage is 98%): (100+X)×98/100=100 or (100+X)-[(100+X)×(2/100)]=100

For okra seeds X=2,04 and total seeds calculated as 73.4+(73.4+2.04/100) (Table 1.).

Table 1. Amounts of Total Seeds Used in Each Container and 1 m^2 (g)

	Sparse sowing		Dense sowing	
	0.0972 m^2 (g)	1 m^2 (g)	0.0972 m^2 (g)	1 m^2 (g)
Okra	74.9	832.25	224.7	2496.76
Spinach	36.96	410.71	110.89	1232.14
Leek	16.61	184.57	49.83	553.73
Carrot	12.37	137.52	37.13	412.58
Cress	19.41	215.77	58.25	647.33

When the temperature in the greenhouse exceeded 30°C, the greenhouse windows were opened and the fan that provided air flow into the greenhouse was turned on. To prevent the increase in light intensity, 40% protection mesh shading material was used 1 week after emergence.

Microgreens were harvested after the cotyledons were fully grown, at the time of the first growth of true leaves (Gioia et al., 2023). Among the harvested microgreens, okra microgreens may contain small amounts of nutrient medium residues due to their hairy structure. For this reason, some okra microgreens were cleaned with the help of shaking and air circulation. It has been observed that okra microgreens do not immediately lose their hard structure even if washed with gently flowing water. Additionally, microgreens are often marketed together with nutrient media. In this case, their freshness is preserved, and the harvesting and cleaning process is done by the consumer.

Parameters of Study:

Morphological Parameters:

Yield (g/m^2): Harvested microgreens were cut from the root collar and weighed on a scale with 0.1 precision, and the yield per m^2 (g/m^2) was determined.

Microgreen weight (g): It was determined by weighing 20 plants from each replicate one by one on a scale with a sensitivity of 0.001.

Root collar width (mm): It was determined by measuring 20 plants from each replicate one by one using a caliper with a sensitivity of 0.01.

Plant Length (mm): It was determined by measuring 20 plants from each replicate one by one using a caliper with a sensitivity of 0.01.

Chemical Parameters:

Total Soluble Solids (%): Total soluble solids were recorded by a hand refractometer, with 3 readings in each repetition.

Ascorbic Acid Content (mg/100g): Ascorbic acid (vitamin C) contents of microgreens were determined spectrophotometrically by the 2.6 dichlorophenol indophenol method (Shimadzu UV-VIS - 1800 spectrophotometer) according to the method of Pearson and Churchill (1970). 175 ml of 0.4% oxalic acid was added to 25 g of sample. The samples were left to filter on Whatmann No:2 filter paper for approximately 10 minutes. L1 value was determined by reading Oxalic acid/Pure Water: 1/10 solution versus Oxalic acid/2.6 Dichlorophenol indophenol: 1/10 solution at 520 nm transmittance value. In addition, with samples taken from each filtrate for each sample; The L2 value for the samples was determined by reading the filtrate/Pure Water:1/10 solution versus the filtrate/2.6 Dichlorophenol indophenol:1/10 solution at 520 nm transmittance. Ascorbic acid content was calculated with the help of this formulation.

The experiment was set up according to the randomized design, with 3 replications.

Statistical analyzes in the experiment were performed using analysis of variance and the LSD ($P<0.05$) test to compare the differences between the averages of the data.

RESULTS and DISCUSSION

Table 2. Comparison of Some Vegetable Microgreens Grown Under Different Sowing Densities in Terms of Yield and Morphological Parameters

Microgreens	Sowing Density [c]	Yield (g/ m^2) [a]	Microgreen weight (g) [a]	Root collar width (mm) [a]	Plant Length (mm) [a]
Okra (*Abelmoschus esculentus* L. cv. Sultani) [b]	Dense	9984.6 a	0.44 a	1.886 a	101.58 a
	Sparse	4673.9 b	0.45 a	1.85 a	103.87 a
Cress (*Lepidium sativum* L. cv. Tere) [b]	Dense	2661.2 cd	0.036 d	0.526 cd	42.06 e
	Sparse	2050.4 d	0.043 d	0.566 d	40.86 e
Spinach (*Spinacia oleracea* L. cv. Matador) [b]	Dense	3122.1 c	0.13 b	0.96 b	66.02 b
	Sparse	2240.4 d	0.14 b	0.996 b	67.96 b
Leek (*Allium porrum* L. cv. Hotanlı) [b]	Dense	996.9 e	0.063 c	0.453 de	105.2 a
	Sparse	730.1 e	0.08 c	0.51 cd	113.61 a
Carrot (*Daucus carota* L. cv. Nantes) [b]	Dense	743.5 e	0.033 d	0.416 e	61.05 bc
	Sparse	719.8 e	0.04 d	0.5 cd	57.1 c
$P<0.05$ LSD =		697.95	0.0185	0.0812	7.7224

a, b, c, d, e or combined representations: Different letters in same column shows the statistical difference, [b]: Microgreen cultivars, [a]: Yield and morphological parameters, [c]: Sowing density (Dense or sparse)

When the yield values were examined, it was determined that the yield values obtained in okra, cress and spinach microgreen varieties in dense sowing applications were higher than sparse sowing applications ($P<0.05$). The highest yield was obtained from okra microgreens that were sown densely. The lowest yield was obtained from leek and carrot microgreens in the same statistical group ($P<0.05$) (Table 2.). It is known that in case of dense planting, there is an increase in competition regarding the use of external resources and the space required for growth.

Thuong, V. T. and Minh, H. G., (2020) conducted three different nutrient media and seed planting density applications (82.36 g/m^2, 109.81 g/m^2, 137.27 g/m^2 164.72 g/m^2) in red radish (*Raphanus sativus*) microgreens in their study. In their study, they used a seed planting machine and applied fertilizing solution. In their study (Thuong and Minh, 2020), they obtained the highest fresh weights (between 1102.38-1318.01 g/m^2) at a seed planting density of 109.81 g/m^2. As seen in the study (Thuong and Minh, 2020), the highest yield was not obtained from the highest seed sowing density. However, the yield was found to be lower than 109.81 g/m^2 in subjects sowed more densely than 109.81 g/m^2. In our study, only the yield of okra, spinach and cress microgreens increased as the seed sowing density increased (P<0.05). It can be said that the increase in yield is much less than the increase in the amount of seeds planted, which is similar to the study of Thuong and Minh, (2020). In addition, the issue of how yield will be affected when seed is planted less densely under the conditions in which our study was conducted may be a new research topic. At the same time, it is seen that okra microgreens are not included in some of the studies conducted using various microgreens (Gioia et al., 2023; Gerovac and Craver et al., 2016).

Wieth et al. (2019) applied different nutrient solutions at different doses to purple cabbage (*Brassica oleracea* var. capitata f. rubra) microgreen, which they planted manually at a planting density of 102 g/m^2. They also applied fertilization containing macro and micro nutrients. In their study, they determined the fresh shoot yield as 1111.31 g/m^2 and the shoot length as 5 cm. The yield amounts were examined both in our study and in the studies (Thuong and Minh, 2020; Wieth et al., 2019) and it was seen that microgreens are not a product that can be described as low-yield. Gioia et al. (2023) planted Cressida cress (*Lepidium sativum* L.) microgreens (germination percentage 92%) at a density of 3 seeds per cm^2 and obtained a yield of 994.7 g/m^2. In our study, it was observed that the yield value of okra microgreen was especially high.

It was observed that microgreen weights in sparse and dense sowing applications were statistically similar (P<0.05). It was determined that the highest weight value was obtained from okra and the lowest weight value was obtained from carrot microgreens (P<0.05) (Table 2.). The weights of microgreens, except okra microgreens in our study; were found to be similar to the weight of the control subject (0.075 g) in the study conducted on arugula microgreens by Murphy and Pill (2010).

When the root collar width values of microgreens were examined, it was seen that the root collar width of cress, leek and carrot microgreens was statistically (P<0.05) higher in sparse planting applications compared to dense planting applications (Table 2.). It is undesirable for the root collars of microgreens to be too thick. However, the eating quality of microgreens of all subjects was found appropriate.

When the plant height parameter was evaluated, it was seen that the highest plant height values were obtained from leeks and the lowest plant height values were obtained from cress microgreens (P<0.05). It was determined that the plant height value in the subject with sparse sowing, only in carrot microgreens, was slightly higher than the subject with dense sowing (P<0.05) (Table 2.).

Table 3. Comparison of Some Vegetable Microgreens Grown Under Different Sowing Densities in Terms of Ascorbic Acid and Total Soluble Solids Parameters

Microgreens	Sowing Density [c]	Ascorbic Acid (mg/100g) [a]	Total Soluble Solids (%) [a]
Okra (*Abelmoschus esculentus* L. cv. Sultani) [b]	Dense	119.18 dc	5.6 a
	Sparse	112.69 e	5.4 a
Cress (*Lepidium sativum* L. cv. Tere) [b]	Dense	145.33 a	3.46 c
	Sparse	144.26 a	3.26 c
Spinach (*Spinacia oleracea* L. cv. Matador) [b]	Dense	117.05 dc	2.66 d
	Sparse	115.06 de	2.73 d
Leek (*Allium porrum* L. cv. Hotanlı) [b]	Dense	124.61 b	2.7 d
	Sparse	126.07 b	2.5 d
Carrot (*Daucus carota* L. cv. Nantes) [b]	Dense	105.58 f	4 b
	Sparse	107.03 f	3.93 b
P<0.05 LSD =		3.5806	0.4321

a, b, c, d, e, f or combined representations: Different letters in same column shows the statistical difference, [b]: Microgreen cultivars, [a]: Ascorbic acid and total soluble solids, [C]: Sowing density (Dense or sparse)

In the study, cress microgreens had the highest ascorbic acid values (Dense sowing: 145.33, Sparse sowing: 144.26 mg/100g) and carrot microgreens had the lowest ascorbic acid values (Dense sowing: 105.58, Sparse sowing: 107.03 mg/ 100g) ($P<0.05$) (Table 3.). In a study (Xiao et al., 2012), total ascorbic acid content in 25 different microgreens ranged from 20.4 (*Rumex acetosa* L.) to 147 (*Brassica oleracea* L. var. capitata) mg/100g. In the study (Xiao et al., 2012), the amount of ascorbic acid in magenta spinach (*Spinacia oleracea* L.) variety was 41.6 mg/100 g.

It was observed that the amount of ascorbic acid contents obtained by dense sowing applications in okra and spinach microgreens were statistically higher ($P<0.05$) than the amount of ascorbic acid contents obtained by sparse sowing applications (Table 3.). This increase was more evident in okra microgreen. In one study (Barzegar et al., 2016), the amount of ascorbic acid in okra decreased with water deficit stress and in another study (Younis et al., 2024), it increased with drought stress. However, more data are needed regarding whether the increase in the amount of ascorbic acid as a result of dense planting in our study is due to water stress. In addition, the most positive effect of dense planting in terms of yield was seen in okra microgreens. At the same time, microgreens have different characteristics than mature plants.

Total soluble solids content is a parameter used to estimate the amount of sugar in the product. Okra microgreens had the highest amount of total soluble solids content (5.6-5.4%), while spinach (2.66-2.73%) and leek (2.7-2.5%) microgreens had similar and the lowest amounts of total soluble solids content ($P<0,05$) (Table 3.). In the study (Wieth et al., 2019), the amount of water-soluble dry matter (°brix) of the control subject in purple cabbage microgreen was determined as 5.03%. In our study, only okra microgreens had values close to this value in terms of total soluble solids content.

Conclusion

The results show that, the yield amount was found higher in okra, spinach and cress microgreens in dense sowing applications compared to sparse sowing. In dense sowing, three times more seeds were planted than sparse sowing. However, except for okra microgreens, the yield in dense sowing could not even reach to twice of sparse sowing. In okra microgreens, the yield obtained in dense sowing was slightly more than twice from the yield value obtained in sparse sowing. In the study, it can be said that sparse sowing instead of dense sowing in the current growing condition is more suitable in terms of saving the amount of seeds to be used. It can also be said that okra microgreens give the most suitable result for dense planting.

In addition, producers may have surplus seeds. Especially in okra production, producers utilize the remaining fruits in the field as seed source. However, they may not be able to find enough irrigable land or labor force to farming from these increasing seeds. In order to evaluate these seeds, it can be recommended that they can be used in microgreen production.

In conclusion cress microgreens had the highest ascorbic acid content and obtained average values in terms of yield and total soluble solids content. However, the highest amount of total soluble solids and yield were obtained from okra microgreens. At the same time, the ascorbic acid content of okra microgreen was not the lowest among the microgreens.

We conclude that, okra microgreen was the leading microgreen variety in the study with its high yield increasing with dense planting.

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