



Effects of density and cultivation method on ratio and yield of essential oil in basil (*Ocimum basilicum* L.)

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Abstract

Basil (*Ocimum basilicum*) belongs to the Lamiaceae. It is mostly used for nutrition in Iran, while it also has numerous pharmaceutical properties. The plant has some aromatic compounds. In order to investigate effects of density and cultivation method on the amount of extractable essential oil, an experiment was done on green and purple cultivars in a farm with area around 200m². Two methods of cultivation were experimented: cultivation in one row and in two rows. Two types of densities were experimented: 40, 60, 80, 100 plants per m² based on factorial design experiment in completely randomized blocks. Significant differences between means values were determined using multivariate Duncan test at 5% by MSTATC software. Correlation coefficients were calculated by SPSS software. Data analysis of variance showed that the cultivar has significant influence on the percentage amount of essential oil and the cultivation method impacts the yield of essential oil. Plant density has significant influence on the percentage and yield of essential oil. Results showed that the highest percentage and yield of essential oil was obtained in the cultivation of purple basil in two rows with a density of 40 plants per m².

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Introduction

Basil (*Ocimum basilicum* L.) belongs to the family of Lamiaceae that is mostly used for nutrition in Iran. Moreover, it has numerous pharmaceutical properties. Some farmers use the boiled leaves of basil as insecticides for cabbage and tomato (Josephwort, 2000). The vegetative body of basil contains essential oil extracts. Basil extract is a liquid with yellow or greenish color, with aroma and has special weight of 0.905 to 0.930. Depending on the climate conditions of the plant cultivation location, the amount of this extracted essential oil varies between 0.5 and 1.5%, and 8 to 10 kg of the essential oil is obtained in one hectare of planted area (Farooqi, 2005). To extract essential oil and oil, the plant is harvested in flowering stage. To use its fresh leaves, they are picked from 10-15 cm below the tip of long stems (Simon, 1995). Basil essential oil should be kept in a closed container, in cool places and away from light (Prakash, 1990).

Although secondary metabolites are made under genetic process, but their synthesis is affected by environmental factors. Environmental factors cause variations in herb growth and the quality and quantity of their metabolites (Omidbaigi, 1995). The density has great effect on the yield of medicinal plants as the distance between plants influences the environmental factors light, humidity and soil nutrients (Malekouti and Balali, 2004). Heidari *et al.* (2008) found that peppermint had more growth in the first harvest than the second and the plant had the highest yield in essential oil in the first harvest; but the amount of essential oil is less in the first harvest than in the second.

Hajseyedhadi *et al.* (2001) obtained the best yield for essential oil in chamomile by planting it in dense area of (20 cm × 50 cm). Their results also showed that the density of bushes in an area has no effect on the amount of flower essential oil. Naghdibadi *et al.* (2002), studied planting oregano in 3 different area density of (15 cm, 30 cm, and 45 cm) also studied harvesting in different time of the year and concluded that the best wet, dry and essential oil yield is obtained from the plant distance of 15 cm. They

expressed that seasonal climate changes have significant effects on the rate of the essential oil, but no significant differences were observed statistically for the distance of the plants as the only factor, while the cultivation distance together with seasonal climate changes had significant effects on the quality and quantity of oregano.

Arabaci and Bayram (2004), in a three-year research found that in applying nitrogen fertilizers on basil, the highest biologic and essential oil yield is for the rate of about 1% by applying 50 kg of nitrogen fertilizer in one hectare of the area, but the highest amount of oil is obtained in no nitrogen condition (control).

Isabella & Barbieri (2006) considered growth, functions and essential oil rate in basil plants under the effects of different application rates of nitrogen fertilizers and found that different amounts of nitrogen could be effective on the growth of basil, but the amount of essential oil is not affected in this regard.

Dadvand and Reza (2006) showed that plant density has significant effects on the dry material and essential oil yield of basil, but it has no significant effects on the rate (percentage) of essential oil, although due to increased yield of the dry substance, the increase in essential oil yield has become significant for the unit of area. They also found that although using nitrogen fertilizers has negative effects on the rate of essential oil, but up to 100 kg/hectare of nitrogen fertilizers increase the yield of essential oil and dry matter in a unit of area. The increasing rate of essential oil yield has been due to increasing yield of the dry matter.

By a pot experiment on basil, Chris and coworkers (1997), found that by increasing the number of plants from nine to 16 in m² of area, the biologic yield show the ascending trend, but the weight of plants decreases by their numbers, in case it is more than eight and they determined that the number per unit area has no effect on the rate of essential oil extraction. Panic and Kosciuska (1990), in some examinations in two years, found that the highest

percentage of essential oil is obtained through the cultivation spacing of 10×50 cm.

The aim of this research was to investigate effects of density and cultivation method on the amount of extractable essential oil on green and purple cultivars of basil ocimum in order to obtaining maximum yield.

Materials and methods

This research was done in Imam Khomeini Higher Education Center (IHEC) farm located in the city of Karaj with the area of 200m², in spring and summer 2010.

Plant materials

Two types of healthy seeds of green and purple basil with high purity and nominal strength that were prepared from the gene bank of Seed and Plantlet Breeding Institute (SPBI) were used.

Preparation and sampling

Manure was homogeneously applied to all the plots, as the base fertilizer for the fertility of soil and before cultivation. Then the seeds were sown and covered by soft soil. Sampling from grown plants was done according to standards. Extracting essential oil was done by hydro distillation and Clevenger.

Measurements

Percentage of essential oil and yield of essential oil were two main parameters that measured according to density, cultivation method and cultivar in basil.

Yield of essential oil calculated as following:

$$Y = (\text{percentage of essential oil} \times \text{biomass}) / 100$$

Statistical analysis

The experiment was carried out with 3 replications in a completely randomized block design. The cultivars (green and purple) were cultivated in two methods (one and two-row) and with density in 4 different levels (40, 60, 80 and 100 bushes per m²). Significant differences between means were done using multivariate Duncan test at 5% by MSTATC software. Correlation coefficient was calculated by SPSS software.

Results

A) Effect of treatments on percentage (ratio) of essential oil

The simple effect of species (type) and plant density and the cross effect of cultivation type and cultivar become significant in 1% level. The purple basil (with the mean of 0.29) showed higher rate than green basil with the average of 0.23. Regarding the rate of essential oil extraction, the one and two-row cultivations were placed in one statistical group and had no significant differences (Table 1).

Table 1. Simple analysis of variance for percentage and yield of essential oil in basil

Variation resources	Degree of freedom	Mean squares	
		Percentage of essential oil	Yield of essential oil
Replication	2	0.5289 ^{n.s}	0.205 ^{n.s}
Cultivar	1	3.0830 ^{**}	0.718 ^{n.s}
Cultivation type	1	0.05775 ^{n.s}	3.603 [*]
Cultivar× cultivation type	1	2.93783 ^{**}	1.411 ^{n.s}
Density	3	7.82416 ^{**}	1.640 [*]
Density × cultivar	3	0.65125 [*]	0.448 ^{n.s}
Cultivation type × density	3	0.66515 [*]	0.541 ^{n.s}
Cultivation type × cultivar × density	3	0.37694 ^{n.s}	1.723 [*]
Error	30	0.19283	0.533

** : significant at 0.01 * : significant at 0.05 n.s : non significant

Comparison of means showed that density of 40 and 60 plants in m² had higher levels with the mean values of 0.43 and 0.32, respectively, as compared to the densities of 80 and 100 plants. Also, the least rate of extract was for the density of 100 plants per m² with the mean value of 0.16%.

Results showed that basil cultivars grouped in different statistical regarding different cultivation intervals and for different rates of essential oil, such that the green basil in two-row cultivation with mean value of 0.26 had higher level than one-row cultivation with the mean value of 0.20. Purple basil and its two-row cultivation with the mean value of 0.33% had significant priority as compared to one-

row cultivation. As a whole, purple basil with 2-row cultivation and mean of 0.33% has the highest rate of essential oil and green basil with one-row cultivation and the mean of 0.26% had the lowest rate of extracted essential oil. Purple basil density in 40 plants per m², with the average rate of 37% prepared the highest rate of extract. Also, green and purple basil with the density of 100 plants per m² and the average rate of 0.19% produced the lowest amount of extract. Cultivation type in different compaction were grouped in different statistical classes for their rate of extract, as the two-row cultivation in 40 plants per m² compaction and the average rate of 0.36% produced the highest rate of extract, but the one-row cultivation with compactions of 80 and 100 bushes per m² and the average rates of 0.20 and 0.19%, respectively, had the lowest rate of essential oil production. The same rates of compactions in two-row cultivation allocated the average rates of 0.21 and 0.19 to itself.

Results, also, showed that purple basil produced the highest rate of essential oil in two-row cultivation in density of 40 plants per m² and the mean value of 0.44%. Hence, for the highest rate of essential oil of purple basil, the 2-row cultivation method and the density of 40 plants per m² is recommended.

Analysis of correlation coefficients showed that the rate of essential oil against yield of it has positive correlation in 1% level; it means that when the rate of essential oil increases, the yield also increases.

The two-row cultivation was placed in different statistical groups due to yield of the essential oil and by the average of 19.10 kg/hectare had significant priority compared with the one-row cultivation with the average values of 35.33 kg/hectare.

Comparison of the mean values showed that compactions of 40 and 60 bushes per m² with the mean amounts of 46.98 and 46.08 kg/hectare had significant priorities as compared to other compaction values. Also, the lowest yield of the essential oil was related to 100 plants / m² with the average of 24.49 kg per hectare (Table 2).

Table 2. Simple comparison of mean values for percentage and yield of essential oil in basil

Treatment	Mean of essential oil
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		Percentage	Yield (kg/hec)
Cultivar	Green	0.23 b	36.99 a
	Purple	0.29 a	37.98 a
Cultivation type	One-row	0.25 a	35.35 b
	Two-row	0.26 a	39.10 a
Density	40	0.34 a	46.98 a
	60	0.32 a	46.08 a
	80	0.21 b	31.14 b
	100	0.16 c	24.49 c

Table 3. Comparison of mean values of triple cross effects of percentage and yield of essential oil in basil

Cultivar	Cultivation type	Density	Mean	
			Percentage	yield
Green	One-row	40	0.20 fg	29.40 d
		60	0.18 g	29.21 d
		80	0.17 g	27.72 d
		100	0.17 g	27.88 d
	Two-row	40	0.31 de	48.20 abc
		60	0.30 de	47.19 abc
		80	0.29 de	47.03 abc
		100	0.27 de	45.90 abc
Purple	One-row	40	0.35 bcd	42.52 abc
		60	0.31 cde	38.03 bc
		80	0.30 de	37.59 bc
		100	0.26 ef	34.39 c
	Two-row	40	0.44 a	54.56 a
		60	0.42 ab	53.13 ab
		80	0.40 ab	51.84 ab
		100	0.38 abc	52.06 ab

Green basil in two-row cultivation with mean value of 42.40 kg/hectare was better than one-row cultivation with the mean value of 31.70 kg/hectare. Purple basil in two-row cultivation with mean value of 45.47 kg/hectare had the highest rate of yield for the essential oil and both cultivars of green and purple basil plants in one-row cultivation with the mean value of 31.70 and 32.29 kg/hectare allocated the lowest rate of yield for the essential oil to itself. Purple basil had the highest rate of production in the compaction with 40 bushes per square meter with the average amount of 45.54 kg per each hectare. Also, green and purple basil with the compaction of 100 bushes in an m² produced the lowest yield of the essential oil by the mean values of 32.14 and 26.67 kg per each hectare.

The type of two-row cultivation and density of 40 plant/m² with the mean value of 48.67 kg/hectare had the highest essential oil yield.

Moreover, the one-row cultivation with 80 and 100 plants density in every m² had the lowest yield for the essential oil in both types of cultivation.

Results showed that the cultivar of purple basil had highest rate of production in two-row cultivation in comparison with 40 bushes per m² with the average

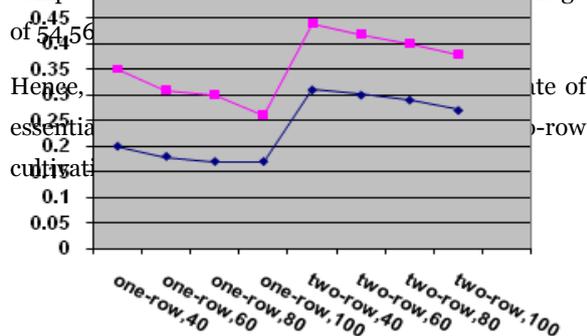


Fig. 1. Comparison of means in triple cross effects about essential oil percentage on two cultivars of basil by two cultivation methods and different plant densities

Discussion

The effective factor in the yield of basil plant is the proper density of plants in unit area. Increased density of higher than a suitable rate increases the length of the plant and competition between the plants for absorbing light increases to cause the growth in their height. The growth in height decreases the seed yield and provides problems such as verse. Reduction in competition results in reducing yield. Reduction in competition between plants with applying proper cultivation model causes the plant to access the existing condition in soil and in such conditions, adequate water and food will be reached to the plants and due to adequate amount of light, maximum absorption of it will be obtained to provide optimum yield (Malekouti and Balali, 2004). To obtain the highest rate and yield of essential oil in basil, studies have been done in this research for the best compaction of the plant.

Literature review showed that no research had been so far published for comparing the green and purple

cultivars of basil. To the best of our knowledge, this research is the first study.

One-row or two-row cultivation of basil has also been studied and reported insufficiently. But, recently the two-row cultivation is emphasized in some references (Rezaienejad *et al.*, 2001). Although the two-row cultivation is a new method in these plant species, but due to getting close to square cultivation and rapid cover of the interval between cultivation rows and reducing the evaporation rate and hence increasing the efficiency in water consumption, this method is better than one-row cultivation. Moreover, the two-row cultivation increases the efficiency in using light. The two-row cultivation reduces the competition between plants and optimizes the use of resources. Also the farming activities and mechanization facilitates it (Drazic & Pavlovic, 2005). In this research, the two-row cultivation, with low density showed most of the effects in yield of essential oil.

The obtained results showed that plant density has significant effect on the percentage and yield of the basil essential oil. The density factor becomes significant on the percentage of the essential oil at 1% level and also becomes significant on the yield of it at 5% level. These results are due to the fact that at low density, the competition between the plants reduces and hence, each plant has more space and produces more leaves. Therefore the rate of produced essential oil increases at low densities. Thus, by increasing the plant density the yield of essential oil increases. Moreover, it should not be neglected that increase in plant density will also affect the dry weight that, in turn, could affect the yield of essential oil. Also, the essential oil ratio of plants under extra light is more than the ratio for the plants of ordinary light and biosynthesis of the essential oil is highly dependent on light conditions. Hence, by increasing the rate of biosynthesis, production of the extract could in overall increase (Letchamo and Gosselin, 1995). In this research, the yield of essential oil has been achieved at low density (40 plants in m²) and the results could be interpreted. Arabaci and Bayram (2004) did also conclude in their 2-year experiment that although by the first year, the cultivation density

had no significant effect on the percentage of the extract, but the density is effective during the 2nd year. In a research on the effect of density on percentage and yield of the essential oil of coriander, Akbarinia and colleagues (2006) found that the density of 40 plants per each square meter provides the highest ratio of essential oil; while in reducing density from 50 bushes to 30 bushes in a square meter could increase the yield of the essential oil. Density of 30 bushes per square meter will have the highest yield of essential oil in coriander. In this research, by reducing the density from 100 to 40 bushes in a square meter, the yield of the essential oil increases. It is consistent with other researches.

In a research on basil plant, Dadvand and Reza (2006) stated that the cultivation compaction had significant effect in 1% level on the performance of dry matter and extract in a unit area. Although compaction has had no significant effect on the ratio of the extract, but it has caused the increase in the performance of the extract in a unit area, due to increasing in the performance of the dry matter. Also, nitrogen fertilizers have had significant effect at 1% level on all the considered parameters. Although using nitrogen fertilizer had negative effect on the ratio of essential oil, but up to 100 kg/hectare of nitrogen fertilizer increased the yield of essential oil and the dry matter in the unit area. This increase in the yield of essential oil was due to the increase in the yield of dry matter. In the present study, plant density had significant effects on the percentage and yield of the essential oil, such that by increasing the density, the percentage and yield of essential oil was reduced and the highest ratio is allocated the compaction with 40 bushes per square meter. Akbarinia *et al.* (2006) reported that at high plant density, they compete for absorbing the light with each other that leads to growth of the height of the plant. It is in conformity with the present study that plant height would increase by increasing the density from 40 to 100 plants per m².

In summary, the highest percentage of essential oil and its yield were obtained in purple basil by two-row cultivation with the density of 40 plants. It was in conformity with our findings and with regard to the

odor and hot tasting of basil, indicating the priority of purple basil. Cultivation of the purple basil on one-row and with the compaction of 40 bushes per m² requires more time for flowering, seeding and ripening period. Generally speaking, it was observed during the study that the growth of purple basil is slower than the green basil.

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