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Investigating the Effect of Bush Density on the Yield and Leaf Essence of Green Basil Landraces (*Ocimum Basilicum)*

Masumeh Soheila Atghaei* [1], Abazar Rajabi[2], Saeed Sadeghzadeh Hemayaty [2]

[1]*M.Sc in Medicinal Plants, Islamic Azad University, Jiroft Branch, Iran
atghaeimasoome@yahoo.com
[2]Assistant Professor (PhD), Sugar beet Seed Institute, Karaj, Iran

Abstract*: In order to investigate the effect of plant density on the essential oil content and yield of four basil landraces collected from Esfahan, Fars, Tehran and Khorasan, an experiment was conducted in Bam, Iran in 2011. The experiment was carried out as a split plot based on a Randomized Complete Block Design with four replications. Treatments included four plant densities (7, 9, 13 and 22 plants per m^2) as main plot factor and four basil landraces (Khorasan, Tehran, Esfahan, Fars) as subplot factor. The results indicated that Esfahan landrace at all plant densities, Fars landrace at the plant densities of 22 and 9 plants per m^2, Khorasan landrace at the plant densities of 22, 13 and 9 plants per m^2 and Tehran landrace at the plant density of 13 plants per m^2 had the highest essential oil yield. The landrace and plant density interaction was significant for leaf dry matter yield, essential oil content and essential oil yield. Esfahan landrace at the plant densities of 7 and 9 plants per m^2 and Khorasan landrace at the plant density of 7 plants per m^2 had the highest essential oil content (0.78%). The lowest essential oil content in Fars (0.45%), Esfahan (0.43%) and Tehran (0.41%) landraces was obtained at the plant density of 22 plants per m^2. In Esfahan landrace, the increased leaf dry weight at the higher plant densities was so high that compensated for the lower essential oil content and finally the essential oil yield at the all plant densities of this landrace gained the highest values (6.8-8.6 kg/ha).*

Keywords: *leaf dry weight, plant density, landrace*

1. INTRODUCTION

In fact, Permanent development is the creation of balance between development and environment. Since chemical materials have a destructive effect on human health and nature, minimizing these materials, is one of the purposes of permanent development. Side effects of chemical materials, environmental issues and gradual tend of tendency toward natural products caused a greater attention to herbal medicine. But lack of chemical medicine use in herbal medicine planting would be achieved while the yield of the influential material increases as far as an economic justification is acquired for the planting of herbal medicine. Therefore, every factor which can improve the quantity and quality of influential materials plays a role in permanent development. Green Basil (*Ocimum basilium*) a gramineous, annual and odorous from the family of labiatae (mint) has more than 60 varieties. It is planted in a wide area in France, America, Indonesia, Morocco, Spain, Egypt, Pakistan and North Africa (Omidbeigy,1375). This plant is planted in many places in Iran, and the most planted area was Fars, Isfahan, Kerman and Tehran provinces in 1386 (Shariat 1382). The use of this plant in fresh eating form, vegetables and secondly as spices and medicine exist (Omidbeigy, 1379). In a study the highest yield of Basil was acquired in rows of 15 cm (EL-Gendy et al., 2001). Also it is found that the highest amount of essence is acquired in rows of 30 cm (Gill, BS. & Band Hawa, GS., 2000). In another study it is reported that the highest yield of the wet matter was in 4197Kg/ha. The yield of the dry matter was (107 kg/ha.), the yield of the leaf (671 kg/ha.), the amount of the essence (0.826 percent of the dry matter of plant) and the yield of essence (5.164 kg/ha.) in planting pattern of 20*20 was acquired. (Arabic, D. & Bayram, E., 2004). In a study by planting distance of 25, 15, 35, 40 cm they reported that increasing the space caused an increase in branches, leaves, the weight of dry plants and decreases the height, also the highest amount of essence is acquired in a distance of 35 cm (EL-Gendy et al., 2001) in order to study the influence of different patterns of planting on the yield of 2 influential matter of the plant *Foeniculum Vulgar* Var. *Soroksary* an experiment was done in Karaj agriculture faculty in 2008. This study was based on complete random blocks plot in 3 repetitions and 5 bushes distance of 30, 25, 20, 15, 10 cm was studied. The distance between the rows in all treatments was 40. The influential matter was extracted from the seed. The highest amount of influential matter was 3/53 % which was acquired from the least density of the plants (the distance of

bushes on the row: 30cm) and the least amount of essence was 3/1 % which was acquired from the highest density (bushes distance on the rows is 10 cm), (Khorshidi, J. et al., 2009). the influence of density in the level of (20*50, 30*50, 40*50) and influence of nitrogen fertilizer in 4 levels on the *silybum marianum* showed that decreasing the density and increasing nitrogen and the gene fertilizer increased the diameter and the number of capitols in plant significantly. Also increasing nitrogen fertilizer and density has a positive relationship with the height of the plant and seed yield in unit. Although increasing density, causes a decrease in yield function in single bush. In this study parameters like the height of the plant, the number of capitols, the diameter of the main stem of each bush and seed function was calculated in hectare.

The effect of density on the height of *silybum marianum* was in a way that in the density of 40*50 cm2 (50000 bushes in hectare) had the least height statistically but two other densities did not have a significant difference. The highest yield of seed in unit in the density of 40*50 cm2 (50000 bushes in hectare) and was acquired 1023 kg/m2 (Gholibeygian et al., 1384) (Rahimmalek et al., 1386) reported that results of this study in the fifth gardening science conference in Iran on the important features of local herbal yarrow in Iran. The seeds of 20 samples of yarrow from 4 different species were gathered. These species were planted along with a sample from Holland in Isfahan industrial university. *Achillea bierstenii* sample from Lorestan province had the highest essence and leaf surface. In general a relationship was observed between the essence percentage and the leaf surface and the weight of dry and wet matter did not differ significant in the present study, the influence of density and mass on the yield and essence percentage of BASIL leaf in Bam region was studied in order to use the best mass in the best density for planting in the region for medical use.

2. MATERIALS AND PROCEDURE

The experiment was done in agriculture year of 1390. The experiment was carried out as a split plot based on a Randomized Complete Block Design with four replications. The density of the plant by four levels including 7, 9,13, 22 plants in squared metre was elected as main plot and of local Basil landraces from Tehran, Isfahan, and Khorasan was considered as secondary plot. Isfahan Research centre provided the seeds. Each secondary experimental unit included 5 planting row by the distance of 30 cm and the length of 2 meters. The distance of the blocks was 1 metre after planting, the farm was irrigated. Irrigation from planting to germinating has been done every alternative days and then once 3 years. Weeding was done manually, since the seeds were native. The seeds features were measured before planting. First 16 Petridish were selected and numbered, also the name of the seed was written on that. The bottom of the dishes was filled with washed soil up to 1-2 cm. Then it was covered by filter paper. In each Petri dish 100 Basil seeds were located on the filter paper and over them another paper was located. The surface of the pages become wet by wet piset and were located inside the germinator , therefore the growing power, germinating rate, the percentage of purity of seed and the percentage of live seeds capable of germinating, was calculated for each landrace separately. These features were analyzed in complete random blocks with 4 treatments in 4 replications. Harvesting in 1 phase was done using clipper. Based on that, while the flowering of plot reached 50 percent, harvesting was done. The level of harvesting including 3 intermediate rows was planted after omitting 25 cm from the beginning and end of every row. After harvesting leaf, stem and flower were separated. Drying the samples was done outdoors for 72 hours. Then a sample of each was located in Aven and after calculating the dry matter weight, the dry matter of rest of the plot was calculated. For reaching essence Celevenger model distiller was used. The volume of the essence of each sample was high and since in great volume, eye error decreases, the volume of gathered sample essence was calculated carefully. Then for sodium sulphate dewatering was used test tube with the essence was weighed and the percentage of essence in dry matter was calculated and regarding the dry matter percentage the yield of essence was acquired. For analysis of variance and calculating the mean of data SAS V. 8 software was used and for drawing the diagram excel program was used. Comparing the mean featured was performed using multi ranged Duncan.

3. RESULTS

Because of locating of the seeds, growth power, degree of purity of seeds, the percentage of live seed capable of germinating and the rate of germinating was determined. These features were analysed in complete random plot with 4 treatments and 4 replications. The results showed that different seed landraces had a significant difference in all mentioned features in probability level of 1%. (table1). Comparing the mean of laboratory features showed that considering the average speed of germinating, although the landraces were close together they were located in 2 groups in a way that 2 landraces of

Kerman and Isfahan were located in the high group and Fars and Tehran were located in low group. Considering growth power the investigated landraces were close together and the average growth power for all was more than 85%. The landraces were located in 2 groups. The landraces of Khorasan, Tehran and Fars composed the high group and the landrace of Isfahan composed the low group. The mean of seed purity in all landraces was more than 90% but statistically landraces of Tehran, Fars and Khorasan were located in the high group and Isfahan in lower group (table 3). Based on that, the planted seeds were planted for creating the final density in their germinating amount in a way that after placing the plants, the final density was executed by distributing.

The effect of local landrace on the length of stem become significant in probability level of 1% (table2). Comparing means showed that local Basil of Tehran, Isfahan and Fars were not different and had the highest bush height and local Basil of Khorasan which was significantly different from other 3 treatments, had the least plant height (table4). The effect of local landrace on the number of side branches in the plant become significant in probability level of 5% (table2).

Comparing means showed that the number of side branches in the plant in local landraces of Khorasan and Fars were the most and landraces in Tehran and Isfahan the least (table4). The effect of density on the leaf number of plants in probability level of 1 % becomes significant (table2). Comparing the means showed that densities of 13, 22 plants in squared meters had a fewer number of leaves in plant compared to the density of 9 and 7 (table5).

The mutual influence of density and local landrace on the area of leaf in probability level of 1% become significant (table2). Comparing means showed that Fars and Isfahan landraces in the density of 7 plants in squared metes did not differ significantly from Isfahan landraces by density of 9 plants and they had the greatest leaf area between treatments (table 6). Khorasan landrace by the density of 22 plants in squared meters had the least leaf area. Totally it can be said that in local landraces of Khorasan and Fars the leaf area decreased by increasing density significantly. In Isfahan mass also surface in density of 7 and 9 plants in squared meters were the same statistically and the leaf area for the density of 15 and 25 plants were not different. In Tehran leaf area was not different for densities of 7 and 9, but leaf area in 7 and 13 plants in squared meters was significantly different (table 6). The mutual influence of local landrace and density on essence percentage in probability of 1, was significant (table 2). Khorasan landraces (by density of 7 plants in squared meters) and Isfahan (by density of 789 plants in squared meters) did not have a significant difference, and had the highest amount of essence. After that there was Khorasan landrace (density of 9 plants in squared meters), which had a significant difference with the rest of treatments. Then the percentage of Fars essence (by density of 7 plants in squared meters) and Khorasan (by density of 13 plants in squared meters) was placed which did not differ, but the difference with the rest of the treatments was significant. Between treatment components (Tehran landrace by density of 7, 9 and 13) Fars mass (by density of 9, 13) Isfahan (by density of 13) did not have a significant difference (figure 5). In brief if each Basil local landrace would be studied separately, the results would be as follows: in Khorasan local mass, increasing density decreases essence percentage significantly. In Fars case, the same results were acquired, but essence percentage in density of 13, 22 plants in squared meters did not have a significant difference. Also the density of 22 had a significant difference with density of 7 and 9 regarding essence percentage. In local landrace of Isfahan, density of 7 and 9 did not have a significant difference and by increasing density, essence percentage decreased significantly. The mutual influence of density and local landrace on the yield of dry matter in probability level of 5 % was significant (table2). Comparing means showed that Fars, Isfahan and Khorasan landrace (in density of 22 bushes) and Tehran landrace (in density of 13 plants in squared meters) had the highest dry matter yields but these 4 treatments had not a significant difference. The least yield was related to Tehran, Khorasan, Fars and then Isfahan density of 7, although the difference in these densities was not significant. They are different from other treatments (figure 5). The mutual influence of density and local landrace on the yield of essence in probability level of 1 was significant (table2). Comparing means showed that the essence yield of Isfahan landrace in all densities, Fars mass in 22 and 9 and Khorasan in 22, 13, 9, Tehran in 13 plants /m2 had the highest essence yield, although the difference was not great (table 6). High essence yield can be the result of increase in essence percentage or increasing dry matter yield or both. The purpose of choosing better landrace of Basil herbal medicine with the best planting pattern was maintaining genetic (inherited) supply and also increasing quantity and quality of its production. Considering the influence of environmental factors, geographic

conditions and ecologic states of growth location on the quality and quantity of the plant, careful studies about the role of the above mentioned factors and their relationship with yield and the quality of plant is necessary. Therefore choosing the better mass is done regarding the ecologic needs in growth place and optimizing growth in agricultural ecosystem. The results of the study showed that Isfahan local landrace in all densities and Khorasan and Fars in some densities had a better compatibility with Bam region for planting. It could produce the highest essence yield and the highest amount of that.

Table1. *Analysis of variance*

Mean squares					
Variance source	Degrees of Freedom	speed of germinating	Germination capacity	degree of purity of seeds	percentage of live seeds capable of germinating
landrace	3	0.117**	50.563**	16.179**	0.011**
within	12	0.003	7.813	0.088	0.001
C.V		1.97	2.92	0.31	2.91

***significant in probability level of 0/01*

Table2. *Analysis of Variance for Land Features*

Mean squares								
Variance source	Degrees of Freedom	length of plant	the number of side branches in the plant	leaf number of plants	the yield of dry matter	essence percentage	leaf area	the yield of essence
replications	3	31.541n.s	126.678n.s	6195.244*	252874.656n.s	41.188**	1.586**	11.197 n.s
plant density	3	12.180n.s	52.918n.s	14008.444**	2439909.212**	1905.354**	28.576**	14.231 n.s
kha	9	10.193	80.458	1140.9	133626.376	4.146	0.133	4.218
landrace	3	97.426**	116.899*	5313.605n.s	67186.347n.s	859.688**	21.176**	7.626**
plant density .landrace	9	11.722n.s	16.116n.s	3874.975n.s	115607.759*	166.757**	1.438**	4.811**
khb	36	8.51	32.019	2789.48	40003.328	12.462	0.171	1.590
c.v		7.09	19.36	19.25	16.06	6.13	3.92	18.33

*n.s: not significant- *significant in the probability level of 0/05. **: significant in the probability level of 0/0!.*

Table3. *Analysis of Variance for Laboratory Features*

Treatments	speed of germination (day)	Germination capacity(percentage)	degree of purity of seeds(percentage)	percentage of live seeds capable of germinating
fars	2.49b	98.5a	96.83a	95.38a
khorasan	2.74a	97.25a	97.03a	94.36a
tehran	2.52b	96.5a	94.96b	91.64a
esfahan	2.84a	90.5b	92.70b	83.9b

Similar letters in each column shows no significant difference between means

Table4.comparing *means of some features in different herbal green basil landraces*

Treatments	length of plant	the number of side branches in the plant
Esfahan	42.27a	27.19b
Tehran	42.91a	26.91b
Khorasan	37.49b	32.61a
Fars	41.89a	30.2ab

Similar letters in each column shows no significant difference between means

Table5. *Comparing mean effect of different patterns of implanting on the number of leafs per bush*

Treatments	leaf number of plant
D_1	245.45b
D_2	253.5b
D_3	302.62a
D_4	292.1a

Similar letters in each column shows no significant difference between means

D_1: plant density one (22 plants per m^2)

D_2: plant density two (13 plants per m^2)

D_3: plant density three (9 plants per m^2)

D_4: plant density four (7 plants per m^2)

Table6. *Comparing the mean mutual effect of density and landrace on some herbal features of green basil*

Treatments	Leaf area	essence percentage	the yield of dry leaf	the yield of essence
D_1v_1	10.16def	43.25f	1822.40ab	7.93abc
D_1v_2	8.65g	41f	1532.90bcd	6.26bcd
D_1v_3	7.11h	48.25e	1600.40abc	7.79abc
D_1v_4	9.86f	45.75ef	1960.97a	8.96a
D_2v_1	10.56cde	50.75ed	1340.95cde	6.84abcd
D_2v_2	9.85f	50ed	1614.95abc	8.13abc
D_2v_3	9.17g	62.50c	1187.45defg	7.41abcd
D_2v_4	10.65cd	49.75ed	1139.50efg	5.80cde
D_3v_1	12.97a	77.75a	1107.25efg	8.57ab
D_3v_2	9.95ef	51ed	1131.90efg	5.88cde
D_3v_3	10.14edf	70.25b	1054.17efg	7.40abcd
D_3v_4	11.52b	54.25d	1276.02cdef	6.94abcd
D_4v_1	13.14a	78.50a	898.62fgh	7.02abcd
D_4v_2	10.52cde	55d	662.72h	3.66e
D_4v_3	11.12bc	78.25a	789.30gh	6.18bcd
D_4v_4	13.32a	65.25c	810.37gh	5.28de

Similar letters in each column shows no significant difference between means

D_1: plant density one(22 plants per m^2), D_2: plant density two(13 plants per m^2),D_3: : plant density three(9 plants per m^2), D_4: : plant density four(7 plants per m^2), V_1: Esfahan landrace, V_2: tehran landrace, V_3: khorasan landrace, V_4: fars landrace.

4. DISCUSSION

Effective factors in essence yield of Green Basil leaf are the yield of leaf dry matter and essence percentage of the leaf. Totally the process of change in leaf dry matter yield in various densities and landraces shows that by increasing density, the yield of leaf dry matter increase in all landraces (table 6). Dry leaf yield is one of the factors which influence leaf essence yield, and this confirms the results of Dadvand et al,. experiment (1387) who found that plant density has a significant effect on Basil essence yield in first harvest, second harvest and total yield in unit and by increasing density because of increase in dry matter yield in flat unit increased in a way that the most yield of essence in unit in first harvest 7/02, in the second 5/99 lit/ha. in density of 266666 plants /ha. and the least was in the first and second harvest respectively 4/19, 4/9 lit/ha. in density of 160000 plants /ha. Arabasi and Bayrami (2004) in *Ocimum basilicum*, Naghdi badi et al., (2004) in *Thymus vulganis*, Heydari et al., (1387) in peppermint (Labiatae) and Khorshidi et al., (2009) in *Foeniculum vulgare* in their experiments found that by increasing the density, dry matter yield increases, the result of the present study confirms these results about the effect of density on dry matter yield. It could be said that by increasing the distance between bushes, the growth of a single bush increases because of decreasing the competition for absorbing light and water and nutrition. But increasing growth due to that, could not compensate for increase in growing tissue resulting from the number of plants in unit, as a result in higher density the yield of dry matter increases. The next factor which can be effective on essence yield is the percentage of essence of the leaf. Isfahan landraces and Khorasan (density of 7) and Isfahan landrace (in density of 9) had the highest essence percentage (table 6). It was observed that by increasing density, the essence percentage in all landraces decreased, which is in agreement with EL-Gendy et al., (2001), Damato & Bianco (1994), and Arabasi & Bayram (2004). In lower densities the competition between bushes is less than higher densities. There is more opportunities for the growth of a single plants; therefore, in lower densities, more leaves are produced but an increase in leaf number cannot be a reason for an increase in essence percentage since essence percentage is dependant on morphologic features (caused by the genetic of each mass) and on the other hand

dependant on physiologic features of the leaf. One of the physiologic features is leaf surface. Based on previous researches by Drazic, Pavlovic (2005) and Hornok (1980) there is a relationship between leaf surface and the amount of essence, in a way that by increasing the distance between plants there will be higher space for single bush, the leaf surface and the number of secretory corks increases and consequently essence percentage increases (cited from Heydari et al., 1387); therefore, beside dry matter yield which can be effective on essence yield is essence percentage. Simon et al., (1990) reported essence percentage in various varieties of Green Basil as 0/53, 0/78, 0/83, 0/5, 0/62 percent which confirms essence percentage of Basil landraces in the present study. Also the results of the present study confirm the results of the experiment by Abdolrahman (2009) who reported essence as much as 0/33 to 0/47 percent. Considering the range of changes of essence percentage in Isfahan landrace from 0/43 to 0/78 percent, Fars landrace from 0/45 to 0/65 percent, Fars landrace from 0/45 to 0/65 percent, Khorasan 0/48 to 0/78 percent, Tehran landrace 0/47 to 0/55 percent, differed dependent on density it could be said that Isfahan and khorasan masses were the optimized essence regarding essence percentage. In fact executing appropriate planting pattern on Isfahan and Khorasan masses suitable Green Basil essence yield could be acquired. Since one of the most important purposes of odorous herbal plants planting is increasing essence yield with the best economic justification. And considering different patterns of planting for Isfahan mass had the same essence yield and in lower density, development of pests and illnesses and protection operation will be easier and less costly, and farm administrators will be done more carefully, thus for planting this landrace the least density is proposed (7 plants /m2). This issue is considered for planting all masses, therefore for Khorasan and Fars landraces the density of 9 and for Tehran 13 is proposed.

SUGGESTIONS

1. It is advised that the essence of unanalysed landraces and the components of that be determined.
2. It is proposed that density of more than 22 plants in squared meter in Isfahan landrace be evaluated, since the probability of increasing essence yield in higher densities exists.
3. In order to select and produce improved species with higher essence and yield, it is proposed that Isfahan and Khorasan landraces be used in improving programs.

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