Effects of Sowing Date, Plant Density and Nitrogen Fertilizer on Yield, Yield Components and Various Traits of *Calendula officinalis*

¹H.R. Ganjali, ²A. Ayeneh Band, ³H. Heidari Sharif Abad and ⁴M. Moussavi Nik

¹Islamic Azad University, Science and Research branch, Khuzestan, Iran
²Shahid Chamran University, Ahwaz, Iran
³Seed and Plant Improvement Institute, Karaj, Iran
⁴Zabol University, Zabol, Iran

Abstract: In order to investigate the effect of different levels of sowing date, plant density and nitrogen fertilizer on different growth and productive variables of *Calendula Officinalis*, an experiment was conducted using split plot factorial on the basis of randomized complete block design with three replications in Zaheden region during 2008-2009. Main plots were consisted of sowing date in 3 levels, 1th, 15th and 30th of April and sub plots included plant density in 3 levels (6×50, 8×50, 12×50 cm²) and amount of nitrogen fertilizer in 2 levels (50, 100 kg ha⁻¹). The variables which examined to consist of plant height, number of auxiliary branches, auxiliary branches length, length and width of leaves, peduncle length, auxiliary stem diameter, main stem diameter, flower diameter, wet flower weight, dry flower weight, number of flower, number of petal, Leaflet length, leaf number, plant weight, stem and root weight and amount of essence. The result of main effects of compound variance analysis including of year, sowing date, plant density and nitrogen fertilizer showed that sowing date on April 1st, (12×50) plant density and 50 kg ha⁻¹ of nitrogen fertilizer, had the most effect in increasing the traits. Also, the interaction effects of date × year, date × density, date ×N fertilizer, date × density × fertilizer, date × density ×N fertilizer × year, year ×density, year ×N fertilizer, density × N fertilizer × year, density × N fertilizer were significant at 5% probability level in all traits. In general, the result of study demonstrated that using of suitable sowing date together with density and amount of nitrogen fertilizer increase yield, yield components and essence of calendula.

Key words: *Calendula Officinalis* · Sowing date · Plant density · Nitrogen fertilizer

INTRODUCTION

In order to decrease harmful effects of chemical medicines, using herbal medicine has been increased in recent years [1,2]. In past, herbal medicine collected from nature for shopping in market was enough, but a number of common species are in danger of extinction because of increasing the request of consuming and more using of natural resources. Extinction of natural species, great request of market and simple obtaining to the herbal sources were a number of reasons developed the cultivation of herbal medicine. However, low yield of medicinal plants persuaded researchers to increase yield. The best and economic way is to achieve the performances in order to increasing yield, using the proper cultivars and variety,

best sowing date, optimum plant density and etc. [3]. Statistics of recent years shows that despite increasing in chemical medicine, producing and using of herbal medicine increases in the world. As, one-third of medicines originate from herbal medicine and it continuously increasing [4,5]. According to the report of global bank, marketing value of herbal medicine will reach to 5 billion dollars by 2050 [5].

Calendula Officinalis L. is an oil plant, annuals, the origin is the west of Asia and Mediterranean and many times cultivates as an ornamental plant before its medicine properties known as an herbal medicine. The plant was first cultivated as an herbal medicine in Europe in 17th century and now there is in Germany, Czech Republic, Slovakia, Austria and Switzer Land, Hungary and recently in Egypt and Syria [1,6].

Corresponding Author: H.R. Ganjali, Department of Agriculture, Islamic Azad University, Science and Research Branch, Khuzestan, Iran, Tel: +09151415572, E-mail: hrganjali@yahoo.com.

Calendula Officinalis use in medical (treating gastric and intestinal disease coetaneous wounds and an anti inflammation medicine), cosmetic, in various creams and nutritional in coloring the foods like cheese and butter. Also, the oil extracted from the seeds has industrial and pharmaceutical application [7-9]. Recently, some evidences have been discovered the positive effects of its essence on HIV [10].

Plant density and nitrogen fertilizer are two effective factors to canopy establishment and also they play an important role in light absorption, resulted in increasing more qualitative and quantitative production [11].

Regarding the limitations to obtain the optimum sowing date and nitrogen application rates in medicinal plants one of the priorities to achieve sustainable agriculture systems is determination of best sowing date and nitrogen rates [12]. Moreover, using fertilizers, especially nitrogen, may increase the crop yield but also may decrease the effectiveness of the medicinal plants or change the composition of the effective substances. Therefore, consider to qualitative and quantitative properties in medicinal plant is important than the other crops [13]. As, in recent years great attention has been drawn to improve the effectiveness of these plants through increasing their essences [14,15].

The aime of this study is to evaluate (i) the effects sowing dates and nitrogen fertilization on different growth and productive characteristics of *Calendula officinalis* (ii) effective substances of the flower including the essence (iii) and the extract of the plant in three phases of budding including: beginning, middle and the end of the budding (90, 150 and 210 days after cultivation, respectively).

MATERIALS AND METHODS

The study was carried out on Sistan Agricultural Research Center, Iran (61ù 41ˣ E, 30ú 54ˣN) during 2008-2009. Results of climate and soil characteristics of the field experiment have been shown in Tables 1 and 2, respectively. Main plots were consisted of sowing date in 3 levels, 1th,15th and 30th of April and sub plots included plant density in three levels (6×50, 8×50, 12×50 cm²) and amount of nitrogen fertilizer in two levels (50, 100 kg ha⁻¹) as factorial with three replication. Plots were plowed before sowing date and disked before planting. Plots were sown with a cone seeder and were 4 m long and 2 m wide, with 4 rows 0.5 m apart. Weeds were controlled mechanically.

Samples were gathered from the middle rows of each plot and 10 plants selected randomly. Amount of effective substances of 200 grams of fresh flower including the essence and the extract of the plant in 3 phases of budding (beginning, middle and the end of the budding, 90, 150 and 210 days after planting) collected and sent to the laboratory. Samples were dried in a forced-air oven at 40° C for 72 h. The essence of the samples extracted using the method of vapor distilling with a "clevenger" and the extract obtained using 70% ethanol.

Data were analyzed by analysis of variance [16]. When significant differences were found (*P=0.05*) among means, Duncan's multiple range test (DMRT) were applied.

Table 1: Monthly average temperature, relative humidity and wind speed recorded at Agricultural Climatology Research Station of Zahak during 2008 and 2009

Year	Soil (cm)	PH	EC (mmhos cm⁻¹)	N (%)	P (ppm)	K (ppm)	Sand	Silt	Clay
2008	0-30	8	7.8	0.053	7.8	190	63	20	17
2009	0-30	8	7.75	0.061	7.83	185	63	20	17

Table 2: Soil characteristics of the experimental location at 2008 and 2009

Year		2008	
Month	Temperature	Relative humidity %	Wind speed.m.sec⁻¹
April	24.2	50.2	4.5
May	34.3	42.5	3.3
June	34.7	30.2	7.2
July	37.2	24	8.5
August	42.3	24.1	10.2
Year		2009	
Month	Temperature	Relative humidity%	Wind speedm.sec⁻¹
April	22.3	50.3	4.1
May	29.1	46.7	3.3
June	31.4	35.4	6.7
July	37.42	24.4	8.5
August	45.6	20.1	10.3

RESULTS AND DISCUSSION

The results of analysis of compound variance showed that there is significant difference between years, plant height, auxiliary branch length, leaf width, auxiliary stem diameter, flower diameter, dry flower weight, flower number and leaflet length at 5% and 1% probability level (Table 3). Mean comparison of different traits was also shown in table 4, 5 and 6. Mean comparison showed that the traits of plant height, auxiliary branch length, auxiliary stem diameter, flower diameter, dry flower weight and leaflet length was more than in second year compared to the first year. This could be resulted from the difference in environmental condition of the second year cause to increasing the average production of these traits in *Calendula officinalis.*

Analysis of compound variance of sowing date in Table 3 showed that there are significant differences between the treatment in number of auxiliary branch, auxiliary branch length, leaf width, peduncle length, auxiliary stem diameter, flower number, stem and root weight, leaf let length, leaf number and leaf weight. Results in Table 4 showed that sowing date of 1st April and 15th April resulted in an increase branch number, leaf weight, root and stem weight, auxiliary stem diameter, flower number, petal number and leaf number. Results of Boroumand Reza Zadeh [17] on herbal medicine of "Ammi" confirmed the effect of sowing date on above traits. They indicated that sowing of herbal medicine in proper date causes the plant entirely finishes its growth period but delay in sowing date decreased yield and essence.

Table 3: Results of analysis of variance combined across years, sowing date, plant density and nitrogen fertilizer.

S.O.V	df	Stem and root weight	Leaf weight	Leaf number	Leaflet length	Petal number	Wet flower number	Dry flower weight	Wet flower weight
Rep(R)	2	0.5ns	9.58ns	0.96	0.01ns	50.45ns	1858.ns	20.76ns	1421.ns
Years(Y)	1	0.034	0.48	4.98	0.14**	1.12	71.74**	4.24**	12.56
R×Y	2	0.06	52.54	20.38	0.05*	454.3	7.45	0.76	36.62
Sowing date (S)	2	0.14*	4.62	8.89	0.10**	714.8	437.6**	13.23**	130.7**
P×Y	2	0.004**	1.46	0.24	0.006	2.4**	0.005	0.074	0.31
error	8	0.087	2.25	8.12	0.009	445.4	87.64	3.91	56.4
Density(D)	2	0.13**	3.72	21.68**	0.015	386.**	87.59	0.31	63.63**
D×Y	2	0.005**	6.68*	0.42**	0.03	5.62	0.87	0.01	1.71
D×S	4	0.05**	9.67**	36.26**	0.10**	94.69	294**	3.45*	99.13*
D×S×Y	4	0.003	1.7	0.71	0.009	2.52	0.37	0.1	0.6
Nitrogen (N)	1	0.018	0.21	21.69**	0.19**	163.**	45.37	0.14	29.62
N×Y	1	0.001	1.51	1.33	0.001	2.08	0.29	0.16	0.95
N×S	2	0.069*	4.16*	1.05	0.051	106.8*	230.87*	4.17**	81.32*
N×S×Y	2	0.003	0.73	1.23	0.012	4.11	1.61	0.02	0.12
F×D	2	0. 5**	6.07**	19.52*	0.02	37.8	174.67*	7.57**	106.96*
N×D×Y	2	0.001	0.27	0.38	0.008	6.81	0.21	0.06	0.027
N×D×S	4	0.096*	4.14*	7.81	0.028	316**	12.75	3.49*	95.68**
N×D×S×Y	4	0.001	0.82	0.4	0.005	6.26	0.1	0.11	0.345
Error	60	0.032	2.06	9.49	0.039	66.95	95.33	1.25	36.73

Diameter flower	Main stem diameter	Auxiliary stem diameter	Peduncle length	Leaf width	Leaf length	Auxiliary branches length	Auxiliary branches number	Plant high	Essence
4.75ns	0.95 ns	0.17 ns	29.02 ns	18.4 ns	242. ns	52.94 ns	48.19 ns	124.9 ns	ns2×10-5
2.37**	0.05	0.024**	0.51	0.19**	2.69	5.55**	2.167	27.04**	*1.2×10-4
1.87	0.1	0.25*	1.52	0.08	2.75	1.26	341.4	0.23	4.6×10-4
0.56**	0.15	0.1*	1.839*	0.12**	1.85	7.09*	360.23*	15.94	*3.7×10-6
0.05	0.01*	0.01	0.045	0.007	0.13	0.05	51.68	0.45**	3.4×10-6
0.07	0.21	0.027	1.35	0.08	2.92	6.73	237.45	16.76	9.6×10-7
0.67**	0.14**	0.09	**4.98	0.067	15.83	12.69**	**422.35	1.5	*1.8×10-5
0.3	0.01	0.02	0.029	0.03**	0.488	0.103	24.73	0.759	*1.2×10-6
0.67**	0.18**	0.07	2.52**	0.18**	12.1**	5.94**	60.88**	4.91	*2.6×10-6
0.01	0.003	0.01	0.015	0.043	0.109	0.32	28.74	0.74	9.1×10-7
1.56	0.01	**0.09	0.17	0.001*	0.31	5.74**	8.05	3.529	*1×10-5
0.68	0.005	0.018	0.039	0.001	0.46	0.089	0.078	0	1.1×10-5
0.05	0.08	0.4**	0.79	0.17**	7.23**	58.26**	34.62	3.64	*2.9×10-6
0.2	0.06	0.024	0.023	0.001	0.08	0.66	0.54	0.377	2.3×10-5
0.13	0.03	0.11*	0.14	0.20**	14.0**	6.31*	92.7	2.659	*5.6×10-5
0.09	0.01	0.011	0.004	0.02	0.11	0.96	2	0.013	3.4×10-7
0.081	0.10*	0.16**	0.578	0.14**	6.60**	3.06	382.93*	12.04*	*2.6×10-6
0.098	0.003	0.008	0.018	0.014	0.089	0.44	5.58	0.359	4.8×10-6
0.21	0.05	0.05	0.842	0.086	2.24	3.33	171.5	6.228	1.2×10-7

*, ** Significant at 0.05 and 0.01 probability levels, respectively. NS=non significant at p> 0.05

Table 4: Mean comparison of main effects on Calendula to sowing dates, plant densities and nitrogen fertilizer during 2006-2008.

Main Effect	Stem and root weight (gr)	Leaf weight (gr)	Leaf number	Leaflet length (cm)	Petal number	Wet flowers number	Dry flowers weight (gr)	Wet flowers weight (gr)	Diameter flower (cm)
Sowing date	**	n.s	n.s	**	n.s	**	**	**	**
1-Apr	0.72a	5.25	10.89	1.67a	36.69	59.18a	6.81a	27.29a	2.93a
15-Apr	0.59b	4.53	11.88	1.50b	41.69	58.77a	6.76a	25.14ab	2.66b
30-Apr	0.67a	4.83	11.45	1.61a	32.8	53.36b	6.22b	24.30b	2.90a
Plant density	**	n.s	**	n.s	**	n.s	n.s	**	**
6*50	0.59b	4.62	11.68a	1.58	33.77b	55.74	6.54	25.73a	2.65b
8*50	0.68a	4.75	10.53b	1.54	37.08ab	54.22	6.48	23.17b	2.83ab
12*50	0.71a	5.23	12.01a	1.57	40.33a	57.34	6.66	23.8b	2.91a
N Fertilize	n.s	n.s	**	**	**	n.s	n.s	n.s	n.s
50kg	0.65	4.91	11.85a	1.52b	38.29b	56.42	6.6	24.77	2.68
100kg	0.67	4.83	10.96b	2.1a	45.83a	55.12	6.53	23.72	2.92
Years	n.s	**	**	**	n.s	**	**	n.s	**
2008	0.64	4.94	11.19	1.52b	36.96	54.95b	6.36b	23.9	2.65b
2009	0.68	4.8	11.62	1.6a	37.16	56.58a	6.76a	24.59	2.94a

Stem diameter (cm)	Auxiliary Stem diameter (cm)	Peduncle length (cm)	Leaf width (cm)	Leaf length (cm)	Auxiliary branches length (cm)	Auxiliary branches number (cm)	Plant high (mg)	Essence
**	n.s	n.s	**	n.s	**	**	n.s	*
1.80a	0.37	2.77	1.89a	8.92	7.79a	52.94a	24.72	0.0033a
0.91b	0.36	2.04	1.80b	8.47	7.06b	46.61ab	23.43	0.0028ab
0.91b	0.26	2.22	1.78b	8.64	7.94a	49.65b	24.37	0.002b
**	n.s	**	**	**	**	**	n.s	*
0.86b	0.27	1.90b	1.87	8.48	7.89a	47.91b	24	0.0022b
0.91a	0.27	1.70b	1.806	9.42	6.78b	47.60b	24.13	0.0025b
0.92a	0.36	2.42a	1.803	8.14	7.72a	53.68a	24.39	0.0039a
n.s	**	n.s	n.s	n.s	**	n.s	n.s	*
0.86	0.93a	1.97	1.82	8.63	8.19a	49.46	24	0.0034a
0.89	0.27b	2.054	1.83	8.73	7.23b	50.01	24.35	0.0032b
n.s	**	n.s	**	n.s	**	n.s	**	*
0.857	0.28b	1.94	1.78b	8.52	7.24b	49.59	23.67b	0.0026b
0.9	0.31a	2.08	1.87a	8.84	7.69a	49.88	24.67a	0.0031a

Means with similar letters in each column are not significantly different at 5 % level of probability. (Duncan)

Table 5: Mean comparison of interaction effects between sowing date × plant density, sowing date × nitrogen fertilizer and plant density × nitrogen fertilizer on Calendula during 2006-2008.

Treatment		Stem and root weight(gr)	Leaf weight(gr)	Leaf number	Leaflet length(cm)	Petal number	Wet flower number	Dry flower weight(gr)
Sowing date	Plant density	**	**	**	**	n.s	**	**
1April	6*50	bc0.61	bc4.35	bc10.41	abc1.567	31.75	cde52.40	A6.75
1April	8*50	a0.80	a6.02	abc11.50	ab1.65	35.91	de51.81	Ab6.55
1April	12*50	ab0.74	ab5.37	14.75a	1.8ab	42.41	b58.3	a6.82
15Apri	6*50	c0.52	bc4.39	abc11.48	ab1.592	37.75	b57.58	Ab6.24
15Apri	8*50	bc0.55	c3.55	c9.80	c1.408	44.5	bcd56.45	6.48ab
15Apri	12*50	ab0.71	ab5.66	a14.36	bc1.525	42.83	a65.27	6.47ab
30April	6*50	abc0.65	ab5.15	ab13.14	ab1.83	31.83	bc57.25	6.5ab
30April	8*50	ab0.69	bc4.68	bc10.29	abc1.567	30.83	bcd54.40	6.42ab
30April	12*50	ab0.67	5.08ab	bc10.92	a1.692	35.75	e48.43	5.7b
Sowing date	N Fertilizer	**	**	n.s	n.s	**	**	**
1April	50	b0.66	ab5.03	11.46	1.49	43.4a	60.5a	6.78ab
1April	100	a0.77	a5.46	10.32	1.65	43.3a	59.4a	6.63ab
15April	50	b0.60	ab4.96	12.40	1.45	a42.88	a59.37	6.72ab
15April	100	b0.58	b4.10	11.36	1.56	a40.5	a60.17	6.81a
30April	50	b0.67	ab4.75	11.70	1.61	b32.33	b54.72	6.2ab
30April	100	b0.66	ab4.92	11.2	1.61	b33.27	b52.01	6.14b
Plant density	N Fertilizer	**	**	**	n.s	n.s	**	**
6*50	50	ab0.62	ab5.14	a12.86	1.550	35.83	a58.34	6.60ab
6*50	100	b0.56	b4.11	b10.49	1.611	31.72	53.55b	6.4ab
8*50	50	0.65ab	ab4.62	ab10.76	1.47	37.16	ab55.31	6.93ab
8*50	100	a0.72	ab4.88	b10.30	1.611	27	b53.13	6.03b
12*50	50	ab0.68	5.35a	12.01ab	1.54	41.88	57.04a	6.21ab
12*50	100	a0.74	a5.48	ab12.08	1.60	38.77	a59.08	7.12a

Table 5: Continued

Wet flower weight (gr)	Flower diameter (cm)	Stem diameter (cm)	Auxiliary stem diameter (cm)	Peduncle length (cm)	Leaf width (cm)	Leaf length (cm)	Branches length (cm)	Branches number	Plant high (cm)	Essence (mg)
**	**	**	n.s	**	**	**	**	**	n.s	*
Ab24.67	b2.50	c0.64	0.28	ab2	a2.03	ab9.2	ab6.99	b49.06	24.5	0.002ab
Ab24.18	a3.09	0.75bc	0.26	ab1.72	ab1.79	ab9.27	ab7.05	b45.17	24.45	0.002ab
25.3a	ab2.90	a1.00	0.26	b1.59	2.12a	11a	9a8.8	a64.58	25.67	0.0025a
A26.28	b2.50	abc0.86	0.27	ab1.86	ab1.75	bc7.99	ab7.79	b46.48	23.7	0.002a
Ab23.93	ab2.76	a1.00	0.29	b1.6	ab1.93	a10.36	b6.47	b50.75	23.16	0.001b
A25.21	ab2.71	abc0.87	0.52	a2.66	b1.71	9.07ab	a6.93	b42.60	23.43	0.0022a
a26.25	ab2.94	ab0.90	0.25	ab1.84	ab1.84	bc8.25	a8.95	b48.20	24.26	0.001b
b21.40	ab2.64	ab0.96	0.25	ab1.80	b1.68	bc8.63	b6.8	b46.88	24.79	0.002b
a25.25	a3.12	ab0.88	0.29	a3.02	ab1.83	ab9.058	ab8.05	ab53.88	24.05	0.002a
**	n.s	**	**	n.s	**	**	**	n.s	n.s	
28.7a	2.67	bc0.81	0.47a	1.75	ab1.85	b8.38	a8.78	51.87	24.65	0.002b
28.4a	3	0.90a	0.4a	1.79	a1.94	a9.46	8.70a	54.01	24.8	0.0024a
abc23.93	2.57	bc0.84	a0.43	1.846	ab1.76	ab8.50	cd7.01	46.04	22.9	0.002b
ab26.35	2.75	a0.98	b0.29	2.23	ab1.84	ab8.5	bcd7.12	47.88	23.96	0.002b
a27.36	2.79	ab0.93	b0.28	2.32	ab1.86	ab8.99	ab8.00	50.47	24.45	0.001b
c21.24	3.01	abc0.90	b0.28	2.12	b1.70	ab8.3	abc7.88	48.83	24.28	0.002b
**	n.s	n.s	**	n.s	**	**	**	n.s	n.s	
a25.41	2.52	0.76	ab0.27	1.84	1.76ab	ab8.94	a7.93	47.78	24.01	0.0026a
a26.06	2.77	0.85	b0.26	1.96	ab1.79	b8.017	a7.84	48.05	24	0.001b
a25.68	2.77	0.92	b0.26	1.73	b1.74	b8.67	ab7.46	45.66	23.65	0.002ab
b20.66	2.88	0.89	b0.26	1.67	ab1.86	a10.17	b6.11	49.55	24.62	0.002ab
26.1a	2.73	0.91	0.38a	2.33	1.97a	9.2a	8.1a	54.94	24.34	0.002ab
a24.44	3.09	0.93	a0.45	2.52	ab1.82	10.2a	7.98a	52.43	24.43	0.002ab

Means with similar letters in each column are not significantly different at 5 % level of probability. (Duncan)

Table 6: Mean comparison of interaction effects between sowing date × plant density × nitrogen fertilizer on Calendula during 2006-2008.

Sowing date	Plant density	N Fertilizer	Stem and root weight (gr)	Leaf weight (gr)	Leaf number	Leaflet length (cm)	Petal number	Wet flower number (gr)	Dry flower weight (gr)	Wet flower weight (gr)
	Treatment		**	**	n.s	n.s	**	n.s	**	**
1-Apr	6*50	50	0.65bc	5.4abc	12.53	1.45	29.33e	54.26	6.46abc	21.95bcde
1-Apr	6*50	100	0.57c	3.2cd	10.16	1.68	34.16cde	50.53	7.05ab	27.4ab
1-Apr	8*50	50	0.74bc	5.1abc	10.4	1.58	41abcde	53.88	7.3ab	27.25ab
1-Apr	8*50	100	0.87a	6.95a	10.27	1.71	31.5de	49.75	5.8bc	29.12bcde
1-Apr	12*50	50	0.71b	6.5ab	11.66	1.45	49.3a	57.36	7.6ab	20.8bcde
1-Apr	12*50	100	0.88a	6.18ab	12.13	1.55	45.5abc	59.3	7.05ab	26.8abc
15-Apr	6*50	50	0.49c	5abcd	13.2	1.58	45abc	58.96	6.58abc	26.7abc
15-Apr	6*50	100	0.55c	4bcd	10.82	1.6	30.5de	56.2	6.1bc	25.8ab
15-Apr	8*50	50	0.57c	4bcd	11.13	1.33	41abcde	56.73	6.65abc	25.2cd
15-Apr	8*50	100	0.53c	3.1d	10.32	1.48	47.8ab	56.18	6.31abc	22.66abcd
15-Apr	12*50	50	0.75b	6.11ab	12.22	1.45	42.5abcd	62.41	6.93ab	19.8bcde
15-Apr	12*50	100	0.67bc	5abcd	12.02	1.6	43abcde	68.13	8.017a	30.56de
30-Apr	6*50	50	0.72b	5abcd	13.7	1.61	33.16cde	61.8	6.95ab	27.55a
30-Apr	6*50	100	0.57c	5abcd	12.58	1.55	30.5de	51.71	6.11bc	24.95ab
30-Apr	8*50	50	0.62bc	4abcd	9.9	1.5	30de	55.33	6.85abc	24.6abcd
30-Apr	8*50	100	0.76b	4bcd	10.68	1.63	31.66de	5.48	6bc	18.21e
30-Apr	12*50	50	0.68bc	4bcd	11.51	1.73	33.83cde	47.03	5.10c	29.93a
30-Apr	12*50	100	0.66bc	5abcd	10.33	1.65	37abcde	49.88	6.13bc	20.56cde

Table 6: Continued

Flower diameter (cm)	Main stem diameter (cm)	Auxiliary stem diameter (cm)	Peduncle length (cm)	Leaf width (cm)	Leaf length (cm)	Auxiliary branches length (cm)	Auxiliary branches number	Plant high (cm)	Essence (mg)
n.s	**	n.s	n.s	**	**	n.s	**	**	*
2.3	0.55c	0.26c	2.1	2.12a	8.96bcd	7.18	44.23cd	abcd24.40	0.002ab
2.71	0.73bc	0.3bc	1.91	1.95ab	9.43abc	6.68	53.9abcd	abcd23.70	0.001b
2.96	0.75bc	0.26c	1.61	1.75ab	7.988cd	7.9	45.46cd	abcd23.86	0.002ab
3.21	0.76bc	0.25c	1.83	1.84ab	10.56ab	6.21	44.88cd	abcd25.03	0.001b
2.75	1.13a	0.38a	1.53	1.68ab	8.21bcd	9.15	65.91a	a25.68	0.002ab
3.06	0.98abc	0.29bc	1.65	2.03ab	8.39bcd	7.21	63.25ab	a25.66	0.002ab
2.5	0.81abc	0.26c	1.67	1.86ab	9.16bcd	7.51	46.3cd	d22.43	0.002ab
2.5	0.91abc	0.28c	2.05	1.65b	6.81d	8.06	46.67cd	abc24.98	0.001b
2.71	1.01ab	0.26c	1.66	1.78ab	8.96bcd	6.81	49.7bcd	d22.26	0.001b
2.81	1.03ab	0.31bc	1.53	2.08ab	11.76a	6.13	51.76abcd	abcd24.06	0.001b
2.5	0.71bc	0.36a	2.2	1.65ab	7.38cd	6.7	42.08cd	abcd24.02	0.001b
2.93	1.03ab	0.28c	3.13	1.78ab	6.76d	7.167	43.13cd	cd22.85	0.003a
2.76	0.91abc	0.26c	1.76	1.9ab	8.7bcd	9.11	52.83bcd	25.21ab	0.001b
3.11	0.90abc	0.25c	1.92	1.78ab	7.8cd	8.78	43.58cd	23.31bcd	0.002ab
2.56	1.01ab	0.26c	1.93	1.70ab	9.08bcd	7.66	41.76bcd	24.81abc	0.002ab
2.63	0.91abc	0.25c	1.66	1.66ab	8.18bcd	6	52bcd	24.76abc	0.002b
2.96	0.88abc	0.33b	3.26	2ab	9.2bcd	7.23	56.8bcd	23.33bcd	0.001b
3.28	0.88abc	0.25c	2.78	1.66ab	8.91bcd	8.86	50.91bcd	24.78abc	12*50

Means with similar letters in each column are not significantly different at 5 % level of probability. (Duncan).

Results of analysis of variance shown in Table 3 also showed that the significant differences at 5% and 1% level between plant densities used in this experiment. These traits were auxiliary branch number, auxiliary branch length, length of peduncle, auxiliary stem diameter, main stem diameter, flower diameter, stem and root weight, flower weight, number of leaf and leaf weight. 12×50 plant density had the most effect on *Calendula officinalis,* increased auxiliary branch number, peduncle length, auxiliary stem diameter, main stem diameter, leaf and root weight, flower diameter, petal number and leaf number. Also 8×50 plant density had better effect compared to 6×50. Results from Rezazadeh [17], Naqdibadi *et al.,* [18] and Pala-Paul *et al.,* [6] Showed that the best density for *Calendula officinalis* was 10×50.

Nitrogen fertilizer had significant effect on auxiliary branch length, auxiliary stem diameter, petal number, leaflet length and leaf number (Table 3). Niyakan *et al.,* [19] reported that nitrogen application affects on wet and dry flower weight, leaf surface and amount of essence in *Calendula officinalis.* Application of 50 kg N ha^{-1} increased auxiliary branch length, auxiliary branch diameter, leaf number while application of 100 kg N ha^{-1} increased petal number and leaflet length.

Interaction effects of year and sowing date in plant height, main stem diameter, stem and root weight and petal number. There is a significant difference for the traits of auxiliary branch length, leaf with, auxiliary stem diameter, main stem diameter, wet flower weight, stem and root weight, petal number and leaflet length in interaction of sowing dates and plant density. The results reported by Sinclair and Horie [20] showed that interaction effects of sowing dates in different plant densities were significant.

1st April at 12×50 plant density produced the highest values in the most different traits, especially amount of essence. Boroumand Reza Zadeh [17], Franz *et al.,* [21] and Moodi [2] showed that different sowing date with different plant density had different effects on the traits. In other words, using the optimum sowing date at high density produced the most essence in other herbal medicine.

Sowing date in April 1st and application of each nitrogen fertilizer caused increase in auxiliary branch length, leaf length, leaf width, flower diameter, root and stem weight, petal number and leaflet length (Table 5). According to Fariborzi [22] and Albadavi *et al.,* [23] different sowing date and nitrogen application increased growth and developed aspect of blossoming in *Calendula officinalis.*

The results in Table 5 showed that using 8×50 and 12×50 density with 50 and 100 kg N per ha increased

flower number, wet flower weight, leaf number, leaf weight, leaf length and width and auxiliary stem diameter. Moodi [2], Cromack and Smith [24] and Vos and Putin [25] also showed increasing of herbal density and nitrogen application increased leaf surface and leaf number in *Calendula officinalis.*

There are significant differences in interaction between sowing date×plant density x nitrogen fertilizer for plant height, branch number, leaf length, leaf width, main stem diameter, stem and root weight, wet flower weight, dry flower weight and petal number (Table 3). At April 1 and 15 sowing dates with 12×50 plant density and 50 or 100 kg N ha⁻¹ were the best combination affected of morphologic and physiologic characteristic of *Calendula officinalis* (Table 6). Similar results were reported by [22, 23, 26].

The result of compound variance analysis for essence, as the most important traits measured in this experiment, showed that sowing date had significant effect on this trait. 1ˢᵗ April sowing date produced the most amount of essence in *Calendula officinalis.* 12×50 and 6×50 plant densities also produced the highest and lowest essence in the experiment, respectively. Boroumand, Reza Zadeh [17], Naqdibadi *et al.,* [18] and Pala-Paul *et al.,* [6] reported that essence of medicinal plants increased in optimum plant density.

Effect of different levels of nitrogen fertilizer indicated that application of high level of nitrogen fertilizer resulted in decreasing amount of essence and the most amount of essence obtained in less application of nitrogen fertilizer. As, 50 kg N ha⁻¹ produced the most essence in this experiment. Interaction of 1ˢᵗ April sowing date × high plant density (12×50) and less amount of nitrogen fertilizer (50 kgN/ha) produced the most essence (Table 6).

CONCLUSION

The results of this study demonstrate that suitable sowing date together with optimum plant density and a nitrogen fertilizer increased yield component and essence of *Calendula officinalis*. It recommends carry out the experiment under irrigation levels, soil types and other conditions.

ACKNOWLEDGMENT

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