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Changes in seed and oil yield of *Calendula officinalis* L. as affected by different levels of nitrogen and plant density

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ABSTRACT

In order to study the effect of different levels of nitrogen and plant density on seed and oil yield of calendula (*Calendula officinalis* L.), an experiment was conducted at research field of Faculty of Agriculture, Lorestan University, Khorramabad in 2009. The experiment was carried out as a split-plot arrangement based on a randomized complete block design with three replications. Nitrogen level was the main factor with four levels (0, 50, 100 and 150 kg/ha) and plant density was the sub-factor with three levels (50, 33 and 25 plants/m^2). Number of heads/plant, number of seeds/plant, 1000-seed weight, weight of seeds/plant, seed yield, oil content and oil yield were recorded. Results of analysis of variance (ANOVA) showed that nitrogen levels had no significant effect on different parameters ($P>0.05$). However, Duncan's Multiple Range Test showed a significant difference among means in all parameters ($P<0.05$). Application of 100 kg/ha nitrogen showed the highest seed and oil yield with 118.919 and 18.152 g/m^2, respectively. Moreover, ANOVA showed that plant density had a very significant effect on number of heads/plant, number of seeds/plant and weight of seeds/plant ($P<0.01$). According to the results, as plant density decreased, number of heads/plant, number of seeds/plant and weight of seeds/plant increased. ANOVA also showed significant effect of the interaction between nitrogen levels and plant densities on weight of seeds/plant, seed and oil yield ($P<0.05$). The highest seed and oil yield (124.472 and 19.564 g/m^2, respectively) was found with application of 100 kg/ha nitrogen and 25 plants/m^2 which were not significantly different from those with application of 150 kg/ha nitrogen and 33 plants/ m^2. Considering the importance of less application of chemical fertilizer in agriculture, 100 kg/ha nitrogen and 25 plants/m^2 could be recommended for producing desirable seed and oil yield in calendula under Khorramabad (Iran) climatic conditions.

Key words : *Calendula officinalis* L., nitrogen fertilizer, oil yield, plant density, seed yield

INTRODUCTION

Calendula officinalis (Asteraceae) is an annual herb with yellow to orange flower, native to Mediterranean region. It is also known as pot marigold, a name historically associated with its use in soups and stews to combat illnesses (Ramos *et al.,* 1988). Seeds of calendula contain 18-20% oil which includes 50-60% of 18-carbon fatty acids and 28-30% linoleic acid (Martin and Deo, 2000). Calendula is also recorded in the world's reliable pharmacopeia as one of the most important medicinal plants.

In one hand, nitrogen fertilizer is known as an effective environmental factor on productivity of agricultural and medicinal plants. On the other hand, 22000 kilocalories unrenewable energy is consumed to produce one kilogram nitrogen fertilizer. Cheapness of energy has caused low price of nitrogen fertilizer and its irregular use. Moreover, underground water and river pollution due to nitrate must be taken into consideration. Also, using an appropriate plant density is necessary for maximum utility of existing factors. Thus, many researches have been done to determine the best level of nitrogen fertilizer and plant density and their effects on vegetative and generative parameters of agricultural and medicinal plants. However, much little has been carried out on calendula. Arganosa *et al.* (1998) investigated the effects of six levels of nitrogen (0, 20, 40, 60, 80 and 100 kg/ha) on calendula. They found that desirable biologic yield, seed yield, oil yield and 1000-seed weight

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were obtained from the application of 80 kg/ ha nitrogen and maximum oil content was obtained from the application of 40 kg/ha nitrogen. Similar results were also reported by Sohrabi *et al.* (2007) on sweet fennel, Farahani *et al.* (2007) on coriander and Abaszadeh *et al.* (2007) on Melissa. Dolatshahi (2008) investigated the effects of three levels of nitrogen (0, 100 and 200 kg/ha) on calendula and found that the desirable plant height, number of stems/plant, fresh and dry weight of aerial plant organs, flower diameter and 1000-seed weight were obtained with the application of 200 kg/ha. Desirable stem diameter and number of petals were found with the application of 100 kg/ha nitrogen. Similarly, Rahmani *et al.* (2008) studied the effects of four levels of nitrogen (0, 30, 60 and 90 kg/ha) on calendula and concluded that the application of 90 kg/ha nitrogen produced maximum seed yield, oil yield, 1000 seed weight, the number of seeds/head and head diameter, whereas the maximum oil per cent was obtained in 60 kg/ha nitrogen.

In this paper, we aimed at studying the effect of different levels of nitrogen and plant density on seed and oil yield of calendula under the climatic conditions of Khorramabad, Iran.

MATERIALS AND METHODS

This study was conducted at research field of Faculty of Agriculture, Lorestan University, Khorramabad, Iran (33°29/ N and 48°22/ E, 1125 m above sea level) in 2009. The field soil properties of the top 300 mm, taken just before sowing, were silty clay loam with pH of 7.65, K 355 mg/kg, P 16.8 mg/kg, total N 0.07%, organic carbon 0.77%, EC 0.73 dS/m, $CaCO_3$ 31.7% and base saturation 44%. Two hundred kg/ha triple super phosphate was added to the soil just before sowing (after soil analysis).

The experimental design was split-plot with three replications. The main plots were nitrogen with four levels (0, 50, 100 and 150 kg N/ha) and the sub-plots were plant density with three levels (20 × 10 cm, 20 × 15 cm and 20 × 20 cm resulted in 50, 33 and 25 plants/ m, respectively). Each sub-plot (experimental unit) had 4 m length and 1.2 m width in which six lines of the plants were cultivated.

The experimental field was well prepared through deep plough, good harrowing, levelling, ridging and thereafter, dividing the experimental land into main and sub-plots by construction irrigation canals and alleys. Calendula seeds were sown manually as the usual dry planting method in March. After emergence of seedling, plants were thinned according to the desired plant densities. Nitrogen fertilizer in the form of urea (46% N) was applied in two equal splits, half at 35 days after sowing and remaining half at 65 days after sowing. Weeds were controlled manually. All the intercultural operations were done in proper time. At the harvest time, 10 plants were selected at random from each sub-plot to collect data on number of heads per plant, number of seeds per plant, weight of seeds per plant and 1000-seed weight and different yield contributing characters. Seed yield was recorded from the 2.0 m^2 of each sub-plot. Seed oil was extracted using soxhelt and petrolium ether as an organic solvent. Oil content was expressed on dry weight basis.

The data were subjected to analysis of variance using the MSTAT- C software. Mean comparison was done using a conventional Duncan's multiple rang test.

RESULTS AND DISCUSSION

Results of analysis of variance (ANOVA) showed that nitrogen fertilizer had no significant effect on all studied parameters (Table 1). However, Duncan's multiple range test showed a significant difference among means in all studied parameters ($P<0.05$). Application of 100 kg/ha nitrogen resulted in the highest number of seeds per plant, weight of seeds per plant, seed and oil yield (870.789 seeds, 4.590 g, 118.919 g/m^2 and 18.152 g/ m^2, respectively). The highest number of heads per plant (32.411) was found with application of 100 kg/ha nitrogen which was not significantly different from those with application of 150 kg/ha nitrogen. The highest 1000-seed weight (5.420 g) was found with application of 100 kg/ha nitrogen which was not significantly different from those with application of 150 kg/ha nitrogen. The highest oil content (15.217%) was found with application of 100 kg/ha nitrogen which was not significantly different from those with application of 150 kg/ha nitrogen (Table 2).

Moreover, ANOVA showed that plant density had a very significant effect ($P<0.01$) on number of heads per plant, number of seeds

Table 1. A summary of ANOVA of the effects of nitrogen levels and plant densities on different parameters of Calendula (Mean squares)

Source of variation	d. f.	No. of heads/plant	No. of seeds/plant	Weight of seeds/plant	1000-seed weight (g)	Seed yield (g/m^2)	Oil content (%)	Oil yield (g/m^2)
Replicates	2	0.355	829.547	0.119	0.066	129.759	1.468	3.241
Nitrogen (A)	3	41.580^{ns}	41271.901^{ns}	1.521^{ns}	0.382^{ns}	1132.720^{ns}	3.359^{ns}	44.316^{ns}
Error A	6	41.755	32368.233	0.952	0.191	1270.744	4.155	38.909
Density (B)	2	226.572**	329336.7375**	8.846**	0.142^{ns}	87.279^{ns}	0.255^{ns}	2.693^{ns}
A × B	6	22.289^{ns}	17982.6552^{ns}	1.235*	0.113^{ns}	306.396*	0.500^{ns}	12.373*
Error	16	10.921	10412.237	0.385	0.126	108.131	0.554	3.749
CV	-	11.00	13.16	15.06	6.76	9.90	5.06	12.54

*,**Significant at P<0.05 and P<0.01 level, respectively.
NS : Not Significant.

Table 2. Effect of nitrogen levels and plant densities on different parameters of Calendula

Treatment	No. of heads/plant	No. of seeds/plant	Weight of seeds/plant	1000-seed weight (g)	Seed yield (g/m^2)	Oil content (%)	Oil yield (g/m^2)
Ntrogen levels (kg/ha)							
0	27411B	711.900B	3.660C	5.320B	91441.C	13.827B	12.726C
50	29356AB	763.622B	4.312AB	5.395A	104.955B	14.792S	15.598B
100	32411A	870.789A	4.590A	5.420A	118.919A	15.217A	18.152A
150	30.967A	754.989B	3.926BC	4.975B	104.989B	14.986A	15.286B
Plant densities ($plants/m^2$)							
50	25.100B	606.142C	3.192C	5.157A	106.631A	14.852A	15.070A
33	31.725A	782.167B	4.291B	5.308A	108.188A	14.703A	15.974A
25	33.283A	937.250A	4.88A	5.368A	103.409A	14.561A	15.287A

Values followed by the same letter(s) are not significantly different at P<0.05.

per plant and weight of seeds per plant (Table 1). The highest number of seeds per plant and weight of seeds per plant (937.250 seeds and 4.884 g, respectively) were found in density of 25 plants/m^2. The highest number of heads per plant (33.283) was found in density of 25 plants/m^2, which was not significantly different from density of 33 plants/m^2 (Table 2).

ANOVA also showed significant effect (P<0.05) of the interaction between nitrogen levels and plant densities on weight of seeds per plant and seed and oil yield (Table 1). Mean comparison also showed significant effect of the interaction between nitrogen levels and plant densities on all studied parameters (P<0.05). The highest number of seeds per plant, weight of seeds per plant and oil content (1063.200 seeds, 5.507 g and 15.620%, respectively) were found with application of 100 kg/ha nitrogen and 25 plants/m^2. The highest seed yield and oil yields (124.472 and 19.564 g/m^2, respectively) were found with application of 100 kg/ha nitrogen and 25 plants/m^2 which were not significantly different from those with application of 150 kg/ha nitrogen and 33 plants/m^2 (Table 3). The highest number of heads per plant with 36.633 heads was found with application of 100 kg/ha nitrogen and 25 plants/m^2 which was not significantly different from those with application of 100 kg/ha nitrogen and 33 plants/m^2 and 150 kg/ha nitrogen and 25 plants/m^2 (Table 3). Moreover, the highest 1000-seed weight (5.697 g) was found with application of 50 kg/ha nitrogen and 25 plants/m^2 which was not significantly different from those with application of 100 kg/ha nitrogen and 25 plants/m^2 and 100 kg/ha nitrogen and 33 plants/m^2 (Table 3).

Results showed that application of nitrogen caused increase in all studied parameters. Higher seed yields were achieved with application of higher levels of nitrogen fertilizer. The increase in seed yield with increasing nitrogen levels might be due to the role of nitrogen in activating the growth and yield components. Similar results were reported by Schatz *et al.* (1999), Mojiri and Arzani (2003), Rahmani *et al.* (2008) and Saleem *et al.* (2010). The increase in growth characters and yield components with increasing nitrogen levels might be due to the role in nitrogen in stimulating vegetative growth. Nitrogen is a constituent of the function of nucleic acids and nucleotides that are essential to the metabolic function of plant (Al-Thabet, 2006). Although nitrogen is not a part of oil structure of calendula, higher levels

Table 3. Interaction effects of nitrogen levels and plant densities on different parameters of Calendula

Treatment		No. of heads/plant	No. of seeds/plant	Weight of seeds/plant	1000-seed weight (g)	Seed yield (g/m^2)	Oil content (%)	Oil yield (g/m^2)
Nitrogen levels (kg/ha)	Plant densities ($plants/m^2$)							
0	50	26.787BC	642.733CD	3.696DE	5.381AB	91.220DE	14.067BCD	12.865D
	33	27.067BC	628.567CD	3.672DE	5.423AB	89.162E	13.787CD	12.297D
	25	28.400BC	862.733B	3.614DE	5.156AB	93.942CDE	13.627D	13.016CD
50	50	24.200B	631.067CD	3.330DE	5.268AB	110.332ABCD	15.130ABC	16.697ABC
	33	31.667AB	760.067BC	4.232BCD	5.220AB	101.172BCDE	14.930ABCD	15.131BCD
	25	32.200AB	899.733AB	5.375AB	5.697A	103.361BCDE	14.317ABCD	140.965BCD
100	50	24.733C	622.700CD	3.147DE	5.192AB	113.587ABC	15.330AB	17.485AB
	33	35.867A	926.467AB	5.118ABC	5.576A	118.700AB	14.700ABCD	17.408AB
	25	36.633A	1063.200A	5.507A	5.493A	124.472A	15.620A	19.564A
150	50	24.700C	528.067D	2.596E	4.787B	99.387BCDE	14.883ABCD	13.232CD
	33	32.300AB	813.567BC	4.141CD	5.014AB	123.717A	15.393AB	19.061A
	25	35.900A	923.33AB	5.041ABC	5.124AB	91.863DE	14.680ABCD	13.565CD

Values followed by the same letter(s) are not significantly different at $P<0.05$.

of nitrogen resulted in higher oil content. Moreover, higher seed yield due to application of higher levels of nitrogen fertilizer was also reported by Sawan *et al.* (2006), Azizi and Kahrizi (2008), Parvaneh and Ehsanzadeh (2008) and Rahmani *et al.* (2008).

The results showed that as plant density decreased, number of heads/plant, number of seeds/plant and weight of the seeds/plant increased. This could be due to less competition among plants for environmental factors i. e. light, water and nutrient. Similar results were reported by Rezvani Moghadam *et al.* (2002) on sesame.

The effect of interaction between nitrogen and plant density on weight of seeds per plant, seed and oil yield was significant. Higher levels of nitrogen and lower plant densities resulted in higher seed and oil yield. The highest seed and oil yields (124.472 and 19.564 g/m^2, respectively) were found with application of 100 kg/ha nitrogen and 25 plants/m^2 which were not significantly different from those with application of 150 kg/ha nitrogen and 33 plants/m^2.

In conclusion, considering the importance of less application of chemical fertilizer in agriculture, 100 kg/ha nitrogen and 25 plants/m^2 could be recommended for producing desirable seed and oil yield in calendula under Iran (Khorramabad) climatic conditions.

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