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Original Article

The Effect of Planting Seasons on Quantitative and Qualitative Characteristics of Black cumin *(Nigella sativa* L.)

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Abstract

Concerns related to the rise of disposition to the application of the medicinal plants throughout the world have gone up on their cultivation and production processes. In order to study the effect of planting seasons on morphological traits, yield, oil content and oil composition of black cumin, an experiment was conducted under field condition in Mashhad, Iran in 2012-2013 as a randomized complete block design with two treatments and three replications. The treatments were included spring (April 2013) and autumn November 2012) planting dates. During growth seasons, the phonological stages in both autumn and spring were recorded. The results showed that the species could be planted well in spring and autumn. Also, results showed that with the delay in sowing from spring to autumn, plant height, branch number per plant, plant dry weight, LAI, number of capsules per plant, number of seeds per capsule, seed number per plant, seed weight, and seed yield and oil content significantly decreased. Major constituents of fatty oil were determined as linoleic, palmitic and oleic acid. Spring sowing dates and maximum change was seen in linoleic acid from 55.71% in autumn to 55.5% in spring. Growth periods from sowing to fruiting stage were 187 and 103 days in autumn and spring sowing date, respectively.

Keywords: Black cumin, Sowing date, Yield, Oil content, Fatty acids

Introduction

The ever-increasing tendency to the use of medicinal plants in the world has grown concerns about their cultivation and production processes. As medicinal plants are more compatible with the nature, special interest and attention has recently been given to herb therapy, and use of medicinal plants, being limited by the rise of pharmaceutical drugs, has become again common and widespread due to a number of reasons [1]. Among the promising medicinal plants, *Nigella sativa* L., a dicotyledon of the Ranunculaceae family, is an amazing herb with a rich historical and religious background [2]. *N. sativa* is found wild in southern Europe, northern Africa, and Asia Minor. The seeds of *N. sativa* are the source of the active ingredients of this plant. *N. sativa* seeds are rich in the unsaturated and essential fatty acids. Chemical characteristics, as well as fatty acid profile of the total lipids, were revealed that the major unsaturated fatty acid is linoleic acid, followed by oleic acid .The seeds are used for the treatment of cold, cough, fever, toothache, gastrointestinal disorders, etc [3-5]. Determining suitable planting date plays an important role in conformation of plant growth stages with desirable environmental conditions which results in maximum yield. Planting date has a considerable effect on seed yield by influencing the yield components so that late planting decreases secondary branches/plant and pods/plant and finally causes a remarkable reduction in seed yield [6]. One of the most important factors of management in producing agricultural products is choosing the proper date for cultivation. The choosing of proper date for cultivation is crucial due to the maximum use of natural resources during the growth season. In very

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early cultivation, the low temperature of soil and frost damages causes a weak establishment for plants in spring. So much delay in cultivation due to the shortening the growth period of plants and the possibility of coincidence in the blossoming time with high temperatures will have adverse effects on the plant growth. The effect of environmental factors on the physiological stages of the plants causes the difference in time of cultivation for different areas even in an area due to the genetic differences among items. The determining factors for a desirable cultivation date in every area are the proper temperature of soil for germination, the rate of ample growth of the plants before blossoming, not coinciding the blossoming time with high temperature and the coolness of the end of season. In the terms of the effect for cultivation date on the plant establishment, the control of weeds, diseases and pests, harvest time and the product quality, being aware of the most proper time for cultivation in every area to promote the quality and quantity of the product is inevitable [7-10]. Fanaei *et al* (2007) reported that the autumn planting rather had the highest yield of *N. sativa* El-Mekawy (2012) that delayed sowing decreased seed yield of *N. sativa* Kizil *et al* (2008) reported that the winter planting dates rather spring planting dates had the highest morphological traits and yield and oil and linoleic, oleic, palmitic acid of *N. sativa* L. Zahtab Salmasi *et al* (2003) studied the effect of three sowing date on *Pimpinella anisum* L. and concluded that delayed sowing decreased plant height and fruit yield. Adamsen and Coffelt (2005) found that reproductive efficiency appeared to change with planting date and in general, October and November planting dates produced seed with higher oil content than December planting dates of rape. The studied the effect of autumn cultivation under climatic conditions of Mashhad of *N. sativa* showed that autumn cultivation did not carry out. Therefore, the present study was carried out to evaluate the effects of planting date seasons on quantitative and qualitative characteristics of *N. sativa*

Material and Methods

The experiment was carried out in field of Horticulture Department, Ferdowsi university of Mashhad, Iran (Long. 61°16´ E., Lat. 33°52´ N) in 2012-2013. The average long-time minimum and maximum temperature is 8.8 and 22.4 °C with average annual precipitation of 254.3 mm and average minimum and maximum relative humidity of 39 and 55%, respectively. The regional climate is cold and arid. A randomized complete block design with three replications was carried out to evaluate the effect of spring and autumn planting dates on *N. sativa*. The plants were planted in November 2012 and April 2013. Also, the plants were planted directly by seed. The seeds were primed 24 hrs in distilled water before culture. The plants were grown in four row plots, with two meters long. During the growing period, weeds were controlled by hand as needed. Before testing, a farm was selected to determine the chemical properties of soil and then, soil sampling was conducted. The soil mixture used in the study had a Clay loam texture, pH 7.9 and electrical conductivity 1.21 dS/m. It contained 0.3 percent N, 19.7 mg/kg P and 334 mg/kg K. In order to determine of morphological traits including plant height, branch number per plant, plant dry weight, LAI (leaf area index), 10 plants were randomly selected from the middle of each plot and these traits were measured. In order to calculate yield and yield seed ingredients when the harvest time arrived, the plants yellowed and capsules dried, having removed the side effects of such characteristics as number of capsules per plant, number of seeds per capsule, seed number per plant, seed weight, and seed yield, were measured. GDD was calculated as:

$$GDD = \sum_{di}^{dn} \left(\frac{Tmax + Tmin}{2} \right) - \text{Tb}$$

Where GDD is the growing degree day, *T*b the threshold temperature for growth (ºC), T min the minimum air temperature (ºC), and T max is the maximum air temperature ºC). Tb of black cumin was considered as 7.8 ºC respectively. The plots were harvested by hand on July 2012, in the spring planting dates, and June 2013, in the autumn planting dates.

Oil Extraction and Fatty Acids Determination

In order to calculate seed oil, an area of was harvested from the middle of each plot. Also, 20 g seed was selected from each plot to determine their oil percentage by Soxcelle method. Then, it was multiplied by seed yield to have oil yield. Fatty acids composition was determined by gas liquid chromatography (GC). The methyl esters were prepared using the method described by khodaparast *et al* [11]. 100 µl of sodium methoxide (0.5 M) was added to 50 µl sample in 1 ml of n-hexane. The mixture was shaken vigorously for 15 min, allowed to stand and separate of spring and autumn planting dates. The gas chromatographic analysis of Younglin-Acme 6000 type XPB-70 was performed on Helium gas chromatograph equipped with a PIF detector. The column used was a 120 m. Temperatures of injector and detector and oven were 250 and 280 ºC, and 180 ºC respectively. The identification of FAMEs was based on comparison of their relative RF (retention times) values with those of authentic standards.

Statistical Analysis

For the variance analysis of test data and drawing graphs, Excel and SAS software was used. All the averages were compared at the 5% level, according to LSD test.

Result and Discussion

Morphological

The results of analysis of variance for morphological traits showed that plant height, branch number per plant, plant dry weight, and LAI (Leaf area index) number of *Nigella sativa* L. were significantly affected by planting dates (Table 1). Means comparison indicated that the delay in planting dates decreased plant height, branch number per plant, plant dry weight and LAI, respectively (Table 2). With a cultivation delay, the height of bushes decreases due to the reduction of the growth period of plants and blossoming as well as through coincidence with hot days. The average minimum and maximum temperature is 8.8 and 22.4 °C and average minimum and maximum relative humidity of 39 and 55%, and average light is 3038 hours respectively. The delay in cultivation can be due to the temperature of growth period in plants and daytime lengthening which results to reducing the growth period and decreasing the leaves of plants [12-14]. The so early cultivation along with increasing the period time of the plants growth leads to the increasing of blossoming branches. In spring cultivation due to the establishing of plants to the blossoming stage and stopping the growth of the main branch, the nutrition will be transferred to lateral branches for growth resulting in more minor. Also, the delay in cultivation because of improper environmental conditions especially the temperature and sunlight causes less blossoming branches in the black cumin [15,16]. The reduction in the weight of bushes along with the delay in cultivation can be attributed to the increasing of temperature during the growth period of plants as well as the daytime lengthening which leads to reducing the growth period, decreasing the minor branches and lowering the photosynthesis level of the plants which finally reduces the dry bushes [17,18]. These results are in agreement with the results of Filippo *et al* (2005) and Zahtab Salmasi *et al* (2003). These variations could be attributed to weather conditions prevailing during vegetative growth, flowering and maturity and accelerated maturity at shorter growth period available for building and accumulation of food material. In addition to high temperature, low humidity and high solar radiation thought autumn season led to long time of stomatal closer and reduced photosynthesis.

Yield and Yield Ingredients

The results showed that yield of *Nigella sativa* L. was significantly affected by spring and autumn planting dates (Table 1). Means comparison indicated that the delay in planting dates decreased number of capsules per plant, number of seeds per capsule, seed number per plant, seed weight, and seed yield, respectively (Table 2). The seed function is the result of a set of parts. Since the black cumin is a late-blossoming and limited-growth plant which the flowers and plants grow at the bottom of the branches, the number of capsules follows the number of blossoming branches [19]. The delay in cultivation causes the reduction of blossoming branches and coincidence of the black cumin blossoming with the warm weather resulting to the flowers recession and the reduction of the capsule numbers [20]. The delay in cultivation leads to reduction of the growth period, decreasing the leaves, and lowering the photosynthesis level of the plant which finally results to the reduction in producing generative organs including the number of seeds in the capsule of black cumin. The reduction of the growth period of plants in the spring time results in existing the plants with small growth body which have entered the generative stage by increasing daytime in spring despite the insufficient growth [21,22]. Therefore, the aggregation of the dry bushes of plant in blossoming has not been desirable leading to the weight loss of the seed. It seems that in autumn cultivation, the lengthening of the growth period of the plant improves the plant growth resulting in more photosynthesis materials which finally leads to the increase in producing the generative organs including the number of seeds in the plant. The reason for the seed function increase in the autumn cultivation is probably due to better growth, canopy improvement, and proper use of sunlight as well as high photosynthesis [23-25]. The dryness tension due to high temperature and soil vaporization was the reason for the blossoming time with high temperature and flower recession and abortion as the main reasons for reducing the seed function in spring [26,27]. These results are in agreement with the results of Saddam *et al* (2012) and Adamsen *et al* (2005) and verma *et al* (2012).

Oil Aamount and Oil Yield

The effect of planting date treatments on oil amount and oil yield was significant at 1% level (Table 1). Means comparison indicated that the delay in planting dates decreased oil amount and yield oil, respectively. The highest oil percentage of 29.22% was recorded with autumn cultivation. Also, the highest oil yield (168.8 kg/he) was recorded with autumn cultivation (Table 2). The average minimum and maximum temperature was 8.8 and 22.4 °C and average minimum and maximum relative humidity of 39 and 55%, and average minimum and maximum light (3038 hours) respectively. The change of cultivation rate along with daytime period, maximum and minimum temperatures and relative humidity and other environmental conditions during the growth season affects the biological function, growth period, and phenological stages as well as the quality and quantity of materials; the following oil is a functional the seed and percent of its oil. Therefore, any change in the functional seed and oil percentage influences the oil function. Hence, in the autumn cultivation due to the lengthening of the growth period, the number of major and minor blossoming stems has been increased and leads to producing more follicle in the black seeds and increasing the function of the seed which finally increases the function and percentage of oil in surface level. On the other hand, the autumn cultivation caused that the main time for blossoming of black seeds no to be coincided with severe heat. The severe heat in summer has a negative effect on the qualitative and quantitative function of medicinal plants [28-30].

Table 1 Analysis of variance (mean squares) components of *Nigella sativa* L.

Source of variation	df	Height plant	LAI	Plant dry weihgt	Branch number per plant	Capsule per plant	Seeds per plant	Seeds weight per plant	Seed yield	Oil	Oil yield
Block	2	46.71^{ns}	0.0001^{ns}	0.001^{ns}	0. 11^{ns}	0. 02^{ns}	1833. 82^{ns}	0.001^{ns}	19889.32^{ns}	6.12^{ns}	1155. 74^{ns}
planting seasons	2	41.44**	0.005**	0. 25**	6.77**	11.44**	137171. 37**	0.021**	6496.3**	17.9**	20177.44**
Error	4	5.49	0.0002	0.006	1.11	1.35	1405.41	0.001	3466.66	0.38	73.92
CV		13.5	13.34	13.05	15.76	19.15	31.97	3.38	8.05	9.91	11.83

*Is significant at the 5% level.**Is significant at the 1% level. ^{ns} not significant.

Table 2 Comparison of average yield and morphological traits and oil amount under planting date seasons of *Nigella sativa* L.

Treatments	Height Plant (cm)	LAI	Plant dry weight (g)	Branch number per plant	capsule per plant	Seeds per plant	Seeds weight per plant (g)	Seed yield (kg/he)	Oil (%)	Oil Yield (kg/he)
Autumn	44.33 a	0.32 a	2.11 a	10 a	9.86 a	630 a	2.14 a	706 a	22.29 a	168.6 a
Spring	37.5 b	0.26 b	1.56 b	7 b	8.63 b	512 b	2.02 b	601 b	18.77 b	120.6 b

Similar letters in each column indicate no significant difference at P<0.05

In the spring cultivation due to the shortening period of growth, the growth period of plants reduces so that it causes the reduction in producing major and minor stems and reducing the photosynthesis level which finally leads to the reduction of oil function. It seems that the rise in oil level in autumn cultivation due to proper temperature leads to lack of high irrigation within a lengthening growth period as well as more production of seed function which finally increases the oil level [30-32]. These results are in agreement with the results of Kizil *et al* (2008) and Adamsen *et al* (2005) and verma *et al* (2012).

The Oil Components

The results from the chemical analyses of the black cumin oil showed 19 different components (Table3). Three important elements of the black cumin oil include linoleic acid, oleic acid, and palmitic acid which in the autumn cultivation constitute 91.16 percentage and in the spring cultivation 89.97 percentage. The highest increase of 55.71% in the linoleic acid was recorded at autumn cultivation respectively. Again the highest increase of 25.12% in the oleic acid was recorded at autumn cultivation respectively. The highest increase of 10.75% in the palmitic acid was recorded at autumn cultivation respectively. Although the effective materials of medicinal plants are made by genetic processes, their production is obviously influenced by the environmental factors. Therefore, the most appropriate conditions needed for producing the aforementioned materials should be obtained by the effective environmental factors and applying the agricultural techniques so that the plants that can be cultivated some different seasons such as the black cumin can have a special place in the mass production of the agricultural products. [33-35].

The results of fatty oil analyses of this study, are compatible with Nickavar *et al* results, that the main fatty acids of the fixed oil were linoleic acid (55.6%), oleic acid (23.4%) and palmitic acid (12.5%). Kizil *et al* (2008) reported that *N. sativa* fatty oil contained unsaturated fatty acid, oleic acid (38.76%), linoleic acid (37.56%) and linolenic acid (1.88%). Oleic acid amounts of this study are lower than that of Kizil *et al* (2008) while linoleic acid percentages are higher than those of aforementioned reports. In addition, El Sayed *et al* (1997) reported major fatty acids of *N. sativa* were linoleic (57%), oleic (23.4%), palmitic acid (12.3%) and unsaturated fatty acids (84.9%). Data obtained from this study is in relation with saturated and unsaturated fatty acid amounts reported by El Sayed *et al* (1997).

Table 3 Fatty acids percentage (%) of black cumin at different planting date seasons

Row	Components	Autumn cultivations	Spring cultivations
1	c12:0(luarate)	0.025	0.003
2	c14:0(myristate)	0.15	0.13
3	c16:0(palmitate)	10.75	9.77
4	c16:1(palmitoleate)	0.23	0.19
5	c17:0(heptadecanoic)	0.08	0.08
6	c17:1(heptadecenoic)	0.07	0.1
7	c18:0(stearate)	3.14	3.42
8	c18:1c(oleate)	24.74	24.7
9	c18:2c(linoleate)	55.71	55.5
10	c20:0(arachidate)	0.26	0.31
11	c18:3c(linolente)	0.34	0.24
12	c20:1(gadoleate)	0.48	0.55
13	c20:2(Eicosadienoic)	3.75	3.99
14	c21:0(Heneicosanoic)	0.04	0.06
15	c22:0(behenate)	0.06	0.06
16	c22:1(erucate)	0.04	0.11
17	22:2(Docosadienoic)	0.02	0.13
18	c24:0(lignoceric)	0.03	0.05
19	c24:1(nervonic)	0.01	0.01

Table 4 Process grow and phenology spring and autumn cultivation under climatic conditions of Mashhad

cultivation season	Full grow	2untile4 leaf	4until6 leaf 4	8untile10 leaf	Budding	Full budding	Flowering	Capsule fomation	physiological maturity
	Days	Days	Days	Days	Days	Days	Days	Days	Days
spring	12	23	26	44	140	157	174	187	195
autumn	14	20	25	41	56	67	81	103	113

Phenological Stages

The table three shows the phonological stages of the black cumin in the spring and autumn cultivations under Mashhad climatic condition. In the black cumin like other plants, the beginning of the generative growth is influenced by the temperature, daytime period, and other factors. Therefore, the necessary number of days needed for beginning the generative stage and other phonological stages is influenced by these factors. Today researches believe that we should not only consider the number of the necessary says for the beginning of the phonological stage but also we had better regard the level of collective and thermal units from the cultivation time to occurrence of any stage. In this research the phenology of the black seed is influence by the cultivation date. The black cumin is one of the most suitable plants for cultivation in Mashhad during spring and autumn so that it has a good percentage for cultivation. The autumn cultivation of the black cumin in Mashhad was successful; in the spring cultivation the number of days for giving ripe fruit was 103 days and in the autumn cultivation 187 days. The black seed was identified as a suitable plant for cultivation in spring and autumn; the autumn cultivation was superior to the spring cultivation.

Conclusion

According to the obtained results, it can be included that the autumn cultivation of the black seed due to the better establishment in the autumn it causes the early phenology of spring and increasing the phonological period along with a better growth and more seed function in the surface level. The qualitative analyses of the oil indicate that the area conditions are suitable for the components of the effective materials of the seed. Also, the results of this study show that there is a possibility for the autumn cultivation of the black cumin under the climatic condition of Mashhad.

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