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Original Article

Effect of Sowing Date and Nitrogen Rate on Morphological Traits, Seed Production and Essential Oil of *Pulicaria gnaphalodes* (Vent.) Boiss.

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ABSTRACT

Pulicaria gnaphalodes (Vent.) Boiss. is a medicinal plant perennial and herbaceous belong to Asteraceae family which grows in some parts of Iran. In order to investigate the effect of two sowing dates (May 3 and 23) and nitrogen rates (0, 80, 160, and 240 kg N/ha) on morphological traits, yield and yield components and essential oil yield of *P. gnaphalodes* aerial parts, a factorial experiment based on a randomized complete block design with three replications was carried out in research field of Birjand branch, Islamic Azad University, Birjand, Iran in 2018. The results of analysis of variance showed that the simple and interaction effects of sowing date and nitrogen rate significantly affected plant height, number of main branch, number of capitols per plant, seed yield, dry weight and percentage of essential oil produced by the aerial parts at 1% level. In addition, the number of secondary branches, canopy diameter, number of seed per capitols and 1000-seed weight were affected by the nitrogen content at 1% level. The effect of sowing date on the number of main branches and canopy diameter was significant at the 1% level and on 1000-seed weight was significant at 5% level. Means comparison of simple effect of sowing date showed that plant height, number of main branches per plant, number of secondary branches per plant, canopy diameter, number of capitols per plant, 1000-seed weight, seed yield, and dry weight of the aerial parts had a significant superiority in the sowing date of May 3 compared to May 23, by 15.7, 19, 12.1, 10, 51.4, 15.8, 88.3 and 33.1%, respectively. Increasing the application of nitrogen to 240 kg N/ha also significantly increased the mentioned traits by 24, 41.2, 17.6, 29.3, 82.6, 115.3, 36.2, and 187.3%, respectively compared to 0 kg N/ha (control). Means comparison of interaction effect between sowing date and nitrogen rate showed that the maximum plant height (68.50 cm), number of main branches per plant (17 branches per plant), number of capitols per plant (170), seed yield (322.87 kg/ha) and dry weight of the aerial parts (1808.52 kg/ha) were obtained in the treatment of the sowing date of May 3 with application of 240 kg N/ha.

INTRODUCTION

The medicinal plant *Pulicaria gnaphalodes* (Vent.) Boiss which belongs to Asteraceae family, is a perennial and herbaceous plant with straight upward stem, small bright yellow flowers, and small clumps of inflorescences [1]. The genus *Pulicaria* includes 80 species widely distributed from Europe to North Africa and Asia [2]. These species exist in Iran, Egypt, Afghanistan, and Pakistan. Five species of this genus (*P. gnaphalodes, P. Arabica, P. vulgaris, P. dysenterica and P. salvifolia*) grow in different parts of Iran in the eastern, western, southern, central, and northwestern regions [3,4]. However, unfortunately no measure has been taken regarding the cultivation of this valuable medicinal plant. Different species of the genus *Pulicaria* have anti-inflammatory and anti-leukemic functions, and are used in traditional medicine as a diuretic and antipyretic. They are also used for reducing the symptoms of flu and cold and treatment of intestinal disorders, inflammation, colic and cough. They are also employed as anti-flatulence [2,4]. In addition, they have anticonvulsant, antibacterial, anti-diarrheal, and antihistamine properties and are used in the treatment of heatstroke. They have also antimicrobial, antifungal, anti-malarial, and insecticidal functions and are used to relieve skin disorders [2,4,5].

Iran has rich resources of medicinal plants and is considered one of the best regions in the world in terms of climate, geographical location, and growth context for these plants [6]. Given the importance of medicinal plants in the treatment of diseases and their limited natural habitats, Asaadi and Heshmati [7] emphasized the necessity of planning for cultivation and domestication of these plants. The domestication of medicinal plants means the cultivation of medicinal and aromatic wildflower species under agronomic conditions that, if successful, there is no necessary for collecting this plant in nature. According to Chen *et al*. [8], investigating the potential of cultivation and domestication of medicinal plants in different areas and achieving the appropriate farming strategies will increase the quantity and quality of active ingredients of medicinal plants, which reduces the pressure on natural population and prevents the extinction of these plants. Ghavam and Azarnivand [6] reported that in order to prevent the extinction of a native species of high medicinal value and to develop its growth, some measures should be taken in cultivation and domestication of that native species. The first effective step in this path is to identify the ecological needs of the plant to increase success rate. In this regard, one of the important agronomic factors is sowing date which affects the quantitative and qualitative yield of active ingredients of medicinal plants through the vegetative and reproductive growth period and the time when these stages are exposed to different environmental conditions [9]. Emongor [10] reported that sowing date has a significant effect on the medicinal composition and yield through affecting growth, metabolic activities, and dry matter yield of medicinal plants. He added that awareness of the most appropriate planting time for each region is necessary to improve the quantity and quality of the product. The appropriate sowing date is when the plant has sufficient time to complete the vegetative and reproductive growth stages before the end of the growing season. Awareness of the optimal crop conditions, especially determining the sowing date is one of the first research needs for the cultivation of medicinal plants. Sowing date varies depending on species, parts to be harvested, and climatic conditions in each region [11]. Investigating the effect of sowing date in *Cichorium intybus* L. showed that sowing date had a significant effect on the dry weight of this plant, and *Cichorium intybus* L. had the highest and lowest leaf phenolic compounds on sowing dates of May 22 and March 21, respectively [11]. In addition to sowing date, meeting the fertilizer needs of plants, including nitrogen, is one of the important factors affecting the growth and achieving proper and economical yield in medicinal plants [12]. Rahmati *et al*. [13] examined the effect of nitrogen fertilizer on vegetative growth of *Matricaria recutita* and found that increasing the application of this fertilizer stimulated vegetative growth and increased dry weight of *M. recutita*. In a study on the effect of 45, 90, 135, and 180 kg N/ha on *Brassica napus* L., Siadat *et al*. [14] reported that the largest number of pod per plant, 1000-seed weight, and seed yield were obtained with 180 kg N/ha, while the largest number of seed per pod was obtained with 45 kg N/ha. Given the importance of agricultural information necessary for success in the domestication and cultivation of medicinal plants, the present study aimed to investigate the effect of sowing date and rate of nitrogen fertilizer on traits of *P. gnaphalodes* in farm conditions in Birjand region.

MATERIALS AND METHODS

This experiment was carried out in 2018 in the research farm of Birjand Branch, Islamic Azad University of with geographical coordinates of Latitude: 32°52′58″ N Longitude: 59°13′16″ E Elevation and 1480 meters above sea level. Climatically, the experiment location was an arid region based on Amberge climatogram. The average 15-year rainfall in this region was 176 mm, with maximum and minimum temperatures of 39.1 and -17 °C, respectively. Climatic conditions during the experiment period are given in Table 1. Based on the results of sampling in 0–30 cm depth of soil before planting, the soil texture of the experimental farm was of silty loam, with soil salinity of 2.3 Ds/m, acidity of 7.4 and nitrogen, phosphorus, and potassium (available in soil) of 3.8, 11.6, and 75.2 mg/kg, respectively.

Table 1 Mean monthly temperature and relative humidity at the research field of the Islamic Azad University of Birjand, South Khorasan Province, Iran in 2018

Month	Temperature (C^o)			Relative humidity (%)			Rainfall
	Minimum	Maximum	Average	Minimum	Maximum	Average	
May	12.4	27.7	20.05	14	54	17.5	15.5
June	17.8	35	26.4	8	27	13.5	0
July	20.9	36	28.45	6	21	19	0
August	19.1	35.4	27.25	8	30	14	0
September	12.7	32.6	22.65	6	22	25.5	0

The research was conducted as a factorial experiment based on a randomized complete block design with three replications in research field of Birjand branch, Islamic Azad University, Birjand, Iran in 2018. The research factors included two sowing dates (May 3 and 23) and four nitrogen rates (0, 80, 160, and 240 kg N/ha from urea fertilizer source). Cultivation was performed through transplanting.

Seeds of *P. gnaphalodes* (Vent.) Boiss. collected from Nofererst habitat in November 2017. Nofererst habitat is located in Bagheran rural district of Birjand city in the southeast of South Khorasan with geographical coordinates of Latitude: 32°43′51″ N Longitude: 59°26′10″ E Elevation. After collecting seeds, they were first disinfected with sodium hypochlorite 3% for 30 seconds and washed several times with distilled water and then 21-day cold treatment (stratification) was applied at 5 °C in order for breaking dormancy and seed germination. Seeds were sown at a depth of 1 cm into the soil and the seed germination was determined in the laboratory before planting (95 %). On April 13 and May 2, seeds were cultivated in the seedling tray at 25 °C, respectively. In four-leaf stage on May 3 and 23, respectively, the seedlings were transferred to the main farm and transplanted at a distance of 40 cm on the row. Each experimental plot had 6 planting lines with a length of 4 meters and a row spacing of 50 cm. The distance between experimental plots of two not planted rows was considered (1m). Bed preparation operations including plowing in winter 2017, leveling and setting out two perpendicular discs before planting were carried out in late April, and finally, furrows was performed with a furrower on May 1. Weeding was performed three times (at the establishment stage, at the beginning of the flowering stage, and at 50% flowering stage) by hand. After establishing the plants in main farm, nitrogen rates were applied as top-dress twice during the growing season. In order to examine the morphological traits (plant height, number of main branches, number of secondary branches and canopy diameter) and the number of capitols per plant in the flowering stage (September), 10 plants per plot were selected randomly, considering the marginal effect. Plant height and canopy diameter were measured with a ruler, and the number of main and secondary branches and the number of capitols per plant were counted. Simultaneously with physiological examination of seeds, 50 capitols were randomly harvested from the two midlines of each plot, considering the marginal effect. After winnowing the seeds and counting them by hand, the number of seeds per capitol was determined. Also, in order to determine seed yield per unit area during the growing season, the capitols of middle one square meter plants identified in each experimental plot were harvested by hand. After cleaning the seeds, seed yield was obtained using a digital scale with an accuracy of 0.001 g, and 1000-seed weight was found by counting one thousand seeds by hand and weighing them using a digital scale. The dry weight of the aerial parts per unit area was also obtained by selecting 10 plants randomly in each experimental plot, considering the marginal effect. It should be noted that to determine the percentage of essential oil, middle one square meter plants of each experimental plot were harvested at a height of 5 cm above the soil, at the flowering stage, and dried in the shade at room temperature (25 °C). Then, essential oil was extracted from these plants with water distillation method and by the use of a Clevenger apparatus for four hours, under exactly the same conditions. After dehydration and weighing, the percentage of essential oil was calculated [15]. Essential oil yield was obtained by multiplying the percentage of essential oil in the yield of the aerial parts. The data obtained from the experiment was analyzed using SAS software, the means were compared at the statistical level of 5% using Duncan's multiple range test.

RESULTS AND DISCUSSION

Morphological Traits

The results of analysis of variance showed that the simple effect of sowing date and nitrogen rate on all morphological traits including plant height, number of main branches, number of secondary branches, and canopy diameter, and also the interaction effect of sowing date and nitrogen rate on plant height and number of main branches were significant ($P < 0.01$) (Table 2).

Comparing the means of simple effect for sowing date showed that the maximum plant height (63.95 cm) and the number of secondary branches per plant (816.41 branches per plant) achieved on May 3 were significantly higher than the related results on May 23, with means of 15.7 and 19%, respectively (Table 3). Also, the number of secondary branches and canopy diameter in the first sowing date had a significant advantage compared to the second one, with means of 12.1and 10.2%, respectively (Table 3). In the second sowing date, it seems that the delay in planting date accelerated the plant to the reproductive phase; making it did not have the necessary opportunity for photosynthesis and allocation of sufficient photosynthetic material to the vegetative parts, which resulted in a significant decrease in morphological traits. In the first sowing date, prolonged vegetative period in early planting improved vegetative growth and increased foliage production, which resulted in better light absorption and availability of photosynthetic materials. As a result, morphological traits including plant height, number of main and secondary branches per plant and canopy diameter in the first sowing date was significantly superior to the second one. The obtained result is consistent with that of Tabiei and Baradaran [16] on *Securiger securidaca* L. The results of Seghatoleslami and Ahmadi Bonkdar [17] in the study on *Trigonella foenum-graecum* also showed that delay in sowing date from February 28 to April 28 reduced plant height by 6.9 cm.

According to means comparison of simple effect for nitrogen application showed that the increase in nitrogen application increased the plant height; so that the maximum plant height (65.92 cm) was observed at 240 kg N/ha; which had a significant superiority of 24, 14.6 and 6.5% than control (non-application of nitrogen) and application of 80 and 160 kg N/ha, respectively (Table 3). The lowest number of main and secondary branches per plant was observed in the absence of nitrogen application. The application of 80, 160, and 240 kg N/ha significantly increased the number of main branches per plant by 41.2, 11.7, and 12.9%, respectively than control. Also the number of secondary branches per plant increased by 17.6, 23.6, and 29%, respectively, comparing with non-application of nitrogen (Table 3).

The results also showed that with increasing the application of nitrogen from 0 to 240 kg N/ha, the diameter of canopy significantly increased by 29.3% compared to the non-application of nitrogen (Table 3). It seems that with increasing nitrogen application, due to the greater accessibility of plant roots to this necessary element and the positive effect of nitrogen on stimulating plant vegetative growth, longitudinal growth potential, branching, and main and secondary branches per plant increased and the plant was able to increase its canopy diameter by producing more foliage.

The content of nitrogen in the plant also has a significant effect on the growth rate, because the main function of nitrogen is cell proliferation and increasing cell length and internodes. It seems that nitrogen increased the production of photosynthetic materials by providing suitable conditions, and made it possible to produce higher plants. In a study on the effect of nitrogen rates (0, 200, 300, and 400 kg N/ha) on *Perovskia abrotanoides* L., it was reported that the largest number of secondary branches was obtained in the treatment of 400 kg N/ha [18]. Also, with increasing nitrogen application from 0 to 75 and 150 kg N/ha in *Trigonella foenum graecum*, an increase of 11.8 and 23.5% was reported in the number of main stem branches, respectively [19]. Increasing the height of *Carthamus tinctorius* L. as a result of increasing the content of nitrogen was also reported by Tahmasbizadeh *et al*. [20], so that the application of 138 kg N/ha with an average height of 88.9 cm yielded the maximum height.

Table 2 Analysis of variance for the effect of planting date and nitrogen fertilizer on morphological traits of *P. gnaphalodes* (Vent.) Boiss.

Source of Variation	df	Mean squared (MS)			
		Plant height	Number of main branches	Number of sub branches	Canopy diameter
Replication	2	0.875 ^{ns}	0.2917 ^{ns}	2225 ^{ns}	52.21 ^{ns}
Sowing date (A)	1	450.667 **	35.0417 **	46552 **	410.44 **
Nitrogen (B)	3	182.125 **	22.4861 **	41077 **	575.42 **
A× B	3	59.361 **	0.3750 **	4134 ^{ns}	49.01 ^{ns}
Error	14	8.661	0.7679	1311	30.68
Coefficient of variation (CV%)		4.8	6.3	4.7	6.5

^{ns}, * and **: non-significant, significant at 5% and 1% levels of probability, respectively

Table 3 Means comparison of simple effects of planting date and nitrogen fertilizer on morphological traits of *P. gnaphalodes* (Vent.) Boiss.

Treatment	Plant height (cm)	Number of main branches	Number of sub branches	Canopy diameter (cm)
Sowing date				
May 3	63.96 a	15.17 a	816.42 a	89.37 a
May 23	55.29 b	12.75 b	728.33 a	81.10 b
Nitrogen (kg/ha)				
0	53.17 d	11.33 c	657.00 c	74.83 c
80	57.50 c	14.33 b	772.87 b	79.87 c
160	61.92 b	14.17 b	812.07 ab	89.50 b
240	65.92 a	16.00 a	847.51 a	96.75 a

Means with same letter(s) in each column were not significantly different at 5% level.

Table 4 Analysis of variance for the effect of planting date and nitrogen fertilizer on yield components, seed yield and essential oil of *P. gnaphalodes* (Vent.) Boiss.

Source of Variation	df	Mean squared (MS)						
		Number of capitols per plant	Number of seed per capitol	1000-seeds weight	Seed yield	Dry weight of aerial parts	Percentage essential oil	Essential oil yield
Replication	2	172.2 *	44.792 ^{ns}	0.0003 ^{ns}	297.9 ^{ns}	2476 ^{ns}	0.000001 ^{ns}	0.5405 **
Sowing date (A)	1	11484.4 **	73.500 ^{ns}	0.003 *	40578.9 **	500586 **	0.025676 **	9.088 **
Nitrogen (B)	3	4075.2 **	325.44 **	0.01 **	40337.1 **	1270608 **	0.008337 **	8.393 **
A× B	3	935.8 **	1.83 ^{ns}	0.001 ^{ns}	6786.4 **	91632 **	0.002037 **	1.148 **
Error	14	34.2	33.45	0.001	162.4	5642	0.000139	0.0352
Coefficient of variation (CV%)	-	5.9	8.7	19.6	9.5	7.4	7.1	10.8

^{ns}, * and **: non-significant, significant at 5% and 1% levels of probability, respectively

In addition, the interaction effect of sowing date and nitrogen content showed that the sowing date of May 10 and the application of 138 kg nitrogen yielded the maximum plant height [20].

Comparing the means of interaction effect between sowing date and nitrogen rate showed that the maximum plant height (68.50 cm) and the number of main branches per plant (17 branches per plant) were achieved in the sowing date of May 3 with nitrogen application of 240 kg/ha. Although this was not significantly differing from the sowing date of May 3 with nitrogen application of 160 kg N/ha, it had a significant advantage compared to other treatments (Table 6). It seems that in the sowing date of May 3, the plant was exposed to cooler weather during the vegetative growth period and the length of vegetative growth period increased; however, on late sowing date, due to an increase in temperature, the plant completed its vegetative growth faster and loses the opportunity for branching and maximizing height. On the other hand, the increase in nitrogen application may have increased the vegetative growth of the plant by increasing the durability and leaf area and using more sunlight. In a study on *Brassica napus* L., Rahnama [21] concluded that as the temperature increased during the plant growth period, the length of vegetative period decreased.

Yield and its Components

The results of analysis of variance showed that the simple and interaction effects of sowing date and nitrogen rate affected the number of flowers per plant, seed yield and dry weight of *P. gnaphalodes*. ($P < 0.01$). In addition, the number of seed per capitol and 1000-seed weight were affected by nitrogen rate ($P < 0.01$), and the effect of sowing date on 1000-seed weight became significant ($P < 0.05$) (Table 4).

Comparing the means of simple effect of sowing date showed that the maximum number of capitols per plant, 1000-seed weight, seed yield and dry weight of the aerial parts had a significant superiority in the sowing date of May 3 compared to May 23, with means of 56.4, 15.8, 88.3 and 33.1%, respectively (Table 5). It seems that a 20-day delay in the sowing date from May 3 to May 23 resulted in decreased plant height and number of main and secondary branches through shortening the vegetative growth period (due to high temperatures at this stage) and reducing the photosynthetic potential of the plant and finally the number of capitols per plant was significantly reduced (Table 3). In other words, due to suitable temperature conditions and sufficient opportunity in the first sowing date, the plant had more secondary branches and much height. As a result, having more leaf area (stronger source) and allocating more photosynthetic material to the reproductive organs, the plant provided suitable conditions for increasing reproductive parts and subsequently, increasing the number of capitols per plant. Decreasing in number of reproductive units per plant with delayed planting was also reported in *Lathyrus sativus* L. [22] and *Coriandrum sativum* [23].

1000-seed weight is affected by the genetic structure of the plant, environmental conditions and their interactions, and consists of two components including seed filling rate and length of seed filling period [24]. It seems that the period of seed filling was longer in the first planting date than in the second one, resulting increased allocation of dry matter to seed in the first sowing date, which was a reason for the increase in 1000-seed weight. In other words, a significant reduction in 1000-seed weight in the second sowing date compared to the first one can also be attributed to the reduced photosynthetic capacity of *P. gnaphalodes* (Vent.) Boiss. due to colder weather and shorter days during the current photosynthesis period and to the reduction of photosynthetic assimilates transfer rate to the seeds. Significant weight loss of 1000 seeds with delay in planting was also reported in a study on *Carthamus tinctorius* L. by Samadi Firozabadi and Yazdani [25], which is consistent with the results of the present study.

It seems that in the first planting date, due to the prolongation of the plant growing season and the increase in the duration of the plant's use of sunlight and nutrients and the production of main and secondary branches and leaf area of the plant increased and thus produced more photosynthetic assimilates and dry matter. Finally, with a significant increase in the number of capitols per plant and 1000-seed weight (Table 3), seed yield and dry weight of the aerial parts increased significantly. Increased seed yield with early planting was also reported in a study on *Trigonella foenumgracum* L. by Seghatoleslami and Ahmadi Bonakdar [17], which is consistent with the results of the present study. In other study, Zahtab Salmasi *et al*. [26] found that delay in planting *Pimpinella anisum* due to the coincidence of vegetative growth period with increasing temperature and that shortening of the growth period reduces leaf area index and photosynthetic rate of the plant and ultimately, its yield. Delay in planting *Cuminum cyminum* L. also reduced the number of seed per umbel and subsequently, seed yield [27]. Acosta *et al*. [28] also showed that delay in planting reduced leaf area index (LAI), leaf area durability (LAD), crop growth rate (CGR), net assimilation rate (NAR) and dry matter production in *Phaseolus vulgaris* L., in their study, Boroomand-Rezazadeh *et al*. [29] found that delay in planting *Trachyspermum copticum L.* reduced biomass and seed yields per unit area.

Comparing the means of simple effect of nitrogen rate showed that the increase in nitrogen application increased the number of capitols per plant, number of seed per capitol, 1000-seed weight, seed yield, and dry weight of the aerial parts, so that with increasing nitrogen application from 0 to 240 kg N/ha, the mentioned traits significantly increased by 82.6, 27.2, 115.3, 362.2 and 187.3%, respectively (Table 5). It should be noted that although in case of the number of capitols per plant, seed yield and dry weight of the aerial parts, different rates of nitrogen application were divided into separate statistical groups, in case of the number of seed per capitol and 1000-seed weight, there was no statistically significant difference between the application rates of 160 and 240 kg N/ha (Table 5). Moreover, comparing the means of interaction effect between sowing date and nitrogen rate showed that the maximum number of capitols per plant (n: 170), seed yield per unit area (322.87 kg/ha) and dry weight of the aerial parts (1808.52 kg/ha) in the treatment of the sowing date of May 3 with the application of 240 kg N/ha had a significant superiority compared to other treatments (Table 6). Significant decrease in the number of branches per plant under delayed planting conditions and significant increase in the number of branches per plant with increasing nitrogen application (Table 3) may be considered as the main reason for the superiority of this treatment in above mentioned traits in the present study.

In both sowing dates, with increasing nitrogen application, seed yield increased significantly, and different rates of nitrogen fertilizer were placed in separate statistical groups; however, the treatments of the second sowing date with the application of 160 and 240 kg N/ha and the treatment of the first sowing date without application of nitrogen in case of the number of capitols per plant, and the treatments of the first sowing date without application and application of 80 kg N/ha and the treatment of the second sowing date with the application of 80 kg N/ha in case of the dry weight of the aerial parts were placed in similar statistical group (Table 6). It should be noted that with increasing nitrogen application from 0 to 240 kg N/ha in the first and second sowing dates, the number of capitols per plant increased by 2 and 1.6 times, respectively, seed yield per unit area increased by 4.9 and 4.5 times, respectively, and dry weight of the aerial parts increased by 2.8 and 2.9 times, respectively. It seems that the application of nitrogen provided the necessary conditions to increase in capitols number per plant and seed number per capitol by increasing the potential for vegetative growth and photosynthesis assimilates production (Table 3). In addition, since nitrogen is effective in pollination through providing the protein needed by pollen to move throughout the style and reach the ovule [30], probably with increasing nitrogen application, the percentage of flower and fruit formation increases, and hence increased number of capitols per plant and seed per capitol may be justified. It may also be concluded that the increase in the number of capitols per plant under nitrogen consumption indicates a limitation of source (leaf area and photosynthetic capability of the plant) under nitrogen deficiency conditions due to which there would be competition among different organs of each plant in receive assimilates, leading to the abortion of many of the flowers formed. However, with the increase in nitrogen application, the severity of abortion decreased, so that with increasing nitrogen application from 0 to 240 kg N/ha, there was a significant difference in the number of capitols produced per plant. In a study conducted by Shokhomgar [31], four fertilizer rates of 0, 50, 150 and 200 kg N/ha were examined in *Trigonella foenum graecum* and the positive effect of nitrogen on the number of pods per plant was reported.

Regarding the positive effect of nitrogen application on 1000-seed weight, it can be said that with sufficient access to nitrogen fertilizer, seed filling period and photosynthesis rate increases probably due to increased leaf area duration [32] and as a result, more photosynthetic assimilates is transferred to the seeds and 1000-seed weight increases. In other words, seed weight is a function of the rate and length of its filling period from two sources of current photosynthesis and remobilization in the plant, and it seems that with increasing nitrogen application, these components had more speed and duration, and as a result, 1000-seed weight increased significantly. Mental and Patel [32] also reported that as the application of nitrogen increased, the number of pod per plant, number of seed per pod and 1000-seed weight of *Trigonella foenum graecum* increased. They said that nitrogen plays a vital role in metabolic processes leading to increased flowering and fruiting in the plant. Therefore, increased nitrogen application improves the number of reproductive units per plant and unit area.

Improving the yield of *P. gnaphalodes* as a result of nitrogen fertilizer application may be a sign of proper nitrogen uptake by the plant, which leads to improved vegetative growth and better use of sunlight due to increased duration and leaf area index [33] and hence increased rate of photosynthesis and transfer of photosynthetic assimilates from source to sink [22]. In addition, since *P. gnaphalodes* is an indeterminate plant, the increase in vegetative growth, number of capitols per plant and 1000-seed weight (Table 3) as a result of increasing nitrogen application, leads to increasing seed yield and dry weight of the aerial parts. Shokhomgar [31] showed that the change in nitrogen application had a significant effect on biomass and seed yield of *Trigonella foenum graecum*, and the highest biomass and seed yield was related to the application of 150 kg N/ha (highest rate). There is a lot of evidence that nitrogen has a direct effect on photosynthesis content per unit area of leaf [34]; therefore, the more suitable conditions are provided for nitrogen uptake by plant, the more photosynthesis and yield may be expected. Similar results were reported by Mental and Patel [32] in study on the effect of increasing nitrogen application on vegetative and yield traits of *Trigonella foenum graecum.* In a other study, it was reported that the application of nitrogen fertilizer could increase biomass yield in *Momordica charantia* L. [35].

Percentage and Yield of Essential Oil

The results of analysis of variance showed that the simple and interaction effects of sowing date and nitrogen rate was significant on the percentage and yield of essential oil produced by the aerial parts at 1% level (Table 4).

Table 5 Means comparison of simple effects of planting date and nitrogen fertilizer on yield components, seed yield and essential oil of *P. gnaphalodes* (Vent.) Boiss.

Treatment	Number of capitols per plant	Number of seeds per capitol	1000-seed weight (g)	Seed yield (kg/ha)	Dry weight of aerial parts (kg/ha)	Percentage of essential oil	Essential oil yield (L/ha)
Sowing date							
May 3	121.33 a	68.58 a	0.198 a	175.36 a	1161.92 a	0.199 b	2.312 a
May 23	77.58 b	65.08 b	0.171 b	93.12 b	873.07 b	0.133 a	1.161 b
Nitrogen(kg/ha)							
0	71.83 d	58.83 b	0.111 c	50.91 d	545.51 d	0.118 c	0.644 d
80	86.00 c	62.67 b	0.173 b	87.62 c	746.85 c	0.165 b	1.233 c
160	108.83 b	71.00 a	0.213 a	163.12 b	1210.60 b	0.172 b	2.081 b
240	131.17 a	74.83 a	0.239 a	235.32 a	1567.03 a	0.209 a	3.275 a

Means with same letter(s) in each column were not significantly different at 5% level.

Table 6 Means comparison of interaction effect of planting date and nitrogen fertilizer on some of morphological traits, yield components, seed yield and essential oil of *P. gnaphalodes*

Sowing date	Nitrogen rate (kg/ha)	Plant height (cm)	Number of main branches	Number of capitols per plant	Seed yield (kg/ha)	Dry weight of aerial parts (kg/ha)	Percentage of essential oil	Essential oil yield (L/ha)
May 3	0	62.17 bc	12.67 c	86.00 d	71.57 e	637.77 e	0.125 de	0.797 ef
	80	59.83 cd	15.33 b	97.00 c	100.23 d	741.02 e	0.215 b	1.593 d
	160	65.33 ab	15.67 ab	132.33 b	206.77 b	1460.37 b	0.210 b	3.067 b
	240	68.50 a	17.00 a	170.00 a	322.87 a	18085.52 a	0.245 a	3.331 a
May23	0	44.17 e	10.00 d	56.67 f	30.257 f	453.24 f	0.112 e	0.508 f
	80	55.17 d	13.33 c	75.00 e	75.00 e	752.68 e	0.115 de	0.866 e
	160	58.50 cd	12.67 c	85.33 d	119.37 d	960.83 d	0.133 d	1.278 d
	240	63.33 bc	15.00 b	92.33 cd	137.77 c	1325.55 c	0.173 c	2.293 c

Means with same letter(s) in each column were not significantly different at 5% level.

Essential oil percentage and yield in the first sowing date had a significant superiority of 49.6 and 43.6%, respectively, as compared the second sowing date Essential oil percentage and yield in the first sowing date had a significant superiority of 49.6 and 43.6%, respectively, as compared the second sowing date (Table 5). In general, the increase in the percentage and yield of essential oil in the first sowing date was due to longer growth period and better vegetative growth of *P. gnaphalodes*, which is in agreement with the results of Ebadi *et al*. [36] on *M. recutita* L.

In addition, with increasing nitrogen application from 0 to 240 kg/ha, the percentage and yield of essential oil increased significantly by 1.77 and 5.1 times, respectively (Table 5). Many essential oil compounds of medicinal plants increase with increasing available nitrogen because nitrogen plays a role in the structure of these compounds [37]. In study of Rahmati [13] also found that the essential oil percentage of *M. recutita* increased to 84% under the influence of nitrogen application, which confirms the results of the present study. The essential oil yield is a function of the percentage of the essential oil and the dry yield of the aerial parts. On the other hand, both these traits were significantly reduced by delayed planting and decreased nitrogen application (Table 5). So, significant reduction in the yield of essential oil with delayed planting and reducing nitrogen consumption may be justified.Means comparison of interaction between sowing date and nitrogen rate showed that the highest percentage and yield of essential oil produced by the the aerial parts *P. gnaphalodes* were obtained in the treatment of sowing date of May 23 with the application of 240 kg N/ha, which had a significant superiority compared to other treatments (Table 6). It should be noted that with increasing nitrogen application from 0 to 240 kg N/ha in the first and second sowing dates, the percentage and yield of essential oil increased by 1.96 and 1.56 times, respectively. This indicates that the longer growth period of the plant along with the increase in available plant nitrogen stimulated biomass production and increased the percentage and yield of essential oil. In study on *M. recutita*, Farhodi *et al*. [38] reported that with increasing nitrogen fertilizer application, the percentage and yield of essential oil increased.

CONCLUSION

The results showed that the delay in planting and the reduction in nitrogen fertilizer application had a negative effect on capitols production capacity per unit area and 1000-seed weight mainly through reducing branch production per plant and led to reduced yields of seed, aerial parts, and essential oil of *P. gnaphalodes* (Vent.) Boiss. In general, the results of the present study showed that the treatment of sowing date of May 3 with the application of 240 kg N/ha to produce maximum yields of seed, aerial parts and essential oil of *P. gnaphalodes* (Vent.) Boiss. can be considered in Birjand region.

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