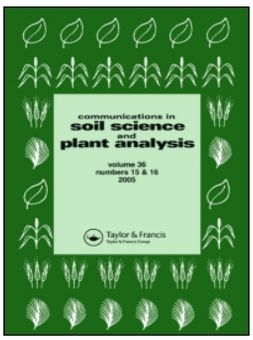

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Responses of Anise Medicinal Plant Species in Terms of Essential Oil Contents and Concentrations to Different Planting Times and Various Nitrogen Fertilizer Sources under Semi-Arid Climatic Conditions

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ABSTRACT

Essential oil content and concentration of anise plant planted in different times under various sources of nitrogen was evaluated in this study. The experiment was conducted in a split plot based on a randomized complete block (RCB) design (different sources of fertilizer as the main plot and planting dates as subplots) with three replications in the Agricultural Research Station at Ferdowsi University of Mashhad in 2015–2016. Treatments included: planting times (sub plot factor): (27th of October, 27th of November, 1st of March, 10th of March, and 8th of April) and nutritional sources (main plot factor): (Cow Manure, Chemical Fertilizer, and mixture of Cow Manure + Chemical Fertilizer). The results showed that the anise plant essential oil qualitative characteristics were significantly influenced by treatments. Also, interaction between the fertilizer treatments and planting dates on the essential oil content and concentration of the plants were significant. The highest percentage of plant essential oil (3.71%) was found in the cow manure treatment, and the lowest percentage of essential oil (2.95%) was obtained in the chemical fertilizer. The highest essential oil yield (16.09 kg/ha) was found in the mixed treatment of the cow manure and the chemical fertilizer, and the chemical fertilizer treatment had the lowest rate of essential oil yield (11.41 kg/ha). The highest percentages of the plant oil (14.31%) and the plant oil yield (77.46 kg/ha) were found in the integrated treatment of the cow manure and the chemical fertilizers. The lowest amounts of the plant oil (12.19%) and the plant oil yield (41.28 kg/ha) were found in the chemical fertilizer. According to the percentage and the yield of the essential oil, the best planting time and fertilizer treatment was the one on 10th of March and using integrated cow manure and chemical fertilizer.

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Chemical fertilizer; essential oil; medicinal plant; nutrient management; organic manure

Introduction

The undesirable side effects of chemical drugs use necessitate consideration of the medicinal plants use and interest in production of these plant species with regard to increasing demand for natural products throughout the world (Malik, Suryapani, and Ahmad 2011). Despite the improvements and the wide use of chemical drugs, there is still a tendency toward the use of medicinal plants, and in some regions of the world, they are an integral part of the health systems and the markets, and compared to the chemical drugs, the medicinal plants are much more available (Zhang 1998).

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Anise (*Pimpinella anisum* L.) is an annual medicinal plant species belonging to the Apiaceae family. The seeds of anise contain 1.5–5% aromatic essential oil. Anise seed yield, depending on the climatic conditions and the harvest time, is between 0.4 and 1 ton per hectare (Omid Bygi 2000).

Cultivation of medicinal plants has a special place in the Iranian traditional agricultural systems. In addition to providing domestic consumption and revenue, it plays an important role to make diversity and sustainability. Anise seed is used as a carminative, stomachic, anti-cough, and enhancing breastmilk. In the food industry, anise is used as a natural flavoring and aromatic agent, and its oil is used in perfumes and pharmaceuticals (Omid Bygi 2000)

Since the excessive use of chemical fertilizers causes problems and damage to the environment, in recent years, much attention has been given to the use of organic fertilizers and animal manure (Astaraei and Koocheki 1996)

Growth, development, and production of crops are influenced by genetic and agronomic factors. Choosing the optimum planting time in each area based on the climatic conditions of the region and appropriate nutritional systems are important factors affecting crops in order to achieve maximum economic yield (Thomas et al. 1990).

Many studies are carried out to determine the optimum planting time for anise and its responses to different sources of fertilizers. For example, Zehtab Salmasi, Javanshir, and Aliary (2001) compared the effect of planting dates in April and May for anise, and they found the plant height, harvest index, number of umbrellas, seed number, seed weight, and seed yield eventually reduced with delay in planting time (compared April to May). In a similar study, Azizi (1998) found the most suitable time for planting anise is in late March. It was reported in this study the planting postponement after this date reduced yield by 30–40%. In the case of other plants belonging to this family, Rahimian Mashhadi (1992) reported that delayed planting date resulted in the loss of yield and yield components of cumin (*Cuminum cyminum*).

The results of Akbarinia et al. (2005) showed that fall planting of anise and ajowain (*Trachyspermum ammi* L.) is not recommended due to the death of the emerged seedlings in Qazvin weather, but it is effective on the yield of fennel belonging to the family of anise.

Due to the fact that in the sustainable agricultural systems, product quality is as important as its quantity, the response of plants to different management practices, including their production without the use of synthetic inputs, is important. Hamzei, Najari, and Salimi (2014) reported that the integrated application of chemical and biological fertilizers can reduce production costs and increase the quality and quantity of anise. Kamaestani et al. (2013), in their experiment on different sources of fertilizers on anise, stated that the application of mycorrhizal combined with chemical fertilizers produced the maximum amount of essential oil (3.5%) and the percentage of the essential oil improved 60% compared to the control. The above researchers (Kamaestani et al. 2013) also reported that the use of integrated nutritional treatments compared with the single application of each of them has more effect on the improvement of the plant essential oil. Single application of fertilizer sources' traits on essential oil percent reduced it 8%, and integrated application increased it by 7%. Fall planting increased the growing period, and as a result, the plant produced more vegetative organs and yield and was able to feed a larger sink and, therefore, produced more yield (Hornok 1992). Also, the late winter and spring rains in most parts of the country delay the land preparation and planting time in spring. Randhawa, Gill, and Raychaudhuri (1992) reported that anise planting in early November increased grain yield, plant height, and number of the umbrellas. Taysi, Vomel, and Ceylan (1977) reported the highest anise yield was obtained in January and early February. The yield loss associated with delayed sowing dates of coriander and dill also has been confirmed by other researchers (Gupta 1982).

Cultivation of anise plant has been common in Khorasan Province, but the farmers are not aware of the ecological requirements of the plant, especially the optimum planting time. Delay in planting time has resulted in reduced production and minimum yield. It seems that planting time of anise has been changed due to climate change in recent years, and the response of this species to autumn planting is better than that in winter and spring.

The objectives of this experiment were to determine the optimum planting time for anise plant and to find the impact of nutritional use of this plant in order to advise farmers and to determine the level of decline in yield with delay in planting time.

Materials and methods

This field study was carried out in the Agricultural Research Station at Ferdowsi University of Mashhad, located 10 kilometers east of Mashhad (latitude 36°20′N, longitude 45°45′E). Before conducting the experiment, soil samples were taken from a depth of 0–30 cm and, together with the organic fertilizers, were analyzed for the physical and chemical properties (Tables 1 and 2). The experiment was performed in a split plot based on a randomized complete block (RCB) design (different sources of fertilizer as the main plot and planting dates as subplots) with three replications. Treatments include: planting times (sub plot factor): (27^{th} of October, 27^{th} of November, 1^{st} of March, 10^{th} of March, and 8^{th} of April) and nutritional sources (main plot factor): (Cow Manure, Chemical Fertilizer, and mixture of Cow Manure + Chemical Fertilizer). For the purpose of this experiment, Farm land was left fallow in the past 2 years, and the preparation of the land was based on the respective treatments. Cow manure was spread on the soil surface and immediately incorporated into the soil by a shovel.

Plots' dimensions were 2.4 × 2 m, and the distance between the plots was 120 cm, and one meter (1 m) between the replications. Distance between the planting rows was 60 cm and between the plants on the row was 10 cm, planting depth was 1 to 2 cm, and the plant density was 66 plants per square meter (m^2). Cultivation was double stack. Based on the laboratory analysis of soil samples, fertilizer recommendations per hectare for anise were given as 100 kg triple super phosphate, 150 kg potassium sulfate, and 100 kg urea. Then, the amount of nitrogen was recommended as an index for the amount of cow manure. Based on the amount of chemical nitrogen fertilizer (100 kg/ha), the amount of cow manure was calculated 16 tons per hectare.

It should be noted that according to the available sources, nutrients in cow manure are not entirely free in the first year, and only 40% to 50% are available and can be used by plants in the first year of the application (Pimentel 1993). Therefore, the amount of the cow manure was considered twice the amount of the nitrogen fertilizer. Organic fertilizer was added to the soil a month before planting and was mixed by a shovel.

All of the phosphorus and potassium and 1/3 of the nitrogen as chemical fertilizers were added to the plots in one application. The remaining (2/3) nitrogen fertilizer was used in two stages (seedling stage and at the beginning of the reproductive stage) as top dressing. Weeding and thinning of the plants were done manually by workers. Irrigation was done in 7-day intervals during the growing season.

The plant harvest was done on the first of August 2015, when the seeds were fully ripe. After harvesting, yield and yield components (plant height, umbels, umbelets, and number of seeds per umbrella, seed yield, biological yield and harvest index) were measured. In order to determine the percentage of essential oil, 50 g seeds were taken randomly from each plot, and the essential oil was

Table 1. Chemical and physical properties of the soil.

Soil texture	Organic carbon %	Total nitrogen %	P (mg/kg)	K (mg/kg)	pH	EC (dS/m)
Loam	0.59	0.063	13.2	135	7.24	1.21

Table 2. Chemical analysis of the cow manure.

Total nitrogen %	P (mg/kg)	K (mg/kg)	EC (dS/m)	pH
0.57	0.09	1.1	6.1	6.8

determined by distillation method and using Clevenger (Arganosa, Sosuskli, and Slikard 1998). The time required to extract the essential oil of the anise seeds is 4 h. To determine the oil percent, 2 g seeds were used from each plot. The seeds were grind, and the oil was extracted using fully automatic soxtec (SOXTEC SYSTEM HT6) soxhlet method following Stancheva et al. (2010) procedures.

The data analysis was performed using the Minitab Ver. 16 software, and the means comparison was done by Duncan's Multiple Range test.

Results and discussion

The results presented in Table 3 show that the anise plant qualitative characteristics were significantly influenced by treatments. The results of the analysis of variance of the data also indicate that interactions between the fertilizer treatments and planting dates on all characteristics of the plants were significant (Table 3).

Mean comparison effects of fertilizer treatments showed the highest percentage of plant essential oil (3.71%) was found in plants treated with cow manure and the lowest percent of essential oil (2.95%) was obtained in plants treated with the chemical fertilizer, which was not significantly different than those treated with the mixed cow manure and chemical fertilizer (Table 4). The results in Table 4 show that the highest essential oil yield (16.09 kg/ha) was found in the plants treated with the mixture of cow manure and chemical fertilizer, but it was not significantly different than those treated with only cow manure. Plants treated with chemical fertilizer had the lowest rate of essential oil yield (11.41 kg/ha) (Table 4).

The highest percentages of the plant oil (14.31%) and plant oil yield (77.46 kg/ha) were obtained in plants treated with the integrated cow manure and chemical fertilizers, which were not significantly different than those treated with only cow manure. The lowest plant oil percentage (12.19%) and plant oil yield (41.28 kg/ha) were obtained in plants treated with the chemical fertilizer (Table 4).

It seems that improvement of the soil physical and chemical properties by the application of the cow manure leads to the increased nutrient availability and ultimately will lead to an increase in the yield and crop quality. Because of the slow growth rate of anise plant, and also because of the simultaneous release of the elements in the cow manure and the plant's maximum nutrient requirement, the yield of the crop increased and its quality improved. It also seems that the integrated treatment of cow manure and chemical fertilizer caused plant at the beginning of the

Table 3. Analysis of variance (mean squares) of the quality traits of anise medicinal plant in response to planting date and fertilizer applications.

Source of variation	Df	Essential oil	Seed oil	Essential oil yield	Seed oil yield
Block	2	0.3527	1.998	62.10	1.334
Fertilizer	2	2.3341**	19.619**	57.98**	6.3210**
Error	4	0.1754	0.201	16.17	4.897
Planting time	4	6.9375**	115.611**	91.249**	6.8444**
Planting time* fertilizer	8	3.6411**	37.576**	22.149**	1.2577**
Error	24	0.0830	1.679	60.15	4.632

**Significant at 0.01 probability level.

Table 4. Mean comparison of the effects of fertilizer treatments and planting time on the quality of the anise plant.

Treatment	Essential oil %	Seed oil %	Essential oil yield (kg/ha)	Seed oil yield (kg/ha)
Cow manure	3.71^{a}	13.97^{a}	15.56^{a}	63.44^{ab}
Chemical fertilizer	2.95^{b}	12.19^{b}	11.41^{b}	48.21^{b}
Integrated cow manure+chemical	3.14^{b}	14.31^{a}	16.09^{a}	77.46^{a}
27^{th} of October	2.68^{c}	16.34^{a}	15.79^{b}	98.47^{a}
27^{th} of November	3.13^{b}	14.76^{ab}	17.63^{a}	83.58^{ab}
1^{st} of March	2.50^{c}	15.42^{ab}	9.11^{b}	56.71^{b}
10^{th} of March	4.63^{a}	13.60^{b}	20.54^{a}	58.26^{b}
8^{th} of April	3.38^{b}	7.33^{d}	8.68^{c}	18.17^{c}

In each column, the mean values followed by the same letters are not statistically different at the 0.05 probability level.

growing season take advantage of the nutrient elements released from the chemical fertilizer and afterward plant used the elements released from the cow manure in order to produce higher yield. Darzi, Haj Sed Hady, and Rejali (2012), evaluating the effects of the organic manure and nitrogen fixing bacteria on essential oil of coriander (*Coriandrum sativum*), reported that the maximum essential oil was obtained with applying cow manure. In another study on the effect of organic and chemical nitrogen fertilizers on fennel (*Foeniculum vulgar* Mill) essential oil, Delfieh, Modarres-Sanavy, and Farhoudi (2016) found that replacing 50% required nitrogen with cow manure in fennel could lead to improving seed quality similar to that with treatment with 100% chemical fertilizers.

Evaluation of the effects of the planting dates on the essential oil of anise plant showed that the highest percentage of essential oil (4.63%) was observed on the planting date of 10th March. The lowest percentage of the essential oil (2.68%) was observed on the 27th of October planting date (Table 4). The highest yield of essential oil (20.54 kg/ha) was found on the 10th of March planting date (8.68 kg/ha), and the lowest one was observed on 8th of April planting date (Table 4).

The moderate environmental stresses in medicinal plants can improve quality of the products as well as the quality of the secondary compounds. It seems that autumn planting dates due to the longer growing season and the availability of more water in the environment as well as less exposure time to stress reduced essential oil of this plant, although the plants produced more biomass. However, winter planting date due to less rainfall and mild stress lead to improved plant quality, while the plants produced less biomass compared to the autumn planting time with longer growth period. Because of the reduced length of the growing season and early plant maturity, the biomass production and yield decreased in spring planting times.

As shown in the results presented in Table 4, oil percentages of the plant on the planting date of 27th of October were the highest (16.34%) and the lowest (7.33) on the 8th of April planting date. Mean comparison effects of the planting date on plant oil yield showed the highest oil yield (98.47 kg/ha) on 27th of October, and the lowest (18.17 kg/ha) was on the 8th of April planting dates (Table 4). Since oil is the first component of the plant, any factor that increases it and affect its quantitative traits can also have effect on the plant oil percentage and yield. Availability of water and nutrients in the environment and the long growing season caused the plant have higher production by better use of the available water and nutrients. A short growing season and early spring planting date decreased the quality and the quantity of the products. Taysi, Vomel, and Ceylan (1977) reported that the highest anise yield was observed in January and early February.

The interaction effects showed that the highest percentage of the essential oil (6.5%) was obtained on 10th of March planting date with the cow manure treatment, but there was not statistically significant difference between the integrated cow manure and the chemical fertilizer treated plants. Also, anise plant had the lowest essential oil percentage (1.57%) on the planting date of the 1st of March with the cow manure treatment (Table 5).

The highest oil yield (33.41 kg/ha) was obtained in anise plants planted on 10th of March and treated with the integrated cow manure and the chemical fertilizer, and the lowest yield (3.57 kg/ha) was obtained in plants treated with the chemical fertilizer and planted on the 8th of April (Table 5).

The results in Table 5 also show that the highest percentage of oil (23.29%) was found in anise plants planted on 27th October and treated with the integrated cow manure and chemical fertilizer. The lowest percentage of oil (6.67%) was obtained in plants treated with the integrated cow manure and chemical fertilizer and planted on the 8th of April (Table 5). The highest oil yield (158.91 kg/ha) was observed in anise plants planted on 27th of October and treated with the integrated cow manure and chemical fertilizer. The lowest rate was observed in anise plants planted on 8th of April and treated with the chemical fertilizer (Table 5).

Table 5. Mean comparison of the interaction between fertilizer treatments and the planting time on the quality of the anise plant.

Treatment	Essential oil %	Seed oil %	Essential oil yield (kg/ha)	Seed oil yield (kg/ha)
27th October, cow manure	3.07^{ced}	13.40^{cd}	15.69^{bcd}	68.84^{bc}
27th November, cow manure	3.09^{bc}	19.00^{b}	24.57^{ab}	119.03^{ab}
1st March, cow manure	1.57^{g}	14.3^{cd}	6.33^{bcd}	54.56^{bc}
10th March, cow manure	5.60^{a}	15.00^{bcd}	20.07^{bc}	53.71^{bc}
8th April, cow manure	4.40^{b}	8.33^{ef}	11.14^{cd}	21.08^{c}
27th October, chemical fertilizer	2.63^{def}	12.33^{ef}	15.98^{bcd}	67.66^{bc}
27th November, chemical fertilizer	2.93^{de}	12.70^{d}	15.43^{bcd}	68.59^{bc}
1st March, chemical fertilizer	3.97^{bc}	17.29^{bc}	13.72^{bcd}	60.13^{bc}
10th March, chemical fertilizer	2.90^{def}	11.60^{de}	8.15^{cd}	32.97^{c}
8th April, chemical fertilizer	2.3^{efg}	7.00^{f}	3.76^{d}	11.69^{c}
27th October, chemical+ cow	2.33^{efg}	23.29^{a}	15.70^{bcd}	158.91^{a}
27th November, chemical+ cow	2.57^{def}	12.59^{de}	12.89^{bcd}	63.10^{bc}
1st March, chemical+ cow	1.97^{fg}	14.83^{bcd}	7.29^{cd}	55.44^{bc}
10th March, chemical+ cow	5.40^{a}	14.20^{cd}	33.41^{a}	88.09^{abc}
8th April, chemical+ cow	3.45^{bcd}	6.67^{f}	11.15^{cd}	21.75^{c}

In each column, the mean values followed by the same letters are not statistically different at the 0.05 probability level.

Conclusions

According to the results of this study, using organic fertilizers, especially cow manure as a suitable nourishment source, has a significant effect on increasing quality of the yield of the anise medicinal plant. So, in all traits, use of cow manure separately or mixed with the chemical fertilizers had a substantial effect on increasing the yield and the yield components compared with using only chemical fertilizers. Therefore, cow manure or its mixture with the chemical fertilizers can be considered as an alternative for chemical fertilizers in the production systems of this plant. Also, due to the high yield and improved quality of the anise plant planted on 10th of March compared to other planting dates, and since the main purpose of anise production is its essential oil production, cultivation of this plant is recommended at this date. Planting after this date decreases the crop yield and oil content. Also, according to the percentage and the yield of the essential oil, the best planting date was the one on 10th of March and using the integrated cow manure and chemical fertilizer.

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