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The Effects of Different Fertilizer Applications on Yield and Quality of Anise (*Pimpinella anisum* L.)

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Abstract

This study was conducted in the experimental field of the Faculty of Agriculture at Eskişehir Osmangazi University in 2022. The aim of the study was to investigate the effects of different organic and inorganic fertilizers (compound fertilizer, vermicompost fertilizer, bat guano, bacterial application, and commercial organic liquid fertilizers such as Micro and Forceful) together with a control application on the morphological and yield criteria of organically grown anise plants under dry conditions in Eskişehir.

There was no statistically significant difference in plant height and essential oil content. Umbel number, seed yield per plant, seed yield per decare, and thousand seed weight parameters were found to be statistically significant at the 5% level. The highest umbel number (13.93), seed yield per plant (1.17 g/plant), seed yield per decare (46.8 kg/da), and thousand seed weight (3.43 g) were obtained from the Micro application. The highest essential oil content (4.56%) was obtained from the Micro application, and the lowest (3.92%) was obtained from the compound fertilizer application. The trans-anethole ratio, which is the most important component of essential oil, was determined to be 89.4-91.5%, and the highest ratio was obtained from the bat guano application.

Key words

Anise, *Pimpinella anisum*, Organic fertilization, Seed yield, Essential oil.

Introduction

The Apiaceae family consists of vegetables such as carrots, celery and medicinal and aromatic plants such as anise, parsley and coriander and is represented by 434 genera and about 3780 species worldwide. (McCormick, 2006; Wang et al., 2022). In Turkey, the Apiaceae family is represented by 104 genera and 486 species. Four of these genera in Turkey are endemic and monoecious. 182 species belonging to 45 genera are endemic. The endemism rate of the Apiaceae family in Turkey has been determined to be 37% (Duran et al., 2015). Anise (*Pimpinella anisum* L.), which belongs to the genus *Pimpinella* in the Apiaceae family, is an economically important medicinal and aromatic plant cultivated in our country. (Baydar, 2016). It is believed that the origin of anise is the Middle East and ancient Egypt (Hemphill and Hemphill, 1988). Anise grows naturally in India, Iran, Egypt and many warm climate regions around the world (Ceylan, 1987; Besharati-Seidani et al., 2005).

Anise is an annual herbaceous plant with 2n=20 chromosomes. The plant has a highly variable number of branches, and at the end of each branch, there is a structure called an umbel, which consists of clusters of flowers called umbellet, ranging from 8 to 15 in number. Each umbel produces 100-200 seeds, and the weight of 1000 seeds is approximately 2-3 g. The number of branches, umbels, umbellet, and seeds per umbel are important characteristics that affect the yield of anise (Baytop, 1971; Doğan et al., 2018; Baydar, 2020). In this study, vermicompot, bat guano, bacterial application, micro and forceful organic liquid commercial fertilizers that can be used in organic aniseed cultivation and chemical compound fertilizer containing nitrogen, phosphorus and potassium were used to represent common farmer practice in order to provide verified information to farmers and entrepreneurs. Different fertilizer applications have been compared by looking at the morphological characteristics, seed yield, essential oil ratio, and trans-anethole ratio values of the product obtained as a result of this fertilization.

Materials and Methods

This study was conducted in 2022 at the experimental field of Osmangazi University Faculty of Agriculture in Eskişehir. In the experiment set up according to a randomized complete block design with three replications, row spacing was set as 35 cm and each plot had a row length of 3 m and a plot width of 1.4 m. To prevent fertilizer applications from affecting each other within a single repetition, a distance of 50 cm was left between each application. The Denizli population of anise seeds were obtained from the Denizli Commodity Exchange and sown in a previously prepared field on March 29th, considering the climatic and soil conditions. The seeds were sown at 1 cm depth at a rate of 1.5 kg per decare. The climatic data for the production season and long years are given in Table 1., and the soil analysis of the experimental area is given in Table 2. Soil analysis was carried out at the Soil Science and Plant Nutrition laboratories of Eskisehir Osmangazi University, Faculty of Agriculture.

Table 1. Climate data (MGM, 2023)

Months	Long Years			2022		
	Temp. (°C)	Precipitation (mm)	Humidity (%)	Temp. (°C)	Precipitation (mm)	Humidity (%)
January	1	44.63	83	-0.1	26.4	79.2
February	3.3	29.49	74.3	3.3	36.5	76.2
March	7.1	30.46	66.7	1.4	25.6	67.6
April	11.6	32.43	61.2	13.2	8.1	51.7
May	16.5	39.66	59.8	16.4	25.1	54.5
June	20.2	63.11	60.5	19.9	78.2	65.5
July	23.1	15.53	54.8	21.7	18.9	55.1
August	23.3	16.36	55	23	33.5	64.8
September	19	14.01	58.5	18.8	4.8	56.8
October	13.4	25.14	68.7	12.7	20.4	70.9
November	7.6	24.09	74	9.4	9.9	67.5
December	2.9	30.06	82.3	5.9	19.2	84.9
Total		364.97			306.6	
Mean	12.4	30.41	66.6	12.1	25.55	66.2

When the April 2022 precipitation data is analyzed, lower precipitation is observed compared to the April precipitation data of many years. The low rainfall occurred during the first month after sowing, causing delayed germination of the seeds and low emergence rates, which affected the yield. The monthly rainfall amount obtained between March and September, which is important for vegetative growth, in 2022 (194.2 mm) is below the long year's monthly rainfall amount (221.56 mm) for the same months. The

average temperature value (16.3 °C) obtained in March and September of the experimental year was lower than the long years average temperature (17.2 °C) for the same months. The average humidity value (59.4%) in March and September of the experimental year was similar to the long year's average humidity value (59.5%) for the same months.

Table 2. Soil properties of the experimental area

Electrical Conductivity (dS/m)	pH	$CaCO_3$ (%)	Organic Matter (%)	Sand (%)	Silt (%)	Clay (%)
0.21	8.29	5.81	1.15	35.56	30.18	34.26

According to the soil analysis conducted at the Eskişehir Osmangazi University, Faculty of Agriculture, Soil Science and Plant Nutrition laboratories, the experimental site had an electrical conductivity value of 0.21 dS/m, indicating that the soil was non-saline. The pH value was 8.21, indicating that the soil was slightly alkaline. The CaCO3 value was 5.81%, indicating that the soil was moderately calcareous. The organic matter content was low, with a value of 1.15%, and the soil texture was clay-loam. (Gözükara, 2021).

NPK 10-18-12 (20 SO3) + ME (No details about micro element content on the package) compound fertilizer were applied to the soil at a rate of 5 kg N per decare along with the sowing process. Vermicompost fertilizer (0.73% N, 0.38% P, 0.55% K) was applied in powder form at a rate of 442 kg per decare along with sowing (Adiloğlu vd, 2015). Bat guano has been diluted with enough water and applied to the field at a rate of 476 kg per decare, along with the sowing. *Bacillus pumilus* bacteria-derived biomobilizer-P fertilizer was applied with sowing by slightly wetting the seeds before sowing and smearing them with this fertilizer Bacteria was applied by infecting the seed with 1.5 grams of bacteria per kilogram of seed before sowing. The amino acid-based "Forceful" commercial organic liquid fertilizer (3% organic nitrogen) and the commercial "Micro" organic liquid fertilizer containing micro elements (0.2% B, 0.3% Cu, 0.8% Fe, 0.4% Mn, 0.3% Zn) was applied to the leaves by diluting with water to 1190ml per decare, once during the vegetative growth period and again at the beginning of the flowering period. Forceful and micro were prepared as 1.25% solution and 400 ml was applied to each plot. These amounts were determined based on the manufacturer's recommended amounts on the package according to the products.

In order to determining the essential oil content, water distillation was performed using a 1000 ml Clevenger apparatus with a flask heater. Anise seeds were ground to 10 grams using a Bosch brand coffee grinder, 15 minutes prior to the distillation process and placed in the flasks. Then, 200 ml of distilled water was added to each flask and the distillation process was carried out for 3 hours. Gas Chromatography/Mass Spectrometry was used for identifying the components of the essential oil obtained from anise, and Gas Chromatography was used for determining the relative percentages. An extract prepared with hexane (10% v/v) was injected into the system at a split ratio of 40:1 and 1 µl (Karık, 2020). These procedures were conducted at Anadolu University, Plant, Drug and Scientific Research Application and Research Center.

Results and Discussion

The analysis of variance table for all the parameters measured in the study is shown in Table 3.

Table 3. Analysis of variance for measured parameters mean squares

Variation Sources	Degrees of Freedom	Plant Height	Umbel Number	Seed Yield per Plant	Seed Yield per Decare	Weight of 1000 seeds	Essential Oil Ratio
Repetition	2	189.270	3.042	0.053	84.310	0.263	0.537
Fertilizers	6	16.755	12.595*	0.140*	224.082*	0.178*	0.129
Error	12	11.532	0.397	0.007	11.928	0.048	0.453

*: Statistically significant at the 0.05 level.

Plant Height (cm)

The changes in average plant heights according to the applications are given in Table 4. The highest plant height (40.23 cm) was obtained from the micro fertilizer application, while the lowest plant height (33.63 cm) was obtained from the vermicompost application.

In the study conducted in Damavand ecological conditions in 2009, the highest plant height (50.1 cm) was obtained from the application of 10 t/ha vermicompost. When phosphorus-solubilizing bacteria were used, the highest plant height was found to be 47.4 cm (Darzi et al., 2012). In the fertilizer study conducted under Iran conditions, the highest plant height (42.92 cm) was obtained from the application using cow manure, while the lowest plant height (39.40 cm) was obtained from the application using compound fertilizer (Faravani et al., 2013).

Number of Umbel

The highest number of umbels (13.93) was obtained from the application of micro fertilizer, while the lowest number of umbels (8.76) was obtained from the application of compost fertilizer.

Under the study conducted in Damavand ecological conditions, the highest umbel number (33.2) was obtained from the application using 10 t/ha vermicompost. When phosphorus-solubilizing bacteria were used, the highest umbel number was found to be 29.5 (Darzi et al., 2012).

In another fertilization study conducted in Iran, the highest number of umbrellas (31.56) was obtained from the application using cow manure, while the lowest number of umbrellas (23.89) was obtained from the application using compound fertilizer (Faravani et al., 2013).

In general, the number of umbels obtained in this study, ranging from 8.76 to 13.93, was lower than those obtained in studies conducted in Iran. Similarly to plant height, the low number of umbels (8.76-13.93) obtained in this study can be explained by the prolonged drought period after sowing and delayed emergence, leading to a weak seedling development phase.

Seed yield per plant (g/plant)

The highest seed yield per plant (1.17 g/plant) was obtained from the application using micro fertilizer, while the lowest seed yield per plant (0.47 g/plant) was obtained from the application using vermicompost. The difference between the treatments was found statistically significant at 5% level.

Seed yield per decare (kg/da)

The highest seed yield per decare (46.08 kg/da) was obtained from the application of micro fertilizer, while the lowest seed yield per decare (19.07 kg/da) was obtained from the application of vermicompost fertilizer. The difference between the treatments was found statistically significant at 5% level. In the fertilizer study conducted on anise plant in Belgrade, Serbia, four different applications were tested: Baktofil (80 l/ha), fertikare (250 kg/ha), organic fertilizer (10 t/ha), and control. In 2004, the highest seed yield (88.7 kg/da) was obtained from the baktofil application, while the lowest seed yield (39.7 kg/da) was obtained from the control (Jevdjovic and Maletic, 2006). In the study conducted in Serbia in 2011 and 2012, the highest yield of was obtained when 40 kg of compound fertilizer (15-15-15 NPK) was applied per hectare. Regarding the application of organic fertilizers, the highest yield was obtained when 500 kg of vermicompost was applied per hectare (Acimovic, 2013). The low average seed yields (19.07-46.08 kg/da) found in this study are due to some negative weather conditions during the germination and early seedling development period. Since seed yield is highly influenced by the genotype of the plant as well as by ecology and agronomic practices, a lot of variation can be observed.

Weight of 1000 seeds (g)

The highest weight of 1000 seeds (3.43 g) were obtained from the application of micro fertilizer, while the lowest thousand grain weight (2.77 g) was obtained from the control application. The difference between the treatments was found statistically significant at 5% level. In the fertilization study of anise plant conducted in Belgrade, Serbia, in 2003, the highest weight of 1000 seeds (2.43 g) were obtained from the application of baktofil (commercial), while the lowest weight of 1000 seeds (2.14 g) were obtained from the application of organic fertilizer. In 2004, the highest weight of 1000 seeds (2.62 g) were obtained from the application of organic fertilizer, while the lowest weight of 1000 seeds (2.47 g) were obtained from the application of baktofil. (Jevdjovic and Maletic, 2006). In the study conducted under Damavand ecological conditions, the highest weight of 1000 seeds (2.16 g) were obtained from the application of 5 t/ha vermicompost, while the lowest weight of 1000 seeds (2.11 g) were obtained from the control application. (Darzi et al., 2012). In the study where organic fertilizers were tested under Iranian conditions, the weight of 1000 seeds (3.88 g) were obtained from the control application, while the lowest weight of 1000 seeds (3.00 g) were obtained from the applications using cow manure and vermicompost (Faravani et al., 2013). The weight of 1000 seeds values found between 2.77-3.43 g in this study were generally similar to the values obtained by other researchers.

Essential Oil (%)

The highest essential oil content (4.56%) was obtained from the micro fertilizer application, while the lowest essential oil content (3.92%) was obtained from the vermicompost application. There was no statistical

difference between the treatments at 5% level. In the fertilization study conducted in Iran, the highest essential oil content (4.1%) was obtained from the application of compound fertilizer, while the lowest essential oil content (3.69%) was obtained from the application of vermicompost. (Faravani et al., 2013). In a fertilization study conducted in Belgrade, Serbia, the highest essential oil content (3.90%) was obtained from the application of biohumus, while the lowest essential oil content (3.64%) was obtained from the control group. (Acimovic, 2015). In the fertilizer study conducted on anise in Aydın ecological conditions in 2015, the highest essential oil content was obtained from the Çeşme ecotype with a commercial fertilizer x organic fertilizer application and two irrigations, with a rate of 1.86%. The researchers also recorded that the ratio of trans-anethole, main compound of the essential oil, varied between 97.5-98.49% depending on the application (Doğramacı and Arabacı, 2015). In Iran, a study was conducted to increase the essential oil content, yield, and trans-anethole ratio in the essential oil of anise by applying three different doses of vermicompost (0, 5, and 10 tons/ha), an Azotobacter mixture, and two different doses of zeolite (0 and 4.5 tons/ha). The results showed that vermicompost and zeolite applications were effective in increasing the essential oil content, yield, and trans-anethole ratio in the essential oil (Khalesro et al., 2012).

Compounds of Essential Oil

The results of the essential oil component analysis of all applications are given in Table 5. While trans-ethanol was the common main component in all treatments, the ratios were lowest in compound fertilizer (89.4%) and highest in bat guano (91.5%). It can be said that the effect of chemical and organic fertilizers on the main constituent of anise essential oil is not significant. In addition, estragol between 1.5-2.7%; γ-Himakalen between 1.7-2.7%; zingiberen between 0.7-0.8%; trans-Psödoizoöjenil-2-metilbutirat between 2.1-3.3%; were determined as other important components in all treatments.

Table 4. Mean values of all measured parameters

Parameters	Control	Compound Fertilizer	Vermicompost	Bat Guano	Bacterial Practice	Forceful	Micro
Plant Height(cm)	39.53	35.43	33.63	39.06	37.33	38.03	40.23
Number of Umbel (no)	12.93_{ab}	8.76_{c}	9.83_{c}	12.43_{b}	9.86_{c}	13.37_{ab}	13.93_{a}
Seed Yield per Plant (g/plant)	0.77_{bc}	0.74_{bc}	0.47_{d}	0.66_{bc}	0.61_{cd}	0.8_{b}	1.17_{a}
Seed Yield per Decare (kg/da)	30.93_{b}	29.73_{b}	19.07c	26.5_{b}	24.53_{bc}	31.83_{b}	46.8_{a}
Weight of 1000 Seeds (g)	2.77_{c}	3.16_{abc}	3.3_{ab}	2.97_{bc}	3.23_{ab}	2.87_{bc}	3.43_{a}
Essential Oil Ratio (%)	4.19	3.92	4.23	4.39	4.07	4.16	4.56
Trans Anethole (%)	90.6	89.4	90.2	91.5	90.1	89.7	90.9

The difference between the means shown with different letters is statistically (0.05) significant.

Table 5. Compounds of essential oil

Compound*	Control	Compound Fertilizer	Vermicompost	Bat Guano	Bacterial Practice	Forceful	Micro
Estragol	2.3	2.0	2.7	1.5	2.5	1.8	2.1
γ-Himakalen	2.2	2.7	1.9	1.7	2.3	2.6	2.2
Zingiberen	0.7	0.7	0.7	0.7	0.7	0.8	0.7
Trans-anethole	90.6	89.4	90.2	91.5	90.1	89.7	90.9
Trans-Psödoizoöjenil-2-metilbutirat#	2.1	3.0	2.5	2.6	2.5	3.3	2.2
Epoksipsödoizoöjenil-2-metilbutirat#	0.4	0.5	0.5	0.5	0.4	1.4	0.5
Total:	98.3	98.3	98.5	98.3	98.6	99.6	98.6

*1-5 Main Compounds # Identified from spectrum similarity

Conclusion

In this study conducted on aniseed in order to create a wide literature on plant basis in organic agriculture, which has an important place in crop production, although the organic fertilizers used increased the yield, there was no statistical difference compared to the control application in fertilizers other than micro element fertilization. It is thought that the insufficient and irregular amount of precipitation in 2022 is the reason for this difference. It has been observed that the late and insufficient germination due to insufficient and irregular rainfall reduces the yield and quality by being under the pressure of weed, which are nature's own children resistant to difficult conditions. Repeating this experiment for another year under Eskişehir conditions, or repeating it under different site conditions, will show the effect of climate on the experiment.

As mentioned before, late germination and low germination rate cause weed pressure in organic cultivation of anise plant. Until early germinating anise varieties are developed, it is believed that cultivating anise organically on a large scale will be challenging because pesticide use is not allowed, and controlling weeds through mechanical means would require a significant amount of labour.

Although climate change has become visible in recent years, when we look at the past few years and beyond, the lack of literature on the chemical and organic fertilization of anise plant and other related topics was observed during the research. Therefore, it is recommended to conduct more studies on anise plant considering climate change.

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Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

The contribution of the authors is equal.

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