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ORIGINAL RESEARCH

Variability in fennel fruit essential oil, fixed oil and their compositions under organic and inorganic fertilizers

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Abstract

Purpose: *Foeniculum vulgare* (fennel) is one of the most important medicinal and aromatic plants well known for its essential oil and fruit value. The research on adding vermicompost and sheep manure to the soil is an important area of applied agricultural research in sustainable development. The objective of our study was to compare the fixed and essential oil composition of fennel in organic and conventional farming systems to find out the impact of organic farming system.

Method: In this study, different doses of vermicompost and sheep manures (10, 15, 20 t/ha) and ammonium sulphate (40, 80, 120 kg/ha) were applied by sowing and compared to a control group (no manure). Essential oils were extracted in Neo-clevenger apparatus, fixed oils were extracted in Soxhlet device. These oil components were quantified using Gas chromatography coupled to mass spectrometry with a flame ionization detector.

Results: The obtained results showed that the application of 10 t/ha sheep manures not only increased the content of essential oils, but also increased the concentration of the main components, such as camphor and *p*-anisaldehyde. While the highest fixed oil was achieved in the application after 120 kg/ha ammonium sulphate, the application of 10 t/ha sheep manure had a higher fixed oil content than vermicompost manure. The maximum petroselinic acid content was obtained from vermicompost manure.

Conclusion: The present study clearly indicated that inorganic fertilizer could be replaced by organic manures due to the higher positive impacts on essential oil and essential oil components, fixed oil and fatty acids.

Keywords: Fennel, Chemical properties, Organic manure, Essential oil, Fixed oil

Introduction

Fennel (*Foeniculum vulgare* L.), a member of the Apiaceae or umbelliferae, is an annual or perennial plant commonly used as a vegetable, spice, and aromatic plant. According to the literature, *Foeniculum vulgare* L. is divided into two types of cultivars: sweet fennel or var. dulce and bitter fennel or var. vulgare (Khammassi et al. 2018). The essential oil extracted from the fruit can be used as a flavoring additive in foods, pharmaceuticals and cosmetics (Yaldiz and Camlica 2019).

Compared to other plants in the Umbelliferae family, fennel is known to have a more relaxing and stimulating effect such as gastrointestinal issues, hormonal disorders, reproductive, and respiratory diseases. Its use as a carminative is increasing in the low-level digestive system disorders. Traditionally, fennel has been used for other purposes, including animal health control (Mwale et al. 2006).

Increased market demand for fennel has improved its cultivation and the crop has become extremely important. There is a growing demand for fennel without pesticides and chemical fertilizers (Malhotra and Vashishtha, 2008). In addition, environmental and agricultural practices in fennel cultivation affect fennel yield and quality (Shahat et al. 2011). For this reason, recent developments in agricultural systems have led to models of organic farming and generalize the development of organic farming. Because the application of chemical fertilizers has negative impacts on environment and biodiversity (Pretty and Bharucha 2014).

Agricultural systems, especially techniques to increase soil fertility in marginal areas, must always be improved. One of the most important agricultural techniques used to increase the yield and quality of conventional crops is fertilization (Yaldiz and Camlica, 2023). The nitrogen fertilizer adequately develops the production, yield and quality properties of fennel. It was reported that growth and yield traits of fennel increased with the N fertilizer (Ehsanipour et al. 2012). As opposed, Kandeel et al. (2002) and Chatzopoulou et al. (2006) reported that that application of N had no marked impact on yied components of fennel. Along with the aim of reducing fertilization costs by using renewable energy forms, the harmful effects of chemical fertilizers and pesticides have shifted researchers' attention to organic amendments such as vermicompost (VM) that can increase the production of crops and protect the environment from harmful pests without polluting the environment.

Among organic fertilizers, VM is better than traditional composted organic fertilizers in terms of improving soil health. It suppresses soil-borne pathogens that can manipulate the physicochemical and microbiological environment of the soil (Sahni et al. 2008). As a matter of fact, VM contains antagonistic organisms to control plant and soil pathogens because it is microbially rich, therefore it is an effective biocontrol. In addition, the presence of humic acid fraction in an organic additive makes it agronomically efficient and environmentally friendly (Senesi et al. 2007). Sheep manure, another animal-derived organic fertilizer used in our study, can be used as soil conditioners to improve organic C, increase nutrient uptake and promote plant growth. It plays a role in buffering soil acidification and protecting essential cations and increasing water use efficiency (Zhang et al. 2015). It contains nitrogen, potassium and carbon like inorganic fertilizers (Ansah et al. 2019).

Pharmacological use of fennel components, metabolite identification, phytochemical content, as well as ethnobotanical use in society still attract the attention of many researchers around the world (Rather et al. 2016). However, research on quality and efficient fennel cultivation for industrial purposes is very limited. On the other hand, there are not many scientific studies on the cultivation of organic or good agricultural practices within the scope of sustainable agriculture in fennel cultivation. So, in this study, fennel was grown with different doses (10, 15 and 20 t/ha) of vermicompost and sheep manure, ammonium sulfate (40, 80, 120 kg/ha) fertilizers with a control (no fertilizer or manure). We have chosen fennel for our study because it is one of the most popular culinary fruits worldwide, and is often commercially produced in field condition, used by local people for medicinal purposes or as a nutrient.

In this study, the essential oil, fixed oil amounts, essential oil components and fatty acids of fennel fruit grown in different doses of sheep, vermicompost and ammonium sulfate fertilizer were compared. This study has the potential to lead the production and market of high value-added secondary metabolites instead of raw drugs by increasing the quality and quantity of valuable compounds of fennel grown in different doses of sheep, vermicompost and ammonium sulfate fertilizers and their trade.

Material and method

Growing conditions and treatments

The field experimental site was located at the Tefenni District of Burdur (37° 18′ 34.2072” N, 29° 46′ 28.9740” E), Türkiye which is located at about 1163 elevation. The seeds of the fennel were obtained from Tefenni District of Burdur, Türkiye, in the year 2020. In this study, doses of the sheep manure (SM) and ammonium sulphate (AS) were applied as reported by previous studies (Meena et al. 2007; Bucagu et al. 2017). Additionally, the doses of VM were established in accordance with the previous studies and suggestion of a commercial company (Bayram et al. 2021).

Fennel fruits were sown according to the randomized complete block design (RCBD) with three repetitions on 26 March 2020. Each plot included five rows, with 20 cm between each plant and 40 cm between each row. One meter was applied as space between the blocks. The nutrient composition of the experimental soil with VM and SM fertilizers were determined before their uses in experiment. SM has the highest organic matter, electrical conductivity (EC), mineral element contents than VM, and VM has higher pH and total nitrogen content than SM (Table 1).

The experimental soil textures were determined and shown in Table 1. The climatic data of the vegetation period was noted as 16.83 °C temperature, 29.8 mm rainfall, and 52.78% humidity (Çelik 2023).

Table 1. The properties of experimental soil and used organic fertilizers.

Properties	Unit	Soil	Vermicompost	Sheep manure
pH		8.1	8.4	7.7
$CaCO_3$	%	8.7	-	-
EC	dS/m	536.0	5.3	6.1
Sand	%	61.0	-	-
Clay	%	23.0	-	-
Silt	%	16.0	-	-
Organic matter	%	1.2	41.2	63.4
P	mg/kg	21.0	23000.0	14166.7
K	mg/kg	272.0	15000.0	18900.0
Ca	mg/kg	6382.0	-	42266.7
Mg	mg/kg	1345.0	-	7361.7
Fe	mg/kg	5.1	-	1368.0
Mn	mg/kg	4.7	-	154.4
Zn	mg/kg	0.3	181.6	599.6
Cu	mg/kg	0.9	52.4	77.0
Moisture	%	-	28.8	7.7
Organic carbon	%	-	21.8	-
C/N	%	-	10.7	-
Organic nitrogen	%	-	1.9	-
Total humic and fulvic acid	%	-	34.8	-
Total Nitrogen	%	-	2.0	1.4

Full dose of SM (10, 15, 20 t/ha), and VM (10, 15, 20 t/ha) were applied 2 weeks before sowing in field conditions. Half of AS (20, 40, 60 kg/ha) and total of diammonium phosphate (DAP) (150 kg/ha) were applied at the sowing time. The remaining AS (20, 40, 60 kg/ha) was added to related plot before the flowering time. Fennel was regularly irrigated to demonstrate good progress in its period vegetation. Fennel fruit harvests were carried out in August 2020. In the study, there were ten treatments described in Fig. 1.

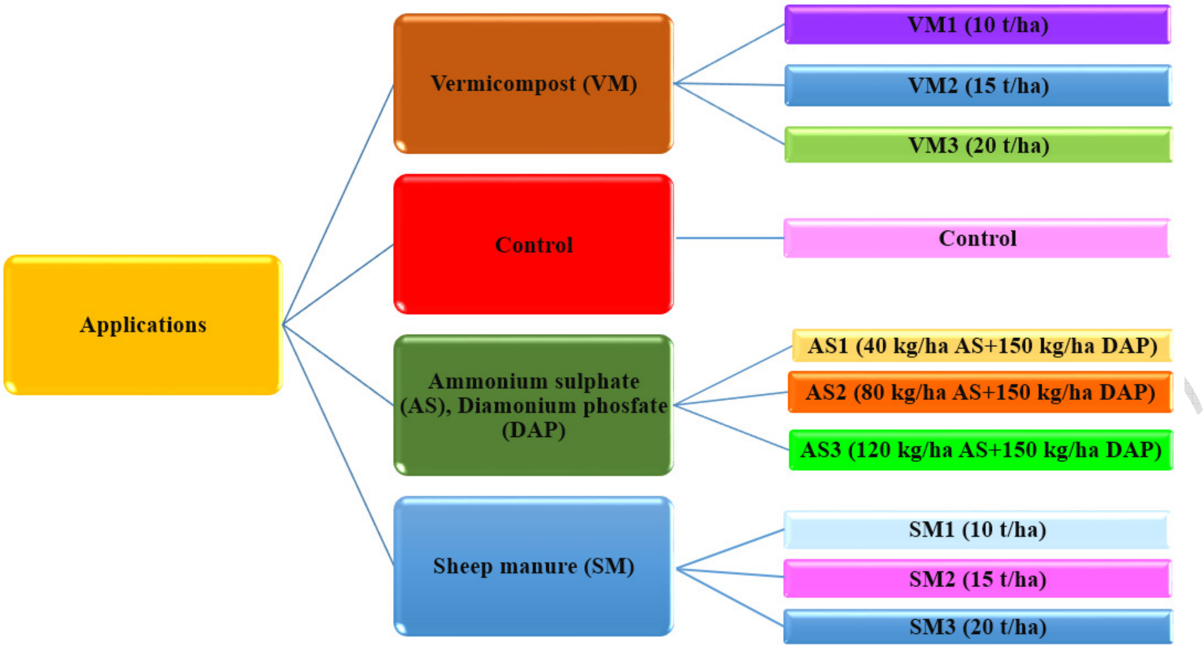

Fig. 1 Treatment codes and description of the study

Isolation of essential oil and fixed oil

The essential oil and fixed oil contents of the fennel fruits grown under SM, VM and AS fertilizers were determined. The essential oil contents of the the dried fennel fruits (200 g) were subjected to distillation with water for 3 hours using a clevenger type apparatus according to TS 8882 method. Anhydrous sodium sulfate was used to dry the isolated essential oils and stored at 4 °C until further use. Essential oil yields were estimated following each sample dried weight. To determine the fixed oil contents, the fennel genotype fruits were crushed to 5 g and extracted at 60 °C with a Soxhlet extractor for 8 hours using n-hexane as a solvent. After oil extraction the solvent was removed by a rotary evaporator, and the oil content was weighed to determine the fixed oil content depending on a dry weight basis and expressed as percentages (Yaldiz and Camlica, 2019; Camlica and Yaldiz, 2024).

Determination of essential oil components

Essential oil compositions of fennel fruit grown under SM, VM and AS fertilizers were measured. Essential oils were analyzed using Agilent Technologies 7890A (Santa Clara, CA, USA) in combination with a flame ionization detector and mass spectrometry (model 5975C) and HP Innowax capillary column (60.0 m × 0.25 mm × 0.25 µm). The extracted essential oils were diluted with hexane (dilution ratio 1:50). GC-MS/FID analysis was performed in 50:1 split mode. The inlet injector and detector temperatures were set at 1 µL and 250 °C, respectively. The oven temperature was ramped from 60 °C to 250 °C at a rate of 10 °C/min, held for 20 minutes, and then held at 250 °C for 8 min. Helium (purity 99.9%) was the carrier gas at a flow rate of 1 mL/min. The mass scan ranged from 35 to 450 amu and the ionization mode was electron trigger mode (70 eV). The GC-FID peak areas were used to electronically determine the relative percentages of the components. The libraries from WILEY, NIST and FLAVOR were used to determine the components of essential oils (Yaldiz and Camlica, 2022).

Analysis of fatty acids

The analysis of fatty acids of the fennel fruits grown under SM, VM and AS fertilizers were reported by previous studies (Yaldiz and Camlica 2019; Camlica and Yaldiz 2021). Fatty acid methyl esters (FAMEs) were analyzed according to IUPAC-International Union of Pure and Applied Chemistry (1987) using a Shimadzu GC-2010 gas chromatograph (Shimadzu Corporation, Tokyo, Japan) equipped with a flame ionization detector (FID) and a Rtx-2330 capillary column (60 m × 0.25 mm) with a thickness of 0.2 µm. The detector temperature was set to 240 °C. The GC oven temperature was programmed at 140 °C for 5 min as the initial time. Then, the temperature was increased at a rate of 4 °C/min up to 260 °C and kept constant for 20 min. Helium was used as the carrier gas (1 ml/min). The FAMEs were determined by comparing retention times with reference standarts (mixture FAME Mix, SUPELCO, which included 37 FAMEs). Methyl-undecanote from Sigma Aldrich Chemical Co. was used as an internal standard to quantify FAMEs. The results were reported as percentages.

Statistical analyses

The statistical analysis of the study was conducted by using XLSTAT program. The statistical differences and importance were found between the means of the examined properties at 5% level with LSD (Least Significant Difference) test. PCA analysis was also performed to determine the relationship between properties.

Results and Discussion

Essential oil content

The results showed statistical significant differences for the essential oil (EO) content. The EO content varied with the used VM, SM and AS doses. The essential oil contents of fennel fruits were between 2.40 and 3.35% (Table 2). The highest essential oil content was found in the SM1 application, followed by the VM3 (3.27%) applications. Both VM and SM doses significantly increased the essential oil content of fennel fruits compare to control. The lowest essential oil content was obtained in the control (2.40%).

In addition, organic and chemical fertilizer applications on fennel were determined with more essential oil contents and produced similar results. The changes in the content of essential oil showed slight differences between the applications of fertilizers (VM, SM and AS) and their respective doses. The absence of fertilizer, represented by control applications, significantly lowered the essential oil content in fennel compared to applications of organic manure or chemical fertilizers.

These results were previously confirmed by Kapoor et al. (2004) and Darzi (2008) on *Foeniculum vulgare* that organic manure increased essential oil yields. In a similar study, Yaldiz and Camlica (2023) determined the effect of different doses of different organic manure on the essential oil content of sage in pot and field experiments. They found that sage plants treated with sheep manure at rates of 7.5 t/ha and 12.5 t/ha accumulated higher levels of essential oils under both pot-based experiment and field conditions. Similarly, Valiki et al. (2015) investigated the effect of different doses of vermicompost (5, 10, 15 and 20 t/ha) and NPK fertilizer on fennel essential oil (*Foeniculum vulgare* Miller) and stated that the application of 15 t/ha vermicompost showed a more positive influence on the recorded properties of fennel essential oil than other treatments. The VM has more nutrient contents growth-promoting substances (enzymes, antibiotics and growth hormones). So, the various effects of chemical and organic fertilizers on the essential oil of fennel may be mainly attributed to the difference in their composition (Vadiraj et al. 1998).

Mimica-Dukic et al. (2003) noted that the EO yield of fennel ranged from 2.82% to 3.38%, Moradi et al. (2011) reported a range from 2.2% to 2.9%, and Mohamed and Abdu (2004) found it to be between 2.30% to 3.53%. Our essential oil results were similar with these previous studies (Mimica-Dukic et al. 2003; Mohamed and Abdu 2004; Moradi et al. 2011). In contrast, Karataylı (2020) noted that the yield of obtained EOs ranged from 1.60% to 2.00% and Anwar et al. (2005) noted the essential oil yield of fennel as 2.81%. The results of the present study were slightly higher than those reported by Karataylı (2020) and Anwar et al. (2005). The obtained fennel fruit essential oil contents of this study were different from previous studies. These differences can be explained by factors such as the applications and doses, ecological condition, genotype besides growing conditions.

Chemical composition of the essential oil

The result of the study showed differences in the main essential oil compositions of fennel fruits. According to essential oil composition analysis, total EO compositions of fruits were noted between 84.46-93.36% with trans-anethole (35.08-44.68%), camphor (16.98-20.49%), limonene (4.35-16.91%), p-anisaldehyde (1.83-13.13%) and terpinene (5.37-9.13%). These essential oil (EO) components were found to constitute approximately 77.29% to 86.56% of the total EO content in the examined properties (Table 2).

In fennel fruits, the maximum concentration of trans-anethole was obtained after applying SM2 (44.68%), followed by SM1 (40.56%) and AS2 (42.18%), whereas the lowest trans-anethole was obtained after applying VM2 (35.08%) and SM3 (36.46%). The content of trans-anethole was higher in SM applications than in AS and VM applications (Table 2). The trans-anethole content increased in fennel fruits with organic manures and inorganic fertilizer except for SM3, VM2 and VM3 applications.

The obtained results of the study showed that application of the manures and fertilizer provides the enhancement of the essential oil content besides contents of the trans-anethole, camphor, limonene and anisaldehyde. Moreover, it was revealed that application of up to 20 t/ha of VM effectively increased the production of EO components. This may be due to the overall stimulatory effect of organic manure on the plant's secondary metabolic pathways (Pandey et al. 2015). Also, other components i.e., camphor concentration increased with the use of SM1 and SM2 and then fell after SM3 application. Similarly, it was seen that AS1 and AS2 doses increased the camphor concentration and then decreased after AS3 application. So, a maximum increment was observed in SM1 (20.49%), followed by AS2 (20.22%) and SM2 (20.11%) applications. In addition, the lowest camphor yield was obtained from VM1 (16.98%), and followed by SM3 (17.31%) applications (Table 2). Our results were greater than Şanlı et al. (2008) (1.04-2.47%), Yaldiz and Camlica (2019) (0.33-3.33%) and Delfieh et al. (2016) (1.0-2.0%).

The recorded results showed significant differences among the applied fertilizers at $p < 0.05$ for the limonene content (Table 2). Limonene was identifed at the highest level after applying AS1 (16.91%), followed by AS3 (16.60%) and control (15.79%) applications, whereas the minimum concentration of limonene was obtained after applying SM1 (4.35%). AS applications of up to 120 kg/ha increased the limonene yield of fennel fruit, which is in consistent with the essential oil and major components of dill which increased at a nitrogen dose of up to 120 kg/ha (Ozliman et al. 2021). Also, *p-Anisaldehyde* had higher concentrations in the treatment with SM1

(13.13%), followed by VM3 (7.90%), and AS2 (5.46%). Furthermore, the lowest *p-Anisaldehyde* content was obtained from AS1 (1.83%) and AS3 (2.25%).

The contents of *p-anisaldehyde* were higher in SM applications than in AS and VM applications. We also found that *p-anisaldehyde* yield could be effectively raised by SM application up to 10 t/ha. This may be caused by the general promoting effect of organic fertilizers on the secondary metabolic pathways of plants (Pandey et al. 2015). Delfieh et al. (2016) reported that the *p-Anisaldehyde* content in fennel fruits ranged from 0.83% to 1.75%, which is quitely lower than our result. In contrast, Kan et al. (2006) observed that *p-Anisaldehyde* content fennel fruits varied from 6.1 to 21.3%, which is partly higher than our result. In addition, our results were comparable with the results described by Şanlı et al. (2008) who found that the *p-Anisaldehyde* content ranged from 2.56 to 13.82%.

As seen in Table 2, the values of terpinene varied between 5.37-9.13% in different organic and chemical fertilizer, and the highest terpinene rate was found in the VM2 (9.13%) applications, followed by the SM1 (6.96%). Among the organic and chemical fertilizer applications, VM applications had the highest terpinene contents. The percentage components of the remaining 12 compounds varied from 0.09 to 5.59%. This study identified α-pinene (0.22-2.28%), anisyl acetone (0.34-5.59%), and germacrene (0.11-3.23%) as the minor components of fennel fruit oils. The highest anisyl acetone and germacrene values were obtained for SM3 application, whereas the highest pinene content was obtained for AS1 application (Table 2).

Our results were greater than that reported by Delfieh et al. (2016), in which determined the trans-anethole contents of fennel fruits ranged from 25.0% to 35.23% in different organic and chemical fertilizers. Furthermore, Moradi et al. (2011) reported that organic (compost and vermicompost) and two biological fertilizers significantly increased the percentages of essential oil and essentiail oil components. It was also reported that compost and VM interaction showed the highest EO yield with anethole concentration and the lowest values in fenchone, limonene and estragole concentrations in total essential oil content. Similarly, Darzi et al. (2009) and Abdelaziz et al. (2007) stated that compost and vermicompost supply plant nutrient requirements by gradually releasing the elements that increase the anethole content of sweet fennel essential oil, resulting in improved plant essential oils quality. Anwar et al. (2005) reported that using vermicompost on basil (*Ocimum basilicum*) led to increase in limonene and methyl chavicol content which improved the quality of essential oil. Furthermore, Channabasanagowda et al. (2008) determined that vermicompost has a significant effect in improving plant growth and development as it has slow nitrogen release due to slow mineralization throughout plant growth. Younesian et al. (2013) evaluated the effect of different biological (*Glomus mosseae, Glomus intraradiceae*), organic (cow manure) and inorganic (urea N) fertilizers on the essential oil content of sweet fennel. They found that the highest essential oil yield (24.6 l/ha) were obtained in cow manure treatment, the highest anethole (81.3%) and the lowest (8.53%), estragole (3.05%) and limonene (3.23%) in essential oil content were obtained in *Glomus intraradiceae* treatment. In another study, Khalesro et al. (2012) used three different doses of VM (0, 5 and 10 t/ha) and two different doses of zeolite (0 and 4.5 t/ha) to determine the essential oil content in anise. It was reported that the application of VM and zeolite effectively increased essential oil content, yield, and trans-anethole concentration in essential oil.

Variability of EO composition from the reported previous findings may be expressed by different methods and conditions of extraction, genotypic difference, soil texture and plant secondary metabolite pathways, besides organic and chemical fertilizers (Pandey et al. 2015).

Table 2. Essential oil and its compositions of fennel grown under organic and chemical fertilizer

EOC (%)/Applications	RT (min)	SM1	SM2	SM3	VM1	VM2	VM3	AS1	AS2	AS3	Control
Essential oil yield (%)		3.35^{a}	2.90^{ab}	3.05^{ab}	2.95^{ab}	3.05^{ab}	3.27^{ab}	3.11^{ab}	3.13^{ab}	3.04^{ab}	2.40^{b}
α-pinene	11.37	0.22^{e}	1.08^{cd}	0.92^{cd}	1.70^{b}	1.03^{cd}	1.05^{cd}	2.28^{a}	0.67^{de}	1.37^{bc}	1.68^{b}
Sabinene	12.82	0.09^{e}	0.44^{cd}	0.41^{cd}	0.58^{bc}	0.34^{d}	0.44^{cd}	0.86^{a}	0.34^{d}	0.58^{bc}	0.67^{ab}
Myrcene	13.11	0.13^{f}	0.42^{def}	0.29^{ef}	0.61^{be}	0.64^{ad}	0.52^{be}	0.95^{a}	0.44^{cf}	0.75^{abc}	0.77^{ab}
a-phellandrene	13.78	0.37^{c}	0.75^{ab}	0.66^{b}	0.98^{a}	0.68^{b}	0.74^{ab}	0.93^{ab}	0.82^{ab}	1.01^{a}	1.00^{a}
Limonene	14.54	4.35^{f}	12.49^{cde}	13.23^{be}	15.07^{a-d}	13.50^{ad}	9.88^{e}	16.91^{a}	12.02^{de}	16.60^{ab}	15.79^{abc}
4-Ethyl-oxylene	14.73	0.37^{e}	0.74^{bcd}	0.55^{de}	0.67^{cd}	0.76^{bc}	0.71^{bcd}	1.01^{a}	0.65^{cd}	0.77^{bc}	0.88^{ab}
1,8-Cineol	14.98	0.10^{d}	0.52^{abc}	0.43^{bcd}	0.71^{ab}	0.59^{abc}	0.38^{cd}	0.76^{a}	0.41^{bcd}	0.67^{abc}	0.77^{a}
Terpinene	15.47	6.96^{bc}	5.37^{c}	6.45^{bc}	7.97^{ab}	9.13^{a}	6.41^{bc}	6.87^{bc}	6.36^{bc}	7.44^{ab}	6.46^{bc}
Fenchon	17.87	0.31^{a}	0.12^{b}	0.06^{d}	0.06^{d}	0.12^{bc}	0.09^{bcd}	0.04^{d}	0.08^{bcd}	0.04^{d}	0.07^{cd}
Thujone	18.5	0.47^{a}	0.26^{b}	0.27^{b}	0.29^{b}	0.43^{a}	0.29^{b}	0.28^{b}	0.30^{b}	0.28^{b}	0.25^{b}
Camphor	20.01	20.49^{a}	20.11^{a}	17.31^{cd}	16.98^{d}	19.95^{a}	18.82^{abc}	19.18^{ab}	20.22^{a}	18.82^{abc}	18.18^{bcd}
Estragole	20.8	0.15^{b}	0.13^{b}	0.12^{b}	0.19^{b}	0.20^{b}	0.31^{b}	0.12^{b}	0.12^{b}	0.53^{a}	0.14^{b}
Trans-anethol	23.63	40.19^{ab}	44.68^{a}	36.46^{ab}	40.64^{ab}	38.44^{ab}	35.08^{b}	39.03^{ab}	42.18^{ab}	41.45^{ab}	38.77^{ab}
***p*-anisaldehyde**	24.08	13.13^{a}	3.80^{cd}	3.84^{cd}	2.79^{cd}	2.75^{cd}	7.90^{b}	1.83^{d}	5.46^{bc}	2.25^{d}	2.46^{cd}
Anisyl acetone	27.48	0.97^{b}	1.17^{b}	5.59^{a}	0.57^{b}	0.58^{b}	1.43^{b}	0.39^{b}	2.56^{ab}	0.34^{b}	1.16^{b}
Germacren D	28.47	0.11^{b}	0.25^{b}	3.23^{a}	0.23^{b}	0.20^{b}	0.13^{b}	0.31^{b}	0.15^{b}	0.17^{b}	1.67^{ab}
Dillapiole	32.56	0.47^{b}	0.32^{b}	1.28^{a}	0.31^{b}	0.23^{b}	0.28^{b}	0.50^{b}	0.32^{b}	0.29^{b}	0.20^{b}
Total (%)		88.88	92.65	91.10	90.35	89.57	84.46	92.25	93.10	93.36	90.92

SM: Sheep manure, VM: Vermicompost, AS: Ammonium sulphate, Means followed by the same letters in each row are not significantly different according to the least significant difference (LSD) test ($P < 0.05$)

Fixed oil content

As seen in Table 3, the fixed oil content (FO) was affected by organic and chemical fertilizer applications. The FOs obtained from fennel genotypes were found to be statistically significantly different ($p < 0.05$), ranging from 17.39 to 33.61% among the different fertilizer applications. There was a difference of approximately two-fold between the highest and the lowest values. The highest FO was achieved in the AS3 application, followed by control (31.71%) and SM1 (31.24%) applications. The lowest FO was found for VM3 application (17.39%) (Table 3).

In the present investigation, it was found that the significant differences were seen among the used organic and chemical fertilizers in terms of FO yield at $p < 0.05$ (Table 3). Among the organic manures, the highest FO yield was obtained from SM compared the VM applications. Generally, the lowest doses of VM and SM applications have the highest FO yield. In addition, the highest fixed oil values were obtained at the highest dose of AS applications.

The FO yield ranged from 17.39 to 33.61% which was significantly higher than the findings of Reiter et al. (1998) who reported that the FO yield of fennel ranged from 10.15 and 14.6%. Likewise, Bayrak (2006) reported that FO yield of fennel ranged from 12.71% to 16.61%. Such differences in the FO content of fennel may be due to different agro-climatic conditions, in different geographical regions and fertilizer applications.

Fatty acids of the fixed oil

In fennel fruit oil, 15 fatty acids have been determined and given in Table 3. The percentages of the main fatty acids were as follows: petroselinic acid (4.99-34.92%), cis-10-heptadecanoic acid (13.35-26.45%), linolenic acid (8.41-14.63%), *cis-4,7,10,13,16,19* docosahexaenoic (8.33-13.96%), caproic acid (%5.13-11.16) (Table 3).

Petroselinic acid (34.92%) was found as the major component in VM3 applications, followed by 31.10% in VM2 applications. The lowest *petroselinic* acid content was found in SM1 (4.99%) application (Table 3). The amount of *petroselinic* acid observed in VM applications were more than those in SM and AS applications.

Significant quantity of *caproic* acid was found in different fertilizer applications ranged from 5.13% in SM3 applications to 11.16% in VM3 application. Other fatty acids such as linolenic acid was identifed at the highest level after applying 20 t/ha SM (14.63%) and the control (14.53%) application, followed by 15 t/ha VM applications, whereas the lowest concentration was obtained from 40 kg/ha AS (8.41%) and 15 t/ha VM applications. So, SM applications have a positive effect on linolenic acid content. Also *cis-4,7,10,13,16,19 docosahexaenoic* acid had higher concentrations in the treatment with 10 t/ha SM (13.96%), followed by 10 t/ha VM (13.33%) and 20 t/ha SM (12.68%). The lowest concentration was obtained from 15 t/ha VM (8.33%) and 20 t/ha VM (8.75%) applications.

In fennel fruits, all treatments resulted in similar percentages of *heptadecanoic* acid, whereas the SM2 application provided the highest *heptadecanoic* acid content at 26.45% (Table 3). In addition, SM3 (23.39%) and AS1 (23.07%) applications have higher *heptadecanoic* acid content than other fertilizer applications.

The proportion of linoleic acid in all fertilizers is between 4.03% and 7.77%, accounting for less than 10% of the total fatty acids. The highest value was achieved with SM3, then SM1 (6.92%) and SM2 (6.69%) and the lowest value found in VM2. The palmitic acid content was also between 1.19-3.52%. The highest value was obtained in SM3 application, followed by VM3 (3.12%) application and the lowest was obtained in VM1 application. Also, arachidonic acid was detected only in control (3.56%), SM1 (1.45%) and VM2 (1.31%) application.

Furthermore, *cis-5.8.11.14.17-eicosapentaenoic* acid changed from 1.82% to 2.80%, the highest value was obtained in SM1 application, the lowest was obtained SM2 application. In addition, *capric* acid ranged from 1.14% to 4.88%, the highest value was obtained in VM2 application, the lowest was obtained SM2 application.

Izadi-Darbandi et al. (2023) reported that the main components of fennel fatty acid profile in Iran condition were *petroselinic/oleic* acid (52-64%), linoleic acid (26-39%), *palmitic* acid (0.3-4.1%), *stearic* acid (1.3-2.4%), *linolenic acid* (0.6-3.6%), and *myristic* acid (0.35-1.07%), which were similar to our study. Likewise, Reiter et al. (1998) stated that *petroselinic* acid is the major isomer in fennel fruit.

Table 3 showed that the fixed oil content of fennel included highly monounsaturated to polyunsaturated and saturated fatty acids. These fatty acids can reveal the screening indicator or quality of fatty oil because of using edible oil (Vidrih et al. 2009; Alameldin et al. 2017). On the other hand, it was recorded that the major 50 fennel fatty acid compositions were 52-64% oleic acid, 26-39% linoleic acid, 0.3-4.1% palmitic acid, 1.3-2.4% stearic acid, 0.6-3.6% linolenic acid and 0.35-1.07% myristic acid (Bahmani et al. 2021).

It has been reported that the fatty acid compositions of fennel as concentrations of palmitic, arachidic, and stearic acids were affected directly by temperature. The increasing temperature values had negative effects on petroselinic and oleic acid contents (Mustiga et al. 2019). In addition, Bahmani et al. (2021) reported that the expression patterns of oil biosynthesis genes are well-paralleled with oleic acid and linoleic acid in relation to environmental factors. Also, these factors may shift the pathway more toward one of the components and reduce the other one's production (negative correlation between petroselinic and linoleic acids).

Principal component analysis (PCA)

A size reduction method, which is called PCA, was used to determine the examined properties. All of the total variations have been derived from 9 principal component axis and eigenvalues, variability values (%) and cumulative values (%) are given in Table 4. Scree Plot (Graphical representation of Eigenvalues) showed the eigenvalues in Fig. 2. Nine eigenvalues were determined. However, first five eigenvalues were used in study. Because previous studies were reported that eigenvalues greater than 1 (PCAs with eigenvalues >1.0) are reliable and more informative than original variables (Iezzoni and Pritts 1991; Mohammadi and Prasanna 2003). The eigenvalues were found as 3.633 for PC1, and 2.670 for PC2, 2.296 for PC3, 1.677 for PC4, 1.205 for PC5. The first principal component had 27.948% of the total variation (PC1). The second principal component (PC2) explained 20.535% of the total variation. The third principal component had 17.660% of the total variation (PC3). The rest of PCs had the 22.165% (PC4=12.900 and PC5=9.265%) in total variations.

Table 3. Fixed oil and fatty acid compositions of fennel grown under organic and chemical fertilizers

FOC (%)/Applications	RT (min)	SM1	SM2	SM3	VM1	VM2	VM3	AS1	AS2	AS3	Control
Fixed oil content		31.24^{ab}	22.16^{c}	23.43^{c}	24.88^{bc}	22.36^{c}	17.39^{c}	20.62^{c}	21.76^{c}	33.61^{a}	31.71^{ab}
Petroselinic acid (C18:2 n12)	4.75	4.99^{d}	17.63^{bcd}	12.69^{cd}	17.92^{bcd}	31.10^{ab}	34.92^{a}	19.52^{ad}	24.96^{abc}	14.16^{cd}	21.31^{abc}
Caproic acid (C6:0)	5.20	10.15^{ab}	9.44^{ab}	5.13^{b}	8.64^{ab}	6.95^{ab}	11.16^{a}	7.35^{ab}	5.46^{ab}	10.52^{ab}	9.28^{ab}
Caprilic acid (C8:0)	5.93	2.61^{c}	-	-	2.84^{b}	2.37^{d}	-	2.41^{d}		2.68^{c}	3.52^{a}
Capric acid (C10:0)	7.18	4.39^{a}	1.14^{c}	-	4.56^{a}	4.88^{a}	-	3.93^{ab}	3.06^{b}	3.32^{b}	3.91^{ab}
Undecanoic acid (C11:0)	8.45	-	-	0.92^{c}	2.00^{a}	0.95^{c}	-	1.25^{b}	-	-	1.26^{b}
Lauric acid (C12:0)	9.70	1.24^{c}	1.40^{b}	0.46^{d}	-	1.45^{a}	-	-	-	0.15^{e}	-
cis-10-pentadecanoic acid (C15:1)	17.12	1.88^{b}	1.44^{c}	2.93^{a}	-	-	-	-	-	-	-
Palmitic acid (C16:0)	17.26	1.45^{cd}	1.95^{bcd}	3.52^{a}	1.19^{d}	2.74^{abc}	3.12^{ab}	2.22^{ad}	2.34^{ad}	1.49^{cd}	1.85^{bcd}
Heptadecanoic acid (C17:0)	19.19	20.38^{ab}	26.45^{a}	23.39^{ab}	16.06^{ab}	16.34^{ab}	13.35^{b}	23.07^{ab}	21.09^{ab}	21.60^{ab}	15.23^{b}
Elaidic acid (C18:1n9t)	21.80	3.88^{a}	-	1.55^{b}	-	-	-	-	-	-	1.27^{c}
Linolelaidic acid (C18:2n6t)	23.10	6.92^{a}	6.69^{a}	7.77^{a}	6.51^{a}	4.03^{c}	6.56^{a}	6.22^{ab}	5.93^{abc}	5.96^{abc}	4.41^{bc}
Linolenic acid (C18:3n6)	26.29	14.53^{a}	10.81^{a}	12.86^{a}	11.40^{a}	13.98^{a}	8.50^{a}	8.41^{a}	9.52^{a}	9.93^{a}	14.63^{a}
Arachidonic acid (C20:4n6)	29.26	1.45^{b}	-	-	-	1.31^{c}	-	-	-	-	3.65^{a}
cis-5,8,11,14,17-eicosapentaenoic acid (C20:5n3) EPA	30.97	2.80^{a}	1.82^{c}	2.20^{b}	2.28^{b}	2.01^{bc}	-	2.23^{b}	2.09^{bc}	2.05^{bc}	-
cis-4,7,10,13,16,19-dokosahexaenoic acid (C22:6n3) DHA	34.36	13.96^{a}	10.86^{c}	12.68^{ab}	13.33^{a}	8.33^{d}	8.75^{d}	11.43^{bc}	11.20^{bc}	11.24^{bc}	9.18^{d}
Total (%)		90.63	89.63	86.10	86.73	96.44	86.36	88.04	85.65	83.10	89.50

SM: Sheep manure, VM: Vermicompost, AS: Ammonium sulphate, Means followed by the same letters in each row are not significantly different according to the least significant difference (LSD) test ($P < 0.05$)

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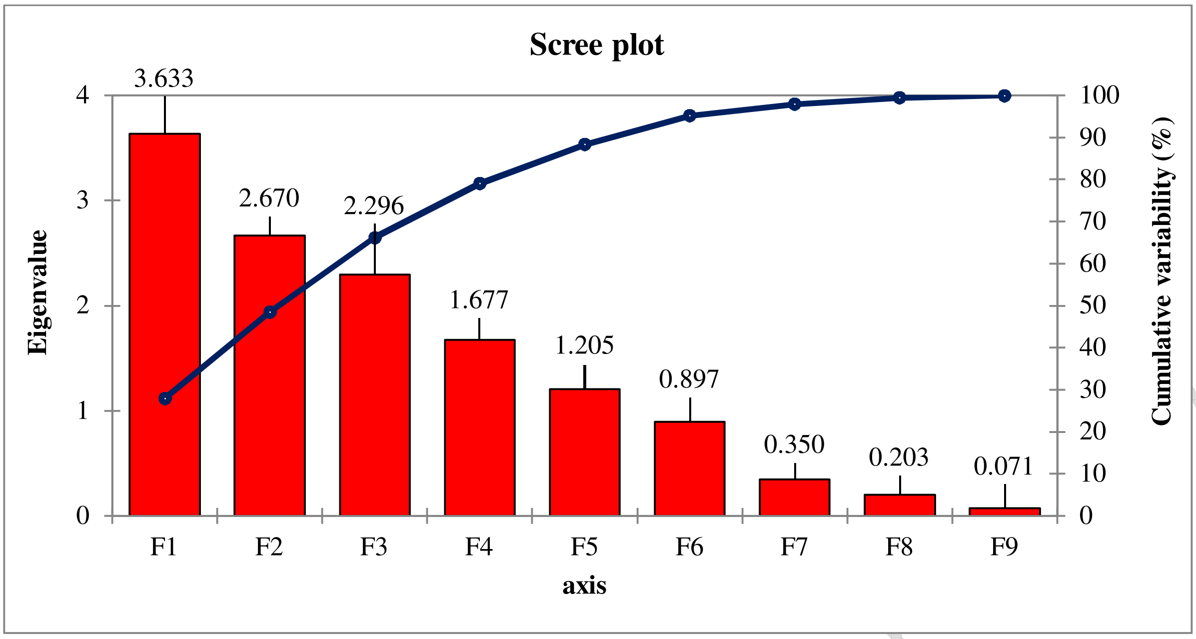

Fig. 2 Graphical representation of eigenvalues

The cumulative ratio of the five primary components in total variation was 88.307%. As a result of the PCA analysis, 9 principal component axes were obtained and these axes represented all of the total variation. The nine principal components explained 100% of the total variation (Table 4).

Table 4. Eigenvalues, variability and cumulative values of examined properties

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	3.633	2.670	2.296	1.677	1.205
Variability (%)	27.948	20.535	17.660	12.900	9.265
Cumulative (%)	27.948	48.483	66.142	79.042	88.307

The PCA showed that the examined properties were divided into the five groups (Fig. 3). Group 1 contained 5 properties and most of them was noted fatty acids. The properties of the first group were found on the positive sides of the component 1. Group 2 had similar examined properties with group 1 and it included five properties and camphor, p-anisaldehyde, essential oil yield with two fatty acids (linolelaidic and caproic acids) took place in this group on the positive side of the PCA1. Rest of the groups had only one properties, and group 3 had a fatty acid (petroselinic acid). Groups 4 and 5 had an essential oil as terpinene and limonene, respectively. PCA results also showed that applications were found close in Fig. 2 except control and VM2 applications. The PC1 was clearly identified with the cis-4,7,10,13,16,19-dokosahexaenoic acid, heptadecanoic acid, petroselinic acid, linolelaidic acid, limonene, and *p*-anisaldehyde contents, while the PC2 was related to essential oil yield, fixed oil yield, linolenic acid, trans-anethol, and caproic acid contents. Most of the factors contributed to PC1 were: SM-1, VM-2 and control applications. On the other hand, the main contributor to PC2 (positive side) was VM-3, whilst AS-3 and VM-1 contributed the negative side.

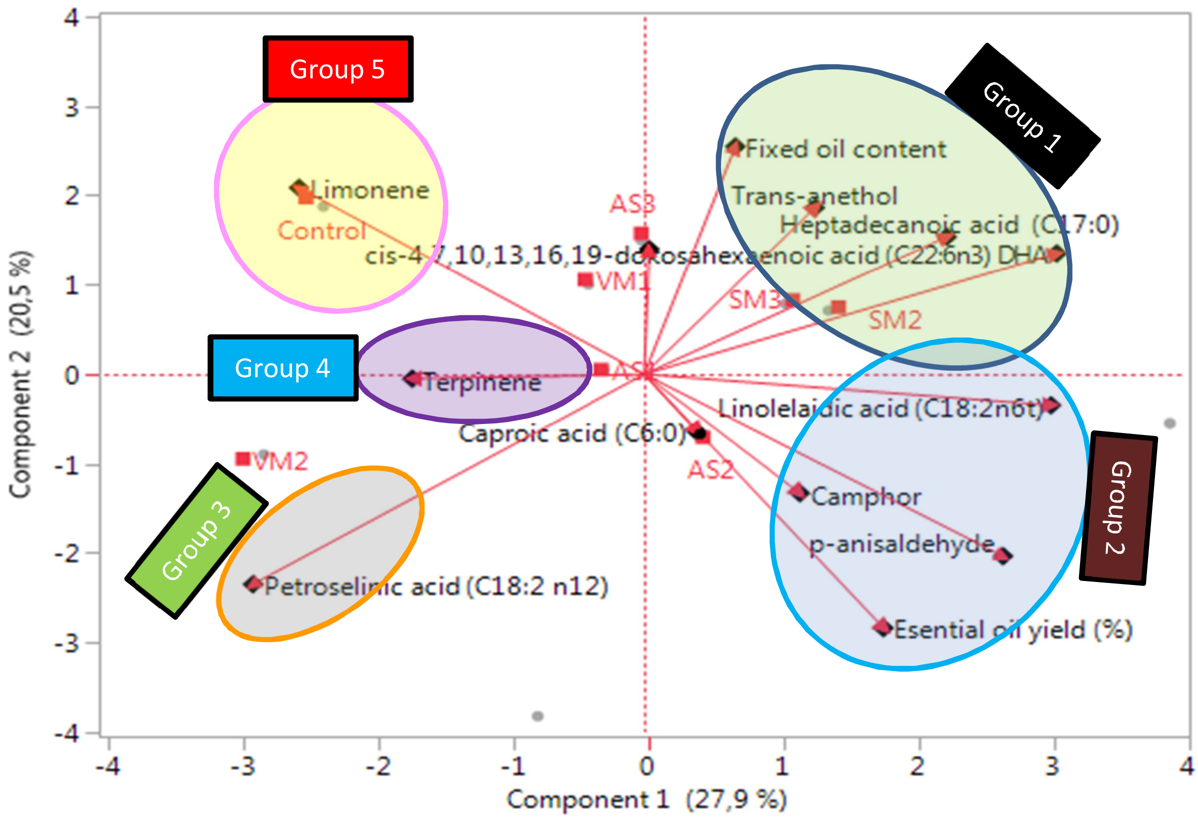

Fig. 3 PCA analysis results of the examined properties of fennel fruits

Conclusion

Fennel is commonly cultivated in tropical and temperate regions for centuries, and it is one of the most important economic crops for export in Türkiye. Organic manures, especially VM, have started to attract attention in agriculture. In this research, where different organic (VM nad SM) manures and chemical (AS) fertilizer were applied. Our results indicated that the efficiency of VM and SM could be increased in producing fennel EO components, when applied up to 20 t/ha. The best results in terms of camphor and *p*-anisaldehyde were obtained from 10 t/ha SM application. Furthermore, the most favorable compound in fennel EO, trans-anethole, had a high proportion in the fruits harvested from SM fertilized fennel. In terms of FO contents, the highest value was obtained from 120 kg/ha AS and 10 t/ha SM applications. Regarding the major acid of the FO, petroselinic and heptadecanoic acids tended to increase up to 20 t/ha applications of the VM and SM. The study extended the advances important of the organic manures existing knowledge, and the SM and VM applications can be used in organic agriculture for the fennel production. Also, the obtained results showed that the organic manures can be used instead of the chemical fertilizer for the health of soil and plants. In conclusion, this study contributes to the existing literature by examining the organic manure applications that may affect the relationship between the manure doses and fennel essential oil, fixed oil and their compositions.

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