

Seasonal Variations in Essential Oil of Dill: A Three Months Study

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Abstract

This study explores the seasonal variations in the essential oil content and composition of dill (*Anethum graveolens*) during the spring season, emphasizing the impact of environmental factors on its bioactive properties. Essential oils from dill are highly valued for their multifaceted medicinal, therapeutic, and culinary applications, primarily due to their rich chemical composition. Understanding the influence of seasonal changes on these oils can significantly enhance their use in food, health, and pharmaceutical industries. Using Gas Chromatography-Mass Spectrometry (GC/MS), the chemical composition of dill essential oils was analyzed across three consecutive harvests during the spring. The results revealed a dynamic shift in the presence and concentration of key compounds, such as α -phellandrene, β -phellandrene, dill ether, carvacrol, germacrene D, dill apiole, and phytol, which some of them increased progressively in later harvests. A notable diversification of bioactive compounds was observed in the later stages of the season, coinciding with variations in environmental factors such as temperature, light intensity, and growth maturity. These factors collectively influenced the biosynthesis of essential oils, optimizing their chemical complexity and potency over time. This study underscores the critical role of seasonal timing in maximizing the nutritional, therapeutic, and functional value of dill essential oils. By identifying the optimal harvest periods and understanding the environmental drivers of these changes, producers can improve the quality, yield, and bioactivity of dill essential oils, supporting their broader application in developing high-value functional foods, natural remedies, and health-promoting products.

Introduction

Dill (*Anethum graveolens*), a globally cultivated herb, is celebrated for its distinctive aroma and wide-ranging medicinal properties (Jana et al., 2010, Mirmohammadmakki et al., 2022, Sharma et al., 2024). Its essential oils are particularly noteworthy due to their extensive applications in the food, pharmaceutical, and cosmetic industries (Chouhan et al., 2017, Cimino et al., 2021, Bolouri et al., 2022). These oils are prized for their bioactive compounds, which contribute to flavor enhancement, therapeutic efficacy, and fragrance formulation (Sharmeen et al., 2021, de Sousa et al., 2024). Understanding how the composition and yield of dill's essential oils vary across seasons, particularly during spring, is critical for ensuring optimal quality, efficacy, and usability (Liao et al., 2021, Rathore et al., 2022). Spring, a season marked by transitional weather patterns and robust plant growth, provides a dynamic environment for dill cultivation (Huot et al., 2014, Walters et al., 2021). Examining the fluctuations in essential oil content and composition of dill over time aims to shed light on the seasonal dynamics that influence its chemical and bioactive profile (Milenković et al., 2024). Such an investigation is pivotal for identifying how environmental factors, phenological development stages, and cultivation practices influence the biosynthesis of essential oils in dill (Aboukhalid et al., 2017, Ghassemi-Golezani et al., 2022). Researchers aim for precise determination of essential oils by utilizing advanced techniques, including gas chromatography-mass spectrometry (GC-MS) (Fan et al., 2017, Filatov et al., 2023, Koljančić et al., 2023). This methodology enables the detailed identification and quantification of the individual components in dill's essential oils, shedding light on their chemical diversity and bioactivity (Mothana et al., 2013, Gamal El-Di Gamal El-Din et al., 2022, Milenković et al., 2024). Beyond chemical

profiling, the study seeks to explore the practical implications of these seasonal variations for various applications (Heavisides et al., 2018, Southwell et al., 2020, Santos et al., 2024). Whether it involves enhancing the sensory properties of culinary creations, formulating effective natural remedies in herbal medicine, or innovating in the realm of fragrances and skincare, understanding the seasonal variability of dill's essential oils is indispensable for maximizing their potential (SharmeeSharmeen et al., 2021, Zhang et al., 2024). Furthermore, the findings of researches, carry significant implications for agricultural practices and crop management (Liu et al., 2018, Tudi et al., 2021). By understanding how seasonal changes affect the quality and quantity of dill's essential oils, farmers can refine their cultivation techniques to optimize yield and potency (Ali et al., 2022, Bunse et al., 2022). This could lead to enhanced crop productivity, economic profitability, and sustainable farming practices (Pretty 2008, Jat et al., 2022). Moreover, documenting the seasonal variations in dill's essential oils contributes to the conservation of this valuable species, helping to preserve its biodiversity and ecological role in natural and cultivated environments (Sharifi-Rad et al., 2017, Rathore et al., 2022).

Iran is a major agricultural producer, ranking sixth globally in vegetable production in 2013. Its agricultural output rose from 4 million tons in 1983 to nearly 16 million tons in 2013 (Mirmohammadmakki et al., 2022). In Iran, the harvesting of greenhouse crops such as cucumbers and tomatoes is mostly done by hand, particularly in provinces like Kerman and Khuzestan. Over half of the nation's vegetable cultivation area is found in seven provinces: Khuzestan, Fars, Hormozgan, Kerman, Hamedan, Zanzan, and Golestan (Mir mohammasmakki & Ziarati 2015). Focusing on the issues raised emphasizes the importance of addressing them.

The scope of this research transcends academic curiosity, offering practical insights for a diverse array of stakeholders, including researchers, producers, consumers, and policymakers. By elucidating the factors driving the seasonal dynamics of dill's essential oils, this study supports informed decision-making in product development, marketing strategies, regulatory policies, and sustainable agricultural initiatives. Additionally, the research contributes to the broader body of knowledge in natural product chemistry, pharmacology, and agronomy, advancing innovation and scientific discourse in these fields. In summary, the determination of dill's essential oil composition over three consecutive springs represents a multidisciplinary effort with broad implications. By uncovering the seasonal variations in the composition and abundance of these oils, this study seeks to unlock valuable insights into dill's aromatic and functional potential. Through rigorous analysis, experimentation, and collaborative inquiry, we aim to harness the power of nature's bounty, enhancing its utility for societal benefit while fostering environmental sustainability.

Materials and Methods

a. Sample Collection and Cultivation of Dill Seed

Dill seeds (*Anethum graveolens*) were obtained from the Ministry of Agriculture, Department of Beans, Vegetables, and Seeds Production in Tehran. The soil used for dill cultivation was sourced from Tehran

city and was sandy-clay with a pH of 6.5. The soil was sieved through a 2 mm mesh before being used to fill pre-prepared pots for vegetable cultivation. Dill seeds were sown in these pots during the first week of March, with regular watering to ensure optimal growth and prevent water stress. Aerial parts of the dill plants were harvested three times in April, May, and June (2022) for essential oil analysis. After collection, the samples were dried at room temperature and ground for further analysis.

b. Essential Oil Extraction

Essential oil extraction was conducted on dried dill leaves using a Clevenger apparatus, in three replicates. Each extraction used 40 grams of dried leaves and lasted for four hours. The essential oils were diluted with n-hexane to ensure optimal separation during analysis. After filtration through Whatman paper and drying with anhydrous sodium sulfate, the extracts were stored in sealed vials at 4°C until analysis.

c. GC-MS Analysis

GC-MS analysis of dill essential oil was carried out using an HP-5 capillary column. In the other words, essential oil constituents were identified by calculating their retention indices using temperature-programmed conditions for (C6 – C26) n-alkanes and the oils on an HP-5 column. Helium was the carrier gas at a flow rate of 0.9 mL/min. The GC oven temperature was initially set at 50°C for two minutes, then increased to 260°C at a rate of 5°C per minute and maintained at 260°C for 10 minutes. Mass spectra were recorded within a range of 40–5000 m/z. Essential oil components were identified using retention indices calculated from n-alkane standards under temperature-programmed conditions.

d. Qualitative and Quantitative Analysis

Mass spectra and retention indices were used to identify the chemical components of dill essential oils by comparing them to the NIST 98 and Wiley 7 libraries and other published sources. Quantitative analysis was based on GC peak areas without correction factors, with component identification verified against standard references (Adams, 2001; Bhuiyan, Begum, & Sultana, 2009). Statistical analysis, including Python 3, and SPSS version 24 were performed to assess variations. The Kovats index was calculated using a homologous series of n-alkanes for accurate identification.

Results and Discussion

Essential oils of dill in three consecutive harvests in the spring of 2022. The amounts of 19, 16, and 8 compounds were identified in each harvest during the corresponding test, respectively from the first to the third harvest. Notably, the amount of essential oils identified decreased from the beginning to the end of the test. However, the main compounds α -Phenellandrene, β -Phellandrene, Germacrene-D, Dill Apiole, Phytol and Croweacin were observed in all three harvests, further validating the thoroughness of our research. As seen in Table 1, a reduction in the amount of compounds in the second and third harvests were observed.

Specifically, some compounds were not observed in the measurement carried out in the second harvest, and the amount of compounds identified in the third harvest was reduced by half compared to the second harvest. The decrease in the compounds found in dill surpassed fifty percent in later assessments, especially in the final test. However, the main substances present in each dill essential oil were still observed in all three harvests.

Table 1
Essential Oil constituents of dill (*Anethum graveolens*) (a. Coriander in the first harvest b. Coriander in the second harvest c. Coriander in the third harvest)

			KI ^a	Presence in Area		
Row	Name	Type	EXPERIMENTAL	A	B	C
1	α -Phellandrene	Terpene	1010	*	*	*
2	m-Cymol	Aromatic Hydrocarbon	1014	*		*
3	β- Phellandrene	Terpene	1041	*	*	*
4	Dill Ether	Benzofuran	1187	*	*	*
5	Carvacrol	Phenol	1305	*		
6	β.-Elemene	Hydrocarbon	1402	*	*	
7	Trans-Caryophyllene	Terpeene	1439	*		
8	R-Nitro-1,5-p-Mentha Diene	Hydrocarbon	1460	*		
9	(R)-2-Nitro-p-Menta-1,5-Diene	Terpene	1462		*	
10	Germacrene-D	Alkene	1509	*	*	*
11	Bicyclogermacrene	Terpene	1522	*		
12	Croweacin	Alkene	1541	*	*	*
13	Cis-Asarone	Phenol	1568	*		
14	Carotol	Terpene	1632	*	*	
15	Dill Apiole	Aromatic compound	1667	*	*	*
16	14-Methyl-1-(3,3-Dimethylbicycl)	Terpenes	1710			
17	3-Methyl-4-Methylenebicyclo(3.2.1)Oct-2-ene	Hydrocarbon	1711		*	
18	5-Epi-Neointermedeol	Terpene	1712	*		
19	n-Butylidene dihydrophthalide	Alken	1772	*		
20	Mintsulfide	Terpenes	1780			*
21	Epi-β-Santalol	Terpenes	1813		*	
22	Neophytadiene	Hydrocarbon	1850	*	*	*
23	2- Pentadecanone. 6,10,14-trimethyl	Terpenes	1856	*		

			KI ^a	Presence in Area		
24	7,10,13-hexadecatrienoic acid	Carboxylic Acide	1910	*		
25	Hexadecanoic acid, Methyl ester	Ester	1933	*		
26	Phytol	Terpene	2127	*	*	*
27	9,12-Octadecatrienoic acid	Carboxylic acid	2155	*		
28	9,12,15-Octadecatrienoic acid, Methyl ester	Ester	2159	*		

Figure 1 is the GC-MS total chromatograms of dill essential oils in the first, second and third harvest.

Similarly, the identified compounds were examined in terms of chemical compound classification, and the results of the studies showed that terpenes and then hydrocarbons accounted for the most significant amount of identified compounds, respectively. In Fig. 3, the amount of identified compounds has been examined in terms of classification in the identified chemical compounds, and the comparison between the identified compounds can be clearly seen in this Figure.

Figure 2 uses the Python programming language to analyze and visualize the data. The data is processed using libraries such as Pandas and NumPy, and valid values are extracted. Also, using Matplotlib, multi-line graphs are drawn to show the changes in different chemical compounds in different months. To display the data trend more accurately, 2nd-degree trend lines were added to each data set. These lines were calculated using the computational tools of Python libraries and displayed in the graph along with the corresponding equations. Different line markers and styles were used to improve readability and make identifying the data easier. This approach provided accurate and understandable information about the trend of changes in values and helped identify key patterns in the data. The analyses also added more credibility and transparency to the research findings.

In Fig. 2, the chemical compositions of dill harvested in three consecutive months of the spring season are compared. Terpene was highest in the first month, lowest in the second month, and increased slightly again in the third month compared to the second month. Phenol was highest in the first month but not detected in the second and third months. The behavior of terpenes, hydrocarbons, and aromatic compounds was similar, so the above compounds were detected in the first and third months, but this amount decreased in the second month. Benzofuran had a Saudi trend in detection. It was detected in all three months, and this amount was higher in the third month. Ester and carboxyl had similar behavior and were only detected in the second month. It should be noted that the amount of phenol had a decreasing trend, and alkene behaved similarly to terpenes, hydrocarbons, aromatic compounds, and alkene. The difference is that the highest amount detected in alkene was in the third month.

In 2021, a similar study was conducted by Mirmohammadmakki et al., where the essential oil compounds of parsley were examined during three consecutive months of the spring season. The

method of this study was similar to the study conducted by Mirmohammadmakki et al., and the same device was used in both studies. It should be noted that in the study conducted by Mackey et al., more compounds were identified than in this study, and also in that study, the highest amount of chemically identified compounds related to the hydrocarbon group was in the first and second harvest and terpenes in the third harvest (Mirmohammadmakki et al. 2021).

According to the observations in Fig. 3, terpene, hydrocarbon, benzofuran, alkene, and aromatic compounds were found in all three harvests carried out in the first, second, and third months. Phenol was detected only in the dill tested in the first harvest, and ester and carboxylic compounds were detected only in the dill harvested in the second month. Notably, phenolic, ester, and carboxylic compounds were not detected in the third harvest.

In a 2021 study by Mohebodini et al., the comparative chemical composition of essential oils in dill (*Anethum graveolens* L.) ecotypes was analyzed, focusing on univariate and factor analysis. The aim of this study was to investigate the chemical composition of essential oils in four ecotypes of Iranian dill. The compositions of essential oils extracted in the ecotypes by this group were different, and they identified 38, 27, 25, and 24 volatile compounds in the four studied samples, respectively. In the present study, the most identified compounds were 19 compounds. The present study was conducted focusing on dill essential oils in three consecutive months of a season to examine changes in this time period. Also, Mohebodini et al. investigated four different ecotypes of dill, while the present study focused on only one species. In the study of Mohebodini et al., compounds such as toluene, α -pathogen, camphene, sabinene, β -pinene, β -myrcene, α -phellandrene, α -terpinene, β -phellandrene, mundane, sabinol, dill ether, carvacrol, germacrene D, dill apiol, neophytadine hexahydrofarnesylacetone and phytol were identified in all ecotypes. Finally, Mohebodini et al. emphasized the need to know the essential oil compositions of the studied ecotypes for appropriate selection for breeding purposes based on phytochemical diversity or use in the pharmaceutical and food industries to extract specific beneficial compounds (Mohebodini et al., 2021).

In 2016 El-Zaeddi et al. researched Volatile compounds of Essential Oils from Different Aromatic Herbs Grown in the Mediterranean Regions of Spain, which was very comprehensive. In this study, El-Zaeddi et al. examined the volatile composition of the essential oils of dill, parsley, coriander, and mint on different harvest dates to determine the most appropriate harvest time for each of these plants. The focus was on different components of the dill plant to examine changes in the composition of its essential oils over a period of time from the beginning to the end of a season. El-Zaeddi et al. used the hydro distillation (HD) method using the Drying system to separate the essential oils. Similar to the present study, volatile compounds were separated and identified using a gas chromatography-mass spectrometry (GC-MS) device. The results of the (quantitative) gas chromatography-flame ionization detector (GC-FID) analysis showed that the main components of the essential oil of dill aerial parts were phellandrene, dill ether, and phellandrene. (Second harvest, fourth week of February 2015). For parsley shoots, main compounds such as p-Mentha triene and -phellandrene were the most common compounds in the essential oil. The main compounds for coriander were E-2-duodenal, dodecanal, and octane; the highest amount was

found in the second harvest. The two main components of mint essential oil were carotenoid and limonene; the highest amount was observed in the first harvest. This study was a study that reported descriptive sensory analysis data of aromatic plants at these optimal and specific harvest dates according to the content of volatile compounds in their essential oils, which was a comprehensive and innovative study in its own right, similar to the present study that examined these compounds throughout a season (El-Zaeddi et al.2016).

In a similar study, Mirmohammadmakki and Hosseini investigated the chemical changes in coriander during three consecutive months of the spring season. They carefully examined and quantified the changes in the chemical composition of coriander during these three consecutive spring months. They used a GC/MS device to extract the extract from Clevenger and to measure the amount of essential oils, and their method was similar to the present study. Their studies and evaluations showed differences in the amount of essential oil, which indicated the effect of the harvesting stages on these compounds. In total, 45 essential oils were measured in the study of Mirmohammadmakki and Hosseini at three harvesting stages. Their studies showed that linalool was the main component of coriander oil. Their study also showed that the sample under study contained more than 20% germacrane, which decreased to less than 2% in subsequent harvests. Also, decanal, cyclohexane, 2-docecen-1-ol, intracanal, neophetadine, oleic acid, and phytol were present in all three harvests. They acknowledged that the coriander samples had significant amounts of essential oil, such as green leafy vegetables. Moreover, Mirmohammadmakki and Hosseini emphasized that, on the other hand, scientists can rely on this type of test to obtain medical extracts of coriander. Finally, Mirmohammadmakki and Hosseini stated that coriander is a valuable sample for use in the medical and food industries (Mirmohammadmakki & Hossaini 2023). In the present study, the first and second harvests (April and May) showed the highest chemical diversity, which could be due to favorable environmental conditions and the high metabolic activity of the plant in the early stages of growth. In the third harvest (June), the number of compounds may have decreased due to plant maturation and decreased metabolic activities. This decrease indicates that the essential oil is more concentrated on stable compounds in the final stages of plant growth. The present study also shows that the continuous presence of terpene, hydrocarbon, benzofuran, and aromatic compounds in all three harvests indicates their stability. This increases the economic and industrial importance of dill essential oils because producers can count on the stable production of these compounds. Dill essential oils can also be a key ingredient in the pharmaceutical, food, and cosmetic industries due to their stable presence in all harvests.

Similar to the present study that investigated dill essential oils, in 2021, Ghassemi-Golezani and Solhi-Khajemarjan experimented with the form of a split-plot design to evaluate changes in the essential oil content of dill (*Anethum graveolens* L.) organs and their response to water changes. Apart from the changes in dill essential oils investigated in both studies, the response of dill aerial organs to water changes was not investigated in the present study. Ghassemi-Golezani and Solhi-Khajemarjan's research on essential oils showed that exogenous salicylic acid increased the percentage of essential oils in all organs of dill, especially under water-limited conditions. Overall, the essential oil yield of dill organs increased with salicylic acid treatment. Investigating the above issue in three consecutive seasons can

provide a good model for a more detailed study of the effect of seasonal changes and irrigation on essential oil content (Ghassemi-Golezani and, Solhi-Khajemarjan 2021).

The changes in the amount of essential oils in dill may be related to the fact that after the first harvest, which occurs during the flowering stage, the plant tends to produce fruits and seeds. As a result, dill collected from regrowth shoots is not typical of the vegetative stage. This is evidenced by the increase in some compounds and the decrease in others, indicating that these changes may depend on the harvesting stage. Additionally, the seasonal conditions during planting and harvesting can significantly impact the quantity and quality of dill's compounds. Therefore, further research should be conducted over the subsequent summer and autumn months to more closely examine and validate or refute these findings.

Conclusion

A recent study conducted over three consecutive spring months (April, May, and June 2022) demonstrated that the compounds found in dill essential oil are influenced by seasonal changes and the plant's growth stages. Early harvests displayed a greater diversity of chemical compounds while focusing on more stable compounds characterized by later harvests. The constant reality of key components, such as essential oils, terpenes, hydrocarbons, benzofurans, and aromatic compounds, indicates the high quality of dill essential oil and its substantial value across various industries. This investigation, alongside the conditions and the practical applications of optimizing harvest timing, is a valuable guide for developing dill-essential oil-based products in food, industrial, and pharmaceutical applications. However, the present research underscores the importance of continuously monitoring seasonal changes in the production of medicinal plants to enhance productivity and lower production costs, empowering industry professionals to make informed decisions. The present study investigated the essential oils of the dill, providing a comprehensive overview of the quantitative and qualitative changes in its chemical compounds during the spring season. It was found that the diversity of these compounds decreased across different harvests. These changes deliberate how diverse growth steps and seasonal environmental conditions affect the plant's metabolism. However, key compounds, like terpenes, hydrocarbons, benzofurans, aromatic compounds, and particularly dill essential oils, were consistently present in all harvests. This stability highlights the biological and economic importance of their potential applications in the pharmaceutical, food, and cosmetic industries. Furthermore, the findings emphasize the need to optimize harvest timing. While early harvests (April and May) are more suitable for producing multipurpose essential oils due to the greater diversity of compounds, the third harvest (June), which concentrates on stable compounds, is more suitable for producing specific essential oils of higher quality. These conclusions can help agricultural programs, like harvesting programming and staggered planting, to enhance productivity and reduce costs in dill cultivation. In conclusion, the present research suggests valuable points in to the chemical compounds found in dill during the spring season. It establishes a solid scientific basis for future studies on medicinal plants. Understanding the seasonal variations in active compounds can improve Various industries cultivation,

extraction, and utilization methods for these natural resources. This kind of information and data could significantly enhance the quality and efficiency of products based on plants.

Declarations

Founding

All the authors declare that this research has received no funding.

Author Contribution

F.M. Writing – original draft , prepared figures, Formal analysis , Methodology , Validation , Visualization
MA.Gh. Corresponding author, Main Supervision , Writing – review & editing , Validation , Visualization ,
Methodology ME.Gh. Main Supervision , Writing – review & editing , Validation , Visualization ,
Methodology V.A. Supervision , Writing – review & editing , Validation , Visualization , Methodology R.E.
Supervision , Writing – review & editing , Validation , Visualization , Data curation , Methodology ,
Software S.A.M. M. Visualization, Data curation, Software

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Figures

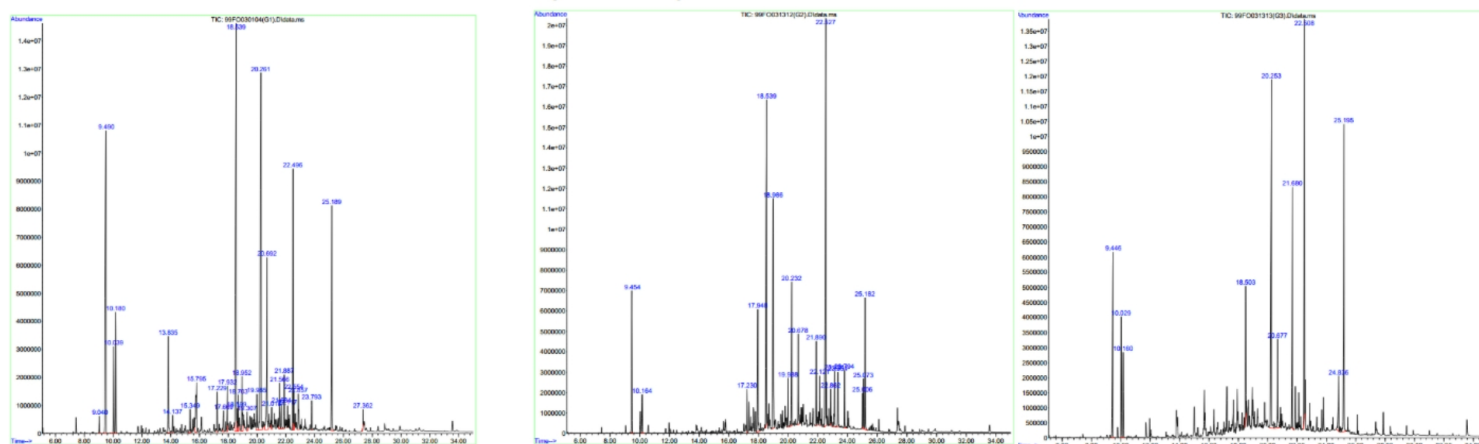


Figure 1

The GC-MS total chromatograms of dill essential oils are as follows: (a) chromatogram of coriander from the first harvest, (b) chromatogram of coriander from the second harvest, and (c) chromatogram of dill from the third harvest.

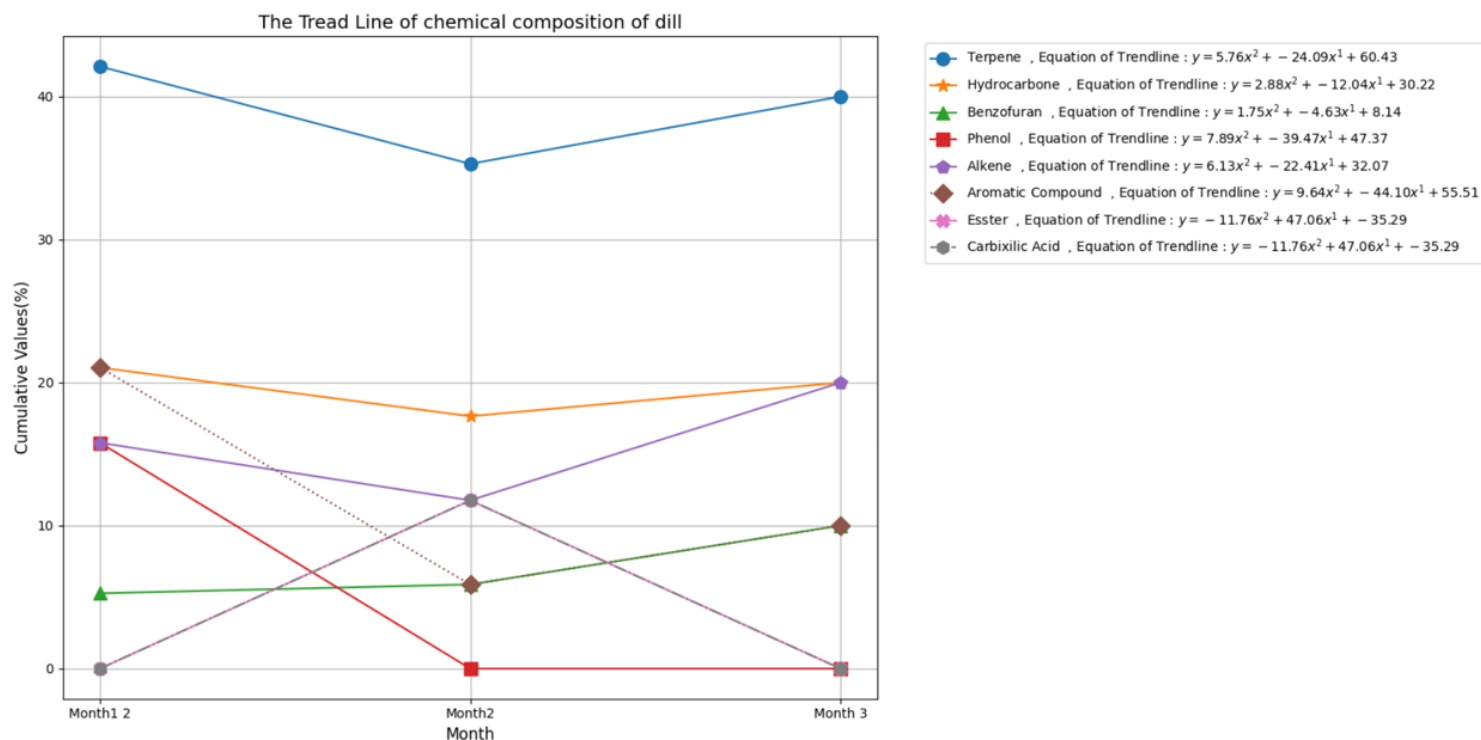


Figure 2

The Tread Line of Dill's chemical composition (Harvested in three consecutive months)

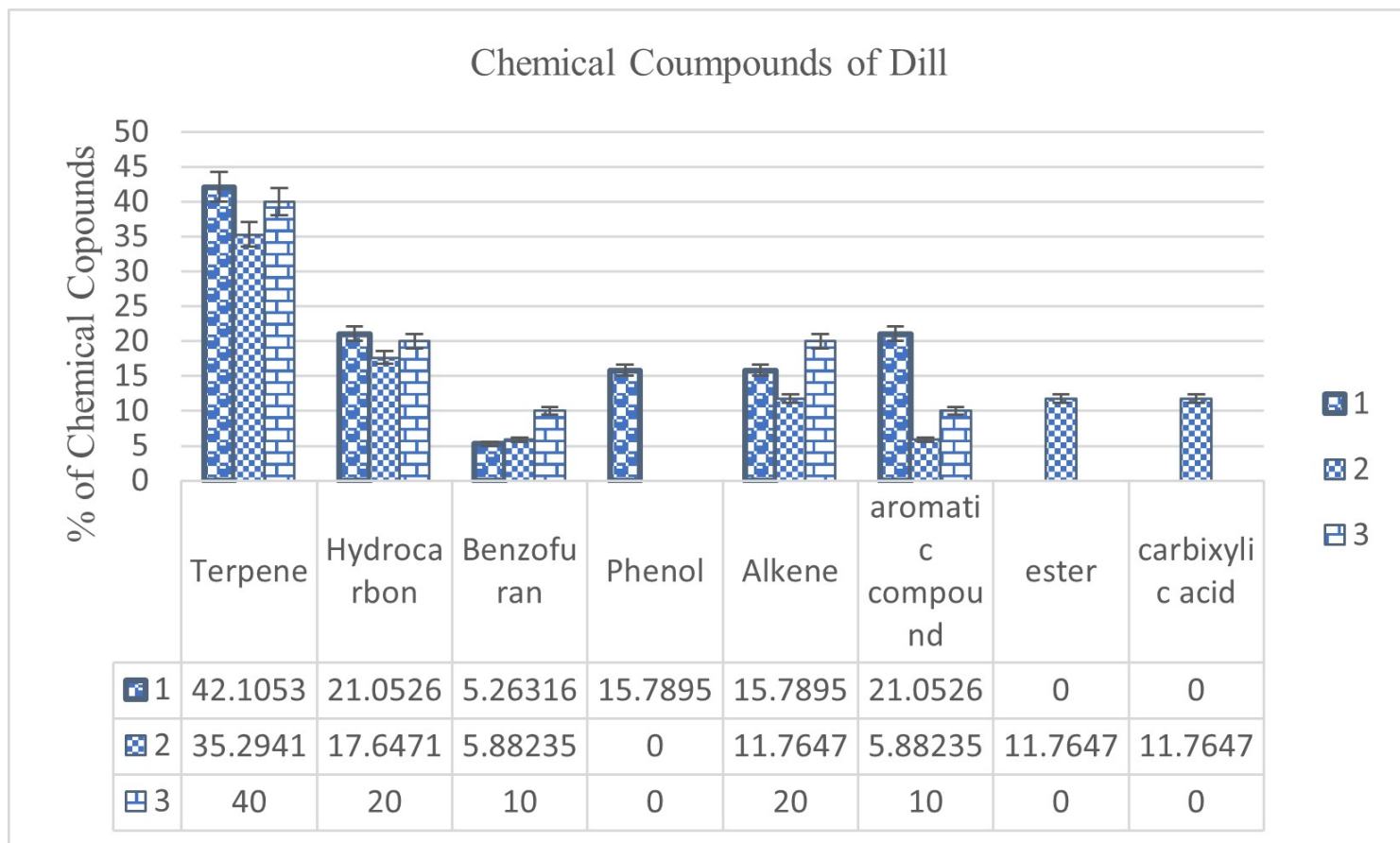


Figure 3

The comparison of chemical composition of essential oils in Sill, (*Petroselinum crispum*.) in the first, second and the third harvest