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Effect of season of harvest on essential oil yield and chemical composition of *Citrus sinensis*

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Abstract

Pulverized fresh and dried leaves of *Citrus sinensis* (500g) from morning harvests during both the rainy and dry seasons were hydro-distilled for 3 hours. The yield of essential oil from fresh leaves during the rainy season was 0.20 % (w/w), whereas in the dry season, it was 0.09 % (w/w). Seasonal variations significantly affected the yield, with higher yields observed in the rainy season. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed that oil from dry season harvest had more compounds than the oil from the dry season harvest. Monoterpenoids were predominant in both oil samples while sesquiterpenoids constituted 12.1 and 27.5% of rainy and dry season harvests respectively. Dominant compounds include limonene (**4**), 3-carene (**3**), alpha-fenchene (**1**) and beta-elemene (**2**). The composition of the oils varied across seasons, affecting their chemical profile.

Keywords: Essential Oils; Seasonal Variations; Yield; *Citrus sinensis*; Alpha-Fenchene (1); Beta-Elemene; Limonene; 3-Carene

1. Introduction

Essential oils are volatile secondary metabolites found in various plants, including *Citrus sinensis*. These oils are influenced by environmental factors such as temperature, humidity, and seasonal variations, which impact both yield and composition. Seasonal changes alter the biosynthesis of monoterpenoids and sesquiterpenoids, leading to variations in chemical profiles. This study investigates how seasonal variations affect the yield and composition of *Citrus sinensis* essential oil, comparing the rainy and dry seasons.

Essential oils are complex mixtures of volatile secondary metabolites found in plants, typically extracted from specific plant parts such as leaves, flowers, stems, bark, seeds, roots, and fruits (Bakkali et al., 2008; Pino, 2012). These oils are known for their distinctive aromas and are composed mainly of terpenoids, including monoterpenes and sesquiterpenes, as well as other bioactive compounds (Isman, 2000; Kamatou et al., 2008). Essential oils play significant roles in plant defense mechanisms, pollination, and ecological interactions (Raina et al., 2015; Rodriguez et al., 2008). Essential oils from plants like *Citrus sinensis* (sweet orange) are stored in secretory structures and are released under environmental stimuli (Schwab et al., 2013). The quantity and composition of these oils vary significantly depending on factors such as species, growth conditions, geographical location, and seasonal variations (Gershenzon & Dudareva, 2007).

1.1. Extraction of Essential Oils

Several techniques can be used to extract essential oils, with the choice of method depending on the plant species and the part from which the oil is extracted. The main extraction methods include:

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- Hydro-distillation – The plant material is submerged in water, and heat is applied to release volatile oils through steam.
- Steam Distillation – Pressurized steam is passed through the plant material, causing the oil to evaporate and later condense into a separate collection chamber.
- Cold Pressing – Used mainly for citrus oils, this method involves mechanically rupturing the oil glands on the fruit peel to release the essential oil.
- Solvent Extraction – A solvent such as hexane is used to dissolve the oil, followed by evaporation to separate the oil from the solvent.
- Microwave-Assisted Extraction – Microwaves generate heat, rupturing plant cells and releasing essential oils with minimal degradation.
- Super-Critical Fluid Extraction (SCFE) – CO_2 is used under high pressure to extract essential oils, resulting in high-purity oils without solvent residues.

For *Citrus sinensis*, cold pressing is the preferred method since the essential oil is primarily stored in oil glands in the peel. Hydro-distillation is also commonly employed for research purposes.

1.2. Chemical Composition of Essential Oils

The chemical composition of essential oils varies based on plant species, growth conditions, and external factors such as seasonal variation (Gershenzon & Dudareva, 2007). Essential oils are primarily composed of monoterpenoids and sesquiterpenoids. Monoterpenoids are the dominant constituents in citrus oils, with limonene, α-pinene, β-pinene, and terpinolene being the most prevalent. These compounds are largely responsible for the characteristic aroma of citrus essential oils (Schwab et al., 2013). Sesquiterpenoids, though present in smaller quantities, contribute to the overall profile, with compounds such as β-caryophyllene and β-elemene being commonly found (Raina et al., 2015). Oxygenated compounds, including citronellol, citronellal, neral, and citral, also play a significant role in the aroma and bioactivity of the oil (Rodriguez et al., 2008). These compounds contribute to the oil's distinctive fragrance and are known for their antimicrobial, anti-inflammatory, and antioxidant properties. Additionally, phenylpropanoids, found in trace amounts, further enhance the oil's antimicrobial and antioxidant activity (Bakkali et al., 2008).

Seasonal variations have a significant impact on the biosynthesis of these compounds, leading to marked differences in the chemical composition of the oils. During the rainy season, higher humidity levels and moderate temperatures tend to favour the production of oxygenated monoterpenes (Tisserand & Young, 2014). In contrast, the dry season often promotes the production of hydrocarbon monoterpenes, which can alter the oil's chemical makeup (Gershenzon & Dudareva, 2007). This variation in composition can have implications on the oil's biological activity (Schwab et al., 2013).

Essential oils from *Citrus sinensis* exhibit a variety of biological properties that make them valuable in multiple applications, including pharmaceuticals, food preservation, and pest control (Rodriguez et al., 2008). These essential oils are particularly known for their antimicrobial activity, with compounds like limonene and citral demonstrating both antibacterial and antifungal effects (Bakkali et al., 2008). In addition, certain terpenes, such as β-elemene and β-caryophyllene, have been found to possess insect-repellent and insecticidal properties, which contribute to the oil's potential use in pest management (Tisserand & Young, 2014). The antioxidant effects of the oil are attributed to the presence of phenolic compounds and oxygenated monoterpenes, which aid in scavenging free radicals (Bakkali et al., 2008). Furthermore, some of the compounds, such as limonene, exhibit anti-inflammatory and potential anticancer properties, further highlighting the therapeutic potential of these oils (Bakkali et al., 2008; Raina et al., 2015). The potency and efficacy of these biological activities can vary depending on seasonal changes that affect the oil's composition (Schwab et al., 2013).

The chemical characterization of essential oils is crucial for understanding their composition and potential applications. Various analytical techniques are employed for this purpose, including Gas Chromatography (GC), which separates volatile compounds based on their interaction with a stationary phase (Rodriguez et al., 2008). Gas Chromatography-Mass Spectrometry (GC-MS) is particularly useful for identifying and quantifying compounds in essential oils, providing a detailed chemical profile (Tisserand & Young, 2014). Fourier-Transform Infrared Spectroscopy (FTIR) is used to determine the functional groups present in the oils, while High-Performance Liquid Chromatography (HPLC) is typically used for analyzing less volatile components (Raina et al., 2015).

For *Citrus sinensis*, GC-MS is the most commonly used technique for identifying seasonal variations in essential oil composition.

1.3. Research Objectives

This study aims to investigate the seasonal variations in the yield and chemical composition of *Citrus sinensis* essential oil by:

- Comparing the essential oil yield from *Citrus sinensis leaves* harvested during the rainy and dry seasons.
- Identifying the key chemical constituents of the essential oils using GC-MS.
- Evaluating the impact of seasonal variation on the relative concentrations of monoterpenoids and sesquiterpenoids.
- By analyzing these factors, this research seeks to contribute to a better understanding of how environmental conditions affect the quality and quantity of *Citrus sinensis* essential oil, aiding industries that rely on natural plant extracts.

1.4. *Citrus sinensis*

There are no reports of sweet oranges occurring in the wild. Generally, it is believed that sweet orange trees originated from Southeast Asia, north-eastern India or southern China (Morton, 1987). They were first cultivated in China around 2500 BC. They are widely grown in Nigeria where it is known as "osan" by Yorubas, "lemu" by Hausas and "ape uta" by the Igbos. Like most citrus plants, sweet oranges do well under moderate temperatures – between 15.5 and 29 °C and require a huge amount of sunshine and water. Oranges, whose flavour could besweet orsour, are commonly peeled and eaten fresh or squeezed for juice. The thick bitter rind is usually discarded, but can be processed into animal feed by desiccation, using pressure and heat. It is also used in certain recipes as a food flavouring or garnish. The outermost layer of the rind can be thinly grated with a zester to produce orange zest. Zest is popular in cooking because it contains the oil glands and has a strong flavour similar to that of the orange pulp. The white part of the rind, including the pith, is a source of pectin and has nearly the same amount of Vitamin C as the flesh (Walton *et al*., 1945).

Figure 1 *Citrus sinensis* plant (Chede, 2012)

Extracts of *Citrus sinensis* contain various phytochemicals. For instance, ethanolic extracts of the pulp contains carbohydrates, alkaloids, tannins, fixed oils, proteins, amino acids, steroids and terpenoids (Chede, 2012). Essential oil can be found in the pulp, peel, rind and leaves of *Citrus sinensis*. Kamal *et al*. (2011) extracted peel essential oil of *Citrus sinensis*. Major compounds were limonene (**4**), myrcene, linalool and α-terpineol. Essential oil of *Citrus sinensis* leaves have been extracted and characterized by various workers (Kumar *et al*., 2011; Sharma and Tripathi, 2006; Abbas *et al*., 2012; Kamal*et al*., 2011; Kasali *et al*., 2011; Usman *et al*., 2016). Monoterpenoids found in the essential oils are; limonene, linalool, trans-β-ocimene, myrcene, sabinene, α-terpinene, terpinen-4-ol, α-terpineol, terpinolene, α-pinene and linalyl acetate (Kumar *et al*., 2011; Hamdan *et al*., 2013).

2. Literature Review

Seasonal variation significantly influences the biosynthesis, yield, and chemical composition of essential oils in plants. Variations in environmental conditions, such as temperature, humidity, rainfall, and photoperiod, impact the metabolic pathways responsible for essential oil synthesis. Several studies have demonstrated that essential oil yield and composition fluctuate with seasonal changes, leading to differences in the concentration of bioactive compounds.

2.1. Effect of Seasonal Variation on Essential Oil Yield

The yield of essential oils is known to vary across different seasons due to environmental factors affecting plant metabolism. Research has shown that essential oil production is generally higher in warmer and more humid conditions. For instance, McGimpsey *et al*. (2006) reported that the essential oil yield from *Thymus vulgaris* leaves was highest in spring compared to winter, summer, and autumn. Similarly, *Origanum vulgare* leaves produced lower essential oil content during the cooler and wetter vegetative period, while a higher yield was obtained during the warmer and drier flowering stage (Skoula & Harborne, 2002).

2.2. Impact of Seasonal Changes on Chemical Composition

The chemical composition of essential oils is influenced by the season, with variations in monoterpenoid and sesquiterpenoid profiles. Monoterpenoids, which contribute to the fragrance and antimicrobial properties of essential oils, are more dominant in the rainy season due to increased enzymatic activity. In contrast, sesquiterpenoids, which are often linked to stress-induced metabolic pathways, are more abundant in the dry season.

A study by Frizzo *et al*. (2004) on *Origanum vulgare* found that thymol and carvacrol contents decreased in autumn and increased in other seasons, with maximum phenol content observed after flowering. Similarly, McGimpsey *et al*. (2006) reported seasonal variation in *Thymus vulgaris* essential oils, with oxygenated compounds being higher in spring compared to summer, autumn, and winter.

2.3. Influence of Environmental Factors on Essential Oil Composition

- Temperature and Humidity – Temperature and humidity affect enzyme activity in the biosynthetic pathways of essential oils. Higher temperatures during the dry season can lead to increased oxidation and polymerization of essential oil constituents, resulting in a lower concentration of volatile monoterpenes.
- Soil Conditions – Abdelrazzaq *et al*. (2013) found that *Salvia officinalis* cultivated under protected soilless conditions produced essential oil rich in camphor, whereas plants grown in open fields yielded oils with higher 1,8-cineole content. This suggests that soil conditions significantly influence enzymatic pathways and oil composition.
- Photoperiod and Light Exposure – Voirin *et al*. (1990) discovered that prolonged exposure to sunlight increased the synthesis of menthol in *Mentha piperita*, while shorter daylight exposure led to higher menthofuran levels.

2.4. Comparative Studies on Seasonal Variation in Essential Oils

Several studies have compared seasonal influences on essential oil yield and composition across different plant species.

- Fennel (***Foeniculum vulgare***) – Marotti *et al*. (1994) observed that fennel seed oil yield was highest at the ripe seed stage, with limonene being dominant in early development and E-anethole prevailing in mature seeds.
- Tea Tree (***Melaleuca alternifolia***) – Postharvest drying of leaves led to increased oil yield due to continued metabolic activity, possibly redistributing oil from the stem to the leaves(Whish & Williams, 1996). This was also demonstrated in *Citrus sinensis*(Usman*et al.,*2016).
- Peppermint (***Mentha piperita***) – The drying process affected the oil's chemical composition, with variations in monoterpenoid levels depending on drying conditions (Asekun *et al*., 2007).

These studies confirm that seasonal variations, along with postharvest handling and environmental factors, play a critical role in determining the quantity and quality of essential oils.

The literature confirms that seasonal variation has a significant impact on essential oil yield and composition. Warmer, humid conditions favor monoterpenoid biosynthesis, leading to higher oil yields, while drier conditions promote stress-induced pathways, resulting in an increase in sesquiterpenoids. The findings from *Citrus sinensis* essential oil align with previous studies, showing significant seasonal variation in both yield and composition. Understanding these changes can enhance the commercial extraction and utilization of essential oils, optimizing production based on seasonal trends.

3. Materials and methods

3.1. Plant Materials

Fresh leaves of *Citrus sinensis* were collected from a selected plantation in Nigeria during both the rainy and dry seasons. The fresh leaves for both seasons were collected in the morning and were used fresh, without drying to avoid the influence of drying and collection time on the yield and chemical composition. The leaves were pulverised and weighed before extraction.

3.2. Oil Isolation

Essential oils were extracted using hydro-distillation in a Clevenger-type apparatus for three hours. To begin, 500 g of pulverized leaves were placed in a distillation flask with an adequate amount of distilled water. The mixture was then heated, generating steam that carried the essential oils through a condenser. The oil was separated from the condensed water using a graduated receiver, after which it was weighed, and the yield was calculated as a percentage of the plant material using the formula:

$$\text{Oil Yield}(\%)=\left(\frac{\text{Weight of Oil Extracted}}{\text{Weight of Plant Material}}\right)\times 100$$

Finally, the extracted oil samples were stored in amber vials at 4°C to prevent oxidation and degradation.

3.3. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The chemical composition of the essential oils was analyzed using GC-MS to identify the presence and concentration of various monoterpenoids and sesquiterpenoids. The analysis was carried out under the following conditions:

- Instrument: Gas chromatograph coupled with a mass spectrometer
- Column: Capillary column (30m × 0.25mm, film thickness 0.25μm)
- Carrier Gas: Helium, at a flow rate of 1.0 mL/min
- Injection Volume: 1 μL, with a split ratio of 1:20

Oven Temperature: Initially set at 60°C for 2 minutes, then increased at a rate of 5°C/min to 250°C and held for 10 minutes

- Mass Spectrometry Conditions:
- Ionization Energy: 70 eV
- Mass Range: 50–500 m/z
- Ion Source Temperature: 200°C

Theidentification of compoundswas achieved by comparing the obtained mass spectrawith data from theNational Institute of Standards and Technology (NIST) library and retention indices from literature sources.

4. Results and discussion

4.1. Yield

The yield of essential oils extracted from *Citrus sinensis* varied significantly between the rainy and dry seasons. These differences are influenced by environmental factors such as temperature, humidity, and soil moisture content, which affect essential oil biosynthesis. The results showed that oil yield was higher during the rainy season (0.2 % w/w) compared to the dry season (0.09 % w/w).The higher oil yield observed during the rainy season suggests that increased humidity and moderate temperatures enhance enzymatic activity related to essential oil biosynthesis. The lower yield in the dry season suggests that water stress and high temperatures negatively impact essential oil production.

4.2. Chemical Composition of *Citrus sinensis* Essential Oil

Table 1 Compounds identified in *Citrus sinensis* essential oils from rainy and dry seasons

	Compounds	**KI**	**% Composition**		**Mass Spectra Data**
			RMF	**DMF**	
1	α-thujene	931	0.4	-	136,121, 105, 93, 77
2	α-pinene	939	1.5	0.9	136, 121, 93, 91, 77
3	α-fenchene (1)	951	12.7	-	136, 121, 93, 79, 41

4	Camphene	953	-	0.2	136, 121, 93, 79, 39
5	β-thujene	971	0.6	0.3	136, 121, 105, 93, 77
6	β-myrcene	991	-	2.3	136, 93, 69, 41, 27
7	2-carene	1001	-	0.7	136, 121, 105, 93, 41
8	α-phellandrene	1005	1.1	0.9	136, 119, 93, 77, 65
9	3-carene (3)	1011	9.1	8.3	136, 121, 105, 93, 91
10	α-terpinene	1018	1.1	-	136, 121, 105, 93, 77
11	ortho-cymene	1020	-	1.8	134, 119, 103, 91, 77
12	Para-cymene	1026	-	0.5	134, 119, 91, 65, 41
13	D-limonene (4)	1031	8.7	6.8	136, 121, 93, 79, 68
14	Cis-β-ocimene	1040	-	6.7	136, 121, 93, 79, 41
15	Isocarvestrene	1047	1.6	-	136, 121, 93, 68, 41
16	Trans- β-ocimene	1050	0.5	0.5	136, 121, 105, 93, 79
17	γ-terpinene	1062	0.5	8.5	136, 121, 105, 93, 77
18	m-cymene	1082	1.3	0.3	134, 119, 103, 91, 77
19	α-Terpinolene	1088	1.4	3.8	136, 121, 105, 93, 79
20	Cis-verbenol	1140	0.3	-	154, 109, 94, 59. 41
21	Isopulegol	1145	0.2	-	154, 121, 81, 55, 41
22	Citronellal (6)	1153	6.1	1.0	154, 136, 121, 95, 69
23	Terpinen-4-ol	1177	2.5	1.9	154, 136, 121, 93, 71
24	α-terpineol	1189	0.6	-	136, 121, 93, 81, 59
25	Decanal	1204	0.3	-	156, 112, 82, 70, 57
26	Citronellol (5)	1228	6.7	3.5	156, 138, 95, 81, 69
27	Neral	1240	4.2	-	152, 137, 109, 84, 69
28	Citral (7)	1240	4.3	0.8	152, 109, 94, 84, 69
29	Geranyl linalool	1244	1.2	-	290, 161, 93, 69, 41
30	Lavandulyl acetate	1289	-	2.8	138, 95, 81, 69, 57
31	Thymol	1290	0.3	-	150, 135, 115, 91, 77
32	Methyl geranate	1323	0.8	0.4	182, 151, 123, 114, 69
33	Citronellyl acetate	1354	2.3	5.0	138, 123, 109, 95, 69
34	β-elemene (2)	1375	2.2	8.6	204, 161, 121, 93, 81
35	β-copaene	1378	-	0.4	204, 161, 119, 105, 93
36	Geranyl acetate	1383	-	2.9	196, 136, 121, 93, 69
37	8-hydroxyneomenthol	1423	-	2.3	
38	γ-elemene	1430	0.3	-	204, 121, 93, 67, 41
39	β-Guaiene	1439	2.0	-	204, 161, 105, 91, 41
40	Humulene	1440	0.8	1.8	204, 147, 121, 107, 93
41	Caryophyllene (8)	1454	2.3	5.2	204, 161, 133, 93, 79

42	Cis-β-farnesene	1458	1.4	1.7	204, 161, 133, 93, 69
43	β-cadinene	1473	-	0.6	204, 189, 161, 105, 91
44	γ-maaliene	1477	-	0.3	204, 161, 119, 93, 79
45	β-selinene	1485	-	0.7	204, 161, 121, 105, 93
46	Valencene	1491	0.2	-	204, 161, 119, 105,93
47	α-selinene	1494	-	0.7	204, 121, 105, 79, 41
48	α-farnesene	1508	-	0.4	204, 119, 107, 93, 69
49	Elemol	1547	-	0.3	204, 161, 93, 59, 41
50	Spathulenol	1576	-	0.5	220, 205, 159, 119, 91
51	Caryophyllene oxide	1581	0.6	1.0	220, 187, 161, 121, 79
52	Eudesmol	1652	0.3	0.4	222, 204,189,149,59
53	α-sinensal	1752	2.0	5.0	218, 134, 93, 79, 55
54	Phytol	1949	1.2	-	296, 123, 81, 71, 57
55	Phytol acetate	2223	-	3.8	340, 278, 123,95, 68
	TOTAL %		83.6	94.5	

RMF: Fresh leaves from morning harvests in the rainy season
DMF: Fresh leaves from morning harvests in the dry season

Table 1 shows the identities, kovats indices, percentage composition and mass spectral data of the constituents of essential oil obtained from fresh *Citrus sinensis* leaves harvested in the morning during the rainy season and dry seasons.A total of 55 compounds were identified; 37 compounds were identified in the rainy season while 40 compounds were identified in the dry season.

The most abundant compound in the rainy season, α-fenchene (**1**) (12.7 %) was absent in the oil from the dry season harvest. β-elemene(**2**) was the most abundant compound (8.6 %) in the dry season and only existed in a minimal quantity (2.2 %) in the oil from the rainy season harvest. 3-carene (**3**) and limonene (**4**) were prevalent in oils from both seasons.

During the rainy season, higher concentrations of monoterpenoidswere observed, particularly α-fenchene (**1**)(12.7 %), 3-carene (**3**) (9.1 %), limonene (**4**) (8.7 %), citronellol (**5**) (6.7 %), and citronellal (**6**) (6.1 %). Notable amounts of citral (**7**) (4.3 %) and neral (4.2%) were also present, while terpinen-4-ol (2.5 %), β-caryophyllene (**8**) (2.3 %), citronellyl acetate (2.3 %) andβ-elemene (**2**) (2.2 %) were found in lower levels. In contrast (Table 2), the dry season showed an increase in sesquiterpenoids, with higher concentrations of β-elemene (**2**) (8.6 %), β-caryophyllene (**8**) (5.2 %) and α-sinensal (5 %). However, citronellal (**6**)and citronellol (**5**) levels were lower, likely due to heat-induced degradation.

Conversely, the dry season resulted in lower oil yields but a higher concentration of sesquiterpenoids, such as β-elemene (**2**), β-caryophyllene(**8**) and α-sinensalindicating a shift towards sesquiterpenoid-dominated profiles.. This increase in sesquiterpenoids suggests that water stress and high temperatures trigger secondary metabolic pathways associated with plant defense mechanisms. Similar findings have been reported in *Origanum vulgare*, where phenolic compound levels varied across seasons, with higher oxygenated monoterpenes in spring and increased sesquiterpenoids in summer and autumn (Kiss A.K, 2012).

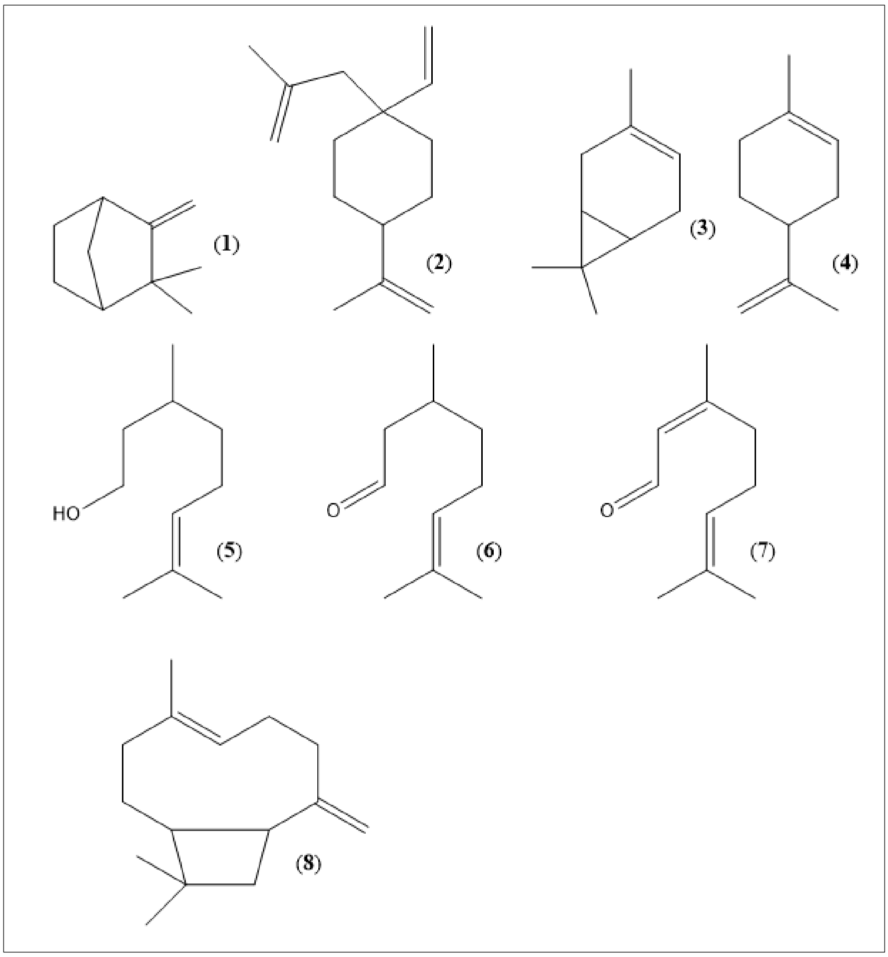

Figure 2 Chemical structures of some notable compounds influenced by seasonal variation in C. sinensis essential oils

Table 2 Major differences in Essential Oil Yield and Composition of *Citrus sinensis* as influenced by season

Parameters	**Rainy Season**	**Dry Season**
Oil Yield (% w/w)	0.2	0.09
No of compounds	37	40
Monoterpenoid Content (%)	69.1	54.9
Sesquiterpenoid Content (%)	12.1	27.5
Major Constituents	α-Fenchene (1) (12.7 %), 3-carene (3) (9.1 %),Limonene (4) (8.7 %), Citronellol (5) (6.7 %), citronellal (6) (6.1 %)	β-Elemene (2) (8.6 %), γ-terpinene(8.5 %), 3-carene (3) (8.3 %),limonene (4) (6.8 %), Cis-β-ocimene(6.7 %), caryophyllene (8) (5.2 %)

These findings confirm thatseasonal variation significantly affects both the yield and composition of *Citrus sinensis* essential oil, with implications for industrial applications suchas flavouring, perfumery, pharmaceuticals, and insecticides**.**

5. Conclusion

The findings of this study indicate that essential oils from *Citrus sinensis* leaves exhibit both qualitative and quantitative variations due to seasonal changes. The rainy season favoured higher oil yields and monoterpenoid production, whereas the dry season resulted in lower yields but a higher concentration of sesquiterpenoids.

These variations have important commercial and industrial implications, as the choice of harvesting season can affect the yield and chemical profile of *Citrus sinensis* essential oils, impacting their use in perfumery, pharmaceuticals, and insecticidal applications.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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