



Essential Oil Variation in *Matricaria chamomilla* L.: The Impact of Harvest Timing and Flower Development

Abdolbaset Mahmoudi^{1b}, Mohamad Norani^{1b}, Sajjad Sedaghat^{1b}, Mohammad-Taghi Ebadi*^{1b}

Department of Horticultural Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

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ABSTRACT

This study investigates the impact of flower harvesting times on the essential oil (EO) yield and composition of *Matricaria chamomilla* L. Flowers were collected at various developmental stages, including early flower opening, full flowering (at 6:00 AM, 9:00 AM, 12:00 PM, 3:00 PM, and 6:00 PM), the end of flowering, and fruit set. EO extraction was performed via hydro-distillation, and volatile components were analyzed using GC and GC/MS. The highest EO yield (0.28% w/w) was obtained at full flowering at 12:00 PM. This peak may be attributed to increased enzymatic activity, light intensity, and diurnal accumulation of volatile compounds. A total of 22 compounds were identified, making up as much as 96.4% of the EO profile. Oxygenated sesquiterpenes, particularly α -Bisabolol oxide A (up to 66.7%), α -Bisabolone oxide A (10.5%), and α -Bisabolol oxide B (6.8%), were predominant at noon and late afternoon. In contrast, *trans*- β -Farnesene peaked in early morning samples, and chamazulene showed no significant variation across times. Comparison with ISO 19332:2020 standards revealed that α -Bisabolol oxide A and α -Bisabolone oxide A exceeded reference values, while chamazulene and *trans*- β -Farnesene remained below typical ranges. The highest proportion of oxygenated sesquiterpenes was observed at 12:00 PM (80.2%), suggesting strong pharmacological potential at this time. Minor compounds such as *cis*-Spiroether and linalool varied slightly by harvest stage. These results demonstrate that both harvest timing and flower developmental stage are critical for optimizing EO yield and therapeutic quality in *M. chamomilla*.

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1. Introduction

Medicinal plants serve as rich reservoirs of secondary metabolites, which are extensively utilized in pharmaceuticals, agrochemicals, flavors and fragrances, natural colorants, bio-pesticides, and food additives (Nouri *et al.*, 2025). *Matricaria chamomilla* L. (*Chamomilla recutita* L.), commonly known as chamomile, is a member of the Asteraceae family. This medicinal plant is native to regions including the Near East, South and East Europe, the Middle East, and Iran (Ebadi *et al.*, 2009a; Rahmati *et al.*, 2010). The plant is characterized by a straight, hollow stem reaching up to 60 cm in height. Its alternately arranged leaves are divided into smaller leaflets. The flowers are arranged in paniculate flower heads (capitula), comprising white ray florets and yellow disc florets (Mahmoudi *et al.*,

2020). Chamomile is widely cultivated due to its therapeutic and economic value, especially for its essential oil (EO), which is extracted from the dried flowers and exhibits notable pharmacological properties. This blue EO is present in the yellow florets at a rate of about 0.5 to 1 percent (Ebadi *et al.*, 2009b). The global chamomile EO market is valued at approximately USD 6.15 billion in 2025 and is expected to reach USD 9.83 billion by 2035, growing at a CAGR of 4.8%. The majority of this demand stems from the cosmetics and aromatherapy sectors, which together account for over 85% of market revenue (Future Market Insights, 2025). In addition to its growing commercial value, the chamomile EO industry faces notable supply chain challenges. Variability in raw material quality, influenced by factors such as

* Corresponding author.

E-mail address: mt.ebadi@modares.ac.ir

cultivation practices and climatic conditions, often leads to inconsistencies in EO composition (Ebadi et al., 2009b).

Chamomile EO is widely recognized for its calming and soothing effects, making it a popular choice in aromatherapy (Shah, 2023). Its aromatic properties are believed to alleviate stress, reduce anxiety, and promote a sense of relaxation (Alvarado-García et al., 2024; Ebrahimi et al., 2022). Applications include diffusion into the air or incorporation into bathwater for therapeutic benefits (Shutes and Galper, 2022). Its therapeutic efficacy is largely attributed to its bioactive constituents, including chamazulene, α -bisabolol and its oxides, and apigenin-7-glucoside, which contribute to its anti-inflammatory, antioxidant, antimicrobial, and sedative activities (Akram et al., 2024; Mahajan et al., 2023). In addition to its psychological benefits, chamomile EO is frequently employed in skincare for its ability to support healthy skin and manage conditions such as eczema and dermatitis (Jahan and Happy, 2022; Sah et al., 2022). Recent research has further highlighted the wound-healing, gastroprotective, and anticancer potential of specific EO constituents (Al-Ghanim et al., 2023; Gladikostić et al., 2023). The economic significance of chamomile EO spans personal care and cosmetic products, aromatherapy and spa treatments, herbal and medicinal applications, flavoring agents, and beverages. Its role in natural and organic product sectors, as well as international trade, underscores its versatility and value (Bolouri et al., 2022; Formisano et al., 2015). Moreover, the production and commercialization of chamomile EO contribute significantly to economic development, offering employment opportunities for farmers, distillers, manufacturers, and distributors involved in its supply chain (Ghareeb et al., 2022; Mirzoieva et al., 2021).

The timing of flower harvesting plays a pivotal role in determining the yield and quality of EO in *M. chamomilla* (Kumar et al., 2023; Salehi and Hazrati, 2017; Yadav et al., 2022). Research consistently demonstrates that harvest timing significantly influences both the quantity and composition of EOs in medicinal plants (Hosseinabadi et al., 2024; Kumar et al., 2020; Moradi et al., 2021; Ostadi et al., 2020). Several studies have documented that both diurnal variation and flower developmental stage lead to significant fluctuations in the levels of major EO

constituents, such as α -bisabolol oxide A, chamazulene, and trans- β -Farnesene (Mehriya et al., 2022; Padalia et al., 2017).

Optimal harvesting of chamomile flowers occurs at the full bloom stage, typically in the early morning hours. During this time, cooler temperatures help preserve volatile compounds, leading to the highest concentration of EO and retaining the aroma and therapeutic properties of the plant (Rathore and Kumar, 2021; Makeri and Salihu, 2023; Katekar et al., 2022). Conversely, harvesting later in the day, when temperatures are higher, can reduce EO content due to the evaporation or degradation of volatile compounds (Kumar et al., 2020; Yeşil and Özcan, 2021).

Environmental factors such as sunlight exposure also influence the chemical composition of chamomile EO. Prolonged exposure to sunlight prior to harvesting can increase the concentration of chamazulene, a blue-colored compound formed during distillation, which contributes to the distinctive blue color of the oil (Mehriya et al., 2022; Rawat et al., 2022). While increased levels of chamazulene may enhance certain desirable properties of the oil, excessive sunlight exposure can lead to alterations in the oil's overall composition, potentially affecting its quality and color. (Belcadi et al., 2023). Similarly, oxygenated sesquiterpenes such as α -Bisabolol oxide A and B tend to peak under specific thermal and photoperiodic conditions, suggesting that harvest timing and environmental interactions are critical in modulating EO profiles.

Moreover, the developmental stage of the plant significantly impacts EO yield and composition (Hazrati et al., 2022). Although the early work by Franz et al. (1978) laid the groundwork for understanding the influence of developmental stages on chamomile EO composition, more recent studies have expanded this knowledge. For example, Rathore and Kumar (2021) reported that flower maturity significantly affects the concentration of major bioactive constituents, with fully opened flowers often yielding higher levels of oxygenated sesquiterpenes. These findings underscore the importance of precise harvest timing aligned with floral development to optimize both yield and therapeutic quality of the EO.

Environmental factors such as temperature and light intensity are also critical for EO biosynthesis. Das and Prakash (2023) emphasized that these factors can

influence the stability and yield of EOs in aromatic plants. Harvesting time during the day further contributes to variations in EO yield and composition (Zamani et al., 2023; Labruzzo et al., 2017). Kumar et al. (2020) found that harvesting chamomile flowers at different times of the day leads to changes in the oil's chemical composition, with certain compounds reaching their peak concentrations at specific times. For instance, α -Bisabolol oxide A may peak at midday, while compounds like *trans*- β -Farnesene show higher levels during early morning harvests. Such temporal dynamics are relevant for optimizing both the quantity and quality of EOs. Collectively, these findings highlight the need to carefully consider environmental factors, developmental stages, and the time of harvest to optimize EO yield and quality in *M. chamomilla*.

Despite the growing body of literature, further research is needed to clarify how phenological stages and environmental factors interact, particularly with respect to diurnal variation and its influence on both major and minor EO constituents. Additionally, fewer than five peer-reviewed studies have compared compositional changes in chamomile EO with international quality standards such as ISO 19332:2020 (ISO, 2020). Therefore, this research aimed to investigate the effects of both flower developmental stage and time of day on EO yield and the concentration of key bioactive compounds in *M. chamomilla*. By identifying optimal harvest parameters and comparing the chemical profile with ISO benchmarks, this study offers novel insights to enhance the efficiency, consistency, and therapeutic quality of chamomile EO for pharmaceutical and commercial applications.

2. Materials and methods

2.1. Plant materials

Seeds of *M. chamomilla* were sourced from Pakaan Bazr Company, which supplies the commonly used variety cultivated by farmers in central and southern provinces of Iran, including Isfahan and Fars. No specific improved or genetically modified cultivar was used in this study. The plants were grown under standard agronomic conditions without the application of fertilizers, pesticides, or growth regulators. Regular irrigation and manual weeding were employed to support healthy plant development under natural conditions. The experiment was conducted at the research farm of the Faculty of Agriculture, Tarbiat

Modares University, located in Tehran Province, Iran (51°09'48"N, 35°44'30.3"E). Flowers of *M. chamomilla* were collected at different developmental stages, including early flower opening, full flowering (at 6:00 AM, 9:00 AM, 12:00 PM, 3:00 PM, and 6:00 PM), the end of flowering, and fruit set. The chamomile samples were harvested during May 2024, which coincides with the peak flowering period in the region. Physico-chemical properties of research farm soil were presented in Table 1.

Table 1. Physico-chemical properties of research farm soil

Texture	pH	N (%)	S	K	P	Mg	Ca	Fe
			(mg kg ⁻¹)					
Sandy loam	7.7	0.2	50.5	34.1	10	0.6	11.6	4.1

Sampling was performed based on a completely randomized design with three replications per treatment. For each developmental stage and time point, flowers were collected from at least ten randomly selected plants, ensuring even distribution across the field. Field density was consistent across the plot (approximately 25 plants m²), and all sampled plants were of uniform height and phenological stage to reduce variability. For the early flowering and fruit set stages, sampling was conducted at a fixed time (9:00 AM) to avoid diurnal variation and ensure comparability across stages. All samples were collected under uniform environmental conditions to minimize variability. Following collection, the flowers were air-dried under shaded conditions at room temperature (approximately 22–25°C) for 3 days until reaching a constant weight.

2.2. Isolation and analysis of EO

Approximately 50 grams of air-dried *M. chamomilla* flowers were finely chopped and immersed individually in 500 mL of distilled water. EO extraction was performed via hydrodistillation using a Clevenger-type apparatus for 3 hours. The extracted EOs were separated from the distillate, dried with anhydrous sodium sulfate, and stored at 4°C for subsequent analysis. The EO content was calculated on a weight/weight (w/w) basis. All samples were analyzed within one week of extraction to minimize any potential changes in composition due to storage.

Gas chromatography (GC) analysis was performed using a system (7890B, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization

detector (FID) and an HP-5 fused silica capillary column (30 m length, 0.32 mm inner diameter, 0.25 μ m film thickness). Helium served as the carrier gas at a constant flow rate of 1.1 mL min⁻¹. The oven temperature was initially set at 60°C and held for 3 minutes, then increased at a rate of 3°C/min to 240°C, where it was held for 10 minutes. Each EO sample (1 μ L) was injected in split mode with a split ratio of 100:1. For GC/MS analysis, the same chromatographic conditions were applied using a gas chromatograph coupled with a Trace mass spectrometer (Thermoquest–Finnigan, San Jose, CA, USA). The ionization voltage was 70 eV, with the ion source and interface temperatures maintained at 200°C and 250°C, respectively.

Compound identification was achieved by comparing the mass spectra of the EO components with those in the Wiley 7.0 and Adams mass spectral libraries, using a match quality threshold of $\geq 80\%$ similarity. Retention indices were calculated using the linear interpolation method based on a homologous series of n-alkanes (C8–C24) analyzed under identical conditions. Published retention index data and reference materials (Adams, 2007) were also consulted for compound confirmation.

2.3. Statistical analysis

The collected data were analyzed using analysis of variance (ANOVA) based on a completely randomized design with three replications. Statistical analyses were performed using the SAS Statistical Package Program (version 9.0). The PROC UNIVARIATE procedure in SAS was used to evaluate the assumptions of ANOVA, confirming that the residuals followed a normal distribution. Mean comparisons were conducted using the least significant difference (LSD) test at a 5% significance level, enabling pairwise comparisons to determine statistically significant differences between treatment means.

3. Results and discussion

3.1. EO content

Analysis of variance revealed that harvest time significantly influenced the EO content of *M. chamomilla*. The EO content varied across the eight harvesting times (Fig. 1), with the highest yield recorded at the full flowering stage (12:00 noon), reaching 0.28% w/w (T3). This result suggests that

optimal harvesting occurs at this specific time, likely due to factors such as temperature, light intensity, and physiological processes during the flowering stage (Jerca et al., 2024). In addition, other contributing factors may include increased enzymatic activity related to secondary metabolite biosynthesis, diurnal fluctuations in volatile compound accumulation, stomatal conductance influencing volatile release, and circadian regulation of terpene synthase gene expression. These combined biochemical and environmental influences may explain the observed peak in EO yield at midday (Liebelt et al., 2019; Hazrati et al., 2022).

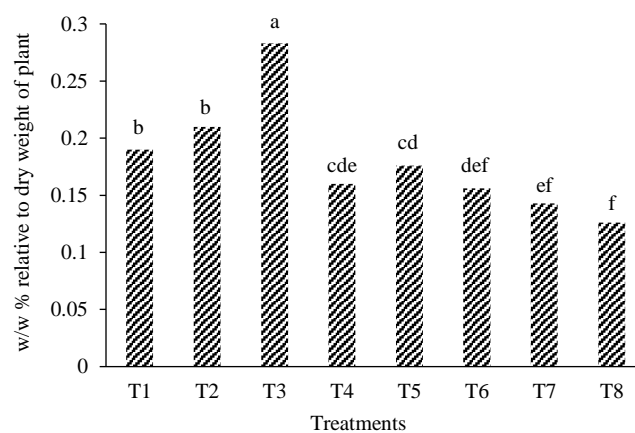


Figure 1. EO content in *M. chamomilla* during different harvest times. Full flowering stage: T1–T5 (T1: At 6 am, T2: At 9 am, T3: At 12 noon, T4: At 3 pm, T5: At 6 pm), T6: Early flower opening, T7: End of flowering stage, T8: Fruit set.

These findings align with previous studies indicating that the EO content of *Ocimum basilicum*, *O. americanum*, and *O. kilimandscharicum* (camphor type) also peaked at full flowering stages around noon (Padalía et al., 2017). Similarly, Salehi and Hazrati (2017) reported variations in chamomile EO content when harvested at different times throughout the day. Their study showed EO percentages of 0.812%, 1.11%, 0.952%, 0.806%, 0.908%, and 0.652% for harvest times of 8:00–10:00 AM, 10:00 AM–12:00 PM, 12:00–2:00 PM, 2:00–4:00 PM, 4:00–6:00 PM, and 6:00–8:00 PM, respectively, with the highest yield observed between 10:00 AM and 12:00 PM.

Similarly, maximum EO yields at full flowering stages have been reported for other medicinal plants, including *Origanum vulgare* ssp. *hirtum* (Baranauskienė et al., 2013; Król et al., 2019), *O. vulgare* ssp. *vulgare* (Baranauskienė et al., 2013), and *Thymus vulgaris* (Moradi et al., 2021). Conversely, the

lowest EO yield for *M. chamomilla* in this study was recorded during the fruit set stage. Similar reductions in EO yield at the fruit set stage have been observed in *Diphasia klaineana* (Boua et al., 2021) and *Origanum onites* L. (Ozkan et al., 2010).

The decline in EO yield observed during the fruit set stage may result from physiological resource allocation toward seed development, which usually decreases the availability of precursors for secondary metabolite synthesis. Additionally, metabolic shifts at this stage may suppress pathways involved in producing volatile compounds, leading to a reduction in EO production. The differences observed in EO content can be linked to a combination of environmental conditions during the growing season (Benomari et al., 2023; Norani et al., 2023).

3.2. EO composition

The EOs of *M. chamomilla* were analyzed using GC and GC/MS. The total identified compounds, along with the primary classes and subclasses of the oil constituents, are presented in Table 2 and Fig. 2. In total, 22 compounds were identified and quantified across eight harvest times: early flower opening, full flowering stage (6 AM, 9 AM, 12 noon, 3 PM, and 6 PM), end of flowering stage, and fruit set. These compounds accounted for 92.4%, 96.4%, 95.6%, 93.6%, 94.3%, 95.6%, 94.1%, and 95.7% of the oil, respectively. The major constituents identified in EOs across all harvest times were α -Bisabolol oxide A (44.8%–66.7%), *cis*-Spiroether (8.9%–14.1%), α -Bisabolone oxide A (5.5%–10.5%), α -Bisabolol oxide B (3.0%–6.8%), and *cis*- α -Santalol (1.1%–4.3%). The highest levels of α -Bisabolol oxide A were recorded at full flowering stages at 12:00 PM (66.4%, T3) and 3:00 PM (66.7%, T4), suggesting that peak light intensity may enhance its biosynthesis. Likewise, α -Bisabolol oxide B (6.8%) and α -Bisabolone oxide A (10.5%) reached their maximum concentrations in the 6:00 PM harvest (T5), possibly due to the cumulative effects of light-driven metabolic activity throughout the day.

Interestingly, *trans*- β -Farnesene exhibited its highest concentration during the early flowering stage at 6:00 AM (T1) and dropped significantly at later time points. This compound is known to play a role in plant defense and signaling, particularly in response to environmental factors regulated by circadian rhythms. It is likely that cooler morning temperatures and early

light exposure stimulate its synthesis and emission (Arimura et al., 2009). In contrast, the elevated levels of oxygenated sesquiterpenes such as α -Bisabolol oxide A and α -Bisabolone oxide A during midday and afternoon harvests may be associated with increased photosynthetic activity and light-dependent enzymatic pathways that promote the accumulation of these bioactive components (Figueiredo et al., 2008; Irmisch et al., 2012). Furthermore, the relatively higher amounts of *cis*-Spiroether observed in early-day samples may reflect its involvement in early stress signaling or plant–environment interactions (Dudareva et al., 2013). Some monoterpenes, such as linalool, which showed a declining trend later in the day, are known to be light-sensitive and more volatile, which could explain their reduced abundance during afternoon harvests (Sangwan et al., 2001).

The relatively stable levels of chamazulene across different harvest times observed in this study are noteworthy, particularly given its known sensitivity to thermal and oxidative conditions during distillation. Some studies have reported similar patterns; for example, Kumar et al. (2020) found minimal variation in chamazulene content across various growth stages and harvest times, suggesting that its formation is largely dependent on the precursor matricin and distillation conditions rather than on environmental or developmental factors. In contrast, other studies have shown greater variability in chamazulene levels due to factors such as drying temperature or distillation method (de Almeida et al., 2020). These contrasting findings underscore the complexity of chamazulene biosynthesis and the need for further research to clarify the key drivers of its fluctuation in *M. chamomilla* EO.

Although several studies have examined EO composition in *M. chamomilla*, few have addressed how chemical profiles vary with both developmental stage and time of harvest. For instance, Ghareeb et al. (2022) reported bisabolol oxide A (33.2%–47.3%), bisabolone oxide A (1.3%–12.4%), chamazulene (1.6%–14.0%), and bisabolol oxide B (1.2%–20.6%) as dominant constituents at full flowering stages in Egypt. Similarly, Salehi and Hazrati (2017) demonstrated that the abundance of compounds such as *trans*-anethole, α -Bisabolone oxide A, and α -Bisabolol oxide B increased in midday harvests, while chamazulene, α -Bisabolol oxide A, and *trans*- β -Farnesene were more prevalent in morning samples.

Table 2. Chamomile EO components (%) during different harvest times

No	RI ^a	Components	T1	T2	T3	T4	T5	T6	T7	T8
1	945	α -Fenchene	-	-	0.03	0.03	0.02	0.03	0.02	-
2	1007	<i>iso</i> -Sylvestrene	0.1	0.1	0.03	0.02	0.04	0.02	0.04	0.08
3	1024	1,8-Cineole	0.1	0.2	0.1	0.07	0.1	0.08	0.1	0.2
4	1026	Limonene	0.01	0.1	-	-	0.01	0.01	0.01	-
5	1063	Artemisia ketone	0.1	0.2	0.04	0.03	0.05	0.02	0.05	0.1
6	1083	Fenchone	0.03	0.1	0.03	0.03	0.02	0.05	0.02	-
7	1195	hydrate Methyl chavicol	0.5	0.4	0.2	0.2	0.2	0.2	0.2	0.04
8	1198	Shisofuran	1.2	-	0.2	0.1	0.2	0.2	0.2	0.2
9	1239	Carvone	2.6	0.1	0.06	0.06	0.05	0.1	0.05	-
10	1244	Carvotanacetone	0.08	0.2	0.06	0.08	0.1	0.06	0.2	0.06
11	1454	<i>trans</i> - β -Farnesene	11.8^a	0.8 ^b	0.1	0.1	0.2	0.2	0.3	0.4
12	1554	β -Vetivenene	2.1	2.6	2.0	2.1	1.8	1.7	1.9	2.5
13	1642	γ -Cadinol	0.7	0.4	0.8	0.4	0.3	0.3	0.6	0.6
14	1656	α -Bisabolol oxide B	4.4 ^c	5.1 ^{bc}	3.7 ^{cd}	3.0 ^d	6.8^a	5.6^b	4.3 ^c	3.7 ^{cd}
15	1674	<i>cis</i> - α -Santalol	2.6	1.1	3.4	2.7	2.7	3.3	4.0	4.3
16	1684	α -Bisabolone oxide A	6.8^c	5.8^c	5.5^c	6.7^c	10.5^a	8.2^b	8.7^b	7.5^b
17	1730	Chamazulene	0.7	1.3	1.3	1.0	1.1	1.0	0.9	1.3
18	1748	α -Bisabolol oxide A	44.8^d	60.2^b	66.4^a	66.7^a	56.6^c	61.2^b	57.6^c	56.0^c
19	1779	14-Hydroxy- α -muurolene	0.7	0.2	0.4	0.3	0.5	0.6	0.9	0.5
20	1879	<i>cis</i> -Spiroether	9.2^c	14.1^a	8.9^c	9.1^c	11.1^b	10.7^b	11.5^b	9.8^c
21	1890	<i>trans</i> -Spiroether	0.8	1.3	0.8	0.8	0.2	0.4	0.2	0.4
22	1934	7-Isopropyl-1,4-dimethyl-2-azulenol	2.4	2.1	1.6	2.8	1.7	1.6	2.3	8.0
Total			91.7	96.4	95.7	96.3	94.3	95.6	94.1	95.7

* RI: retention indices according to the normal alkanes between C8-C24. The bold type face means the compounds have the highest value. Full flowering stage: T1-T5 (T1: At 6 am, T2: At 9 am, T3: At 12 noon, T4: At 3 pm, T5: At 6 pm), T6: Early flower opening, T7: End of flowering stage, T8: Fruit set.

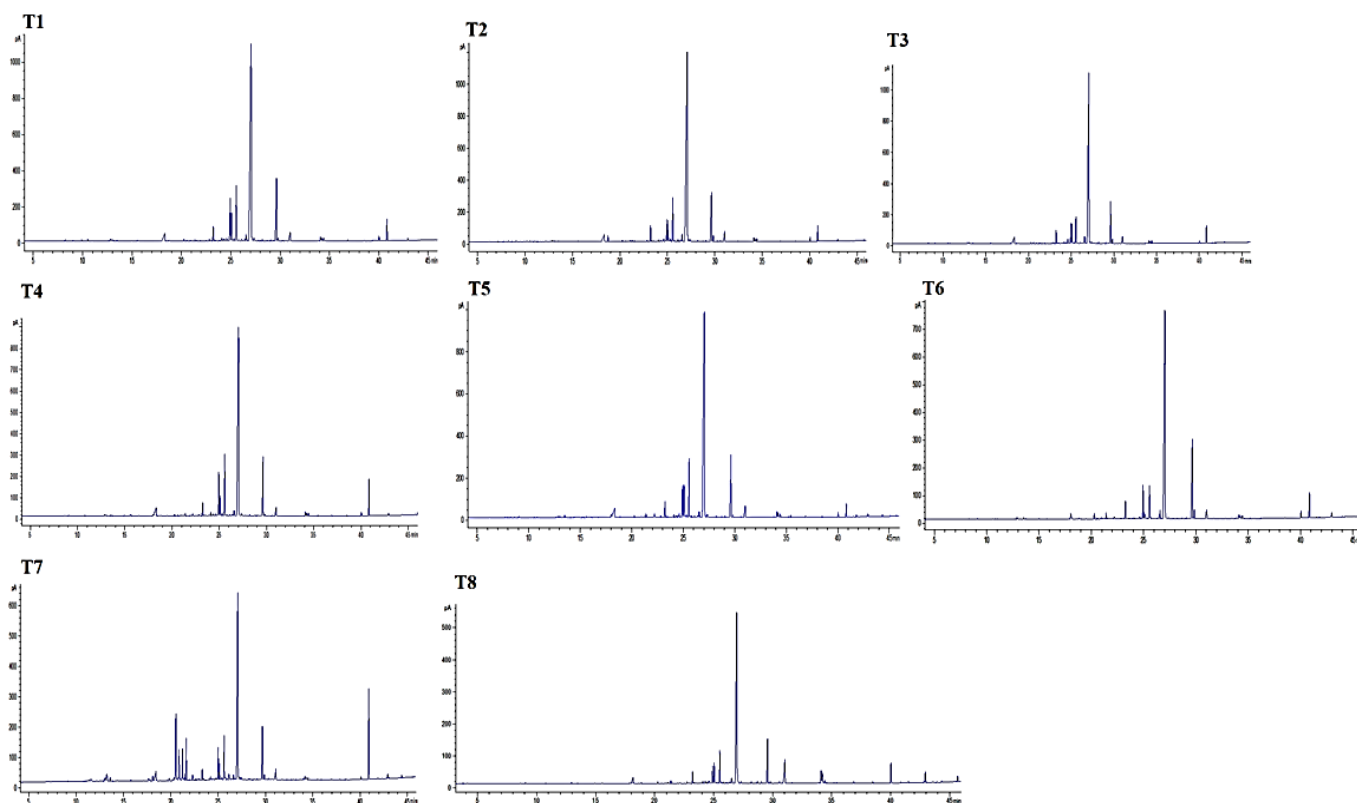


Figure 2. GC profile of EO in *M. chamomilla* during different harvest times. Full flowering stage: T1-T5 (T1: At 6 am, T2: At 9 am, T3: At 12 noon, T4: At 3 pm, T5: At 6 pm), T6: Early flower opening, T7: End of flowering stage, T8: Fruit set.

The variations observed in our study are consistent with previous findings and can be attributed to a range of factors, including environmental conditions, plant developmental stage, genotype, and extraction methods

(Benomari et al., 2023; Norani et al., 2023). Oxygenated sesquiterpenes remained the dominant class across all treatments (Figs. 3 and 4), contributing significantly to the oil's anti-inflammatory and wound-

healing potential (Al-Ghanim et al., 2023). Additionally, minor components such as linalool, myrcene, and *cis*-Spiroether, although present in lower concentrations, play important roles in the aroma and potential therapeutic effects of chamomile EO (Gladkostić et al., 2023). These constituents are sensitive to both harvest timing and postharvest handling.

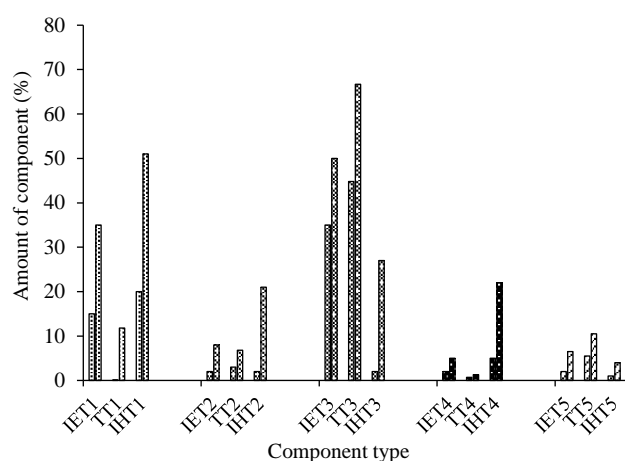


Figure 3. The comparison between ISO 19332 standard of main components in EO of *M. chamomilla* (Egyptian and Hungarian types) and tested type in this study. Each coupled column is representative of minimum and maximum of special component. (IET (ISO Egyptian Type), TT (Tested Type) and IHT (ISO Hungarian Type). The numbers from 1 to 5 are demonstrators of *trans*- β -Farnesene, α -Bisabolol oxide B, α -Bisabolol oxide A, Chamazulene, α -Bisabolone oxide A, respectively.

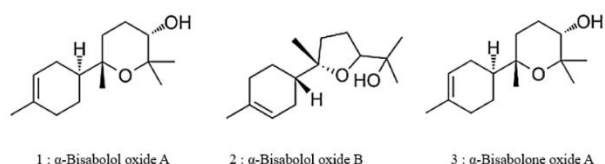


Figure 4. Structure of active oxygenated sesquiterpenes, including α -Bisabolol oxide A, α -Bisabolol oxide B and α -Bisabolone oxide A of *M. chamomilla* EO.

Overall, the chemical composition of chamomile EO is influenced by a complex interplay of factors including species, cultivation practices, distillation conditions, environmental parameters (e.g., temperature, light), and harvest timing. These factors not only affect total yield but also determine the relative abundance of pharmacologically important compounds (Chen et al., 2021; Crişan et al., 2023; Moradi et al., 2021). Irmisch et al. (2012) emphasized that terpene composition in chamomile is highly responsive to developmental stage, highlighting the importance of choosing harvest time based on target compounds.

In this study, comparisons were made with ISO 19332:2020 for the main components of *M.*

chamomilla EO, focusing on Egyptian and Hungarian types (Table 3). The *trans*- β -Farnesene content ranged between 15–35% in the Egyptian type and 20–51% in the Hungarian type, while in this study, it ranged from 0.1% to 11.8%, notably below the ISO 19332.

Table 3. Chamomile EO composition in ISO 19332 standard

Component	Egyptian type (%)	Hungarian type (%)
<i>trans</i> - β -Farnesene	15-35	20-51
α -Bisabolol oxide B	2-8	2-21
Bisabolone oxide A	2-6.5	1-4
α -Bisabolol	1-10	15-40
Chamazulene	2-5	5-22
α -Bisabolol oxide A	35-50	2-27

The α -Bisabolol oxide B content was within the ISO 19332 range (Egyptian type: 2–8%, Hungarian type: 2–21%), with levels in this study ranging from 3–6.8%. Conversely, the α -Bisabolol oxide A content exceeded the ISO 19332 (Egyptian type: 35–50%, Hungarian type: 2–27%), with levels ranging from 44.8–66.7% in the tested type. Similarly, α -Bisabolone oxide A levels (5.5–10.5%) in this study surpassed the ISO 19332 limits (Egyptian type: 2–6.5%, Hungarian type: 1–4%). However, chamazulene content in this study was significantly lower than the ISO 19332 values (Fig. 3).

These differences may be attributed to a combination of genetic and environmental factors. The chamomile samples analyzed in this study were derived from commonly used local landraces in Iran, which may differ genetically from the standardized cultivars typically used in Egypt and Hungary. Additionally, environmental variables such as temperature, soil type, altitude, and light exposure—known to influence secondary metabolite biosynthesis—could also account for deviations in EO composition. Further controlled studies using identical cultivars across regions would be needed to fully disentangle genetic versus environmental influences.

These deviations from ISO 19332:2020 standards could have important implications for the commercial use of the oil. For example, the lower *trans*- β -Farnesene and chamazulene content may reduce acceptability in markets that strictly adhere to ISO-defined chemical profiles, particularly in pharmaceutical or cosmetic formulations where consistency is critical. Conversely, the elevated levels of α -Bisabolol oxide A and α -Bisabolone oxide A may enhance the oil's therapeutic value and appeal in specific applications, but they may

also necessitate further standardization or labeling adjustments to meet export and quality assurance criteria. Overall, alignment with or deviation from ISO thresholds can influence not only regulatory compliance but also consumer trust and pricing in the global market.

The results of the study of the chemical groups of EO components (Table 4) showed that Monoterpene hydrocarbons remained constant across all harvest stages, ranging from 0.1% to 0.2%, regardless of the time of day or growth stage. This indicates that this group is minimally affected by developmental or environmental changes during harvesting. The highest proportion of oxygenated monoterpenes was observed during T1 (4.5%, at 6 am), decreasing sharply as the day progressed, reaching its lowest levels in T3 (12 noon) and remaining low in subsequent harvests.

Table 4. Chamomile EO components groups (%) in different harvest times

Group	T1	T2	T3	T4	T5	T6	T7	T8
Monoterpene hydrocarbons	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
Oxygenated monoterpenes	4.5	1.0	0.6	0.5	0.6	0.7	0.6	0.5
Sesquiterpene hydrocarbons	13.9	3.4	2.1	2.2	2.0	1.9	2.2	2.9
Oxygenated sesquiterpenes	60.0	72.8	80.2	79.8	77.4	79.2	76.1	72.6
Others	13.1	18.8	12.6	13.7	14.1	13.7	14.9	19.5
Unknown	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.1

The sharp decline in oxygenated monoterpenes from early morning to midday may be attributed to their high volatility and sensitivity to temperature. Monoterpenes are known to evaporate rapidly under rising temperatures, which can lead to reduced concentrations in later harvests (Sangwan et al., 2001). Additionally, temperature-related modulation of enzymatic pathways—such as those involving terpene synthases or monooxygenases—may influence the biosynthesis rate of these compounds during the day (Dudareva et al., 2013). These factors likely contribute to the higher accumulation of oxygenated monoterpenes in the early hours and their subsequent decline as the day progresses.

The sesquiterpene hydrocarbons were most abundant in the early morning of the full flowering stage (T1, 13.9%) and showed a steep decline by T2 (3.4%) and later harvest stages. By the time of fruit set (T8), sesquiterpene hydrocarbons slightly increased again to 2.9%, possibly due to shifts in biosynthesis

during the maturation process. Oxygenated sesquiterpenes consistently dominated the EO composition, reaching their peak during T3 (80.2%, at 12 noon). Afterward, there was a gradual decline through the later times of the full flowering stage (T4–T5), the end of flowering stage (T7, 76.1%), and the fruit set stage (T8, 72.6%). This trend suggests that mid-day harvests during the full flowering stage (T3) optimize the yield of oxygenated sesquiterpenes, which are often linked to the oil's therapeutic properties. Similar results have been reported by researchers, including studies by Kumar et al. (2020), El Mihaoui et al. (2022), and Gasic et al. (1991). These studies highlight the significant influence of harvest timing and developmental stages on the yield and chemical composition of chamomile EO. The biosynthesis of oxygenated sesquiterpenes begins with the mevalonate (MVA) pathway, which generates farnesyl pyrophosphate (FPP) as a key precursor. This intermediate is then converted into various sesquiterpene skeletons by sesquiterpene synthases, followed by oxidation reactions—often catalyzed by cytochrome P450 monooxygenases—that introduce oxygen-containing functional groups, resulting in the formation of bioactive compounds such as α -Bisabolol oxides and α -Bisabolone oxide A (Dudareva et al., 2013; Irmisch et al., 2012).

The "Others" group of compounds exhibited noticeable variability across different developmental stages and harvest times. The lowest proportion was observed at T3 (12.6%), which corresponded with the peak concentration of oxygenated sesquiterpenes. In contrast, the highest proportion was recorded during the fruit set stage (T8, 19.5%). This pattern suggests that as the plant transitions from flowering to fruit development, the accumulation of less volatile constituents or potential degradation products may increase. These compounds may include oxidized derivatives of major constituents, intermediate metabolites, or heavier non-volatile sesquiterpenes and lactones that are not fully identified in the GC/MS analysis due to their low volatility or lack of reference spectra. Additionally, late-stage metabolic shifts or oxidative breakdown of sensitive components under prolonged field exposure could contribute to this increased chemical complexity (de Almeida et al., 2020). Further targeted analysis is needed to better characterize the nature of this fraction.

Based on the comparison of key compounds (Table 5), the chamomile EO samples in this study most closely aligned with the Type B chemotype, particularly in harvest times T2, T6, and T7, where α -Bisabolol oxide B and α -Bisabolol oxide A fell within the upper ranges reported for Type B. However, the Chamazulene levels in the samples were consistently lower than the ranges reported for all chemotypes. Due to the absence of data for α -Bisabolol, a complete classification was not possible, but the overall trends strongly indicated a closer resemblance to the Type B chemotype.

Table 5. Chemotypes of chamomile based on their key EO components (Lawrence, 2001)

Compounds	Type A	Type B	Type C	Type D
	(%)			
α -Bisabolol oxide B	22.43-58.85	5.27-8.79	4.37-15.41	10.23-24.2
α -Bisabolol oxide A	4.74-15.68	31.07-52.25	2.13-18.5	9.62-25.83
α -Bisabolol	4.37-15.41	8.81-12.92	24.18-77.21	8.49-19.58
Chamazulene	2.7-17.69	5.4-7.95	1.45-14.9	1.91-7.89

The predominance of Type B chemotype in this study is consistent with findings by Nouri et al. (2025), who reported that a native Iranian landrace of chamomile cultivated in southern Iran (Abadeh, Fars Province) exhibited high levels of α -Bisabolol oxide A and chamazulene, characteristic of the Type B profile. This suggests that under Iranian agro-climatic conditions, especially with native or locally adapted landraces, the Type B chemotype may be relatively common. However, deviations in compound levels, such as the consistently low chamazulene in our study, could be due to differences in environmental stress, genetic background, or postharvest handling. It is important to acknowledge certain limitations of this study. The experiment was conducted at a single geographic location and during one growing season, which may limit the generalizability of the results.

4. Conclusion

This study demonstrated that both flower maturation and harvest timing significantly influence the composition and pharmacological potential of *M. chamomilla* essential oil. The optimal harvest was identified at full flowering during midday (T3), when EO yield and oxygenated sesquiterpenes, including α -

Bisabolol oxide A, α -Bisabolol oxide B, and α -Bisabolone oxide A, reached their highest levels. In contrast, chamazulene content remained consistently low and below the ISO 19332:2020 standard, highlighting potential genetic or environmental influences. While midday harvests optimize therapeutic components, practical constraints in large-scale farming may necessitate broader harvest windows. Environmental variability, genetic background, and chemotypic differences should also be considered, and further comparative studies across genotypes, environments, and related species are recommended. Overall, these findings provide actionable insights for improving harvest strategies, enhancing EO quality, and guiding sustainable cultivation and breeding practices.

Conflict of interests

The authors have no conflict of interest to declare.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All co-authors have read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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