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Irrigation Effects on Volatile Profile and Essential Oil Yield of Guayule During Flowering

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Abstract: Guayule (*Parthenium argentatum* A. Gray) has the potential to be an alternative source of rubber if its co-products can be exploited on an industrial scale. Among the co-products that have garnered interest are the essential oils (EOs), which can reach relatively high yields. In the present study, the production and profile of EOs from two guayule accessions, AZ-3 and AZ-5, across different flowering stages (5 months) were analyzed under two irrigation regimes (100% and 50% of crop water evapotranspiration) and compared with control plants that received no additional water, (considered as a water-stress condition). The results showed that the extracted EO yield was consistently higher in the AZ-3 accession than in the AZ-5, especially under water-stress conditions, and that the flowering stage significantly affected the yield irrespective of the accession. Furthermore, differences in EO composition were observed between accessions, with AZ-3 containing more monoterpenes and AZ-5 containing more sesquiterpenes. The yields obtained underline the economic potential of guayule EO production, especially under water-stress and flowering conditions, and position it favorably against other aromatic plants. These results provide valuable insights for optimizing guayule cultivation to increase EO yields, with both economic and environmental benefits.

Keywords: guayule; volatile extracts; essential oils; water supply; phenological floral stage

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1. Introduction

The global EO market was valued at USD 23.74 billion in 2023 and is expected to grow at a compound annual growth rate of 7.9% through 2030, indicating a promising global market outlook in the coming years [1]. Therefore, the EOs industry is focused on the search for new applications or on the production of EOs from new raw materials [2]. In case of the production of new EOs, it is noteworthy that the yield and chemical composition of EOs and, consequently, their quality are significantly affected by several factors besides the plant species, such as the geographical origin and environmental parameters [3]; the part of the plant used, the age or developed stage, or the agronomical practices carried out are also crucial [4–7].

Guayule EOs have not been extensively studied, and the extraction yield of their EOs varied from 0.8% to 2.8%, depending on the part of the plant extracted and the accession [8–10]. Such extraction yields might be considered modest when compared with other industrially productive plants, such as *Lavandula latifolia* (4.6% in leaves and flowers) [11]. There are not many studies related to the production of the essential oils of other related spices closed to the guayule family or genus, but those that have been found to have lower EO yields, for example, *Artemisia annua* (0.3%) and *Parthenium hysterophorus* (0.04%) and *Tanacetum parthenium* (L.) EOs (0.45–0.7%) [12,13], have been studied.

Several studies in the last 40 years have demonstrated that agronomic management practices influence the production of rubber and co-products from guayule. For example, it has been reported that plant biomass directly increases with irrigation, while rubber yield tends to decrease [14,15]. Similar studies investigating bioactive guayulin and argentatin co-products in the resin have shown that their content is dependent on water and nitrogen supply [16,17]. With respect to guayule EOs, the findings of one study with CAL-7 accession reported that the stress caused by reducing the amount of water up to 75% (no further data were provided) increased the EO content in flowers from 0.4 to 1.0% [18]. The composition of volatiles was also dependent on the irrigation treatment; for example, α-pinene increased from 5.8% to 20.6% under moderate water stress, and significant increases were also observed for germacrene D (1.8 to 12.1%) and bicyclogermacrene (3.1 to 16.5%) [18]. No studies have been carried out on the production of guayule EO during its flowering period, but in the case of *Origanum vulgare* L., the EO yield increased with flowering from 2.5% to 4.0% [19], and an even higher yield was observed for *Lavandula angustifolia* (1.5 to 4.6%) [20].

Efforts are underway to make guayule crop [21,22] more efficient to be industrially exploited, but this will only be fully realized if other chemical fractions (co-products) of the shrub that are different from rubber can be commercially exploited as well [23–28], and the latest proposal for its use is the production of essential oils (EOs). For this reason, the aim of the present work was to carry out a detailed study of EO accumulation in two guayule accessions (AZ-3 and AZ-5), considering the hypothesis that the guayule EOs yield and volatile profile will be modified when the plant is subjected to different water treatments during its flowering period.

2. Materials and Methods

2.1. Germplasm

Two guayule accessions, AZ-3 (*P. argentatum x non mariola*) and AZ-5 (*P. argentatum*), were obtained from the USDA-ARS National Plant Germplasm System and were planted in May 2019.

2.2. Field Location and Sampling

The trial field was located in Santa Cruz de la Zarza (Toledo, Spain), on a 0.8 ha experimental plot located at coordinates 39°57′34.9200″ N, 3°10′18.1560″ W, with an average altitude of 775 m above sea level. It is the same plot and experimental design as described previously [17]. The agro-climatic conditions were characterized by a developing arid climate, relatively cold winters, and warm summers. The mean annual temperature is 15.4 °C, and the lowest mean temperature is ~9.5 °C during the coldest month. The average annual precipitation is 240 mm, concentrated in the spring and autumn, causing winters to be somewhat dry and summers very dry.

As is common in crops where different parts of the plant are at simultaneous phenological stages, for the guayule in this study, multiple BBCH codes were recorded to accurately describe the developmental stage of the crop together with the ten weekly samplings that were carried out from 25 April 2023 to 25 August, as follows: S1 (25 April 2023; BBCH 23, 34, 55), S2 (3 May 2023; BBCH 23, 35, 52), S3 (9 May 2023; BBCH 23, 35, 55), S4 (17 May 2023; BBCH 23, 35, 55, 63), S5 (24 May 2023; BBCH 23, 35, 55, 66, 77), S6 (6 June 2023; BBCH 23, 35, 55, 66, 77, 86), S7 (21 June 2023; BBCH 23, 35, 55, 66, 77, 86), S8 (5 July 2023; BBCH 55, 66, 77, 86), S9 (19 July 2023; BBCH 66, 77, 86), and S10 (25 August 2023; BBCH 66, 77, 86). Three homogeneous representative plants were randomly selected for each treatment.

2.3. Irrigation Parameters

The estimation of crop water requirements was calculated following the methodology described in FAO-56, using the method for determining crop evapotranspiration (ETc) by means of the dual crop coefficient [29]. The ET0 was calculated using the Penman–Monteith equation, as recommended by FAO-56. This method integrates weather parameters to estimate the amount of water needed to replace the moisture lost due to evapotranspiration from a well-watered, grass reference crop.

Each irrigation treatment was determined through a detailed water balance calculation for each phenological period. The crop water requirement (ETc) was computed by adjusting ET0 with the dual crop coefficient (Kc) approach, considering both crop transpiration (Kcb) and soil evaporation (Ke). The irrigation treatments were designed to meet either 50% or 100% of the ETc, applying water twice a week during critical growth stages such as sprouting and flowering (Table 1). Water applications were adjusted accordingly, based on the calculated ETc for each growth period. For the control treatment, no additional irrigation was provided beyond the effective summer rainfall. This lack of supplemental water created a controlled water-stress environment, particularly during periods of high evaporative demand, which allowed us to evaluate the impact of water deficit on the crop under local climate conditions.

Table 1. Guayule crop evapotranspiration for the three irrigation conditions tested.

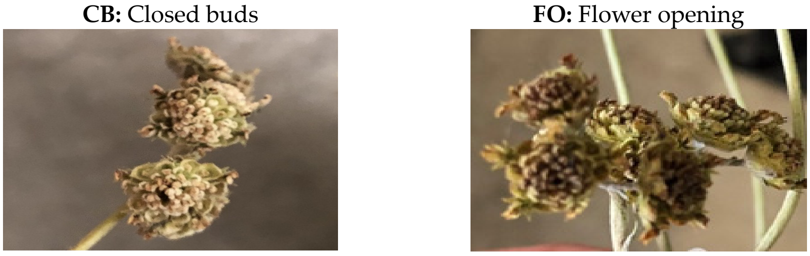
Plant Stage	Period of Days	Effective Rainfall (mm)	0% ETc		50% ETc		100% ETc	
			Irrigation (mm)	Mean Deficit (mm)	Irrigation (mm)	Mean Deficit (mm)	Irrigation (mm)	Mean Deficit (mm)
Dormancy	90	23	0	25	0	25	0	25
Sprouting	2	2	0	56	43	54	85	50
Flowering	191	191	0	93	555	34	1109	24
Senescence	130	130	0	49	0	32	0	32
Total	365	346	0	63	597	35	1194	29

2.4. Processing and Essential Oil Extraction

Each plant was harvested at 5 cm from the soil surface, immediately weighed (fresh weight), then dried at 60 °C for 48 h, and reweighed to determine the dry weight. The flowers of each harvested plant were counted manually, distinguishing four types of flowers: closed buds (CB), flower opening (FO), flower fully mature (FFM), and flower senescing (FS) (Figure 1).

CB: Closed buds

FO: Flower opening



FFM: Flower fully mature

FS: Flower senescing

Figure 1. Types of flowering stages in *Parthenium argentatum* A. Gray. The first stage is closed buds (CB), followed by flower opening (FO), flower fully mature (FFM), and, finally, flower senescing (FS).

For the laboratory analysis, 200 g of each of the three plants (including stems, flowers, and leaves) were mixed together to obtain a representative sample. The procedure was carried out in duplicate. The samples for each batch were crushed in a Thermomix for 15 s at speed 6 (Thermomix TM 31, Vorwerk, Madrid, Spain). Extraction of EOs was performed by steam distillation using 200 g of crushed plant material and 700 mL of distilled water in a Clevenger-type steam distillation apparatus for 2 h. The EO was collected in an Eppendorf tube and centrifuged at 15,000 rpm for 2 min to improve phase separation, and the resulting volume was measured with a precision pipette. All samples were distilled in duplicate.

2.5. Volatile Identification by GC-QTOF-MS

Samples of EOs were analyzed following the method described by González-Navarro et al. [8]: They were diluted 1:20 (EO/methanol) and then injected into a GC 7280 (Agilent, Santa Clara, CA, USA) gas chromatograph system equipped with a HP5MS column (30 m × 0.25 mm; 0.25 µm; Agilent 19091S-433UI) connected to an accurate mass QTOF-MS (Agilent 7200) for identification. The sample was introduced into the GC using an autosampler at a volume of 1 µL in split mode (1:100). The temperature of the injector was 250 °C, and the helium carrier gas was set to a constant flow of 1 mL min^{-1}. The oven temperature was set to 70 °C (10 min), 3 °C min^{-1} to 95 °C, 4 °C min^{-1} to 170 °C, 20 °C min^{-1} to 300 °C, and then maintained at 300 °C (2 min). The transfer line temperature was 300 °C. The nitrogen collision gas was fixed at 1 mL min^{-1}, ionization energy at 70 eV, and the temperature of the electron ionization source at 260 °C. Mass spectra were acquired with a scan range of 35–350 m/z. Compound identification was performed with the NIST mass spectral library (version 14) using MassHunter qualitative analysis software (version 10.0, Agilent).

2.6. Data Analysis

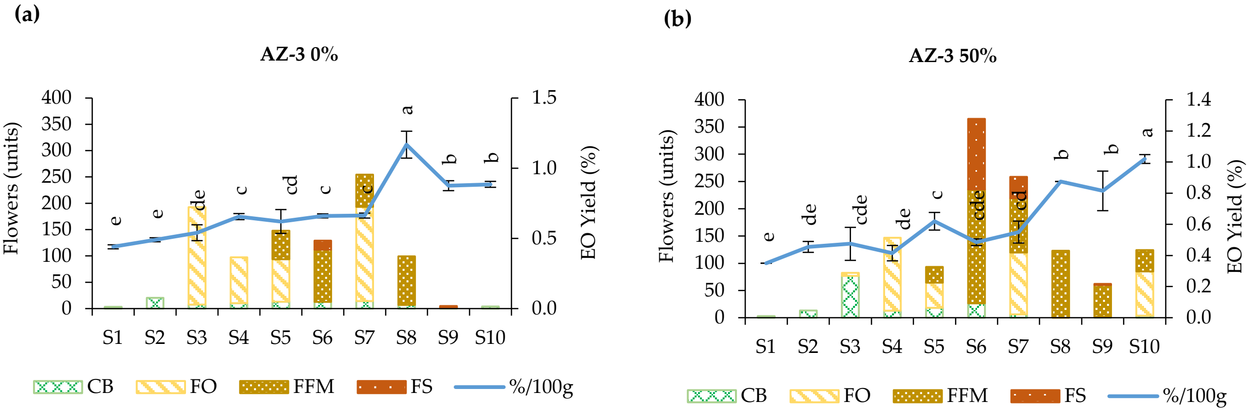
IBM SPSS Statistics v25 [30] was used to analyze the EO yields and the results of volatile profiling across the ten different samplings. Analysis of variance (ANOVA) was used to test for differences in yield between accessions within the same sampling period as well as differences between the samplings for each accession. Both the composition of chemical families and volatile evolution along time were compared by ANOVA in the different germplasms using Tukey's test at 95% confidence level. The compounds identified in EOs (40) were analyzed by principal component analysis to select those that could explain the main differences in the profiles among both accessions and samplings.

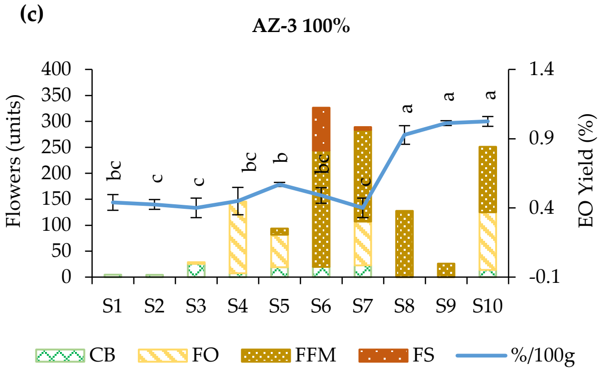
3. Results

AZ-3 and AZ-5 guayule accessions were grown in the same plot with homogeneous soil and climatic conditions and the same plant maturity and were harvested at the same time. The total annual effective rainfall (346 mm) corresponds to the average year in this Spanish area, which is commonly concentrated during spring, as shown in Table 1, corresponding to the flowering guayule vegetative stage with 191 mm of effective rain and, to a lesser extent, during the senescence plant period with 130 mm of rain. As shown in Table 1 and looking to the most significant vegetative stages, it was observed that during flowering, the situation with higher water stress was at 0% ETc (93 mm) in comparison to the 50 and 100% ETc mean water deficit.

3.1. Flowering and Yields of Essential Oils

As shown in Figure 2, the EO yield in accession AZ-3 differed between the two irrigation treatments and the control and showed a clear relationship with flowering stage.





(d)

	S1: 25 April	S2: 3 May	S3: 9 May	S4: 17 May	S5: 24 May
0%	A	A	A	A	A
50%	A	A	A	B	A
100%	A	A	A	AB	A
	S6: 6 June	S7: 21 June	S8: 5 July	S9: 19 July	S10: 25 August
0%	A	A	A	A	B
50%	B	AB	B	A	A
100%	B	B	B	A	A

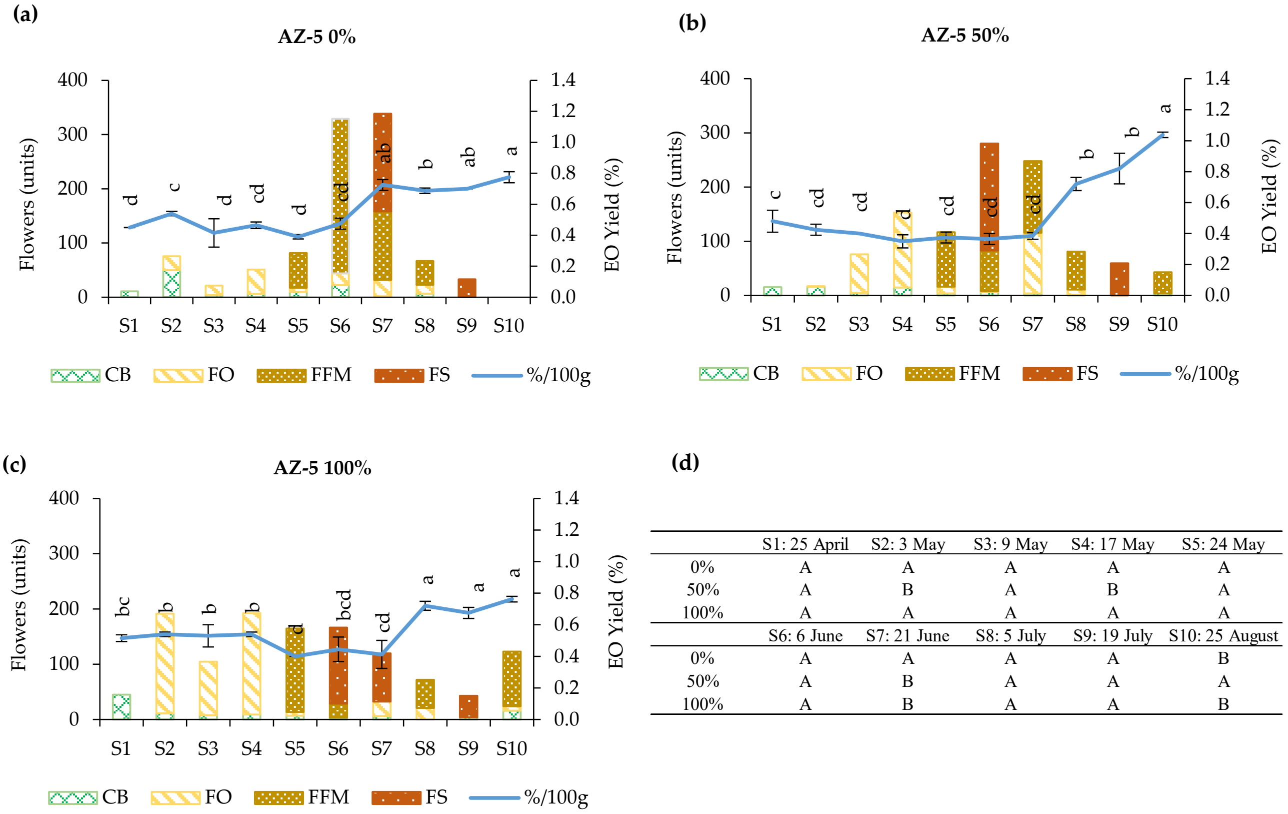
Figure 2. Quantity of flowers (left *Y* axis) related to yield (right *Y* axis) of essential oils in AZ-3 at (**a**) 0% irrigation, (**b**) 50% irrigation, and (**c**) 100% irrigation. (**d**) ANOVA comparing differences between yield in the same sampling time for different irrigation regimes. Note: CB, closed buds; PO, flower opening; FFM, flower fully mature; FS, flower senescing. S1 to S10 all represent 2023. Different letters indicate significant differences at 95% confidence level by Tukey's test. Lowercase letters refer to an ANOVA comparing differences between yield in the same irrigation regime for different sampling times Uppercase letters refer to an ANOVA comparing differences between yield in the same sampling but different irrigation.

Independently of irrigation, the EO yield showed an overall trend for increase from sampling 1 (S1) to S5, reaching 0.5–0.6% (Figure 2). This coincided with the progression of flower maturity, starting with closed buds at S1 and S2, and with flower opening appearing at S3 for shrubs receiving 0% and 50% irrigation (Figure 2a,b) and one week later for shrubs receiving 100% irrigation (Figure 2c). Fully mature flowers started to appear at S5 in all irrigation schemes.

Control plants (0% irrigation) showed a constant yield of EOs between S4 and S7 (Figure 2a), whereas under 50% irrigation, the EO yield remained constant from S5 to sampling S7 (28 days), at which time a second flowering took place in both cases (S7). Plants at 100% irrigation showed a significant decrease in EO content, reaching from 0.6% (S5) to 0.4% at S7 (Figure 2c). At S8, the EO extraction yield in control plants reached a value of 1.2%, at which time most flowers became mature. This was followed by a significant decrease in EO yield 15 days later, when flowers disappeared (S9), which remained constant over the next month (S10) with 0.9% yield (Figure 2a). For plants at 50% irrigation (Figure 2b), a significant increase in EO yield was observed from S7 to S8, which remained constant to S9 (15 days later), and a new increase in EO yield occurred at S10 with the appearance of new flower opening. For the 100% irrigation (Figure 2c), the highest yield was achieved at S8 (1%) and remained constant for a further month (S10). From S6 to S8 (Figure 2d), differences in EO yield were observed across treatments, with control plants showing the highest yield. No significant differences were observed between the groups at

S9, but at S10, the 50% and 100% groups showed the highest yield, the opposite of what was observed at S6 and S8.

In AZ-5 accession, there were differences between irrigation managements: 100% irrigation had a significant effect on the flowering growth (S2 to S4) (Figure 3). Independently of the management fully mature flowers did not appear until S5, when the proportion of opening flowers and closed buds was lower, evolving to the appearance of senescing flowers 15 days later (S6) and resulting in a consistent extraction yield from S1, reaching 0.3–0.4%. For control plants (0% irrigation), an increase in EO extraction yield (0.7%) was observed in S7 and remained constant until the last sampling (Figure 3a), in which no flowers were collected. Differences were observed from S8 onwards for plants irrigated at 50% (Figure 3b) and 100% (Figure 3c). The EO extraction yield remained constant in the 100% group (0.7%) (Figure 3c), whereas it continued to increase in the 50% irrigation group until S10, with a maximum yield of 1.1% (Figure 3b). Notably, there was a second flowering at S7 for the 50% irrigation group, which may be related to the high extraction yield obtained.



(d)

	S1: 25 April	S2: 3 May	S3: 9 May	S4: 17 May	S5: 24 May
0%	A	A	A	A	A
50%	A	B	A	B	A
100%	A	A	A	A	A
	S6: 6 June	**S7: 21 June**	**S8: 5 July**	**S9: 19 July**	**S10: 25 August**
0%	A	A	A	A	B
50%	A	B	A	A	A
100%	A	B	A	A	B

Figure 3. Quantity of flowers (left *Y* axis) related to yield (right *Y* axis) of essential oils in AZ-5 at (**a**) 0% irrigation, (**b**) 50% irrigation, and (**c**) 100% irrigation. (**d**) ANOVA comparing differences between yield in the same sampling time for different irrigation regimes. Note: CB, closed buds; PO, flower opening; FFM, flower fully mature; FS, flower senescing; S1, 25 April 2023; S2, 3 May 2023; S3, 9 May 2023; S4, 17 May 2023; S5, 24 May 2023; S6, 6 June 2023; S7, 21 June 2023; S8, 5 July 2023; S9, 19 July 2023; S10, 25 August 2023. Different letters indicate significant differences at 95% confidence level by Tukey's test. Lowercase letters refer to an ANOVA comparing differences between yield in the same irrigation regime for different sampling times. Uppercase letters refer to an ANOVA comparing differences between yield in the same sampling but different irrigation.

The biomass of AZ-3 increased throughout the trial, with significant differences between the sampling periods (Figure S1). For the control (0% irrigation) plants, the greatest biomass was reached at S8 (1.85 kg plant^{-1}), while additional irrigation increased the individual plant weight to well over 2 kg at the end of the sampling (S10) (Figure S1). The biomass dynamics and EO yield were expressed as EOs yield/production (L ha^{-1}), as shown in Table 2. The maximum EO yield for AZ-3 without irrigation was 175 L ha^{-1} at S8. However, irrigation extended the EO extraction period with respect to the optimum obtained without irrigation. In this case, irrigation (at any dose) provided a significant improvement of 153–154 L ha^{-1} compared with the control value of 133 L ha^{-1} at the end of the flowering period (S10). The performance of AZ-5 was completely different, performing better at S10 with 50% irrigation than in the other conditions.

Table 2. Yield (L ha^{-1}) of essential oils produced by AZ-3 and AZ-5 accessions across 10 samplings.

Sampling	**AZ-3**			**AZ-5**		
	0% **Yield (L ha^{-1})**	**50%** **Yield (L ha^{-1})**	**100%** **Yield (L ha^{-1})**	**0%** **Yield (L ha^{-1})**	**50%** **Yield (L ha^{-1})**	**100%** **Yield (L ha^{-1})**
S1	66.00 ± 2.12 ab	52.50 ± 0.00 b	66.00 ± 8.49 ab	67.50 ± 0.00 ab	72.00 ± 10.61 ab	77.25 ± 3.18 a
S2	73.50 ± 2.12 ab	68.25 ± 5.30 ab	63.75 ± 5.30 b	81.00 ± 2.12 a	63.75 ± 5.30 b	81.00 ± 2.12 a
S3	81.00 ± 8.49 a	71.25 ± 15.91 a	60.00 ± 10.61 a	62.25 ± 13.79 a	60.00 ± 0.00 a	79.50 ± 10.61 a
S4	98.25 ± 3.18 a	62.25 ± 7.42 b	67.50 ± 14.85 b	69.75 ± 3.18 b	52.50 ± 6.36 b	81.00 ± 2.12 ab
S5	93.00 ± 12.73 a	93.00 ± 8.49 a	85.50 ± 2.12 a	58.90 ± 2.12 b	56.25 ± 5.30 b	60.00 ± 0.00 b
S6	99.00 ± 2.12 a	72.75 ± 3.18 b	73.50 ± 8.49 b	71.25 ± 5.30 b	54.75 ± 5.30 b	66.75 ± 11.67 b
S7	99.37 ± 2.65 b	82.50 ± 10.61 ab	60.00 ± 10.61 b	108.75 ± 5.30 a	57.75 ± 3.18 b	61.87 ± 13.26 b
S8	**175.12 ± 14.32 a**	131.25 ± 0.00 bc	139.12 ± 10.08 b	103.12 ± 2.65 c	108.00 ± 6.36 c	108.00 ± 4.24 c
S9	131.25 ± 5.30 ab	122.25 ± 19.09 ab	**151.87 ± 2.65 a**	105.00 ± 0.00 b	123.00 ± 14.85 ab	101.25 ± 5.30 b
S10	132.75 ± 3.18 b	**153.00 ± 4.24 a**	**153.75 ± 5.30 a**	116.25 ± 5.30 c	**155.62 ± 2.65 a**	114.37 ± 2.65 c

Note: S1, 25 April 2023; S2, 3 May 2023; S3, 9 May 2023; S4, 17 May 2023; S5, 24 May 2023; S6, 6 June 2023; S7, 21 June 2023; S8, 5 July 2023; S9, 19 July 2023; S10, 25 August 2023. L ha^{-1} means liter per hectare. Tukey's test was used to assign lettercase significance. Different letters refer to an ANOVA comparing differences between the same sampling with different irrigation conditions in all accessions at 95% level of confidence. Words in bold refer to maximum values.

3.2. Profiling of Essential Oil Volatiles

Forty volatile compounds were identified and mainly grouped into the terpene family (73.5% in AZ-3 and 75.6% in AZ-5) and the sesquiterpene family (26.6% in AZ-3 and 24.4% in AZ-5). These values are averages of each compound independent of sampling and composition (see Tables S1 and S2).

A multivariate analysis was performed to determine whether sampling or irrigation factors influenced the volatile EO profile of the two guayule accessions (Table 3). Analysis of the sampling factor revealed only three compounds showing no significant differences: two terpenes, namely 2-thujene (C5) and linalyl formate (C19), and the sesquiterpene germacrene D (C29). The content of these compounds varied from 3.9 to 8.2% for C5 and between 2.5 and 5.2% for C29 and was negligible for linalyl formate (<1%). Eight of the forty volatiles identified (3 terpenes and 5 sesquiterpenes) showed no significant difference for the accession factor, while fifteen of them were included in this category when the irrigation factor was considered (10 terpenes and 5 sesquiterpenes). When the double-factor interaction was considered, more sesquiterpenes showed no significant differences in comparison with the single-factor analysis. The sampling × accession × irrigation triple interaction showed that only 12 compounds would be responsible for the significant differences: 8 terpenes and 4 sesquiterpenes (Table 3).

The EOs volatile composition at the stage where both accessions registered the maximum yield were compared (Figure 4), showing that AZ-3 contained more monoterpenes, and AZ-5 contained more sesquiterpenes. No response was observed for the volatiles C13 (sabinol), C16 (vervenone), and C37 (viridiflorol) that could not be quantified but were identified in the selected conditions, and the content of C15 and C17, although quantifiable, was very low (Figure 4). For the remaining compounds, it could be observed that the content of C1 (santolina triene), C6 (sabinene), C7 (β-myrcene), C8 (β-E-ocimene), C11 (3-carene), and C26 (caryophyllene) was significantly higher in AZ-3 than in AZ-5.

Table 3. Multivariate analysis of the essential oil profiles.

		Sampling	**Accession**	**Irrigation**	**Sampling × Accession**	**Sampling × Irrigation**	**Accession × Irrigation**	**Sampling × Accession × Irrigation**
C1	Santolina triene	**	***	***	**	NS	***	NS
C2	α-thujene	**	***	***	NS	NS	NS	NS
C3	α-pinene	***	***	***	**	NS	**	NS
C4	Camphene	**	***	NS	***	***	**	***
C5	2-thujene	NS	***	NS	NS	NS	**	NS
C6	Sabinene	***	***	***	NS	NS	NS	NS
C7	β-myrcene	***	***	**	***	***	***	***
C8	β-E-ocimene	***	***	NS	***	***	***	**
C9	β-terpinene	***	***	***	**	***	NS	***
C10	Limonene	***	***	***	NS	NS	**	**
C11	3-carene	***	***	***	***	***	NS	***
C12	2-methy-2-bornene	***	***	NS	**	***	NS	NS
C13	Sabinol	**	NS	NS	NS	NS	NS	NS
C14	Verbenol	***	NS	NS	NS	NS	NS	NS
C15	Myrtenol	***	**	NS	NS	NS	NS	NS
C16	Vervenone	**	NS	NS	NS	NS	NS	**
C17	Myrtenyl isovalerate	***	***	NS	***	NS	NS	NS
C18	Bornyl acetate	***	***	***	***	***	NS	***
C19	Linalyl formate	NS	***	NS	NS	NS	NS	NS
C20	α-guaiene	***	***	***	NS	NS	NS	NS
C21	α-cubebene	**	***	**	NS	NS	NS	NS
C22	Modephene	***	***	***	**	NS	NS	NS
C23	α-isocomene	***	***	***	***	NS	NS	**
C24	β-copaene	***	NS	***	**	NS	NS	NS
C25	β-isocomene	***	***	***	**	NS	NS	NS
C26	Caryophyllene	***	***	***	NS	**	***	NS
C27	Humulene	***	NS	***	NS	NS	**	NS
C28	Aromandrene	***	**	NS	**	NS	NS	NS
C29	Germacrene D	NS	NS	***	NS	NS	NS	NS
C30	Bicyclogermacrene	***	***	***	**	***	***	***
C31	δ-cadinene	***	***	NS	NS	NS	NS	NS
C32	Elemol	***	**	***	**	NS	NS	NS
C33	Nerolidol	***	***	**	NS	NS	NS	NS
C34	γ-muurolene	***	***	**	**	NS	**	NS
C35	Spathulenol	***	***	NS	NS	**	***	***
C36	Caryophyllene oxide	***	***	NS	NS	NS	NS	NS
C37	Viridiflorol	***	***	**	***	***	***	***
C38	γ-eudesmol	**	NS	NS	NS	NS	NS	NS
C39	α-copaen-11-ol	***	***	***	NS	**	NS	NS
C40	β-eudesmol	**	NS	***	NS	NS	NS	NS
Volatiles with significant differences		37	32	25	19	12	13	12
NS Total Volatiles		3	8	15	21	28	27	28
NS Terpenes		2	3	10	10	13	13	12
NS Sesquiterpenes		1	5	5	11	15	14	16

, and * indicate significant differences between groups of $p < 0.01$, and $p < 0.001$. NS, not significant. Terpenes and sesquiterpenes represent the amount of each family that is not significant.

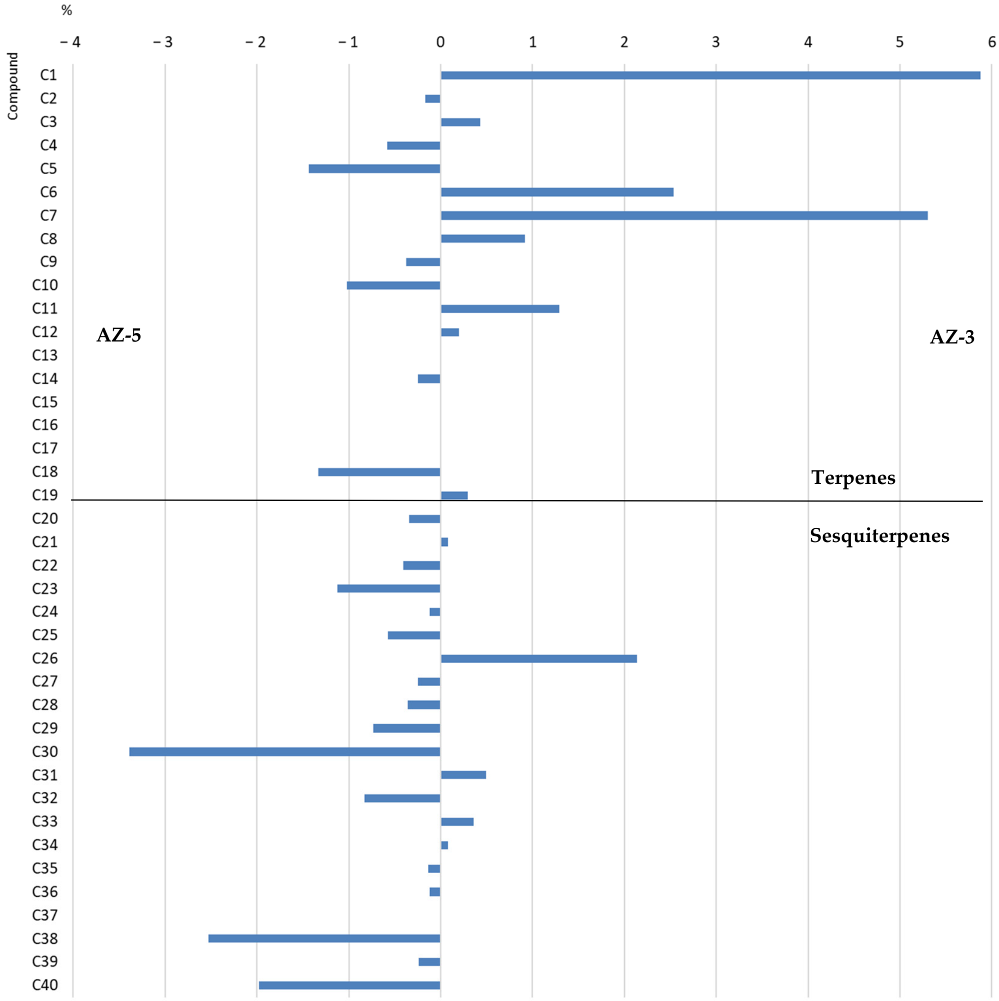


Figure 4. Comparison of the maximum yields of essential oils for AZ-3 (0% irrigation) and AZ-5 (50% irrigation).

4. Discussion

The guayule accessions of the present study (AZ-3 and AZ-5) are different from the CAL-7 accession used in the only similar study carried out in 2008 [18]. Under the homogeneous growth conditions used in this study, differences in essential oil yields and flower maturity across accessions could be directly attributed to irrigation levels (Figures 2 and 3). It is important to note that, however, as shown in Table 1, the average daily deficit of the 50% ETc treatment was only slightly higher (34 mm) than that of the 100% ETc treatment (24 mm), which implies that this year's rainfall satisfied a large part of the crop's water demand, especially in the latest treatment. A similar trend was observed during plant senescence, although water requirements at this stage are lower (0% ETc with 49 mm of effective rain).

Irrigation influenced flower development and, consequently, essential oils yield, particularly as flowers reached full maturity: S8 and S9 for AZ-3 (Figure 2) and S10 for AZ-5 (Figure 3). When all samplings and irrigation treatments were compared in AZ-3 accession (Figure 2d), a stabilization or increase in the EOs yield under irrigation conditions of 100% and 50%, respectively, was observed, and this effect could be attributed to the fact that the flower maturity cycle (S8 and S9) had ended, and a new cycle started at S10. This was not observed at 0% irrigation, as a second flowering cycle did not occur.

The flowering period of AZ-5 accession started earlier than for AZ-3, and maturation commenced in S2 (Figure 3). When comparing all the samplings in AZ-5, (Figure 3d), different yields were observed, but it is significant that the 50% and 100% treatments showed the lowest yield at S7 compared to the control group, which coincided with greater quantities of flowers and a higher degree of maturity, accompanied by higher yield values. Finally, at S10, the highest yield occurred in the 50% group, with fully mature flowers in contrast to the 0% group. Contrastingly, the 100% management group started to flower a second time, presumably because it had more water. Overall, this analysis suggests a link between the number and maturity of flowers and EO production.

A comparative analysis of extractions yields revealed that EO yield was higher in AZ-3 than in AZ-5. The yield achieved with 0% irrigation was especially significant (1.2% yield at S8), and when irrigation was applied, the highest yield was achieved two weeks later, between S9 and S10 (1.0 and 1.1%). Thus, in relation to flowering, the results suggest that guayule flowers need to be fully mature or senescing to achieve a higher yield of EOs.

The guayule EO yields obtained are consistent with other findings for CAL-7 [18], in which the highest yield recorded was under water stress (1.0%), supporting the idea that moderate stress conditions optimize EO yields [18]. This amount is consistent with the yield for AZ-3 in the present study, whereas some water was needed (50%) for accession AZ-5 to achieve the same yield (1.0%) compared with that for 0% irrigation (0.8%), and too much water (100%) did not necessarily mean a greater response, as the EO yield decreased to 0.8%. A recent study [8] also demonstrated extraction yields of the same order for other guayule accessions, including AZ-2 (1.0%), CFS18-2005 (0.9%), CAL-7 (0.8%), and CFS18-2005 (0.7%), at the end of the summer, although no additional irrigation was applied.

In terms of biomass, it was clearly observed that plant growth was greater in AZ-5 than in AZ-3, with a biomass higher than 2.30 kg plant^{-1} at control and 100% irrigation at the end of the sampling (S10) (Table S1), although at 50% irrigation, the highest biomass was achieved at S9 with 2.92 kg plant^{-1}. The EOs yield/production (L ha^{-1}) (Table 2) revealed that accession AZ-3 achieved the highest EO yields. In the absence of irrigation, the maximum performance of AZ-3 was 175 L ha^{-1} at S8, which not only represents an economic benefit for the industry but also considers water-use efficiency and environmental conservation. It is clear that irrigation extended the EO extraction period with respect to the optimum obtained without irrigation. However, the results suggest that the trial with AZ-5 should perhaps have been extended to verify its maximum productive capacity, as under these conditions, the production of 156 L ha^{-1} was very similar to that of AZ-3 under the same treatment.

In sum, the amount of guayule EOs that can be obtained at an industrial level is sufficiently important to make the crop highly profitable when compared, for example, with other aromatic plants in production, such as lavender EOs, with yields of 61–180 L ha^{-1} depending on the accession [31,32]. Given the similar results with the two accessions at 50% irrigation in S10, the interest that the different volatile profiles of the two accessions may generate in the industry may be decisive for the grower.

The volatile profile of the extracted EOs was consistent with a previous study characterizing 15 guayule accessions, including industrial-scale processing of AZ-3 [8]. The major compounds in the AZ-3 guayule accession were C1 (santolina triene), C3 (α-pinene), C6 (sabinene), and C10 (limonene). A similar profile was noted in AZ-5 with the exception of C1, the content of which was very low (Tables S1 and S2).

The volatile EO profile of the two guayule accessions (Table 3) did not show any significant differences considering the studied factors (samplings and irrigation managements). But if the study was reduced to the key points, that is, the time at which the accessions produced their greatest EO yield, which was at S8 (5 July 2023) for AZ-3 with no water supply and S10 (25 August 2023) for AZ-5 with 50% irrigation, then differences were observed among accessions (Figure 4). AZ-3 contained more monoterpenes, and AZ-5 contained more sesquiterpenes, which means that such different volatile profiles may be exploited differently by specialized applications within the EO industry. Without considering the previous study with AZ-3 [8], in the present study, there were many more volatiles in number and quantity than those reported in previous studies [9,10,18]. These differences from previous studies may be attributed to advances in chromatographic techniques and mass identification, which now allow for more detailed characterization of volatile compounds. The content of C1 (santolina triene) in AZ-3 (10–15%) is clearly different from that reported for the CAL-7 accession (0.1–0.8%) [18], and it more closely matches that of AZ-5 (values up to 5% depending on the sampling). Contrastingly, the previously reported contents of α-pinene (C3) (5.8–60%), camphene (C4) (0.1–1.2%), limonene (C10) (5.9%), and germacrene D (C29) (1.8–12.1%) [9,10,18] are similar to those of the present study, for example, C3 ranging from 16.3% to 28.5% (AZ-3) and 20.1% to 41.0% (AZ-5); C4 ranging from 0.6% to 1.4% (AZ-3) and 0.8% to 2.5% (AZ-5); C10 ranging from 6.4% to 10.4% (AZ-3) and 8.7% to 11.8% (AZ-5); and C29 ranging from 2.5% to 5.2% (AZ-3) and 2.8% to 5.7% (AZ-5). While accession AZ-3 may be more interesting for the producer due to its higher EO yield, AZ-5 may attract attention because of its higher content of sesquiterpenes, which are known to have strongest antioxidant potential and bioactivity.

5. Conclusions

The AZ-3 guayule accession consistently showed higher EO yields than AZ-5, which was particularly notable under water-stress conditions (93 mm effective rain, 0% ETc). Additionally, the flower maturity stage significantly influenced the EO content. The volatile profiles of both accessions were dominated by terpenes, although this differed in quantity and type between AZ-3 and AZ-5. The latter contained a higher content of bioactive sesquiterpenes, which may be more attractive to industry. The significant EO yields obtained from guayule, especially under specific irrigation and flowering conditions, highlight the crop's potential for profitability when compared with other aromatic plants EOs in the market.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14122107/s1, Figure S1: Biomass (kg) of plants for (a) AZ-3 accession and (b) AZ-5 accession; Table S1: (a) Average values (%) of the compounds identified in the essential oils of AZ-3 depending on the water applied. (b) Average values (%) of the compounds identified in the essential oils of AZ-3 depending on the water applied; Table S2: (a) Average values (%) of the compounds identified in the essential oils of AZ-5 dependent on the water supplied. (b) Average values (%) of the compounds identified in the essential oils of AZ-5 depending on the water supplied.

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