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Article

Diversity of *Crithmum maritimum* L. from Salento Coastal Area: A Suitable Species for Domestication

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Abstract: *Crithmum maritimum* L., known as sea fennel, is an aromatic halophyte typical of the cliffs and coastal areas of the Mediterranean Sea and Atlantic Ocean. Their phytochemicals have been of great interest in the food and pharmaceutical industry. In this work, we analyzed, by SPME/gas chromatography coupled with mass spectrometry, the chemical variability of *C. maritimum* accessions in terms of volatile organic compounds. *C. maritimum* seeds were collected from different coastal sites in Salento, Southern Apulia, Italy, and subsequently cultivated ex situ. Several volatile compounds produced by *C. maritimum* leaves were detected, and, among them, D-limonene was found to be emitted at high levels by plants of all accessions representing the main compound, while other monoterpenes were produced at low levels. Moreover, the phenylpropene volatiles dillapiol and apiol (designated together as (dill)apiol) were emitted at variable amounts with different accessions. The correlation among groups based on volatile compounds has been analyzed using hierarchical cluster analysis, which has revealed three main groups based on (dill)apiol presence and its total amount in the cultivated plants of different geographic origins, confirming intraspecies biodiversity. Moreover, we have evaluated the seed germination and seedling development of *C. maritimum* in controlled conditions. We found no dormancy and a high germination rate for all samples analyzed. The chemo-diversity evidenced in cultivated plants obtained from seeds collected at different locations on the Salento peninsula is probably related to variations in climate resulting from different exposures along the coast. These findings highlighted the importance of *C. maritimum* as a suitable candidate for cultivation because it can tolerate harsh conditions/stresses and also has a possible use besides food and pharmaceuticals and for the restoration of coastal environments.

Keywords: *Crithmum maritimum*; volatile organic compounds; phenylpropenes; monoterpenes; biodiversity

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1. Introduction

Wild halophytes have been traditionally used since ancient times in different areas. In recent years, growing interest in the use of these plants has derived from the identification of many metabolites with health and nutritional properties. Recently, several studies dealt with the biology and origins of “sea fennel” or “rock samphire” *Crithmum maritimum*, their cultivation, chemical composition, and “omic” analyses showing their abundant contents, including beneficial nutrients for human health [1]. Traditionally, it has been included, in addition to several wild vegetables, in the Mediterranean diet, as reported by ethnobotanical studies [2]. Over the centuries, the use of the plant has decreased, but in recent years, more studies have highlighted its importance as an “emerging vegetable crop” [3].

Sea fennel, belonging to the Apiacae family represents a perennial facultative halophyte, growing in rocky sea cliffs, rarely in sands and gravel; it has a very wide distribution, occurring along the European Atlantic coasts, the Mediterranean and Black Sea coast, and Northwest Africa, where the plants are exposed to several abiotic stress, such as fluctuating soil salinity and drought [4]. *C. maritimum* developed morphological, physiological, and biochemical adaptations to tolerate salt excess; therefore, it was proposed as a potential crop for sustainable agriculture requiring few inputs as a "cash crop" [1]. Although it is able to survive in saline environments, its optimum growth occurs in salt-free or low-salinity grounds [5]. The aerial parts, rich in mineral elements, possess high nutritional values [6]; in addition, the species is employed in the food and pharmaceutical industries because of its high antioxidant power [7]. The phytochemical composition of aerial parts is variable depending on geographic distribution [8] and can be influenced by different abiotic stresses [9]. Chemical compositions are additionally influenced by different factors besides genotype, i.e., variable environmental factors [10].

The wide utilization of this species in several fields (medicine, food, agriculture, and environment) comes from the presence of several mineral elements, bioactive compounds, polyphenols, fatty acids, and flavonoids. Moreover, the ability to overcome water, salinity, and temperature stresses makes it suitable for growing in marginal lands and unproductive for traditional agronomic crops and in degraded coastal habitats [11]. Essential oils, widely used, release numerous volatile organic compounds (VOCs), including a large variety of chemical compounds classified as secondary metabolites.

Different factors, such as genetics (species, subspecies, and even varieties), physiological (development stage, organs), drying [12,13], storage, and even the method of volatile matter isolation, can significantly influence VOC profiles [14]. Nevertheless, VOC profiles in plants were strongly influenced by the environment [15]; therefore, the interaction between climate and genotype can affect the essential oil quality and yield [16].

Quantifying the metabolic diversity in plant populations is crucial for the efficient selection of plants for domestication programs. It is interesting to identify promising species with high adaptability capacity in the Mediterranean area, characterized by negative effects on agriculture due to climate change, including drought, soil erosion, desertification, and loss of biodiversity [17].

In the last decade, the increasing demand for sea fennel phytochemicals for industrial purposes has led to the development of efficient cultivation methods [18].

Based on these assumptions, we tested the seed's germination and cultivation outside the natural environment of *C. maritimum*, employing seeds harvested from different sites along the Salento coasts, Apulia region, Italy. Moreover, we analyzed the VOC profiles of plants produced from the same seeds and grown ex situ under the same conditions to explore the chemical variability of *C. maritimum* accessions.

2. Materials and Methods

2.1. Plant Material and Experimental Conditions

Crithmum maritimum L. seeds were harvested in autumn 2022 from plants growing in six different localities of Salento area, Puglia, selected for different growth environments (Figure 1). Salento's climate is typically windy and Mediterranean, with alternating mild winters, hot springs, torrid summers, and warm autumns; with regard to winds, the Salento peninsula is exposed to the Mistral and Tramontana (cool, dry winds) to the east, while on the Ionian side, to the west, it is exposed to the Scirocco (hot, humid wind). Rainfall is almost always below 500–700 mm. For each site, some climate data and the geographical coordinates, recorded with a GPS device, are reported in Table S1. A voucher (003/12/2022/halophyte) of samples analyzed was deposited in the Database of Botanical Garden, University of Salento, Lecce. The seeds were germinated in a greenhouse located in the Botanical Garden of the University of Salento. The seedlings were produced in polystyrene plug trays (cells with a diameter of 2.5 cm and volume of 21 mL) filled with peat. After growing in plug trays for 45 days, seedlings were transferred to 10 cm diameter plastic containers (0.5 L). A mixture composed of a peat-based substrate (Brill® 3, Brill Substrate GmbH & Co., Georgsdorf, Germany) and perlite (Agrilit 3, Perlite Italiana, Corsico-Milano, Italy), in a 2:1 (v/v) ratio, was used as growing substrate. The pots were placed on benches and grown using an ebb-and-flow hydroponic system. Plants were grown for 75 days with nutrient solution (NS) prepared with pre-collected raining water and containing (mg L^{-1}) 119 nitrogen, 117 potassium, 16 phosphorus, 24 magnesium, 116 calcium, 54 sulfur, 1.12 iron, 0.27 manganese, 0.13 zinc, 0.27 boron, 0.03 copper, and 0.01 molybdenum, resulting in electrical conductivity of 1.8 dS m^{-1}, pH 6.3; NO_3-N:NH_4-N at a percentage ratio of 84:16 was used as the nitrogen source. The experiment was organized in a fully randomized design with three replications for each geographic site, with every replica consisting of nine pots.

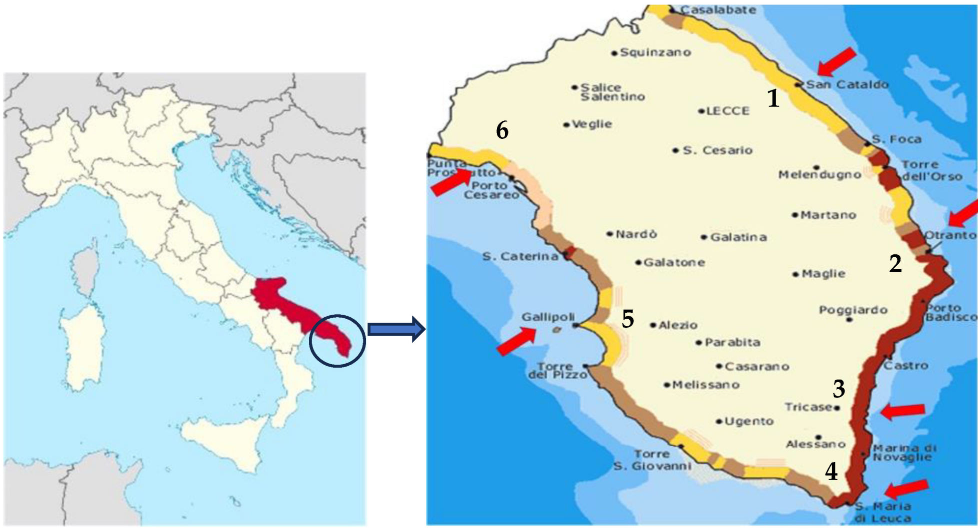

Figure 1. Geographical location of Apulia and Salento in relation to the Italian peninsula and indication of the places (red arrows) along the Salento coast where the seeds of the different *C. maritimum* accessions were collected.

For the analysis of volatile compounds, the apical leaves of 6 cultivated plants were collected in July 2023 and processed as described below.

2.2. Germination Rate

Seed germination was measured by evaluating emergence of the radicle [19]. Five days after harvesting, the seeds were selected and purified by washing with sodium hypochlorite solution and then rinsed three times with demineralized water. For each sample, four replicates of 30 seeds were sown in Petri dishes containing filter paper soaked with demineralized water. The plates were placed in a phytotron at 25 °C with a photoperiod of 16 h light (light intensity of 10 kLx) and 8 h dark. Every day, the seeds with a protruding radicle were counted and removed from the dish. About three weeks after sowing time, the course and germination rate were evaluated. The germination parameter was determined for each seed accession and was expressed as mean of the 4 replicates with standard error. In Figure 2, the procedure for germination of *C. maritimum* seeds and ex situ cultivation was schematized.

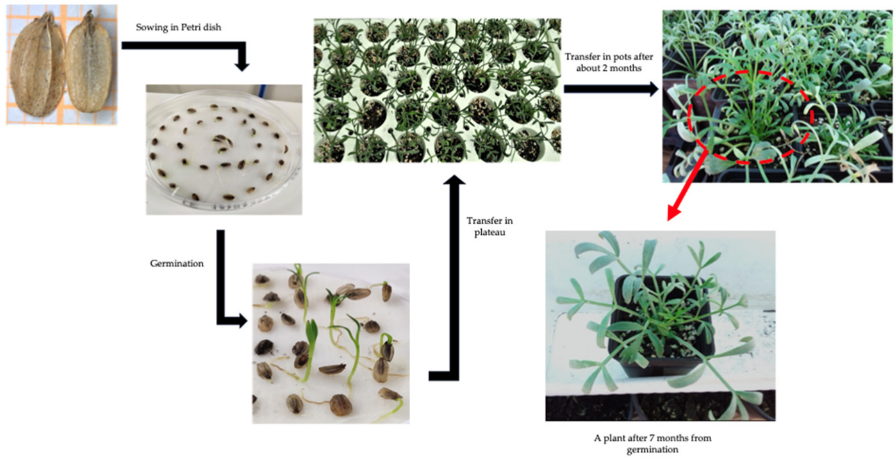

Figure 2. Scheme of procedures for seed germination and ex situ cultivation of *C. maritimum*.

2.3. Analysis of the Volatile Compounds

The analyses were carried out by solid-phase microextraction (SPME) methodology, as described previously by Negro et al. [20] and Dimita et al. [21]; the apical leaves of *Crithmum* plants, produced from seeds collected in the six different locations and grown in pots, were collected (1 g of fresh weight) and immediately sealed into 20 mL SPME vials (Agilent Technologies, Palo Alto, CA, USA) by metal screw-caps with pre-notched, Teflon-silicone septa, which is used for trapping the volatile organic compounds. The vials were then placed at 40 °C for 10 min in a thermostatically controlled bath to allow the evaporation of the compounds; hereafter, a SPME syringe was inserted, and the fiber (50/30 µm Divinyl-benzene/Carboxen/Polydimethylsiloxane, Supelco/Merck KGaA, Darmstadt, Germany), which was previously conditioned for 5 min at 235 °C in the gas-chromatograph injector, was exposed for 10 min to absorb the volatile compounds. Subsequently, the fiber was inserted into the injector port of gas chromatography with a mass-spectrometry detector (Agilent 7890B coupled with MS single quadrupole Agilent 5977A), and the desorption of the volatile compounds was performed at 235 °C for 4 min. At this point, the chromatographic run was started with an Agilent HP-5 MS column (30 m × 0.25 mm, 0.25 µm) (where temperature was raised from 60 °C to 230 °C, with a constant increase of 3 °C/min), with a helium (purity > 99.999%) constant flow of 1.0 mL/min. Compounds were identified by library search and analytical standards if available. The mass spectrum of an unknown compound was searched in a data-processing system. Substances with a score above 800, both for identity and purity, were putatively identified after comparing the detected compound with the one in the NIST Computational Chemistry Comparison and Benchmark database. Retention index (RI) was obtained, as reported by Zhao et al. [22], being employed as a reference of the retention times of a series of C_8–C_{20} alkanes separated under the GC-MS conditions mentioned above.

All the statistical analyses described were carried out using the percent content value of each compound on the total VOCs, considering the mean value of the three technical replicates for each compound.

2.4. Agglomerative Hierarchical Cluster (AHC) Analysis of Volatile Organic Compounds

Data preprocessing and statistical analyses were conducted using XLSTAT for Windows (Version 2021.3.1). Metabolite concentration data were imported, and an agglomerative hierarchical clustering analysis was performed, utilizing Ward's minimum variance method to determine the clusters. The dendrogram representation of the clusters was generated, providing a visual summary of the similarity (Euclidean distance) between the metabolite profiles from the six different locations.

3. Results

3.1. Seed Germination

The germination rates of seeds collected from six different sites along the Salento coast are shown in Figure 3. Germination was completed in approximately three weeks, and no dormancy was observed; the lowest germination was recorded for seeds collected in Gallipoli, but the value remained above 50%, while those from Tricase, Otranto, and Torre Lapillo showed the highest germination values (above 80%); finally, the seeds from Santa Maria di Leuca and San Cataldo showed slightly lower germination rates, about 79% and 78%, respectively.

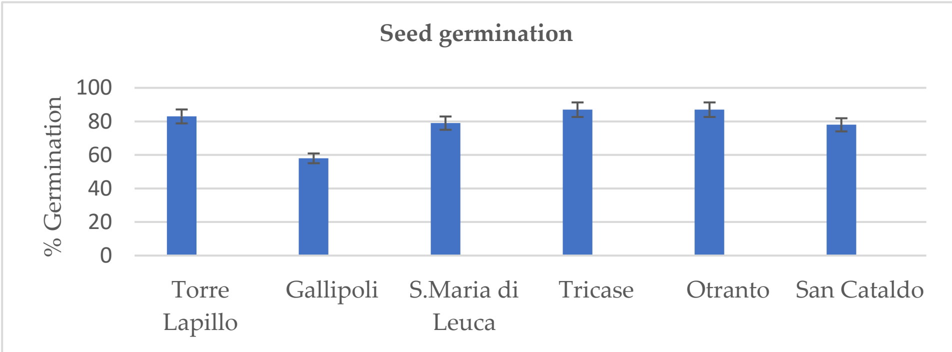

Figure 3. Germination rates of seeds of six different *C. maritimum* accessions of Salento coast. Bars indicate standard error.

3.2. Volatile Organic Compounds

The GC-MS analysis of sea fennel leaf VOCs highlighted different chromatographic profiles among different *C. maritimum* accessions grown in a greenhouse, allowing us to identify more than 25 volatile organic compounds (Figure 4).

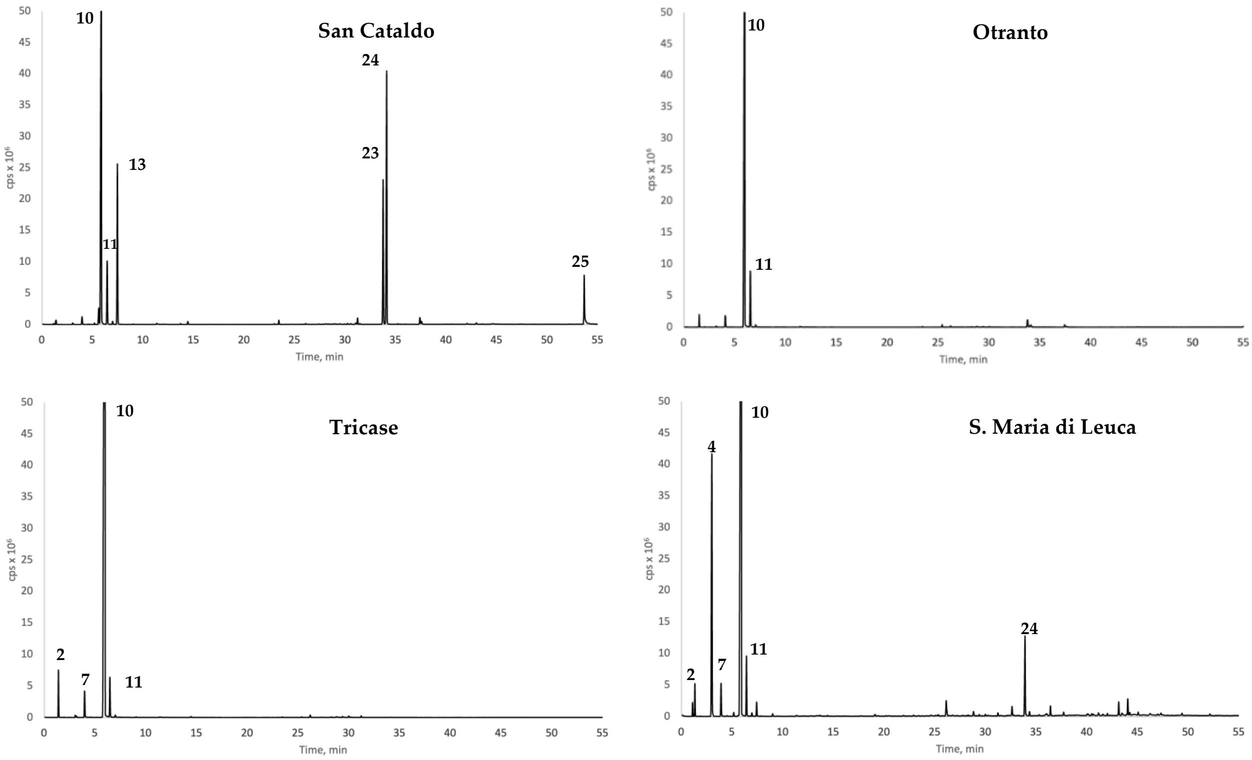

Figure 4. *Cont.*

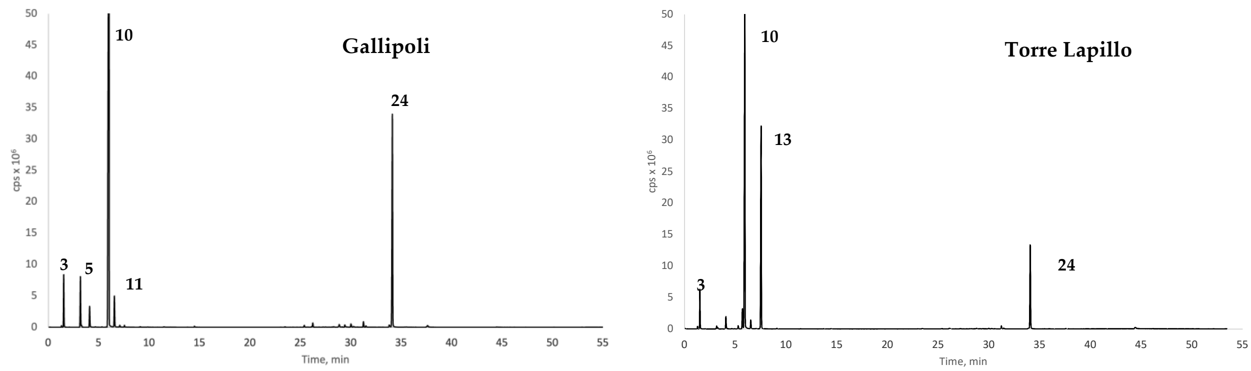

Figure 4. Representative chromatograms showing the main VOCs emitted by aerial parts of *C. maritimum* from six sites analyzed, San Cataldo Otranto, Tricase, S. Maria di Leuca, Gallipoli, Torre Lapillo. The numbers refer to the most significant compounds. The peak identified with n. 10 is out of scale.

The peak area values (%) of the identified volatile compounds are reported in Table 1. The predominant compound was D-limonene, which is present at a high level in all chromatograms, up to a value of about 92% for the Tricase accession. The content of monoterpenes cis/trans β-Ocimene and β-Mircene was low in all samples; the amount of phenylpropanoids dillapiol (4,5-dimethoxy-6-prop-2-enyl-1,3-benzodioxole) and apiol (4,7-dimethoxy-5-prop-2-enyl-1,3-benzodioxole), both indicated as (dill)apiol because our chromatographic system did not allow us to distinguish between the two compounds (having just a methoxy group positioned differently on the benzene ring), was high for the San Cataldo accession (about 34%), and Gallipoli and Torre Lapillo accessions showed an amount of about 20% and 12%, respectively. These compounds were detected at the lowest levels in samples from Otranto and S. Maria di Leuca and undetected for Tricase.

Table 1. Main VOCs emitted by *C. maritimum* leaves from six different accessions (San Cataldo, Otranto, Tricase, S. Maria di Leuca, Gallipoli, and Torre Lapillo) in Salento coast. Numbering of volatile compounds respects the order of elution after the analysis of aerial parts of plants. Major compounds are those with a peak area of >4% of the total.

No.	Compound Name	Peak Area (%)						RI[a]	RI[b]
		San Cataldo	Otranto	Tricase	S. Maria di Leuca	Gallipoli	Torre Lapillo		
1	Unknown				0.6		0.3	960	-
2	Δ^3-Carene	0.4		2.6	1.5			978	1005
3	Cyclofenchene		1.2			3.5	4.1	981	946
4	Sabinene			0.2	18.0			985	978
5	β-Phellandrene					3.9	0.4	1001	1004
6	β-Pinene			0.1				1005	990
7	β-Myrcene	0.7	1.2	1.8	1.9	1.7	1.7	1013	992
8	α-Terpinene						0.4	1021	1014
9	Cymene						2.9	1024	1020
10	D-Limonene	37.8	76.3	92.0	64.7	66.4	49.3	1028	1031
11	(c/t) β-Ocimene	5.0	5.6	2.4	3.1		1.0	1032	1032
12	(c/t) β-Ocimene	0.3	0.3	0.2		2.4		1034	1032
13	γ-Terpinene	13.3			0.8		27.9	1062	1064
14	Allocimene		1.2					1122	1131
15	α-Terpineol	0.3		0.1				1182	1190
16	α-Copaene	0.3						1376	1376
17	β-Caryophyllene		0.3					1418	1417
18	α-Bergamotene			0.2	1.2	0.4		1427	1438
19	α-Cedrene			0.1				1433	1446
20	β-Bisabolene			0.1				1498	1505
21	β-Sesquiphellandrene			0.1		0.3		1522	1524

Table 1. *Cont.*

No.	Compound Name	Peak Area (%)						RI[a]	RI[b]
		San Cataldo	Otranto	Tricase	S. Maria di Leuca	Gallipoli	Torre Lapillo		
22	G ermacrene B	0.5		0.1		0.5	0.4	1556	1558
23	(Dill)apiol	11.9	0.9					1678	1681
24	(Dill)apiol	22.5	0.3		5.0	20.2	11.6	1681	1681
25	Panaxynone	4.2						2022	2018
Total compounds identified %		97.2	87.3	100	96,8	99,3	100		
Monoterpene hydrocarbons		57.5	85.8	99.3	90.6	77.9	88		
Oxygenated monoterpenes		0.3		0.1					
Sesquiterpenes hydrocarbons		0.8	0.3	0.6	1.2	1.2	0.4		
Phenylpropanoids		34.4	1.2		5.0	20.2	11.6		

The values given are the averages of three repetitions, and standard error values are not reported but were all values within 5%. RI[a], Retention index calculated, RI[b], Retention index data from the NIST library (https://webbook.nist.gov/chemistry/, accessed on 28 December 2023).

The γ-terpinene was observed in samples collected from San Cataldo and Torre Lapillo, and sabinene was found to be emitted only from the leaves of S. Maria di Leuca accession.

A compound found at a low level only in plants from San Cataldo was Panaxynone, a polyacetylene also known as Falcarinone, with a level of about 4%.

Agglomerative Hierarchical Cluster Analysis on VOC Composition

A hierarchical cluster analysis (HCA) was performed for the evaluation of the volatile fingerprints of *C. maritimum* from different sites and to evaluate the chromatographic differences in the volatile compound contents. Ward's algorithm was employed in the analysis. Clusters and sub-clusters are visualized in a dendrogram plot (Figure 5). As shown in Figure 5, two well-separated clusters are visualized: a cluster was represented by plants from San Cataldo, which were distinguished from other *Crithmum* groups for high (dill)apiol content (>30%), the second cluster, characterized by a lower (dill)apiol amount, grouped the other five accessions/sites analyzed. Moreover, this cluster was divided into two subclusters: one comprises Gallipoli and Torre Lapillo accessions with a (dill)apiol content between 10 and 30%, and the other Tricase, Otranto, and S. Maria di Leuca groups, with phenylpropanoid amounts of <10%.

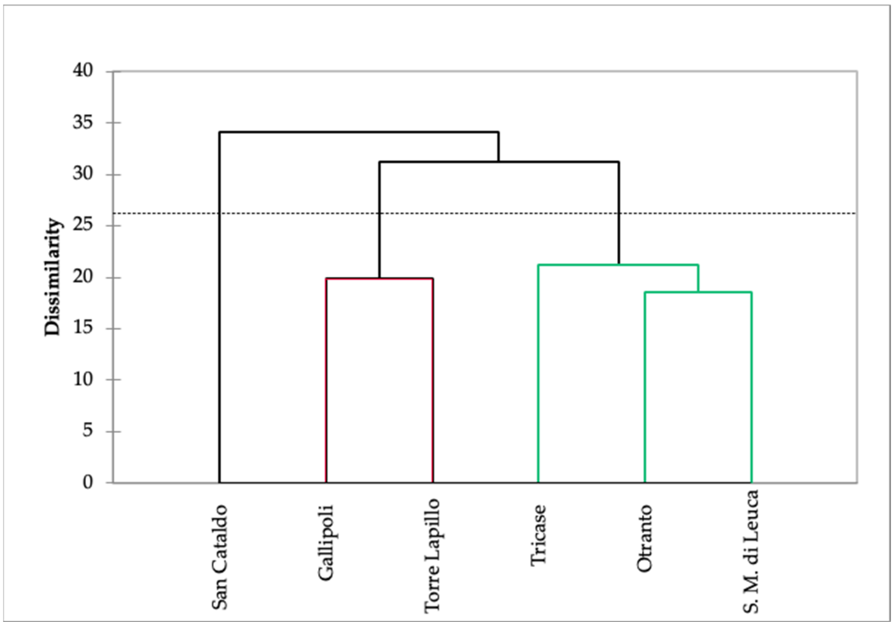

Figure 5. Dendrogram of agglomerative hierarchical cluster analysis of *C. maritimum* from six sites in Salento coastal area with remarks on the volatile organic compounds.

4. Discussion

Given the interest in the exploitation of *C. maritimum* to enhance the production ex situ, we evaluated the germination efficiency. Our results showed that the seeds have a high germination rate (average value of about 78%) and no significant dormancy in controlled conditions, suggesting a great potential for introduction in culture despite the reported need for improved germination for *C. maritimum* cultivation [23]. A similar level of germination was obtained by Nimac et al. [24]. However, differences in germination between seeds collected from different sites (Figure 3) suggest intraspecific variability in germination patterns reported for other species [25,26] and for *C. maritimum* seeds from Italy or from the England Atlantic coast [27]. These results highlight an important trait for the Salento accessions that should help *C. maritimum* cultivation on a large scale.

Comprehensive information about the presence of different metabolites could represent a prerequisite to investigating the biodiversity of organisms belonging to the same species to develop domestication programs. In this study, the different composition of VOCs produced by *C. maritimum* plants obtained from seeds collected in six sites along Salento coasts was evaluated. Through gas chromatography, 25 volatile chemical compounds were identified; most of them are monoterpenes (Table 1), sesquiterpenes, and phenylpropanoids, such as (dill)apiol. We observed quantitative and qualitative differences in the mix of volatiles released from distinct plant accessions.

Terpenoid VOCs play an important role in direct and indirect plant defense [28]. In recent years, many studies reported that sea fennel contains a wide variety of chemical constituents, such as phenolic compounds [29], and volatile compounds, such as limonene, α-pinene, p-cimene, γ-terpinene, and β-myrcene, have been found in essential oils from different plant portions [30,31].

In this work, data obtained by the GC analysis of VOCs emitted by the apical leaves of plants grown in a greenhouse (within the Botanical Garden of the University of Salento) show a common feature in D-limonene as the most abundant compounds: more than 60% in Otranto, Tricase, S. Maria di Leuca, and Gallipoli accessions, nearly 50% for Torre Lapillo samples, and about 38% for plants from San Cataldo. In wild plants growing in Central Italy and in Sicily, limonene represents a major essential oil component [8,32], suggesting that *Crithmum* plants growing in Italy belong to a chemotype characterized mainly by this monoterpene. Similarly, a great amount of D-limonene was found in essential oils from Turkey [33] and Croatia [34] plants.

Data obtained from AHC analysis highlighted that there are two main clusters, probably related to different chemotypes: one named, according to the review of Renna [3], the phenylpropanoid-type, typical of the San Cataldo site, which has, in addition to monoterpenes, a high level of (dill)apiol, and the other, namely monoterpene hydrocarbon-type, characterized by the prevalence of different combinations of D-limonene, c/t β-Ocymene, β-mircene, γ-terpinene, and several cyclic monoterpenes at low concentrations.

However, the high variability in the phenylpropanoid content observed in the six accessions (Table 1) led us to focus on two subclusters obtained from AHC analysis, one related to Gallipoli and Torre Lapillo samples, showing a (dill)apiol amount between 10 and 20%, the other including Tricase, Otranto, and S. Maria di Leuca samples, represented by plants with a (dill)apiol concentration less than 10%. Therefore, we assume the presence of three probable chemotypes: the first, named chemotype I (high (dill)apiol), represented by San Cataldo accession, characterized by a (dill)apiol content > 30%; the second one, named chemotype II (medium (dill)apiol), with a (dill)apiol content between 10 and 20%, including Gallipoli and Torre Lapillo accessions; and the third, named chemotype III (low (dill)apiol), for Otranto, S. Maria di Leuca, and Tricase accessions with a (dill)apiol amount < 10%. An interesting finding from the data is that (dill)apiol has not been detected in the Tricase samples.

In a study carried out to characterize the chemical composition of a Portuguese *C. maritimum* essential oil, Pateira et al. [35] identified two chemotypes based on the dillapiol amount in extract: chemotype 1 with dillapiol > 14.5% and chemotype 2 with dillapiol < 5.8%. Numerous *Apiacae* species were characterized by a high content of volatile phenylpropanoids as (dill)apiol [36]. In *Piper lanceaefolium*, different chemotypes were identified based on the (dill)apiol content [37].

The comparison of the chemical composition of *C. maritimum* plants from Apulia with other Italian regions evidenced that a phenylpropanoid-type chemotype of *Crithmum*, characterized by a significant amount of dillapiol, obtained by the hydrodistillation method, was found only in Sardinia [38].

Due to the presence of several monoterpene compounds in all samples, we compared our data with those from previous studies about essential oil characterization in *C. maritimum* with different geographic origins, such as Cyprus [39], France [8], Spain [40], Turkey [33], and Greece [18,41]. In agreement with our findings, some authors have found that the most abundant volatile compounds identified in *C. maritimum* leaves were terpenoids with different combinations of essential oils [42,43].

An interesting finding in the data is that, among terpenoid compounds, we detected a significant amount of sabinene, a cyclic monoterpene, for Maria di Leuca accession, while only traces were detected for Tricase accession. These data, together with the absence of (dill)apiol, could explain why, in cluster analysis (Figure 5), the Tricase sample diverged from Otranto and S. Maria di Leuca. A combination of sabinene and limonene as the major compounds in S. Maria di Leuca was a common trait with the essential oil extracts from plants growing in Dalmatia (Croatia) [34].

Moreover, the evaluation of all compounds identified in Table 1 showed an important variation in the minor compounds, which contribute together with different combinations of major compounds to the determination of intraspecific variability. It is likely that, over time, varying soil and environmental conditions have influenced the chemical composition of the chemotypes highlighted, although grown in a small area like Salento.

A result worth noting is that in plants from San Cataldo, we detected panaxynone (also known as falcarinone), a polyacetylene previously isolated from almost all species belonging to several families, including Apiaceae. This is the first time that it was identified in *C. maritimum*. Previous studies reported the identification in *Panax ginseng* and *Dacus carota* roots [44,45]; Meot-Duros et al. [9] highlighted the presence of falcarindiol in *C. maritimum* plants from Britanny. Falcarinone, together with falcarinol and falcarindiol, belongs to highly bioactive compounds with cytotoxic, anti-inflammatory, and potential anticancer properties [46].

Among the compouns identified in the plants of Salento, it is interesting that some of them determine aromatic characteristics, which are employed, adding to food or cosmetic products. D-limonene, found in all Apulian accessions, occurring more commonly in nature as the fragrance of oranges, is a flavoring agent in food manufacturing and some medicines; it is also used as a botanical insecticide [47,48].

γ-terpinene has strong antioxidant activity, has a wood/lemon/lime odor, and is widely used in the food, flavor, soap, cosmetic, pharmaceutical, and perfume industries [49]. The spicy sabinene contributes to the savory flavor of black pepper and the earthy taste of carrots. It is popular in aromatherapy and is a major constituent of essential oils with therapeutic qualities, such as carrot seed oil and tea tree oil [50].

The phenylpropene volatiles dillapiol and apiol impart one of the characteristic aromas of dill (*Anethum graveolens*) weeds [51]. Dillapiol is a phenylpropanoid with one methylenedioxy and two methoxy groups; in apiol, a methoxy group is positioned differently on the benzene ring. It is known that dillapiol showed insecticide activity inhibiting cytochrome P450 monooxygenases acting on insect metabolism, blocking the insect catabolism of xenobiotics as plant toxins [52,53]. Razzaghi-Abyaneh et al. [54] evidenced that dillapiol and apiol inhibit the ability of *Aspergillus parasiticus* to synthetize aflatoxins. Apiol has been demonstrated to inhibit human colon cancer cells [55].

β-ocimene, found in all accessions analyzed, belongs to acyclic monoterpenoids, and it has a citrus, green woody aroma along with a green floral or woody taste. It is an approved food additive and is used in perfumery products. Ocimene occurs naturally in several plants and fruits, including basil, coriander, mint, and mangos, and it is also a constituent of the pheromones of several insects [56]. It has many biological functions, such as its use in pesticides and as a defense against herbivores [57].

From a sensorial point of view, VOC analysis can explain some interesting attributes of *C. maritimum*, translating into some notes of celery, common fennel, and peel of green citrus with a pungent aftertaste [58].

Overall, the variability observed presents good practice to fulfill the demand of food and pharmaceutical industries. Therefore, different chemotypes of *C. maritimum* may be employed as needed in many fields, depending on the combination and amounts of VOCs.

5. Conclusions

The intraspecies diversity based on qualitative and quantitative metabolites is an efficacious approach to understanding the interaction plant environment, especially in wild plants, which have the ability to adapt to challenging climatic conditions. *C. maritimum* L. is considered an emerging crop for its physiological potentialities, high nutritional value, and biological effects. The high germination rate and the growth of seedlings in open fields suggest that *C. maritimum* could be cultivated in soils with non-agronomical importance to produce a source of high quality for industrial purposes.

As a result, we suggested that in Salento, there are three main *C. maritimum* chemotypes depending on (dill)apiol content, according to the clusters and subclusters obtained from AHC analysis: a chemotype I (high (dill)apiol) with a content > 30%, a chemotype II (medium (dill)apiol) between 10 and 30%, and a chemotype III (low (dill)apiol) < 10%.

Although we have considered three different chemotypes based on (dill)apiol content, we found a high diversity, in terms of terpenoids identified among the six *C. maritimum* accessions of plants, each characterized by a specific VOC composition.

Our findings provide a better understanding of the phytochemistry of this species useful for domestication programs and the commercial cultivation of aromatic plants. Further studies are needed (i) to deepen the chemical peculiarities of each accession through the analysis of essential oils in different organs during the life cycle and (ii) to evaluate the optimal conditions that have a high yield in terms of valuable metabolites.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10010081/s1, Table S1: GPS coordinates and characteristics of six sites of Salento, Apulia region in Italy, considered in this study; Figure S1: Time course of seed cumulative germination (%) rates of *C. maritimum*. The results are presented for plants from different sites. Each line represents a replicate of 100 non dormant seeds.

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References

1. Atia, A.; Barhoumi, Z.; Mokded, R.; Abdelly, C.; Smaoui, A. Enviromental eco-physiology and economical potential of the halophyte *Crithmum maritimum* L. (Apiaceae). *J. Med. Plants Res.* **2011**, *5*, 3564–3571.
2. Tardío, J.; de Cortes Sánchez-Mata, M.; Morales, R.; Molina, M.; García-Herrera, P.; Morales, P.; Díez-Marqués, C.; Fernández-Ruiz, V.; Cámara, M.; Pardo-de-Santayana, M.; et al. Ethnobotanical and food composition monographs of selected Mediterranean wild edible plants. In *Mediterranean Wild Edible Plants*; Sánchez-Mata, M., Tardío, J., Eds.; Springer: New York, NY, USA, 2016; pp. 273–470. [CrossRef]

3. Renna, M. Reviewing the prospects of sea fennel (*Crithmum maritimum* L.) as emerging vegetable crop. *Plants* **2018**, *7*, 92. [CrossRef]
4. Böer, B.; Khan, M.A.; Marcum, K.B. World Halophyte Garden: Economic Dividends with Global Significance. In *Sabkha Ecosystems. Tasks for Vegetation Science*; Khan, M.A., Böer, B., Öztürk, M., Al Abdessalaam, T.Z., Clüsener-Godt, M., Gul, B., Eds.; Springer: Dordrecht, The Netherlands, 2014; Volume 47, pp. 335–336. [CrossRef]
5. Kraouia, M.; Nartea, A.; Maoloni, A.; Osimani, A.; Garofalo, C.; Fanesi, B.; Ismaiel, L.; Aquilanti, L.; Pacetti, D. Sea Fennel (*Crithmum maritium* L.) as an emerging crop for the manufacturing of innovative foods and nutraceuticals. *Molecules* **2023**, *28*, 4741. [CrossRef]
6. Nabel, N.; Boudries, H.; Chougui, N.; Loupassaki, S.; Souagui, S.; Burlo, F.; Hernandez, F.; Carbonell-Barrachina, A.A.; Madani, K.; Larbat, R. Biological activities and secondary compound composition from *Crithmum maritimum* aerial parts. *Int. J. Food Prop.* **2017**, *20*, 1843–1855. [CrossRef]
7. Sanchez-Faure, A.; Calvo, M.M.; Perez-Jimenez, J.; Martín-Diana, A.B.; Rico, D.; Montero, M.P.; Gomz-Guillen, M.D.C.; Lopez-Caballero, M.E.; Martinez-Alvarez, O. Exploring the potential of common iceplant, seaside arrowgrass and sea fennel as edible halophytic plants. *Food Res. Int.* **2020**, *137*, 109613. [CrossRef]
8. Pavela, R.; Maggi, F.; Lupidi, G.; Cianfaglione, K.; Dauvergne, X.; Bruno, M.; Benelli, G. Efficacy of sea fennel (*Crithmum maritimum* L., Apiaceae) essential oils against *Culex quinquefasciatus* Say and *Spodoptera littoralis* (Boisd.). *Ind. Crops Prod.* **2017**, *109*, 603–610. [CrossRef]
9. Meot-Duros, L.; Cérantola, S.; Talarmin, H.; Le Meur, C.; Le Floch, G.; Magné, C. New antibacterial and cytotoxic activities of falcarindiol isolated in *Crithmum maritimum* L. leaf extract. *Food Chem. Toxicol.* **2010**, *48*, 553–557. [CrossRef]
10. Cohen, I.; Zandalinas, S.I.; Fritschi, F.B.; Sengupta, S.; Fichman, Y.; Azad, R.K.; Mittler, R. The impact of water deficit and heat stress combination on the molecular response, physiology, and seed production of soybean. *Physiol. Plant.* **2021**, *172*, 41–52. [CrossRef] [PubMed]
11. Accogli, R.; Tomaselli, V.; Direnzo, P.; Perrino, E.V.; Albanese, G.; Urbano, M.; Laghetti, G. Edible halophytes and halo-tolerant species in Apulia region (Southeastern Italy): Biogeography, traditional food use and potential sustainable crops. *Plants* **2023**, *12*, 549. [CrossRef]
12. Renna, M.; Gonnella, M. The use of the sea fennel as a new spice-colorant in culinary preparations. *Int. J. Gastr. Food Sci.* **2013**, *1*, 111–115. [CrossRef]
13. Giungato, P.; Renna, M.; Rana, R.; Licen, S.; Barbieri, P. Characterization of dried and freeze-dried sea fennel (*Crithmum maritimum* L.) samples with headspace gas-chromatography/mass spectrometry and evaluation of an electronic nose discrimination potential. *Food. Res. Int.* **2019**, *115*, 65–72. [CrossRef]
14. Mancianti, F.; Ebani, V.V. Biological activity of essential oils. *Molecules* **2020**, *25*, 678. [CrossRef] [PubMed]
15. De Agostini, A.; Robustelli della Cuna, F.S.; Cortis, P.; Cogoni, A.; Sottani, C.; Soddu, F.; Sanna, C. Volatile organic compounds (VOCs) diversity in the Orchid *Himantoglossum robertianum* (Loisel.) P. Delfore from Sardinia (Italy). *Diversity* **2023**, *14*, 1125. [CrossRef]
16. Figueiredo, A.C.; Barroso, J.G.; Pedro, L.G.; Scheffer, J.J.C. Factors affecting secondary metabolite production in plants: Volatile components and essential oils. *Flavour Fragr. J.* **2008**, *23*, 213–226. [CrossRef]
17. Migliorini, P.; Gkisakis, V.; Gonzalvez, V.; Raigón, M.D.; Bàrberi, P. Agroecology in Mediterranean Europe: Genesis, State and Perspectives. *Sustainability* **2018**, *10*, 2724. [CrossRef]
18. Zafeiropoulou, V.; Tomou, E.-M.; Douros, A.; Skaitsa, H. The effect of successive harvesting on the volatile constituents of two essential oils of cultivated populations of sea fennel (*Crithmum maritimum* L.) in Greece. *J. Essent. Oil Bear. Plants* **2021**, *24*, 1–11. [CrossRef]
19. Nascimento, J.P.B.; Vieira, D.C.M.; Meiado, M.V. Ex situ conservation of Brazilian Cacti. *Gaia Sci.* **2015**, *9*, 111–116.
20. Negro, C.; Dimita, R.; Min Allah, S.; Miceli, A.; Luvisi, A.; Blando, F.; De Bellis, L.; Accogli, R. Phytochemicals and Volatiles in Developing *Pelargonium* 'Endsleigh' Flowers. *Horticulturae* **2021**, *7*, 419. [CrossRef]
21. Dimita, R.; Min Allah, S.; Luvisi, A.; Greco, D.; De Bellis, L.; Accogli, R.; Mininni, C.; Negro, C. Volatile compounds and total phenolic content of *Perilla frutescens* at microgreens and mature stages. *Horticulturae* **2022**, *8*, 71. [CrossRef]
22. Zhao, Y.Z.; Li, Z.G.; Tian, W.L.; Fang, X.M.; Su, S.K.; Peng, W.J. Differential Volatile Organic Compounds in Royal Jelly Associated with Different Nectar Plants. *J. Integr. Agric.* **2016**, *15*, 1157–1165. [CrossRef]
23. Meot-Duros, L.; Magné, C. Effect of salinity and chemical factors on seed germination in the halophyte *Crithmum maritimum* L. *Plant Soil* **2008**, *313*, 83–87. [CrossRef]
24. Nimac, A.; Lazarević, B.; Petek, M.; Vidak, M.; Šatović, Z.; Carović-Stanko, K. Effects of Salinity and Seed Priming on Germination of Sea Fennel (*Crithmum maritimum* L.). *Agric. Conspec. Sci.* **2018**, *38*, 181–185. Available online: https://hrcak.srce.hr/203017 (accessed on 24 November 2023).
25. Bischoff, A.; Müller-Schärer, H. Testing population differentiation in plant species- how important are environmental maternal effects. *Oikos* **2010**, *3*, 445–454. [CrossRef]
26. Del Vecchio, S.; Mattana, E.; Acosta, A.T.R.; Bacchetta, G. Seed germination responses to varying environmental conditions and provenances in *Crucianella maritima* L., a threatened costal species. *Comp. Rend. Biol.* **2012**, *335*, 26–31. [CrossRef]
27. Marchioni-Ortu, A.; Bocchieri, E. A study of the germination responses of a Sardinian population of sea fennel (*Crithmum maritimum*). *Can. J. Bot.* **1984**, *62*, 1832–1835. [CrossRef]

28. Abbas, F.; Ke, Y.; Yu, R.; Yue, Y.; Amanullah, S.; Jahangi, M.M.; Fan, Y. Volatile terpenoids; multiple functions, biosynthesis, modulation and manipulation by genetic engineering. *Planta* **2017**, *246*, 803–810. [CrossRef]
29. Jallali, I.; Megdiche, W.; Oueslati, S.; Smaoui, A.; Abdelly, C.; Ksouri, R. Changes in phenolic composition and antioxidant activities of the edible halophyte *Crithmum maritimum* L. with physiological stage and extraction method. *Acta Physiol. Plant.* **2012**, *34*, 1451–1459. [CrossRef]
30. Atia, A.; Debez, A.; Barhoumi, Z.; Abdell, C. Localization and composition of seed oils of *Crithmum maritimum* L. *(Apiaceae) Afr. J. Biotechnol.* **2010**, *9*, 6482–6485.
31. Burczyk, J.; Wierzchowska-Renke, K. Geographic and Environmental Influences on the Variation of Essential Oil and Coumarins in *Crithmum maritimum* L. *J. Herbs Spices Med. Plants* **2002**, *4*, 305–311. [CrossRef]
32. Ruberto, G.; Baratta, M.T.; Deans, S.G.; Dorman, H.J.D. Antioxidant and antimicrobial activity of Foeniculum vulagare and *Crithmum maritimum* essential oils. *Planta Med.* **2000**, *66*, 687–693. [CrossRef]
33. Senatore, F.; Napolitano, F.; Ozcan, M. Composition and antibacterial activity of the essential oil from *Crithmum maritimum* L. (Apiaceae) growing wild in Turkey. *Flavour Fragr. J.* **2000**, *15*, 186–189. [CrossRef]
34. Politeo, O.; Popovic, M.; Bratincevic, M.V.; Kovacevic, K.; Urlic, B.; Mekinic, I.G. Chemical profiling of Sea Fennel (*Crithmum maritimum* L., Apiaceae) essential oils and their isolation residual waste-water. *Plants* **2023**, *12*, 214. [CrossRef] [PubMed]
35. Pateira, L.; Nogueira, T.; Antunes, A.; Venâncio, F.; Tavares, R.; Capelo, J. Two chemotypes of *Crithmum maritimum* L. from Portugal. *Flavour Fragr. J.* **1999**, *14*, 333–343. [CrossRef]
36. Chizzola, R. Essential oil composition of wild growing Apiaceae from Europe and the Mediterranean. *Nat. Prod. Commun.* **2010**, *5*, 1477–1492. [CrossRef] [PubMed]
37. Mundina, M.; Vila, R.; Tomi, F.; Tomàs, X.; Ciccío, J.F.; Adzet, T.; Casanova, J.; Canigueral, S. Composition and chemical polymorphism of the essential oils from *Piper lanceaefolium*. *Biochem. Syst. Ecol.* **2001**, *29*, 739–748. [CrossRef] [PubMed]
38. Marongiu, B.; Maxia, A.; Piras, A.; Porcedda, S.; Tuveri, E.; Goncalvess, M.J.; Cavaleiros, C.; Salgueiros, L. Isolation of *Crithmum maritimum* L. volatile oil by supercritical carbon dioxide extraction and biological assays. *Nat. Prod. Res.* **2007**, *21*, 1145–1150. [CrossRef] [PubMed]
39. Polatoglu, K.; Karakoc, O.C.; Yucel Yucel, Y.; Gucel, S.; Demirci, B.; Baser, K.H.C.; Demirci, F. Insecticidal activity of edible *Crithmum maritimum* L. essential oil against Coleopteran and Lepidopteran insects. *Ind. Crops Prod.* **2016**, *89*, 383–389. [CrossRef]
40. Sanchez-Hernandez, E.; Buzon-Duran, L.; Andres-Juan, C.; Lorenzo-Vidal, B.; Martin-Gil, J.; Martin-Ramos, P. Physicochemical characterization of *Crithmum maritimum* L. and *Daucus carota* subsp. *gummifer* (Syme) Hook.fil. and their antimicrobial activity against apple tree and grapevine phytopathogens. *Agronomy* **2021**, *11*, 886. [CrossRef]
41. Pasias, I.N.; Ntakoulas, D.D.; Raptopoulou, K.; Gardeli, C.; Proestos, C. Chemical composition of essential oils of aromatic and medicinal herbs cultivated in Greece-Benefits and Drawbacks. *Foods* **2021**, *10*, 2354. [CrossRef]
42. Senatore, F.; De Feo, V. Essential oil of a possible new chemotype of *Crithmum maritimum* L. growing in Campania (Southern Italy). *Flavour Fragr. J.* **1994**, *9*, 305–307. [CrossRef]
43. Jallali, I.; Hannachi, H.; Zaouali, Y.; Smaoui, A.; Abdelly, C.; Ksouri, R. *Crithmum maritimum* L. volatile compound's diversity through Tunisian populations: Use of a plant organ-based statistical approach for chemotype identification. *Chem Biodivers.* **2023**, *20*, e202300827. [CrossRef]
44. Murata, K.M.; Iida, D.; Ueno, Y.; Samukawa, K.; Ishizaka, Y.; Kotake, T.; Matsuda, H. Novel polyacetylene derivatives and their inhibitory activities on acetylcholinesterase obtained from *Panax ginseng* roots. *Nat. Med.* **2017**, *71*, 114–122. [CrossRef]
45. Purup, S.; Larsen, E.; Christensen, L.P. Differential effects of falcarinol and related aliphatic C(17)-polyacetyenes on intestinal cell proliferation. *J. Agric. Food Chem.* **2009**, *57*, 8290–8296. [CrossRef]
46. Christensen, L.P. Bioactive C17 and C18 acetylenic oxylipins from terrestrial plants as potential lead compounds for anticancer drug development. *Molecules* **2020**, *25*, 2568. [CrossRef] [PubMed]
47. PubChem. Available online: https://pubchem.ncbi.nlm.nih.gov/compound/limonene (accessed on 24 November 2023).
48. Mursiti, S.; Lestari, N.; Wahyu Ningsih, T. The activity of D-limonene from sweet orange peel (*Citrus sinensis* L.) extract as a natural insecticide controller of bedbugs (*Cimex cimicidae*). *Orient. J. Chem.* **2019**, *35*, 1420–1425. [CrossRef]
49. PubChem. Available online: https://pubchem.ncbi.nlm.nih.gov/compound/gamma-Terpinene (accessed on 24 November 2023).
50. Weedmaps. Available online: https://weedmaps.com/learn/the-plant/sabinene (accessed on 23 November 2023).
51. Koeduka, T.; Watanabe, B.; Shirahama, K.; Nakayasu, M.; Suzuki, S.; Furuta, T.; Suzuki, H.; Matsui, K.; Kosaka, T.; Ozaki, S. Biosynthesis of dillapiole/apiole in dill (*Anethum graveolens*): Characterization of regioselective phenylpropene O-methyltransferase. *Plant J.* **2023**, *113*, 562–575. [CrossRef] [PubMed]
52. Scott, J.G. Cytochrome P450 and insecticide resistance. *Insect Biochem. Mol. Biol.* **1999**, *29*, 757–777. [CrossRef] [PubMed]
53. Belzile, A.S.; Majerus, S.L.; Podeszfinski, C.; Guiller, G.; Durst, T.; Arnason, J.T. Dillapiol derivatives as synergists: Structure-activity relationship analysis. *Pest. Biochem. Physiol.* **2000**, *66*, 33–40. [CrossRef]
54. Razzaghi-Abyaneh, M.; Yoshinari, T.; Shams-Ghahfarokhi, M.; Rezaee, M.B.; Nagasawa, H.; Sakuda, S. Dillapiol and Apiol as specific inhibitors of the biosynthesis of aflatoxin G_1 in *Aspergillus parasiticus*. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 2329–2332. [CrossRef]

55. Wu, K.H.; Lee, W.J.; Cheng, T.C.; Chang, H.W.; Chen, L.C.; Chen, C.C.; Lien, H.M.; Lin, T.N.; Ho, Y.S. Study of the antitumor mechanisms of apiole derivatives (AP-02) from *Petroselinum crispum* through induction of G0/G1 phase cell cycle arrest human COLO 205 cancer cells. *BMC Complem. Altern. Med.* **2019**, *19*, 188. [CrossRef]
56. FooDB. Available online: foodb.ca/compounds/FDB001465 (accessed on 24 November 2023).
57. Farré-Armengol, G.; Filella, I.; Llusià, J.; Peñuelas, J. β-Ocimene, a key floral and foliar volatile involved in multiple interactions between plants and other organisms. *Molecules* **2017**, *22*, 1148. [CrossRef]
58. Renna, M.; Gonnella, M.; Caretto, S.; Mita, G.; Serio, F. Sea fennel (*Crithmum maritimum* L.): From underutilized crop to new dried product for food use. *Gen. Res. Crop Evol.* **2017**, *64*, 205–216. [CrossRef]