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Variability in *Crithmum maritimum* L. Essential Oils' Chemical Composition: PCA Analysis, Food Safety, and Sustainability

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Abstract: In this study, four accessions of *Crithmum maritimum* L., not previously studied, collected in Isola delle Femmine (Italy) (S43), Croatia (S44), Montenegro (S45), and Israel (S46) were investigated. The volatile profile of essential oils was evaluated using GC–MS and 38 compounds were identified. All the analyzed samples show a composition characterized essentially by monoterpene hydrocarbons (94.0–97.6%), with limonene, γ-terpinene, β-phellandrene, α-pinene, and *p*-cymene as the principal compounds. In addition, a comprehensive review of the composition of *C. maritimum* essential oils that have been studied thus far was conducted. To evaluate the similarity between samples, principal component analysis (PCA) and Hierarchical Cluster Analysis (HCA) were utilized. To evaluate the possibility of addressing food value to natural species that can strengthen sustainable food policies, it appears necessary to consider the previous safety of the dietary intake of *C. maritimum*. A matrix plot analysis of the content of dillapiole, a toxic constituent, in the samples was performed. The results of the statistical analysis show the presence of six clusters indicating some differences between *C. maritimum* accessions from different locations. Regarding dillapiole content, the four accessions discussed in this paper showed dillapiole values of less than 2%, suggesting the healthiness of sea fennel from these locations, while the highest values were found in samples from France, Portugal, and Tunisia.

Keywords: Apiaceae; ecological sustainability; limonene; dillapiole; statistical analysis; plant by-products

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1. Introduction

Crithmum maritimum L. (Apiaceae) is an edible perennial halophyte plant.

This plant, commonly found on rocky coastal areas, piers, breakwaters, and, rarely, on sandy beaches, is known by several names including rock sapphire, sea fennel, and marine fennel [1]. This wild plant grows all along the Mediterranean coast and it is especially common in countries such as France, Turkey, Tunisia, Italy, Croatia, Greece, and Spain; it is also found along the Atlantic coast in Portugal [2]. The plant has fleshy, hollow stems that can grow up to 50 cm tall and small green or yellowish-green flowers that bloom in summer [3]. Some recent experiments have shown the possibility of introducing the species in cultivation in small pots on different substrates, demonstrating the possibility of producing vegetative material with specific compositions [4].

Crithmum maritimum has a unique fennel-like aroma and flavor and is used in culinary dishes as a flavoring herb, but it is also used in traditional medicine and cosmetics [5]. This halophyte plant is used for many applications in folk medicine, but it can also be adopted as a main tool in some approaches based on ecological transition. The medicinal application of *C. maritimum* varies depending on the used part [6]; specifically, aerial parts are used in the form of infusion, decoctions, and juices to prevent or alleviate many diseases such as gastrointestinal disorders [7], inflammatory and skin problems [8,9] infectious diseases [10,11], and liver and genitourinary diseases [7,9,10]. These healthy properties of sea fennel are also approved by the Italian Ministry of Health [11] and make it an interesting food. The edible use of *C. maritimum* is typical in many countries, where the fresh leaves and young branches of sea fennel are pickled and used as a condiment in salads, sometimes replacing capers [10]; this condiment is prepared, for example, in Salento (Italy) where it is called 'salissia'. It is a very versatile dish, typically paired with fish. Sea fennel can be used both fresh and dried. The fresh leaves of the sea fennel are used as an aromatic herb in addition to soups, sauces, and salads. Recently, an innovative food use of *C. maritimum* has been identified; that is, as a new spice-dye useful for various gastronomic products [7]. From a nutritional point of view, according to the literature, sea fennel has lower water content and a higher total lipid and protein content compared to common fennel. The total carbohydrate content appears to be the same for both species. However, fennel has a lower sugar content and a relatively higher fiber content compared to common fennel. Additionally, it is a source of several minerals, including potassium, sodium, calcium, and magnesium [12], as well as other micronutrients such as vitamin C [7,13]. Sea fennel is mainly composed of hydroxycinnamic acids, with caffeic acid and its derivatives being the most abundant [11,12]. The polyphenolic content varies based on the vegetation period. This halophyte has high phenolic contents compared to other crop species [14].

The biological characterization of the genetic populations of sea fennel found in different Mediterranean areas, and the definition of food properties, represent a system of valorization of plant by-products, as well as soil conservation, reinforcing sustainable development policies. This process finds wide applicability in food policies related to climate change mitigation, which passes through the enhancement of genetic resources with very high suitability. Following the actions proposed by the Agenda 2030 Sustainable Development Goals, all projects developed with a view to germplasm enhancement have positive reflections, especially in relation to climate crisis mitigation. *Crithmum* maritimum, from this point of view, represents an important sustainable resource in Mediterranean conditions because it associates its growth with conditions of water shortage, low soil fertility, and high salinity, characteristics that are of a limiting but typical growth environment for the effects of the climate crisis.

Characterization of sea fennel populations, therefore, becomes a resilience tool, as it can enhance geographic areas where water is becoming an unprecedented critical factor. Several studies have been conducted on extracts and oils of sea fennel due to its culinary and health-promoting potential. The fixed oil extracted from *C. maritimum* L. seeds contains approximately 44% oil, primarily composed of oleic acid (78.6%), linoleic acid (15.4%), and palmitic acid (4.8%). The oil obtained from sea fennel seeds [15] is of high quality and comparable to other oils like olive and canola. The oil extracted from the leaves of *C. maritimum* has a unique composition, containing significant amounts of fatty acids from both the ω-3 and ω-6 series. A hydro-ethanolic extract of *C. maritimum* leaves collected in France [16] exhibited a rich phytochemical profile, with abundant soluble polyphenols that displayed high quantitative and qualitative variability. The extract contained eighteen compounds, primarily chlorogenic acids and flavonoids. Notably, cirsiliol, a flavonol, was identified for the first time in *C. maritimum*. An assessment of the food safety of this plant would benefit from an objective evaluation of potential toxic constituents to humans.

Essential oils showed the presence of many bioactive compounds responsible for different biological properties such as antibacterial, antioxidant, insecticidal, acaricidal, anti-tumor, anti-inflammatory, mosquicidal, vasodilatory, and cholinesterase inhibitory properties [17–21]. The phenolic fraction of *C. maritimum* aerial parts collected in the Tighzert region, Algeria, composed of hydroxycinnamic acids, exhibited scavenging activity against DPPH and ABTS+ [12]. A study [22] revealed the insecticidal potential of *C. maritimum* essential oil against mosquitoes and agricultural pests. Essential oils from various plant samples collected from different locations were tested on *Culex quinquefasciatus* Say and *Spodoptera littoralis* larvae. The most efficient essential oils were obtained from the seeds and aerial parts of plants in the French region. LD_{50} values of 62.3 and 71.7 µg/larva were estimated for *S. littoralis*, respectively, and LC_{50} values of 13.7 and 15.6 µL/L were estimated for *C. quinquefasciatus* larvae, respectively, when exposed to these essential oils. Another study [18] investigated the insecticidal activity of the essential oil of *C. maritimum* collected in Cyprus. The oil was tested on *S. exigua* larvae at different stages of development and showed high fumigant and contact insecticidal activity against the main insects found in stored products, namely *S. oryzae* and *O. surinamensis*. Due to the edible nature of this plant species, the essential oil may be considered safe for use in food.

In addition to the essential oil properties, other activities of derivatives obtained from *Crithmum* have also been investigated. The residual water from hydrodistillation, which is usually discarded, has also shown promising antioxidant activity due to the presence of hydroxycinnamic acids and flavonoid glycosides [10]. In another study [23], some biological properties of the ethanol extract of different parts of *C. maritimum* were further analyzed and compared with those of the essential oil. Essential oils and extracts with a high content of secondary plant compounds, limonene, and chlorogenic acid, had different influences on the biological properties studied. The essential oils were found to be very effective against cholinesterase enzymes. Additionally, the sea fennel flower extract demonstrated a positive vasodilatory effect.

In this research, the essential oils of four accessions of *C. maritimum* were studied. We performed Hierarchical Cluster Analysis (HCA) and Principal Component Analyses (PCA) to find similarities between our samples and those reported in the literature. Furthermore, in order to improve knowledge of the food safety of this plant, the content of the unsafe metabolite dillapiole was analyzed in all samples.

2. Materials and Methods

2.1. Plant Material

The flowering aerial parts (leaves, stems, and flowers) of four accessions of *C. maritimum* were collected on the beach at the following different localities: Isola dell Femmine, Palermo, Italy, (38°11′01″ N 13°14′06″ E 3 m s/l) in August 2023 (S43); Sakarum Beach, Dugi Otok, Croatia (44°08′03″ N 14°52′24″ E 1 m s/l) in July 2023 (S44); Drobni Pi-jerak, near Budva, Montenegro (44°08′03″ N 14°52′24″ E 3 m s/l) in July 2023 (S45); and Caesarea (Israel), (32°29′58″ N 34°53′27″ E 7 m s/l) in June 2023 (S46). All samples, identified by Prof. Vincenzo Ilardi, were stored in the University of Palermo Herbarium (Voucher No. 109780-109781-109782-109783, for S43, S44, S45, and S46, respectively).

2.2. Isolation of Volatile Components

The fresh samples were ground in a Waring blender and then subjected to hydrodistillation for three hours, following the standard procedure described in the European Pharmacopoeia [24]. The oils were dried over anhydrous sodium sulphate and stored in sealed vials under N_2 at −20 °C, ready for GC–MS analyses. Samples S43, S44, S45, and S46 yielded 0.85%, 0.14%, 0.24%, and 0.1% oil (*w*/*w*), respectively.

2.3. GC and GC–MS Analysis

Analysis of essential oil was performed according to the procedure reported by Porrello et al. [25]. The percentage values in Table 1 were calculated using the TIC from MS. The identification of peaks was carried out by comparison with their mass spectra and relative retention indices with WILEY275, NIST 17, ADAMS, and FFNSC2, as well as using Kovats indices (KIs).

Table 1. Composition (%) of *Crithmum maritumum* essential oils collected in Isola delle Femmine (S43), Croatia (S44), Montenegro (S45), and Israel (S46).

No.	Compounds [a]	KI [b]	KI [c]	S43 [d]	S44 [d]	S45 [d]	S46 [d]
1	*β*-Thujene	920	929	0.5	0.2	0.4	0.4
2	*α*-Pinene	931	936	3.6	4.9	0.5	15.3
3	Camphene	953	950	tr	0.10	tr	0.17
4	*β*-Phellandrene	971	977	4.1	8.0	28.3	0.6
5	*β*-Myrcene	981	986	nd	nd	nd	0.8
6	*β*-Pinene	982	996	0.9	0.9	1.8	0.8
7	Octanal	1005	1007	nd	0.2	nd	0.3
8	*α*-Phellandrene	1010	1011	nd	nd	0.3	nd
9	*α*-Terpinene	1017	1022	0.3	0.1	1.6	0.2
10	*p*-Cymene	1029	1037	13.6	nd	nd	nd
11	Limonene	1032	1049	nd	79.0	50.0	43.1
12	*cis*-*β*-Ocimene	1041	1056	nd	tr	nd	1.6
13	*γ*-Terpinene	1059	1064	49.0	1.4	4.0	27.0
14	Terpinolene	1089	1085	tr	0.2	0.9	0.2
15	*cis*-Sabinene hydrate	1101	1099	tr	tr	0.4	nd
16	*cis*-*p*-Menth-2-en-1-ol	1122	1124	tr	tr	0.5	nd
17	*trans*-allocimene	1131	1131	tr	0.2	nd	0.2
18	*cis*-Limonene oxide	1134	1137	nd	0.2	0.1	tr
19	*trans*-*p*-Menth-2-en-1-ol	1145	1143	nd	tr	0.2	nd
20	Terpinen-4-ol	1180	1180	0.6	1.2	5.7	0.1
21	*α*-Terpineol	1187	1193	0.2	0.2	0.4	tr
22	*cis*-Dihydrocarvone	1195	1195	nd	0.1	nd	nd
23	*cis*-Piperitol	1211	1207	nd	tr	0.2	nd
24	*trans*-Carveol	1217	1219	nd	0.1	tr	nd
25	Thymol methyl ether	1235	1250	24.5	nd	nd	0.5
26	Carvacrol	1298	1294	0.1	nd	nd	nd
27	*β*-Bourbenene	1382	1373	nd	nd	nd	0.1
28	Damascenone	1384	1374	nd	nd	0.1	nd
29	*β*-Elemene	1392	1403	nd	nd	nd	0.1
30	*β*-Caryophyllene	1415	1431	tr	tr	nd	0.2
31	*γ*-Elemene	1425	1446	tr	tr	nd	0.7
32	Germacrene D	1480	1496	tr	nd	nd	1.3
33	Cuparene	1502	1498	nd	tr	tr	0.2
34	*β*-Bisabolene	1506	1502	tr	0.1	0.1	nd
35	*β*-Sesquiphellandrene	1519	1518	0.1	0.1	0.1	nd
36	*β*-Himachalene	1527	1524	nd	nd	tr	0.3
37	Spathulenol	1574	1567	tr	0.2	tr	tr
38	Dillapiole	1622	1618	tr	tr	1.6	tr
	Monoterpene Hydrocarbons			72.0	95.1	87.8	90.6
	Oxygenated Monoterpenes			25.4	1.9	7.5	0.6
	Sesquiterpene Hydrocarbons			0.1	0.3	0.2	2.8
	Oxygenated Sesquiterpenes			-	0.2	-	-
	Other			-	-	1.8	-
	Total			97.6	97.3	97.2	94.0

[a] Components listed in order of elution on a DB-5MS apolar column; [b] KIs based on the literature (https://webbook.nist.gov/, accessed on 15 January 2024), [c] Experimental KIs on a DB-5MS apolar column; [d] Area (%) values (tr < 0.01%); nd: not detected.

2.4. Statistical Analysis

Principal component analysis (PCA) was performed on the dataset (46 samples, Table 2) containing the following five variables: monoterpene hydrocarbons (MHs), oxygenated monoterpenes (MOs), sesquiterpenes hydrocarbons (SHs), and oxygenated sesquiterpenes (OSs) and others (Os) and were based on a variance/covariance matrix. When ranges for statistical analysis were provided, the average between them was utilized.

Table 2. The main components (>2%) and classes of *Crithmum maritimim* L. aerial part essential oils, obtained using HD, as reported in the literature.

Sample	Origin	v.p.	Compounds	MH	OM	SH	OS	O	Ref.
S1	Algeria, Bejaia	July	γ-terpinene (50.5), thymol methyl ether (33.6), *p*-cymene (12.6)	65.3	33.7				[12]
S2	Croatia, Dalmatia	June	limonene (58.4), sabinene (26.5), terpinen-4-ol (5.6), γ-terpinene (2.8)	93.4	5.6				[5]
S3	France, Corsica, Mandriolu		γ-terpinene (42.2), β-phellandrene (20.3), dillapiole (7.9), thymol methyl ether (7.3), *p*-cymene (6.4), sabinene (5.2), α-pinene (2.2)	77.5	7.3			7.9	[26]
S4	France, Brittany, Finistere	September	dillapiole (55.7), γ-terpinene (14.0), thymol methyl ether (11.8), sabinene (4.7), myristicin (4.4), *p*-cymene (3.5), α-pinene (2.3)	26.5	13.0			60.4	[22]
S5	France, Brittany, Finistere	September	γ-terpinene (33.0), thymol methyl ether (22.0), dillapiole (17.5), *p*-cymene (8.7), α-pinene (6.4), sabinene (6.0)	57.3	23.7			18.1	[21]
S6	Greece, Chios	June–August	γ-terpinene (22.8), limonene (20.4), sabinene (15.1), β-phellandrene (8.6), *cis*- β -ocimene (8.1), carvacrol methyl ether (6.2), β-pinene (2.8), terpinen-4-ol (2.5)	84.5	6.9	1.8	0.1		[27]
S7	Greece, Larissa	April/May	β-phellandrene (15.5–30.9), γ-terpinene (17.5–19.6), sabinene (15.8–17.6), *p*-cymene (16.7), thymol methyl ether (7.6–9.3), terpinen-4-ol (4.8), α-terpinene (3.9), α-phellandrene (2.6), myrcene (2.2–2.3)	79.4–81.7	14.2–16.1	0.2–3.0	0.2–0.3	0.3–2.0	[8]

Table 2. *Cont.*

Sample	Origin	v.p.	Compounds	MH	OM	SH	OS	O	Ref.
S8	Greece, Magnesia		sabinene (49.4), γ-terpinene (31.4), α,β-pinene (9.6), limonene (2.7)	95.8	1.5				[28]
S9	Greece, N. Euboea, Artemisio	August	limonene (43.5), sabinene (21.7), γ-terpinene (19.2), α-pinene (7.5), terpinen-4-ol (3.0)	93.9	4.3	0.3			[29]
S10	Greece, Crete, Agia Marina	June–August	sabinene (38.0), limonene (12.8), β-phellandrene (18.5), γ-terpinene (17.1), terpinen-4-ol (3.9)	93.5	6.3				[27]
S11	Greece, Crete	June–August	γ-terpinene (35.4), sabinene (21.8), thymol methyl ether (19.7), limonene (6.4)	76.8	22.4				[27]
S12	Greece, Kos Island		sabinene (35.6), β-phellandrene (22.5), γ-terpinene (18.7), thymol methyl ether (10.9), terpinen-4-ol (3.1)	81.8	14.2	0.51			[30]
S13	Greece, Melos	June–August	γ-terpinene (33.3), limonene (28.5), thymol methyl ether (13.7), α-pinene (7.7), *cis*-β-ocimene (6.5), sabinene (3.3), bicyclogermacrene (2.8)	80.8	14.0	3.1	0.2	0.2	[27]
S14	Italy, Campania, Naples	June–August	γ-terpinene (39.4), carvacrol methyl ether (25.8), dillapiole (11.5), isoterpinolene (6.5), *p*-cymene (6.2)	55.9	26.6			11.5	[27]
S15	Italy, Campania, Sorrento	April	γ-terpinene (36.6), thymol methyl ether (28.8), *p*-cymene (9.6), β-pinene (7.5), α-pinene (4.7), dillapiole (0.2)	64.7	30.9	1.6		0.8	[31]
S16	Italy, Liguria	May	γ-terpinene (66.2), thymol methyl ether (13.5), α-pinene (7.4), *p*-cymene (5.2), 1,8-cineole (4.8), dillapiole (1.0)	78.9	18.3			1.0	[32]

Table 2. *Cont.*

Sample	Origin	v.p.	Compounds	MH	OM	SH	OS	O	Ref.
S17	Italy, Liguria	June	γ-terpinene (47.1), 1,8-cineole (15.6), thymol methyl ether (14.2), *p*-cymene (4.8), sabinene (4.4), dillapiole (1.1)	59.2	29.8			1.1	[32]
S18	Italy, Liguria	July	γ-terpinene (68.0), thymol methyl ether l (17.7), *p*-cymene (5.7), 1,8-cineole (2.4), dillapiole (0.5)	77.6	20.1			0.5	[32]
S19	Italy, Liguria	September	γ-terpinene (41.1), sabinene (30.0), thymol methyl ether (12.0), *p*-cymene (5.7), dillapiole (1.6)	79.8	12.9			1.6	[32]
S20	Italy, Liguria	December	γ-terpinene (48.8), thymol methyl ether (16.2), dillapiole (9.5), *p*-cymene (8.8), sabinene (5.1), α-pinene (4.0), 1,8-cineole (2.2)	66.7	18.4			9.5	[32]
S21	Italy, Marche, Camerano	April	γ-terpinene (50.0), thymol methyl ether (18.2), *p*-cymene (8.9), limonene (8.9), sabinene (5.0), α-pinene (2.6), dillapiole (2.5)	77.7	19.1			2.5	[11]
S22	Italy, Marche, Senigallia	August	limonene (38.4), γ-terpinene (19.9), sabinene (12.4), dillapiole (8.1), *cis*-β-ocimene (4.8), carvacrol methyl ether (4.2), terpinen-4-ol (3.1), *p*-cymene (2.6)	83.3	7.9	0.2		8.3	[33]
S23	Italy, Sicily, Catania	May	thymol methyl ether (25.5), γ-terpinene (22.9), limonene (22.3), *p*-cymene (4.3), α-pinene (3.2)	56.7	26.1	2.2		3.9	[34]
S24	Italy, Sicily, Palermo, Addaura	June	thymol acetate (14.4), β-myrcene (13.7), *p*-cymene (11.7), β-phellandrene (6.6), α-pinene (5.5), 2,3,4-trimethylacetophenone (5.3), camphene (5.2), terpinen-4-ol (3.5), 2-methyl-6-(2-propenyl)phenol (3.3), bornyl acetate (2.7), thymol (2.6)	45.1	40.0	1.9	0.5	2.5	[35]

Table 2. *Cont.*

Sample	Origin	v.p.	Compounds	MH	OM	SH	OS	O	Ref.
S25	Portugal, Viana do Castelo	May–October	dillapiole (14.5–46.6), γ-terpinene (16.8–32.9), sabinene (7.0–21.2), thymol methyl ether (10.1–14.4), *p*-cymene (3.4–7.7), α-pinene (2.2–5.9), *cis*-β-ocimene (2.4–3.1), terpinen-4-ol (2.0–2.9)	36.4–64.9	13.1–20.9			14.5–45.4	[36]
S26	Portugal, S. Martinho do Porto	May–October	γ-terpinene (29.5–44.1), sabinene (17.4–31.5), thymol methyl ether (13.7–18.5), *p*-cymene (4.1–8.3), *cis*-β-ocimene (2.5–7.5), terpinen-4-ol (3.6–6.0), α-pinene (2.0–4.7), dillapiole (2.0–2.3), α-terpinene (2.0)	70.4–80.3	18.7–26.3			0–2.3	[36]
S27	Portugal, Boca do Inferno	May–October	γ-terpinene (24.9–38.1), sabinene (22.8–34.4), thymol methyl ether (12.4–17.5), terpinen-4-ol (3.1–7.8), *p*-cymene (3.8–7.2), dillapiole (2.5–5.8), *cis*-β-ocimene (2.2–5.4), α-terpinene (2.0–2.5)	67.8–77.7	19.2–25.5			1.7–5.8	[36]
S28	Portugal, Praia da Ilha do Pessegueiro	May–October	dillapiole (20.2–41.5), γ-terpinene (20.4–35.2), sabinene (8.6–22.0), thymol methyl ether (11.7–15.1), *p*-cymene (3.9–5.6), terpinen-4-ol (2.0–5.2), *cis*-β-ocimene (2.0–4.7), α-pinene (2.2–3.4)	40.3–60.5	15.9–19.1			20.3–41.5	[36]
S29	Portugal, Almograve	May–October	γ-terpinene (36.0–43.0), sabinene (18.5–25.7), thymol methyl ether (13.1–16.6), *p*-cymene (5.0–7.5), *cis*-β-ocimene (2.8–6.7), terpinen-4-ol (3.4–5.6), α-pinene (2.5–4.6), dillapiole (0.1–1.5)	76.9–81.9	18.7–22.3			0.1–1.6	[36]
S30	Portugal, Setubal		sabinene (35.3), γ-terpinene (29.9), thymol methyl ether (18.9), *p*-cymene (5.2), *cis*-β-ocimene (4.0)	78.0	21.5			0.4	[37]

Table 2. *Cont.*

Sample	Origin	v.p.	Compounds	MH	OM	SH	OS	O	Ref.
S31	Portugal, Nazaré		γ-terpinene (33.6), sabinene (32.0), thymol methyl ether (15.7), *p*-cymene (3.9), terpinen-4-ol (3.4), *cis*-β-ocimene (2.7), dillapiole (0.1)	78.0	20.1	0.1		0.1	[10]
S32	Spain, San Sebastian	June–August	dillapiole (35.1), thymol methyl ether (20.4), γ-terpinene (14.9), sabinene (8.1), *p*-cymene (2.2)	28.1	23.5	0.2		36.3	[27]
S33	Tunisia, Kelibia	August	dillapiole (40.2), thymol methyl ether (20.6), γ-terpinene (19.3), β-phellandrene (6.6), *p*-cymene (5.7), terpinen-4-ol (2.3),	33.7	22.9			40.2	[17]
S34	Tunisia, Monastir	August	thymol methyl ether (40.4), γ-terpinene (30.6), dillapiole (14.3), *p*-cymene (9.9), sabinene (2.2),	42.1	40.8	1.0		14.3	[17]
S35	Turkey, Bolu	August	sabinene (26.9), limonene (24.2), γ-terpinene (19.3), *p*-cymene (5.3), terpinen-4-ol (9.0), methyl chavicol (3.4), dillapiole (0.1)	81.1	16.5		0.3	1.2	[38]
S36	Turkey, Silifke	June/July	thymol methyl ether (8.1–29.8), γ-terpinene (8.2–25.5), terpinen-4-ol (2.7–21.2), dillapiole (1.9–21.0), sabinene (13.0–20.5), β-phellandrene (6.3–12.8), α-terpineol (3.3), *p*-cymene (4.4–5.8), *cis*-β-ocimene (4.6–5.3), limonene (2.2–2.7)	40.4–68.3	12.4–54.3	0–1.3	0.1–1.8	1.9–21.1	[39]
S37	Turkey, Bodrum	June/July	γ-terpinene (8.8–35.2), thymol methyl ether (7.7–17.2), limonene (15.4–15.9), *cis*-β-ocimene (2.2–12.7), spathulenol (0–8.4), *p*-cymene (4.5–7.2), β-phellandrene (4.9–6.4), phytol (0–6.0), nonacosane (0–5.4), dillapiole (0–0.7)	22.5–78.8	10.9–19.6	0.9–4.1	0.1–13.8	0.1–14.9	[39]

Table 2. *Cont.*

Sample	Origin	v.p.	Compounds	MH	OM	SH	OS	O	Ref.
S38	Turkey, Gazipasa	April	*p*-cymene (27.1), thymol methyl ether (9.2), γ-terpinene (8.3), cryptone (4.0), limonene (3.9)	41.4	16.8	0.1		12.7	[39]
S39	Turkey, Sipahili	June	γ-terpinene (35.5), β-phellandrene (21.4), sabinene (12.6), thymol methyl ether (8.4), *p*-cymene (8.3), limonene (4.6), *cis*-β-ocimene (2.5)	89.5	20.1	0.2			[40]
S40	Turkey, Yesilovacık	June	γ-terpinene (32.4), β-phellandrene (22.3), dillapiole (9.7), sabinene (9.1), thymol methyl ether (8.6), *p*-cymene (7.6), *cis*-β-ocimene (3.2)	79.7	9.3	0.3		9.7	[40]
S41	Turkey, Antalya	June	β-phellandrene (30.0), thymol methyl ether (24.6), *cis*-β-ocimene (14.3), *p*-cymene (12.8), estragole (3.1), fenchone (2.5), limonene (2.1)	64.1	35.2	0.4			[41]
S42	Turkey, Mersin	June	γ-terpinene (24.3), dillapiole (20.6), β-phellandrene (13.7), sabinene (11.7), thymol methyl ether (8.7), *p*-cymene (7.0), α-terpineol (3.5), *cis*-β-ocimene (3.1)	64.8	13.3			20.6	[41]

MH = monoterpene hydrocarbons; OM = oxygenated monoterpenes; SH = sesquiterpene hydrocarbons; OS = oxygenated sesquiterpenes; O = others; v.p.= vegetative period.

Similarly, Hierarchical Cluster Analysis (HCA) was used to test the similarity among the different samples in relation to the contents of their chemical constituents.

HCA was carried out using unweighted pair-group average (UPGMA) algorithms and the Euclidean similarity index. The PCA and the HCA were chosen to visualize similarities or differences between samples and to summarize the multivariate nature of the data.

The statistical analysis of dillapiole was performed using the data from Table 2. When ranges for statistical analysis were provided, the average between them was utilized.

All statistical analyses and graphs were performed using PAST 4.04 software [42].

3. Results and Discussion

3.1. Essential Oils Composition

The hydrodistillation of *C. maritimum* aerial parts collected in Isola delle femmine (S43) gave a pale-yellow oil. Overall, 23 compounds were identified, representing 97.6% of the total components, listed in Table 1 according to their retention indices on a DB-5MS column and classified into five classes based on their chemical structures. Monoterpene hydrocarbons (72.0%) were the principal metabolites with γ-terpinene (49.0%), *p*-cymene (13.6%), and β-phellandrene (4.1%) as the main ones. Oxygenated monoterpenes were the second most abundant class (25.4%), totally represented by thymol methyl ether (24.5%). Sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and other classes were practically absent.

The oil of the other population, collected in Croatia (S44), also gave a pale-yellow oil. In this case, 28 compounds were identified, representing 97.3% of the total components (Table 1). Also in this case, monoterpene hydrocarbons represent the main class (95.1%), but the composition of this oil is quite different from that of the previous sample (S43). In fact, limonene is the major compound (79.0%) followed by β-phellandrene (8.0%), while γ-terpinene accounts for only 1.4% of the total composition. The remaining classes were present in limited amounts.

The chemical composition of *C. maritimum* essential oil from Montenegro (S45) showed 25 compounds, representing 97.2% of the total components (Table 1). The metabolites occurring in this oil are very similar to those of S44, although their percentages vary somewhat. In fact, in S45, the main class was represented by monoterpene hydrocarbons (87.8%), with limonene (50.0%) and β-phellandrene (28.3%) as principal constituents of the oil. The amount of γ-terpinene (4.0%) is slightly higher with respect to S44 (1.4%). Oxygenated monoterpenes were the second most abundant class (7.5%), with terpinen-4-ol being the principal metabolite (5.7%).

The hydrodistillation of *C. maritimum* aerial parts collected in Israel (S46) gave a pale-yellow oil. Overall, 26 compounds were identified, representing 94.6% of the total components, listed in Table 1. Similar to the other essential oils analyzed in this paper, monoterpene hydrocarbons represent the main class (90.6%) of S46, with limonene (43.1%), γ-terpinene (27.0%), and α-pinene (15.3%) as principal metabolites.

The comparison of the analyzed data (Table 1) showed a good similarity between the samples; in fact, S43, S44, S45, and S46 have monoterpene hydrocarbons as their main class, with quite comparable percentages. However, the oil from Isola delle Femmine (S43) shows γ-terpinene as the main component, unlike the other samples where limonene is the main compound.

Several reports have been published on the chemical composition of the essential oils from the aerial part of *Crithmum maritimum* (Table 2). A comparison of the essential oils investigated in this work with those reported in the literature (Table 2) shows some very interesting points, which will be further discussed later with the PCA analysis (Figure 1).

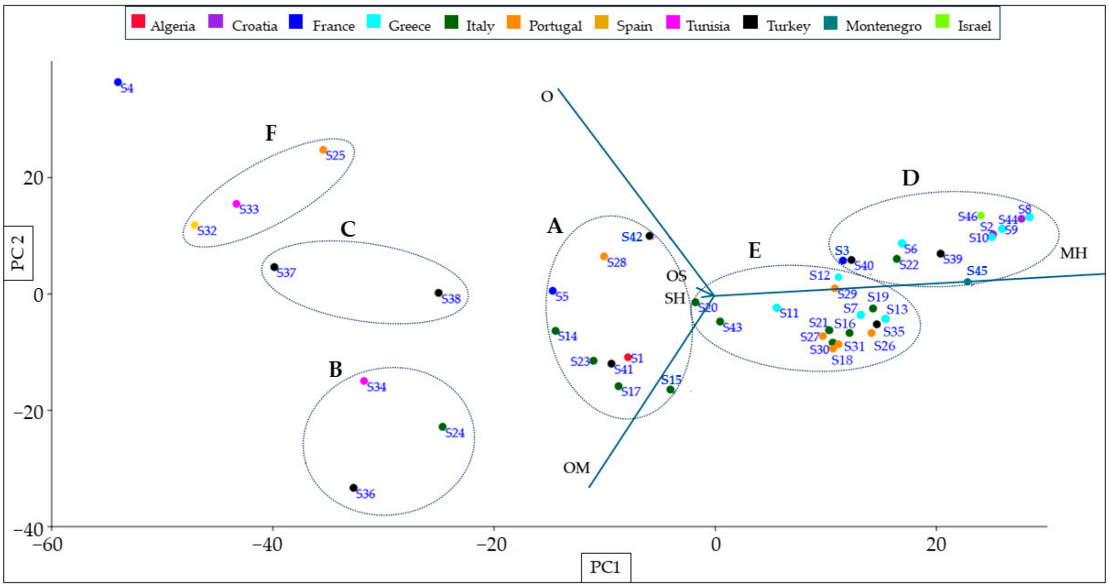

Figure 1. Principal component analysis (PCA) of the essential oil composition of various accessions of *Crithmum maritimum*, based on the principal classes of compounds. The vectors displayed represent the eigenvectors of the covariance matrix.

In particular, samples S44 and S45 show a volatile profile quite similar to that of sample S2 of *Crithmum maritimum* collected in Dalmatia (Croatia) [5]. Indeed, this essential oil, which is characterized by hydrocarbon monoterpenes, shows a high limonene value (58.4%), followed by γ-terpinene (2.8%); however, it has a high amount of sabinene (26.5%), which is totally absent in samples S44 and S45. Essential oil S46, although it has limonene as the main component, differs from the previous ones by a higher percentage of γ-terpinene (27.0%) and α-pinene (15.3%); this composition seems similar to sample S9 (Greece, N. Euboea, Artemisium) [29], which has 19.2% γ-terpinene and 7.5% α-pinene, respectively. Again, the sample described in the literature (S9) shows the presence of sabinene, which is absent in sample S46.

Sample S43, on the other hand, has a similar composition to sample S15 (Sorrento, Italy) [31], in that both show γ-terpinene as the principal compound, 49.0% and 36.6%, respectively, followed by thymol methyl ether (24.5% and 28.8%) and finally p-cymene (13.6% and 9.6%).

3.2. PCA and HCA Analyses of the Essential Oil Composition of Crithmum maritumum Accessions

PCA (Figure 1) and HCA (Figure 2) analysis revealed that there is a consistent grouping of the samples.

The analyses were carried out considering the classes' compounds with a significant contribution, according to the loading plot obtained by principal component analysis (PCA) for monoterpene hydrocarbons (MHs), oxygenated monoterpenes (OMs), sesquiterpene hydrocarbons (SHs), oxygenated sesquiterpenes (OSs) and other compounds (Os).

For the *C. maritimum* essential oils, as shown in the graph (Figure 1), all variables affected PC1 and PC2. In fact, PC1 (72%) was mainly represented by MHs in the positive score and with a minor contribution by OMs, Os, SHs, and OSs in negative scores; meanwhile, PC2 (23%) was mainly represented by a positive score of MHs, OSs, and Os and in negative score by OMs and SHs.

HCA based on the Euclidean distance between groups indicated six clusters (from A to F, Figure 2), identified by their essential oil chemotypes with a similarity $\leq$25.

Cluster A was composed of S1, S5, S14, S15, S17, S23, S28, S41, and S42 samples. For these samples, the MHs class is the main one, with values slightly above 50% of the total composition (55.9–65.3%), with the residual part being characterized by the OMs class (13.3–35.2%) and the Os class (0–20.6%). This cluster shows the complete absence of OSs and a very low percentage of the SHs class (0–2.2%).

Cluster B, which includes S24, S34, and S36 samples, shows a composition characterized by a similar percentage of MHs and OMs with values between 40.4–45.1% and 40.0–54.3%, respectively.

A second small cluster (cluster C) was represented by only two samples, S37 and S38. It was characterized by a high content of MHs (23.1–41.4%), medium levels of OMs (9.5–16.8%), OSs (0–15.5%), and Os (12.7–13.4%). The SHs class was present in a very limited amount.

Another representative group (cluster D) includes twelve samples (S2, S3, S6, S8, S9, S10, S22, S39, S40, S44, S45, and S46) and it is characterized by the highest content of MHs (77.5–95.8%) followed by a low–medium percentage of OMs (0.6–10.1%).

Cluster E represents the biggest in the graph containing the samples S7, S11, S12, S13, S16, S18, S19, S20, S21, S26, S27, S29, S30, S31, S35, and S43. Similar to the previous cluster (D), cluster E shows a higher amount of MH compounds (66.7–81.8%) but, in this case, the group is characterized by a medium–high amount of OMs (12.9–25.4%). For both clusters D and E, the classes SHs, OSs, and Os show similar values. This similarity between the two groups emerges from the HCA graph (Figure 2) when considering a similarity $\leq$ 35.

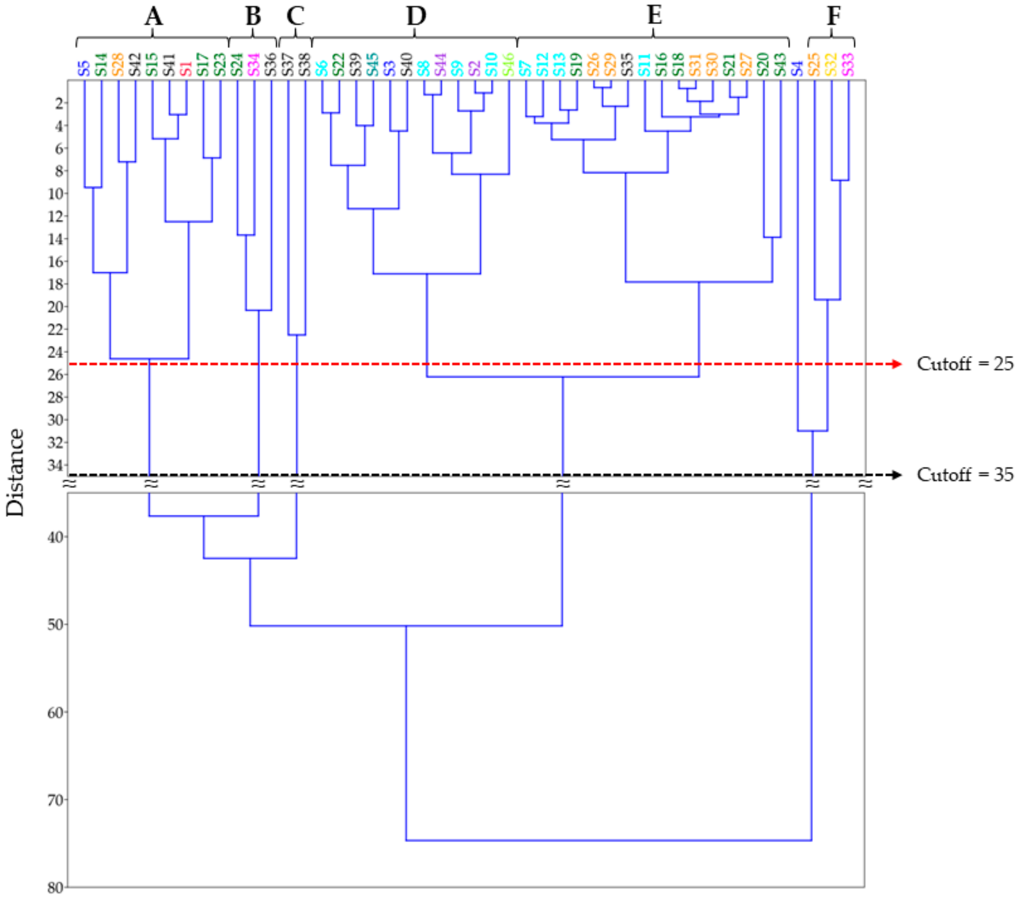

Figure 2. Dendrogram obtained using HCA, based on the Euclidian distances between groups of A, B, C, D, E, and F. The dotted red line corresponds to a cutoff of 25; while the dotted black line corresponds to a cutoff of 35.

Finally, cluster F represented by S25, S32, and S33 samples was characterized by the highest value of Os (36.2–44%). Samples belonging to this group also show almost null values of SHs and Oss, as well as moderately high amounts of MHs (28.1–41.1%) and OMs (14.1–23.5%). Sample S4 does not fit into any cluster, according to the HCA graph with a similarity ≤ 25. If, however, similarity ≤ 35 is considered, the latter sample joins cluster F. In fact, its composition, characterized by 60.4% of Os, 26.5% of MHs, 13.0% of OMs, and null values for SHs and OSs, appears like the other three samples that constitute this cluster.

When present, dillapiole represents the majority compound of the class ‘other’.

Dillapiole is a phenylpropanoid (Figure 3, $C_{12}H_{14}O_4$, molecular weight 222.23) found for the first time in Indian dill, *Anethum sowa* Roxb. ex Fleming (Apiaceae). The dillapiole is a viscid, colorless substance and is found to have a synergic action on pyrethrins (used in insecticides), making it more effective over synthetic synergic compounds like piperonyl butaoxide [43]. The dillapiole is toxic to human consumption at more than 5% in essential oil and, thus, the quality of essential oil is considered better if the dillapiole content in the oil varies between 0 and 5% [44]. In a study of EFSA [45] on the toxicity of dill oil, the maximum daily intake of dillapiole in μg/kg body weight per day was calculated at the level of use of the additive in feed considered safe for dogs and cats. The calculated intake values for this compound were 0.0133 μg/kg body weight per day for dogs and 0.0114 μg/kg body weight per day for cats.

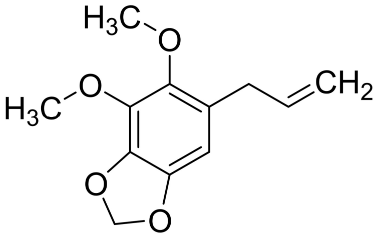

Figure 3. Dillapiole structure.

Due to this toxicity, the distribution of this phenylpropanoid in the different samples was investigated.

A box and jitter plot (Figure 4) was used to display all dillapiole percentage data obtained from the essential oil fraction of *C. maritimum* aerial part samples from different countries. In this plot, the 25–75 percentiles are drawn using a box; minimum and maximum are shown at the end of the thin lines (whiskers), while the median is marked as a horizontal line in the box fitting.

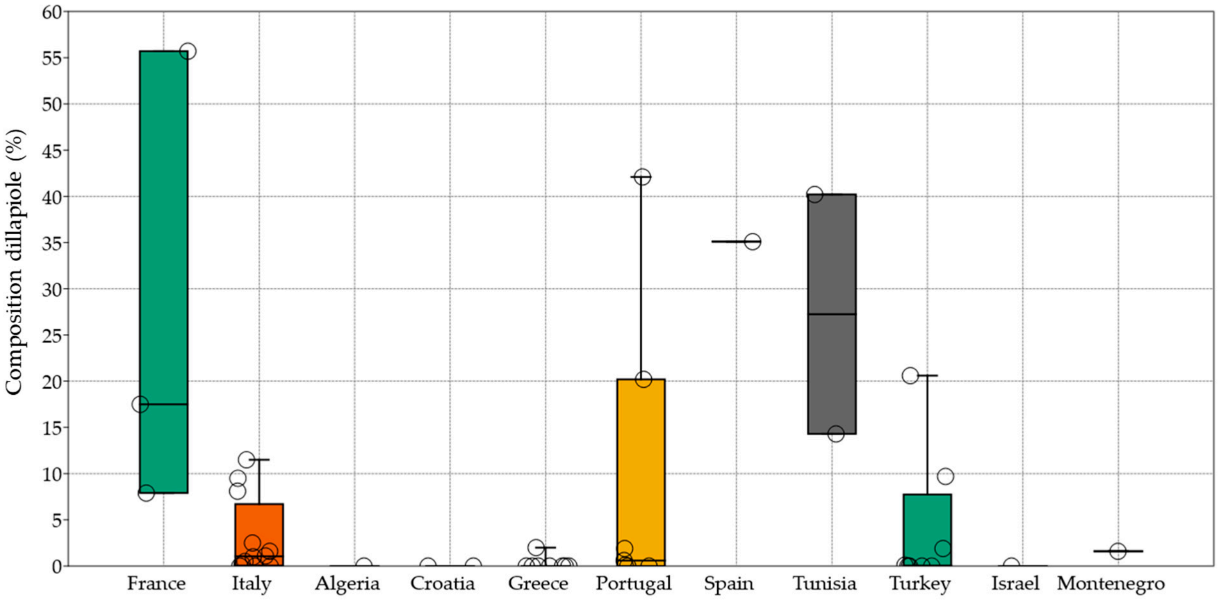

Figure 4. Box and jitter plot showing the percentage composition of dillapiole found in *C. maritimum* aerial parts samples from different countries. The 25–75 percentiles are drawn using a box; the minimum and maximum are shown at the end of the thin lines (whiskers); and the median is marked as a horizontal line in the box plot.

The box and jitter plot (Figure 4) shows a complete geographical distribution of this molecule, also considering the different number of samples whose composition has been characterized so far in the literature.

Similarly, the matrix plot (Figure 5) was used to observe, through the chromatic variation (from red to yellow), the maximum percentage compositions of dillapiole between samples from different countries.

The matrix plot (Figure 5) shows that France has the highest percentage of dillapiole in *C. maritimum* essential oil (55.7%), followed by Portugal (42.1%), Tunisia (40.2%), and Spain (35.1%). Plants from the other countries studied have significantly lower or no dillapiole levels. Looking at the distribution of dillapiole in different countries, a particular geographical trend can be observed. In fact, essential oils from species collected in countries further east in Europe have low or no dillapiole levels overall, in contrast to those from Western Europe—countries with high levels. Samples S43, S44, S45, and S46, the subject of this article, show values consistent with this geographical trend, with dillapiole percentages ranging from 0 to 1.6%. However, more data are needed to confirm this trend.

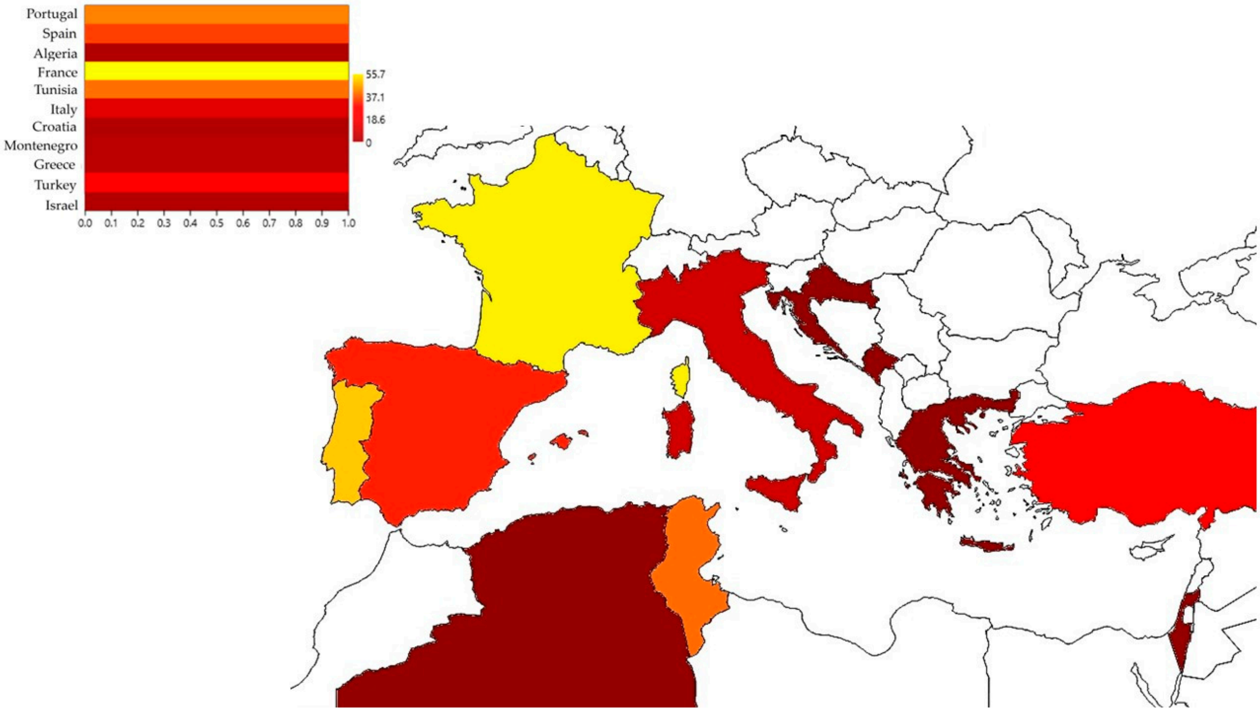

Figure 5. Matrix plot of dillapiole percentage of *C. maritimum* essential oils from different countries.

4. Conclusions

In the present work, the essential oil chemical composition of four accessions of *Crithmum maritimum*, not previously studied, was investigated. The volatile profile of all samples was characterized by a large amount of monoterpene hydrocarbons. The edible use of *C. maritimum* suggests the need to expand knowledge on the composition of this plant, to highlight the presence of compounds that are harmful to humans. In this study, the presence of dillapiole, a toxic compound, was assessed in plants harvested from different locations in Europe and the Middle East. It is advisable to ingest sea fennel that contains minimum dillapiole values. PCA analyses based on the different chemical classes and other statistical analyses are useful tools for a comprehensive investigation, also leading to an understanding of the diversification of sea fennel from different countries. These results are particularly important if we also want to envision the development of new systems for the enhancement of wild species transferred to a sustainable food system, as they allow for the strengthening of resilience pathways developed along the lines of the agroecology principles aimed at reducing waste and preserving environmental resources.

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