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Quality Variation of the Moldovan *Origanum vulgare* L. ssp. *vulgare* L. and *Origanum vulgare* L. ssp. *hirtum* (Link) Ietsw. Varieties in Drought Conditions

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Citation: Gonceariuc, M.; Muntean, M.V.; Butnaraş, V.; Duda, M.M.; Benea, A.; Jelezneac, T.; Vornicu, Z.; Cotelea, L.; Botnarenco, P. Quality Variation of the Moldovan *Origanum vulgare* L. ssp. *vulgare* L. and *Origanum vulgare* L. ssp. *hirtum* (Link) Ietsw. Varieties in Drought Conditions. *Agriculture* **2021**, *11*, 1211. https://doi.org/10.3390/agriculture11121211

Academic Editor: Ilaria Marotti

Received: 1 November 2021
Accepted: 22 November 2021
Published: 1 December 2021

Abstract: In this paper, we have comparatively analyzed two subspecies of *Origanum vulgare* (*O. vulgare* ssp. *hirtum* and *O. vulgare* ssp. *vulgare*) in climatic conditions with higher than usual temperatures from the Institute of Genetics, Physiology and Plant Protection Chisinau, Rep. Moldova. The aim of the research was to study the producing capacity, content, composition of essential oil, as well as the polyphenols content in *O. vulgare* varieties, their drought testing and the selection of promising cultivars. Seven *O. vulgare* ssp. *vulgare* (Ov) varieties and fourteen *O. vulgare* ssp. *hirtum* (Oh) varieties were used as the biological material. The essential oil (EO) separated by hydrodistillation was analyzed by GC-MS techniques. The polyphenolic content was assessed using spectrophotometric techniques. The presence of a direct correlation between the content, production of essential oil and content of polyphenols has been highlighted. Thus, the varieties of *O. vulgare* ssp. *hirtum* demonstrate a higher content of essential oil than the varieties of *O. vulgare* ssp. *vulgare*, while the polyphenol content, on the other hand, is higher for the varieties of *O. vulgare* ssp. *vulgare* than for those of *O. vulgare* ssp. *hirtum*. This research has resulted in the selection of two varieties, i.e., "Savoare" of *O. vulgare* ssp. *hirtum* and "Panacea" of *O. vulgare* ssp. *vulgare*.

Keywords: *Origanum vulgare* ssp. *vulgare*; *Origanum vulgare* ssp. *hirtum*; variety; essential oil; polyphenols; productivity

1. Introduction

The Lamiaceae family, especially the species of the genera *Mentha*, *Thymus* L., *Origanum* L. and *Salvia* L., are important due to their antibacterial, antifungal, antioxidant, antiviral and flavoring properties supported by their complex and diversified chemical composition, which can change in relation to the ecological and environmental conditions, even within the same species [1–5].

Origanum vulgare L. (oregano) is an important species due to its antibacterial, antifungal, antioxidant, antiviral and flavoring properties supported by its complex chemical composition. Oregano is a perennial herbaceous species of Mediterranean origin from the *Lamiaceae* family known and used since antiquity [6–11]. Oregano can be used in the development of a sustainable agroecosystem and for the reconstruction of over-cropped land [12]. Due to its biological specificity and significant economic importance, *Oregano* has been included in the List of Priority Species in Europe [12].

The relevance of the study of oregano is also determined by the importance of the species as a medicinal, aromatic and spicy plant, supported by the chemical composition, which includes polyphenols, essential oil, vitamins, bitter substances, etc. [9,10,13–15], synthesized and accumulated in the aerial part of the plant [9,14]. Oregano essential oil is recognized for its bactericidal inhibitor, antimicrobial [16], antiviral and antifungal activity [17,18], as well as antioxidant and anti-lipase actions [16,19–21]. Recent investigations have confirmed that *O. vulgare* compounds synthesized by the species have strong antioxidant [13,15,19,21–23], anti-inflammatory, antidiabetic [24], hepatoprotective [22] and anti-cancer [17] actions. The properties of oregano essential oil are of potential interest to the food industry [12,15] as an antimicrobial, antioxidant additive [21,25–27], preservative in food products [12,16,21,26–29], cosmetics and pharmaceuticals [17].

In conditions of drought and high temperatures, the metabolism of plants can change, and the chemical composition can be influenced, including the content of volatile oils [30,31].

The *O. vulgare* species comprises a lot of biotypes and forms with a strictly local distribution, which are distinguished by their biomorphological and biochemical diversity accentuated in the particular area of prevalence [11,12,21,23,32–38]. Six *O. vulgare* subspecies are described and identified: *O. v.* ssp. *vulgare* L.; *O. v.* ssp. *glandulosum* (Desf.) Ietsw.; *O. v.* ssp. *virens* (Hoffmanns and Link) Ietsw.; *O. v.* ssp. *hirtum* (Link) Ietsw; *O. v.* ssp. *gracile* (K. Koch) Ietsw. (=*O. tyttanthum*); *O. v.* ssp. *viridulum* (Martrin-Donos) Nyman [14,39] with different geographical distributions.

O. vulgare ssp. *vulgare* and *O. vulgare* spp. *hirtum* (Link) Ietsw. are cultivated in our country.

The aim of our study was to comparatively analyze the differences between two subspecies of *Origanum vulgare*: *O. vulgare* ssp. *hirtum* (Greek oregano) and *Origanum vulgare* ssp. *vulgare* (common oregano). The varieties of both subspecies have high producing capacity and quality, and are adapted to the cultivations in Moldova, established in accordance with the provisions of the project of the State Program 20.80009.5107.07 "Reducing the consequences of climate change by creating, implementing varieties of medicinal and aromatic plants drought, frost, winter, disease resistant, which ensures sustainable development of agriculture and guarantees high quality raw material predestined to the perfumery, cosmetics, pharmaceuticals and food industry".

2. Materials and Methods

The complete experiments were conducted on the experimental field of the Institute of Genetics, Physiology and Plant Protection Chișinău on a leached chernozem with a humus content of 2.2%. At the beginning of March, the experiments were fertilized with NPK, 25 g/m^2 (250 kg/ha). The maintenance of the experiments was done manually (manual plows). Harvesting was done manually, with a sickle, by cutting the aerial part of the plants.

According to the data of the Chisinau Meteorological Station (with observations of 126 years), the average annual air temperature in 2020 was +12.7 °C (3.2 °C higher than the multiannual average)—the largest deviation in the history of weather observations in Chisinau (Table 1). In the Republic of Moldova, the year 2020 can be characterized by a high thermal regime. The high thermal regime and the significant deficit of precipitation in the Chisinau area between January and August 2020 determined the appearance of atmospheric and pedological drought.

The biological material involved fourteen *O. vulgare* ssp. *hirtum* (Greek oregano) genotype clones and seven *O. vulgare* ssp. *vulgare* (common oregano) genotype clones vegetatively multiplied and selected according to the quantitative characteristics, including essential oil content and, thus, becoming clone varieties. They are termed 'varieties' in the text. In the year 2020, the varieties were tested in competitive variety testing in 4 replicates in the third year of vegetation according to the regulations of the Ministry of Agriculture and Food Industry of the Republic of Moldova [40].

Table 1. Monthly and annual temperature (° C) and precipitation (mm), year 2020, Chișinău. Meteorological Station Rep. of Moldova.

Months												
I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**Anuală**
0.6	4.4	7.8	11.3	14.4	21.5	22.8	23.5	20.2	14.2	4.6	2.9	12.7 °C
9	23	19	4	69	86	85	5	75	81	32	74	562 mm

The productivity of the clone varieties was assessed by determining the production of raw matter and standard humidity 60% (t/ha) and calculating the production of essential oil (kg/ha) and efficiency (kg/t, kg of essential oil isolated from a ton of fresh raw material). To determine the essential oil content, the samples (the aerial part of the plant) were collected in the morning twice per season during the full flowering phase. The essential oil was isolated from 100 g samples in two repetitions by hydrodistillation for 60 min. The oil content was recalculated to standard humidity (60%) and dry matter. Following distillation, the essential oil was dried over anhydrous sodium sulfate and stored at 4–6 °C. Qualitative and quantitative analysis of the essential oil was conducted using GC coupled with mass spectrometry (GC-MS): gas chromatograph—Agilent Technologies 7890; mass selective detector 5975C Agilent Technologies with a quadruple, capillary column (30 m/0.25 mm/0.25 µm) with HP-5 ms non-polar stationary phase. The injector and detector temperatures were 250 °C and 280 °C, respectively, using a temperature gradient from T1 = 70 °C (2 min), T2 = 200 °C (5 °C/min) to T3 = 300 °C (20 °C/min, 5 min). Mobile phase: helium 1.1 mL/min, injected volume of essential oil—0.03 µL, split rate—1:50. MSD scanning: 30–300 am, 15 min; 30–450 pm, 23 min. The identification of chromatographic peaks was performed using the software package AMDIS™, coupled with the NIST database.

The total phenolic content (TPC) was estimated for varieties with high essential oil content by spectrophotometric techniques by the Folin-Ciocalteu (F-C) method with some modifications [41]. The samples were prepared by liquefying 10 mg of the extract in 10 mL of ethanol 70% to yield a concentration of 1 mg/mL. Each sample (1 mL) was mixed with 5 mL of the F–C reagent (1:10, *v*/*v*) in a 25 mL volumetric flask. After 5 min, 4 mL of the sodium carbonate 7.5% (*m*/*v*) solution and 20 mL water were added, and the mixture was agitated. After 60 min, the absorbance of the mixture was measured using a UV-VIS spectrophotometer (Metertech SP8001) at 765 nm against the blank. A calibration curve was prepared using gallic acid (GA) as the standard with concentrations of 0.02–0.12 mg/mL. TPC was revealed as gallic acid equivalents in milligrams per g of the extract. All samples were measured in triplicate.

Data analysis was carried out using RStudio software [42], version 1.4.1106, and associated packages. The basic statistics of the results were analyzed individually based on the separation imposed by the two subspecies studied and the varieties of each, the formulas used being from the "psych" package [43]. The variance analysis and the exploration of differences between the oregano varieties under study were based on the "agricolae" package [44]. The least significant differences (LSD) test in this package allows the assessment of significant differences due to the species and varieties, with multiple comparisons indicating the level of maintenance of these differences at a level of $p < 0.05$ [45,46]. The analysis of the total similarity of the varieties was performed by cluster analysis, the "ape" package allowing the evaluation of the complete link-age [47,48] and highlighting the genotype belonging to a species. The final exploration of the results was performed based on a principal component analysis (PCA) with the "vegan" package [49], allowing a spatial projection of varieties in the form of a point containing the entire available dataset and association with the vectors represented by the parameters studied [50].

3. Results

The research was carried out in drought and hot weather conditions in 2020, which is considered by the State Hydro Meteorological Service of the Republic of Moldova to be the year with the highest average annual temperatures in the last 126 years. The deficit of atmospheric deposits in 2020 was more drastic in January, April, June and August, constituting 12–40% of the multiannual average in these months (Table 1).

Previous evaluations have confirmed a phenotypic diversity and pronounced variability of quantitative traits in *O. vulgare* ssp. *hirtum* and *O. vulgare* ssp. *vulgare* both in the genotypes of each subspecies and in the significant differences between the subspecies regarding content and qualitative and quantitative composition of essential oil [10,14].

O. vulgare ssp. *hirtum* formed plants with a height of 55.8–63.1 cm depending on the variety, while the plants of *O. vulgare* ssp. *vulgare* had an average height of 59.4–66.1 cm. The length of the inflorescences, as in previous years, was longer in *O. vulgare* ssp. *vulgare*, and the inflorescences in *O. vulgare* ssp. *hirtum* were shorter and more compact with more branches. Pronounced variability was also found in the number of floral stems per plant, this index being from 49.5 to 121.9 for *O. vulgare* ssp. *hirtum* varieties and 67.8–107.2 for *O. vulgare* ssp. *vulgare*. Quantitative characters influenced the yields of raw matter (the aerial part of the plant), calculated to the standard humidity (60%), as well as the yields of dry pharmaceutical and spicy *herba* (humidity 13%).

3.1. Productivity of O. vulgare ssp. hirtum and O. vulgare ssp. vulgare Varieties

In conditions of heat and acute drought, the varieties of *O. vulgare* ssp. *hirtum* formed a fresh raw matter production of 3.3–9.3 t/ha with an essential oil content ranging from 1.209 to 1.641% at 60% humidity, or 3.023–4.102% calculated for dry matter (Table 1, Figure 1).

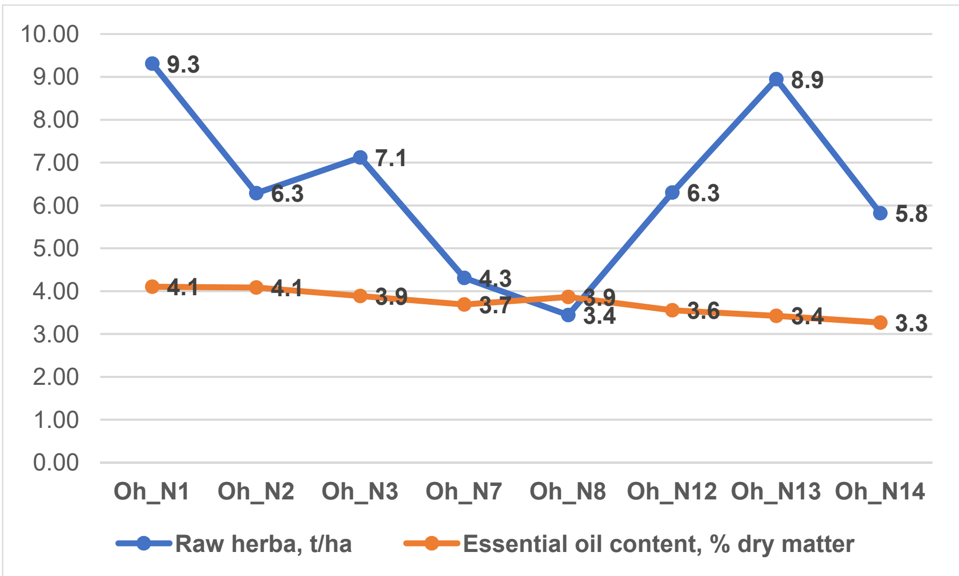


Figure 1. Variability of raw matter and essential oil content in *O. vulgare* ssp. *vulgare* varieties

The production of fresh matter in *O. vulgare* ssp. *vulgare* varied between 2.5 and 9.7 t/ha depending on the variety (Figure 2). The production of dry spicy and pharmaceutical *herba* ranged from 0.68 to 1.51 t/ha depending on the variety. The content of essential oil was highly variable and quite low, with 0.028–0.107% in fresh raw (60% humidity) matter or 0.078–0.267% (dry matter).

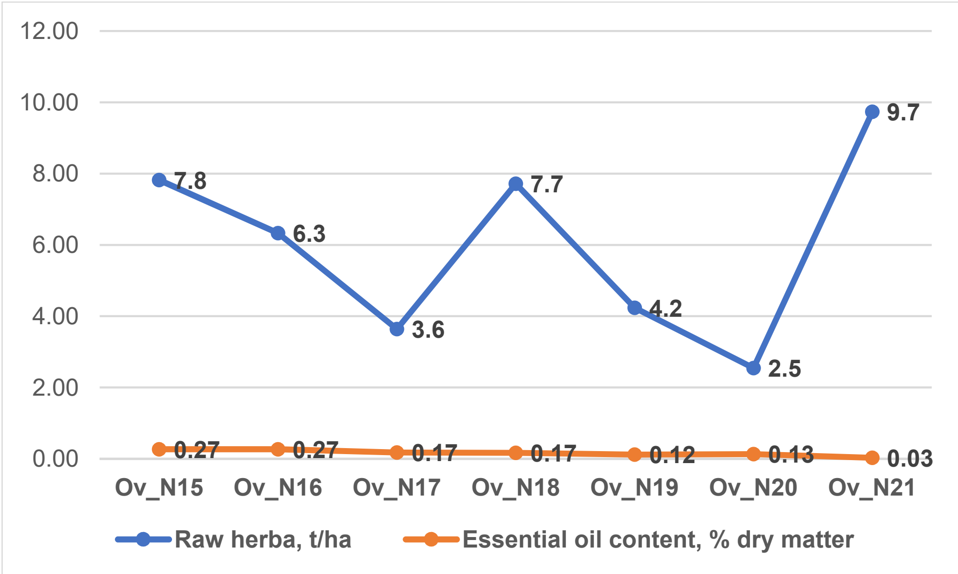


Figure 2. Variability yields of raw matter and essential oil content in *O. vulgare* ssp. *vulgare* varieties.

The varieties of *O. vulgare* ssp. *hirtum* are distinguished by high yields of essential oil and sustained by the high content of essential oil. This quality index of the varieties for this subspecies varied from 45.9 kg/ha in variety no. 9 to 152.6 kg/ha for variety number 1 (Figure 3). The yield of varieties of *O. vulgare* ssp. *hirtum* was high and varied from 12.1 to 16.4 kg of essential oil per ton of raw material in different varieties.

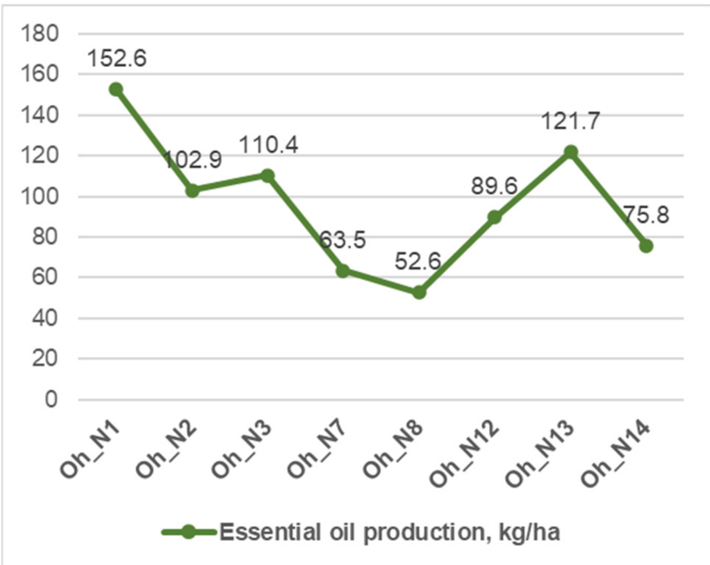


Figure 3. Variability of essential oil production in *O. vulgare* ssp. *hirtum* varieties (kg/ha).

The production of essential oil varied from 1.35 to 8.35 kg/ha (Figure 4), the yield being 0.28–1.07 kg per ton of raw material in the varieties of *O. vulgare* ssp. *vulgare*.

This research resulted in two varieties selected. Variety number 1 of *O. vulgare* ssp. *hirtum* turned into the variety *Savoare* and variety number 15 of *O. vulgare* ssp. *vulgare* turned into the *Panacea* variety.

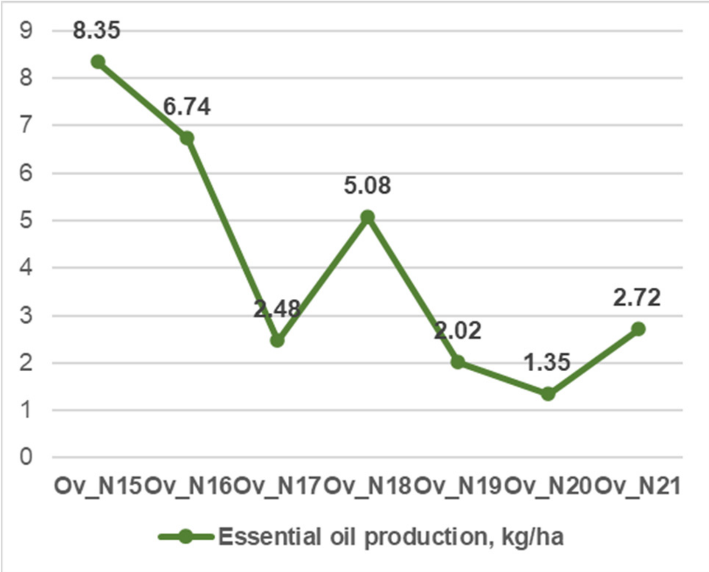


Figure 4. Variability of essential oil production in *O. vulgare* ssp. *vulgare* varieties (kg/ha).

Savoare, patent application v2020 0021, a *O. vulgare* ssp. *hirtum* variety (Figures 1 and 3, no. 1) is distinctive due to the high yields of fresh raw matter of 9.3 t/ha and dry (humidity 13 %) pharmaceutical *herba* production of 1.85 t/ha. The essential oil content is 1.641% at standard humidity and 4.102% for dry matter. The variety's efficiency yield was 16.4 kg/t.

Panacea, patent application v2020 0020, an *O. vulgare* ssp. *vulgare* variety (Figures 2 and 4, no. 15) is distinguished by the production of fresh raw matter of 7.8 t/ha with an essential oil content of 0.107% at standard humidity of 60%, and 0.267% recalculated to dry matter, the efficiency being 1.07 kg of essential oil per ton of raw material. The variety ensures the production of pharmaceutical *herba* (humidity, 13%) of 1.51 t/ha.

3.2. Qualitative and Quantitative Composition of Essential Oil

Qualitative and quantitative analyses of essential oil (GC-SM) isolated from *O. vulgare* ssp. *hirtum* varieties have revealed a different number of components ranging from 15 to 28, these constituting 98.39–100.0% of the essential oil (Table 2).

The major component in the essential oil for all the *O. vulgare* ssp. *hirtum* varieties evaluated is phenolic monoterpenoid, carvacrol at concentrations of 74.84% (no. 5)–86.26% (no. 14), followed by γ-terpinene monoterpenes (3.20–8.76%) and p-Cymene (2.66–4.48%). The third component regarding the concentration in the essential oil of most varieties is bicyclic sesquiterpene β-Caryophyllene (1.32–3.08%), except for variety number 9 in which γ-terpinene is followed by another sesquiterpene, i.e., thymol (3.72%), a carvacrol isomer, followed by p-Cymene (2.82%) and β-Caryophyllene (2.29%).

Table 2. Variation of the qualitative and quantitative essential oil composition of the *Origanum vulgare* ssp. *hirtum* Moldovan varieties.

Components	Rt, min	Sample Area %							
		1 *	**2**	**3**	**7**	**8**	**12**	**13**	**14**
α-Thuene	4.27	0.93	1.08	1.01	1.02	0.65	0.84	0.57	0.49
α-Pinene	4.41	0.34	-	0.37	0.38	0.24	0.32	0.21	0.19
Camphene	4.70	0.08	0.39	0.08	-	-	-	-	-
β-Phellandrene	5.17	0.28	-	0.31	0.36	0.26	0.38	0.23	0.11
β-Pinene	5.25	0.35	0.31	0.37	0.37	0.37	0.44	0.32	0.22
β-Mircene	5.49	1.28	0.31	1.17	1.28	0.98	1.26	0.96	0.87
α-Phellandrene	5.81	0.17	1.31	0.16	0.18	0.14	0.17	0.13	0.13
β-Terpinene	6.09	0.77	0.18	0.7	0.82	0.67	1.28	1.02	0.62
p-Cymene	6.28	2.88	0.86	2.99	3.03	2.67	4.48	3.12	2.78
Sylvestrene	6.38	0.22	2.66	0.22	0.27	0.18	0.25	0.20	0.19
trans-Ocimene	6.53	-	0.32	-	-	-	-	-	-
cis-Ocimene	6.79	0.06	-	0.08	-	0.07	0.09	0.07	0.06
γ-terpinene	7.09	3.54	-	3.20	3.95	3.41	8.76	7.49	3.64
4-Thujanol	7.29	0.75	3.99	0.81	0.78	0.98	0.83	0.75	0.63
Linalool	8.08	0.35	0.58	0.58	0.38	0.76	0.51	0.43	0.44
Borneol	9.82	0.26	-	0.21	-	0.27	0.36	0.40	0.29
4-Terpineol	10.13	0.40	-	0.51	0.52	0.50	0.40	0.40	0.34
Thymol methyl ester	11.86	0.14	-	0.13	-	0.12	0.05	0.06	0.10
Thymol	13.14	0.33	-	0.34	-	0.37	0.39	0.44	0.35
Carvacrol	13.78	83.91	-	82.87	83.12	83.82	76.46	80.62	86.26
Thymol acetate	15.23	-	84.27	0.08	-	0.09	0.05	0.08	0.10
β-Caryophyllene	16.46	2.10	-	2.34	2.49	1.95	1.75	1.48	1.66
α-Caryophyllene	17.27	0.11	2.59	0.12	-	0.12	0.15	0.14	0.17
Germacrene D	17.95	0.35	-	0.80	0.65	0.63	0.17	0.11	0.08
β-Bisabolene	18.56	0.11	0.78	0.22	0.39	0.30	0.22	-	0.21
δ-Cadinene	18.92	0.07	0.37	0.12	-	0.09	-	-	-
Spathulenol	20.18	-	-	0.11	-	0.16	-	-	-
Caryophyllene oxide	20.41	0.10	-	0.07	-	0.09	0.07	0.05	0.10
Components number		25	15	28	17	26	24	23	24
Identification, %		99.88	100	99.97	99.99	99.89	99.68	99.28	99.99

* variety Savoare.

The *O. vulgare* ssp. *vulgare* varieties evaluated are also distinguished by a different number of components identified in the essential oil, ranging from 12 (no. 18) to 33 (no. 19); the identification rate was 95.48–100.0% (Table 3). The major components in the *O. vulgare* ssp. *vulgare* essential oil were sesquiterpenes. The highest concentration was that of Germacren D (21.63–33.02%) followed by β-Caryophyllene (12.85–15.79%) or β-Farnesene (8.57–16.60%), bicyclogermacren (2.75–10.0%). Another sesquiterpene-Spatulenol (2.33–6.64%) was present only in the essential oil of four of the varieties evaluated. All the samples of essential oil have been shown to contain terpenic alcohol linalool (2.47–9.28%), as well as its ester, linalyl acetate (2.36–4.91%), which is present in the essential oil of six varieties. The presence of Cadinol and α-Cadinol was attested, their total making 5.56–9.05%. We could ascertain that germacren D/β-caryophyllene/β-farnesene are the major chemotype in the *O. vulgare* ssp. *vulgare* varieties evaluated. It is worth mentioning that carvacrol was contained only in the essential oil of five varieties of *O. vulgare* ssp. *vulgare*, the highest concentration being 11.43% (Table 3, no. 20). In the essential oil of four varieties, carvacrol was a minor component (2.10–3.64%), and in two varieties, it was completely missing.

Table 3. The qualitative and quantitative essential oil composition of the *Origanum vulgare* ssp. *vulgare* Moldovan varieties.

Components	Rt., min	Sample, Area%						
		15 *	**16**	**17**	**18**	**19**	**20**	**21**
Sabinene	5.17	2.69	1.37	0.72	-	1.84	1.44	1.05
1-Octen-3-ol	5.22	-	0.49	0.27	-	0.28	-	0.41
β-Mircene	5.48	-	0.15	-	-	0.34	-	-
α-Phellandrene	6.10	-	-	-	-	0.21	-	-
p-Cymene	6.27	0.33	0.46	0.23	-	0.77	-	0.32
trans-Ocimene	6.54	1.97	1.78	0.96	-	2.14	1.43	1.21
cis-Ocimene	6.79	2.64	1.86	0.72	-	0.87	-	1.33
γ-terpinene	7.07	0.67	0.51	-	-	0.62	-	0.35
4-Thujanol	7.29	0.90	0.64	0.22	-	0.18	-	0.69
Linalool	8.08	2.47	6.31	7.06	4.91	4.50	9.28	5.74
Borneol	9.82	-	0.21	-	-	-	-	-
4-Terpineol	10.13	1.63	2.21	1.52	-	3.34	3.33	2.13
α-Terpineol	10.47	0.34	0.67	0.66	-	0.68	1.44	0.59
Linalylacetate	12.15	-	2.96	3.03	4.28	2.78	4.91	2.36
Lavandulyl acetate	13.05	-	0.56	0.54	-	0.45	1.12	0.54
Carvacrol	13.39	-	3.02	3.64	-	2.10	11.43	2.35
β -Bourbonene	15.55	1.21	1.22	0.95	1.98	1.03	1.50	1.37
Elemene	15.71	0.69	-	0.50	-	0.55	-	0.59
β-Caryophyllene	16.50	15.78	12.85	15.30	6.61	8.77	8.89	14.38
β-Cubebene	17.08	0.52	-	0.26	-	0.27	-	-
α-Caryophyllene	17.28	1.76	1.42	2.24	-	1.64	1.69	1.55
(+)Aromadendrene	17.50	1.46	1.27	0.34	-	0.58	-	0.82
Germacrene D	18.08	31.13	28.82	24.69	33.02	25.97	21.63	32.04
β -Bergamotene	18.20	0.54	-	0.39	-	0.55	-	-
Bicyclogermacrene	18.36	2.96	2.82	3.01	10.00	6.39	5.81	2.75
β -Farnesene	18.60	11.41	8.57	12.21	16.60	15.56	9.83	9.42
Cadinene	18.74	0.62	-	0.74	-	0.38	-	0.33
Cadinene	18.92	2.01	2.27	2.38	2.71	1.79	2.02	2.41
cis Bisabolene	19.36	0.84	0.31	0.54	-	-	-	-
GermacreneD-4ol	20.18	-	-	4.88	4.03	3.47	3.45	6.28
(-)Spatulenol	20.24	6.55	5.54	-	6.64	2.04	2.33	-
Caryophyllene oxide	20.41	1.35	1.36	1.81	-	1.26	2.15	1.48
Cubenol	21.09	0.73	-	0.35	-	-	-	0.73
Cadinol	21.62	2.57	2.46	3.02	4.03	3.16	2.79	2.33
α -Cadinol	22.00	2.99	3.19	3.60	5.02	2.75	3.53	3.56
Trans-Phytol	30.07	0.21	0.18	0.28	-	0.30	-	-
Number identified components		28	29	31	12	33	20	28
Total, identified components, %		98.97	95.48	97.06	99.83	97.56	100	99.11

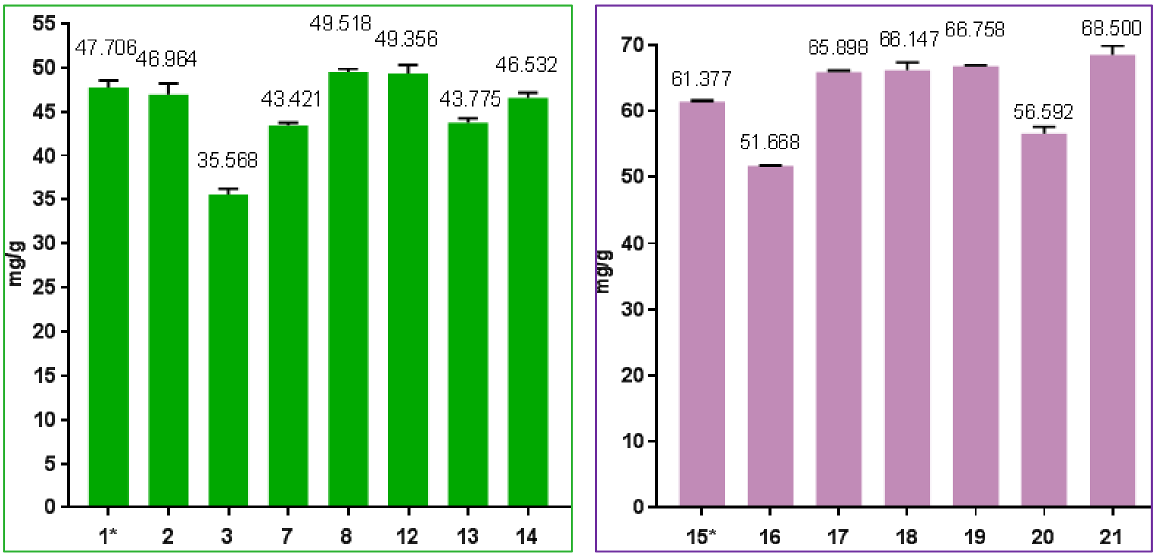
* variety Panacea.

3.3. Polyphenols Content

The phenotypic and biomorphological distinctness, especially the diversity according to content and the qualitative and quantitative composition of the essential oil of the *O. vulgare* ssp. *hirtum* and *O. vulgare* ssp. *vulgare* genotypes [10,14] and varieties aroused our interest to study the polyphenols—their content in the genetic material of both subspecies.

The findings demonstrated the presence of a correlation between the essential oil and polyphenols contents. Thus, all *O. vulgare* ssp. *hirtum* varieties synthesize and accumulate a much higher content (3.023–4.102%, dry matter) of essential oil than *O. vulgare* ssp. *hirtum* varieties (0.078–0.267%, dry matter).

The content of polyphenols, on the other hand, was much lower in *O. vulgare* ssp. *hirtum* (Figure 5a), the varieties accumulated from 35.506 mg/g (no. 3) to 49.518 mg/g (no. 8), than the varieties of *O. vulgare* ssp. *vulgare*, in which polyphenols accumulated in much larger quantities ranging from 51.668 mg/g (no. 16) to 68.500 mg/g (no. 21) (Figure 5b).



(**a**) (**b**)

Figure 5. The polyphenols content (mg/g) in varieties of *Origanum vulgare* ssp. *hirtum* Ietsw. (**a**), and *Origanum vulgare* ssp. *vulgare* L. (**b**). (**a**) 1*—variety Savoare; (**b**) 15*—variety Panacea.

As for the varieties selected, we observed that "Savoare" of *O. vulgare* ssp. *hirtum*, in which the essential oil content was 1.641% at standard humidity and 4.102% at dry matter, accumulated 47.706 mg/g of polyphenols (Figure 5a, nr 1*). "Panacea" of *O. vulgare* ssp. *vulgare* with an essential oil content of only 0.107% at standard humidity and 0.267% recalculated to dry matter synthesized and accumulated 61.377 mg/g of polyphenols (Figure 5b, no. 15).

The data analysis confirms the presence of significant differences, both at the general level of the two species studied and at the variety level of each species (Tables 4 and 5).

In the case of *Origanum vulgare* ssp. *hirtum* (Table 4), the recorded value of polyphenols was within the range of 35.51–49.52 mg/g, with the minimum and maximum associated with the varieties Oh_N3 and Oh_N13. Significant differences were recorded for this parameter at four levels, between the value of 49 and the values of 46–47, 43 and 35 mg/g, respectively.

Table 4. Content of essential oil and polyphenols in raw material of the *Origanum vulgare* ssp. *hirtum* (Oh) varieties.

Oh Varieties	Essential Oil Content % in:		Polyphenols Content, mg/g	Raw Materials Production, kg/ha
	Fresh Raw Matter	Standard Humidity		
Oh_N1 *	1.43 ± 0 a	1.64 ± 0 a	47.7 ± 0.82 ab	9308.67 ± 11.02 a
Oh _N2	1.37 ± 0.01 b	1.63 ± 0.02 a	46.97 ± 1.25 b	6287 ± 54.06 d
Oh _N3	1.27 ± 0 d	1.56 ± 0 b	35.51 ± 0.65 d	7119.67 ± 21.96 c
Oh _N7	1.23 ± 0 f	1.48 ± 0 c	43.42 ± 0.33 c	4309.33 ± 12.06 f
Oh _N8	1.29 ± 0 c	1.55 ± 0 b	43.78 ± 0.46 c	3442.67 ± 37 g
Oh_N12	1.29 ± 0 c	1.42 ± 0 d	49.36 ± 0.96 a	6300.67 ± 29.14 d
Oh_N13	1.25 ± 0.01 e	1.37 ± 0 e	49.52 ± 0.35 a	8948 ± 80.07 b
Oh_N14	1.11 ± 0 g	1.31 ± 0 f	46.53 ± 0.63 b	5820.67 ± 19.73 e
F test	1056.66	873.83	114.07	7815.42
p.val	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

* Variety Savoare, by different letters present significant differences according to LSD test ($p < 0.05$).

Table 5. Content of essential oil and polyphenols in raw material of the *Origanum vulgare* ssp. *vulgare* (Ov) varieties.

Ov Varieties	Essential Oil Content, % in:		Polyphenols Content, mg/gg	Raw Materials Production, kg/ha
	Fresh Raw Matter	Standard Humidity		
Ov_N15 *	0.09 ± 0.01 a	0.11 ± 0 a	61.38 ± 0.19 b	7819.33 ± 21.01 b
Ov_N16	0.09 ± 0 a	0.11 ± 0 a	51.67 ± 0.08 d	6329.33 ± 35.85 d
Ov_N17	0.06 ± 0 b	0.07 ± 0 b	65.9 ± 0.22 f	3638.33 ± 29.26 f
Ov_N18	0.06 ± 0 b	0.07 ± 0 bc	66.14 ± 1.19 c	7715.33 ± 16.17 c
Ov_N19	0.04 ± 0 bc	0.05 ± 0 cd	66.76 ± 0.13 e	4235 ± 11.79 e
Ov_N20	0.04 ± 0.02 bc	0.05 ± 0.02 bc	56.59 ± 1.03 g	2544 ± 6 g
Ov_N21	0.02 ± 0 c	0.03 ± 0 d	68.5 ± 1.36 a	9732.67 ± 12.22 a
F test	183.25	55.93	183.25	45,210.09
p.val	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

* Variety Panacea, by different letters present significant differences according to LSD test ($p < 0.05$).

The influence of variety in determining this parameter was determined to be very significant. Essential oils have much clearer differences than polyphenols extracted from raw matter, with a maximum (1.43) in Oh_N1 and a minimum of 1.11 in Oh_N14. In this case, the influence of variety was about nine times higher than in the case of polyphenols (F = 1056.66).

The standardization of the essential oil value normalized the data, maintaining approximately the same trend as in the case of values extracted from raw matter. There was a reduction in significant differences between genotypes, especially in the case of Oh_N1 and Oh_N2, where the significance disappeared.

In contrast, the differences observed in the *raw material* production indicate the variation of this parameter (F = 7815.42). The differences recorded are very significant between varieties, showing a clear hierarchy. The *raw materials* maximum was 9308.67 kg, compared to the minimum of only 3442.67 kg.

For *O. vulgare* ssp. *vulgare* (Table 5), multiple comparisons indicate a strong hierarchy of parameters depending on the variety, the ANOVA test having the maximum value in the case of raw materials production (9732.67, $p < 0.001$). Essential oils from raw matter were present in small quantities, and significant differences were recorded only when comparing the Ov_N15 and Ov_N16 varieties with the other ones. The same trend was maintained in the case of standardization of essential oils, with a slight amplification of the differences maintaining the maximum of the two genotypes. Raw material production indicates a very large variation between varieties, the maximum being 9732.7 kg (Ov_N21), by 2000 kg higher compared to the two varieties (Ovv_N15 and Ov_N18). The varieties Ov_N17 and Ov_N20, with 3638.33 kg and 2544 kg, respectively, present an interesting case, both being significantly lower compared to the level of 4000 kg/ha. Polyphenols vary significantly in the range of 51.67–68.5 mg/g, the differences being significant between all the varieties analyzed.

The multiple comparisons applied to the two subspecies—*Origanum vulgare* ssp. *hirtum* vs. *O. vulgare* ssp. *vulgare* allow a clear hierarchy of their productive potential (Figure 6) based on the significant differences registered. The polyphenol minimum obtained in the experiment was observed in the Oh_N3 variety, followed by a significantly higher value of two genotypes of the same species (Oh_N7 and Oh_N8). The maximums recorded were for the species *O. vulgare* ssp. *Vulgare*; all the varieties tested for this parameter had inferior results compared to the *O. vulgare* ssp. *hirtum* species. The same trend was observed in the case of essential oils, the minimum being found for the species *O. vulgare* ssp. *vulgare*, and with a clear separation from the *O. vulgare* ssp. *hirtum* species. Only *raw materials* production allows a mixed classification of the productive potential of the two subspecies. The minimum recorded was for the variety Ov_N20, significantly lower than that in Oh_N8 and Ov_N17, respectively. The recorded maxima place the varieties

Ov_N21 and Oh_N1 on the first two positions, with significant differences between the recorded yields.

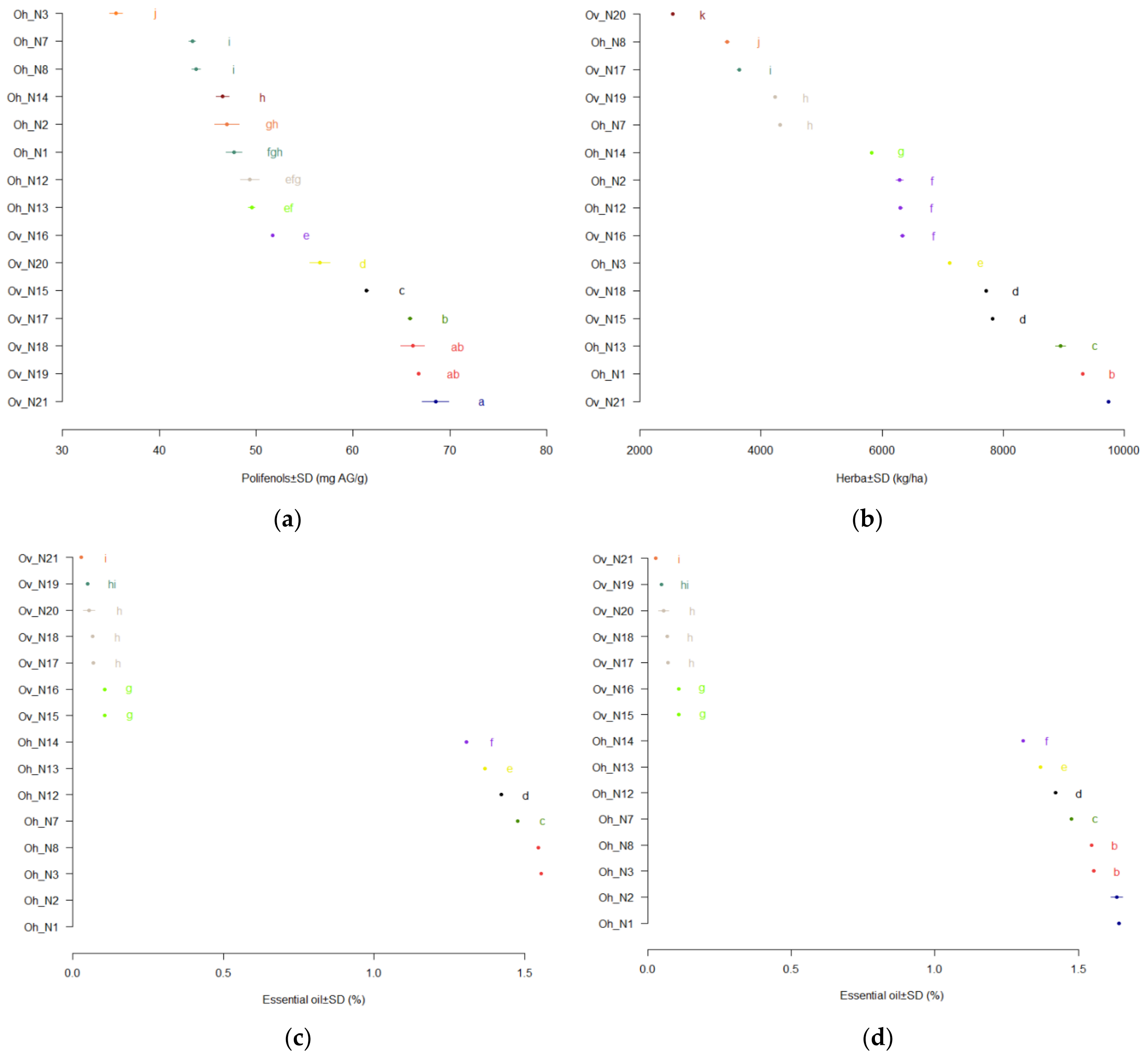


Figure 6. Comparative analysis of the production of essential oil, polyphenols and *raw material* in O. *vulgare* ssp. *hirtum* (Oh) and *O. vulgare* ssp. *vulgare* (Ov) varieties (**a**) F_{spp} = 5544.40, $p < 0.001$; F_{var} = 148.39; $p < 0.001$; (**b**) F_{spp} = 310,518.82, $p < 0.001$; F_{var} = 281.04, $p < 0.001$; (**c**) F_{spp} = 463,640.02, $p < 0.001$; F_{var} = 513.72, $p < 0.001$; (**d**) F_{spp} = 2051.44, $p < 0.001$; F_{var} = 15,247.06, $p < 0.001$, by different letters present significant differences according to LSD test ($p < 0.05$).

Cluster analysis provides an image of the stability of the two subspecies in terms of the total quantum of the parameters analyzed (Figure 7). However, three groups of varieties with similar parameters could be identified at the level of each subspecies.

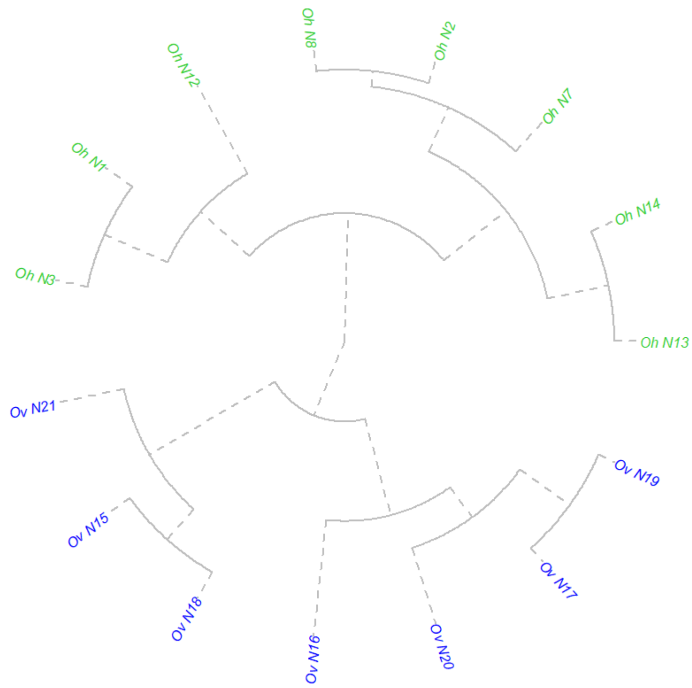


Figure 7. Cluster analysis of the parameters recorded in *O. vulgare* ssp. *hirtum* vs. *O. vulgare* ssp. *vulgare*.

A cluster was formed by the varieties Oh_N13 and Oh_N14, another formed by the varieties Oh_N7, Oh_N2 and Oh_N8, and a combined cluster formed by the varieties Oh_N3 and Oh_N1, to which the easy-to-differentiate variety Oh_N12 was associated, in the case of *O. vulgare* ssp. *hirtum*.

As for *O. vulgare* ssp. *vulgare*, the varieties followed each other hierarchically, the complete linkage indicating a much more visible hierarchy. The varieties Ov_N19 and Ov_N17 formed a stable cluster, to which Ov_N20 and Ov_N16 were gradually attached. The two varieties attached have transient characteristics towards the second cluster formed by Ov_N18 and Ov_N15. In this last cluster, the transition is represented by the Ov_N21 variety.

The principal compound analysis (PCA) provides a spatial image of the two species and the direction of the gradients formed by the parameters studied (Figure 8), with a variance fully explained by Axis 1 (100%). The Ov_N16 variety was closest to the middle of the order, close to Ov_N12, Ov_N14 and Ov_N2, respectively. All these varieties were associated with the gradients of essential oils and had high stability in the analyzed samples.

The *raw material* production had a weak gradient without association, the varieties Ov_N13, Ov_N1 and Ov_N8 being present on the one side. These varieties had a direct correlation with both the *raw material* and the essential oils.

On the other side of the *raw material* gradient were the varieties Ov_N15, Ov_N18 and Ov_N21, all three being correlated with the production of polyphenols. The varieties Ov_N19, Ov_N17 and Ov_N15, poorly correlated with polyphenols, are visible in the last quadrant of the ordering.

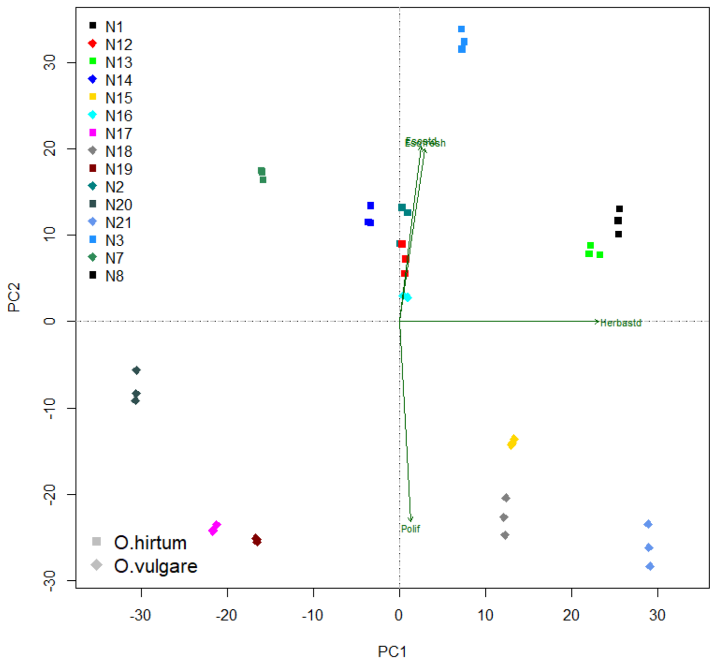


Figure 8. PCA analysis of the parameters recorded in *O. vulgare* ssp. *hirtum* vs. *O. vulgare* ssp. *vulgare*.

4. Discussion

This paper presents the results of a complex study conducted in 2020 in drastic conditions of drought and very high temperatures on a lot of 21 varieties of *Origanum vulgare*, of which 14 are part of *O. vulgare* ssp. *hirtum* and 7 belong to *O. vulgare* ssp. *vulgare*.

Research conducted in the Republic of Moldova has shown phenotypic diversity and a pronounced variability of quantitative traits of *O. vulgare* ssp. *hirtum* and *O. vulgare* ssp. *vulgare* in both the varieties of each subspecies and significant differences between subspecies in terms of the content and the qualitative and quantitative composition of essential oil [10,14] (F = 1056.66). Similar biodiversity has been described by many researchers in other countries on *Origanum vulgare* species and biotype origin [11,12,32–34,36–38,51]. For example, the number of compounds in the essential oil of Moldovan *O. vulgare* ssp. *hirtum* genotypes vary from year to year. In 2014, genotypes were described as having the number of compounds in the essential oil varying between 24 and 31, the identification rate being 98.07–99.90% [14]. In 2015, the number of compounds in essential oil was 18–25, depending on the genotype, this constituting 99.87–100.0% of the total essential oil [10]. In 2020, the varieties of *O. vulgare* ssp. *hirtum* also revealed a different number of compounds ranging from 15 to 28, this constituting 98.39–100.0%.

Research in other geographical areas attested to the presence of a different number of compounds in the essential oil of *O. vulgare* ssp. *hirtum* in wild flora samples. Meanwhile, 19 compounds were found in the essential oils of the materials collected in Greece [52] and Argentina [29]. Materials from Sicily [53] have shown 53 compounds in the essential oil of this subspecies. Some authors [54] identified 64 compounds in the materials growing wild in Southern Italy; others [55] while have found 35 compounds. Samples of essential oil with 28 compounds are also described for this subspecies in Italy [56]. In Montenegro, 30 compounds were attested in the essential oil of *O. vulgare* ssp. *hirtum* [57].

The major compounds in the essential oil separated from the Moldovan *O. vulgare* ssp. *hirtum* varieties in this work, as already mentioned, were phenolic monoterpenoid carvacrol (74.84–86.26%), followed by monoterpenes γ-terpinene (3.20–8.76), p-cymene (2.66–4.48%), thymol (3.72%) and bicyclic sesquiterpene β-caryophyllene (1.32–3.08%). In *O. vulgare* ssp. *hirtum* from the wild flora of Montenegro, the dominant compound was also carvacrol (74.3%), not followed by thymol [57]. The same major compounds were identified in Greek oregano by several authors [11,16,18,34,36,49,53,55,56,58–60].

Several authors mention thymol as a major compound in *O. vulgare* ssp. *hirtum* [13,36,38,53,56,61]. In our studies, thymol was identified as a minor compound at relatively low concentrations (0.33–3.72%), completely missing in the essential oil of two of the varieties. The previous findings [10,14] showed an even lower concentration of thymol (0.17–0.24%). Similar data were obtained by Sarrouet et al. in breeding material originating from Samothraki Island of Greece [60]. The presence of carvacrol and thymol, followed by γ-terpinene and p-cymene, were found in the *O. vulgare* ssp. *hirtum* essential oil by other authors [36,62,63].

The *O. vulgare* ssp. *hirtum* essential oils are known to have pronounced antioxidant [13,57,64,65], protective action [66], as well as antimicrobial, antibacterial, antifungal properties and the ability to inhibit bacterial growth [15,18,54,64,65]. Due to these important characteristics, this essential oil is used as a preservative in food [27,67].

Studies have demonstrated the importance of carvacrol for human health [63] and that carvacrol and thymol are responsible for antioxidants [18,19,29,68] and anti-inflammatory actions [68]. The essential oil rich in carvacrol is very promising and has a high potential in use for animal health and nutrition [63]. Recent studies report a positive effect of *O. vulgare* ssp. *hirtum* essential oil on skin disorders, highlighting the most successful pharmaceutical formula used to treat skin conditions and anti-Alzheimer activities [59].

Our *O. vulgare* ssp. *vulgare* varieties provided variable yields of raw material in drought and heat conditions ranging from 2.5 to 9.7 t/ha. The essential oil content also varied depending on the variety and makes 0.03–0.11% at standard humidity of 60%, or 0.071–0.267%, recalculated for dry matter. Compared to *O. vulgare* ssp. *hirtum, O. vulgare* ssp. *vulgare* varieties accumulate much less essential oil. The multiple comparisons applied to the two subspecies—*O. vulgare* ssp. *hirtum* vs. *O. vulgare* ssp. *vulgare*—allow a clear hierarchy of their productive potential (Figure 6), based on the significant differences recorded.

The qualitative composition of the essential oil of our *O. vulgare* ssp. *vulgare* varieties differed from variety to variety, comprising 12–33 constituents at different concentrations (Table 3). The results published by authors in other countries also vary. In Lithuania, Mockute et al. [58] identified 42 compounds, while Shafiee-Hajiabad et al. [62] recorded 17 compounds. In Montenegro, Stešević [57] reported 40 to 48 compounds in the essential oil of this subspecies.

The findings regarding the quantitative composition of the *O. vulgare* ssp. *vulgare* essential oil published by other authors is different. In the material from the wild flora of Montenegro, the concentration of compounds varied considerably: germacrene D (15.4–27.9%), β-caryophyllene (7.7–14.6%), α-terpineol (4.8–17.8%), linalyl acetate (0.5–9.6%), linalool (3.0–8.8%), thymol (0.2–8.3), 4-ol terpinene (1.5–8.3%) [57], but the germacrene D, β-caryophyllene and linalool contents were much lower than those in the essential oil of our *O. vulgare* ssp. *vulgare* varieties. In Poland [11], the major constituents of the essential oil of common oregano were sabinene, but in Lithuania [58], the major constituents of the essential oil of this subspecies were β-ocimene, germacren D,β-caryophyllene and sabinene, the concentration being much lower (10.0–16.2) in germacren D compared to our findings. As for β-caryophyllene, the data were similar. Other researchers in Lithuania [62] reported higher concentrations of trans-sabinene hydrate, α-caryophyllene and germacren D in the samples of the National German Genebank. Some authors indicated high concentrations of sabinene or ocimenes in this species depending on the cultivation conditions [69].

Origanum vulgare ssp. *vulgare* L. accumulates a very low content of essential oil (0.03–0.11% at standard humidity) and is used more as a medicinal pharmaceutical plant in infusions and teas. However, the essential oil of this subspecies is considered, like other essential oils, to have higher bioactivities and can be used as a new source of antioxidants [13], antimicrobials and antibacterials. Weglarz et al. [10] considered this plant to be a rich source of rosemary acid, a compound known for its extremely high antioxidant properties and can be used in natural medicine. *O. vulgare* subsp. *vulgare* has shown strong free radical scavenging activity, reducing potency, antimicrobial and acetylcholinesterase, butylcholinesterase, α-amylase and α-glucosidase [20]. The antioxidant action of the essential oil of *O. vulgare* ssp. *vulgare* is mentioned by several researchers [20,21,23,41,67]. Given that the major component of the essential oil is germacrene D, we can consider that this essential oil certainly has antimicrobial and insecticidal action. The research of De Falco et al. [69] confirms the antimicrobial action of the essential oils of *O. vulgare* ssp. *vulgare* L.

It should be mentioned that our study and those of many other researchers indicate the presence of a considerable difference between *O. vulgare* ssp. *hirtum* and *O. vulgare* ssp. *vulgare* in terms of both the content and qualitative and quantitative composition of the essential oil. Accentuated variation is attested not only between subspecies, but also between varieties or origins of both subspecies.

Our research has shown the presence of a direct correlation between the content, yields of essential oils and content of polyphenols in the varieties of *O. vulgare* ssp. *vulgare* and those of *O. vulgare* ssp. *hirtum* as well. However, *O. vulgare* ssp. *hirtum* varieties have a higher essential oil content than *O. vulgare* ssp. *vulgare* varieties, while the polyphenols content, on the other hand, is higher in *O. vulgare* ssp. *vulgaris* (51.668–68.500 mg/g) than in the *O. vulgare* ssp. *hirtum* varieties (35.568–49.518 mg/g).

The importance of polyphenolic compounds for maintaining human health and food systems [68,70–72] include uses in oxidative stress, antioxidant, anti-inflammatory effects, [73] and the prevention and treatment of cancer [74]. Polyphenols have important effects in protecting the body against external factors and the cleansing of reactive oxygen species appearing as a consequence of some diseases [73]. Studies on living (animals, humans) and epidemiologic organisms show that different polyphenols have antioxidant and anti-inflammatory properties [73] that could have preventive and/or therapeutic effects for hypertensive conditions, diabetes, cardiovascular diseases, neurodegenerative disorders, cancer and obesity [71,73]. The beneficial action of polyphenolic compounds is also evident in the prevention and treatment of allergic diseases [75].

Thus, *O. vulgare* ssp. *hirtum* and *O. vulgare* ssp. *vulgare* varieties as sources of both valuable essential oils and imported polyphenols can be successfully employed for pharmaceutical, medical and food purposes to maintain health and treat many diseases.

5. Conclusions

O. vulgare ssp. *hirtum* Ietsw. and *O. vulgare* ssp. *vulgare* L. varieties were evaluated in drought and heat conditions. The variation of the indices of the quantitative character values, including the content and qualitative and quantitative composition of essential oil, as well as the producing capacity, were confirmed for both *O. vulgare* L. subspecies.

The presence of a direct correlation between the content, production of essential oil and the content of polyphenols has been highlighted. The varieties of *O. vulgare* ssp. *hirtum* demonstrate a higher content of essential oil (1.31–1.64% at standard humidity; 3.12–4.10%, dry matter) than the varieties of *O. vulgare* ssp. *vulgare* (0.03–0.11% at standard humidity; 1.31–1.64%, dry matter), while the polyphenol content, on the other hand, was higher (51,668–68,500 mg/g) for the varieties of *O. vulgare* ssp. *vulgare* than for those of *O. vulgare* ssp. *hirtum* (35.568–49.518 mg/g).

The research has resulted in the selection of two varieties, i.e., "Savoare" of *O. vulgare* ssp. *hirtum* (Patent application v2020 0021) and "Panacea" of *O. vulgare* ssp. *vulgare* (Patent application v2020 0020). The "Savoare" variety of *O. vulgare* ssp. *hirtum* is characterized by the following quality characteristics: polyphenol content, 47.71 mg/g; essential oil content, 1.555% at standard humidity, 3.877%, dry matter; with the following compounds: carvacrol, 83,917; γ-terpinene, 3.54%. Production capacity: *herba* production at standard humidity, 8.9 t/ha; pharmaceutical *herba* production at 13% humidity, 1.88 t/ha. Essential oil production, 138 kg/ha. Efficiency, yield—15.5 kg EO/t of raw material. The "Panacea" variety of *O. vulgare* ssp. *vulgare* has the following quality characteristics: polyphenol content, 61.38 mg/g; essential oil content in the raw material, 0.107% at standard humidity; 0.267%, dry matter. Major compounds in essential oil: germacren D, 31.13%; β-caryophyllene, 15.785%; α-Farnesene, 11.41%. Production capacity: Production of raw *herba* at standard humidity, 7.8 t/ha. Dry *herba* production at 13% humidity, 1.51 t/ha; essential oil production, 8.35 kg/ha; efficiency, 1.1 kg/t.

Author Contributions: Conceptualization, M.G., M.V.M. and M.M.D.; methodology, M.G., A.B., V.B., M.V.M., M.M.D, T.J. and L.C.; software, M.M.D., M.G., L.C. and V.B.; validation, M.M.D., A.B., M.G. and V.B.; formal analysis, M.G., A.B., T.J., Z.V., V.B. and P.B.; investigation, M.G., A.B. and V.B.; resources, M.G., M.V.M. and M.M.D.; data curation, M.G., A.B., V.B., T.J., Z.V. and P.B.; writing—original draft preparation, M.G., M.M.D. and M.V.M.; writing—review and editing, M.G., M.M.D. and M.V.M. First author (M.G.), and the corresponding authors (M.V.M.) and (M.M.D.) have contributed equally to this paper, both being considered as first authors. All authors have read and agreed to the published version of the manuscript.

Funding: Research was carried out within the project of the State Program from Republic of Moldova 20.80009.5107.07 "Reducing the consequences of climate change by creating, implementing varieties of medicinal and aromatic plants drought, frost, winter, disease resistant, which ensures sustainable development of agriculture and guarantees high quality raw material predestined to the perfumery, cosmetics, pharmaceuticals and food industry".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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