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Article

Chemotypes of Species of the Genus *Thymus* L. in Carpathians Region of Ukraine—Their Essential Oil Qualitative and Quantitative Characteristics and Antimicrobial Activity

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Abstract: The study of the R&D in this paper is to determine the range of essential oils (EOs) in the raw materials of species of the genus Thymus of the natural flora in the Carpathian region and their antimicrobial activity. It was found that the component range of EO in species of the genus Thymus depends on the microclimatic conditions of the population. The range of essential oils in the raw material of *Th. Serpyllum* and *Th. Pulegoides* is 7–9 mL and *Th. Marschallianus* is 3.5 mL. The research found that the plants *Th. Serpyllum* and *Th. Pulegoides* that grow in sunny habitats have an aromatic mono- and bicyclic monoterpenoid chemotype (K/α-T-neol/ G/p-C/B), with total dominance of carvacrol and p-cymene. The populations of *Th. Serpyllum*, which grow on the edges of sparse pine forests, and populations of *Th. Pullegioide*, with denser plant cover and which grow in meadows, have an acyclic and bicyclic monoterpene chemotype (G/α-T-neol/B/K). Plants that grow in the communities of meadow-steppe vegetation have the following chemotypes: *Th. Serpyllum*—L/K/G with 63% of linalool, *Th. Pullegioides*—G/α-T-neol/L/B, and *Th. Marschallianus*—α-T-neol/K/L/α-T-nen/G/B. Of these, the dominant chemotypes are α-terpineol and carvacrol (28:6.5%). A wide spectrum of antimicrobial activity was registered in samples of *Th. Pullegoides* and *Th. Serpyllum* that have an aromatic-monoterpenoid chemotype. Essential oils of *Th. Pullegoides* were dominated by carvacrol, and p-cymene had the highest fungicidal action (41.00 ± 1.0%). Plant populations of *Th. Pullegoides* and *Th. Serpyllum* with the aromatic-monoterpenoid chemotype are suggested by R&D to be of use in the pharmaceutical industry. They have high contents of natural components, which are effective in a wide spectrum of antimicrobial activity. The EO of *Th. Marschallianus* had the lowest influence on the inhibition of bacterial and fungal reproduction.

Keywords: chemotype; essential oils (EOs); biochemical content; antimicrobial activity; biodiversity

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1. Introduction

Species of the genus *Thyme* (*Thymus* L.) are rich in essential oils, exhibit antimicrobial and antioxidant properties, and are widely used in ethnic and traditional medicine, aromatherapy, cosmetology, and cooking. Thyme has been used as a spice since ancient times in many countries of Southern Europe (Mediterranean) and Asia.

The investigation of the quantitative content and component composition of essential oils, depending on the environmental conditions of growth, and their antimicrobial and antifungal activities, depending on the chemotype of the population, are urgent issues today.

The component composition of essential oils depends, first of all, on geographical distribution and climatic conditions [1]. There is regularity in nature: species that grow in xerothermic conditions, in addition to a specific morphological and anatomical structure, have the ability to accumulate substances of secondary synthesis in greater quantities. It was found that in regions with a warm climate, species of the genus *Thymus* are dominated by a chemotype with a predominant content of aromatic monoterpenes (p-cymene, thymol, and carvacrol) [2–5], while in countries with a cool climate, the chemotype is monoterpenes and their oxygen derivatives and sesquiterpenes.

Thus, six chemotypes of *Th. Pulegioides* have been described in the Mediterranean, thymol, carvacrol, geraniol, linalool, fenchone, and alpha-terpenyl acetate, of which phenolic chemotypes predominate [2]. In the aerial organs of *Th. Serpyllum*, collected in the Kumaon and Uttarakhand regions of the Himalayas (India), 29 essential oil components were identified, of which approximately two-thirds of the composition was thymol, r-cymene, thymol methyl ester, and carvacrol methyl ester [3,4]. Thymol (32.2% and 27.4%) was the main compound in oils *Th. Vulgaris* and *Th. Pulegioides* in different thyme species grown in the Botanical Garden in Romania, with carvacrol (25.8%) in the oil of *Th. Serpyllum* and terpinyl acetate (47.6%) in *Thymus Glabrescens* oil [5].

In a temperate climate, the chemotype of monoterpene hydrocarbons is formed. In Lithuania, the essential oils of *Th. Serpyllum* ssp. *serpyllum* were combined into five monoterpene chemotypes, namely 1,8-cineole, germacrene B, (E)-β-ocimene, α-cadinol, and cis-p-ment-2-en-1-ol [6]. Herba *Th. Serpyllum* grown in Estonia had an essential oil yield of 0.6–4.4 and 1.9–8.2 mL/kg-^{1}. The following chemotypes of acyclic (myrcene) and bicyclic (borneol) monoterpenes and acyclic (E)-nerolidol) and bicyclic sesquiterpenes (caryophyllene oxide) are distinguished in it. Thymol and carvacrol, which are mentioned in the *European Pharmacopoeia* as the main components, are not the main components of the essential oil grown in Estonia [7]. The composition of the essential oil of *Th. camphoratus* grown in the Lviv Botanical Garden was dominated by bicyclic monoterpenoids—borneol and fenhol [8].

In the composition of essential oils of the raw material of *Th. Pulegioides* collected in Croatia were acyclic monoterpenoids, geraniol and linalool, followed by aromatic thymol, γ-terpinene, thymol methyl ester, and borneol, the amount of which changed during development. Due to the high content of geraniol in the essential oils of *Th. Pulegioides*, it may be interesting as an aromatic material for the perfume industry or for food flavoring [9].

The content of essential oils depends on the altitude above sea level [10]. In the composition of oil from the population of *Th. Serpyllum* in the Altai Mountains, located at an altitude of 150 m above sea level, monoterpenes predominated; from the population located at 500–700 m above sea level, half of the essential oil (EO) composition was aromatic oils—carvacrol, *p*-cymol, and other monoterpenes—γ-terpinene, and 1,8-cineole. Similar results were obtained from populations of *Th. Pulegioides* in Monte Pisani (Santagallo, Tuscany, 650 m.a.s.l.). Two populations had a thymol chemotype, and the third, with a typical lemon smell, contained more geraniol, with an essential oil level of 1.05 g [11].

The accumulation and qualitative composition of essential oils is affected by the method of their distillation [12]. Volatile substances of *Th. Serpyllum* were obtained by hydrodistillation (HD), simultaneous hydrodistillation and extraction (SDE), and static gas chromatographic mass spectrometry (HS) analysis. Monoterpenes were the most dominant in all three techniques (84.8–94.2%). The essential oil profiles obtained by HD and SDE were similar, with oxygenated monoterpenes being the most common (up to 75.4%). HS was dominated by monoterpene hydrocarbons (94.2%). The main compounds were α-terpinyl acetate (HD and SDE) and myrcene (HS).

In addition to essential oils of different component compositions, the synthesis of which depends on temperature conditions, because p-cymene and γ-terpinene are precursors of thymol, *Th. Serpyllum* contains various phenolic compounds. Fourteen phenolic compounds were detected in it, five of which were phenolic acids and nine of which were flavan-3-ols [13]. Tested samples of *Th. Serpyllum* contained rosmarinic acid (4–23 mg/g) and luteolin-7-*O*-β-D-glucuronide, which were the main components, while protocatechinic, 3-*O*-caffeylquinic, and caffeic acids (0.19–0.62 mg/g) were secondary components [14].

In the raw material of *Th. Marschallianus*, flavonoids were found: luteolin, quercetin, apigenin, and their derivatives, as well as rosmarinic acid and methyl rosmarinate. Significantly higher amounts of flavonoids were detected in the sample collected in the spontaneous flora, while for rosmarinic acid, a higher amount was detected in the cultivated sample. Both samples showed promising antibacterial activity, especially against Gram-positive organisms [15]. The most characteristic components of *Th. Marschallianus* were rosmarinic acid, protocatechuic acid, luteolin 7-*O*-glucoside, and apigenin 7-*O*-glucuronide, and GC-MS analysis of the volatile fraction revealed the presence of thymol and carvacrol. In addition, sesquiterpenoids, fatty acids, and their ethers were detected [16].

When analyzing random amplified polymorphic DNA (RAPD) of thymol and sesquiterpene chemotype plants, *Th. Pulegioides* revealed a partial correlation between molecular and chemical evaluations [17].

The *European Pharmacopoeia 8.0*, 2018 [18], includes raw material of *Thymi herba* species *Thymus Vulgaris* or *Th. Zygis*. In the *State Pharmacopoeia of Ukraine*, 2014 [19], in addition to these species, which are cultivated in the south of Ukraine, is the herb *Serpylli herba* of the natural flora species *Th. Serpyllum*. However, this name is often used in addition to *Th. Serpyllum* and other types of natural flora—*Th. Pulegioides* and *Th. Marshallianus*. For this reason, confusion often arises when preparing medicinal plant raw materials.

The aim of the R&D in this paper is to determine the range of essential oils and their qualitative and quantitative characteristics in the raw materials of genus Thymus and its species, which are growing in the natural flora of the Carpathian region in Ukraine. The study of their antimicrobial and antifungal activities was an important part of the experimental works.

2. Materials and Methods

2.1. Collection of the Plant Material

The object of the research was the medicinal plant material (MPM) of species of the genus Thymus (*Thymus* L.): pine thyme (*Th. Serpyllum* L.), which is included in the *State Pharmacopoeia of Ukraine*, and the non-official species flea thyme (*Th. Pulegioides* L.) and Marshall's thyme (*Th. Marschallianus Willd.*), the raw materials of which are used under the name *Herba serpylli*. *Th. Pulegioides* and *Th. Marschallianus* are promising essential oil medicinal plants, but they are often confused or considered to be admixtures of the morphologically close species *Thymus Serpyllum*, due to their morphological similarity.

The material for research (MPM) was selected in the Lviv region. Raw material of *Th. Serpyllum* was collected in three places of growth in Roztochchja, in the Yavoriv National Park (see the Herbarium at the website: https://yavorivskyi-park.in.ua/en/ (accessed on 16 December 2022)): on the crystalline outcrops of the Bila skelja near the village of Lelehivka (population No 1.1), in communities of psamophilous vegetation on the outskirts of the village of Strach (population No 1.2), and in a pine forest meadow near the village of Trostyanets, Mykolaiv district (population No 1.3). Raw material of *Th. Pulegioides* was collected in communities of grass-herbaceous vegetation in three places of growth: in the eastern outskirts of the city of Pustomyty (population No 2.1), and two in the Yavoriv National Park near the village of Yameljnya (population No 2.2) and the village of Vereshchytsja in the Havrylove tract in the communities of meadow-steppe vegetation (population No 2.3). Individuals of *Th. Marschallianus* were collected on Pishchana Gora (Lion Mountain) in the "Znesinnja" RLP, Lviv (population No 3.1) (Figure 1) (Atlas of the Lviv region: https://geoknigi.com/load.php?id=3 (accessed on 16 December 2022)) [20].

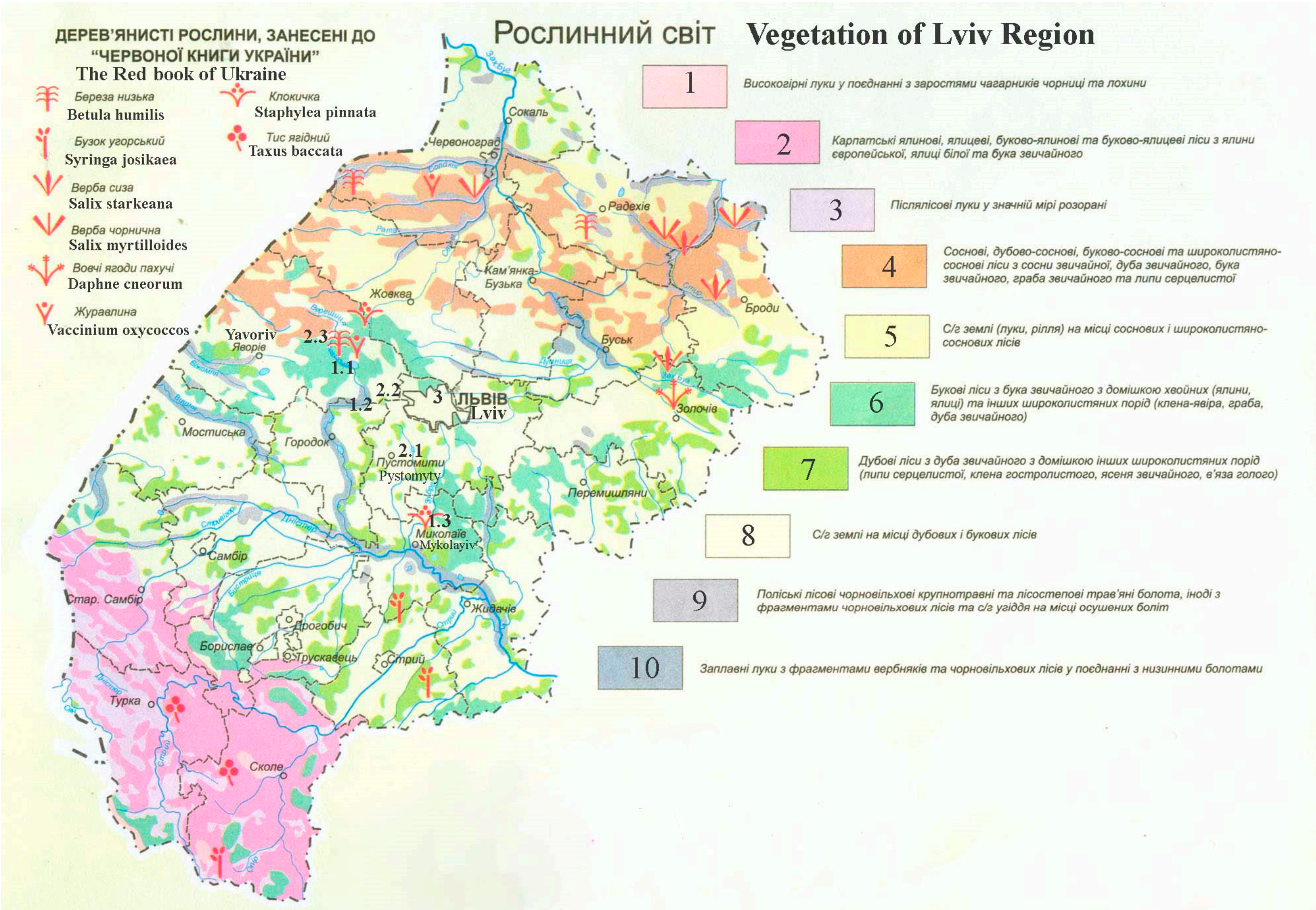

Figure 1. The distribution of species of the genus Thymus in vegetation of Lviv Region. *Th. Serpyllum*: 1.1—population No 1.1; 1.2—population No 1.2; 1.3—population No 1.3. *Th. Pulegioides*: 2.1—population No 2.1; 2.2—population No 2.2; 2.3—population No 2.3. *Th. Marschallianus*: 3—population No 3. Legend: 1 High-altitude meadows combined with thickets of huckleberries and blueberry bushes. 2 Carpathian spruce, fir, beech–spruce, and beech–fir forests from European spruce, white fir, and ordinary beech. 3 Post-forest meadows are largely plowed. 4 Pine, oak–pine, beech–pine, and broadleaf-pine forests of ordinary pine, ordinary oak, ordinary beech, ordinary hornbeam, and heart-leaved linden. 5 Agricultural land (meadow, arable land) in the place of pine and broadleaf-pine forests. 6 Beech forests from ordinary beech with an admixture of conifers (spruce and fir) and *other broad-leaved species (sycamore maple, hornbeam, and ordinary oak)*. 7 Oak forests from ordinary oak with admixture of other broad-leaved species (heart-*leaved linden, sharp-leaf maple, ordinary ash, and bare elm)*. 8 Agricultural land in the place of oak and beech forests. 9 Polissja forest of black alder coarse-grass and forest-steppe grass swamps, sometimes with fragments of black alder forests and agricultural land in place of drained swamps. 10 Floodplain meadows with fragments of willow and black alder forests in combination with lowland swamps.

2.2. *Thymus* Oil Distillation

Each sample of dry *Thymus* herb with weight of 20 g was grounded in a blender. The EO from this raw material was prepared by hydrodistillation (2 h) in a Clevenger-type apparatus according to the *European Pharmacopoeia 8.0* [18]. Hexane (1 mL) was used as a collecting solvent. The essential oils were stored under N_2 at + 4 °C in a dark space before their GC-FID/MS analysis.

2.3. Analyses of the Essential Oils

The determination of the qualitative composition of essential oils was carried out by GC/FID gas chromatography using a GC-SCION 456. The following operating conditions were used: column SOLGEL-WAX, 60 m × 0.25 mm i.d., thickness of the film 0.25 µm, carrier gas nitrogen regulated to the current of 1 mL/min, the temperature of injection to the detector FID 220 °C and 250 °C, respectively.

The components were identified according to their retention time (RT) (min), and the obtained values were compared with literature data. Standards for comparison of essential oils were provided by Extrasynthesis Ltd. (Lyon, France) and Sigma-Aldrich (St. Louis, USA). Research results are presented in percentages. The percentage of single chromatographic peaks was measured as the ratio of the single peak area to the total peak area. Detailed descriptions of component determination of all *Thymus* species including with RT and chromatographic profiles were given by Hrytsyna et al., 2020 [8].

Analysis of GC/MS was conducted using gas chromatograph Varian 450-GC connected with Varian 220-MS. The division was made using FactorFourTM: capillary column DB-WAX, 30 m × 0.25 mm i.d., thickness of film 0.25 µm. The injector of 1177 type was heated at a temperature of 220 °C. The injection equaled 1 µL of the solution of 1:1000 n-hexane/diethyl ether. Helium was used as carrier gas during constant velocity of the current in the column of 1.2 mL/min. Temperature of the column was set as follows: the initial temperature for 10 min was 50 °C, then to 100 °C at 3 °C/min; isothermic temperature was held for 5 min, and then continued to 150 °C at 10 °C/min. Total time for the analysis of one sample was 54.97 min. The constituents were identified by comparing their mass spectrum with the spectra kept in NIST 02 (library of software) or mass spectra from the literature (Adams, 2007) [21], and also the comparison of their indices obtained with the standards was performed. Statistical analysis was conducted using reliable intervals at the level of $p < 0.05$ with our calculation through mean statistical deviation and standard error.

2.4. Method for the Testing of the Antimicrobial Activity

The antimicrobial activity of EO was studied at the Microbiological Laboratory of the Department of Genetics, Plant Physiology and Microbiology, Uzhhorod National University. The sensitivity of microorganisms against the EOs was determined by agar diffusion test using typical *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922), *Streptococcus pyogenes* (ATCC19615), and *Candida albicans* (ATCC885-653) [22]. As positive controls were used gentamicin (10 mg/disk) for Gram-negative bacteria, ampicillin (10 mg/disk) for Gram-positive bacteria, and nystatin (100 UI) for *Candida*. As negative control DMSO was used. The bacterium inocula 100 µL in the physiological solution were adjusted to the equivalent of 0.5 McFarland (5×10^8 KFU/mL) standard, and evenly spread on the surface of Muller–Hinton agar (incubated at 37 ± 2 °C for 24 h); yeasts were spread on SDA agar (incubated at 35 ± 2 °C for 48 h). The 20 µL extracts were introduced into wells 6 mm in diameter. The diameters of the inhibition zones were measured in millimeters, including the diameter of the well. The antimicrobial effect was assessed by the presence of the growth inhibition zone. Each antimicrobial assay was performed at least three times.

2.5. Statistical Analyses of the Data

For the results of experiment, we used statistical software ANOVA with the calculation of averages, error, and standard deviation. Differences were considered significant at $p < 0.05$.

3. Results

The *European Pharmacopoeia 8.0* (2018) [18] includes raw material of *Thymi herba*. These are whole leaves and flowers of *Th. Vulgaris* L. or *Th. Zygis* L. with an essential oil content of at least 12 mL/kg, in which the sum of thymol and carvacrol is at least 40 percent of the essential oil. In the *State Pharmacopoeia of Ukraine*, 2014 [19], species that are cultivated in the south of Ukraine and the herb *Serpylli herba* of the natural flora species *Th. Serpyllum* are also included. In the herb *Serpylli herba*, the content of essential oil is not less than 3.0 mL/kg, and in the national article is not less than 1.5 mL/kg.

As a result of the research, it was found that individuals from all three research populations of *Th. Serpyllum* contain fairly large contents of essential oil (Table 1). The largest amount of essential oil (0.9 ± 0.05%, or 9 mL/kg) was from plants from the communities of thermophilic vegetation on the outcrops of crystalline rocks of the Bila skelja (population 1.1). In second place in terms of essential oil content (0.8 ± 0.05%, 8 mL/kg) was the population on the edge of a sparse pine forest on the Torton sandstones (population 1.3). Plants growing in the plant communities of psammophilous vegetation had the lowest content of essential oil—0.7 ± 0.05% (7 mL/kg of dry raw material) (population 1.2) (Table 1). In all research populations of *Th. Pulegioídes*, raw material contained 0.8 ± 0.05% (8 g/kg, calculated on anhydrous raw material), 0.85 ± 0.05%, and 0.9 ± 0.05% essential oil. The content of essential oil in the raw material of Marshall's thyme (*Th. Marschallianus*) was 0.35 ± 0.05% (Table 2). The total number of identified main components of EO ranged from 67.2 to 90.6% (Tables 1 and 2).

Table 1. The component composition of essential oils of *Th. Serpyllum* from different places of plant population growth (x ± SD).

Population		**1.1**		**1.2**		**1.3**	
EO Content (%, *v*/*w*, Expressed in Dry Weight)		**0.90 ± 0.05**		**0.70 ± 0.05**		**0.80 ± 0.05**	
GC Analysis (%)	**RT (min)**	**1***	**2***	**1***	**2***	**1***	**2***
β-myrcene	12.84	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
1,8-cineole	14.18	trace **	trace	0.1 ± 0.1	0.1 ± 0.1	trace	trace
limonene	14.74	trace	trace	trace	trace	trace	trace
α-terpinene	16.0	3.0 ± 0.5	2.5 ± 0.5	4.0 ± 0.5	3.5 ± 0.5	4.0 ± 0.5	3.2 ± 0.5
p-cymene	16.77	2.0 ± 0.5	2.0 ± 0.5	10.0 ± 1.0	9.1 ± 1.0	1.6 ± 0.2	1.6 ± 0.2
terpinolene	17.91	trace	trace	trace	trace	trace	trace
α-thujone	22.5	0.1 ± 0.1	0.1 ± 0.1	1.6 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
linalool	24.54	63.0 ± 2.0	63.0 ± 2.0	0.4 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
bornyl acetate	25.26	0.5 ± 0.1	0.3 ± 0.1	4.5 ± 0.5	3.5 ± 0.5	1.4 ± 0.2	1.0 ± 0.2
β-caryophyllene	26.51	2.5 ± 0.5	2.0 ± 0.5	3.5 ± 0.5	3.0 ± 0.5	4.0 ± 0.5	5.5 ± 0.5
borneol	29.25	1.3 ± 0.2	1.7 ± 0.2	7.5 ± 0.5	7.5 ± 0.5	8.0 ± 0.5	9.5 ± 1.0
α- terpineol	29.92	2.5 ± 0.5	2.2 ± 0.5	20.2 ± 1.0	20.0 ± 1.0	17.0 ± 1.0	18.3 ± 1.0
fenchol	32.09	0.2 ± 0.1	0.2 ± 0.1	2.0 ± 0.5	2.0 ± 0.5	4.0 ± 0.5	3.0 ± 0.5
geraniol	33.19	5.0 ± 0.5	4.5 ± 0.5	15.0 ± 1.0	15.1 ± 1.0	35.0 ± 2.0	35.0 ± 2.0
thymol	42.65	0.6 ± 0.1	0.8 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	trace
carvacrol	45.53	6.0 ± 0.5	6.0 ± 0.5	20.0 ± 1.0	18.0 ± 1.0	6.0 ± 0.5	6.5 ± 0.5
Content of the main EOs (%)		87.0	85.6	89.5	83.5	82.7	85.1

Note: 1*—GC-SCION 456 SOLGEL-WAX gas chromatograph with a column length of 60 m; 2*—GC-Carlo Erba VEGA DB-WA gas chromatograph with column length 30 m; ** <0.05%.

Table 2. The component composition of *Thymus* essential oils from different places of growth plant species (x ± SD).

Type		*Th. Pulegoides*						*Th. Marschtallianus*	
Population		**2.1**		**2.2**		**2.3**			
EO Content (%, v/w, Expressed in Dry Weight)		**0.90 ± 0.05**		**0.85 ± 0.05**		**0.80 ± 0.05**		**0.35 ± 0.05**	
GC Analysis (%)	**RT (min)**	**1***	**2***	**1***	**2***	**1***	**2***	**1***	**2***
β-myrcene	12.84	0.7 ± 0.1	0.7 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	3.5 ± 0.5	3.5 ± 0.5
1,8-cineole	14.18	0.1 ± 0.1	0.1 ± 0.1	trace	trace	trace	trace	0.4 ± 0.1	0.3 ± 0.1
limonene	14.74	trace **	trace	trace	trace	trace	trace	0.9 ± 0.1	0.8 ± 0.1
α-terpinene	16.0	8.0 ± 0.5	7.5 ± 0.5	1.6 ± 0.2	1.5 ± 0.2	2.5 ± 0.5	2.0 ± 0.5	4.0 ± 0.5	4.0 ± 0.5
p-cymene	16.77	6.0 ± 0.5	6.0 ± 0.5	3.0 ± 0.5	3.2 ± 0.5	1.6 ± 0.2	1.6 ± 0.2	1.0 ± 0.2	1.0 ± 0.2
terpinolene	17.91	trace	trace	trace	trace	1.8 ± 0.2	2.0 ± 0.5	2.2 ± 0.2	2.4 ± 0.2
α-thujone	22.5	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
linalool	24.54	0.6 ± 0.1	0.6 ± 0.1	trace	trace	13.0 ± 1.0	12.0 ± 1.0	4.5 ± 0.5	3.5 ± 0.5
bornyl acetate	25.26	3.0 ± 0.5	3.0 ± 0.5	1.0 ± 0.2	1.0 ± 0.2	0,3 ± 0.1	0.4 ± 0.1	4.0 ± 0.5	3.0 ± 0.5
β-caryophyllene	26.51	2.5 ± 0.5	3.5 ± 0.5	4.0 ± 0.5	5.0 ± 0.5	3.0 ± 0.5	4.0 ± 0.5	2.0 ± 0.2	1.0 ± 0.2
borneol	29.25	7.0 ± 0.5	8.0 ± 0.5	10.0 ± 1.0	11.0 ± 1.0	6.0 ± 0.5	7.5 ± 0.5	2.5 ± 0.5	2.5 ± 0.5
α-terpineol	29.92	13.0 ± 1.0	11.0 ± 1.0	20.0 ± 2.0	21.0 ± 2.0	15.0 ± 1.0	15.0 ± 1.0	28.0 ± 1.0	30.1 ± 1.0
fenchol	32.09	3.5 ± 0.5	3.5 ± 0.5	4.0 ± 0.5	4.0 ± 0.5	2.5 ± 0.5	2.5 ± 0.5	1.2 ± 0.2	1.2 ± 0.2
geraniol	33.19	22.0 ± 2.0	22.0 ± 2.0	33.0 ± 2.0	33.0 ± 2.0	15.0 ± 1.0	15.0 ± 1.0	3.5 ± 0.5	2.5 ± 0.5
thymol	42.65	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	2.0 ± 0.5	2.0 ± 0.5	2.2 ± 0.2	2.2 ± 0.2
carvacrol	45.53	17.0 ± 1.0	18.0 ± 1.0	9.5 ± 0.5	9.5 ± 0.5	3.5 ± 0.5	4.0 ± 0.5	6.5 ± 0.5	9.0 ± 0.5
Content of the main EOs (%)		84.8	85.3	87.7	90.6	67.7	69.3	67.2	67.7

Note: 1*—GC-SCION 456 SOLGEL-WAX gas chromatograph with a column length of 60 m; 2*—GC-Carlo Erba VEGA DB-WA gas chromatograph with column length 30 m, ** <0.05 %.

When analyzing the component composition of essential oils, it turned out that the raw materials of *Th. Serpyllum* collected on the lawn of a pine forest on the Torton sandstones and in communities of psamophilous vegetation on sandy soils in Roztochchja (populations 1.2 and 1.3) are similar in their component composition of EO: geraniol, α-terpineol, borneol, and carvacrol, p-cymene (G/α-T-neol/B/K) in the ratio 35:17:7.5:6% (population 1.3) and K/α-T-neol/G/p-C/B in the ratio 20:20:15:10:9.5 (population 1.2) (Table 1; Figure 2). Other essential oils were less than 1–4%: β-myrcene, α-terpinene, α-thujone, bornyl acetate, β-caryophyllene, fenchol, and tenths of thymol. For 1,8-cineole, limonene, terpinolene, and linalool (population 1.2) there were only traces (<0.05%). Plants *Th. Serpyllum* from Lelehivka, which grew on the outcrops of crystalline rocks of the Bila skelja in the community of meadow-steppe vegetation, differed from other populations. Their raw material contained 63% linalool (an acyclic monoterpene) and only 6% of carvacrol, 5% of geraniol, and 2.5% of α-terpineol (L/K/G) in the composition of essential oils, while there were traces of linalool in raw materials from other populations.

Therefore, all populations are dominated by monoterpene volatile compounds; in particular, for population 1.3, located near a pine forest, the mono- and bicyclic terpenoids-carvacrol (G/α-T-neol/K/B/p-C) chemotype is characteristic. Linalool (L/G/K) chemotypes were found in the plants of population 1.1 on the crystalline outcrops of the Bila skelja overgrown with pine trees. However, in population 1.2, which is located in a sunny meadow in the communities of psammophilous plants, it is aromatic, mono- and bicyclic terpenoids (K/α-T-neol/ G/p-C/B) chemotype, with a significant content of aromatic monoterpenes—carvacrol and p-cymene (30%) (Figure 2). Since in the populations 1.2. and 1.3 about 20% is α-terpineol, which is the genetic precursor of carvacrol and p-cymene (aromatic compounds), then we can say that high summer temperatures are not enough for their complete transformation [23].

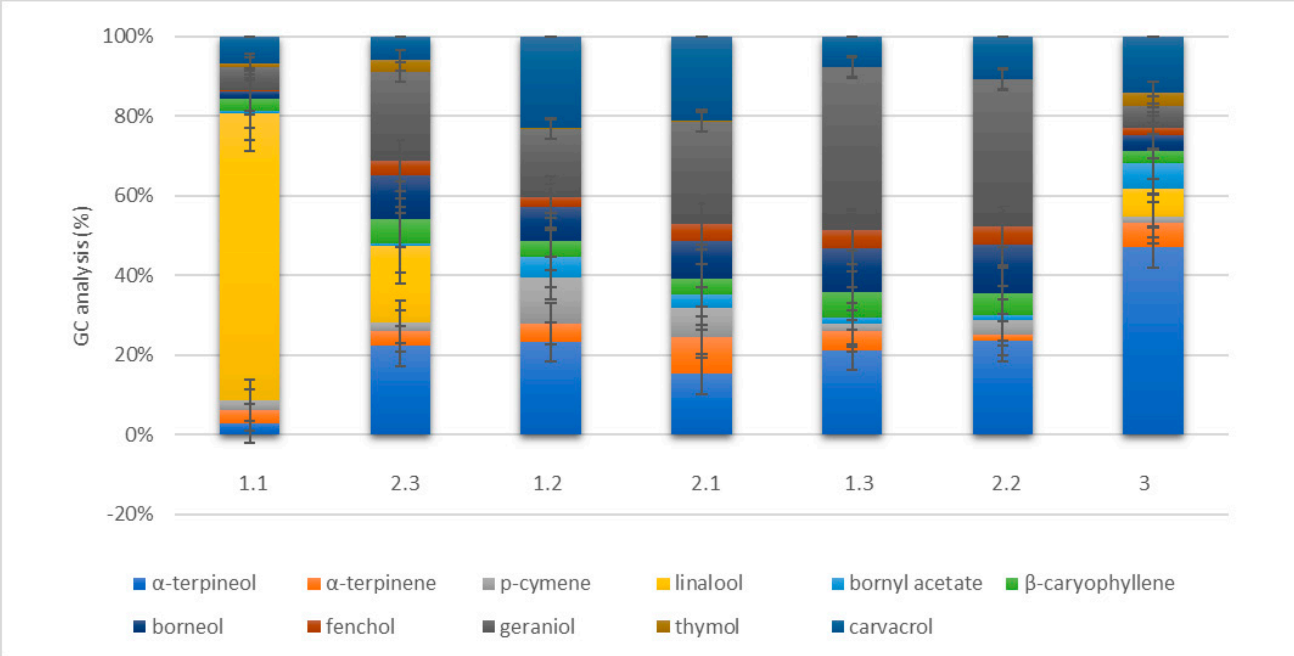

Figure 2. The component composition of essential oils of species of the genus *Thymus* under different growth conditions. Legend: 1.1—population of the *Th. Serpyllum* in the community of meadow-steppe vegetation on the crystalline outcrops of the Bila skelja near the village of Lelehivka in Roztochchja (Yavoriv National Park) (chemotype L/K/G); 1.2—population of the *Th. Serpyllum* in a community of psamophilous vegetation on the outskirts of the village of Strach (chemotype K/α-T-neol/G /p-C/B); 1.3—population of the *Th. Serpyllum* in a community of psamophilous vegetation in a pine forest meadow near the village of Trostyanets, Mykolaiv district (chemotype G/α-T-neol /B/K); 2.1—population of the *Th. Pulegioides* of grass-herbaceous vegetation on the eastern outskirts of the city of Pustomyty (chemotype K/G/α-T-neol/α-T-nen/B/p-C); 2.2—population of the *Th. Pulegioides* of grass-herbaceous vegetation near the village of Yameljnya (Yavoriv National Park) (G/α-T-neol /B/K chemotype); 2.3—population of the *Th. Pulegioides* in the community of meadow-steppe vegetation in the Havrylove tract near the village of Vereshchytsja (Yavoriv National Park) (G/α-T-neol/L/B); 3—population of the *Th. Marschallianus* collected on Pishchana Gora (Lion Mountain) in the "Znesinnja" RLP, Lviv (α-T-neol/K/L/α-T-nen/G/B-at chemotype).

In another species, *Th. Pullegoides*, raw materials were selected from plants growing in populations of the meadow-grass vegetation (Table 2). Monoterpenes and their oxygen derivatives dominate the EO composition of raw materials from all populations. Thus, the raw material collected in population 2.1 from the sunny eastern outskirts of the city of Pustomyty had the highest content of aromatic essential oils (carvacrol and p-cymene, 17 + 6%; geraniol content, 22%; and α-terpineol, 13%). The plants had high contents of α-terpinene (8%) and borneol (7%) (chemotype K/G/α-T-neol/α-T-nen/B/p-C). The EO contents of bornyl acetate, β-caryophyllene, α-thujone, β-myrcene, borneol, and fenchol ranged from 4 to less than 1%, while thymol was 0.2% and only in the population from Vereshchytsja, 2%. Traces of such essential oils as 1,8-cineole, limonene, terpinolene, and linalool were detected. Raw material of *Th. Serpillum* had a similar chemotype with a large percentage of aromatic compounds from the solar psammophilous population 1.2 (Figure 2).

In plants from population 2.2. from the outskirts of the village of Yameljnya, there were more monocyclic alcohols; in particular, there was twice as much geraniol (33%) and α-terpineol (20%) compared to carvacrol (9.5%) and bicyclic monoterpene borneol (10%), and much less α-terpinene and p-cymene (G/α-T-neol/B/K chemotype) (Table 2). All other oils were approximately similar in quantitative content. Population 2.2, where individuals of *Th. pullegoides* compete with forbs, was determined by the geranial-α-terpineol-borneol-carvacrol chemotype, similar to the population of *Th. Serpillum* from the edge of the pine forest of Trostyanets village (population 1.3) (Figure 2).

Plant raw material of *Th. Pulegoides*, collected on the edge of the forest in a community of meadow-steppe vegetation in the Havrylove tract near the village of Vereshchytsja, had 15% of geraniol and α-terpineol and 13% of linalool, traces of which were present in other populations, and 6% of borneol. Aromatic monoterpenes were only 5.5% of the composition (carvacrol and thymol). The monoterpenoid (geranial-α-terpineol-linalool-borneol) (G/α-T-neol/L/B) chemotype is characteristic of the plant material from this population. In the population of *Th. serpillum* from the Bila skelja, the greater half of the EO composition was linalool (Figure 2).

Therefore, individuals of *Th. Pulegoides* from populations 2.1. and 2.2 are also characterized by the geranial-α-terpineol-borneol-carvacrol chemotype. The most aromatic essential oils of p-cymene and carvacrol are in population 2.1—aromatic-geraniol-α-terpineol-α-terpinene-borneol (G/K/α-T-neol/α-T-n/B/p-C) chemotype in the ratio of 22:17:13:8:7:6%. Individuals of population 2.2 have a geranial-α-terpineol-borneol-carvacrol (G/α-T-neol/B/K) chemotype in the ratio of 33:20:9.5:10%, whereas population 1.3 from Vereshchytsja has geraniol-α-terpineol-linalool-borneol (G/α-T-neol/ L/B) in the ratio of 15:15:13:6% (Figure 2).

The content of essential oils in raw material of *Th. Marschallianus* growing on Pishchana Gora ("Znesinnja") was the lowest (0.35 ± 0.05%), about 2–2.5 times compared to other species. The qualitative composition was dominated by α-terpineol (28%) and carvacrol (6.5%), while linalool, α-terpinene, geraniol, bornyl acetate, which were abundant in other species, accounted for only 3–4.5% each (α-T-neol/K/L/α-T-nen/G/B-at chemotype) (Table 2; Figure 2).

A comprehensive comparison of the qualitative and quantitative compositions of the essential oil of individual species of the genus *Thymus* in the Carpathians region of Ukraine is illustrated in Figure 2. These are the first results from Ukraine that determine *Thymus* chemotypes that can be used for tests of therapeutic use.

The research shows that the highest levels of antimicrobial activity were shown by the samples of *Th. pullegoides* (population 2.1) and *Th. Serpyllum* (population 1.2), which had an aromatic-monoterpenoid chemotype, and population 2.2 with a mono-bicyclic-terpenoid-carvacrol chemotype (Tables 3 and 4). At the same time, a wide range of activity of these samples should be noted for both Gram-positive and Gram-negative bacteria, as well as microscopic fungi of the genus *Candida.* It was found that essential oils had the most pronounced antimicrobial effect on microscopic fungi of the genus *Candida*, as the zones of growth retardation in the analysis of all samples ranged from 11.66 ± 0.58 to 41.00 ± 1.00. The most pronounced antimycotic effect was characteristic for raw materials obtained from population 2.1., *Th. pullegoides*, with an aromatic-monoterpenoid chemotype. High activity against *S. pyogenes* was characteristic of the sample of *Th. Marschtallianus* and for the sample from population 1.3, *Th. Serpyllum*, with a monoterpenoid chemotype. Regarding *E. coli*, a sample from population 1.2, *Th. Serpyllum*, had an aromatic-monoterpenoid chemotype.

Table 3. The influence of EO from raw materials of *Th. Serpyllum* from different habitats on typical strains of microorganisms: inhibition zones, mm, M ± m.

	Population		
	1.1	**1.2**	**1.3**
Test microorganisms	Inhibition zone, mm, M ± m		
Staphylococcus aureus ATCC 25923	11.17 ± 0.29	13.17 ± 0.29	11.50 ± 0.50
Escherichia coli ATCC 25922	15.66 ± 0.58	19.83 ± 0.76	9.66 ± 0.58
Streptococcus pyogenes ATCC 19615	11.67 ± 0.58	14.00 ± 0.25	18.50 ± 0.87
Candida albicans ATCC 885-653	11.17 ± 0.29	29.66 ± 1.52	22.66 ± 0.58

Table 4. Antimicrobial and fungicidal action of raw materials of *species of the genus Thymus* from different habitats: inhibition zones, mm, M ± m.

	Th. Pulegoides (Population)			***Th. Marschtallianus***
	2.1	**2.2.**	**2.3**	**3**
Test microorganisms	Inhibition zone, mm, M ± m			
Staphylococcus aureus ATCC 25923	14.50 ± 0.50	13.50 ± 0.50	13.50 ± 0.50	8.67 ± 0.58
Escherichia coli ATCC 25922	14.50 ± 0.50	15.33 ± 0.58	10.83 ± 0.76	0
Streptococcus pyogenes ATCC 19615	15.83 ± 0.29	13.33 ± 0.58	15.50 ± 0.50	18.83 ± 0.76
Candida albicans ATCC 885-653	41.00 ± 1.00	29.00 ± 1.00	11.66 ± 0.58	0

Therefore, a wide spectrum of antimicrobial activity was recorded in samples of *Th. pullegoides* and *Th. Serpyllum* with an aromatic-monoterpenoid chemotype.

Extracts made from plants of population 2.2, *Th. Pulegoides*, which was dominated by monoterpenoid essential oils, which are the precursors of aromatic ones, had slightly lower antimicrobial activity and almost half as much fungicidal activity (29.00 ± 1.00%). Raw materials obtained from population 1.2., *Th. Serpyllum*, with a carvacrol-geraniol chemotype, had a similar fungicidal action (29.66 ± 1.52%) (Tables 3 and 4; Figure 3). All other populations with the monoterpenoid-bicyclic chemotype had moderate antimicrobial activity.

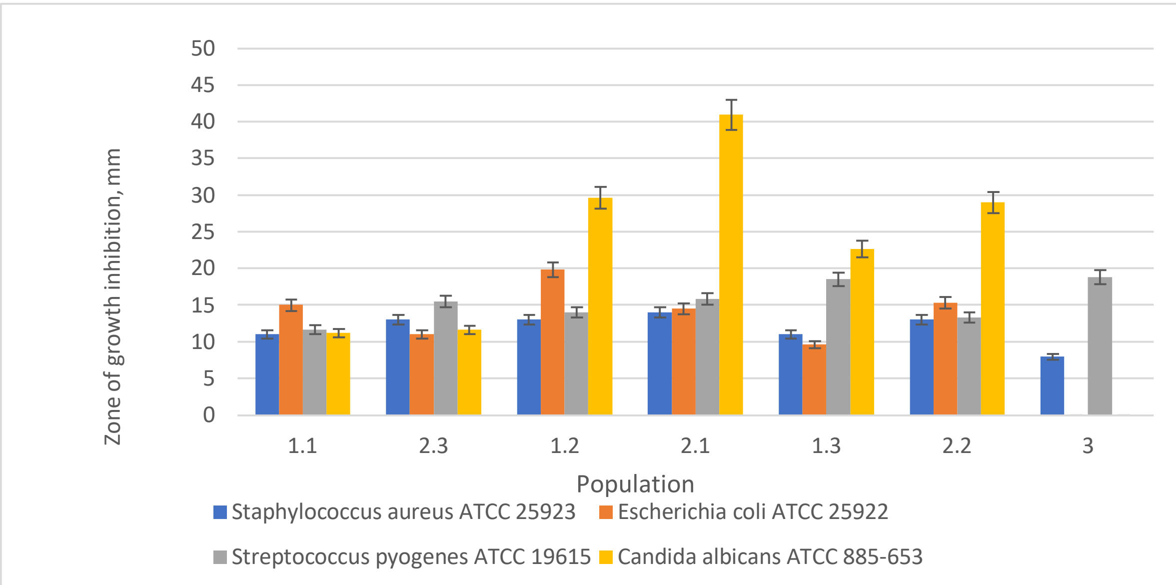

Figure 3. Antimicrobial and fungicidal action of raw materials of species of the genus *Thymus* from different habitats, M ± m. Legend: notations are similar as in Figure 2.

Plants with acyclic monoterpene chemotypes, linalool *Th. Pulegoides* and linalool *Th. Serpyllum*, had low antimicrobial and especially fungicidal activity.

The antimicrobial action on *S. aureus* ATCC 25923 and *S. pyogenes* ATCC 19615 was similar to that of other populations, while on *Escherichia coli* ATCC 25922 it was somewhat lower, and there was a completely low fungicidal effect on *Candida albicans* ATCC 885-653.

The essential oils of *Th. Marschallianus* had the least antimicrobial action, while they had no influence on *C. albicans* ATCC 885-653 and *E.coli* ATCC 25922. Perhaps this can be explained by the low content of geraniol (3.5%), which is abundant in other species and aromatic monoterpenes.

4. Discussion

In the literature, considerable attention is paid not only to the quantitative content, but also to the component composition of essential oils and its dependence on environmental conditions, as well as to the antimicrobial and fungicidal properties of MPM with different component compositions of essential oils.

Therefore, the component composition of essential oils in species of the genus *Thymus* depends, first of all, on the microclimatic conditions of the population. Therefore, in populations of *Th. Serpyllum* growing shaded by a pine forest, or *Th. Pulegoides* in the formed cereal communities and shaded by cereal grasses, characteristic mono- and bicyclic aromatic chemotypes G/α-T-neol/K/B/p-C (*Th. Serpyllum*) and G/α-Teol/B/K (*Th. Pulegoides*) are present, with a slight content of carvacrol (Figure 2: 1.3; 2.2).

Populations in *Th. Serpyllum* and *Th. Pulegoides*, which grow on sunny lawns, have an aromatic mono- and bicyclic monoterpenoids chemotype: K/α-T-neol/G/p-C/B in the first species and G/K/α-T-neol/α-T-nen/B/p-C in *Th. Pulegoides*, with the total dominance of aromatic monoterpenes (carvacrol and p-cymene) (Figure 2: 1.2; 2.1).

In *Th. Serpyllum* plants growing on the crystalline outcrops of the Bila skelja there are linalool (L/G/K) chemotypes. A high content of linalool is found in the population of *Th. Pulegoides* from Vereshchytsja (Havrylove tract) (G/α-T-neol/L/B) (Figure 2: 1.1; 2.3). Both species grow in a group of unique meadow-steppe vegetation. In populations of *Th. Pulegioides* on limestone meadows in Germany, 60% of the research individuals also had the linalyl acetate chemotype, and 20% had the β-caryophyllene-germacrene D-β-bisabolene chemotype [24].

The content of essential oil in the raw material of Marshall's thyme (*Th. Marschallianus*) collected in the communities of strongly changed meadow-steppe vegetation on Pishchana Gora was 0.35 ± 0.05%, and α-terpineol dominated the component composition (Figure 2). *Th. pulegioides*, which grows in meadow coenoses with the dominance of meadow cereals and a high Easter load, has a stable content of essential oils.

Raw material obtained from the population of *Th. Serpyllum* and *Th. Pulegioides* growing in sunny open places (population 1.2 and 2.1) has an aromatic-monoterpenoid chemotype and strong antimicrobial and fungicidal action. This can be explained by the fact that the precursor of carvacrol is α-terpineol, and their transformation depends on the amount of photosynthetically active solar radiation. In addition, the amount of EO in *Th. Pulegioides* decreased in cold rainy weather and increased with the growth of photosynthetically active solar radiation from 0.72% to 0.98% [23,25]. The percentage of carvacrol varied between 16.88 and 29.29%, p-cymene from 5.54–11.33%, and γ-terpinene from 20.60–24.43%. Moreover, the amount of carvacrol grew with the increase in photosynthetically active solar radiation during flowering in July, and the highest content was at the stage of fruiting. The percentage of carvacrol precursors—p-cymene and γ-terpinene—decreased. The p-cymene content showed a significant positive relationship with temperature and precipitation during the April–July period and July temperature. The best time to harvest this type of thyme, both in terms of essential oil yield and phenol content, is during or immediately after full bloom. Research conducted in Kazakhstan showed that the highest content of essential oils—0.4–1.4%—was in the aerial part of *Th. Serpyllum* at the stage of full flowering, and the plants had a phenolic chemotype [10]. In the Left Bank Forest-Steppe, the content of essential oil in the raw material of *Th. Pallasianus* changes during the vegetation period of plants from traces to 0.09%, in *Th. Marcshallianus* from 0.35 to 1.3%, in *Th. Serpyllum* from 0.11 to 0.97%, in *Th. Dimorphus* from 0.24 to 0.37%, and in *Th. Pulegioides* from 0.25 to 0.86% [26].

In addition, *Th. Serpyllum* grows in favorable, thermophilic conditions, which contributes to the synthesis of essential oils in the plant organs. Tymchenko et al., 2007 [27], when studying the resource potential of *Th. Serpyllum* in the Volynj region, came to the conclusion that the reason for the decrease in the resources of species of the genus *Thymus* is the Easter load. The species is important for raw materials mainly in areas with poorly developed grass cover, which are not used for pastures, or in areas with disturbed vegetation cover. As the level of turfing of coenoses increases, the development of populations of *Th. Serpyllum* is suppressed. Formation of populations of *Th. Serpyllum* and *Th. polessicus* in the conditions of the Sumy region is a complicated growth under the tent of a pine forest, and in the local growth there are only generative plants [28]. Only the population formed under the conditions of the *Elytrigioso (repenae)—thymosum* (*serpyllae*) communities is complete in terms of its ontogenetic structure. Morphometric indicators also depend on growth conditions [29]. In our research, the raw material of both species, collected on the edge of a pine forest (population 1.3; 2.2), had monoterpenoids (geranial-α-terpineol-borneol-carvacrol) with a lower antimicrobial and fungicidal action (Figure 3).

The component composition of EOs, as research has shown, depends on geographical distribution and climatic conditions. The content of essential oil from raw materials collected on the territory of the Left Bank Forest-Steppe of Ukraine differed from our results [26]. In the phase of mass flowering for *Th. marcshallianus* it is 0.9%, compared to 0.35% in the Lviv district; for *Th. Serpyllum*, on the contrary, it is 0.26% compared to 0.9 in the Lviv district. For *Th. Pulegioides* the contents are the same: 0.8% in both regions. Such a difference in the content of essential oils is obviously due to the fact that *Th. Serpyllum* in the Lviv district grows in its characteristic populations on pine sands at the edges of pine forests, while the steppe species *Th. marcshallianus* has a three times lower content of essential oil than on the Left Bank, which is due both to its growth on sandy soils in a place with a strong anthropogenic load and to the fact that the investigated region is the extreme limit of distribution of this species.

Chemotypes of monoterpene hydrocarbons and their oxygen derivatives and aromatic-monoterpenoid chemotypes that were isolated for MPM of the species investigated by us of the *Thymus genus* (*Th. Serpyllum, Th. Pulegioides L.* and *Th. Marschallianus* Willd.) are also characteristic of the temperate climate of northern European countries [6,7]. Thus, in *Th. Serpyllum L. ssp. serpyllum var. serpyllum* investigated in the Vilnius region (Eastern Lithuania) [30], the following chemotypes were distinguished: 1,8-cineol/caryophyllene oxide and chemotype with a boron carbon skeleton (camphor + borneol + bornyl acetate). The composition of *Th. Pulegioides* EO was dominated by citral-geraniol with a lemon smell and carvacrol chemotypes [31]. The essential oils of plants with an "ether" odor containing 50–70% α-terpenyl acetate belong to the alpha-terpenyl acetate chemotype, which has not previously been observed in the species *Th. Pulegioides* [32]. In further investigations, it was found that only 35% of the population of *Th. Pulegioides* in the Vilnius region (Lithuania) had the α-terpinyl acetate chemotype [33].

In the south of Ukraine, in the Kherson region, two forms of *Th. Serpyllum* had an essential oil content of 1.1% and 0.67%, and the dominant components were thymol (40.70% and 40.29%) and γ-terpinene (12.88 and 23.31%) [34].

When grown in culture, *Th. Pulegioides* collected from various natural environments in Lithuania had the following chemotypes: geraniol/geranium/neral (G/G/N), thymol (T), linalool (L), carvacrol/γ-terpinene/p-cymene (C/γT/pC), and thymol/carvacrol/γ-terpinene/p-cymene (T/C/γT/pC). It was found that with a sudden change in environmental conditions, two types of *Th. Pulegioides* can be distinguished: plants that retain their chemical composition of essential oils and plants that significantly change their chemical composition of essential oils [35].

The composition of the essential oil is stable and determined genetically [36]. When studying in Lithuania the content of essential oils in *Thymus ×Oblongifolius Opiz*, which is a hybrid of *Th. Serpyllum* L. and *Th. Pulegioides* L., eight chemotypes were found: 1-octen-3-ol, (Z)-β-ocimene, geraniol, myrcene, 1,8-cineole, α-terpinyl acetate, germacrene B, and geraniol/germacrene B. It is known that hybrids, in addition to intermediate morphological features, have a diverse chemical composition. When grown in culture for four years, *Th. × oblongifolius* had the same oil composition.

EOs are widely used not only as part of herbal preparations and in cosmetology, but also as aromatic ingredients in a variety of food products, beverages, and confectionery [37].

The antibacterial activity of essential oils makes thyme a potential natural preservative in the food industry [38].

The antimicrobial action of EOs is determined by the chemotype of the population. Our research also confirmed this hypothesis. Thus, raw materials collected from populations 1.2 and 2.1 that had an aromatic-monoterpene chemotype had a stronger antimicrobial action, and medicinal plant raw materials *Th. Pulegoides*, whose EO were dominated by carvacrol and p-cymene, had the highest fungicidal activity (41.00 ± 1.00%) against *Candida albicans* ATCC 885-653 in all samples (Figure 3). However, populations 1.3 and 2.2 with mono- and acyclic monoterpenoid essential oils had a slightly lower antimicrobial action and almost half the fungicidal action (29.00 ± 1.00%). Plants with the linalool chemotype of both species (populations 1.1 and 2.3) had low antimicrobial and especially fungicidal activity. EOs of *Thymus Marschallianus* had the least antimicrobial activity, while they had no influence on *C. albicans ATCC 885-653* and *E. coli ATCC 25922.*

EOs of samples *Th. Pulegioides* collected in Campania and Sicily, in which 36–39% have phenols, in particular thymol, showed antibacterial activity against eight selected Gram+ and Gram− microorganisms: *S. aureus* (ATTC 25923), *S. faecalis* (ATTC 29212), *B. subtilis* (ATCC 6633), *B. cereus* (PCI 213*), P. mirabilis* (ATCC 12453), *E. coli* (ATCC 25922), *S. typhi Ty2* (ATCC 19430), and *P. aeruginosa* (ATCC 27853) [2].

Th. Pulegioides EO of the thymol chemotype (T) had the strongest anti-Pseudomonad activity against seven phytopathogenic *Pseudomonas* species isolated from vegetables: it inhibited the growth of all tested bacteria even up to 84.4% compared to the control. Linalool chemotype (L) essential oils inhibited bacterial growth less than chemotype T, and the geraniol/geranium/neral G/G/N chemotype was the least effective [39].

It was found that the phytotoxic action of *Th. Pulegioides* EO with the α-terpinyl acetate chemotype has a stronger influence on monocotyledonous than dicotyledonous plants. It also showed high antimicrobial activity against fungi and dermatophytes, but less activity against bacteria and *Candida* yeast. Therefore, *T. pulegioides* with the α-terpinyl acetate chemotype may be a potential compound for the development of preventive measures and/or medicines against mycosis [33].

All species of the genus *Thymus* investigated in Romania—*Th. Vulgaris* and *Th. Pulegioides* (thymol chemotype), *Th. Serpyllum* (carvacrol), and *Th. Glabrescens* (terpinyl acetate chemotype)—showed complete inhibition of the growth of *P. aeruginosa*, *Listeria inacua*, and *S. pyogenes* [5].

EOs with high contents of carvacrol and thymol showed antifungal activity against *Candida* (seven clinical isolates and four ATCC type strains), *Aspergillus* (five clinical isolates and two Colección Española de Cultivos Tipo (CECT) and two ATCC type strains) and five clinical dermatophyte strains. The results showed that the EO of *Th. Pulegioides* showed significant activity against fungi, due to the formation of lesions in the cytoplasmic membrane and a significant decrease in ergosterol content [40].

During this experimental work, it was found that the EO of lemon thyme (*Th.* × *Citriodorus* (Pers.) Schreb. var. "Silver Queen") has a pronounced antibacterial action against *S. aureus* ATCC 25923 (diameters of growth retardation were 14.60 ± 1.52 mm) and fungicidal activity of *Candida albicans* ATCC 885–6530 (29.30 ± 2.82 mm). The antibacterial activity of thyme EO was found against *E. coli* (19.60 ± 1.85 mm). It was experimentally proven that it had no bactericidal influence on the test strain of *P. aeruginosa* [41].

Productivity (fresh and dry leaves and branches) of *Th. Serpyllum* in the highlands of Baluchistan was 113 kg/ha^{-1}. The EO content was 0.58% and 0.87% in fresh and dry plant samples. Three different concentrations of leaf extracts (0.5 g, 1 g, and 1.5 g) were used for three different time periods (24 h, 48 h, and 72 h). The action of 0.5 g of the *Th. Serpyllum* extract for 24 h against the causative agent of *E. coli* was the most effective [42].

Th. Serpyllum EO had an antimicrobial action on anaerobic bacteria, lactic acid bacteria, and enterobacteria causing spoilage of chicken thigh meat for 2 weeks. The EO slowed down the growth of the colonies of these bacteria. *Pseudomonas* spp. were detected only in the control group stored in air for 12 and 16 days. The results of these investigations indicate the possibility of using EOs of *Th. Serpyllum* as natural food preservatives and potential sources of antimicrobial ingredients for the food industry [43].

EO of *Th. Serpyllum* L. and its components—thymol and total phenol extracted from plants—had an influence on the growth and production of mycotoxins of *Aspergillus ochraceus*, *A. carbonarius*, and *A. niger.* The minimum inhibitory concentration (MIC) determined for the essential oil and thymol and the selected concentration of phenol inhibited fungal growth and ochratoxin A biosynthesis by more than 60%, depending on the conditions and duration of incubation with fungi. The EO showed the strongest inhibitory effect due to the synergistic or cumulative influence of its components [44].

Th. Marschallianus and its components showed antioxidant activity and effect against *Helicobacter pylori* ATCC 43504 with an MIC (minimum inhibitory concentration) = 0.625 mg/mL [16].

5. Conclusions

It has been proven that the quantitative and qualitative composition of EOs depends on the amount of solar radiation and microclimatic and orographic conditions of the population. Different chemical composition of EOs from plant raw materials of three Thymus species from the Carpathians region of Ukraine were recorded in this research study. There are important differences in all monitored characteristics. Plant raw materials from different habitats of the searched species have a high range of EO contents. The plant populations of *Th. serpyllum* and *Th. Pulegioides*, located in sunny meadow conditions, have an aromatic mono- and bicyclic monoterpenoid chemotype, with significant contents of the aromatic monoterpenes carvacrol and p-cymene. Populations of *Th. Serpyllum*, that is shaded by a pine forest and *Th. Pulegoides*, that is shaded by cereal grasses, has mono- and bicyclic monoterpenoid chemotypes. Plant raw materials *Th. Serpyllum* and *Th. Pullegioides* selected from meadow-steppe vegetation on sandy soils has monoterpenoid chemotype with hight contents linalool. *Th. Marschallianus* were reported to have the lowest EO and α-terpineol dominated the component composition. Antimicrobial activity and a high content of natural components of *Th. pullegoides* and Th. serpyllum with an aromatic-monoterpenoid chemotype determines the promise of their use as raw materials in the pharmaceutical industry.

Author Contributions: M.K. and M.H. processed the experimental data, performed the biochemical and biological analyses, and drafted the manuscript. I.S. devised the project of *Thymus* biodiversity, the main conceptual ideas, and the proof outline. M.S. and O.N. worked out almost all of the technical details and performed the numerical calculations for the suggested experiment with the plant population collection in the Ukraine Carpathians. All authors discussed the results and commented on the manuscript. All authors have read and agreed to the published version of the manuscript.

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