VILNIUS UNIVERSITY

NATURE RESEARCH CENTRE

INSTITUTE OF BOTANY

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THYMUS PULEGIOIDES CHEMOTYPES IN LITHUANIA: DISTRIBUTION, INFLUENCE OF EDAPHIC AND CLIMATIC FACTORS, ALLELOPATHIC CHARACTERISTICS

Summary of doctoral dissertation

Biomedical sciences, ecology and environmental sciences (03 B)

Vilnius, 2018

The research has been carried out during 2013–2017 in Nature Research Centre, Institute of Botany.

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The official defence of the dissertation will be held at the open session of the Council:

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The official defence of the dissertation will be held at the open session of the Council at 11 a.m on 17 of May, 2018 at the Baltoji hall Institue of Botany, Nature Research Centre.
Address: Žaliųjų Ežerų Str. 49, LT - 08406, Vilnius, Lithuania.
The summary of doctoral dissertation was sent on 13 of April, 2018.
The dissertation is available at the libraries of Vilnius University and Nature Research Centre and VU Web site address: https:// *www.vu.lt/lt/naujienos/ivykiu-kalendorius*

VILNIAUS UNIVERSITETAS
GAMTOS TYRIMŲ CENTRAS
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Daktaro disertacijos santrauka

Biomedicinos mokslai, ekologija ir aplinkotyra (03 B)

Vilnius, 2018

Disertacija rengta 2013–2017 metais Gamtos tyrimų centro, Botanikos institute.

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Disertacija bus ginama viešajame disertacijos gynimo tarybos posėdyje 2018 m. gegužės mėn. 17 d. 11 val. Gamtos tyrimų centro Botanikos instituto Baltojoje salėje.
Adresas: Žaliųjų Ežerų g. 49, LT - 08406, Vilnius, Lietuva.

Disertacijos santrauka išsiuntinėta 2018 m. balandžio mėn. 13 d.
Disertaciją galima peržiūrėti Vilniaus universiteto ir Gamtos tyrimo centro bibliotekose ir VU interneto svetainėje adresu: https:// *www.vu.lt/lt/naujienos/ivykiu-kalendorius*

INTRODUCTION

Most plants accumulate essential oils, of oil consistency and mixtures of volatile organic compounds of specific odour. These secondary metabolites help plants to attract pollinators, to protect themselves from herbivores and parasites (THOMPSON et al., 2003; GERSHENZON & DUDAREVA, 2007). Essential oils also have an allelopathic effect, and, therefore, such plants can compete with other plants in the communities (INDERJIT et al., 2011; LINHART et al. 2015). Essential oils are important not only to the plant itself, but also to the human life: they and/or essential oil-bearing plants are often used in pharmaceutics, food industry, cosmetics, perfumery, can be used as insecticides (SOTOMAYOR et al., 2004; KAY et al., 2013; NEZDALI et al., 2014).

The genus *Thymus* is one of the most abundant genera of the Lamiaceae family. This genus includes medicinal, aromatic, essential oil-bearing plants of practical significance: applied in pharmacy, food industry, cosmetics and perfumery. Large thyme (*Thymus pulegioides*) is a medicinal, aromatic, essential oil-bearing plant that spreads throughout Europe (RADUŠIENĖ & JANULIS, 2004; LOŽIENĖ et al., 2007; RADONIC & MASTELIC, 2008). Like other species of the genus *Thymus*, *T. pulegioides* is characterized by chemical polymorphism, i.e. different individuals of the same species synthesize essential oils of different chemical composition. Individuals bearing essential oils of the similar chemical composition belong to the same chemotype. Chemical polymorphism prevents collecting of homogeneous (standardized) raw material in natural habitats, which is very relevant in the pharmaceutical industry, but is a good basis for the selection of individuals, their reproduction and cultivation. According to chemical compounds dominating in essential oils, eight chemotypes of large thyme have been defined: thymol, carvacrol, thymol/carvacrol, geraniol, α-terpinyl acetate, linalool, fenchone and cis-sabinene hydrate (MARTONFI, 1992; GRENDHAl et al., 2008; MICHET et al., 2008). In Lithuania, six chemotypes of *T. pulegioides* have been defined: thymol, carvacrol, thymol/carvacrol, geraniol, α-terpinyl acetate and linalool (MOCKUTĖ & BERNOTIENĖ, 1999, 2001; LOŽIENĖ et al., 2002, 2003). The main components of essential oils of *T. pulegioides*, which are main determinants of *T. pulegioides* chemotypes and are found in the largest amounts – thymol, carvacrol, geraniol, linalool and α-terpinyl acetate are biologically active compounds and have a fairly wide range of applications. The monoterpenic phenol thymol together with isomer carvacrol have wide spectrum of antioxidant and antimicrobial activity, therefore, are widely used in pharmaceutical and food industries (for food preservation) (YANISHLIEVA et al., 1999; DORMAN & DEANS, 2000; PINA-VAZ et al., 2004; LOŽIENE et al., 2007; PIRBALOUTI et al., 2013). The acyclic monoterpene alcohol geraniol and linalool are widely used in cosmetics, perfumery for their intense aroma as well as the insect repellents (MUMCOUGLU et al., 1996; RADONIC & MASTELIC, 2008; BEIER et al., 2013). The α-terpinyl acetate has antibacterial properties (HUGH & TAN, 2005; ZOUAGHI et al., 2015).

Although the chemotype of the plant (and the composition of essential oil accumulated therein) is determined by genetic factors, it has been observed that environmental factors may influence the change in chemotype diversity in space (VERNET et al., 1986; TARAYRE & THOMPSON, 1997; THOMPSON et al., 2003; KAYA et al., 2013). Quantitative and qualitative composition of essential oils can be influenced by climatic conditions (temperature, amount of precipitation, sunshine duration), soil chemical composition, altitude above the sea level (SANGWAN et al., 2001; KAYA et al., 2013; NURZYNSKA-WIERDAK, 2013; FARHAT et al., 2016). The edaphic and climatic conditions in the territory of Lithuania are also different. In the western part of Lithuania, the marine climate is more pronounced, while in the eastern – the continental climate. Climatic sub-districts distinguished in Lithuania differ by the average annual temperature, the amount of precipitation per year, the duration of snow preservation (BUKANTIS, 1994a). Larger amounts of humus, mobile phosphorus, mobile potassium and most of the micro-elements are found in soils in Central Lithuania, while higher acidity of soil is found in Western Lithuania (BUIVYDAITĖ, 2001; LIEKIS, 2001). Therefore, these differences in climatic and edaphic factors in the territory of Lithuania may affect not only the quantitative and qualitative composition of essential oils of *T. pulegioides*, but also different distribution of chemotypes. The influence of edaphic and climatic factors on the prevalence of chemotypes of this species of the *Thymus* genus in the space, on quantitative and qualitative composition of essential oils has not been clarified, and peculiarities of distribution of *T. pulegioides* chemotypes in the territory of Lithuania have not been fully investigated until now.

Monoterpenes synthesized in plants of the genus *Thymus* and released into the environment are potent allelochemicals characterized by allelopathic and/or autoallelopathic effects (TARAYRE et al., 1995; VOKOU et al., 2003; JENSEN & EHLERS, 2010) and may affect the species composition of plant communities (EHLERS et al., 2014; LINHART et al., 2015). Consequently, different *T. pulegioides* chemotypes synthesizing and releasing into the environment the essential oils of different chemical composition can affect differently not only the species of other plants, but also the germination of its own seeds and the development of sprouts, which may also affect the prevalence of *T. pulegioides* chemotypes. Allelopathic and autoallelopathic characteristics of different *T. pulegioides* chemotypes have not been studied until now.

The aim of the study was to determine the influence of edaphic and climatic factors on the composition the essential oils of *T. pulegioides* and distribution of chemotypes; and to evaluate the allelopathic activity of essential oils of different chemical composition.

Main objectives:

1. To determine the influence of edaphic conditions (soil chemical composition, pH) on quantitative and qualitative composition of essential oils of *T. pulegioides* and distribution of chemotypes.
2. To establish the effects of climatic conditions (temperature, amount of precipitation, photoactive solar radiation, sunshine duration) on quantitative and qualitative composition of essential oils of *T. pulegioides* and distribution of chemotypes.
3. To evaluate allelopathic and autoallelopathic activity of *T. pulegioides* essential oils of different chemical composition.

The defended statements:

1. The macro- and microelement composition of the soil as well as pH of the soil influence quantitative and qualitative composition of essential oils of *T. pulegiodes* and distribution of chemotypes.
2. Climatic conditions impact quantitative and qualitative composition of essential oils of *T. pulegioides* and prevalence of chemotypes. The chemotypes of *T. pulegioides* are differently distributed in various climatic sub-districts of Lithuania.

3. Essential oils of different *T. pulegioides* chemotypes (geraniol, carvacrol, α-terpinyl acetate) have different effects on seed germination and the development of sprouts of *T. pulegiodes* and other plant species.

Novelty and relevance. In the present study, the quantitative and qualitative composition of essential oils of *T. pulegioides* growing in different climatic sub-districts of Lithuania and prevalence of chemotypes were evaluated for the first time. Until now, the chemical composition of essential oils of *T. pulegioides* has not been studied in different climatic sub-districts of Lithuania; it has been investigated in more detail in the vicinities of Vilnius (LOŽIENĖ, 1997; MOCKUTĖ & BERNOTIENĖ, 1999, 2001, 2005; LOŽIENĖ et al., 2002, 2003).

MÁRTONFI et al. (1994) have studied the effects of chemical composition of the soil (total nitrogen, ammonium, nitrates, carbonates, carbon, phosphorus, potassium, calcium and magnesium) and soil pH on the distribution of *T. pulegioides* chemotypes in Slovakia. PLUHAR and co-authors (2005) have investigated the influence of soil chemical composition (humus, nitrogen, potassium, cadmium, manganese sulfur, iron and copper) on the quantitative and qualitative composition of essential oils of *T. pannonicus*. Influence of the amount of nitrogen in the soil on quantitative and qualitative composition of essential oils of *T. vulgaris* has also been studied (BARANAUSKIENĖ et al., 2003). However, the effects of soil chemical elements such as aluminum, copper, iron, manganese, sodium, sulphur, silicon, titanium and zinc on qualitative and quantitative composition of essential oils of *T. pulegioides* and prevalence of chemotypes in the species were investigated for the first time.

Most of the studies related to the influence of climatic factors on the chemical composition of essential oils of the genus *Thymus* plants have been performed under laboratory conditions. For example, the effects of different moisture regimes on the composition of essential oils of *T. vulgaris* and *T. hyemalis* have been investigated under laboratory conditions (LETCHAMO et al., 1994; JORDAN et al., 2003; SOTOMAYOR et al., 2004). Similar studies under natural (field) conditions are performed very rarely. Only the data on precipitation and temperature effects on thymol, carvacrol, p-cymene, γ-terpinene, linalool and α-pinen in plants of *T. pannonicus* have been found in the studies performed under field conditions in Algeria (HADEF et al., 2007). Influence of environmental factors on the quantitative composition of essential oils of *T. pulegioides* plants growing (cultivated) under natural (field) conditions has not been investigated until now. The influence of photoactive solar radiation on the quantitative and qualitative composition of essential oils in the plants of the genus *Thymus* growing in natural environment was evaluated for the first time in the present study. Also, the influence of temperature and precipitation on the quantitative and qualitative composition of essential oils of geraniol and α-terpinyl acetate chemotypes was evaluated for the first time in the plants of the genus *Thymus* grown under field conditions.

In the present study, the allelopathic and autoallelopathic effects of essential oils of different *T. pulegioides* chemotypes and their main components were tested for the first time through air and water, and compared with each other.

The obtained results and conclusions are important for fundamental scientific purposes: the chemical composition of essential oils of *T. pulegioides* and distribution of chemotypes was investigated throughout the territory of Lithuania; it was clarified what edaphic and climatic factors and how they influence the synthesis of essential oils of *T. pulegioides* and distribution of different chemotypes.

Practical significance. In previous years, the studies have shown that *T. pulegioides* species can be promising for cultivation, since it accumulates significant amounts of valuable biologically active compounds and can produce a considerable above-ground yield (LOŽIENĖ, 2009). Therefore, the results of this research can be important in the cultivation of different chemotypes of *T. pulegioides*: soil selection, fertilizing of plants, predicting not only the yield of essential oil of different chemotypes, but also the amounts of industrially valuable chemical compounds such as geraniol, carvacrol, linalool in the raw material depending on climatic conditions of the season. By growing under natural conditions, in some places of Lithuania, the individuals of one group of chemotypes will grow better and will synthesize more basic components, while in other places of Lithuania – individuals of another group of chemotypes: in the western part of Lithuania, individuals of phenolic chemotypes will grow better.

Presentation of the results. Four scientific publications were prepared on the dissertation work theme; reports were made at four international and three national conferences.

Volume and structure of the dissertation. The dissertation consists of the following chapters: Introduction, Literature Review, Materials and Methods, Results and Discussion, Summation of Results, Conclusions, References (210 literature sources). Volume of the dissertation is 165 pages, 21 tables and 19 figures. The dissertation is written in Lithuanian, and the summary is English.

Acknowledgements. I express my gratitude to Dr. Kristina Ložienė for all the assistance in preparing the doctoral dissertation. I sincerely thank Sigitas Juzėnas, a lecturer at the Centre for Life Sciences of the University of Vilnius, for consultations on multifactorial statistical analysis. I am also sincerely thanking Dr. Kęstutis Arbačiaukas for consultations on redundancy analysis (RDA). I thank the biologist Giedrė Abrutienė for assistance carrying out allelopathic investigations. I am thankful to Dr. Rita Butkienė for assistance in identifying chemical compounds in essential oils of *T. pulegioides*. I am grateful to Dr. Ričardas Taraškevičius for helping to identify the chemical elements of *T. pulegioides* in the soil of the habitats. I am grateful to Dr. Jolita Radušienė, Dr. Jurga Būdienė and Dr. Juozas Labokas for valuable suggestions and comments. I am also grateful to Dr. Zofija Sinkevičienė for contribution in describing the unfamiliar plant species in *T. pulegioides* habitats. I am thankful to Violeta Ptašekienė for linguistic assistance .

MATERIALS AND METHODS

One hundred and thirty one different habitats of *T. pulegioides* were investigated in Lithuania (Fig.1). The study of the habitats was carried out in all climatic sub-districts of Lithuania: in Aukštaitija climatic sub-district 21 habitats of *T. pulegioides* were studied, in Dzūkija – 20, Mūša-Nevėžis – 40, the Nemunas Lowland – 22, Sūduva – 4, Venta – 5, Žemaičiai – 17. In Pajūris and Pajūris Lowland only one habitat of *T. pulegioides* was found in each climatic sub-district. This species was not found in the Curonian Spit climatic sub-district. The map of climatic sub-districts of Lithuania is presented in Fig. 2.

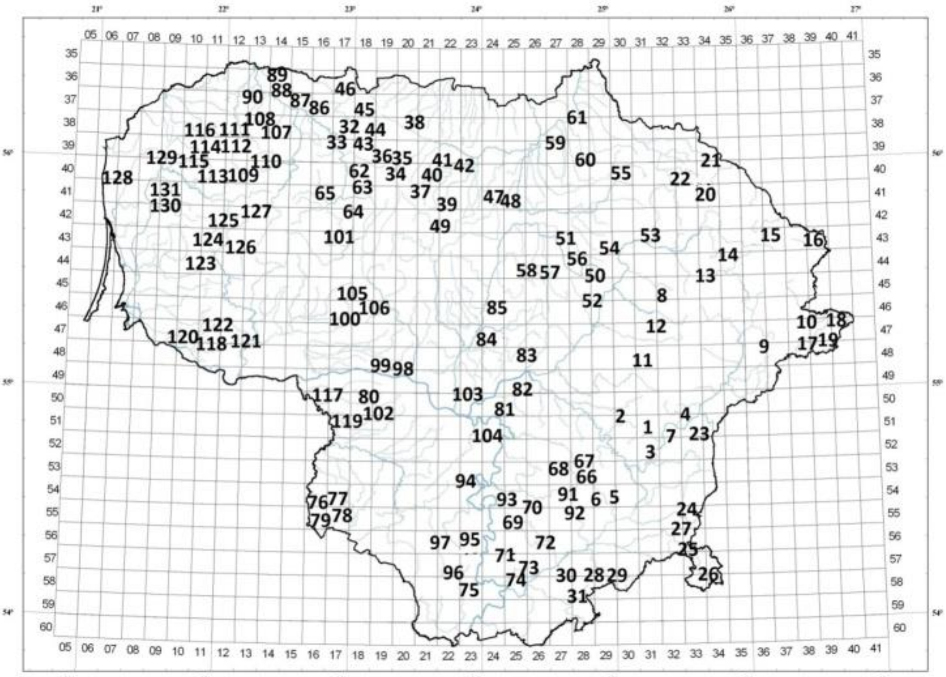


Fig. 1. Distribution of the habitats of *Thymus pulegioides* in Lithuania. The habitats of Aukštaitija climatic sub-district: 1–4, 7–23; Dzūkija: 5, 6, 24–31, 66–75; Mūša-Nevėžis: 32–65, 83–85, 101, 105, 106; the Nemunas Lowland: 80–82, 91–100, 102–104, 117–122; Sūduva:. 76–79; Venta: 86–90; Žemaičiai: 107–116, 123–127, 129, 130; Pajūris: 128; Pajūris Lowland: 131.

Fig.2. Climatic sub-districts of Lithuania according to K.Kaušyla (BUKANTIS, 1994 b).

Each habitat was evaluated topographically (relief, inclination of slope, exposition of slope). The study of meadows was performed according to the methodology of BRAUN-BLANQUET (1964). In each habitat the areas occupied by the herbs, trees, bushes, lichen and moss as well as the whole area of a habitat were evaluated. Relative lightness of the habitats was defined visually. The raw material of *T. pulegioides* was collected during full flowering in July. The plant raw material was collected in the following way: same selected mass of aerial part of *T. pulegioides* was cut from each individual plant growing in the habitat and mixed. The weight was selected depending on each habitat abundance and/or size of individual plants in the habitat: 10 g of aerial part from each individual plant of *T. pulegioides* was cut in abundant and big habitats, but 30–50 g – in small habitats. The samples of soil were collected from each habitat separately and dried at room temperature. Each sample of topsoil was prepared in the following way: 5–9 subsamples (subject to the area of the habitat; each subsample ~ 100 g) were taken from the depth of 10–15 cm (from plant rhizosphere) by the envelope principle with the distance of 1 m from the central point of habitat and mixed (homogenized).

The essential oil of each sample of the plant material (from leaves and flowers; stems were not used) was isolated separately by hydrodistillation in the Clevenger apparatus for two hours (European Pharmacopoeia, 2008). The distillation of essential oil was carried out from two to six repeats in each habitat. Essential oils solutions of 1% were prepared in the mixture of diethyl ether and n-pentane (1:1) for further investigations. The identification of the main compounds of essential oils were based on an GC-2010 Plus instrument equipped with a GC-QP 2010 Plus (Shimadzu) series mass selective detector in the electron impact ionisation mode at 70eV. Separation of compounds was performed on fused silica (100% dimethyl polysiloxane) column (30 m × 0.25 mm ID × 0.25 µm film thickness) (Restek, USA), splitless injection; helium as carrier gas at a flow rate of 1.6 ml/min, injector and detector temperatures 250 °C. GC oven temperature was programmed as follows: initial temperature of 50 °C (isothermal for 7 min) was increased to 250 °C at the rate of 4 °C/min to (isothermal for 5 min) and further increased at the rate of 30 °C/min to 300 °C, the final temperature kept for 2 min. Identification of the investigated compounds was based on the comparison of retention indices (RIs) (ADAMS, 2007), computer mass spectra library (NBS75K) and analytical standards of these (Sigma-Aldrich). The retention indices were determined relative to the retention times of a series of n-alkanes (C_7–C_{30}) with linear interpolation. The quantitative analysis of main compounds was carried out using a FOCUS GC (Thermo Scientific) gas chromatograph with a flame ionisation detector (FID) on the silica capillary column TR-5MS (30 m × 0.25 mm ID × 0.25 μm film thickness) (Thermo Electron Corporation, USA) under the same chromatographic conditions. The percentage amounts of the investigated compounds were recalculated according to the areas of the FID chromatographic peaks assuming that all constituents of the essential oil comprise 100%.

The content of humus in the samples of topsoil was estimated by oxidation method, using potassium bichromate and sulphur acid solution, the content of mobile potassium (K_2O) and mobile phosphorus (P_2O_5) – by flame photometry using 0,2 M HCl solution, the soil pH – electrometrically using 1 M KCl solution. The elements of soil (Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, Co, S, Si, Ti, Zn, Cl) were estimated the by energy-dispersive x-ray fluorescence analysis (EDXRF equipment SPECTRO SCEPOS).

The meteorological data (temperature (C°), rainfall (mm), photosynthetically active solar radiation (PAR) (Mj/m^2) and sunshine duration (h)) were obtained from the meteorological bulletins (2006–2016) of the nearest station of meteorology of the Lithuanian Hydrometeorological Service under the Ministry of Environment. The photosynthetically active solar radiation was calculated by multiplying the total solar radiation a factor of 0.52 (BUKANTIS, 1994 a). For the analysis we used the mean values of these factors (June, July is a period of *T. pulegioides* blooming and $\Sigma_{\text{April–July}}$ is a period from the beginning of vegetation to flowering) obtained in 2006–2016. The meteorological data were analysed in nineteen meteorological stations: Biržai, Dotnuva, Dūkštas, Kaunas, Klaipėda, Kybartai, Laukuva, Lazdijai, Nida, Panevežys, Raseiniai, Šiauliai, Šilutė, Telšiai, Ulmergė, Varėna, Vilnius and Mažeikiai. The average of the above-mentioned meteorological factors was calculated in case several meteorological stations belonged to one climatic sub-district.

Individuals of three different chemotypes (geraniol, linalool and carvacrol) of *T. pulegioides* were grown at the Field Experimental Station of the Nature Research Centre, Vilnius, Lithuania (54°46′ N and 25°17′ E) in 2008–2013. In 2014–2017 the individuals of four different chemotypes were grown ate the Station: four belonged to carvacrol chemotype, two – to geraniol, one – to linalool and one – to α-terpinyl acetate. The aerial parts of separate individuals were collected separately at full flowering stage and were dried at room temperature. The isolation and analysis of essential oils was done annually.

The effect of essential oils of three different *T. pulegioides* chemotypes (geraniol, cavacrol, α-terpinyl acetate) and analytical standards of their main compounds (geraniol, carvacrol, and α-terpinyl acetate) on germination and growth of radicles of *T. pulegioides* was investigated through air and water. For comparison the effect of the mentioned above essential oils and their main chemical compounds was also analysed for germination and growth of radicles of monocotyledons (*Poa pratensis* L. and *Phleum pratense* L.) and dicotyledons (*Trifolium pratense* L. and *Hypericum perforatum* L.). The investigation through air was carried out in the following way: the filter paper was moistened with 15 ml of distilled water and put into Petri dish; the small aluminium containers with 3 μL pure essential oil or pure analytical standard were placed in the centre of Petri dish (on the moistened filter paper). The investigation through water was carried out as follows: filter paper was moistened with 3μL of essential oil or analytical standard dissolved in 1% of Tween 20 and put into Petri dish. By 100 seeds were overspread gradually in each Petri dish (every treatment was carried out in three repeats). Seeds were germinated at room temperature in May. The germinated seeds were counted each experiment day; the experiment was closed, when the seeds ceased to germinate. The total number of germinated seeds was calculated after the experiment and recalculated to the final germination percentage (GP). Mean daily germination (MDG), which is the index of daily germination, was calculated from the following equation MDG = GP/d, where GP – the final germination percentage, d – days to the maximum of final germination. Germination index (GI) was calculated as follows: $GI = \sum G_t/T_t$, where G_t– the number of seeds germinated per day t,T_t– the number of days from the beginning of the experiment. Then radicles were measured in each Petri dish (10 radicles in every Petri dish, 30 radicles per treatment) to establish the length of radicles after the experiment.

The calculation of means, standard deviations (SD), coefficients of variation (CV), minimum and maximum values was carried out. Cluster analysis by Ward's method was performed by grouping the habitats of *T. pulegioides* based on chemotype-determining compounds (carvacrol, thymol, p-cymene, γ-terpinene, carvacrol methyl ether, thymol methyl ether, geraniol, geranial, nerol, neral, linalool, and α-terpinyl acetate). Spearman’s rank correlation coefficients (r) were to used analyse the influence of soil chemical composition and meteorological factors on *T. pulegioides* essential oil yield and composition. Spearman’s rank correlation analysis was also performed to test the relationship between the coverage of *T. pulegioides* and phytocoenological class as well as total herb layer coverage.

PCA was used to analyse the influence of soil chemical composition on the amount of essential oil and the main compounds that form the chemotypes of *T. pulegioides*. Simple linear regression was used to analyse the interaction between meteorological factors and the amounts of essential oils as well as main chemical compounds accumulating in them. One-way ANOVA analysis was used to assess whether the populations of *T. pulegioides* in different climatic sub-districts differed in terms of quantitative and qualitative composition of essential oils and prevalence of chemotypes. Turkey's post-hoc criterion was used to assess the differences. The partial Mantel test was performed to determine whether the temperature and rainfall influence the amount of main chemotype-determining compounds. The Kruskal-Wallis test was performed in order to evaluate whether the germination of seeds of *T. pulegioides* and other plant species was affected differently by essential oils of different *T. pulegioides* chemotypes and analytical standarts. To evaluate whether essential oils of different chemotypes of *T. pulegioides* and analytical standarts differently affected the development of the roots of the studied plant species one-way analysis of variance (one-way ANOVA) was performed. Redundancy analysis (RDA) was performed to find out what part of chemotype-determining compounds could be explained by the environmental factors (edaphic and climatic) and what had greater influence on the prevalence of these chemical compounds (edaphic or climatic factors). The analysis included only those *T. pulegioides* chemotype-determining compounds that showed significant correlation with the investigated edaphic factors or Mantel test showed that temperature and rainfall influence the distribution of these compounds.

Statistical data processing was carried out withthe STATISTICA®7 and MS Excel 2007 software. The partial Mantel test was done by PAST version 3.16. Redudancy analysis (RDA) was performed by BRODGAR version 2.7.5.

RESULTS AND DISCUSSION

The habitats of *T. pulegioides*

The studied *T. pulegioides* habitats (N = 131) were ascribed to meadow communities belonging to four phytocoenological classes: *Molinio-Arrhenatheretea elatioris* R. Tx. 1937, *Festuco-Brometea erecti* Br.-Bl. et R. Tx. 1943, *Trifolio-Geranietea sanguinei* Th. Müller 1961 and *Koelerio-Corynephoretea canescentis* Klika et Novak 1941. More than half (59 %) of the investigated *T. pulegioides* habitats were attributed to grassland communities of the *Molinio-Arrhenatheretea elatioris* class. The coverage of *T. pulegioides* in the studied communities ranged between + and 3 values according to the Braun-Blanquet (1964) scale. In more than half (58.8 %) of the habitats the coverage of *T. pulegioides* reached + value. In the three fourths of the investigated habitats of *T. pulegioides* the grassland coverage varied from 80 % to 95 %. More than half of the *T. pulegioides* habitats occurred on the slopes of southern, southeastern or southwestern exposition with different inclinations.. The Spearman rank correlation analysis showed significant positive correlation between *T. pulegioides* coverage and phytocoenological classes ($r = 0.33$, $p < 0.05$): the sequence of frequencies +>1>2>3 of *T. pulegioides* coverage in the habitats corresponds to that of the following phytocoenological classes: *Molinio-Arrhenatheretea elatioris* > *Trifolio-Geranietea sanguinei* > *Festuco-Brometea erecti* > *Koelerio-Corynephoretea canescentis*, i. e. higher probable of low coverage of *T. pulegioides* is possible in the habitats belonging to the phytocoenological class *Molinio-Arrhenatheretea elatioris* than in the habitats belonging to the *Festuco-Brometea erecti* class.

Significant correlation between phytocoenological classes and main chemical compounds (carvacrol, thymol, geraniol, linalool and α-terpinyl acetate) of the essential oils of *T. pulegioides* was not established. This means that the investigated *T. pulegioides* chemotypes may be prevalent in various plant communities.

Variation in quantitative and qualitative composition of essential oils of *Thymus pulegioides* between different habitats and influence of climatic factors on quantitative and qualitative composition of essential oils and distribution of chemotypes

The habitats *of T. pulegioides* were found in nine (out of ten) Lithuanian climatic sub-districts: in the Curonian Spit climatic sub-district this species was not found. In the investigated 131 habitats of *T. pulegioides,* the mean of essential oil yield was 0.61 ± 0.21 %. The amount of essential oil of *T. pulegioides* growing wild in Lithuania is known to vary from 0.33% to 1.21% (RADUŠIENĖ & JANULIS, 2004). It was established that the highest mean amount of essential oil was determined in Pajūris climatic sub-district (0.75 ± 0.00 %) and the lowest – in Venta climatic sub-district (0.52 ± 0.19 %) (Fig.3).

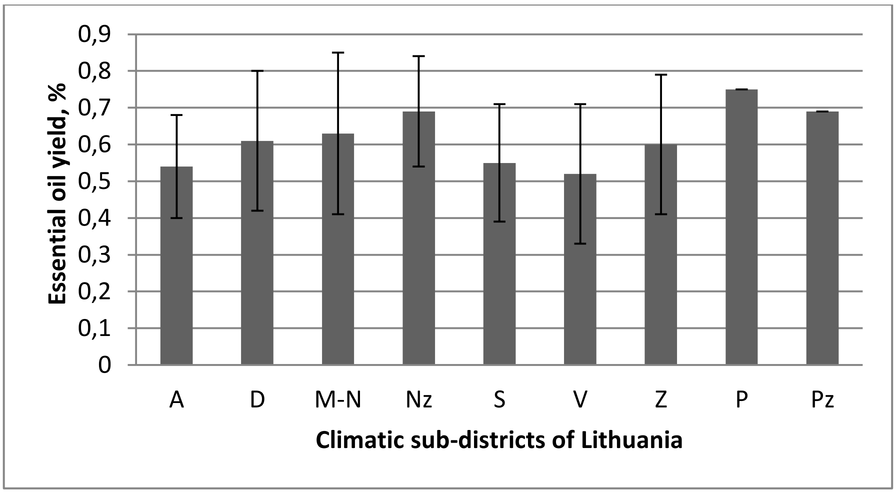


Fig. 3. Mean amount of essential oil in *T. pulegioides* populations in different climatic sub-districts of Lithuania. Explanations: A – Aukštaitija climatic sub-district, D – Dzūkija, M-N – Mūša-Nevėžis, Nz–the Nemunas Lowland, S – Sūduva, V – Venta, Z – Žemaičiai, P – Pajūris, Pz – Pajūris Lowland.

After analysing the chemical composition of essential oils we identified 55 chemical compounds. Literature data suggest that different chemical compounds in the essential oils of *T. pulegioides* vary from 26 to 86 (LOŽIENĖ et al., 2002; MOCKUTĖ & BERNOTIENĖ, 2005; RADONIC & MASTELIC, 2008; DE MARTINO et al., 2009). We established that oxygenated monoterpenes are the main chemical compounds in the essential oils.

Carvacrol was abundant and most frequent chemical compound (17.66 ± 9.43 %) in the essential oils of *T. pulegioides*, and dominated in seven sub-districts of Lithuania (Table 1). Therefore it can be suggested that in Lithuania the individuals of carvacrol chemotype dominate. The one-way analysis of variance (one-way ANOVA) and Tuekey's post-hoc test demonstrated that in Aukštaitija and Sūduva climatic sub-districts, the populations of *T. pulegioides* were significantly different by the amount of carvacrol in the essential oils, in these populations we established the lowest and the highest mean amounts of carvacrol, respectively (Table 1, Fig. 4). The partial Mantel test demonstrated that temperature and rainfall impact the distribution of carvacrol chemotype (R= 0.12; p = 0.02).

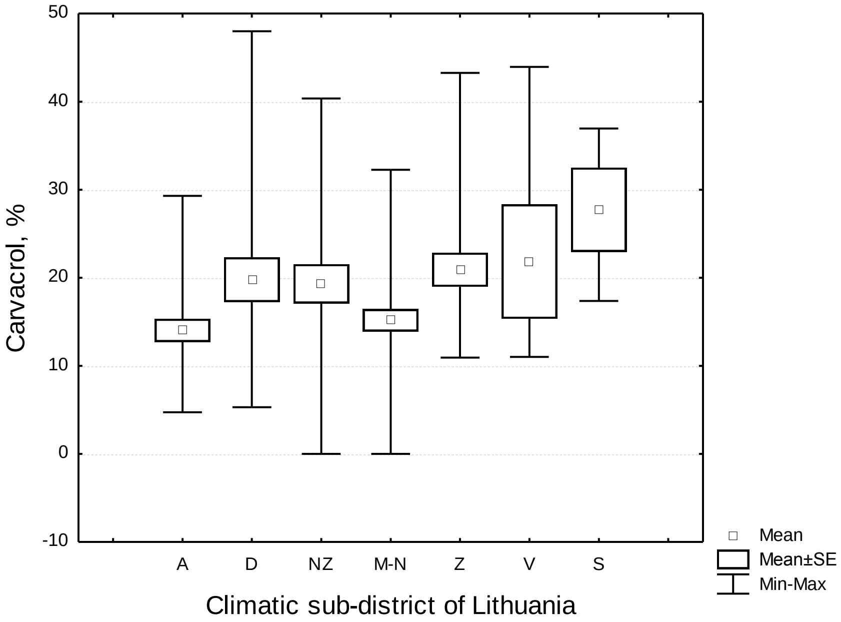


Fig.4. Distribution of the amount of carvacrol in the essential oils of *T. pulegioides* populations in different climatic sub-districts of Lithuania. The climatic sub-districts are marked as in Fig. 3.

The mean amount of thymol (carvacrol isomer) is 5.5 times lower in the essential oils of *T. pulegioides* in Lithuania. The one-way analysis of variance (one-way ANOVA) and Tuekey's post-hoc test demonstrated that in Aukštaitija (in this climatic sub-district the highest amount of thymol in the essential oils was established) and in the Nemunas Lowland climatic sub-districts the populations of *T. pulegioides* significantly differed by the amount of thymol in the essential oils (Table 1). This suggests that in Aukštaitija climatic sub-district the individuals of thymol chemotype of *T. pulegiodes* are more distributed than those of carvacrol. This can be attributed to the fact that the individuals of carvacrol chemotype are more sensitive to cold than the individuals of thymol chemotype (GOUYON et al., 1986; THOMPSON et al., 2003). Namely the lowest temperature in January was observed in Aukštaitija climatic sub-district compared to other climatic sub-districts of Lithuania (BUKANTIS, 1994 a). The amounts of carvacrol and thymol precursors (p-cymene and γ-terpinene) were similar to those of carvacrol. *T. pulegioides* populations in Venta climatic sub-district (very low amount of p-cymene was established in this climatic sub-district) significantly differed from Aukštaitija and Mūša- Nevėžis climatic sub-districts by the amount of p-cymene in the essential oils. In

Venta climatic sub-district the mean of rainfall in June (52.9 ± 22.92 mm), July (62.4 ± 31.50 mm) and average total rainfall in April–July (229.4 ± 72.78 mm) was the lowest compared to other sub-districts of Lithuania which were included in the one-way analysis of variance (one- way ANOVA). The study of the individuals of carvacrol chemotype at the Field Experimental Station demonstrated that there was statistically significant correlation between the amount of p-cymene and total rainfall in April–July ($R = 0.94$, $p < 0.05$). This suggests that higher amount of rainfall stimulated the synthesis of p-cymene. The populations of *T. pulegioides* in Žemaičiai climatic sub-district significantly differed from those in Aukštaitija, Mūša- Nevėžis and Venta climatic sub-districts by the amount of γ-terpinene in the essential oils. The populations in Žemaičiai climatic sub-district distinguished by the highest mean and max value of γ-terpinene in the essential oils compared to other climatic sub- districts (Table 1). In Žemaičiai climatic sub-district the highest mean values of sunshine duration in June, July and total values in April–July were established. Higher amount of light is known to stimulate the synthesis of monoterpene in plants of the *Thymus* genus (SHARAFZADEN, 2012).

The mean amount of geraniol in the studied habitats ($N = 131$) was 2.7 times lower than that of cavacrol (Table 1). This suggests that the individuals of geraniol chemotype are rarer in Lithuania than the individuals of carvacrol chemotype. The highest means of geraniol and geranial were established in Venta climatic sub-district, the highest means of nerol and neral were in Aukštaitija climatic sub-district and the lowest means of these geraniol chemotype characterizing chemical compounds were established in the essential oils of *T. pulegioides* individuals growing in Žemaičiai climatic sub-district. Geraniol chemotype-determining chemical compounds were characterized by higher variation in the amount. By the amount of geraniol chemotype, the main compound geraniol statistically significantly differed in the populations *of T. pulegioides* in Žemaičiai and Venta climatic sub-districts. Žemaičiai climatic sub-district differed by the lowest mean amount of geraniol and Venta climatic sub-district by the highest values (Table 1, Fig.5). The partial Mantel test demonstrated that temperature and rainfall influence the distribution of geraniol chemotype ($R= 0.17$; $p = 0.02$). It also was noticed that in the habitats of *T. pulegiodes* occurring in the eastern and middle part of Lithuania the amounts of geraniol and biogenetically related compounds (geranial, neral, nerol) were higher (Table1, Figs. 2,5).

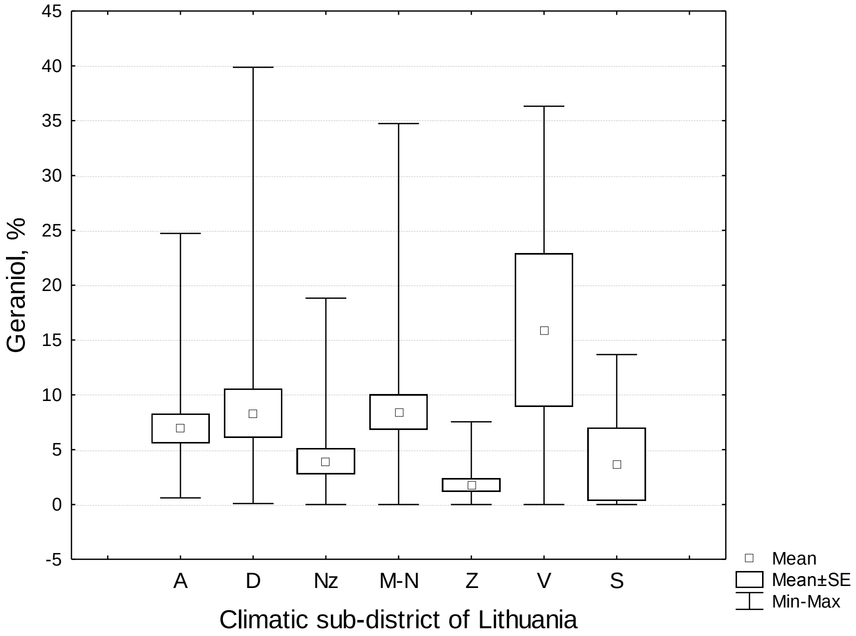


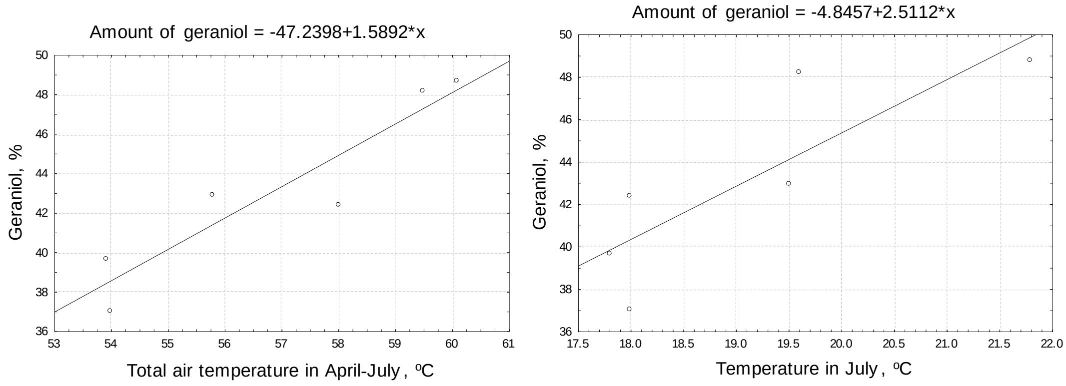
Fig. 5. Distribution of the amount of geraniol in essential oils of *T. pulegioides* populations in different climatic sub-districts of Lithuania. The climatic sub-districts are marked as in Fig. 3.

Table 1.Variations in main chemical compounds of essential oils of *Thymus pulegioides* in different climatic sub-districts of Lithuania and the one-way analysis of variance (ANOVA) of the investigated climatic sub-districts (df = 6) (SD – standard deviation, F – Fisher criterion, p –significance level).

Chemical compound		Climatic sub-district of Lithuania							One-way ANOVA	
		Aukštaitija	Dzūkija	Mūša-Nevėžis	Nemunas Lowland	Venta	Sudūva	Žemaičiai	F	p
Carvacrol	Mean ± SD, %	14.02 ± 6.01	19.78 ± 11.31	15.17 ± 7.98	19.32 ± 10.39	21.84 ± 14.52	27.73 ± 9.55	20.92 ± 7.94	2.73	0.02*
	Min – max, %	4.79–29.30	5.30 –48.00	0.00–32.26	0.00– 40.36	11.02 – 43.95	17.37 –39.95	10.94 – 43.27		
Thymol	Mean ± SD, %	4.74 ± 3.45	1.25 ± 1.69	3.70 ± 6.12	0.64 ± 1.39	0.37 ± 0.45	0.43 ± 0.67	4.52 ± 3.94	3.62	0.00*
	Min – max, %	0.20 –10.94	0.00–5.77	0.00–27.86	0.00–5.73	0.00–1.10	0.00–1.22	0.08–11.64		
p - Cymene	Mean ± SD, %	17.12 ± 7.81	10.67 ± 6.97	12.12 ± 9.58	10.10 ± 8.18	0.99 ± 0.41	9.68 ± 1.23	11.59 ± 4.43	3.53	0.00*
	Min – max, %	3.29–29.93	1.00–28.87	0.39–38.49	0.14–22.60	0.57–1.53	8.32–11.15	5.07–21.35		
γ - Terpinene	Mean ± SD, %	12.97 ± 4.45	16.78 ± 9.46	15.53 ± 9.80	18.93 ± 7.74	10.75 ± 5.40	20.08 ± 2.96	23.92 ± 7.83	3.85	0.00*
	Min – max, %	2.67–21.87	1.69–29.67	0.00–40.10	0.48–27.61	4.78–17.53	17.89–24.29	9.76–42.60		
Geraniol	Mean ± SD, %	6.93 ± 6.20	8.33 ± 10.10	8.43 ± 10.12	3.95 ± 5.57	15.91 ± 15.63	3.68 ± 6.68	1.48 ± 2.52	2.89	0.01*
	Min – max, %	0.60–24.72	0.09–39.87	0.00–34.74	0.00–18.82	0.00–36.32	0.00–13.67	0.00–7.54		
Geranial	Mean ± SD, %	1.39 ± 1.26	1.68 ± 1.87	1.37 ± 1.63	0.52 ± 0.68	1.93 ± 1.88	0.51 ± 0.93	0.41 ± 0.65	2.73	0.02*
	Min – max, %	0.10–4.71	0.00–5.03	0.00–6.57	0.00–2.15	0.00–4.28	0.00–1.89	0.00–1.88		
Nerol	Mean ± SD, %	5.75 ± 5.27	5.73 ± 7.18	4.02 ± 4.66	1.50 ± 2.25	5.19 ± 5.24	1.28 ± 2.23	1.09 ± 1.63	3.30	0.00*
	Min – max, %	0.51–17.17	0.00–20.57	0.00–16.71	0.00–8.78	0.00–12.40	0.00–4.78	0.00–5.39		
Neral	Mean ± SD, %	4.35 ± 4.11	4.32 ± 5.53	3.97 ± 6.21	1.15 ± 1.74	4.10 ± 4.18	1.00 ± 1.89	0.75 ± 1.27	2.29	0.04*
	Min – max, %	0.09–13.37	0.00–15.46	0.00–34.92	0.00–6.61	0.00–9.87	0.00–3.84	0.00–3.95		
Linalool	Mean ± SD, %	0.99 ± 3.13	0.74 ± 1.37	2.45 ± 8.14	4.35 ± 12.93	0.41 ± 0.40	0.36 ± 0.10	0.29 ± 0.15	1.00	0.43
	Min – max, %	0.00–14.25	0.17–6.46	0.16–40.37	0.10–57.75	0.00–1.08	0.25–0.49	0.00–0.50		
α-Terpinyl acetate	Mean ± SD, %	0.48 ± 0.45	0.56 ± 1.18	2.20 ± 6.84	2.90 ± 12.26	0.00	0.01 ± 0.02	0.004 ± 0.01	0.53	0.78
	Min – max, %	0.19–2.34	0.00–4.33	0.00–43.56	0.10–57.50	0.00	0.00–0.04	0.00–0.05		

* – significant differences between climatic sub-districts(selected significance level p<0.05).

The study of the individuals of *T. pulegioides* geraniol chemotype in the Field Experimental Station revealed a statistically significant positive correlation between the amount of geraniol and total air temperature in April–July ($R = 0.90$, $p < 0.05$) and temperature in July ($R = 0.89$, $p < 0.05$) (Fig.6).



A B

Fig.6. Relationship between the amount of geraniol in geraniol chemotype and total air temperature in April–July (A) and temperature in July (B).

The average temperature in July and total air temperature in April–July in the eastern part of Lithuania was higher than in the western part of the country, except for Sūduva climatic sub-district. The individuals of geraniol chemotype are more likely to distribute where the temperature in vegetation period is higher and where the climate is more continental. Likewise under the lower total photosinthetically active solar radiation in April–July the experimental individuals of geraniol chemotype accumulated higher amount of this monoterpene alcohol in essential oils ($R = -0.83$, $p < 0.05$). It can be assumed that higher temperature and lower photosynthetically active solar radiation contribute to the accumulation of geraniol.

The individuals of *T. pulegioides* linalool and α-terpinyl acetate chemotypes are rare in Lithuania: the means of linalool and α-terpinyl acetate were lower than 2 % in the studied habitats ($N = 131$) of *T. pulegioides*. These chemical compounds had the highest variation in the amounts compared to other compounds, because linalool and α-terpinyl acetate chemotypes can segregate in particular habitats more than carvacrol or geraniol chemotypes. The highest means of linalool and α-terpinyl acetate were established in the Nemunas Lowland and Mūša-Nevėžis climatic sub-districts (Table 1). The impact of climatic factors on the accumulation of linalool and α-terpinyl acetate in the essential oils was not assessed.

Literature data suggest that in Lithuania the phenolic (in particular carvacrol) chemotypes of *T. pulegioides* are more distributed (LOŽIENĖ, 1997; LOŽIENĖ et al., 2002; MOCKUTĖ & BERNOTIENĖ, 1999, 2001, 2005). In the countries of Europe, phenolic cavacrol and thymol chemotypes of the genus *Thymus* are more distributed, and thymol and cavacrol are the most abundant chemical compounds in the essential oils (PINTO et al., 2006; GRENDAHL et al., 2008; DE MARTINO et al., 2009; RADULESCU et al., 2009; PAVEL et al., 2010). In essential oils of *T. pulegioides*, considerable amounts of compounds such as myrcene, β-cariophyllene, β-bisabolene, cariophyllene oxide, α-terpinene, borneol, and cis-β-guaiene were also assessed.

Influence of soil chemical composition on quantitative and qualitative composition of *Thymus pulegioides* essential oils and prevalence of chemotypes

The soil acidity in the investigated *T. pulegioides* habitats varied from slightly acid (pH= 5.1) to medium alkaline (pH= 8.3), the amounts of humus – from 0.9 % to 7.2 %, the amounts of mobile potassium – from 35 to 368 mg/kg, and the amounts of mobile phosphorus – from 22 to 680 mg/kg. In Slovakia, previous studies have shown that plants of this species can tolerate soil of different acidity and humus content as well as different amounts of potassium and phosphorus in it (MÁRTONFI et al., 1994, 1996).

It was found that the accumulation of essential oil in *T. pulegioides* can be promoted by higher levels of humus in the soil ($R = 0.18$; $p < 0.05$) and limited by higher amounts of manganese ($R = –0.22$; $p < 0.05$) and cobalt in the soil ($R = –0.32$; $p < 0.05$). It was established that higher amounts of mobile phosphorus ($R = 0.18$; $p < 0.05$) and sulphur ($R = 0.27$; $p < 0.05$) in the soil can stimulate carvacrol accumulation in essential oils of *T. pulegioides,* and higher amounts of natrium in the soil ($R = –0.20$; $p < 0.05$) can limit the accumulation of this chemical compound. The accumulation of γ-terpinene in essential oils of *T. pulegioides* can be positively influenced by higher amounts of mobile phosphorus ($R = 0.23$; $p < 0.05$), whereas p-cymene accumulation can be limited by higher amounts of phosphorus ($R = –0.18$; $p < 0,05$) and sulphur (R =

–0.20; $p < 0.05$) in soil (Fig. 7). Therefore higher amounts of sulphur and mobile phosphorus in the soil can positively influence, whereas higher amounts of natrium negatively impact the distribution of *T. pulegioides* carvacrol chemotype.

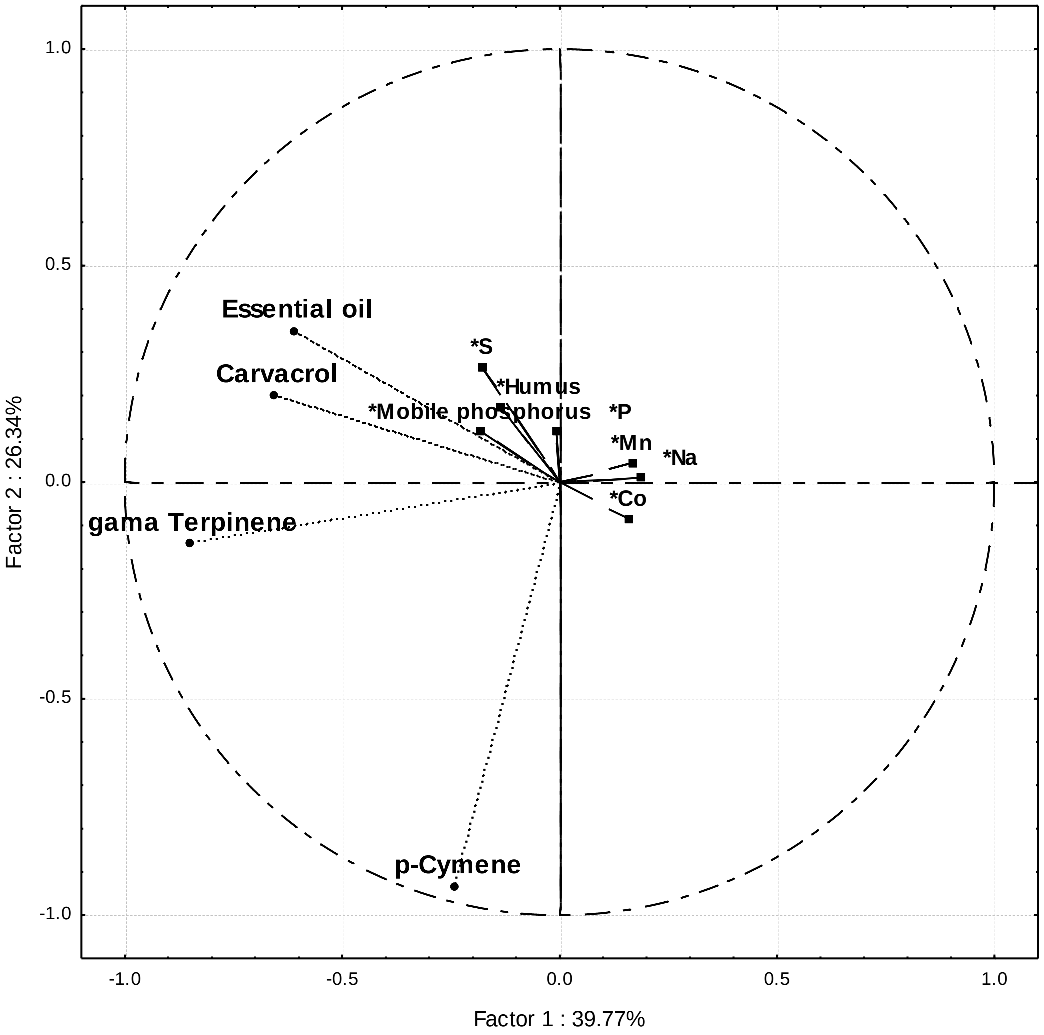


Fig. 7. Impact of humus content, mobile phosphorus and and soil chemical elements :such as manganese (Mn), natrium (Na), sulphur (S), cobalt (Co) and phosphorus on the amount of essential oil in *T. pulegioides* and the content of p-cymene, γ-terpinene and cavacrol in it .

The studies demonstrated that geraniol and geranial negatively correlated with the amounts of humus ($R = -0.19$; $R = - 0.20$; $p < 0.05$) in the soil of *T. pulegioides* habitats. The accumulation of geraniol and biogenetically related compounds (geranial, nerol, neral) may be due to the increased content of natrium or may be limited by higher amounts of chlorine in the soil. Higher amounts of sulphur in the soil can negatively influence the accumulation of geranial, nerol and neral (R = – 0.23; R = –0.25; R = –0.24; $p < 0.05$). Higher amounts of calcium in the soil can limit the accumulation of nerol and neral in essential oils of *T. pulegioides* (R = –0.22; R= –0.19; $p < 0.05$) (Fig.8). It seems that the individuals of geraniol chemotype of *T. pulegioides* can be more widely distributed where there are higher amounts of natrium and lower amounts of humus, chlorine, sulphur and calcium in the soil.

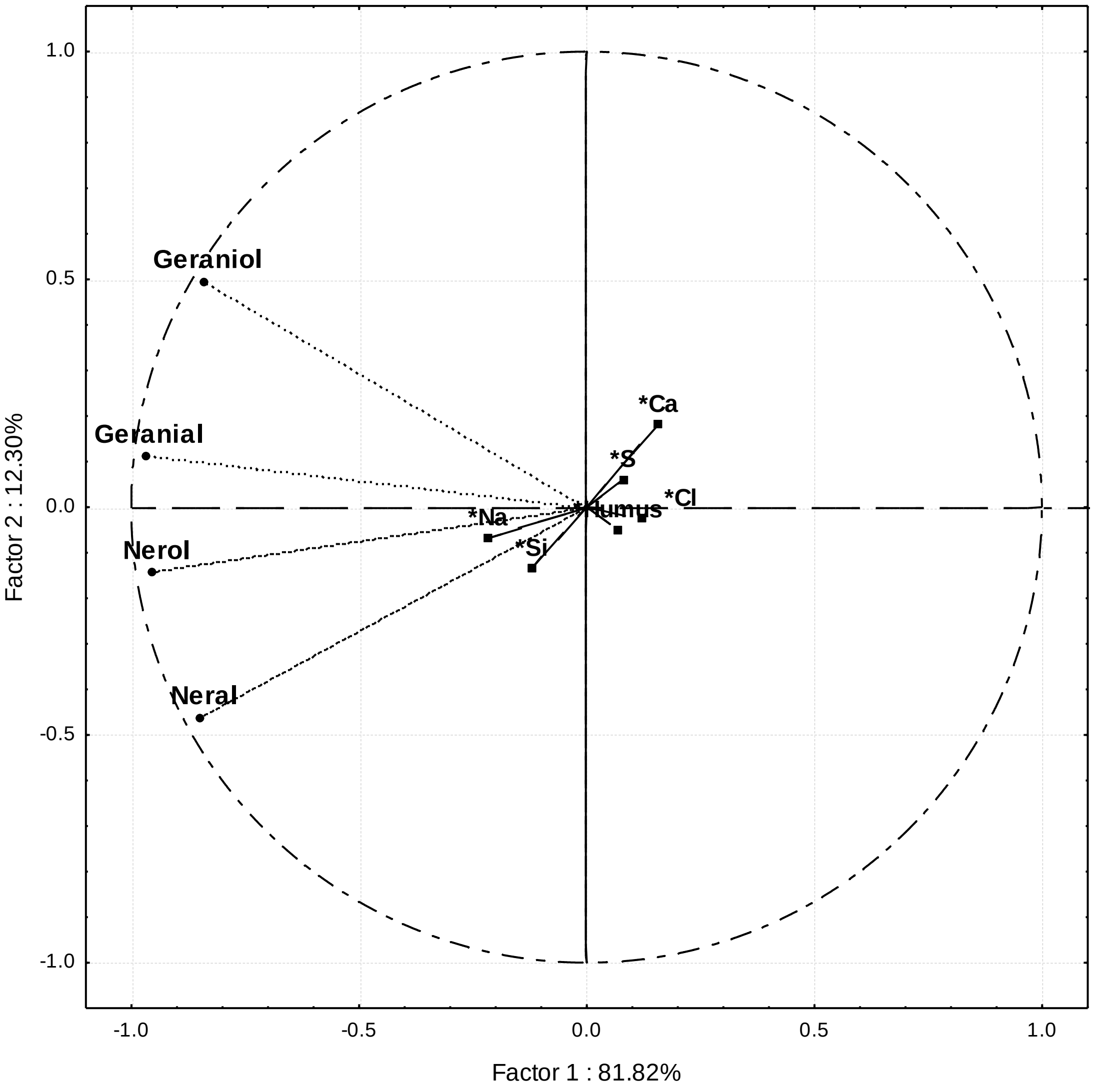


Fig. 8.Impact of humus content and chemical elements of soil, such as calcium (Ca), sulphur (S), chlorine (Cl), natrium (Na) and silicon (Si) on the accumulation of geraniol and biogenetically related compounds (geranial, nerol, neral) in essential oils of *T. pulegioides*.

Influence of edaphic factors on the levels of thymol, linalool, α-terpinyl acetate and on the prevalence of these chemotypes was not established.

Influence of edaphic and climatic factors on distribution of geraniol and carvacrol chemotypes of ***T. pulegioides***

The redundancy analysis (RDA) showed that the studied edaphic and climatic factors could explain the prevalence of 17% of carvacrol and geraniol chemotype-determining compounds of *T. pulegioides.* The first two axes of this analysis explain 13.50% of the results obtained (first axis – 9.58%, second axis – 3.92%) (Fig.9). According to the literature data, the qualitative composition of essential oils is determined by the genetic properties of the plant and the environmental conditions (temperature, rainfall, lightness, chemical composition of the soil) (SANGWAN et al., 2001: BADI et al., 2004; FIGUIREIDO et al., 2008 a; NEZHADALI et al., 2014). Based on the results obtained, it can be assumed that genetic factors have greater influence on the prevalence of carvacrol and geraniol chemotypes of *T. pulegioides*. Thymol, linalool and a-terpinyl acetate chmotype-determining compounds were not included in this analysis since the influence of edaphic and climatic factors on the distribution of these compounds was not established. Edaphic factors had a stronger influence on the prevalence of *T. pulegioides* carvacrol and geraniol chemotypes (based on axial length) than temperature and rainfall (Fig. 9). It is also clear from this graph that the influence of rainfall on the distribution of these chemotypes was lower than that of the temperature. This analysis also confirms that higher content of sulphur and mobile phosphorus (P_2O_5) in the soil has positive,whereas that of sodium has a negative influence on the prevalence of *T. pulegioides* carvacrol chemotype, and that the individuals of geraniol chemotype can be detected more often with higher content of natrium, less frequently with higher content of chlorine in the soil (Fig. 9). As the analysis also confirmed the positive relationship between the content of geraniol and the total temperature in July and April–July, the distribution of *T. pulegioides* geraniol chemotype is positively influenced by a higher temperature, therefore, the individuals of this chemotype are more frequent in the continental part of Lithuania, where the continental climate predominates with characteristic hotter summers.

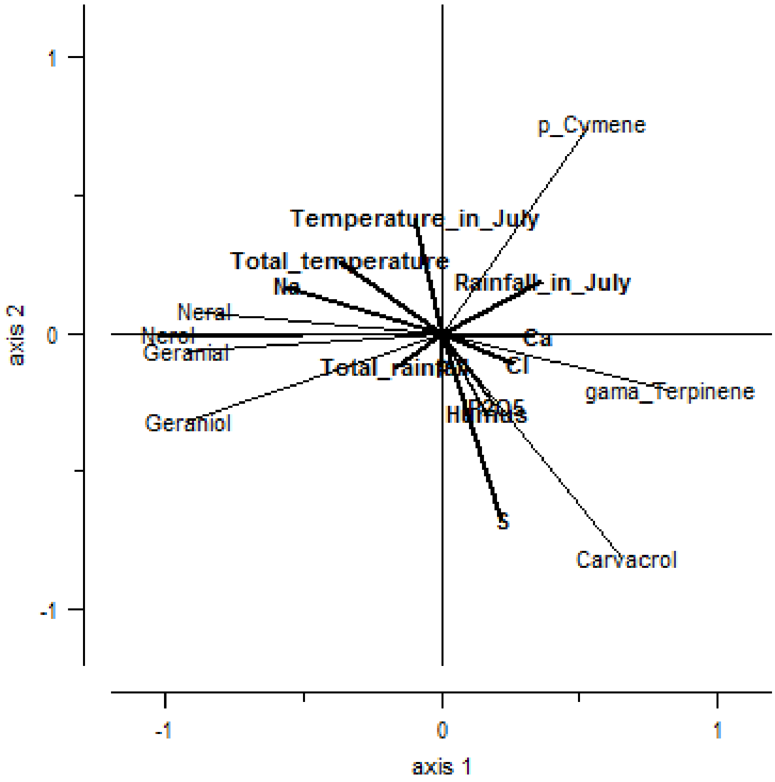


Fig. 9. Dependence of the amounts of *T. pulegioides* geraniol and carvacrol chemotype-determining compounds in essential oils on edaphic and climatic factors (redundancy analysis).

Allelopathic and autoallelopathic effect of essential oils of different *Thymus pulegioides* chemotypes

The study of the effect of essential oils of *T. pulegioides* chemotypes such as geraniol (55.99% of essential oil), carvacrol (23.71%), α-terpinyl acetate (64.22%) and analytical standards of their main compounds (geraniol, carvacrol, α-terpinyl acetate) through the air and water on seed germination of *T. pulegioides* demonstrated that seeds germination was most inhibited under the effect of essential oil of geraniol chemotype through the air. Under the effect of essential oil of *T. pulegioides* α-terpinyl acetate chemotype through the air, seeds germination was equal to control, but the germination index was about two times lower than that of the control. This shows a weaker autoallelopathic effect of essential oil of this chemotype, because germination of seeds requires more time. Comparing the autoallelopathic effect of essential oils of *T. pulegioides* chemotypes through the air, it was found that the effects of essential oil of geraniol chemotype and essential oil of α-terpinyl acetate chemotype were statistically significant. Under effect of essential oils, strong synergistic effect of their components was evident, because the effect of the analytical standards of geraniol, carvacrol and α-terpinyl acetate on *T. pulegioides* seeds germination through the air was not statistically significant. The effect of all tested essential oils and analytical standards through the water was not statistically significant (Table 2).

Table 2. Effect of essential oils of different *Thymus pulegioides* chemotypes and analytical standards of the main compounds characterizing these chemotypes on germination of *T. pulegioides* seeds.

Effect	Chemical compounds		GP ± SD., %	MGD, %	GI, seeds/ day
Control			58.33 ± 13.43	5.83	21.18
Through air	Essential oil	Geraniol chemotype	33.25 ± 9.24^{ac}	3.08^{ac}	4.48^{ac}
		Carvacrol chemotype	43.67 ± 2.13^{a}	4.37^{a}	9.18^{a}
		α-Terpinyl acetate chemotype	58.20 ± 4.46^{ab}	5.30^{ab}	11.62^{ab}
	Analytical standard	Geraniol	48.17 ± 5.88^{A}	4.24^{A}	8.44^{A}
		Carvacrol	44.25 ± 13.07^{A}	4.17^{A}	10.66^{A}
		α-Terpinyl acetate	64.33 ± 11.72^{A}	6.43^{A}	18.47^{A}
Through water	Essential oil	Geraniol chemotype	54.33 ± 3.21$^{a'}$	5.43$^{a'}$	17.08 $^{a'}$
		Carvacrol chemotype	55.00 ± 13.08$^{a'}$	5.50$^{a'}$	17.01$^{a'}$
		α-Terpinyl acetate chemotype	53.33 ± 3.51$^{a'}$	5.67 $^{a'}$	16.91$^{a'}$
	Analytical standard	Geraniol	54.67 ± 3.51$^{A'}$	5.47$^{A'}$	14.33$^{A'}$
		Carvacrol	49.00 ± 7.55$^{A'}$	4.90$^{A'}$	17.96$^{A'}$
		α-Terpinyl acetate	52.00± 7.00$^{A'}$	5.20$^{A'}$	14.36$^{A'}$

Notes: GP – germination percentage, MGD – mean daily germination, GI – germination index, SD – standard deviation. Letters by numbers (3–5 columns) denote significant and insignificant differences: lower letters between essential oils and capital letters between analytical standards. Letters without apostrophes mark the effect through air and letters with apostrophes – effect through water. Different letters denote significant differences and same letters – insignificant differences.

The growth of *T. pulegioides* radicles was most inhibited by analytical standard of carvacrol through the air. By comparing the effect of essential oils through the air, we established that the growth of radicles was also inhibited by the essential oil of carvacrol chemotype. This indicates that this phenolic compound is a potent inhibitor of the growth of *T. pulegioides* radicles. Meanwhile, the seedlings exposed to the essential oil of α-terpinyl acetate chemotype through the air developed better than in control, i.e. stimulatory effect was evident. Under the impact of essential oils through the water, the essential oil of α-terpinyl acetate chemotype and α-terpinyl acetate standard distinguished by the highest inhibitory effect, while the lowest effect was assessed under the impact of essential oil of carvacrol chemotype and carvacrol standard (Fig.10). Monoterpenes accumulated in the species of the *Thymus* genus possess an inhibitory effect, which may vary slightly under different environmental conditions and may be involved in both intra- and inter-species competition (GOUYON et al., 1986; TARAYRE et al., 1995). Our study showed that essential oil of *T. pulegioides* geraniol chemotype had the most inhibitory effect, while the individuals of *T. pulegioides* geraniol chemotype in the territory of Lithuania are less prevalent than those of the carvacrol chemotype (individuals of *T. pulegioides* carvacrol chemotype are the most common in Lithuania).

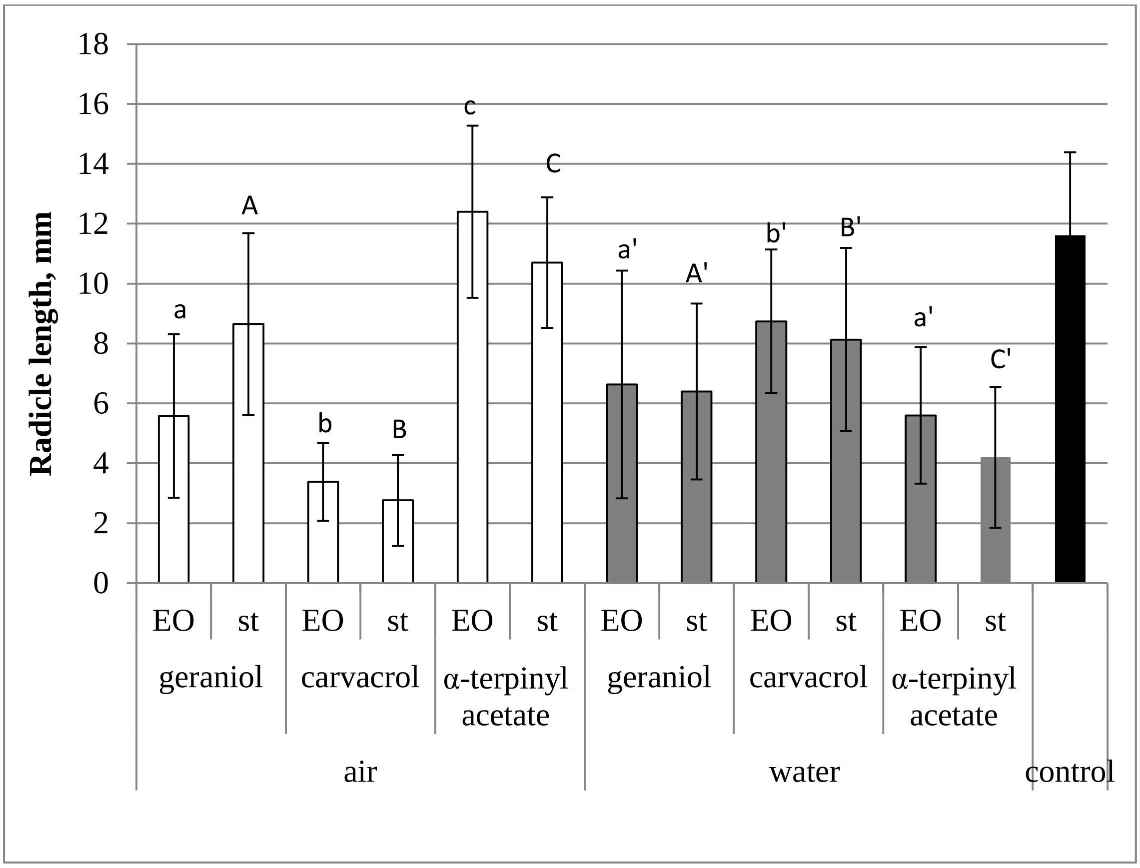


Fig.10. Effect of essential oils (EO) of different *Thymus pulegioides* chemotypes and analytical standarts (st) of the main compounds characterizing these chemotypes on the length of *T. pulegioides* radicles. Statistically significant or insignificant differences are marked as in Table 2.

After studying the effect of essential oils of different *T. pulegioides* chemotypes and analytical standards on seeds germination of other plant species, almost in all cases we determined lower germination rates (compared to control). Only total germination and mean daily germination of the seeds of *Trifolium pratense* when impacted through the water by essential oils of all *T. pulegioides* chemotypes and analytical standards of carvacrol and α-terpinyl acetate were higher than in control. Different plant species reacted differently to the essential oils of the same *T. pulegioides* chemotypes: *Poa pratensis* seeds germination and the growth of radicles was most inhibited by the essential oil of α-terpinyl acetate chemotype, acting through the water. *Phleum pratense* and *Hypericum perforatum* seeds germination was most inhibited by the essential oil of geraniol chemotype, while the growth of radicles was inhibited by the essential oil of carvacrol chemotype. *T. pulegioides* geraniol chemotype had the strongest allelopathic effect (on seed germination), while the growth of radicles was most strongly inhibited by carvacrol chemotype. According to the literature data, phenolic chemotypes of the *Thymus* genus have a stronger toxic effect than nonphenolic (LINGHART & THOMPSON, 1999; STAHL-BISKUP &SAEZ, 2002).

CONCLUSIONS

1. The above-ground part of large thyme (*T. pulegioides*) accumulates on average, 0.61±0.21% of essential oil, the most frequent and most abundant component of which is carvacrol (17.66±9.43%). Carvacrol chemotype dominates in all climatic sub-districts of Lithuania (except for Pajūris and Pajūris Lowland climatic sub-districts). The dominance of carvacrol chemotype in Lithuania does not constitute an exception to the entire natural range of *T. pulegioides* in Europe. The second most abundant chemical compound is geraniol (6.57±8.70%); the chemotype of geraniol dominates in Venta climatic sub-district. The chemotypes of linalool and a-terpinyl acetate are rare in Lithuania.

2**.** The accumulation of essential oil in the plants of *T. pulegioides* is positively influenced by higher levels of humus, and negatively affected by higher levels of manganese and cobalt in the soil. Higher amounts of natrium in the soil can promote, whereas chlorine and sulphur may inhibit the accumulation of geraniol and biogenetically related compounds as well as the distribution of *T. pulegioides* geraniol chemotype. The accumulation of carvacrol and the proliferation of *T. pulegioides* carvacrol chemotype can be stimulated by higher levels of sulphur and mobile phosphorus, and suppressed by higher levels of natrium in the soil.

3. *T. pulegioides* populations in different climatic sub-districts of Lithuania differ (p <0.05) in the amount of thymol, carvacrol and their precursors, and in the amount of geraniol and its biogenetically related compounds accumulated in the individuals of *T. pulegioides*. Higher photosynthetically active solar radiation (PAR) during vegetation period stimulates the accumulation of essential oil in the individuals of *T. pulegioides* carvacrol chemotype. The prevalence of geraniol chemotype is caused by higher temperatures during butonization and vegetation period, while higher content of PAR during vegetation period suppress the distribution of this chemotype; higher amount of essential oil in this chemotype is accumulated at a higher temperature during vegetation period. The accumulation of essential oil in the individuals of linalool chemotype is due to longer sunshine duration in the vegetation period.

4. *T. pulegioides* seeds germination is inhibited mostly by essential oil of geraniol chemotype, acting through the air; the development of *T. pulegioides* sprouts is promoted by the essential oil of α-terpinyl acetate chemotype, acting through the air. The

essential oils of different *T. pulegioides* chemotypes may have different effect on seeds germination of monocotyledons and dicotyledons. It was determined that essential oils of the studied chemotypes almost did not inhibit seeds germination of red clover (*Trifolium pratense*), and most of all inhibited the seeds germination of meadow grass (*Poa pratensis*).

5. It was revealed that the investigated edaphic and climatic factors may explain by 17 % of the prevalence of *T. pulegioides* chemotype-determining compounds. Edaphic factors make greater influence on the prevalence of *T. pulegioides* chemotypes than climatic factors.

REZIUMĖ

T. pulegioides augavietės buvo tirtos 9-iuose (iš 10-ies) Lietuvos klimatiniuose parajoniuose: Kuršių Nerijos klimatiniame parajonyje ši rūšis aptikta nebuvo. Ištyrus 131 augavietę nustatyta, kad jose *T. pulegioides* vidutiniškai sukaupia 0,61 ± 0,21 % eterinio aliejaus, didžiausias vidutinis eterinio aliejaus kiekis buvo nustatytas Pajūrio (0,75 ± 0,00 %), o mažiausias – Ventos klimatiniame parajonyje (0,52 ± 0,19 %). Dažniausias ir gausiausias cheminis junginys eteriniuose aliejuose – karvakrolis (17,66 ± 9,43 %), kuris dominavo beveik visuose klimatiniuose parajoniuose (išskyrus Pajūrio ir Pajūrio žemumos klimatinius parajonius). Antras pagal gausumą cheminis junginys – geraniolis (6,57 ± 8,70 %), kuris dominavo Ventos klimatiniame parajonyje. Linalolio ir α-terpinilo acetato chemotipai Lietuvos teritorijoje yra reti; linalolio ir α-terpinilo acetato vidutiniai kiekiai *T. pulegioides* eteriniuose aliejuose atitinkamai sudaro 1,66 ± 6,59 % ir 1,48 ± 6,96 %.

Nustatyta, kad Lietuvos klimatinių parajonių *T. pulegioides* populiacijos skyrėsi ($p < 0,05$) pagal *T. pulegioides* augaluose sukaupiamų fenolinių junginių (timolio ir karvakrolio) bei jų pirmtakų, pagal geraniolio ir biogenetiškai susijusių junginių bei pagal β-bisaboleno, kariofileno oksido, cis-β-guaieno, α-terpineno kiekius. Pritaikius Tjukio kriterijų buvo nustatyta, kad pagal karvakrolio kiekį *T. pulegioides* eteriniame aliejuje statistiškai reikšmingai skyrėsi Aukštaitijos ir Sūduvos klimatinių parajonių *T. pulegioides* populiacijos: jose buvo nustatyta atitinkamai mažiausia (14,02 ± 6,01 %) ir didžiausia (27,73 ± 9,55 %) karvakrolio vidutinė reikšmė. Didesnis FAR kiekis balandžio–liepos mėnesiais skatina eterinio aliejaus kaupimąsi karvakrolio chemotipo augaluose ($R = 0,89$, $p < 0,05$). Pagal timolio kiekį *T. pulegioides* eteriniame aliejuje statistiškai reikšmingai skyrėsi Aukštaitijos ir Nemuno žemumos klimatinių parajonių populiacijos; Aukštaitijos klimatinio parajonio *T. pulegioides* populiacijose buvo nustatyta didžiausia timolio vidutinė reikšmė. Pagal geraniolio kiekį *T. pulegioides* eteriniame aliejuje statistiškai reikšmingai skyrėsi Žemaičių ir Ventos klimatinių parajonių populiacijos, kur buvo nustatyta atitinkamai mažiausia (1,48 ± 2,52 %) ir didžiausia (15,91 ± 15,63 %) geraniolio vidutinė reikšmė. Geraniolio kaupimąsi ir *T. pulegioides* geraniolio chemotipo plitimą skatina aukštesnė liepos mėnesio temperatūra ($R = 0,90$, $p < 0,05$) ir balandžio–liepos mėnesių suminė temperatūra ($R = 0,89$, $p <$

0,05), o riboja didesnis suminis fotosintetiškai aktyvios saulės spinduliuotės kiekis balandžio–liepos mėnesiais (R = – 0,83, p < 0,05); didesnis eterinio aliejaus kiekis šiame chemotipe sukaupiamas esant aukštesnei balandžio–liepos mėnesių suminei temperatūrai (R = 0,90, p < 0,05). Eterinio aliejaus kaupimąsi linalolio chemotipo individuose skatina didesnė balandžio–liepos mėnesių saulės spindėjimo trukmė (R = 0,83, p < 0,05). Linalolio ir α-terpinilo acetato kiekių sintezei klimatinių faktorių įtaka nustatyta nebuvo.

T. pulegioides eterinio aliejaus kaupimuisi teigiamą įtaką daro didesnis humuso (R = 0,18, p < 0,05), o neigiamą – didesni mangano (R = – 0,22, p < 0,05) bei kobalto (R = – 0,32, p < 0,05) kiekiai dirvožemyje. Natrio kiekio didėjimas dirvožemyje gali skatinti, o chloro ir sieros kiekio didėjimas –slopinti geraniolio ir biogenetiškai susijusių junginių kaupimąsi ir *T. pulegioides* geraniolio chemotipo plitimą. Karvakrolio kaupimąsi ir *T. pulegioides* karvakrolio chemotipo plitimą gali skatinti didesnis sieros (R = 0,27, p < 0,05) bei judriojo fosforo kiekis (R = 0,18, p < 0,05), o slopinti – didesnis natrio kiekis dirvožemyje (R = – 0,20, p < 0,05). Timolio, linalolio ir α-terpinilo acetato sintezei bei šių chemotipų plitimui dirvožemio cheminės sudėties įtaka nustatyta nebuvo.

Tirtieji edafiniai ir klimatiniai faktoriai gali paaiškinti 17 % *T. pulegioides* karvakrolio ir geraniolio chemotipus lemiančių junginių paplitimo. Edafiniai veiksniai turėjo stipresnę įtaką karvakrolio ir geraniolio chemotipų paplitimui nei temperatūra ir krituliai. Kritulių įtaka minėtų chemotipų paplitimui buvo mažesnė nei temperatūros.

Buvo nustatyta, kad labiausiai *T. pulegioides* sėklų dygimą slopina geraniolio chemotipo eterinis aliejus, veikdamas per orą, o skatina grynas α-terpinilo acetatas, veikdamas per orą. Labiausiai *T. pulegioides* daigų vystimąsi slopina grynas karvakrolis, veikdamas per orą, o skatina α-terpinilo acetato chemotipo eterinis aliejus.

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