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EFFECT OF NATURAL FERTILIZATION AND THE TYPE OF SUBSTRATE ON THE BIOLOGICAL VALUE OF THE THYME HERB (*Thymus vulgaris* L.)

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Abstract. One of the oldest medicinal plants in the world is thyme (*Thymus vulgaris* L.). On the biological value of this plant is affected by biotic and abiotic conditions, which include fertilizers and soil type. The aim of the present study was to determine the effect of organic fertilization and the type of substrate on the biological value of the thyme herb. Plants were grown in a greenhouse in a different type substrates: peat and peat + sand, using varying amounts of manure 10, 20, 30 g·dm^{-3} of substrate. In the obtaining raw material was determined the content of essential oil, dry matter, L-ascorbic acid, total nitrogen and proteins. There was a significant increase in the N-total and protein contents in thyme herb after application of increasing doses of examined manure independently of the test substrate. Applied natural fertilization and type of substrate did not affect significantly the content of essential oil in *Thymus vulgaris* L. herb. The highest content of L-ascorbic acid and dry matter was obtained for thyme after application of the lowest dose of manure, regardless of the substrate used.

Key words: essential oil, protein, L-ascorbic acid, nitrogen, manure, medicinal plant

INTRODUTION

In recent years, many herbal plants are discovered anew. This is due to the prevailing global trend of lifestyle change or a return to the natural sources of nutrition and treatment. The impact on this situation are common civilization diseases, decrease in a natural resistance to infections and increased susceptibility to various allergies. The re-learning about herbs is affected by development of the food industry, especially

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the increase in demand for vegetable additives in order to raise the taste and aroma, as well as a favorable impact of their preservative and antioxidant properties [Lawrencet 2001]. Increasingly, herbal plants are introduced into the diet as preparations or herbal mixtures. In the group of spices and herbal medicines, has thyme is fairly important.

Thymus vulgaris L. is a perennial plant belonging to the family Lamiaceae; originating from Mediterranean areas, mainly Spain, southern France and Italy, as well as North Africa [Morales 2002]. Thyme is a plant with therapeutic properties, comprising biologically active substances such as essential oil (0.32–4.9%), flavonoids, tannins, phenolic acids, triterpene compounds, bitter, saponins, sugars, vitamins, and minerals [Baranauskiene et al. 2003, Jabbari et al. 2011]. The greatest concentration of these compounds is recorded in the raw pharmacopoeial material, i.e. the herb. *Thymi herba* and extracted essential oil (*Thymi oleum*) show disinfection, germicidal [Lakis et al. 2012], fungicidal [Omidbeygi et al. 2007], anti-inflammatory [Oceaňa and Reglero 2012], spasmolytic [Śliwińska et al. 2001], expectoration and antioxidant properties, as well as it stimulates the stomach activity and the secretion of digestive juices [Nurzyńska--Wierdak 2015]. Qualitative and quantitative composition variability of biologically active substances produced by the plant depends on genetic [Miguel et al. 2004], ontogenetic [Hudaib et al. 2002], and environmental factors [Porte and Godoy 2008], including fertilization and soil type.

Herbal plants have different requirements for various nutrients, which is associated with the growth and development of a given species. Fertilization, besides a significant impact on the amount of crop yield, also modifies its quality. Natural fertilizers are designed to increase the nutrient contents in the soil, improve air and water relations, improve capacity of the sorption complex of the ground, and promote development of microorganisms [Khalid and Shafei 2005, Nurzyński 2008, Najm et al. 2012]. This type of fertilizer used in the experiment is manure. Natural fertilizers are used in organic production, where synthetic fertilizers and pesticides are not applied, which favors launching the plant's own defense system against pathogens, involving, among others, the production of secondary active substances. Content of nitrogen in the soil also affects the production of these compounds by plants. In the system of organic cultivation, wherein the nitrogen is supplied to the soil in the form of organic fertilizers, its availability is lower. As a consequence, plants in the first instance produce carbon-containing compounds (simple and complex sugars, as well as secondary plant metabolites (vitamins, essential oils). In an environment where the availability of nitrogen is higher, e.g. in a conventional production, due to the use of readily water-soluble nitrogen fertilizers application, plants produce mainly nitrogen compounds such as amino acids, proteins or alkaloids [Coley et al. 1985, Brandt and Molgaard 2001, Kazimierczak et al. 2010].

Soil (subsoil), which is the main source of nutrients, is important in the cultivation of plants. Due to the continuous development of plant production under controlled conditions, the demand for substrates with very specific properties adapted to the needs of cultivation, also increases. Different properties of soil and peat, as well as different systems of factors influencing growth of the root system cause that the mineral soil is not suitable as a subsoil for cultivation in containers [Banach et al. 2013, Onyszko et al. 2015]. High peat for years has been the primary substrate in the cultivation of plants, and also the main component of horticultural substrates, but subsoil that at least in part would be able to replace it, are still sought after. This is due to the deepening peat consumption, contributing to the growth of its price and the protests of environmental groups against the expansive use of peatlands. One possibility to reduce the amount of this material utilization is mixing peat with other cheap and readily available materials such as sand [Rumpel 1998, Kemppainen et al. 2004]. In the present study, two types of substrate were used. The first was acidic peat, which is an organic substrate showing a high water and air capacity with abundant sorption complex. The second was a mixture of peat and sand, i.e. a combination of organic with mineral substrate (sand), which improves the air-water balance for the crop's root system [Nurzyński 2008].

Among important aspects of herbal production, there is both the yield of raw material, as well as its quality, which is expressed, among others, essential oil, dry matter, total nitrogen, protein, and L-ascorbic acid contents.

The aim of this study was to analyze the influence of fertilization using manure and the type of the substrate on the biological value of obtained raw thyme herb.

MATERIAL AND METHODS

The greenhouse experiment with common thyme was carried out in 2014 and 2015 at The Research Station "Felin" belonging to the University of Life Sciences in Lublin (51°25'N 22°56'E) located in south-eastern Poland. Plants were grown singly in pots with a capacity of 2 dm^3 filled with high peat and mixture of peat and sand (1:1). The substrates were limed using fertilization chalk in an amount of 10 g $CaCO_3$ per dm^3 of substrate adjusting pH to 6.5. Each combination included 10 repetitions (a single repetition consisted of one plant). Seeds from PNOS Ożarów Mazowiecki cultivation (without specifying the varieties) were seeded at the turn of February and March in 2014 and 2015 to the peat subsoil. After 2 weeks, plants were planted to the multiplets, and then watered with a preventive fungicide preparation Previcur Energy 840 SL at the concentration of 0.15%, according to the plant protection program. Seedlings were transplanted to their permanent place into pots at the beginning of April 2014 and the end of March 2015. The study was conducted under controlled conditions. During experiments, plants were watered once with the same amount of water and, when necessary, 2–3 times per day. During the growing season, there were no diseases and pests, and therefore protection measures were not used. The harvest was carried out in the first decade of July 2014 and the third decade of June 2015, when plants entered the beginning of flowering. Ground *Thymus vulgaris* L. herb was subject to chemical analyzes.

Experiment with common thyme was founded in bi-factorial pattern, where the first factor was the manure dose: 10, 20 and 30 $g \cdot dm^{-3}$ of substrate, while the second factor consisted of the type of substrate: peat and mixture of peat and sand in 1:1 ratio (1 dm^3 peat + 1 dm^3 sand).

Bovine manure in granulated form was administered once prior to herb planting onto the permanent place. The manure applied in the experiment contained (%): N-total 4.2 (N-organic 3.5 and $N\text{-}NH_4$ 0.7); P_2O_5 3.0; K_2O 2.8; MgO 1.0; CaO 9.0; SO_4 0.5, and in $mg \cdot kg^{-1}$: Fe 1200; Mn 480; Zn 420; Cu 110; B 45; Mo 11.

Fresh plant material was subject to determination of the contents of L-ascorbic acid applying Tillmans method according to Polish Standards [PN-A-04019:1998]. Essential oil content in air-dry herb determined in accordance with the Polish Pharmacopoeia VII [2006] by hydro-distillation. Qualitative and quantitative composition of oil was determined by means of liquid chromatography and mass spectrometry (GC/MS). The Kjeldahl method applying Kjeltec 2002 Distilling System Unit was used to analyze the total content of nitrogen in the dry plant material, and after multiplying the obtained N-total values (%) by the factor of 6.25, the protein content in plant was obtained. Dry matter (%) was determined in fresh mater by the gravimetric method (after drying samples at 105°C to constant weight).

Achieved results were statistically processed by means of variance analysis for dual classification. The significance of differences was shown on a base of Tukeys t-test at the significance level $\alpha = 0.05$.

RESULT AND DISCUSSION

The research results upon the main biological value parameters of the thyme herb are provided in Table 1. Organic fertilization and soil type significantly influenced on the biological value of thyme herb.

Table 1. Influence of the subsoil type and biological value of thyme herb (mean for 2014 and 2015)

Type of substrate	Dose of manure (g·dm-3)	Dry Matter (%)	L-ascorbic acid (mg 100 g^{-1} f.w.)	N-total (% d.m.)	Protein (% d.m.)
peat	10	29.61	64.09	2.39	14,97
	20	26.84	41.43	3.00	18,75
	30	24.79	55.12	2.91	18,22
Mean		27.08	53.52	2.77	17.31
peat + sand	10	35.64	69.83	1.55	9,68
	20	29.73	41.59	2.07	12,97
	30	28.58	57.40	2.24	14,00
Mean		31.32	56.27	1.95	12.22
Mean for dose	10	32.63	66.96	1.97	12,33
	20	28.29	41.46	2.54	15,86
	30	26.69	56.76	2.58	16,11
$LSD_{\alpha = 0.05}$					
type of substrate		2.97	n.s.	0.36	2.24
dose of manure		4.46	16.99	0.53	3.32
interaction		n.s.	n.s.	n.s.	n.s.

n.s. – not significant

Significantly different contents of dry matter in the thyme herb depending on manure dose and substrate type were found. Studied thyme material had a high amount of dry matter, which ranged from 24.79 to 35.64%. The greatest amount of dry matter characterized thyme plants treated with the smallest manure dose, regardless of the substrate applied. It was noted that after the application of increasing doses of manure, crops from peat and from mixed subsoil revealed a decrease in dry matter content.

Thyme herb contained from 41.34 to 69.83 mg L-ascorbic acid per 100 g fresh weight. There has been a decrease in the content of vitamin C under the influence of increasing doses of manure applied, regardless of the substrate type. The richest source of L-ascorbic acid was thyme grown in combination with the lowest organic fertilizer rate (10 $g \cdot dm^3$ substrate) on a mixture of peat with sand (69.83 mg $100 \cdot g^{-1}$ fresh weight). When fertilizing with 20 g of manure, concentration of the component in question was the lowest for tested substrates, respectively 41.59 $mg \cdot 100\ g^{-1}$ fresh weight (peat + sand) and 41.34 $mg \cdot 100\ g^{-1}$ fresh weight (peat). The increase of L-ascorbic acid content after manure fertilization of thyme, lemon balm and mint was noted by Kazimierczak et al. [2011]. Similar results of higher vitamin C content were obtained using organic fertilizer to lemon balm, mint, lovage, thyme, and sage [Kazimierczak et al. 2010]. Similar study results were also recorded by Toor et al. [2006] and Caris-Veynard et al. [2004]; which confirm much higher L-ascorbic acid concentrations in tomato fruits from organic rather than conventional growing. Tests carried out upon organic and conventional cultivation of black currant [Kazimierczak et al. 2007], onions [Hallmann and Rembiałkowska 2006], as well as peppers and tomatoes [Hallmann et al. 2005] also underline that vitamin C content at plants is largely depended on the cultivation method, however plant fertilization is one of the most important factors. The results of cited authors confirm that the use of organic fertilizers contributed to a higher content of vitamin C in plants, while content of increased nitrogen amount in the substrate caused reduced production of secondary metabolites at plants, including synthesis and accumulation of vitamin C [Coley et al. 1985, Kazimierczak et al. 2010]. Concentration of L-ascorbic acid in fresh weight of thyme, depending on the substrate applied, varied in similar way at plants grown on peat and peat with sand mixture. Similar results from 38.0 to 60.0 mg per 100 g of fresh weight of the test component in the container growing on peat, were achieved by Nurzyńska-Wierdak et al. [2012]. L-ascorbic acid concentration in thyme herb was comparable to its content in the dill herb and garden chervil grown on peat substrate in the greenhouse [Frąszczak and Knaflewski 2004, Frąszczak et al. 2006].

Manure used in the experiment is a source of nitrogen and other nutrients for plants. Nitrogen stimulates the plant's vegetative weight increase and is the main building element of proteins and nucleic acids. It is also a structural component of vitamins, nucleotides, alkaloids and chlorophyll [Khalid and Shafei 2005, Najm et al. 2012]. The analyzed plant material contained N-total at the level of 1.55–3% DM. Applying increasing organic fertilization, a significant increase in the nitrogen content, both in thyme grown on peat and on mixed substrate, was observed. A similar amount of this component within the range of 1.89–2.70% N was obtained in organic farming by Seidler-Łożykowska et al. [2006]. Similar nitrogen concentration in thyme herb in biennial cultivation after manure was recorded by Seidler-Łożykowska et al. [2008] (2.45%). The increase in the nitrogen content at plants after manure application was also found for basil and savory [Seidler-Łożykowska et al. 2008], as well as marjoram [Al-Farihat et al. 2011]. Golcz et al. [2003], when studying basil herb, along with Dzida and Jarosz [2006], who tested savory herb, achieved similar results referring to the impact of varied nitrogen rates on a plant. Similar effects of nitrogen contents in onions were observed by Yassen and Khalid [2009] after application of improved bovine manure doses in combination with poultry manure. Nutrition of *Plantago arenaria* plants with increased doses of organic fertilizers caused the increase in N-total contents in dry matter of leaves [Hendawy 2008].

It has been shown the positive effects of increasing organic fertilization on the protein content in studied plants. Concentration of the test parameter in thyme herb changed under the influence of factors studied. The greatest concentration of protein was obtained at plants grown on peat using moderate and the highest dose of manure. By introducing the increasing dose of manure to the subsoil, plants received a greater amount of nitrogen, which is the main constituent of proteins. Dzida [2013], when analyzing the impact of increasing nitrogen doses, observed the increase in protein content at thyme and savory plants. There have been significant difference in the protein content by analyzing the nature of the substrate applied. Plants grown on peat were characterized by a higher amount of protein as compared to those grown on mixed substrate.

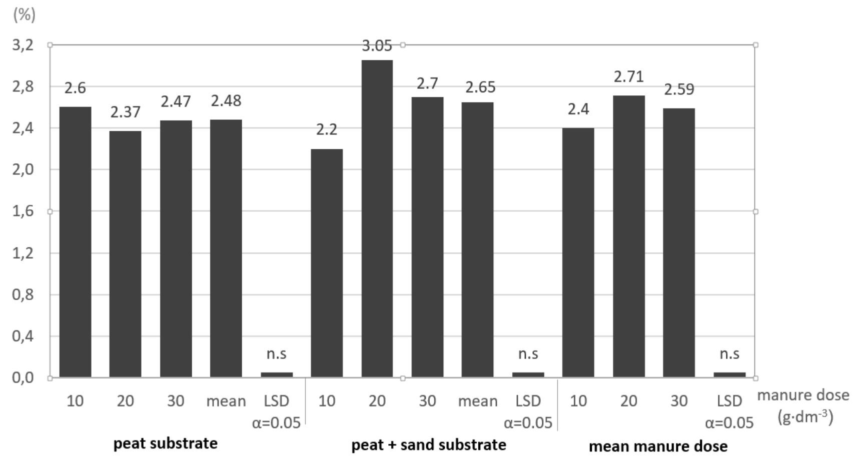

Fig. 1. Essential oil content in thyme herb depending on manure dose and subsoil type

The content of essential oil in the *Thymus vulgaris* L. herb ranged from 2.2 to 3.05% (fig. 1). The natural fertilization and type of substrate did not affect significantly the content of essential oil in thyme herb. But most of the essential oil was reported in thyme after application of 20 grams of fertilizer per dm^3 of substrate (peat + sand (1:1)). The least essential oil in tested raw material was obtained when the thyme was grown on the same substrate and plant was administered with the lowest dose of manure. Thyme oil content obtained was similar to that reported by Seidler-Łożykowska et al. [2008] at thyme plants grown in the position after manure application, which ranged from 2.05% to 3.0%. Higher oil content from organic farming of thyme was also obtained by Seidler-Łożykowska et al. [2006]. Similar value of this component was obtained from the organic cultivation of thyme in different regions of Poland [Seidler--Łożykowska et al. 2009]. There has been no significant effect of manure dose on essential oil content in thyme raw material. However, the upward trend in the parameter content was observed after the application of a double dose of manure as compared to the starting dose at plants grown on a mixture of peat and sand. The increase in the oil content after thyme plant fertilization with bovine and sheep manure as compared to the control was also observed in studies carried out by Hendawy et al. [2010]. The beneficial effect of fertilization with manure on the increase in the content of essential oil in thyme herb was also shown by Heikal [2005] and for marjoram by Edris et al. [2003]. Ateia et al. [2009] found, however, that the use of natural fertilizer in the form of a mixture of compost and sheep manure (3:1) gives a higher yield of essential oil of thyme. Higher content of this component after manure fertilization was also achieved by Seidler-Łożykowska et al. [2008] and Seidler-Łożykowska et al. [2006] for basil, marjoram and savory plants as compared to plants growing on conventional farms. The content of essential oil at fennel has also been elevated due to bovine manure application [Mohamed and Ahmed 2003, Azzas et al. 2009, Osman 2009]. Similar results concerning the tested component under the influence of manure were found for basil, marjoram, mint, hyssop, oregano, and cumin [Aflatuni 1993]. While bovine manure fertilization of lavender on sandy soils was conducive to positive increase in the content of essential oil in comparison to the control plants [Weaam et al. 2015]. Similar studies have shown that fertilization using manure can increase the quantity and change the chemical composition of the oil at some medicinal plants such as fennel [Khalid and Shafei 2005], sage [Kaplan et al. 2009], basil [Biasi et al. 2009], lemon balm [Santos et al. 2009], or onions [Yassen and Khalid 2009]. The increase in oil concentration after fertilizing the plants with organic fertilizers was also found at *Coriandrum sativum* L. [Darzi and Haj Seyed Hadi 2014], *Chrysanthemum balsamita* L. [Marculescu et al. 2002], *Majorana hortensis* L. [Al-Fraihat et al. 2011], as well as *Calendula officinalis* L. [Khalid et al. 2006].

The results of qualitative and quantitative analysis of the main oil constituents are presented in Figure 2. Analysis has shown the presence of 97 compounds in composition of thyme essential oil. The dominant compounds in thyme oil were thymol, p-cymene and γ-terpinene. Similar results upon the occurrence of major compounds in the essential oil of thyme from Southeast regions of Poland were recorded by Zawiślak [2007]. The highest amount of thymol was observed in plants grown on peat substrate after application of 20 g manure. In contrast, the high concentrations of p-cymene (49.14 and 44%) were recorded in thyme herb after application of different manure doses on both investigated substrates, when thymol was present in trace amounts. The content of γ-terpinene as one of the major components of the oil, ranged from 0.05 to 23.44%. Chemical composition of the oil has also revealed the presence of larger quantities of α-terpinene, linalool, and carvacrol. Other compounds identified in thyme oil were obtained in minor amounts.

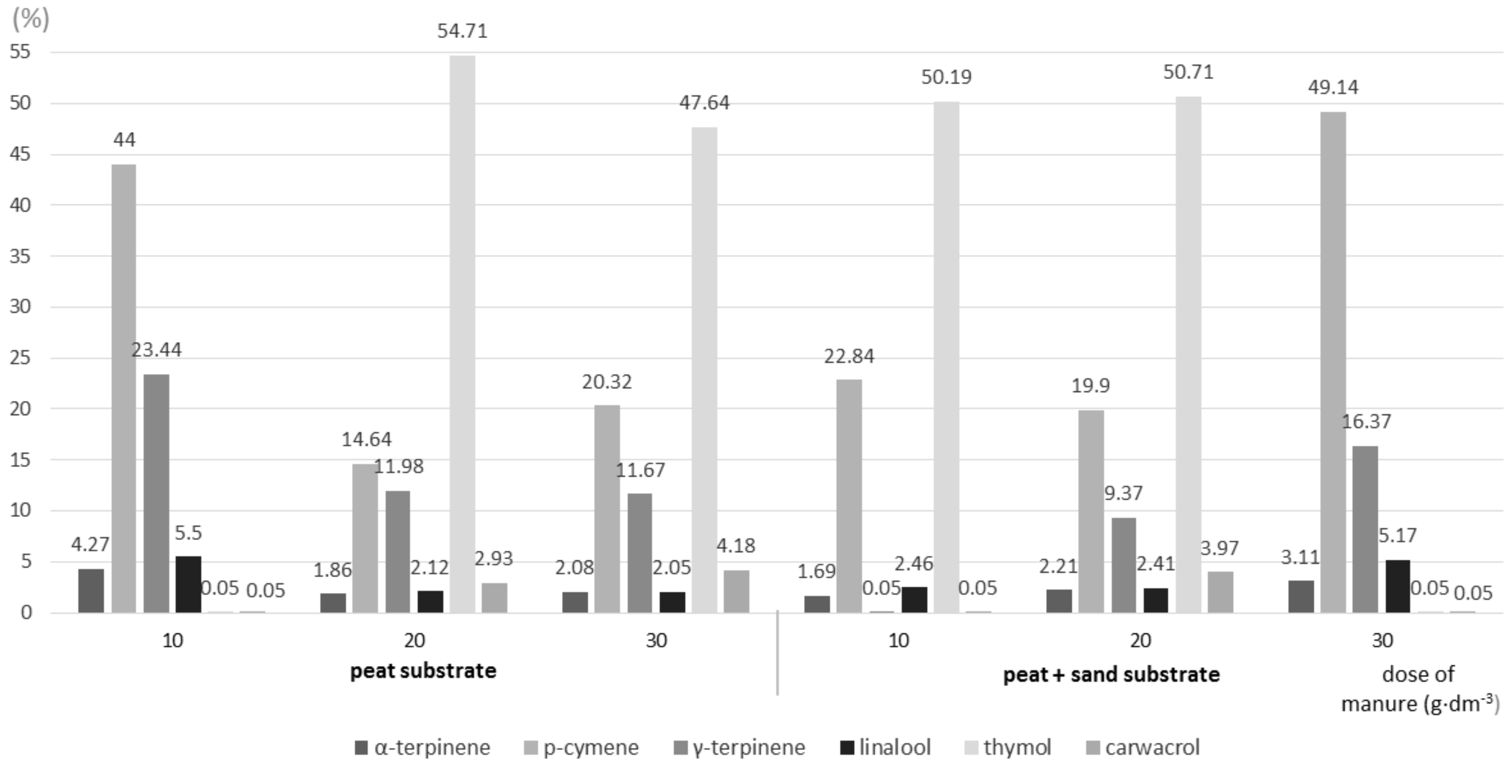

Fig. 2. Influence of natural fertilization and the type of substrate on chemical composition of thyme essential oil

Analysis of the chemical composition showed a wide variation in chemo-type of essential oil in the test species, which does not allow for a clear statement upon the influence of examined factors on the obtained values. On the basis of available literature data, several chemo-types of *Thymus vulgaris* L. essential oil can be distinguished, which is due to the genetic, ontogenetic [Mewes et al. 2008] and environmental variation [Alizadeh et al. 2011]. Marzec et al. [2010], based on literature references, found that for oils abundant in thymol, individual subgroups with higher amounts of different chemicals can be distinguished, like: thymol > p-cymene > γ-terpinene, p-cymene > thymol > γ-terpinene, and p-cymene > γ-terpinene > thymol. In addition, other authors reported higher contents of other chemical compounds in thyme oil under the influence of biotic and abiotic factors, including geraniol, linalool, carvacrol [Thompson et al. 1998, Thompson et al. 2003], neral and citronelol [Raal et al. 2005], as well as camphor and camphene [Imelouane et al. 2009]. Referring own results to findings by Marzec et al. [2010], it can be seen the presence of all three subgroups of thyme oil. The resulting variability may also result from the impact of nitrogen within manure on a plant. Nitrogen is involved in the biosynthesis of essential oil through the influence on photosynthesis, respiration, and is included in the ATP, NADPH and CoA, which are important compounds during the biosynthesis of terpenoids [Sell 2003]. As a result of the aromatization processes, γ-terpinene is converted onto p-cymene, which by means of hydroxylation, leads to the thymol and carvacrol [Mikio and Taeko 1962, Nhu Trang et al. 2006]. Nitrogen available in manure and collected by plants on different types of substrates used, could affect the resulting variability of oil chemo-types. Furthermore, the difference in chemical composition of the oil may arise from genetic variation, as the experiment used seed of the hybrid *Thymus vulgaris* L. without specifying the cultivar. The results allow to conclude that the biosynthesis of essential oil at thyme is influenced by genetic factors, but is also a subject to environmental factors.

CONCLUSION

1. There was a significant increase in the N-total and protein contents in thyme herb after application of increasing doses of examined manure independently of the test substrate.
2. Applied natural fertilization and type of substrate did not affect significantly the content of essential oil in *Thymus vulgaris* L. herb.
3. Qualitative analysis of the thyme raw material showed the presence of three chemo-types of composition the essential oil of different the thymol content in the particular oil samples.
4. The highest content of L-ascorbic acid and dry matter was obtained for thyme after application of the lowest dose of manure, regardless of the substrate used.

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WPŁYW NAWOŻENIA NATURALNEGO I RODZAJU PODŁOŻA NA WARTOŚĆ BIOLOGICZNĄ ZIELA TYMIANKU (*Thymus vulgaris* L.)

Streszczenie. *Thymus vulgaris* L. jest jedną z najstarszych roślin przyprawowych świata. Na wartość biologiczną tej rośliny mają wpływ zarówno warunki biotyczne, jak i abiotyczne, do których zalicza się nawożenie oraz rodzaj podłoża. Przeprowadzone badania miały na celu określenie wpływu zróżnicowanego nawożenia obornikiem (dawki 10, 20, 30 g dm^{-3} podłoża) i różnego rodzaju podłoża (torf, torf + piasek) na zawartość suchej masy, białka, azotu ogółem, kwasu L-askorbinowego oraz olejku eterycznego w surowcu. Stwierdzono istotny wzrost zawartości N ogółem i białka w zielu tymianku po zastosowaniu wzrastających dawek obornika niezależnie od badanego podłoża. Zróżnicowane nawożenie naturalne oraz rodzaj podłoża nie wpłynęły istotnie na zawartość olejku w zielu *Thymus vulgaris* L. Największą zawartość kwasu L-askorbinowego oraz suchej masy otrzymano w tymianku po zastosowaniu najniższej dawki obornika niezależnie od użytego podłoża.

Słowa kluczowe: olejek eteryczny, białko, kwas L-askorbinowy, azot, obornik, rośliny lecznicze

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