

EFFECT OF NATURAL FERTILIZATION AND CALCIUM CARBONATE ON YIELDING AND BIOLOGICAL VALUE OF THYME (*Thymus vulgaris* L.)

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ABSTRACT

Thyme belongs to herbal plants, the yield and quality of which depend on biological factors, agrotechnical procedures and the way of processing and storage. Basic factor differentiating the biological value of plants is fertilization, including manure, as well as plant growth and development, which is significantly influenced by the pH of the substrate, in which the plants are grown. The aim of the study was to determine the effect of manure dose (12.5 and 25 g·dm⁻³) and calcium carbonate (5 and 15 g·dm⁻³) on the yield and biological value of thyme. The fresh thyme mass yield was changed under the influence of the factors used. The highest yield of raw material was recorded after using a high dose of manure and calcium carbonate. Significantly higher concentration of essential oil in thyme was found after feeding the plants with higher dose of manure. The inverse relationship was demonstrated for the amount of L-ascorbic acid that decreased with the increase in manure dose. Calcium fertilizers not only serve to regulate the acidity, but are also a source of calcium for plants. After applying a higher dose of CaCO₃, significant increase in the yield of fresh thyme mass was observed and higher dry matter content was recorded.

Key words: herb, manure, ecological, ascorbic acid, essential oil

INTRODUCTION

Herb plants have been very popular among consumers for centuries. They are a source of many nutrients such as: vitamins, sugars, proteins, amino acids and minerals [Nurzyńska-Wierdak et al. 2012]. Due to the variety of primary and secondary substances in herbal plants, they have found a wide application as a medicinal and spice raw material. They are great disinfectants and antitussive agents [Hałubowicz-Kliza 2007, Kazimierczak et al. 2011]. The abundance and value of thyme are determined by the content of essential oil, phenolics, vitamins, tannins, flavonoids, triterpene compounds, vitamins and minerals. Biological value of

plants depends on biological factors, agrotechnical procedures and the way of processing and storage [Jordán et al. 2006, Kwiatkowski 2007, Seidler-Łożykowska 2007, Król and Kiełtyka-Dadasiewicz 2015]. Agronomic treatments affecting the quality of raw thyme include, among others, plant fertilization and soil/substrate acidity regulation process. The prevailing consumer trend for “healthy food” sets the direction of plant nutrition, using mainly natural and organic fertilizers. A very important element in plant nutrition is also calcium, that affects the growth and development of plants as well as quality of the obtained crop.

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Common thyme (*Thymus vulgaris* L.) is used both in pharmaceutical and food industries, therefore it is aimed at the raw material yield and the biological value of thyme to be of the best quality. Thyme belongs to the Lamiaceae family. It comes from the Mediterranean region, where it grows dry and vegetation-poor areas [Osińska 2006]. This plant shows great adaptability to the conditions of other environments, which results in cultivating it in areas with a temperate climate throughout Europe [Kwiatkowski and Kołodziej 2005]. The wide spectrum of thyme and preparations made on its basis is reflected in gastronomy, medicine and cosmetology [Osińska and Rosłon 2016].

Thyme also found a very wide application in medicine, where it is used as an antibacterial, expectorant, diaphoretic, calming, as well as wounds healing and general strengthening agent. Thyme infusions are used for symptoms of dry cough, hoarseness, as well as difficulties in expectoration and bronchitis. It is used to rinse the mouth and throat in inflammation; sometimes used in the form of compresses and poultices, and as a bath supplement, it heals ulcers, acne and herpes [Osińska 2006, Szumny et al. 2007, Kołodziej 2009, Pióro-Jabrucka et al. 2010, Nurzyńska-Wierdak 2015]. The conducted studies show a wide range of thyme action, especially its stimulating effect on the nervous system and improvement of blood circulation in blood vessels manifested by an increase in the difference between systolic and diastolic pressure. Thyme oil, as a component of some ointments and anti-rheumatic liniments, is also used externally [Steinbrich 1995, Kluszczynska 2001, Kędzia et al. 2012].

Research conducted for many years confirms the thesis that natural and organic fertilizers have positive effect on soil/substrate properties, and thus on the yield. After their application, the soil/substrate structure is plump and richer in nutrients as well as very beneficial microorganisms [Kołodziej 2010]. Natural fertilizers are often called complete fertilizers, because they contain all the necessary nutrients. The most commonly used natural and organic fertilizers in the cultivation of herbs include: manure, green manures and compost.

All plants, both crops and those growing in the natural environment, take nutrients necessary for their proper development from the soil. The amount of components taken depends on the individual needs of individual plant species. Calcium fertilizers are in-

troduced into the soil to reduce acidification, improve physical properties, provide calcium as a nutrient and regulate the uptake of other elements by plants.

The aim of the study was to analyze the effect of manure dose (12.5 and 25 g·dm⁻³) and calcium carbonate (5 and 15 g·dm⁻³) on yielding and biological value of common thyme grown under controlled conditions.

MATERIAL AND METHODS

The experiment involving thyme (*Thymus vulgaris* L.) was carried out in 2014 and 2015 at the Department of Plant Cultivation and Nutrition in the Experimental Farm of the University of Life Sciences in Lublin (51°25'N, 22°56'E). Plants were grown in the greenhouse from March 22 to June 18. The experiment was established in a two-factor scheme. The research factors of the experiment were: manure dose (12.5 and 25 g·dm⁻³) and dose of CaCO₃ (5 and 15 g·dm⁻³). Plants grew individually in 2-liter pots filled with high peat, pH 4.5, limed with calcium carbonate and mixed with manure. The following fertilizers were used in the experiment: bovine manure with a percentage composition: N-tot 4.2, N-org. 3.5, N-NH₄ 0.7, P₂O₅ 3.0, K₂O 2.8, MgO 1.0, CaO 9.0, S-SO₄ 0.5, as well as Fe 1200 mg·kg⁻¹, Mn 480 mg·kg⁻¹, Zn 420 mg·kg⁻¹, Cu 110 mg·kg⁻¹, B 45 mg·kg⁻¹, Mo 11 mg·kg⁻¹ and fertilizer chalk 45% CaO.

Before the experiment was liquidated on March 18, 2015, the plants were measured and the above ground parts of the herb were weighed. In fresh plant material, L-ascorbic acid was determined using the Tillmans method (PN-A-04019 1998), essential oil by hydro-distillation, dry matter content by the drying method, and protein was calculated by multiplying the total nitrogen content in thyme by 6.25 [Klepacka 1997]. The results obtained were statistically processed using variance analysis function. Significance of differences was determined by Tukey's test at the significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Common thyme is one of the oldest aromatic and spice plants valued in Poland and Western and South-

ern Europe. Its adaptive abilities allow it to be grown all over Europe. Herbal plants have different needs for particular nutrients, related to the growth and development of a given species. Fertilization, apart from significant influence on the crop yield, also modifies its quality. Natural fertilizers are designed to increase the content of nutrients in the substrate, improve air-water ratios, improve the sorption capacity of the substrate and promote development of microorganisms [Naghdi et al. 2004, Naguib 2011, Sharafzadeh 2011]. Natural fertilizers are used in organic production, where synthetic fertilizers and pesticides are not allowed, which encourages the plants to launch their own defense systems against pathogens, including production of secondary active substances. The nitrogen content in soil also affects the production of these compounds by plants. In the organic farming system, where nitrogen is supplied to the soil in the form of organic fertilizers, its availability is lower. As a result, plants first produce carbon containing compounds (simple and complex sugars) and secondary plant metabolites (vitamins, essential oils). In an environment where availability of nitrogen is higher, for example in conventional production, plants mainly produce nitrogen compounds, such as amino acids, proteins or alkaloids

due to the use of nitrogen fertilizers readily soluble in water [Coley et al. 1997, Brandt and Molgaard 2001, Kazimierczak et al. 2011].

The available literature lacks sufficient information, as well as research upon the impact of natural fertilization on the biological value of thyme and agrotechnics of this plant.

Grown thyme plants in containers under cover in different ways reacted to the applied doses of manure and calcium carbonate. Own research shows that the dose of manure and CaCO₃ influenced the size of the fresh weight of thyme and its height (Tab. 1). There was a higher yield of fresh thyme mass after using a higher dose of CaCO₃ (133.9 g). It was found that common thyme from container cultivation grew up to 39.3 cm in height, i.e. it reached the standard height (40 cm) given by Steinbrich [1995] and Kluszczynska [2001]. The average content of dry matter in the thyme raw material was 25.6%, which is close to the results of Kazimierczak et al. [2011].

The growing season of thyme from planting to a permanent place till harvesting the raw material was 64 days. Using 12.5 g of manure and 5 g of calcium carbonate per dm³ of the substrate for feeding plants, the largest differences were found in the increase of

Table 1. Effect of manure and CaCO₃ dose on yield of fresh thyme mass and height plants (average of 2014 and 2015 years)

Dose of CaCO ₃ (A) (g·dm ⁻³)	Yield (g·plant ⁻¹)			Height (cm)		
	Dose of manure (B) (g·dm ⁻³)					
	12.5	25	mean for dose CaCO ₃	12.5	25	mean for dose CaCO ₃
5	106.6	126.0	116.3	36.33	39.33	37.83
15	110.8	157.0	133.9	31.50	29.66	30.58
Mean for dose manure	108.7	141.5		33.91	34.50	
LSD _{α=0.05}						
A			14.05			2.537
B			14.05			n.s.
A×B			n.s.			n.s.

n.s. – non-significant differences

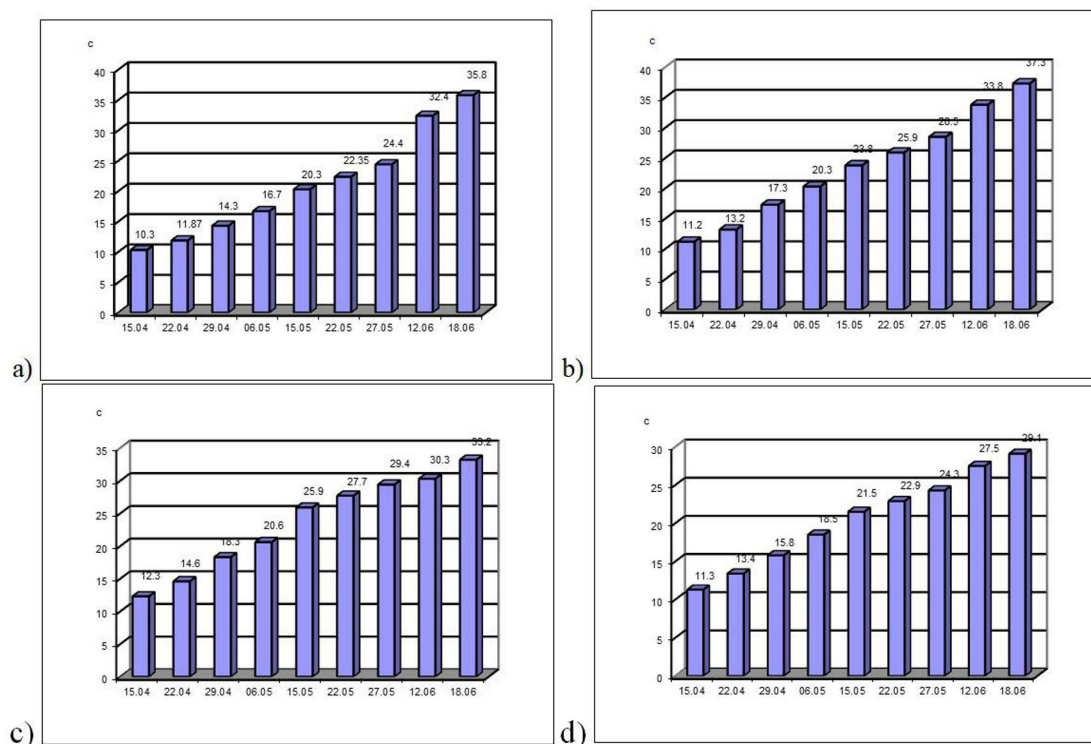


Fig. 1. Dynamics of thyme growth in the growing season after application of: a) 12,5 g·dm⁻³ manure and 5 g·dm⁻³ CaCO₃; b) 25 g·dm⁻³ manure and 5 g·dm⁻³ CaCO₃; c) 12,5 g·dm⁻³ manure and 15 g·dm⁻³ CaCO₃; d) 25 g·dm⁻³ manure and 15 g·dm⁻³ CaCO₃

fresh plant mass between 27 May and 12 June; in other dates during the growing season, the gains were similar (Fig. 1). The use of manure at a higher dose had positive effect on the growth of thyme. All plants from this combination were higher compared to those with lower manure fertilization, and the same dose of calcium carbonate. Measurements of thyme growth during the growing season after applying 12.5 g of manure and 15 g of calcium carbonate per dm³ of substrate indicate regular increases between particular dates of measurement. The largest growth of thyme was recorded between May 6 and May 15, which was 5.3 cm. After applying a higher dose of manure and calcium carbonate, a uniform thyme growth rate was recorded. In comparison to the above-mentioned combinations, the use of higher doses of tested fertilizers caused slower growth of plants, especially in the second half of the growing season. At the harvest time, plants from this combination were the shortest.

The content of L-ascorbic acid in thyme plants (Tab. 2) varied under the influence of the research factors used, i.e. dose of calcium carbonate and manure, ranged from 33.6 to 37.8 mg · 100 g⁻¹ FW and was larger than the content given by Kluszczyńska [2001], and similar to the results obtained by Nurzyńska-Wierdak et al. [2012], which ranged from 11.9 to 74.0 mg · 100 g⁻¹ FW. Skubij and Dzida [2016] research shows that thyme herb contained from 41.34 to 69.83 mg of L-ascorbic acid per 100 g of fresh weight. There was some decrease in the content of vitamin C due to the use of increasing doses of manure, regardless of the substrate type. Based on our own research, it was found that thyme plants grown in a substrate with lower dose of calcium carbonate in combination with lower dose of manure contained the highest amount of L-ascorbic acid (37.8 mg·100 g⁻¹ FW). Similar results of higher content of vitamin C were obtained using organic fer-

Table 2. Effect of manure and CaCO₃ dose on the L-ascorbic acid and protein content of thyme (average of 2014 and 2015 years)

Dose of CaCO ₃ (A) (g·dm ⁻³)	L-ascorbic acid (mg·100 g ⁻¹ FW)			Protein (%)		
	Dose of manure (B) (g·dm ⁻³)					
	12.5	25	mean for dose CaCO ₃	12.5	25	mean for dose CaCO ₃
5	37.8	34.9	36.4	19.4	17.4	18.4
15	36.9	33.6	35.2	17.5	18.3	17.9
Mean for dose manure	37.4	34.2		18.5	17.8	
LSD _{α=0.05}						
A			n.s.			n.s.
B			2.666			n.s.
A×B			n.s.			n.s.

n.s. – non-significant differences

Table 3. Effect of manure and CaCO₃ dose on the essential oil and dry thyme mass (average of 2014 and 2015 years)

Dose of CaCO ₃ (A) (g·dm ⁻³)	Essential oil (%)			DM (%)		
	Dose of manure (B) (g·dm ⁻³)					
	12.5	25	mean for dose CaCO ₃	12.5	25	mean for dose CaCO ₃
5	2.91	3.60	3.25	23.5	25.0	24.3
15	2.87	3.30	3.08	27.8	26.0	26.9
Mean for dose manure	2.89	3.45		25.7	25.5	
LSD _{α=0.05}						
A			n.s.			1.544
B			0.266			n.s.
A×B			n.s.			2.978

n.s. – non-significant differences

tilizer in lemon balm, mint, lovage, thyme and sage plants [Kazimierczak et al. 2011].

Natural fertilizer used in the experiment is a source of nitrogen and other nutrients for plants. Nitrogen stimulates the growth of plant vegetative mass and is the main proteins and nucleic acids building element. It is also a structural component of vitamins, nucleotides, alkaloids and chlorophyll [Ashraf et al. 2005, Karaivazoglu et al. 2007, Nouri et al. 2014, Rothstein and Cregg 2005, Taylor et al. 1982]. In the examined plant material, the N-total content was 3.01–3.42% DM. Lower amount of the tested component in the range of 1.89–2.70% N in organic farming was obtained by Seilder-Łożyskowska et al. [2006] and Seilder-Łożyskowska et al. [2008] (2.45%, respectively). However, the increase in nitrogen content in the plant after using manure was recorded in basil and savory [Seilder-Łożyskowska et al. 2008]. Golcz et al. [2003] by examining the basil herb and Dzida and Jarosz [2006] – savory herb, revealed similar results of the effect of different doses of nitrogen towards plants. *Thymus vulgaris* fertilization with higher doses of organic fertilizers increased growth, yield and essential oil [Hendway et al. 2010].

An ambiguous effect of manure and calcium carbonate doses on the content of protein in thyme herb was found (Tab. 2). In objects with higher dose of calcium carbonate in combination with lower dose of manure, a positive effect on protein concentration was recorded. Skubij and Dzida [2016] reported positive effect of increasing organic fertilization on the protein content in the thyme plants.

According to Rumińska [1984], content of oil in the raw material depends on the variety and origin, and its quantity ranges from 0.7 to 3.5%. Analyzing the effect of manure and calcium carbonate dose on the content of essential oil in thyme, the highest content of essential oil in plants fed with a lower dose of calcium carbonate and a higher dose of manure 3.6% air-dry mass, was recorded. Kołodziej [2006], when analyzing the influence of studied factors, received varied content of thyme oil, which ranged from 0.5% to 2.5%. Cases et al. [2009], studying thyme populations in Spain, showed that the concentration of oil was within very wide limits from 0.13% to 2.95%; similar results were obtained by Marzec et al. [2010]. Whereas Syamasundar et al. [2008] studied the con-

tent of thyme oil that originated from India. Studies have shown that it was characterized by lower content of essential oil, compared to plants from Rio de Janeiro, which contained 1.1% of essential oil [Porte and Godoy 2008]. The obtained data confirm the influence of environmental and genetic factors on the amount of essential oil in thyme herb. Anthropogenic factors affecting the quality of raw material to be grown include the way of cultivation and nutrition of plants. Based on the tests carried out [Dzida 2013] with differentiated nitrogen-potassium nutrition, a significant effect of the dose of the analyzed elements on the content of essential oil of thyme, which varies from 3.3 to 4.25% air-dry mass, was also recorded.

Content of essential oil (Tab. 3) in the *Thymus vulgaris* L. herb depends not only on the dose of manure and the type of substrate, in which the plants are grown. Skubij and Dzida [2016], when analyzing the effect of manure dose and type of the substrate, indicated significant effect of the studied factors on the content of oil in thyme, which ranged from 2.2 to 3.05%. The greatest amount of essential oil was recorded in thyme after applying 20 g of fertilizer per dm³ of the substrate (peat: sand, 1:1). The least amount of oil in the tested raw material was obtained when thyme was grown in the same substrate, and the lowest dose of manure was used for plant nutrition. The obtained content of thyme oil in own research was higher as compared to values obtained by Seilder-Łożyskowska et al. [2008] for thyme plants cultivated on a manure site that ranged from 2.05 to 3.0%. A similar content of oil from organic thyme cultivation was obtained by Seilder-Łożyskowska et al. [2006] lower value of the tested factor was obtained from growing the organic thyme in various regions of Poland [Seilder-Łożyskowska et al. 2009]. The increase of oil content after fertilization of plants with bovine and ovine manure as compared to the control, was also recorded in the studies by Hendawy et al. [2010]. The beneficial effect of manure fertilization on the growth of essential oil content in thyme herb was also demonstrated by Heikal [2005]. Ateia et al. [2009] found, however, that the use of natural fertilization in the form of a mixture of compost and sheep manure (in a ratio of 3 : 1) gives higher yield of thyme essential oil. Seilder-Łożyskowska et al. [2008] and Seilder-Łożyskowska et al. [2006] also reported higher content of the parameter exam-

ined after fertilization with manure for basil, savory and marjoram plants as compared to plants grown on conventional plantations.

CONCLUSIONS

The applied dose of calcium carbonate significantly differentiated the size of fresh mass yield, thyme plant height, as well as the percentage of dry matter content. A significant effect of the manure dose on the fresh weight of thyme as well as the content of L-ascorbic acid and essential oil in the studied plants was also noted. Significantly higher yield of thyme and higher content of essential oil was obtained after using a higher dose of manure (25 g·dm⁻³ medium) compared to half the dose. The applied research factors did not affect the protein content in thyme plants. Manure is a very good source of nutrients for plants.

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