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ORIGINAL PAPER

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INFLUENCE OF NITROGEN DOSE AND HARVESTING DATE ON THE YIELD AND BIOLOGICAL VALUE OF RAW GARDEN SAVORY (*Satureja hortensis* L.) OF SATURN CV.

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ABSTRACT

Garden savory (*Satureja hortensis* L.) is an aromatic spice plant commonly used in the food and pharmaceutical industries. Biological value of this plant is influenced by biotic and abiotic conditions, which include fertilization and date of harvesting. The research was aimed at determining the effect of differentiated nitrogen fertilization (nitrogen doses 0, 4, 8, 12, 16 $g \cdot m^{-2}$) and different harvesting time (initial flowering and full flowering phase) on yield of fresh plant weight, yield of essential oils, dry matter, protein, L-ascorbic acid contents in the raw material of garden savory of Saturn cv. The highest yield of fresh mass and essential oil was recorded in the herb harvested in the initial flowering phase after applying the highest doses of nitrogen. The obtained results allow to conclude that the greatest influence on the content of L-ascorbic acid in the herb had a dose of 4 $g\ N \cdot m^{-2}$ in both examined dates. The highest amount of protein and dry matter was found in savory raw material after applying the highest dose of nitrogen in the full flowering phase.

Key words: medicinal plants, nitrogen fertilization, herb yield, essential oil yield, vitamin C

INTRODUCTION

In recent years, both the food, pharmaceutical and cosmetic industries have been systematically abandoning the use of compounds that do not have their counterparts in nature. Research is being carried out to find a preservative other than parabens, but equally effective, with a pleasant aroma, enriching the taste of the dishes or the effectiveness of the medicines. The potential substitutes are naturally occurring biologically active substances in the environment, such as essential oils, phenolic compounds, or L-ascorbic acid present in herbal plants. They are mixtures of chemical compounds with strong biological activity, usually characterized by a scent commonly recognized as attractive. These substances have numerous therapeutic properties, including antifungal, antibacterial and antioxidant ones, which can be used in products of plant, animal and cosmetic raw materials.

Natural preservatives obtained from plants from *Lamiaceae* family include essential oils. Among many herbal species belonging to the *Lamiaceae* family, a significant spice plant, widespread in many countries around the world is garden savory (*Satureja hortensis* L.). The savory herb is a raw material with multidirectional biological activity. The raw material has both typically spicy properties, because it stimulates secretion of gastric juice and enhances diges-

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tion, prevents from excessive intestinal fermentation and has a carminative, antispasmodic or antidiarrheal effect. It also has antimicrobial, anti-inflammatory and analgesic properties, as well as antioxidant activity. The decisive influence on the chemical composition of garden savory herb is exerted by external factors associated with the type of this species cultivation, especially method of fertilization and harvesting term, which is combined with numerous biochemical processes that take place within the plant during ontogenesis [Sangwan et al. 2001, Çirak et al. 2007, Lee and Ding 2016]. The date of harvest has a significant impact on the content of L-ascorbic acid, chlorophyll, carotenoids, or essential oil in the raw material of various herbal plants [Zawiślak 2011, 2014]. Studies of various species of the genus *Satureja* carried out by Satil and Kaya [2007], Mohammadhosseini and Beiranvand [2013], Jafari et al. [2016] as well as Mozafari et al. [2018] show the distinctiveness of *Satureja hortensis* L., both in terms of morphological and chemical features.

Sustainable supply of nutrients to plants allows obtaining a high quality yield. Nitrogen, the main yielding element, has the greatest influence on the growth of plant biomass [Nouri et al. 2014]. Nitrogen plays the most recognizable role in plant nutrition, because it has a share in the structure of a protein molecule. Nitrogen is found in organic molecules such as purine, pyrimidine, the derivatives of which build nucleic acids RNA and DNA. It has also been shown that nitrogen fertilization not only increases the N content in plant tissues, but also changes the concentration of other nutrients [Taylor et al. 1982, Ashraf et al. 2005, Rothstein and Cregg 2005, Karaivazoglu et al. 2007]. Nitrogen is one of the most important macroelements for plants, which affects both qualitative and quantitative factors of secondary metabolites of plants. Niakan et al. [2004], when analyzing the influence of NPK on the content of dry and fresh mass, the surface of leaves and the amount of essential oil in peppermint (*Mentha pipperita* L.) reported that the applied dose of 200–100–200 kg/ha of NPK contributed to the increase of leaf area and the amount of essential oil. Babalar et al. [2010] studied the effects of nitrogen and calcium carbonate on growth, the amount of rosmarinic acid and yielding of savory of Saturn cv. They showed that the height of plant and content of dry and fresh mass increased significantly after applying 100 kg N/ha in the form of ammonium nitrate. Moumivand et al. [2009] reported that the type of nitrogen fertilizer had significant impact on the height of plants and dry weight of garden savory. Alizadeh et al. [2007] showed that the soil application of 150 kg N/ha and foliar application of 7.5% solution resulted in the highest yield of essential oil, plant growth and the largest number of side branches of savory. Important aspects of herbal production include both the raw material yield and its quality, which is expressed, among others, by the content of dry matter, essential oil, protein or L-ascorbic acid.

The aim of conducted research was to analyze the effect of nitrogen fertilization and date of harvesting the raw material on the yield and biological value of garden savory of Saturn cv.

MATERIAL AND METHODS

The field experiment with garden savory of Saturn cv. was carried out in 2014–2016 in the agricultural farm located in south-eastern Poland, in the Lublin province, Zamch locality (50°18'N, 23°1'E) near Biłgoraj. The experiment was established from a direct sowing using 1 g of seed per m^2. Seeds from cultivars of the Institute of Natural Fibers and Herbal Plants were sown in the mid of May 2014 and the beginning of May 2015 and 2016. The dates of seed sowing were dependent on the course of weather conditions affecting the beginning of vegetation. Garden savory plants grew on medium, podzolic soils developed from loose sands and loamy dust deposits of bonitation class 3b.

In all years, the experiment was established in a two-factor scheme, by means of random blocks in five replications, the surface area of a single microplot was 1 m^2. The first study factor was the dose of nitrogen in the amounts of 0, 4, 8, 12, 16 g $N \cdot m^{-2}$, while second – term of the raw material harvest, i.e. phase of initial flowering and full flowering of plants. Before sowing, half of the dose was used: nitrogen (2, 4, 6, 8 g $N \cdot m^{-2}$) in the form of ammonium nitrate, potassium (5 g $K \cdot m^{-2}$) as potassium salt and magnesium (2 g $Mg \cdot m^{-2}$) in the form of heptahydrate sulfate and a full dose of phosphorus (3 g $P \cdot m^{-2}$) calculated as 20% P superphosphate. After emergence, the second dose was administered: nitrogen (2, 4, 6, 8 g $N \cdot m^{-2}$), potassium (5 g K·m^{-2}) and magnesium (2 g Mg·m^{-2}) in the form of the aforementioned fertilizers.

During the experiment, the plants were manually weeded, not using any herbicides to fight the weed infestation. During the growing season, no diseases or pests were found, therefore no protection measures were applied.

The first harvest was carried out at the beginning of August 2014, mid of August 2015 and the end of July 2016, when plants entered the beginning of flowering phase. The second date of raw material harvest was at the full flowering stage, about 14 days from the moment of initial flowering, i.e.: 20.08.2014, 01.09.2015, 04.08.2016.

For each object, the yield of fresh herb was determined by gravimetric method, dry matter content by the dryer-weight (gravimetric) method. In fresh plant material, the content of L-ascorbic acid was determined using the Tillmans method according to the Polish Standard [PN-A-04019:1998]. The yield of essential oil was calculated according to the formula given by Aliabadi et al. [2008]. Protein content in plants was calculated by multiplying the N-total value (%) by the factor 6.25.

Data on the course of atmospheric conditions in 2014–2016 come from meteorological station of the Experimental Plant of Varieties Evaluation in Nowy Lubliniec (Fig. 1). The course of climatic conditions in individual years was variable, which resulted in its varied impact on field cultivation of garden savory. Year 2014 was characterized by the most favorable atmospheric conditions, both high humidity (significant sum of atmospheric precipitation) and optimal temperature course relative to the development phases of plants. Years 2015 and 2016 were characterized by much smaller sum of rainfall with the participation of higher air temperatures, whereby plants in particular months underwent periodic soil drought. Based on meteorological data, it was found that the average air temperature in the years of research was 14.48°C. In general, after sowing in May, there were abundant rains, encrusting the soil and delaying the plant emergence. In June, the air temperature increased with a significant drop in atmospheric precip itation by more than 49 mm relative to the previous month. In the next period, by the harvest, a systematic increase in temperature and the highest rainfall in relation to the entire growing season in all years of

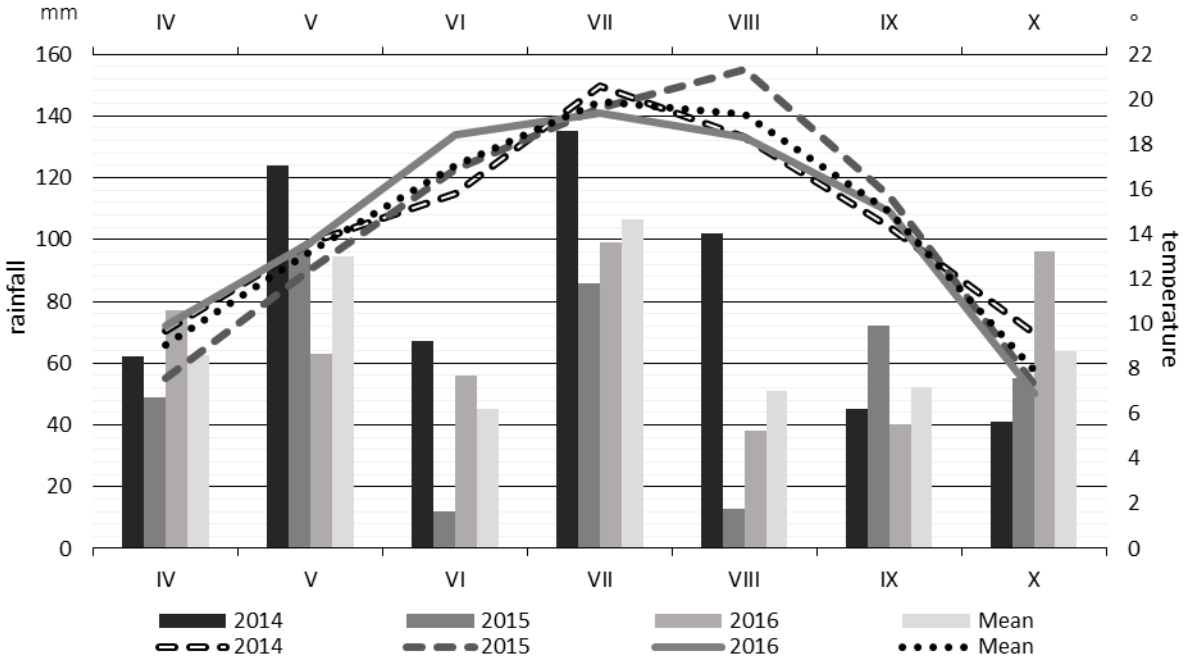

Fig. 1. Average monthly distribution of air temperatures (°C) and sum of atmospheric precipitation (mm) in 2014–2016 during the field cultivation of garden savory

the research were recorded. In August, during the harvest, there was a slight rainfall at a higher temperature; the average for this month was 19.30°C.

The obtained results were statistically processed applying the analysis of variance for double classification. Significance of differences was demonstrated on the basis of the t-Tukey test at the significance level of α = 0.05.

RESULT AND DISCUSSION

Achieved results of studies on the biological value of garden savory herb are presented in Tables 1–4. The applied mineral fertilization and date of harvest significantly affected the yield and research parameters of savory herb's biological value.

Table 1. Influence of nitrogen dose and date of harvest on yield of fresh garden savory herb and dry matter content in the herb (mean for 2014–2016)

Nitrogen dose ($g \cdot m^{-2}$) (B)	Harvest time (A)					
	fresh weight ($kg \cdot m^{-2}$)			yield of dry herb ($g \cdot m^{-2}$)		
	beginning of flowering	full flowering	mean	beginning of flowering	full flowering	mean
0	1.41	1.33	1.37	302.0	284.0	293.0
4	1.60	1.55	1.58	342.0	331.0	336.5
8	1.67	1.94	1.81	356.0	417.3	387.0
12	2.35	1.71	2.03	504.3	365.7	435.0
16	2.14	1.80	1.97	459.7	383.0	421.3
Mean	1.84	1.67		392.9	356.2	
$LSD_{\alpha = 0.05}$						
A			0.150			5.004
B			0.335			11.346
A × B			0.554			19.004

Table 2. Influence of nitrogen dose and date of harvest on the content of L-ascorbic acid and protein in garden savory (mean for 2014–2016)

Nitrogen dose ($g \cdot m^{-2}$) (B)	Harvest time (A)					
	L-ascorbic acid (mg 100 g^{-1} FW)			protein (% DM)		
	beginning of flowering	full flowering	mean	beginning of flowering	full flowering	mean
0	14.82	30.28	22.55	23.87	21.75	22.81
4	44.62	31.24	37.93	22.06	22.19	22.13
8	16.25	13.33	14.79	23.33	21.90	22.62
12	15.97	15.50	15.73	22.32	23.00	22.66
16	20.05	18.25	19.15	24.38	25.45	24.90
Mean	22.34	21.72		23.19	22.85	
$LSD_{\alpha = 0.05}$						
A			n.s.			n.s.
B			7.920			1.525
A × B			13.170			2.536

n.s.– not significan

Table 3. Influence of nitrogen dose and date of harvest on the yield of essential oil in garden savory (mean for 2014−2016)

Nitrogen dose ($g \cdot m^{-2}$) (B)	Harvest time (A)					
	yield of essential oil ($kg \cdot ha^{-1}$)			dry matter (%)		
	beginning of flowering	full flowering	mean	beginning of flowering	full flowering	mean
0	153.0	71.3	112.2	23.69	31.69	27.69
4	191.0	155.0	173.0	23.89	31.67	27.78
8	185.7	171.0	178.3	20.73	34.00	27.37
12	245.3	169.3	207.3	24.38	32.53	28.45
16	274.3	207.7	241.0	22.01	34.07	28.04
Mean	209.7	154.9		22.94	32.79	
$LSD_{\alpha = 0.05}$						
A			4.268			3.471
B			9.676			n.s.
A × B			16.208			n.s.

n.s. – not significant

Table 4. Influence of nitrogen dose and date of harvest on the correlation between test parameters in garden savory

	Dry matter	Yield of essential oil	Protein	L-ascorbic acid
Fresh weight	–0.18688 p = 0.605	0.86546* p = 0.001	0.156264 p = 0.666	–0.40569 p = 0.245
Dry matter		–0.39844 p = 0.254	–0.1248 p = 0.731	–0.05424 p = 0.882
Yield of essential oil			0.450174 p = 0.192	–0.26185 p = 0.465
Protein				–0.40716 p = 0.243

* correlation coefficient significant for $p < 0.05$

Fresh weight yield of *Satureja hortensis* L. of Saturn cv. herb ranged from 1.33 to 2.35 $kg \cdot m^{-2}$. The obtained yield value was similar to results obtained by Mumivand et al. [2011] in garden savory plants of the cultivar tested and grown after the use of various doses of N and Ca, which ranged from 171 to 198 $g \cdot plant^{-1}$. Comparative size of the fresh mass yield from conventional savory cultivation was also obtained by Seidler-Łożykowska et al. [2007]. There was a significant relationship between nitrogen dose used and date of harvesting the raw material for tested parameter. The highest value of the fresh weight yield was recorded in the savory herb harvested in the initial flowering phase after the application of 12 and 16 g per m^2 (2.35 and 2.14 $kg \cdot m^{-2}$, respectively). Mumivand et al. [2011], in the studied garden savory variety, also recorded the highest content of fresh herb mass after the use of higher and highest dose of 100 and 150 $kg \cdot ha^{-1}$ nitrogen in the form of ammonium nitrate, which corresponded to the highest doses used in the present experiment. Positive effect of increasing the doses of nitrogen fertilization on fresh weight yield was also found in garden savory of Saturn cv. [Babalar et al. 2010], thyme [Baranauskiene et al. 2003], sage [Bielski et al. 2011], peppermint [Niakan et al. 2004], sweet wormwood [Ayanoglu et al. 2002], as well as basil [Rao et al. 2007, Biesiada and Kuś 2010]. On the other hand, Golcz and Bosiacki [2008] recorded higher yield of fresh herb mass of thyme after using a lower dose of nitrogen. Similar relationship was found by Dzida and Jarosz [2006] with savory, Dzida [2013] with thyme and Nurzyńska-Wierdak et al. [2012] with basil. There was a significant difference in the yield of savory herb relative to the date of harvesting the raw material. Plants obtained in the initial flowering phase were characterized by 11% higher increase in fresh weight than plants harvested in full flowering (Tab. 1). The impact of the developmental phase on the value of examined parameter was also found in garden savory achieved for a bunch harvest, in which a higher yield was obtained in the first harvest period (July) rather than the second one (August) [Dzida et al. 2015]. Significance of ontogenetic variation to the fresh weight increase in savory was also recorded by Ziomba and Frąszczak [2008], who obtained the highest yield of plants in the initial flowering phase in relation to the phase of full flower buds formation and full flowering phase.

The highest content of dry savory herb was found in the initial flowering phase after the application of 12 g N per m^2 (504.3 $g \cdot m^{-2}$). Achieved yield of dry herb is comparable to results obtained by Sielder-Łożykowska et al. [2007] for herb of garden savory of Saturn cv. from conventional and organic cultivation. A positive effect of nitrogen dose on the yield of dry raw material of garden savory was also found by Babalar et al. [2010], Mumivand et al. [2011], El-Gohary et al. [2015] and Sahzabi et al. [2010].

The tested Saturn cv. savory raw material was characterized by a large amount of dry matter, ranging from 20.73 to 34.07%. Savory of Saturn cv. contained larger amount of dry matter as compared to the PlantCo cultivation without specifying a cultivar, for which after the application of increasing doses of nitrogen fertilization, the amount of dry matter ranged from 16.71 to 21.68% [Dzida 2013]. The highest value of dry matter in own studies was recorded in herb of savory harvested in the full flowering phase after the application of both 8 and 16 g per m^2 (34.0% and 34.07%, respectively). When analyzing the effect of increasing doses of nitrogen (0, 50, 100, 150 kg $N \cdot ha^{-1}$), an increase in dry matter yield of garden savory of Saturn cv. along with the administered dose of nitrogen was obtained [Babalar et al. 2010, Mumivand et al. 2011]. Increasing foliar fertilization of *Satureja hortensis* L. with multiconstituent fertilizer (NPK) [El-Gohary et al. 2015] and increasing soil fertilization with nitrogen under *S. hortensis* [Sahzabi et al. 2010] and *S. montana* L. in the form of urea [Said-Al. Ahl and Hussien 2016] contributed to the increase in dry matter yield of plants and the yield of essential oil. There was significant difference in the content of dry matter relative to the date of the raw material harvest. Plants obtained in the full flowering phase were characterized by 30% higher content of dry matter than those collected at the beginning of flowering. The effect of developmental phase on the yield of dry herb was also found in the mountain savory, in which higher yields were produced by plants harvested in full flowering and after flowering rather than at the beginning of flowering and in the vegetative phase [Nurzyńska-Wierdak et al. 2017]. A similar relationship was also reported in sage [Baranauskiene et al. 2011] and hyssop [Zawiślak 2011]. The importance of a longer development cycle in relation to the date of harvesting of the raw material was noted in common thyme of Sunflower cv., for which during the 180 days of the growing season of these plants, the highest value of dry herb yield was obtained [Król and Kiełtyka-Dadasiewicz 2015].

The yield of essential oil in *Satureja hortensis* L. of Saturn cv. herb ranged from 71.3 to 274.3 $kg \cdot ha^{-1}$. The highest yield of essential oil was recorded after applying a dose of 16 g nitrogen per m^2 in plants harvested at the beginning of flowering. The smallest yield of oil in the examined raw material was achieved in control plants both in the initial phase and in full flowering of plants (153.0 and 71.3 $kg \cdot ha^{-1}$, respectively) in relation to plants fertilized with nitrogen. Obtained yield of essential oil from Saturn cv. garden savory was higher than that recorded by Mumivand et al. [2011], which ranged from 60.891 to 72.723 $l \cdot ha^{-1}$. Literature data show that the content of essential oil in plants of Saturn cv. garden savory varies depending on the cultivation method, its location and weather conditions [Sielder-Łożykowska et al. 2007, Osińska and Rosłon 2016]. The yield of essential oil of the studied cultivar was dependent on both the applied nitrogen dose and development phase of plants. Significant correlation was also found between the fresh mass yield and the yield of essential oil. There has been a gradual increase in the yield of essential oil in relation to the biomass of plants produced after the application of increasing doses of nitrogen. Similar dependence of the effect of nitrogen on yield of oil and biological mass of plants was recorded by Sahzabi et al. [2010] for garden savory, where nitrogen was delivered both to soil and in foliar form. However, in the experiment performed by Alizadeh et al. [2010], there has been a gradual increase in the yield of essential oil of garden savory after delivery of multi-component fertilizer with higher nitrogen content to the plants.

Garden savory herb is characterized by the presence of secondary metabolites with antioxidant properties in its composition, which include L-ascorbic acid participating in the removal of reactive forms of oxygen and nitrogen, which is responsible for maintaining the appropriate redox state of an organism [Byer and Perry 1999]. The raw material of tested species contained from 13.33 to 44.62 mg of L-ascorbic acid per 100 g of fresh weight. Concentration of L-ascorbic acid in savory herb was comparable to the content in lemon balm, mint and thyme herb grown by conventional methods [Kazimierczak et al. 2011]. However, it was lower than the content of this parameter in garden savory herb derived from greenhouse cultivation after the application of differentiated nitrogen fertilization, where the content was in the range from 41.59 to 59.48 mg per 100 g of fresh weight [Dzida 2013]. In the conducted studies, a decrease in the content of mentioned component was recorded due to the application of increasing doses of nitrogen for both examined terms of raw material harvesting. The most abundant source of L-ascorbic acid was the savory herb grown at the lowest fertilization level (4 g $N \cdot m^{-2}$) in the initial flowering phase of plants (44.62 $mg \cdot 100\ g^{-1}$ FW) and in the phase of full flowering of plants (31.24 $mg \cdot 100\ g^{-1}$ FW). Similar results of higher content of vitamin C in the initial phase and full flowering of plants in relation to other developmental stages were obtained in the mountain savory [Nurzyńska-Wierdak et al. 2017]. The use of higher nitrogen doses caused a decrease in the concentration of mentioned component in garden savory herb in both examined dates. As the researchers emphasize, the content of vitamin C in a plant is significantly influenced by the way of cultivation, but one of the decisive factors is the fertilization of plants. The research results confirm that after using fertilizers with a higher nitrogen content in crops, a lower content of vitamin C is obtained [Kazimierczak et al. 2011, Toor et al. 2006, Caris-Veynard et al. 2004, Lee and Kader 2000]. It has been reported that the administration of more nitrogen to the substrate contributes to the reduction of secondary metabolites production in a plant, including the synthesis and accumulation of vitamin C, for the surplus of nitrogen compounds such as amino acids, proteins or nucleic acids [Coley et al. 1985, Kazimierczak et al. 2010].

One of the primary metabolites present in plants is a protein that has building, storage, regulatory and catalytic as well as protective and defensive functions in the plant. The main structural component of proteins are amino acids containing nitrogen in their structure [Kopcewicz et al. 2012]. Formation of protein in the leaves depends not only on the formation and movement of amino acids, but also on the plant's respiratory activity, the level of sugar and the inflow of nitrogen [Nowotny-Mieczyńska and Gołębiowska 1960]. In the conducted studies, a positive effect of increasing nitrogen fertilization on the protein content in the studied plants was demonstrated. The amount of protein in the Saturn cv. savory plants ranged from 21.75 to 25.42% DM. The highest concentration of protein was obtained using the highest dose of nitrogen in both studied phases of plant development. There was a slight difference in the protein content when analyzing the date of the raw material harvest. Savory plants harvested at an earlier stage of development were characterized by a slightly higher protein content. This dependence is related to the optimal supply of amino acids and depends on the rate of plant development. It is usually the highest in young leaves, and decreases with the approximation of the generative phases when the biomass increase is smaller. The obtained results allow to conclude that the presence of protein in garden savory herb is under the influence of ontogenetic factors, but is also subject to the influence of environmental factors.

CONCLUSIONS

In the period of ontogenesis, a number of developmental changes occur in plants manifesting themselves in morphological and physiological processes. The influence of studied factors on the morphological changes of savory, which were visible in the size of the yield and physiological changes that contributed to the modification of the chemical composition of the savory raw material, was recorded. The applied diverse nitrogen fertilization contributed to the increase in the fresh mass yield and the yield of essential oil of savory, while the content of dry matter and protein changed slightly under the influence of studied factor. Significantly the highest concentration of L-ascorbic acid was found when 117 kg of ammonium nitrate was used per ha of cultivation. Plants harvested in the initial flowering phase were characterized by higher yield and more L-ascorbic acid and protein. Plants harvested in full flowering contained more dry matter as compared to those harvested at the first harvest date. In the cultivation of *Satureja hortensis* L., in order to obtain the highest quality yield, it is possible to recommend nitrogen fertilization in a dose of 4–12 $g \cdot m^{-2}$ and the harvest of plants in the initial flowering stage.

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