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PLANT MORPHOLOGICAL PARAMETERS AND YIELD OF WINTER SAVORY DEPENDING ON THE METHOD OF PLANTATION ESTABLISHMENT

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ABSTRACT

Winter savory (*Satureja montana* L.) is an aromatic subshrub of significant medicinal and seasoning qualities. Winter savory herb yield and quality depend on various factors, including environmental and agronomic ones. The aim of the present study was to evaluate the effect of winter savory plantation establishment method (seedlings, herbaceous cuttings) on the morphological traits and raw material yield. A more uniform propagation material with better morphological parameters was produced using generative propagation relative to vegetative propagation. A higher fresh and dry herb yield as well as a higher yield of herb without stems were obtained from plants grown from transplants compared to plants grown from vegetative cuttings. The essential oil content in the studied plants did not vary, but the oil yield was higher in plants grown from transplants compared to those derived from vegetative cuttings.

Key words: *Satureja montana* L., plant propagation, herb yield, essential oil content, essential oil yield

INTRODUCTION

Winter savory (*Satureja montana* L.) is an aromatic subshrub from the Lamiaceae family with significant medicinal qualities. It is a species native to the Mediterranean area, introduced and now found also in different countries of Europe, in Russia, Turkey and North America [Ćetković et al. 2007]. The herbal raw material is the herb (*Saturejae herba*) harvested during flowering for essential oil or in the beginning of flowering for dried seasoning (needed are only dried leaves). The winter savory herb is characterized by varying chemical composition which is largely dependent on the genotype and habitat conditions. Essential oil content shows different variations, ranging from 0.22 to 1.61% [Ibraliu et al. 2010]. The following are mentioned as the main components of winter savory essential oil: carvacrol (63.40%), p-cymene (10.97%) γ-terpinene (3.70%) (Romania) [Trifan et al. 2015], carvacrol (63.4%) and thymol (19.4%) (Croatia) [Čavar et al. 2013]. Winter savory herbal material and essential oil exhibit antioxidant [Čavar et al. 2013, Trifan et al. 2015] and antimicrobial activity [Bezić et al. 2005, Marin et al. 2011, Serrano et al. 2011]. Bezić et al. [2005] suggest that winter savory essential oil has potential as a local antibacterial agent against important human pathogens, in particular against *Staphylococcus aureus* strains resistant to meticillin. Moreover, Marin et al. [2011] showed that *S. montana* essential oil exhibits significant activity against fungi and Gram-positive bacteria. Herbs, in particular herb extracts, are now becoming very attractive not only for modern phytotherapy but also for the food industry as spices and

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food additives. The winter savory herb contains substantial amounts of phenolics and flavonoids and probably its antioxidant activity is associated with their significant content [Hassanein et al. 2014]. Many authors also indicate that thymol and carvacrol are the main components of the antioxidant complex of *S. montana* essential oil [Nurzyńska-Wierdak 2016]. Mihajilov-Krstev et al. [2014] report that, due to its strong antimicrobial activity, winter savory oil can be a natural source of thymol, carvacrol and linalool, compounds used in the treatment of food poisoning. A study by Ćetković et al. [2007] proves that winter savory herb extracts exhibit strong antioxidant activity against hydroxyl and peroxide radicals, which is related to its phenolic content. Furthermore, the investigated extracts show antimicrobial effects.

The varied chemical composition of winter savory results from its polymorphism. Papadatou et al. [2015] demonstrated that in this species some plants are characterized by a high percentage of carvacrol in the oil (61.0%, 56.7%, 51.4%,), while other ones by the presence of monoterpene hydrocarbons (54.4%) or oxidized sesquiterpenes (80.9–89.7%) with a dominant proportion of caryophyllene oxide and spathulenol. These authors also confirmed a relationship between the chemical composition of savory raw material and cultivation conditions. Winter savory plantations are established using direct seeding, transplants or vegetative cuttings [Papadatou et al. 2015]. Savory grown from seed grows and develops more slowly than vegetatively propagated savory. Savory herb yield and quality depend on various factors, including agronomic and environmental ones. Dudaš et al. [2013] report that the percentage of dried leaves in the fresh winter savory herb collected from natural sites (Croatia) is from 21.3 to 30.7% and depends on the location and harvest date. Similarly, the essential oil content in the savory herb is dependent on harvest date and decreases from June to August (from 2.09 down to 1.05%). Damjanović-Vratnica et al. [2011] showed that the *S. montana* essential oil obtained from the herb harvested before flowering exhibited stronger antibacterial activity than the oil extracted from the herb collected during flowering. The contents of the main oil constituents were dependent on harvest date and this content before and during flowering was as follows, respectively: thymol (37.36% and 27.68%), carvacrol (15.47% and 4.40%), γ-terpinene (11.75% and 8.66%), and p-cymene (7.86% and 31.37%) [Damjanović-Vratnica et al. 2011]. A study by Hussain et al. [2016] reveals that essential oil accumulation by winter savory plants also depends on nitrogen and phosphorus fertilization as well as on the number of harvests. The aim of the present study was to evaluate the effect of winter savory (*Satureja montana* L.) plantation establishment method (seedlings, herbaceous cuttings) on the morphological traits and raw material yield of plants grown under temperate climate conditions.

MATERIALS AND METHODS

This study was carried out at the Experimental Farm of the University of Life Sciences in Lublin (51°23'N, 22°56'E) over the period 2006–2010. The climate in Lublin is moderately cold. Lublin's heavy rainfall prevails even in the hottest months (fig. 1). The Lublin region is characterized by good light conditions. The average annual sunshine duration amounts to 1542 hours (average daily sunshine duration reached 4.2 hours) which corresponds to 34.4% of possible sunshine duration. It is 16 hours more in comparison with the average value for Poland for the period of 1951–1975 [Gluza 2000]. The soil in this area is a loamy sand soil with an organic matter content of 1.6%. The experiment was designed as a single-factor experiment in four replicates. Two methods of winter savory (*Satureja montana* L.) plantation establishment were used: from transplants and from stem cuttings. Savory transplants were produced in a greenhouse. Seeds, obtained from Botanic Garden of Maria Curie Skłodowska University in Lublin, were sown about March 20 in germination boxes filled with peat substrate and then covered with a 2 mm sand layer. The seeds germinated after about 2 weeks. The seedlings were pricked out into plug trays. Winter savory cuttings were made from lateral branches collected from 2-year-old mother plants (the same plants from Botanic Garden where the seeds were collected) in the middle of April. Branch seg-

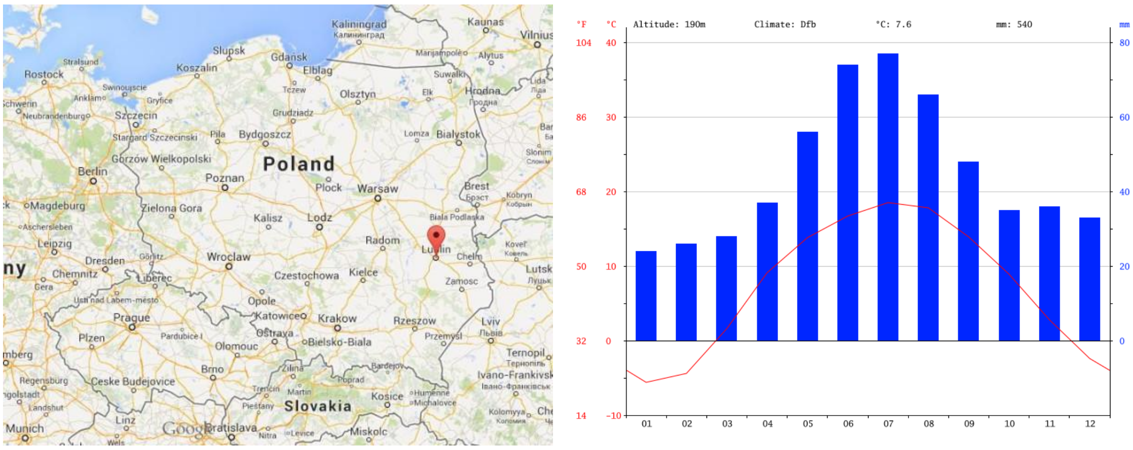

Fig. 1. Location (www.worldeasyguides.com/europe/poland/lublin) and climateogram showing the average annual temperature (line) and precipitation (posts) in Lublin (https://pl.climate-data.org)

ments were placed in plug trays filled with peat substrate. The plants remained in the greenhouse during the entire rooting period. Transplants and cuttings were hardened off for a week, reducing watering and intensively aerating the greenhouse. The plants were planted in the field at the end of May at a spacing of 30 × 30 cm. During plant growth, crop management operations were carried out, which involved regular manual removal of weeds and soil loosening. Biometric measurements were made on 20 randomly selected plants before planting transplants and cuttings in the field. The following plant traits were evaluated: plant height, number of leaves, number of lateral branches, lateral branch length, plant weight, and above-ground weight. The winter savory herb was harvested at the beginning of flowering. Savory plants were cut at a height of 5 cm above soil level. The herb was dried in a drying oven at a temperature of 30°C. The dried herb was passed through a 3 mm mesh sieve in order to obtain herb without stems. Immediately after harvest, fresh herb yield of winter savory was determined, while after drying the raw material – dry herb yield and yield of herb without stems. The percentage of herb yield without stems in dry herb yield was estimated based on the obtained results. The essential oil content in the winter savory herb without stems was extracted by steam distillation in a special apparatus in the conditions described in the European Pharmacopoeia 5th ed. [2005]. Essential oil yield was determined according to Sahzabi et al. [2010] based on the following equation: essential oil yield = essential oil percentage × flowering shoot yield, giving the results in kg·100 m^{-2}. The results were statistically analyzed by variance analysis (ANOVA), determining the significance of differences at the 0.05 level. The coefficient of variation (*CV*), defined as the ratio of the standard deviation to the expected return, was calculated.

RESULTS AND DISCUSSION

The savory propagation material (transplants, stem cuttings) morphologically varied. When analyzing the plant material before planting, it was noted that generatively propagated plants surpassed vegetatively propagated plants in terms of the selected morphological parameters (tab. 1). Analysis of the coefficient of variation allowed us to conclude that winter savory plants showed low variation (V < 20%) in terms of plant height, number of leaves, plant weight and above-ground weight (except for transplants). At the same time, the plants were characterized by

Table 1. Biometric features of winter savory plants before planting in the field (mean for 2006–2010)

Feature	transplants			cuttings		
	mean	*V*%	extreme values	mean	*V*%	extreme values
Plant height (cm)	10.1	12.0	7.6–12.0	9.0	8.2	7.8–10.2
Number of leaves (pcs·plant^{-1})	10.6	11.4	8.4–12.4	9.3	10.4	7.8–11.4
Number of lateral branches (pcs·plant^{-1})	3.6	30.9	2.0–6.0	0.6	81.6	0.0–1.6
Lateral branch length (cm)	0.8	30.3	0.5–1.5	0.1	92.2	0.0–0.5
Plant weight (g·plant^{-1})	1.0	15.8	0.7–1.2	0.7	15.2	0.5–0.9
Above-ground weight (g·plant^{-1})	0.8	24.0	0.3–1.0	0.6	16.8	0.3–0.7

V% – coefficient of variation

Table 2. Beginning of flowering of winter savory plants depending on the method of cultivation

Method of cultivation	2006	2007	2008	2009	2010
From transplants	17 VII	21 VII	23 VII	10 VII	16 VII
From cuttings	13 VII	15 VII	20 VII	12 VII	12 VII

average (transplants) and very high variation (cuttings) in the following traits: number of lateral branches (respectively: V = 20–40% and V > 60%) and lateral branch length. Comparing plants derived from transplants and vegetative cuttings, it can be stated that a more uniform material is obtained from generative propagation than from vegetative propagation. Regardless of the cultivation method, the investigated savory plants bloomed from July 11 to July 21 (tab. 2). Winter savory belongs to plants with a long growing season during which peak flowering and the highest increase in biomass occur in the late summer months [Palacio and Montserrat-Marti 2006]. The transition from the phase of vegetative growth to generative growth usually takes place at the most beneficial time for the plant, which ensures the production of the maximum number of flowers and then seeds [Tretyn and Kopcewicz 2003]. The obtained results demonstrate that the environmental conditions were favorable for the transition of savory plants from the vegetative phase to the generative phase. The small difference in the onset of the flowering stage between years should be noted. Moreover, when comparing plants grown from transplants and vegetative cuttings, it was found that vegetatively propagated plants started flowering 3–6 days earlier (on average July 14) than generatively propagated plants (on average July 17). An exception was the year 2009 in which plants grown from transplants started flowering a day earlier than those derived from cuttings.

In herbal plants, fresh and dry herb yield as well as yield of herb without stems are important parameters in the production of herbal raw material and they depend, among others, on harvest date, plantation fertilization, and drying method [Dudaš et al. 2013, Hussein et al. 2016]. The study by Dudaš et al. [2013] reveals that a decrease in winter savory herb weight during drying is from 58.34% to 66.64% and that the trend of weight reduction during the drying process is dependent on harvest timing. The raw material harvested in late summer contains more dry matter than that from an earlier harvest. After drying and separating lignified stems, from 100 kg of fresh above-ground parts of winter savory on average 24 kg of dry raw material is obtained for pharmaceutical use [Dudaš et al. 2013]. The present study showed the plantation establishment method to have a significant effect on winter savory yield. The average fresh herb yield obtained from plants grown from transplants was 110.52 kg·100 m^{-2} and it was higher by 25.5% compared to the yield found for plants grown from cuttings (tab. 3). Likewise, analyzing the dry raw material, the dry herb yield and yield of herb without stems were found to be significantly higher for the plantation established using transplants. The dry herb yield harvested from plants grown from transplants was on average 33.12 kg·100 m^{-2} and it was higher by 9.97 kg than the yield harvested from plants propagated from cuttings. In growing winter savory from transplants, the yield of herb without stems was higher by 7 kg than that harvested from the plantation established from cuttings (tab. 4).

Table 3. Herb yield of winter savory depending on the method of cultivation

	Method of cultivation	2006	2007	2008	2009	2010	Mean
Fresh herb yield (kg·100 m^{-2})	from transplants	101.21	112.39	124.06	102.06	112.92	110.52
	from cuttings	97.62	77.89	100.94	63.98	71.15	82.31
	mean	99.41	95.14	112.50	83.02	92.03	96,42
	$LSD_{0.05}$	n.s.	12.895	6.667	12.511	8.800	7.266
Dry herb yield (kg·100 m^{-2})	from transplants	33.68	26.44	42.52	31.95	31.01	33.12
	from cuttings	25.05	21.80	26.63	19.42	22.85	23.15
	mean	29.36	24.12	34.57	25.68	26.93	28.13
	$LSD_{0.05}$	7.827	5.773	5.463	2.729	4.911	3.117
Yield of herb without stems (kg·100 m^{-2})	from transplants	21.94	20.84	23.11	18.35	20.20	20.88
	from cuttings	13.88	13.49	15.47	9.66	16.92	13.88
	mean	17.91	17.16	19.29	14.0	18.56	17.38
	$LSD_{0.05}$	3.236	2.543	3.411	1.897	2.286	1.374
Percentage of herb without stems in dry herb (%)	from transplants	65.14	78.81	54.35	57.43	65.14	63.04
	from cuttings	55.40	61.88	55.79	49.74	74.04	59.95
	mean	60.27	70.34	55.07	53.58	69.59	61.49

Table 4. Essential oil content and oil yield depending on the method of cultivation

	Method of cultivation	2006	2007	2008	2009	2010	Mean
Essential oil content (%)	from transplants	1.67	1.38	1.57	2.00	2.07	1.74
	from cuttings	1.61	1.50	1.63	2.08	1.88	1.74
	mean	1.64	1.44	1.60	2.04	1.97	1.74
	$LSD_{0.05}$	n.s.	0.130	n.s.	n.s.	n.s.	n.s.
Essential oil yield (kg·ha^{-1})	from transplants	36.65	29.00	36.40	36.70	41.20	35.99
	from cuttings	22.30	20.60	25.30	20.00	31.90	24.02
	mean	29.32	24.80	30.85	28.35	36.55	30.00
	$LSD_{0.05}$	4.390	5.949	6.011	4.797	7.063	2.105

n.s. – non significant

Dudaš et al. [2013] report that the percentage of leaves in the dried herb of winter savory is initially from 63.94 to 66.83% and subsequently it increases until the flowering period, but later on it decreases. In the present study, the average percentage of herb without stems in the dry herb obtained from plants grown from transplants was 63.04%, whereas in the dry herb harvested from plants grown from cuttings – 59.95% (tab. 3). Large differences in this trait between years were noted (55.07–70.34%), which was probably attributable to climatic conditions. It was also shown that the average percentage of stems in the raw material obtained from plants grown from transplants was 36.96%, while in the case of the herb harvested from plants derived from cuttings it was more than 40% (tab. 3). A study by Dzida et al. [2015] demonstrates that summer savory, which is related to winter savory, is characterized by a variable percentage of herb without stems in total yield (65.00–76.87%), which is dependent, among others, on sowing date.

Essential oil content is an important element of savory raw material quality and depends, among others, on harvest date. This study found the essential oil content in the winter savory dry herb without stems to range from 1.44 to 2.04% in individual years of the experiment. It was shown that the average essential oil content in the herb harvested from plants grown from transplants and from cuttings did not differ significantly, averaging 1.74% (tab. 4). Similar results (1.7%) were obtained by Bezić et al. [2005] under southern Europe conditions. Furthermore, Ibraliu et al. [2010] demonstrated that winter savory growing in agro-climatically diverse habitats in Albania contained from 0.22 to 1.61% of essential oil in the herb. These authors found the highest oil content in the herb of plants growing in the eastern part of Albania, while the lowest one for plants growing in the northern part. Results found in studies conducted in different geographic regions may vary also due to significant chemical polymorphism of *Satureja montana* [Nurzyńska-Wierdak 2016]. When comparing our results with the above cited ones, it can be stated that winter savory has high adaptive capacity and can be successfully grown in temperate climate countries. Likewise, winter savory is considered to be a species with good tolerance to cool climate conditions, both in terms of raw material yield and essential oil content and chemical composition [Galambosi et al. 2002].

In analyzing the essential oil yield, it was proved that winter savory cultivation method significantly affected its level. A higher oil yield was obtained in all years of the study when winter savory was grown from transplants. The average oil yield was 0.35 kg·100 m^{-2} (plants grown from transplants) and 0.24 kg·100 m^{-2} (plants grown from cuttings) (tab. 4). Studies of other authors show that the essential oil content in the winter savory herb exhibits ontogenetic and environmental variations. Dudaš et al. [2013] revealed a decreasing trend in oil content from early summer to the late summer period (respectively: 2.09, 1.58, and 1.05%). In turn, Hussain et al. [2016] showed an opposite relationship. These authors obtained from 0.19 to 0.59% of oil in the winter savory herb harvested in the period from July to September. Damjanović-Vratnica et al. [2011] found a higher oil content in the winter savory herb harvested before flowering (1.9%) than during flowering (1.1%), thus proving that plant growth stage largely affects essential oil efficiency. Likewise, Mastelić and Jerković [2003] found the highest oil content in the winter savory herb before flowering (1.46%) and a decrease in its oil content at full bloom (0,86%).

CONCLUSIONS

1. A more uniform propagation material of winter savory with better morphological parameters is obtained from generative propagation than from vegetative propagation. However, plants derived from vegetative cuttings start to bloom 3–6 days earlier than those grown from transplants.

2. Plants grown from transplants show higher fresh yield, dry herb yield and yield of herb without stems as well as a higher percentage of herb without stems in dry herb compared to plants grown from vegetative cuttings.

3. No differences were found in the essential oil content in the herb of generatively and vegetatively propagated plants. But plants grown from transplants are characterized by higher oil yield than plants from vegetative cuttings.

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