

## VARIATION IN WINTER SAVORY (*Satureja montana* L.) YIELD AND ESSENTIAL OIL PRODUCTION AS AFFECTED BY DIFFERENT PLANT DENSITY AND NUMBER OF HARVESTS

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### ABSTRACT

Similarly to other species of the genus *Satureja* (Lamiaceae), winter savory is valued in traditional medicine in many countries of the world. Most studies on winter savory are primarily focused on the chemical composition and biological activity of its raw material, but few studies concern proper growing conditions. The aim of the present study, conducted on one-year-old plants, was to determine relationships of plant density and number of harvests with selected parameters of raw material yield of winter savory grown in field under temperate climate conditions. The herb of winter savory grown under temperate climate conditions was shown to have an average essential oil content of 1.69%. Yield of herb without stems, the percentage of this herb in dry herb and essential oil content in the herb increase as plant density decreases. On the other hand, essential oil content and yield, do not depend on number of harvests and harvest date.

**Key words:** herb cultivation, environmental variability, raw material yield, essential oil yield

### INTRODUCTION

In recent years, we have observed an increased interest in cultivation of medicinal plants, even in less favorable climates. Medicinal plants offer alternative remedies with tremendous opportunities. WHO reports show that more than 80% of the world's population rely solely or largely on traditional medicinal remedies [Batugal et al. 2004]. Winter savory (*Satureja montana* L.) from the Lamiaceae family is species native to the Mediterranean regions of Europe. The winter savory is an evergreen shrub growing up to a height of 10–50 cm, with strongly branched shoots. The plant produces brown woody stems, glossy, dark green leaves and

lavender/white flowers [Lippert and Podlech 1989]. The aromatic savory herb, collected from natural sites and from cultivated plants, is valued in traditional medicine in many countries of the world [Momtaz and Abdollahi 2008, Jafari et al. 2016, Nurzyńska-Wierdak 2016]. Modern research reveals that extracts and essential oil from the savory herb can be considered to be natural antimicrobial and antioxidant agents that can be used in food production [Serrano et al. 2011, Fathi et al. 2013, Papadatou et al. 2015] and phytomedicine [Ćavar et al. 2013, Valizadeh et al. 2014, Trifan et al. 2015, Jafari et al. 2016].

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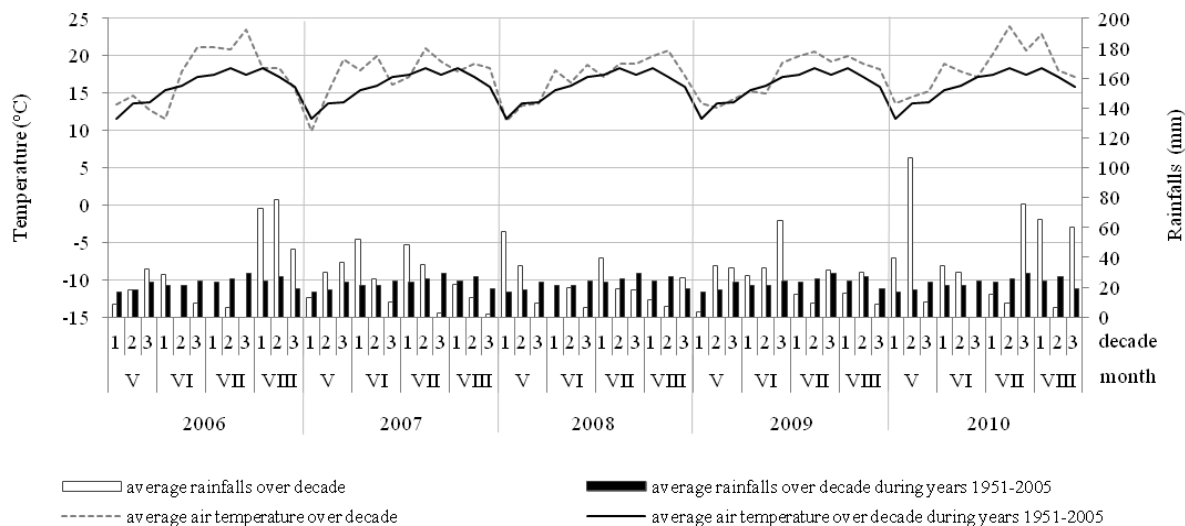
Yield of medicinal and aromatic plants is modified by many genetic, ontogenetic and environmental factors [Farahani et al. 2009, Said-Al Ahl and Hussien 2016]. Some of these relationships, in particular those that can be regulated using agronomic practices, are used in developing recommendations for growing herbal plants [Abbaszadeh et al. 2014, Ahmadi and Hadipanah 2014, Ibrahim et al. 2014, Mansoori 2014]. At the same time, suggestions appear concerning the need to carry out field investigations on the cultivation of medicinal plants and to introduce some non-native species of medicinal plants into cultivation [Singh and Vidyasagar 2015]. Planting density is an important factor that determines micro-environmental conditions in plant production systems [Morteza et al. 2009, Nurzyńska-Wierdak and Dzida 2009, Ahmadi and Hadipanah 2014]. The results of a study by Abbaszadeh et al. [2014] concerning *Satureja sahendica* Bornm. demonstrate that selection of proper planting date and density is important to obtain high raw material and essential oil yield. Hadian et al. [2016] indicate that dry weight and essential oil content in *Satureja khuzestanica* Jamzad increase with decreasing plant density, while herb yield and essential oil yield are highest at the highest density. Harvest date and number of harvests of raw material, particularly in the case of essential oil plants, are strictly related to raw material quality as determined by the quantity of volatile substances [Nurzyńska-Wierdak 2009, Nurzyńska-Wierdak and Dzida 2009, Ibrahim et al. 2014, Singh et al. 2014].

The most effective components of winter savory herb are: thymol, *p*-cymene, carvacrol,  $\gamma$ -terpinene, flavonoids, triterpenes [Momtaz and Abdollahi 2008]. There are many studies on winter savory which are primarily focused on the chemical composition and biological activity of its raw material [Bezić et al. 2005, Ibraliu et al. 2010, Serrano et al. 2010, Čavar et al. 2013, Miladi et al. 2013, Jafari et al. 2016]. Only few papers report results regarding agronomic factors that optimize yield of winter savory plants [Dudaš et al. 2013, Said-Al Ahl and Hussien 2016]. Technology of growing winter savory under temperate climate conditions has not thus far been developed, while most research studies have been conducted in areas with a milder climate.

Some studies demonstrate that wild populations of winter savory strongly vary in morphological and chemical terms and their authors suggest the need to carry out agronomic experiments on wild growing plants [Ibraliu et al. 2010, Dudaš et al. 2013]. Papadatou et al. [2015] revealed that winter savory positively responds to growing conditions and that good quality herbal raw material can be obtained under optimal agro-climatic conditions. Winter savory has lately enjoyed ever greater popularity as a spice, with the interest in the aromatic savory herb enhanced by its valuable biological properties [Serrano et al. 2011, Papadatou et al. 2015, Trifan et al. 2015]. The aim of the present study was to determine relationships of plant density and harvest method with selected parameters of herb and essential oil yield of winter savory grown under temperate climate conditions.

## MATERIALS AND METHODS

This study was carried out over the period 2006–2010 at the Experimental Farm of the University of Life Sciences in Lublin, Poland (51°23'N, 22°56'E). A field experiment was set up as a two-factor experiment (plant density and number of harvests) in a randomized block design in four replicates. The weather conditions are shown in Figure 1. Winter savory (*Satureja montana* L.) seeds were obtained from the Botanical Garden of the Maria Curie-Skłodowska University in Lublin. The study was conducted on one-year-old plants because older plants were characterized by significant stem lignification and weaker biomass increase. Seeds were sown in a greenhouse at the beginning of April and then seedlings were pricked out into plug trays with peat substrate. Plants were planted in the field at the end of May at the following spacings: 30 × 20, 30 × 30 and 30 × 40 cm. The plot area was 1.6, 2.5 and 3.2 m<sup>2</sup>, respectively. Plants were grown on grey-brown podzolic soil derived from loess deposits, with an organic matter content of 1.6%. Based on chemical analysis of the soil, the following fertilization was applied on a per hectare basis: 60 kg N, 50 kg P<sub>2</sub>O<sub>5</sub> and 100 kg K<sub>2</sub>O. Crop management operations carried out in the plantation involved soil loosening and manual weed control. No presence of



**Fig. 1.** Weather conditions during the development of the examined plants along with perennial data

pests or diseases was observed on the savory plants and therefore no chemical plant protection treatments were used.

Two methods of savory herb harvest were used: a double harvest carried out at the flower bud stage and after the stems resprouted (the beginning of July and the middle of August) and a single harvest at the beginning of flowering (the middle of August). The herb was harvested by hand. At the beginning of July, plants were cut at a height of about 6 cm above soil level, while in the middle of August winter savory was cut at about 10 cm above soil level. After harvest the herb was dried in a drying oven at a temperature of 30°C. The drying time was 10 days. Having dried the raw material, dry herb yield was determined, whereas after the dried herb was passed through a 3 mm mesh sieve, yield of herb without stems was determined. Percentage of herb without stems in dry herb was also estimated. Essential oil content in savory herb without stems was determined according to European Pharmacopeia [2005], directly distilling 30 g of dried raw material from 400 ml of water in a 1000 ml flask for 2 hours. Essential oil yield was determined according to the formula given by Sahzabi et al. [2010]. The results were statistically analyzed by two-way analysis of variance (ANOVA),

determining the significance of differences at 0.05 [Oktaba 1968].

## RESULTS AND DISCUSSION

The average yield of fresh savory herb was from 86.81 kg·100 m<sup>-2</sup> (30 × 20 cm spacing) to 89.06 kg·100 m<sup>-2</sup> (30 × 40 cm spacing). It was shown that fresh herb yield was not dependent on plant density (tab. 1). In analyzing savory yields in individual years of the study, on the other hand, a significant relationship was found between plant density and yield, with large differences in yield. The highest average fresh herb yield was obtained in 2007 (114.21 kg·100 m<sup>-2</sup>), whereas it was lower by 58.3% in 2006 and by 34.9% in 2009 (tab. 1). This indicates mutual modifications between agronomic and environmental factors which contribute to changes in the direction of individual types of variation. Yield of herbal raw material and its chemical composition are largely dependent on growing conditions [Nurzyńska-Wierdak 2009, Zawiślak and Dzida 2010, Król and Kiełtyka-Dadasiewicz 2015], which sometimes causes divergent results in individual years and study areas. The results of a study conducted by Abbaszadeh et al. [2014] reveal that the

highest *Satureja sahendica* herb yield is obtained at the highest plant density (20 × 20 cm). Likewise, Ahmadi and Hadipannah [2014] found the highest yield of fresh dragonhead herb at the lowest density of plants in the plantation. Mansoori [2014] reports that fresh and dry peppermint biomass yield significantly increased with decreasing plant density, similarly to plant height and fresh leaf weight. On the other hand, fresh biomass and essential oil yield significantly increased by increasing plant density. In the case of hyssop, Zawiślak [2011] found the highest yield of fresh herb, dry herb and herb without stems from plants grown at the medium density (40 × 40 cm) compared to the lower and higher density.

The present study demonstrated significant differences in fresh herb yield depending on the number of harvests of raw material. The average fresh herb yield from a single harvest was 103.03 kg·100 m<sup>-2</sup> and higher by 28.75% than the raw material from plants cut twice. Analyzing the data from individual years, it was found that in the case of plants cut both once and twice, the highest yield of fresh savory herb was

in 2007 (respectively: 131.30 kg·100 m<sup>-2</sup> and 97.13 kg·100 m<sup>-2</sup>). Harvest date is an important factor that modifies yield quantity and quality of herbal plants and the determination of a proper harvest date and method is of great practical importance. Number of harvests is sometimes dependent on the climatic conditions for cultivation. In Spain the environmental conditions are favorable enough to carry out four harvests of lemon balm per year, but the highest oil content (0.4%) is characteristic for September harvest [Adzet et al. 1992]. A study by Khalid et al. [2009a, b] reveals that the essential oil concentration in lemon balm significantly decreases in successive harvests and its content ranges from 0.12 to 0.25%. The presented data show that under temperate climate conditions a single harvest of winter savory herb in the first year of cultivation is more favorable for obtaining a higher fresh herb yield than a double harvest. This is associated with the fact that in the case of plants harvested twice their growth is interrupted too early, which does not allow for intensive biomass production.

**Table 1.** Fresh herb yield of winter savory depending on plant spacing and number of harvests (kg·100 m<sup>-2</sup>)

Spacing (cm)	Harvest	2006	2007	2008	2009	2010	Mean
30 × 20	double	31.32	92.57	87.89	63.17	94.82	73.95
	single	71.10	119.37	117.55	87.64	102.70	99.67
	mean	51.21	105.97	102.72	75.40	98.76	86.81
30 × 30	double	29.69	102.33	98.74	82.24	79.17	78.43
	single	63.49	122.22	100.29	98.16	111.43	99.11
	mean	46.59	112.27	99.51	90.20	95.30	88.77
30 × 40	double	28.69	96.49	91.00	49.60	73.31	67.81
	single	61.27	152.32	119.85	65.05	153.10	110.31
	mean	44.98	124.40	105.42	57.32	113.20	89.06
Mean	double	29.90	97.13	92.54	65.00	82.43	73.40
	single	65.28	131.30	112.56	83.61	122.41	103.03
	mean	47.59	114.21	102.55	74.30	102.42	88.21
LSD <sub>0.05</sub>							
Spacing (A)		1.451	6.020	1.711	8.711	4.650	n.s.
Frequency of harvest (B)		0.974	4.044	1.149	5.851	3.123	1.766
Interaction (A × B)		2.552	10.590	3.010	n.s.	8.179	4.626

**Table 2.** Dry herb yield of winter savory depending on plant spacing and number of harvests (kg·100 m<sup>-2</sup>)

Spacing (cm)	Harvest	2006	2007	2008	2009	2010	Mean
30 × 20	double	13.94	30.58	29.24	27.22	24.47	25.09
	single	24.33	29.48	29.05	28.67	28.73	28.05
	mean	19.13	30.03	29.14	27.94	26.60	26.57
30 × 30	double	14.06	32.30	27.97	31.17	26.30	26.36
	single	20.42	36.36	26.45	31.43	31.34	29.20
	mean	17.24	34.33	27.21	31.30	28.82	27.78
30 × 40	double	15.13	31.06	24.41	17.31	19.94	21.57
	single	18.43	49.64	30.37	23.49	41.00	32.58
	mean	16.78	40.35	27.39	20.40	30.47	27.07
Mean	double	14.37	31.31	27.20	25.23	23.57	24.34
	single	21.06	38.49	28.62	27.86	33.69	29.94
	mean	17.71	34.90	27.91	26.54	28.63	27.14
LSD <sub>0.05</sub>							
Spacing (A)		1.021	4.151	1.518	3.482	3.299	n.s.
Frequency of harvest (B)		0.685	2.788	1.019	2.339	2.216	1.002
Interaction (A × B)		1.796	7.302	2.670	n.s.	5.804	2.624

**Table 3.** Yield of herb without stems of winter savory depending on plant spacing and number of harvests (kg·100 m<sup>-2</sup>)

Spacing (cm)	Harvest	2006	2007	2008	2009	2010	Mean
30 × 20	double	7.82	20.36	18.79	13.62	17.28	15.57
	single	11.18	20.72	17.69	13.46	20.21	16.65
	mean	9.50	20.54	18.24	13.54	18.74	16.11
30 × 30	double	8.16	23.30	15.30	17.83	16.98	16.31
	single	11.45	22.57	15.90	18.02	21.78	17.94
	mean	9.80	22.93	15.60	17.92	19.38	17.12
30 × 40	double	7.07	21.14	13.82	10.69	15.50	13.64
	single	10.09	32.53	15.28	13.12	28.01	19.80
	mean	8.58	26.83	14.55	11.90	21.75	16.72
Mean	double	7.68	21.60	15.97	14.04	16.58	15.17
	single	10.90	25.27	16.29	14.86	23.33	18.13
	mean	9.29	23.43	16.13	14.45	19.95	16.65
LSD <sub>0.05</sub>							
Spacing (A)		0.671	1.930	1.760	2.210	2.118	0.864
Frequency of harvest (B)		0.451	1.297	n.s.	n.s.	1.423	0.580
Interaction (A × B)		n.s.	3.396	n.s.	n.s.	3.726	1.521

**Table 4.** Percentage of herb without stems in dry herb of winter savory depending on plant spacing and number of harvests

Spacing (cm)	Harvest	2006	2007	2008	2009	2010	Mean
30 × 20	double	56.09	66.57	64.26	50.03	70.61	62.05
	single	45.95	70.28	60.89	46.94	70.34	59.35
	mean	49.66	68.39	62.57	48.48	70.47	60.70
30 × 30	double	58.03	72.13	54.70	57.20	64.56	61.87
	single	56.07	62.07	60.11	57.33	69.49	61.43
	mean	57.05	67.10	57.40	57.26	67.02	61.65
30 × 40	double	46.72	68.06	56.61	61.75	77.73	63.23
	single	54.74	65.53	50.31	55.85	68.31	60.77
	mean	50.73	66.79	53.46	58.80	73.02	62.00
Mean	double	53.44	68.98	58.71	55.64	70.34	62.32
	single	51.75	65.65	56.91	53.33	69.24	60.55
	mean	52.59	67.31	57.81	54.48	69.79	61.43

**Table 5.** Essential oil content depending on plant spacing and number of harvests (%)

Spacing (cm)	Harvest	2006	2007	2008	2009	2010	Mean
30 × 20	double	1.38	1.56	1.80	2.03	1.73	1.70
	single	1.50	1.53	1.60	1.43	1.76	1.56
	mean	1.44	1.54	1.70	1.73	1.74	1.63
30 × 30	double	1.49	1.62	1.81	1.74	1.69	1.67
	single	1.65	1.58	1.52	1.60	1.85	1.64
	mean	1.57	1.60	1.66	1.67	1.77	1.65
30 × 40	double	1.43	1.88	1.89	1.83	1.78	1.76
	single	1.73	1.80	1.90	1.80	1.90	1.83
	mean	1.58	1.84	1.89	1.81	1.84	1.79
Mean	double	1.43	1.69	1.83	1.87	1.73	1.71
	single	1.62	1.64	1.67	1.61	1.83	1.67
	mean	1.52	1.66	1.75	1.74	1.78	1.69
LSD <sub>0.05</sub>							
Spacing (A)		0.118	0.176	0.168	0.130	0.086	0.122
Frequency of harvest (B)		0.169	n.s.	0.148	0.188	0.098	n.s.
Interaction (A × B)		0.241	0.241	0.258	0.227	0.194	0.248

Dry herb yield of winter savory was not dependent on plant spacing, but number of harvests had a significant effect on the value of this yield (tab. 2). The average dry herb yield obtained from a single harvest was 5.6 kg higher than the dry herb yield from plants cut twice. Analyzing the results from the entire study period, it was shown that dry herb yield was significantly higher when the herb was harvested once compared to that obtained in the case of double harvest (tab. 2). Timing of harvest of aromatic herbs affects biomass yield and essential oil content, but depending on environmental factors [Zawiślak and Dzida 2010, Zawiślak 2011]. Said-Al, Ahl and Hussien [2016] found a twice higher weight of dried winter savory herb harvested 4 months after planting compared to 2 months after planting. This indicates a significant increase in savory herb biomass between two harvests of the herb, which is confirmed by these results.

In many species of herbal plants, the herb without stems is the most frequent type of raw material that is used for seasoning purposes. The average yield of herb without stems obtained in this experiment was significantly dependent on number of harvests (tab. 3). When savory was cut once, the yield was higher by 16.32% than in the case of plants harvested

twice. As regards the effect of number of harvests on yield of herb without stems in the successive years of the study, no significant relationship was revealed in the years 2008 and 2009. However, the statistical analysis results show a significant relationship between yield of herb without stems obtained from plants grown at the 30 × 20 cm spacing relative to 30 × 30 cm. The average value of this yield was higher by 6% than in the case of plants grown at the 30 × 30 cm spacing (tab. 3). In all years of the study, the percentage of herb without stems in dry herb was higher for the raw material obtained from plants cut twice (tab. 4). Stems, which are a waste material in the winter savory herb, accounted for about 40% of the dry herb harvested once during the growing season. The percentage of stems in the dry herb harvested twice was lower – 37.68%. The dry winter savory herb obtained from plants grown at the lowest density (30 × 20 cm) was characterized by the lowest percentage of herb without stems in dry herb (60.70%). This percentage increased with increasing plant spacing and was highest (62.0%) in the raw material harvested from plants grown at the 30 × 40 cm density (tab. 4). This indicates that dry herb yield of winter savory grown at a lower density has better parameters

**Table 6.** Essential oil yield depending on plant spacing and number of harvests (kg · 100 m<sup>-2</sup>)

Spacing (cm)	Harvest	2006	2007	2008	2009	2010	Mean
30 × 20	double	0.11	0.36	0.34	0.28	0.29	0.28
	single	0.17	0.31	0.28	0.19	0.35	0.26
	mean	0.14	0.33	0.31	0.23	0.32	0.27
30 × 30	double	0.12	0.38	0.28	0.31	0.29	0.28
	single	0.19	0.36	0.24	0.29	0.40	0.30
	mean	0.15	0.37	0.26	0.30	0.34	0.29
30 × 40	double	0.10	0.39	0.26	0.19	0.27	0.24
	single	0.17	0.34	0.29	0.23	0.53	0.31
	mean	0.13	0.36	0.27	0.21	0.40	0.27
Mean	double	0.11	0.37	0.29	0.26	0.28	0.26
	single	0.18	0.34	0.27	0.23	0.42	0.29
	mean	0.14	0.35	0.28	0.25	0.35	0.27
LSD <sub>0.05</sub>							
Spacing (A)		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Frequency of harvest (B)		n.s.	n.s.	n.s.	n.s.	0.133	n.s.
Interaction (A × B)		n.s.	n.s.	n.s.	n.s.	0.256	n.s.

compared to higher density. For marjoram, Zawiślak and Dzida [2010] demonstrated that herb without stems had a higher percentage in dry herb in the case of plants harvested at the second harvest date (September) relative to those harvested at the first date (July). In turn, hyssop plants grown at the lowest and highest densities had a higher percentage of herb without stems in dry herb compared to plants grown at the medium density [Zawiślak 2011].

Essential oil content and yield depend, among others, on the growing method [Nurzyńska-Wierdak and Dzida 2009, Król 2013, Abbaszadeh et al. 2014, Mansoori 2014]. Winter savory plants investigated were characterized by variable essential oil content in the herb depending on weather conditions in individual years of the study and agronomic practices used (tab. 5). The average oil content in the raw material was 1.69%. With decreasing plant density, the essential oil content in the herb increased. Harvest method was not shown to significantly affect oil content in the savory herb, though such differences were found in all years of the study, except for the second year. The interaction of the investigated factors was also revealed to have a significant effect on this quality characteristic of savory yield. In two years of the study, more essential oil in the herb was obtained in the case of single harvest, while in two other years in the case of double harvest. It seems that these modifications are due to the superimposition of two types of variation: agronomic and climatic. Harvest date of herbal raw materials from essential oil plants determines their chemical composition and biological activity mostly due to ontogenetic variation [Ibrahim et al. 2014, Zantar et al. 2015]. The marjoram herb harvested at the beginning of September contains more oil than that cut in the middle of July [Zawiślak and Dzida 2010]. The average essential oil content found in this experiment was  $0.27 \text{ kg} \cdot 100 \text{ m}^{-2}$  and, except for one year of the study, was not dependent on plant density and herb harvest method (tab. 6). Essential oil content and yield in peppermint increases with increasing plant density [Mansoori 2014]. Plants grown at a higher density accumulate more essential oil than those grown at a lower density [Nurzyńska-Wierdak and Dzida 2009]. Increased

density of pot marigold plants results in a decrease in essential oil content in raw material [Król 2013]. In the case of hyssop, plant spacing was not found to significantly affect essential oil content [Zawiślak 2011]. Planting density has a significant effect on most morphological traits, raw material yield and essential oil content in the herb of *Satureja sahendica* Bornm plants [Abbaszadeh et al. 2014]. Although essential oil content in *S. sahendica* herb is highest in plants grown at the lowest density, oil yield is highest at the highest density. Due to this, these authors indicate that  $20 \times 20 \text{ cm}$  is the optimal planting spacing for *S. sahendica* plants under the climatic conditions of Iran.

## CONCLUSIONS

Herb yield of winter savory grown under temperate climate conditions is modified by agronomic factors. Among the agronomic factors studied, number of harvests and harvest date have a greater effect on fresh and dry savory herb yield than planting density.

Yield of herb without stems, the percentage of this herb in dry herb and essential oil content in the herb increase with decreasing planting density.

Essential oil content and yield of winter savory were not dependent on number of harvests and harvest date.

Proper plant density (planting spacing:  $30 \times 30 \text{ cm}$  and  $30 \times 40 \text{ cm}$ ) as well as harvest date and number of harvests (a single harvest at the beginning of flowering) determine good herb yield from one-year-old winter savory plants grown under temperate climate conditions.

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