Effect of Irrigation on the Production and Secondary Metabolites of Summer Savory (*Satureja hortensis* L. 'Budakalászi')

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Summary

As consequence of the predicted climatic changes, analysis of the effects of drought stress on different plant species seems to be essential. In our study summer savory (*Satureja hortensis* L. 'Budakalászi') was investigated in an open field experiment in the Experimental and Research Farm of the Szent István University in Soroksár. To identify the effect of water supply, irrigated (W) (additional 2 × 20 mm water per week) and non-irrigated control (C) treatments were applied on the plants with 50 × 30 cm growth distance. During the vegetation period water potential (pressure chamber) and the chlorophyll content (SPAD-502) were measured. Fresh and dry mass and leaf ratio were determined when plants were harvested at full flowering stage. The essential oil content was hydro-distilled with a Clevenger-type apparatus according to the Hungarian Pharmacopoeia (7th ed.). The essential oil composition was identified by GC-MS. The effect of the irrigation was obvious for the majority of the examined traits. The chlorophyll content decreased (W: 32.58 SPAD unit; C: 35.70 SPAD unit) while the water potential increased (W: -12.85 bar; C: -21.35 bar) significantly with water supply. The fresh mass (W: 104.00 g per plant) and dry mass (W: 14.09 g per plant) of the watered plants were higher compared to the untreated control (fresh: 60.55 g per plant; dry: 6.58 g per plant). The leaf ratio did not change significantly (W: 51.04%; C: 48.40%). The essential oil composition of savory seems to be independent from the water supply. The main components of the essential oil were carvacrol (50-51%), γ-terpinene (35-36%), and p-cymene (3-4%) in both oils. Irrigation decreased the accumulation level of the essential oil (W: 4.289 ml 100 g^{-1} DM; C: 4.859 ml 100 g^{-1} DM). However, due to higher biomass, the essential oil yield of well-watered plants was higher. Based on this information we may declare that during the cultivation of savory, additional irrigation seems to be necessary.

Key words

drought, irrigation, *Lamiaceae*, stress, water supply

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Introduction

Summer savory (*Satureja hortensis* L.) is a widely known annual herbaceous plant from the *Lamiaceae* family. It is traditionally used as a spice and nowadays the demand is increasing because of its wide application potential as natural antioxidant and antimicrobial agent (Exarchou et al., 2002; Madsen et al., 1996; Pank et al., 2004). The essential oil (EO) content varies between 0.5 and 3.5 ml 100 g^{-1} dry mass (DM) (Hadian et al., 2007; Pank et al., 2004). However, without the stem fraction a higher EO content (5.9%) was also detected (Seidler-Łożykowska et al., 2009). The main components of the essential oil are carvacrol and γ-terpinene (Pank et al., 2004; Svoboda and Greenaway, 2003).

Several papers were published about the effect of drought stress on the secondary metabolites of the *Lamiaceae* species (Baher et al., 2002; Gershenzon et al., 1978; Radácsi et al., 2010; Simon et al., 1992; Zámboriné et al., 2005) but only a few about the genus *Satureja*. Gershenzon et al. (1978) described that *Satureja douglasii* produced lower biomass, shorter stems and smaller leaf area but deeper antocyanic coloration and more glandular trichomes on the leaves as the result of lower soil moisture. However, the monoterpenoid yield per leaf dry mass did not change significantly. Baher et al. (2002) reported that the height, stem fresh and dry weight of summer savory were reduced under low water supply, while the EO content was increased. Differences were not detectable in the EO composition. Radácsi et al. (2016 detected that the moderate drought stress (50% soil water capacity, SWC) caused the highest EO content of summer savory, while the control (70% SWC) and severe water stress (30% SWC) resulted in lower EO accumulation level.

Although in the Central European area precipitation seems to be the primary yield limitation factor in most of our cultivated crops, till now it is not clear what is the real effect of drought on the production and secondary metabolites of the different species. Selmar and Kleinwächter (2013) concluded that the level of secondary metabolites is frequently enhanced by the drought stress, but the stress induced is counteracted by the loss of biomass. The aim of this study was to get a deeper insight into the effects of irrigation on summer savory. The goal was to support growers with appropriate information on the consequences of water supply concerning quantity and quality of the drug.

Material and methods

The plant growing was carried out in the experimental field of Szent István University in Budapest-Soroksár (Hungary) in 2009. Seeds of *Satureja hortensis* L. 'Budakalászi' cultivar were selected from the gene bank of the Department of Medicinal and Aromatic Plants. Seeds were sown into seed trays (27 × 57 cm) in greenhouse in the middle of March. The seedlings with two leaves were transplanted to 0.1 L pots. In the end of second decade of May, thirty individuals for each treatment were planted into the open field plots with 50 × 30 cm spacing (1 irrigated and 1 non-irrigated plot). The soil of the experimental field was sandy, the detailed characteristics of the soil are presented in the Table 1.

To detect the effect of water supply, irrigated (W) and non-irrigated control (C) treatments were applied. The irrigated plot was watered with 20 mm water twice a week, while the non-irrigated ones got only the natural precipitation. A spraying irrigation system was used. The amount of water was checked by a water meter. One meter isolation distance was kept between the irrigated and non-irrigated plots. The climatic parameters of the experiment period are indicated in the Figure 1.

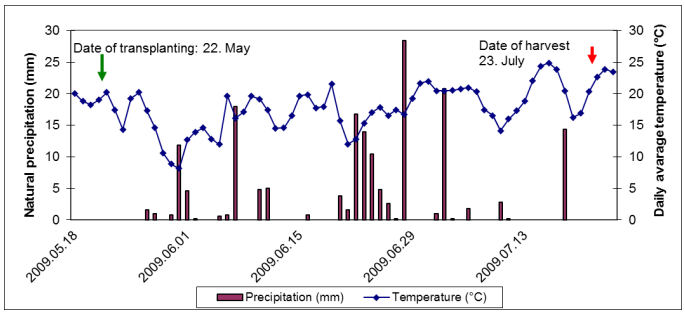

Figure 1. Meteorological conditions in the period of experiment (May 18 - July 23, 2009, Budapest)

The water potential of the plants was determined in the leaves of the second and third nodes under the top of the shoots. The measurements were carried out in nine replications (nine plants) per treatment in full flowering phase, two days after watering, between noon and 2 p.m. in a pressure chamber (Model 610, PMS Instrument Company, Albany, USA). For increasing the pressure in the chamber CO_2 was used.

Chlorophyll content of the leaves indicated by the quantification of green colour intensity was measured by a SPAD 502Plus (Konica Minolta Inc., Japan) equipment. The readings were taken at the third internodes under the inflorescence one day before harvesting. Eight readings were made on each leaf and their mean was calculated. This measurement was repeated in nine replications per each treatment.

Plant height of ten randomly selected individuals was measured from the root neck to the tip of the shoots just before harvesting. Plants were harvested at the full flowering stage (majority of buds opened) in the end of July 2009. After harvesting the fresh mass of the plant individuals was measured. Plants were dried in the shade at room temperature till constant weight, then the dry

Table 1. Characteristic of the soil of the experimental field

pH_{H_2O}	Salt (%)	Humus (%)	NO_3-N (mg kg^{-1})	P_2O_5 (mg kg^{-1})	K_2O (mg kg^{-1})	Ca (%)
6.49	0.039	1.17	1.24	291	36.7	0.489
Mg (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	$CaCO_3$ (%)	
53	106	37.8	1.73	3.47	<1	

mass was recorded. The dry leaves and flowers were separated from the stems and their ratio to the stem was calculated. These measurements were carried out in 10 replicates per treatment. Further laboratory analyses were carried out with bulk samples of the harvested individuals in three replications, however, only the leaf + flower fraction was used.

The EO content was hydro-distilled from leaves and inflorescence (excluded the stems), in three replications per treatment with a Clevenger-type apparatus according to the VII. Hungarian Pharmacopoeia (Pharmacopoeia Hungarica, 1986). The composition of the EO was determined by GC-MS method. GC analysis was carried out using an Agilent Technologies 6890 N instrument equipped with HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm film thickness), working with the following temperature program: initial temperature 60°C, heating at a rate of 3°C /min up to 240°C; the final temperature was maintained for 5 min; injector and detector temperatures: 250°C; carrier gas: helium (constant flow rate: 1 mL min-1); split ratio: 30:1, injection volume 0.2 µL (10%, n-hexane). A mixture of aliphatic hydrocarbons (C9-C23) in n-hexane was injected to calculate the linear retention indices using the generalized equation of van Den Dool and Kratz (1963). For the GC-MS analysis the above mentioned equipment was used with an Agilent Technologies MS 5975 detector. Ionization energy was 70 eV. The mass spectra were recorded in full scan mode, which revealed the total ion current (TIC) chromatograms. The linear retention indices (LRI) and mass spectra were compared with those of commercial (NIST, Wiley) and home-made library mass spectra built up from data obtained from standard (Sigma/Aldrich) pure compounds. The proportions of the individual compounds were expressed as total area percentages.

The results were analysed with the IBM SPSS Statistics 19 software. Means, standard deviations, and two independent sample t-test were applied. Normality of the residuals was proved according the Kolmogorov-Smirnov method. Homogeneity of variances was tested by Levene's method.

Results and discussion

The applied irrigation increased the water potential of the plants (Table 2). Low water potential of leaves appears when the transpiration rate of the leaves is higher than the water absorption rate of the roots (Jarvis, 1976). Low soil water content is one of the most common reasons of the roots' reduced water uptake. In this experiment, lower leaf water potential was measured under non-irrigated control (C). The increasing water saturation level in the soil influenced it positively.

Higher SPAD value (35.70 SPAD unit) was detected in the control plants. Almost 10% lower chlorophyll content was measured under the well-watered (W) circumstances (Table 2). Former findings in connection with drought stress and chlorophyll content are contradictory. Rahimi et al. (2010) detected similar result to these ones in *Plantago ovata* and *Plantago psyllium* plants, while intensive decrease of SPAD value was reported in soy and cotton plants (Inamullah and Akihiro, 2005). In studies performed by Radácsi et al. (2016) even higher SPAD values (up to 65 SPAD unit) were detected in savory under controlled environment. As the result of drought, the photosynthetic activity of plants might decrease.

The irrigation did not influence the plant height of savoury plants. The average height of plants in both treatments was 36 - 37 cm (Table 2).

Table 2. Effect of water supply on the physiological parameters (A) and production (B) of summer savory

	Measured parameter	Treatment		Statistics
		Irrigated (W)	Non-irrigated control (C)	
A	Water potential (bar, mean±SD)	-12.85±0.78	-21.35±1.25	* (p≤0.000)
	Chlorophyll content (SPAD unit, mean±SD)	32.58±2.59	35.70±2.56	NS (p=0.554)
B	Plant height (cm, mean±SD)	37.36±2.01	36.73±3.69	NS (p=0.621)
	Fresh mass (g per plant, mean±SD)	104.00±15.80	60.55±15.72	* (p≤0.000)
	Dry mass (g per plant, mean±SD)	38.82±5.96	28.36±8.02	* (p≤0.002)
	Leaf ratio (%, mean±SD)	51.04±3.46	48.40±4.58	NS (p=0.143)
	Essential oil content (ml 100 g^{-1} DM, mean±SD)	4.289±0.107	4.859±0.187	* (p=0.010)
	Essential oil yield[a] (ml per plant, mean±SD)	0.850	0.667	

* - significant difference (α=0.05); NS – not significant; [a] – calculated data

Although both in the scientific literature (Hoppe, 2012; Momtaz and Abdollahi, 2008) and in the cultivation practice summer savory is described as a species preferring sunny and arid conditions, in this experiment the higher water regime resulted in higher production. Under watered conditions both fresh and dry mass of the plants increased significantly (Table 2). Fresh mass of the control plants (60.55 g per plant) was only the half of the well-watered ones (104.00 g per plant). The same tendency was observed in the case of dry mass (Table 2). Dry mass of differently treated plants was statistically different from each other. However, the ratio of difference was lower than in the case of fresh mass. It shows, that not only the dry matter content of the plants was influenced by the watering but also the water content as well. No significant difference concerning the leaf ratios compared to the total shoot mass was measured among the treatments (Table 2). The average values were between 48 and 51%.

Significantly higher EO content was measured in the untreated control plants (4.859 ml 100 g^{-1} DM) than in the irrigated plants (4.289 ml 100 g^{-1}) (Table 2). However, the calculated essential oil yield per plant showed opposite tendency. Practically, it means that the slightly higher EO content cannot compensate the loss of biomass production.

Thirteen compounds were identified in the distilled EO (Table 3). In each sample, carvacrol and γ-terpinene were the main components of the oil. The water supply did not modify their ratio significantly. Proportion of carvacrol was detected in both samples between 50-51%, while γ-terpinene varied between 34.9% (C) and 35.7% (W). The third largest compound in the essential oil was p-cymene, the common precursor of both carvacrol and γ-terpinene. This tendency was detected in former study of

Radácsi et al. (2016), however, in the climatic chamber the severe drought stress enhanced the number of detected compounds.

Additional water supply influences the majority of the measured parameters of summer savory. Thus, in the cultivation practice under arid conditions irrigation might enhance biomass and EO yield without significant influence on its quality.

Table 3. The effects of water supply on the essential oil composition of summer savory

Component	RT	LRI	Well watered (W)	Untreated control (C)
α-thujene	5.31	928.14	0.64	0.75
α-pinene	5.48	934.88	1.17	1.47
ß-pinene	6.64	980.86	0.61	0.69
ß-myrcene	6.99	994.73	1.21	1.42
α-phellandrene	7.43	1008.87	0.20	0.23
α-terpinene	7.79	1019.28	3.27	3.43
p-cymene	8.09	1027.95	3.24	3.86
limonene	8.19	1030.84	0.34	0.40
γ-terpinene	9.20	1060.02	*35.76*	*34.96*
carvacrol	19.37	1317.71	*50.97*	*50.74*
carvacrol-acetate	22.04	1382.00	1.63	1.06
ß-caryophyllene	23.68	1419.95	0.83	0.82
ß-bisabolene	27.23	1508.25	0.13	0.17
Total			100.00	100.00

RT= retention time, LRI= linear retention index relative to C9-C23 n-alkanes on a HP-5MS capillary column

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