Growth and Essential Oils of *Salvia officinalis* Plants Derived from Conventional or Aeroponic Produced Seedlings

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Summary

Salvia officinalis L. (Lamiaceae) is cultivated in many countries as a valuable medicinal and aromatic plant with antiseptic and spasmolytic effects due to its essential oils, used as ingredient in many phytopreparations. The present study aimed to enhance the growth of *S. officinalis* plants and to improve the essential oil quality, applying hydroponic technology. Seeds of cultivated *S. officinalis* were used as initial material: experimental seeds were germinated on aeroponic vertical system into peat cubes in pots with keramzite and control ones in terrines with ordinary soil. All seedlings were transferred to the greenhouse in pots with sterilized compost and universal soil mixture. The morphometric parameters of both groups, 80 seedlings each, were compared after two months; 36 plants per group were transferred to the field plot, and herbage of 10 randomly chosen plants per group was harvested during the full flowering stage in two consecutive years. Essential oils were extracted on Clevenger apparatus and their composition was analyzed by GC/MS. Conventionally obtained seedlings were significantly higher ($P<0.001$), while aeroponically derived plants increased their biomass by 18.4%, ramified earlier, and developed much more generative shoots ($p<0.05$). Plants' size of the two groups equalized at the second year, essential oils yield being about 1.1% w/v. Some fluctuations in essential oils composition was noticed in the two groups and the two years. To summarize, application of aeroponic system shortened the period from germination to harvest, enhanced plants flowering and reflected on the composition of the essential oils.

Key words

sage; medicinal and aromatic plant; hydroponic technologies; cultivation

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Introduction

Salvia officinalis L. (Lamiaceae) is cultivated in many countries as a valuable medicinal and aromatic plant. Aerial parts of *S. officinalis* are used in the treatment of many diseases such as diabetes, cancer, hot flashes, obesity, diarrhea, as well as for regulation of cholesterol level and for memory improvement (Hamidpour et al., 2013; Abdelkader et al., 2014). Its antiseptic and spasmolytic effects are mainly due to the essential oils accumulated in the aboveground herbage (Mossi et al., 2011). Essential oil of sage was found to have activity against *Candida albicans* strains and could be used as denture cleanser (Sookto et al., 2013). Plants are used as tea or as source of ingredients for many phytopreparations for oral usage as capsules, chewables (e.g. gummies, tablets), liquids, powders, strips or tablets (European Medicines Agency, 2009; Health Canada, 2013). Essential oils quantity and composition are influenced by both phenophase and season (Peshevski et al., 1997). In addition, the yield and the composition of the essential oils depend on the method of herbage drying (Sellami et al., 2012). *S. officinalis* is among the species with commercial importance for Bulgaria, with annual export about 6.2 t *Folium Salviae* and 8.3 t *Herba Salviae* (Evstatieva et al., 2007).

It is considered that soilless cultivation is applicable to almost any terrestrial plant. One of the main benefits of hydroponic gardening is the significant crop yields increase (Texier, 2014). Plants receive balanced nutrients and crop productivity does not depend on the seasons, weather, precipitations, pest infestations, neither on the soil type, fertility, salinity, pH, and soil related weeds, diseases and pests; in addition, water waste and pesticides are avoided. As a part of a project dealing with test of soilless cultivation of medicinal and aromatic plants, experiments with *S. officinalis* were carried out, resulting in establishment of *ex situ* collection of the species in the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences.

The present study aimed to enhance the growth of *Salvia officinalis* plants and to improve the essential oil quality, applying hydroponic technology.

Material and methods

Seeds of cultivated *S. officinalis* were used as initial material. Conventional seedlings production, used as a control, began in November 2014 in terrines with ordinary soil, then in pots, in a greenhouse. Seedlings production on aeroponic vertical system Green Diamond (GH) started in April next year (seeds into peat cubes in pots with keramzite) in a phytotron. The initial nutrient solution consisted of distilled water with 6 ml/L First Feed (Root It), pH 5.5-6.5, EC 1 mS^{-1}. After root system development the solution was replaced with more appropriate one for this stage, containing 0.7 ml/L of each Flora Micro, Flora Grow, and Flora Bloom (GH). In early July all seedlings were transferred into pots with sterilized compost and universal soil mixture in proportion 2:1. At this moment the morphometric parameters of both groups of 80 seedlings each were measured; conventionally grown seedlings being 7-month old, while hydroponic seedlings only 3-month old. After two months in the greenhouse, in September, 36 seedlings from both groups were planted in the experimental field plot. In early June 2016, herbage of 10 randomly chosen plants per group was harvested during the full flowering stage and separately dried at shadowed and airy place in the greenhouse. Comparison of the two groups was made on the base of plant survival, plant flowering, individual assessment of fresh and dry weight, number of vegetative and generative shoots, number of branches and leaves, as well as quantity and composition of the essential oils. Data was statistically analyzed by Excel ANOVA Single Factor.

Essential oils were extracted on Clevenger apparatus by hydro-distillation, from samples of 50 g DW consisting of leaves and florets of separate plants, in a flask with 500 ml water, for 2 h. Oil composition was analyzed by GC/MS. Oil sample analyses were performed on Thermo GC equipped with a Focus DSQ II mass detector and a HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Chromatographic conditions were as follows: helium as carrier gas at a flow rate of 1 mL/min; injection volume was 1 μl, and the split ratio was 1:50. Column temperature was 60°C for 10 min, and programmed at the rate of 3°C/min to 200°C, and finally, held isothermally for 10 min. The injection port was set at 220°C. Significant quadrupole MS operating parameters: interface temperature 240°C; electron impact ionization at 70 eV with scan mass range of 40 to 400 m/z at a sampling rate of 1.0 scan/s. The components were identified by comparing their relative retentions times with the retention times of authentic standards, and mass spectra with National Institute of Standards and Technology (NIST), of the GC/MS system, and literature data (Adams, 2007). Oil analyses were repeated in 2017.

Results and discussion

Seeds in the terrines germinated for three weeks (November 2014), and those on the aeroponic vertical system Green Diamond (GH) – for two weeks (April 2015). At the age of five weeks hydroponic seedlings were about 10-15 cm high with well-developed roots (Fig. 1). Soilless cultivation stimulated development of vigor root system and shoot ramification (Fig. 2, 3).

Table 1. Characteristics of the seedlings at the time of their transferring to pots with sterilized compost and universal soil, 80 plants per group (early July 2015).

Plants	Age of seedlings	*Stem length [cm]	Ramification: first and second rank shoot branches			Root length [cm]
			Number of first rank branches	Number of plants with second rank branches	Total number of second rank branches	
Conventionally grown	7 months	31.6 ± 3.8	8.8 ± 1.9	15 of 80	134	no data
Hydroponically grown	3 months	23.9 ± 2.5	7.8 ± 0.6	19 of 80	140	12.6 ± 1.7

* statistically proved difference $p < 0.01$ (ANOVA Single Factor)


Figure 1. Vertical aero-hydroponic system Green Diamond (GH) with 5-week old seedlings of *S. officinalis*

Seedlings' growth was rapid and at the age of three months the average plant height was 23.9 ± 2.5 cm and about ¼ of all 80 plants had formed branches of second rank (Table 1).

Comparative measurement in early July 2015 showed that the 7-month old conventionally grown plants were significantly higher than the 3-month old hydroponically obtained ones ($P<0.001$) but the ramification of the two groups was similar.

All plants grew well in the experimental field plot (Fig. 4). Differences in branching were notable for one year (Table 2). All 36 hydroponically derived plants bloomed while 11.1% of conventionally grown plants didn't form flowers the first year. Two of the hydroponically derived plants died during wintering. Although the variation concerning the number of vegetative and generative shoots, branches and leaves was important, and plants were not uniform, it is worth to notice that in general the flowering of the hydroponically derived plants was much more expressed, the number of their generative shoots being significantly higher ($p<0.05$). The number of their branches and leaves was much

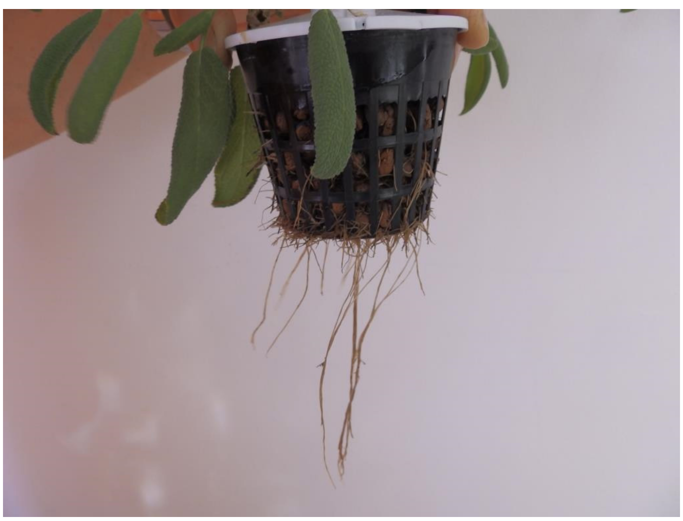
Figure 2. Root system of 10-week old seedling of *S. officinalis* obtained by soilless cultivation

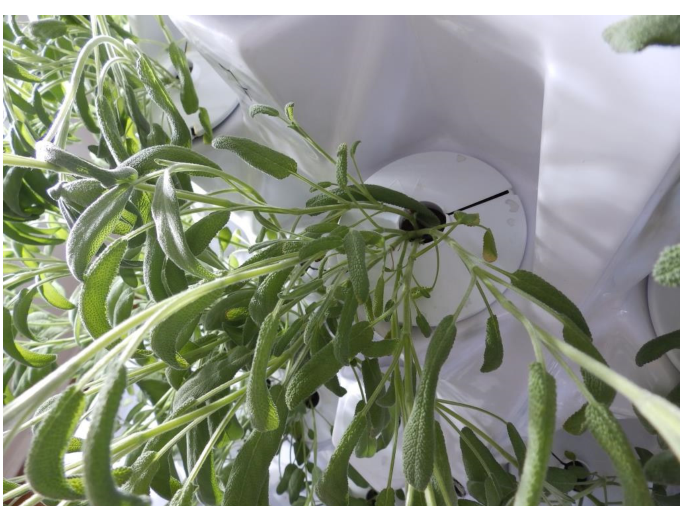
Figure 3. Ramified 10-week old seedling of *S. officinalis* obtained by soilless cultivation with branches of first and second rank


Figure 4. General view of *S. officinalis* experimental plants (conventionally derived plants on the left half, hydroponically derived plants on the right half).

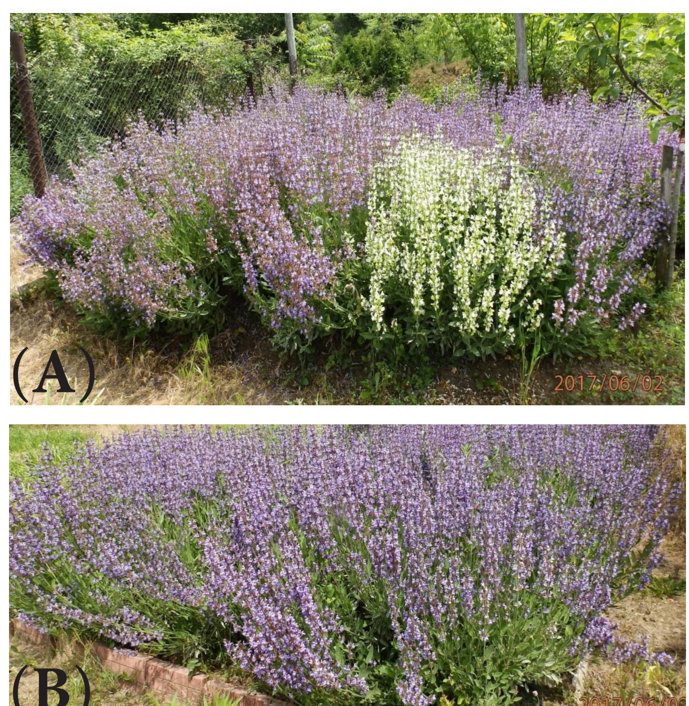

Figure 5. Conventionally (A) and hydroponically (B) derived plants during the full flowering stage in their second year on the experimental field.

Table 2. Plants characteristics at full flowering stage, under controlled cultivation at the experimental field, 36 plants per group (early June 2016).

Plants	Fresh weigth [g]	Dry substance %	Average number of shoots, branches, and leaves per plant				
			Vegetative shoots	Generative shoots*	% of generative shoots*	Branches	Leaves
Conventionally grown	271±113	22.5±1.8	51.7±20.3	22.4±15.9	25.3±21.6	253±136	750±222
Hydroponically grown	321±224	23.6 ± 2.6	48.6±45.1	45.9 ± 38.3	49.6±26.2	293±218	938±568

* statistically proved difference $p < 0.05$ (ANOVA Single Factor)

higher, which resulted in increase of the fresh biomass by 18.4%. Next year all plants increased significantly their size and during the full flowering in May 2017 the average plant fresh weight was 1373 ± 655 g (Fig. 5).

The average essential oils concentration of the harvested in early June 2016 conventionally and hydroponically derived plants were 1.08 and 0.98 % w/v, respectively. The second year, all experimental plants bloomed and the difference in their size decreased, the average essential oils concentration being 1.10 and 1.08 % w/v for the two groups.

The main compounds in the essential oils detected chromatographically and their mean values are presented in Table 3. Alpha-thujone and 1,8-cineole were detected in highest concentrations. Besides the components listed in the Table 3, other volatile compounds were also identified in minor concentrations such as p-cymene, caryophylene oxide, bornyl acetate, etc. Other authors also reported α-thujone and 1,8-cineole as main components of the *S. officinalis oil*, together with camphor (Peshevski et al., 1997; Pop et al., 2013).

In general, essential oils content depends on the phenophase, its maximum being in the stage buttoning to full flowering. Considerable variation in the terpene pattern among the analyzed individuals have been observed, which can be explained by the fact that some of the samples collected during the harvesting consisted of buttons and blossoming florets, while those of other individuals – of blossoming and fade florets. The amount of 1,8-cineole and α-thujone was higher in the hydroponically grown plants in the samples collected in 2016. The sesquiterpene viridiflorol was not found in the hydroponically grown plants while the amount of

Table 3. Composition of *S. officinalis* essential oils in conventionally and hydroponically derived plants, in the first and the second years of their cultivation in the experimental field plot (data indicate the average values calculated for 10 samples from each of two groups)

Compound	RT	2016				2017			
		Conventionally grown		Hydroponically grown		Conventionally grown		Hydroponically grown	
		Area %	in plants (from 10)	Area %	in plants (from 10)	Area %	in plants (from 10)	Area %	in plants (from 10)
α-Pinene	7.08	6.94	10	5.37	7	6.35	10	5.89	9
Camphene	7.47	1.23	10	1.53	10	1.34	9	1.13	10
β-Pinene	8.18	2.65	10	2.33	10	5.11	9	4.63	9
1,8-Cineole	9.62	*14.35*	10	*22.60*	10	*18.97*	9	*16.87*	9
α-Thujone	11.67	*12.93*	5	*22.25*	4	*15.50*	7	*13.03*	7
β-Thujone	11.56	8.90	5	22.63	4	14.76	7	24.55	1
(-)-Camphor	12.60	6.13	10	8.49	7	3.87	8	4.08	7
Borneol	13.20	3.43	9	3.34	9	3.75	8	3.21	9
β-Caryophyllene	19.02	7.22	10	8.80	10	8.19	9	9.44	9
α-Caryophyllene	19.84	6.50	9	6.27	10	4.15	8	5.15	8
Unknown compound	22.68	3.64	7	11.22	10	4.38	4	10.92	10
Manool	23.42	*10.09*	7	*1.19*	10	*10.74*	7	*0.89*	9
Viridiflorol	30.79	*3.33*	9	*0.00*	0	*3.71*	9	*0.00*	0

the diterpene manool was considerably lower in these plants as compared to the conventionally grown ones. These differences could be related to the significantly higher number of generative shoots observed in hydroponically derived plants representing half of all shoots, while in conventionally derived plants their number was two times lower (Table 2). It is known that sage essential oil is extracted from leaves only or from the whole aerial part according to the purpose of its use because of the variation in the components' content. Considerable differences were reported concerning the content of many compounds of sage essential oils in the whole herbage, harvested at the outset of the flowering stage (June) and in leaves, harvested after seed ripening (September) (Evstatieva et al., 1997). Authors indicated that the percentage of some compounds were higher in spring and some in autumn. Thus, the content of 1,8-cineole was much higher in the whole herbage during the flowering, which corresponds with our results, as in the samples of the hydroponically derived plants there was two times more generative shoots in comparison with the samples of conventionally derived plants.

Conclusion

We can summarize that the application of the aeroponic system enhanced plant growth and increased the crop yield for nearly one fifth. The period from germination to harvest was shortened for five months. Flowering was stimulated the first year, which reflected on the quantity and composition of the essential oils. In addition, the results indicate that the way of cultivation influence the pattern of the essential oils, some compounds as manool and viridiflorol missed or were in much lower concentrations than in the samples from conventionally derived plants. Despite the considerably higher herbage yield of the hydroponically derived plants during the first year in the experimental field, the long-term effects are expected to decrease significantly as *S. officinalis* is a perennial crop, and the size of the plants in the two groups equalized during the second year. On the other hand, the unexpected alterations in the oil composition of the hydroponically derived plants that lasted the second year (viridiflorol, manool), and the fluctuations recorded for the main oil compounds (1,8-cineole, α-thujone) pose new questions which have to be further investigated.

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