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Research Article

Effect of Three Different Growth Media on Yield and oil constituents of Sage (*Salvia officinalis*) under Protected Agriculture Conditions

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Abstract

Aromatic plants native to the Mediterranean region and Arabian peninsula are used as cures and in perfumery since times immemorial. Of these aromatic herbs, common sage (*Salvia officinalis*), is known to be bestowed with a multitude of medicinal properties in its leaves or extracted essential oil. However, the production of aromatic plants in the Arabian Gulf countries is limited by the poor soil resources, scarce and saline water and the harsh desert environment. This study aimed to investigate the production potential and oil quantity and quality of sage plant grown on different growth media under greenhouse conditions. Three growth media were used; agricultural soil, compost and hydroponic system where tuff (inert volcanic material) was used as substrate. The result indicated that the high salinity of the agricultural soil limited growth and oil yield in sage. Plants shoot fresh and dry weights, moisture content, and number of leaves and branches were generally lower in sage grown in sand compared with those grown in hydroponic and compost. Chemical constituents of sage shoot was higher in plants grown in hydroponic and compost compared to sand by approximately 50, 64, and 7% for N, P, and K content, respectively, while Na content was higher in sand grown plants by 16% over those in the other growth media. Essential oil chemical constituents concentrations did not change significantly with growth media. This study demonstrated the great potential of commercial production of sage in Arabian Gulf region under greenhouse conditions without compromising the oil quality or quantity.

Keywords: essential oils, sage, hydroponic production, Arabian Gulf.

INTRODUCTION

Agriculture has always played a role of great importance in the health of man and his ecology (Hachemi et al., 2013). Interest in herbal medicine and traditional systems of medicine has increased substantially in both developed and developing countries over the past two decades. Global and national markets for medicinal herbs have been growing rapidly, and significant economic gains are being realized (WHO, 2003).

Salvia officinalis, commonly known as sage, salvia, broadleaf sage, common sage, or garden sage, belongs to the

Lamiaceae (Labiatae) family (Kintzios, 2000; Kintzios et al., 2002). The name Salvia is derived from the Latin verb Salvare which means to save, to heal, or to cure. It is a native of the Mediterranean regions and can be cultivated all over the world (Kintzios, 2000; Chevalier, 2006; Mirjalali et al., 2006). The plant is herbaceous, evergreen perennial shrub. Leaves are small, oval, lanceolate, simple, and covered with hairs that give the plant the grayish appearance (Kintzios, 2000).

Sage is used for the multiple pharmacological effects including antibacterial, antiviral, antioxidative, antimalarial, anti-inflammatory, antidiabetic, cardiovascular, antitumor and anticancer (Coisin et al., 2012).

In folk medicine, sage is used in the treatment of many gastrointestinal disorders, infected wounds, and skin disorder (Mirjalili *et al.*, 2006). It can also be used in the treatment of problems associated with heart and circulatory system, insomnia, as a drug to treat acute arthritic pain in patients with rheumatism (Kintzios, 2000).

The herb contains mainly 1- 2.5% volatile oil which consists mainly of 1,8-cineol, β-caryophyllene, α-humulene, camphor, borneol, β-thujone, α-thujone, manool and viridifloro, (Hayouni *et al.,* 2008; Bernotiene *et al.*, 2007)

According to the high importance of this medicinal plant and its use in folk medicine, this study aimed to investigate the effect of 3 different growth media mainly agricultural soil, compost, tuff -in hydroponic, on growth and yield, essential oil yield, and active constituents of Sage (*Salvia officinalis).*

MATERIALS AND METHODS

A field experiment was conducted in Sultan Qaboos Centre for Developed and Soilless Agriculture Greenhouse and Laboratories, Arabian Gulf University, Manama, Bahrain, during November 2009- April 2010. Sage was transplanted in the greenhouse under controlled environment on November 2009,. Three growing beds, each 16m long, 1m wide, and 30cm deep were divided into 12 plots (each $1m^2$) leaving 30cm between plots. In each plot, 16 uniform plant seedlings were transplanted in 4 rows, with 20cm spacing between rows and plants (16 plants/ plot). The temperature of the greenhouse was maintained at 25 ± 5^oC during the day.

Three different growth media were used for the three different agricultural systems: 1) Agricultural soil; for the conventional agriculture, 2) Tuff (scoria); for soilless agriculture (hydroponics), 3) Compost;

Plants grown in hydroponics were irrigated by automatic drip irrigation system once a day, for 10 minutes, directly from the nutrient solution tank. The nutrient solution used was Hoagland's Nutrient Solution. Plants grown in agricultural soil (sandy soil) and compost were manually irrigated with tap water every other day. NPK fertilizer was added to the plants grown in the agricultural soil twice during the growing season.

After 16 weeks plant shoots were harvested. Shoot height, number of leaves and branches, and shoot fresh and dry weights were determined. Early harvesting of plants grown in sandy soil was done because they were badly affected by salt injury.

Growth Media and Irrigation Water Analysis

Samples of agricultural soil and compost were taken 3 times throughout the growth period and irrigation water (tap water and nutrient solution) were analyzed to determine their nitrogen content using Kjeldahl System (Kjeltec 2400), and phosphorus content using Spectrophotometer (UV Visible VARIAN), while potassium, calcium, and sodium contents were done by the Atomic Adsorption (AA 240 FS-Varian). EC and pH were determined using EC and pH meters.

Chemical Constituents of Plant Shoot

Plant shoot nitrogen content was determined using Kjeldahl System, (Kjeltec), phosphorus was determined by the phosphomolybdate method and the developed color measured using Spectrophotometer (UV Visible VARIAN). Both potassium and sodium were determined using Atomic Adsorption (AA 240 FS-Varian).

Extraction of Essential Oils

After harvesting, plant leaves were oven-dried at 40^oC and ground using mortar and pestle, then subjected to distillation.

Essential oils were extracted by hydrodistillation method using Clevenger-type apparatus by placing plant material in a rounded-bottom flask containing distilled water for 3-4 hours (starting from boiling of water till no more oil was coming out). Essential oils were collected and the percentage of essential oil was determined using the formula described by Rao (1995); Oil (%) = weight of E.O recovered (g)/ weight of dried material (g) * 100

Chemical Identification of Essential Oils

The essential oil was analyzed by GC-MS using a Perkin Elmer Claus 600 C mass selective detector coupled with a PE Claus 600 Gas Chromatograph, PE Elite-5MS capillary column (30 m × 0.2 mm(ID), film thickness 0.25µm). Operating conditions were as follows: carrier gas, helium with a flow rate of 1 mL/min; column temperature, zero min at 80ºC,80–280ºC at 10ºC /min and finally held for 20 min at 280ºC; injector temperature, 250ºC; source temperature, 200ºC; volume injected, 1 µL of the oil in dichloromethane (0.1%); split ratio, 1:20. The MS operating parameters were as follows: ionization potential, 70 eV; ion source temperature, 200ºC; quadrapole 100ºC, solvent delay 3.0 min, mass range 25–600 amu, EI mode.

Experimental design and Statistical Analysis

The experiment was arranged in a completely randomized design with 12 replications. Data obtained were subjected to statistical analysis using the statistical software JMP8 (SAS Corporation, Wisconsin, USA, 2006), and means were separated at $P \leq 0.05$ level of significance.

RESULTS AND DISCUSSION

Growth Media and Irrigation Water Analysis

Table 1 shows that, in sandy soil, there was a gradual increase in nitrogen, phosphorus, and potassium content probably due to NPK fertilizer added in the second and third months after planting. Also, sodium content increased slightly. Electrical conductivity (EC) was highly increased, by 400%, due to salt accumulation in root zone brought by tap water, where Na content is high (Table 1). Electrical conductivity increased to more than 18 dS/m by the 11th week. In compost, nitrogen, phosphorus and potassium content were adequate and decreased slightly along the growing period due to nutrient consumption by the plant. Electrical conductivity increased by 71% due to the increase in salt content brought in by irrigation water and the pH increased by 7% in the sandy soil reducing micronutrients availability.

Table 1. Chemical analysis of growth media (agricultural soil and compost)

Growth Media	Months after Planting	N %	P %	K %	Na %	(EC) dS/m	pH
Sandy soil	1	0.015	0.042	0.017	0.119	3.60	5.97
	2	0.047	0.788	0.140	0.639	8.20	6.33
	3	0.063	0.823	0.302	0.794	18.50	6.37
Compost	1	0.674	0.772	0.007	0.74	0.70	5.88
	2	0.665	0.770	0.004	0.77	0.80	5.95
	3	0.617	0.733	0.003	0.79	1.20	6.05

Analyzes of irrigation water (Table 2) showed that nutrient solution contained optimum and stable amounts of nutrient concentrations that can sustain plant growth, whereas tap water contained negligible amounts of these nutrients, so fertilizers must be added in order to enhance plant growth. Sodium content and EC values were very high causing salinity of the sandy soil, which in turn cause an adverse effect of plant growth.

Table 2. Chemical analysis of irrigation water (nutrient solution and tap water)

Irrigation Water	Concentration (ppm)				E.C (dS/m)	pH
	N	P	K	Na		
Nutrient Solution	170	50	280	-*	2.90	6.40
Tap Water	10	21	1	15.54	0.34	7.28

* not determined

Plant Parameters

Figure 1 shows that the plants growing in the 3 media. Plants growing on the sandy soil are small compared to those growing in hydroponic and compost. Shoot height increased progressively with time in all growth media. However, the rate of increase was more pronounced in both hydroponic and compost grown plants. Significant differences ($p \leq 0.05$) were found in shoot height between plants grown in compost and both those grown in hydroponic and sand, 3 weeks after transplanting, whereas the differences between hydroponically grown plants, and both compost and sand grown plants was significant from week 5 after transplanting. Eleven weeks after transplanting, significant differences were found between plants grown in sand and both those grown in compost and hydroponic, and in week 15 hydroponic exceeded compost. Plant height increased by 57, 617, and 565% for sand, hydroponic, and compost, respectively, after 16 weeks period (Figure 2).

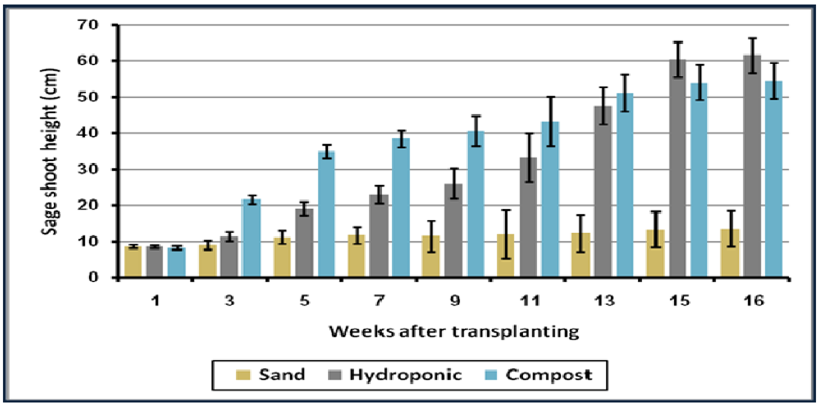

Figure 1. Effect of growth media on sage shoot height (cm) during growing period

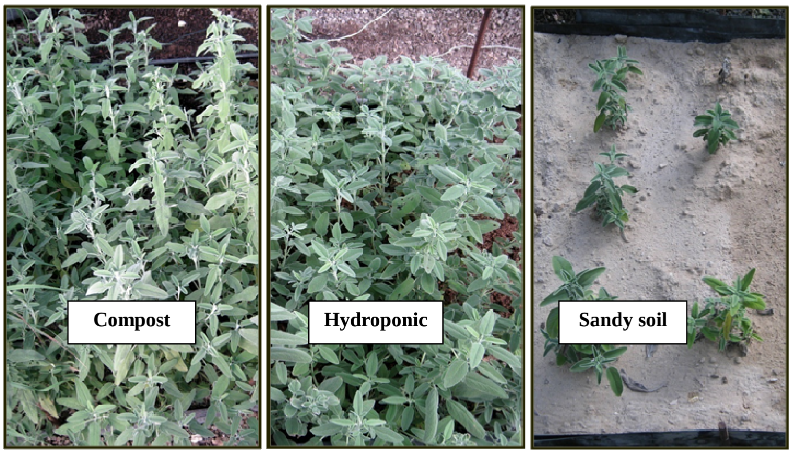

Figure 2. Effect of different growth media on sage growth

Other Plant Parameters

According to Table 3, there were no significant differences (p≤ 0.05) in shoot fresh and dry weights and moisture content between plants grown in hydroponic and compost. Although there were no significant differences in shoot fresh and dry weights between hydroponic and compost, compost exceeded hydroponic in this trait. Regarding the number of branches, significant differences were found between plants grown in compost and hydroponic. Number of branches was higher in plants grown in hydroponic. These results agreed with (Dob et al., 2007; Hashemi et al., 2013; Hayouni et al., 2008)

Table 3. Other Plant parameters

Growth media	Shoot fresh weight (g)	Shoot dry weight (g)	Moisture content %	Number of branches	Number of leaves
Sand	58.00 *b*	21.50 *b*	62.93 *b*	63.25 *c*	3.03 *b*
Hydroponic	1568.28 *a*	350.14 *a*	77.67*a*	188.63 *b*	9.28 *a*
Compost	2000.75 *a*	477.87 *a*	76.12 *a*	537.20 *a*	8.03 *a*
S.E ±	300.19	68.15	10.66	38.51	1.66

Means followed by the same letter are not significantly different at p≤ 0.05 according to DMRT

Chemical Constituents of Plant Shoot

Plant tissue of plants grown in sand contained high sodium content compared to those grown in compost and hydroponic (Figure 3). Ben Taarit *et al.* (2010) reported that significant decrease in sage plant dry weight followed the application of different NaCl levels, causing inadequate photosynthesis that lead to stomatal closure and consequently limited carbon dioxide uptake. There were significant differences (p≤0.10) in plant shoot nitrogen content and significant differences (p≤0.05) in phosphorus and potassium between plants grown in sand and those grown in compost and hydroponic, where nitrogen and phosphorus in plants grown in compost were higher than those grown in sand (Figure 3). Potassium content in shoots of hydroponically grown plants was higher than in those grown on compost and sand. Significant differences in shoot sodium content were found between plants grown in sand and those grown in hydroponic and compost; plants in sand had remarkably the highest value and those in compost had the lowest.

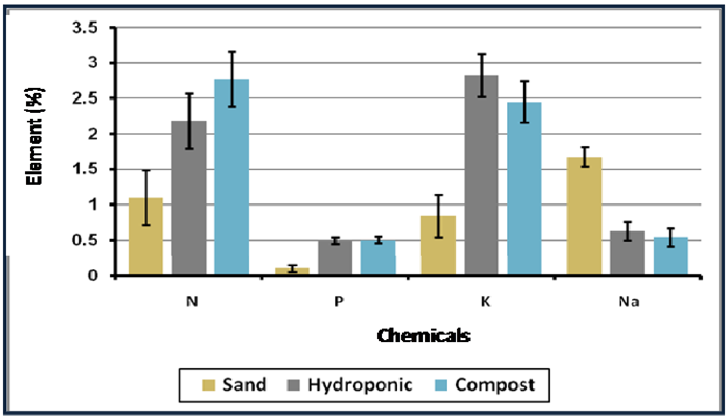

Figure 3. Effect of growth media on percent sage shoot content of some elements

Essential Oil Yield (%)

Essential oil (E.O) extracted from sage leaves is characterized by its pale yellow color and strong refreshing odor. Table 4 shows significant differences (p≤0.10) in sage essential oil yield between plants grown in sand and those grown in hydroponic and compost. Essential oil yield in hydroponically grown plants exceeded those in agricultural soil by 382%.

Table 4. Essential oil percentage in plants grown in different growth media

Growth Media	Sage oil %
Sand	0.34 *b*
Hydroponic	1.64 *a*
Compost	1.10 *a*
S.E ±	0.22

Means followed by the same letter are not significantly different at p≤ 0.05.

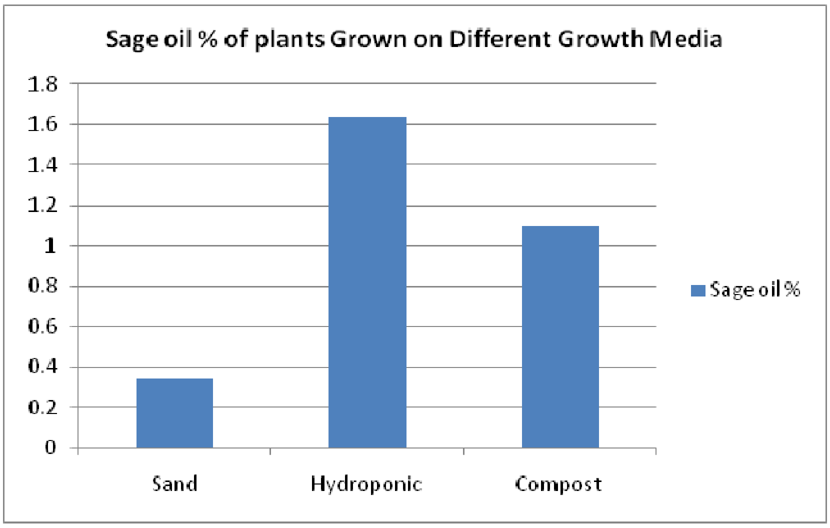

Figure 4. Sage oil % of Plants Grown on Different Growth Media

Essential oil yield (w/w) based on plant dry weight was found to be 0.34% in plants grown in sand, 1.64% in those grown in hydroponic, and 1.10% in those grown in compost. Ben Taarit *et al.* (2010) and Baatour *et al.* (2010) mentioned that increasing soil salinity level strongly influences the essential oil biosynthesis and this may affect synthesis and composition of oil and cause a yield reduction in Laminaceae species.

All sage E.O components in plants grown under greenhouse conditions were higher compared with those grown in field. Similar results were obtained by Bernotiene *et al* (2007) from Eastern Lithuania, Dob *et al.* (2007) from Algeria, Hendawy and Khalid (2005) from Egypt, Mirjalili *et al.* (2006) from Iran, and Bouaziz *et al.* (2009) from Tunisia. There was a wide range of differences in concentration of chemicals, as well as absence of some of them between sage obtained from other countries and with those grown in greenhouse.

Chemical Constituents of Essential Oils

The percentage of essential oil content (w/w) of sage was found to be 0.34% in plants grown in sand, 1.64% in those grown in hydroponic, and 1.10% in those grown in compost (Table 5). The compounds α-phyllandrene (RT= 4.17), α-pinene (4.29), camphene (4.49), β-pinene (4.78), eucalyptol (1,8-cineol) (5.49), thujone (6.51), camphor (7.03), terpinen-4-ol (7.59), bornyl acetate (8.80), β-caryophyllene (10.90), and α-caryophyllene (11.30) are components found in sage oil (RT: retention time). According to Table 5, there were no significant differences ($p \leq 0.05$) between all treatments on their effect on chemical percentages. The component β-pinene was higher in plants grown in sand and hydroponic (14.30 and 16.80%, respectively) than those grown in compost (13.07%). Eucalyptol (1,8-cineol) was slightly lower in plants grown in sand and hydroponic (48.74 and 47.04%, respectively) than those grown in compost (49.28%). The content of β-caryophyllene increased from 7.13 and 8.33% in sand to 8.33 and 8.71% in those grown in hydroponic and compost, respectively.

Table 5. Chemical concentration of sage essential oil extracted from plants grown on the different growth media

Retention time (RT)	Compound	Concentration %		
		Sand	Hydroponic	Compost
4.78	β-pinene	14.30 *a*	16.80 *a*	13.07 *a*
5.49	Eucalyptol (1,8-cineol)	48.74 *a*	47.04 *a*	49.28 *a*
7.03	Camphor	16.16 *a*	15.60 *a*	16.23 *a*
7.59	Terpinen-4-ol	8.05 *a*	6.10 *a*	7.23 *a*
10.90	β-Caryophyllene	7.13 *a*	8.33 *a*	8.71 *a*
11.30	α- Caryophyllene	3.07 *a*	4.03 *a*	3.21 *a*

Means followed by the same letter are not significantly different at p≤ 0.05.

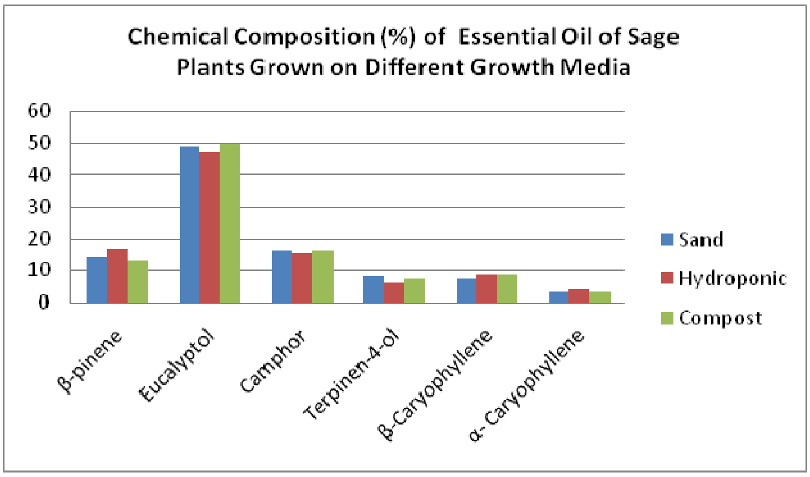

Figure 5. Chemical Composition (%) of Essential Oil of Sage Plant Grown on Different Growth Media

From Figure 4, it is clear that the effect of the three media on individual components is almost the same. Eucalyptol is the major constituent (47-49 %), followed by camphor (*ca* 16%) and β-pinene (13-17%), β-Caryophyllene(*ca* 8.1%) and Terpinen-4-ol (*ca* 7.1%) with lesser amounts of the toxic component α-thujone (5%). The fact that thujone is not the major component might indicate that sage plant used belongs to a different chemotype from Algerian sage reported recently (Djeddi *et al.*, 2012) with an α-thujone content of 24.9%.

Table 6.: Chemical concentration of sage essential oil compared with sage oil in some countries

Retention time (RT)	Compound	Concentration %							
		This study			Other countries*				
		Sand	Hydroponic	Compost	Eastern Lithuania	Algeria	Egypt	Iran	Tunisia
4.78	β-pinene	14.30	16.80	13.07	2.60	1.40	1.17	8.00	4.41
5.49	Eucalyptol (1,8-cineol)	48.74	47.04	49.28	17.60	12.3	1.27	16.8	16.29
7.03	Camphor	16.16	15.60	16.23	2.00	20.40	27.61	7.10	14.19
7.59	Terpinen-4-ol	8.05	6.10	7.23	0.20	0.80	0.25	0.40	-
10.90	β-Caryophyllene	7.13	8.33	8.71	10.80	4.50	-	10.50	-
11.30	α- Caryophyllene	3.07	4.03	3.21	-	-	0.52	-	-

Table 6 shows that the chemical composition of the various compounds does not vary very much among the three different media used in this study. However, when compared to values obtained from different countries, substantial variation were found in each compound. Under controlled growth conditions this study tends to give higher values for each compound expect for the values reported for camphor from Egypt and Algeria.

CONCLUSIONS

Highly valued medicinal plants like sage can be grown successfully under protected agriculture conditions on compost or hydroponic system. Oil yield was greatly improved. Important oil constituents were not affected by the type of growth media. This is particularly important in the GCC region where both soil and water resources are limited. The fact that % of the toxic component **α-**thujone is minimum in the sage plant used in this study is indicative of a different chemotype than thujone-producing chemotypes and a better oil safety profile.

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