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Effects of Salicylic Acid on Quality and Quantity of Essential oil Components in *Salvia macrosiphon*

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ABSTRACT

The essential oil of *Salvia macrosiphon* Boiss use as a folk medicine. The effect of Exogenous application of salicylic acid (0 , 200 , 4oo mgL^{-1}) in early flowering stage on components of essential oils of *Salvia* was evaluated. Its essential oil was analyzed by GC and GC/MS .The SA application increased Linalool from 20.02% (0 mgL^{-1}) to 41.37% (400 mgL^{-1}) , Hexyl isobutanat from 3.17%(0 mgL^{-1}) to 5.6% (400 mgL^{-1}) and decrease δ -cadinen from 5.63% (0 mgL^{-1}) to 3.86% (400 mgL^{-1}) . Some compounds such as Franesol, δ –Amorphene, Caryophyllene oxide, Hexyl octanoate, Beta Eudesmol and α-Bisabolol only detected in control treatment. α – Pinene and 1,8-Cineol only detected in plants treated with SA.The yield of essential oil was 0.23% in (0 mgL-1) and 0.48% (400 mgL-1). The results suggest that SA application manipulated essential oil.

Key words: Sag, Lamiaceae, stress, Essential oil, Linalool

INTRODUCTION

Salvia genus belongs to the subfamily Nepetoideae of Mentheae tribe in Lamiaceae family (Pederson 2000). Numerous species of the genus *Salvia* have been used since ancient times in folk medicine and have been subjected to extensive pharmacognostic research intended to identify biologically active compounds (Lu et al. 2002, Sivropoulou et al. 1997). These species have been found to possess significant biological activities, including antibacterial, antiviral, adstringent, antitumor, spasmolytic, antioxidant, anti-inflammatory, antihydrotic activity and have been also used in the treatment of mental, nervous and gastrointestinal conditions. Sage species are used traditionally in foods and cosmetics preparation as well (Lu et al. 2002, Tepe et al. 2004).There are several reports in the literature on the phytochemical analysis of species belonging to *Salvia*. These scientific studies on Salvia species show the presence of many compounds belonging mainly to the groups of phenolic acids, phenolic glycosides, flavonoids, anthocyanins, coumarins, polysaccharides, sterols, terpenoids and essential oils (Lu et al. 2002, Baser et al. 1998, Ghannadi et al. 1999). The essential oil of *S. ringens and S. tomentosa* exhibit antimicrobial activity against Gram-positive and Gram- negative microorganisms. The yield of the *S.macrosiphon* oil was 0.14% (w/w). The main constituents of the oil were linalool (26.3%), hexyl hexanoate (9.6%), hexyl isovalerate (9.3%), hexyl- 2-methyl-butanoate (8.9%), sclareol (7.2%) and hexyl octanoate (6.1%) (Javednia et al. 2005). The essential oil of *S.macrosiphon* Boiss was prepared by steam distillation . the major constituents were sesquiterpenes (69.5 %), α-Gurjunene (11 %), β-Cubebene (10.6 %) and Germacrene-B (7%) (Matloubi-Moghaddanm et al. 2000).

Chemical composition of volatile compounds from *Salvia rhytidea* Benth. was analyzed, representing 98.2% of total volatiles. The essential oil was characterized by a high content of hydrocarbon and oxygenated monoterpenes. The main constituents were p-Cymene-8-ol (11.9%), Spathulenol (7.3%), Pulegone (6.4%), Sabinene (5.8%), Terpinen-4-ol (5.5%) and α-Copaene (5.3%) (Sajjadi and Ghannadi 2005).

The yields of essential oils of *Salvia bracteata* were 0.22% before flowering stage and 0.28% at full flowering stage. Before flowering stage 17 compounds and at full flowering stage 19 compounds were characterized. β-caryophyllen and γ-muurolene were the highest rates of the compounds (Hooshidari et al. 2006). Some factors such as isolation method (Rohloff et al. 2000, Hachey et al. 1990), environmental condition (nutrient level, temperature) and some stress (Nmeth et al. 1993) may plays a considerable role in the composition and quality of obtained essential oil.

Salicylic acid (SA) is an endogenous growth regulators of phenolic nature which participates in the regulation of physiological process in plant (He et al. 2005). SA plays an important role in the defend responses to pathogen attack and to several abiotic stress (Elizabeth and S. Munn – Bosch, 2008). Exogenous application of SA improved plant tolerance to heat (Det et al. 1998), chilling (Janda et al. 1999),

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and salt stress (Borsoni et al. 2001). Improved heat tolerance in creeping bent grass by application of SA was associated with its protection against oxidative damages. Exogenous SA were effective in inducing secondary metabolites formation in plant cell culture or *in vive* plants (Kiddle et al. 1991).

This study was designed to determine the exogenous application, of SA on quantity and quality composition of essential oil in *S. macrosiphon* in field conditions.

MATERIALS AND METHODS

Plant materials

Seeds of sage were sown in Jefe pot in experimental greenhouse of Sadra near Shiraz, Iran, in September 2008. One mount later obtained seedlings were transferred to fields and distributed homogenously in 9 blocks $3m^2$ which was spread by 2m.

Salicylic acid treatments

Plants at flowering stage (June 2009) were sprayed with distilled water as a Control, and SA at 200 and 400 mgL^{-1} of SA dissolved in distilled water. All sprays solution were sprayed to the point of run off. The experiment was arranged in completely randomized block design with three replications for each treatment. At flowering stage (one week after SA application) the aerial parts of *S.macrosiphon* were harvested and air dried at ambient temperature in the shade. Then hydro-distilled using a Clevengar-type apparatus for 3h. It was dissolve in hexane (Marck), dried over a hydrous sodium sulphate and stored at 3-4c.

Identification of the oil components

GC analysis was carried out using a Varian GC 3600 chromatograph with DB-5 column (30m X 0.25 mm i.d.; 0.25 μm). Oven temperature was performed as follows: 60° C to 260° C at 3°/min; injector temperature 250° C; detector temperature, 260° C; carrier gas, He (0.8 ml/min); split ratio of 1:20. GC-MS analysis was carried out using a Hewlett-Packard 6890 operating at 70 eV ionization energy, equipped with a HP-5 capillary column (phenyl methyl siloxane, 30m X 0.25 mm i.d.) with He as the carrier gas and split ratio 1:20. Retention indices were determined using retention times of n-alkanes that were injected after the essential oil under the same chromatographic conditions. The retention indices for all components were determined according to the Van Den Dool and Kratz (1963) method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RRI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley library or published mass spectra data.

RESULTS AND DISCUSSION

The constituents of the obtained essential oils of *Salvia macrosiphon* are presented in Table 1. Twenty–four components were identified in untreated plants and seventeen components in SA-treat ed plants (Table 1). The differences were supposed to be the effects of SA. Decrease in the proportion of δ–Cadinen and Sclareol have been found according to concentration of SA. Some compounds such as Franesol, δ-Amorphene Caryophyllene oxide, Hexyl octanoate, Beta Eudesmol, α-Bisabolol, α-Muurolol, Decanoic acid, Manoyl oxide and Manool only detected in control (Table 1). According to studies a means of 20 and uttermost 64 compounds have been identified in *Salvia macrosiphon* from Iran (Javednia et al. 2005, Matloubi-moghadam et al. 2000). The main constituents of the oil were Linalool (21.02% - 41.37%), Hexyle isovalerat(14.83% - 14.3%) and Hexyl 2 –methyle buterat (10.64%-9.97%) (Table 1).

Table 1. Composition of the volatile oil of S*alvia macrosiphon* L. from Iran

Peak no.	Components	Salicylic acid			RI	Method of identification
		control	200mgL^{-1}	400 mgL^{-1}		
1	α - Pinene	-	5.62	3.09	978	RI,MS
2	1,8-Cineol	-	2.48	2.7	1033	RI,MS
3	Linalool	**21.02**	**39.61**	**41.37**	1102	RI,MS
4	Hexyl isobutanat	**3.17**	**4.43**	**5.6**	1150	RI,MS
5	Hexyl 2 –methyle buterat	10.46	9.31	9.97	1234	RI,MS

Continued Table 1.

6	Hexyle isovalerat	14.83	13.82	14.3	1243	RI,MS
7	Linalyl acetate	-	1.95	-	1250	RI,MS
8	α-Terpinen	-	-	0.96	1294	RI,MS
9	Ocytyl isobutyrat	1.48	1.77	1.67	1348	RI,MS
10	Beta elemen	-	-	1.5	1386	RI,MS
11	Hexyl- hexanoat	4.80	4.55	-	1392	RI,MS
12	β - Longipinene	**2.02**	**3.07**	**3.08**	1412	RI,MS
13	Octyl 2-methyl butanoate	1.76	1.54	1.84	1434	RI,MS
14	γ -Gourjanene	2.18	1.99	2.25	1474	RI,MS
15	Germacren-D	2.49	1.04	2.06	1480	RI,MS
16	B- Selinene	0.84	1	0.77	1489	RI,MS
17	δ - Selinene	1.1	1.35	1.5	1493	RI,MS
18	Franesol	4.26	-	-	1499	RI,MS
29	δ -Amorphene	3.26	-	-	1512	RI,MS
20	δ -cadinen	**5.63**	**3.20**	**3.86**	1515	RI,MS
21	Caryophyllene oxide	5.07	-	-	1581	RI,MS
22	Hexyl octanoate	1.69	-	-	1584	RI,MS
23	Beta Eudesmol	2.6	-	-	1611	RI,MS
24	α-Bisabolol	1.61	-	-	1622	RI,MS
25	α-Muurolol	0.94	-	-	1645	RI,MS
26	Decanoic acid	1.58	-	-	1920	RI,MS
27	Manoyl oxide	1.36	-	-	1996	RI,MS
28	Manool	2.06	-	-	2055	RI,MS
29	Sclareol	3.89	3.27	3.23	2225	RI,MS

Linalool was significantly increased by SA- treatment (Figure 1). β-Longipinene and Hexyl isobutanat were increased with SA- treatment but δ–Cadinen decreased (Figure 2).

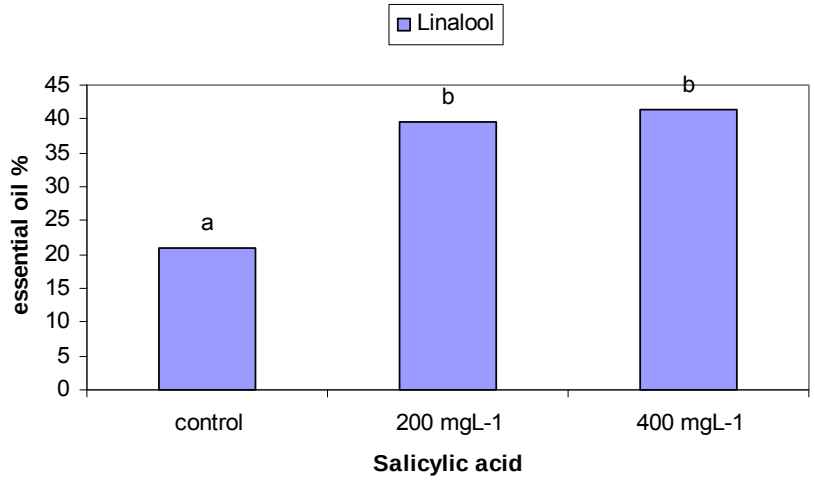

Figure 1. The effects of salicylic acid on Linalool

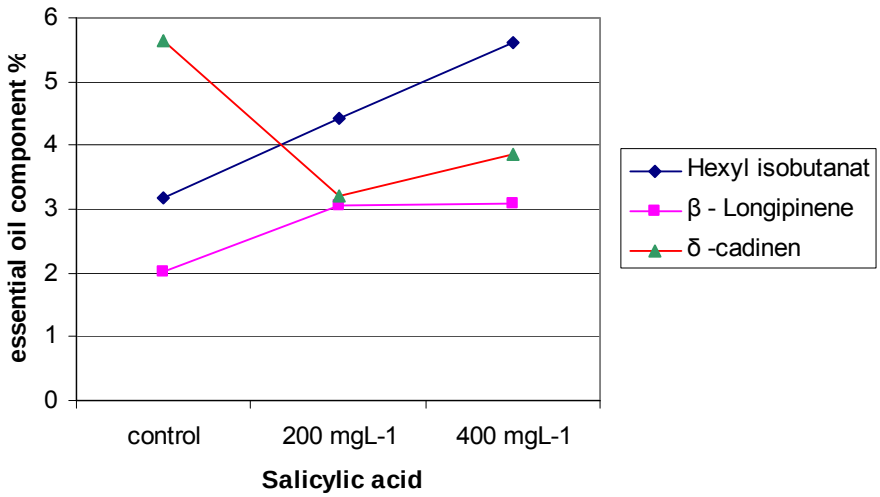

Figure 2. The effects of salicylic acid on some essential oil component

We harvested plants materials in full bloom stage. It may be affected the kind of oils, because it was showed that during the intensive growth period the precursor flow distributes between the cytoplasm (sites of sesquiterpene synthesis) and plastid (sites of monterpen synthesis) while after full development of the cell the majority is utilized in the plastids.

The yield of the *S. macrosiphon* oil was 0.23% in (0 mgL-1), 0.45% (200 mgL-1) and 0.48% (400 mgL-1). SA significantly increased the yield of essential oil (Figure 3).

The reports indicate that the best harvest time to obtain essential oils from *S. fruticosa* with the highest active ingredients is July. Yields from pilot-scale distillations in summer were significantly higher from non-flowering accessions (0.23% FW) than from flowering accessions (0.14%). Oils from flowering and non-flowering accessions had different compositions, with significantly higher levels of thujones, ß-caryophyllene and viridiflorol determined in flowering accessions (Mueller-Riebau, et al. 1997). Yoshida and Sawasaki found that the maximum clary sage oil content was obtained at the end of the blossom period (Yoshida and Sawasaki, 1978).

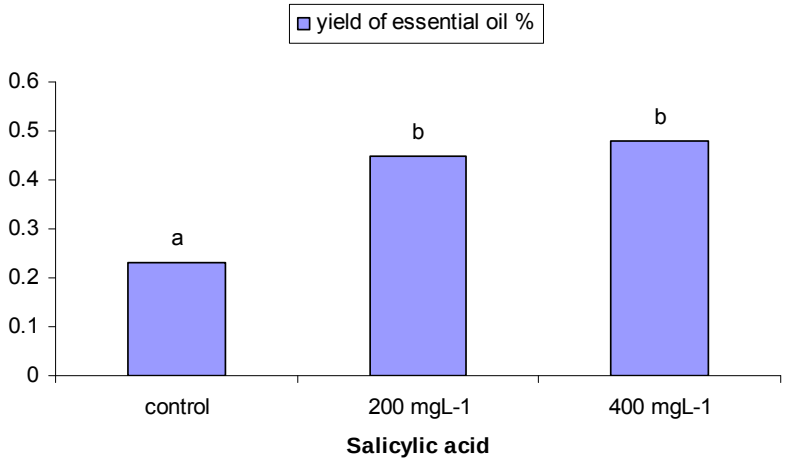

Figure 3. The effects of salicylic acid on yield of essential oil

The stress can change quality and quantity of essential oils. It has been previously assumed that *Salvia* sp. would produce high–quality oils only under stressful conditions (high temperature, drought , low fertility) (Hay 1993). In Thyme, the highest essential oil concentration (0.49 % Fw), Thymol and Myrecen contents of the oil occurred in full sunlight. Exogenous application of SA caused to reduction in chlorophyll levels (Elizabeth and Munn – Bosch, 2008) and the cell with an abundance of endoplasmic reticule and having few plastids my represent the sites of sesqiterpens synthesis and with decrease in monoterpens biosynthesis sites (Lourenco et al. 1999). Among salicylates, free salicylic acid is considered to be the most biologically active from as a mediator of plant stress responses, including disease and systemic acquired resistance (Lee et al. 1995). It has been reported that SA may participate in plant responses to abiotic stress, such as drought stress (Munne -Bosch 2003) and it has a role in controlling gene expression (He et al. 2003) that most of the gene regulated by SA are defense related genes and many of them participate in plant responses to biotic and abiotic stresses. Therefore SA may change secondary metabolites and its pathway by effects on plastid, chlorophyll level and represent conditions stress.

CONCLUSION

The SA like stress manipulated quality and quantity of essential oil of salvia macrosiphon. The yield of essential oil was increased. The useful component such as Linalool was increased. Seventeen components were identified in SA-treated plants. Some components such as α – Pinene and 1,8-Cineol only detected in plants treated with SA.

REFERENCES

Baser KHC, Kurkcuoglu M, Aytac Z (1998). Composition of the essential oil of *Salvia euphratica* Montbret et Aucher ex Bentham var. euphratica from Turkey, Flavour Fragr. J., 13: 63-64.

Borsani O, Valpuesta V, Botella MA (2001). Evidence for a role of salicylic acid in the oxidative damage generated by NACL and osmotic stress in *Arabidopsis* seedling .J. Plant Physiol., 126: 1024-1030.

Dat GF, Lopez – Delgado H, Foyer CH, Scott IM (2000). Effects of salicylic acid on oxidative stress and thermo tolerance in tobacco .J. Plant Physiol., 156: 659-665.

Der Marderosian A (2001). The review of natural products. Missouri: Facts and Comparison Press, p. 636-612.

Elizabeth A, Munn – Bosch S (2008). Salicylic acid may be involved in the regulation of drought –induced leaf senescence in perennials: A case study in field-grown *Salvia officinalis* L. plants. Enviro. Experi. Botany, 64:105-112.

Ghannadi A, Samsam-Shariat SH, Moattar F (1999). Volatile constituents of the flower of *Salvia hydrangea* DC. ex Benth., Daru, 7: 23–25.

Gundlach H, Mueller MJ, Kutchan TM, Zenk MH (1992). Jasmonic acid is a single transducer in elicitor - induced plant cell cultures. Proc .Natl .Acad . Sci .USA, 89: 2389-2393.

Hay RKM (1993) Physiology. In R.K.M. Hay and P.G. Waterman (eds.), Volatile Oil Crops: Their Biology, Biochemistry and Production, Longman, England, pp: 23–46.

He Y, Tang H, Swain W, Green JD, Gack AL, Jan T PS (2007). Networking senescence - regulating pathway by using *Arabidopsis* enhancer traplines.Plant Physiol., 126: 707-713.

He Y,.Liu Y, Cao W, Huai M, Xu B, Huang B (2005). Effects of Salicylic acid on heat tolerance associated with antioxidant metabolism in Kentucky bluegrass Crop Sci., 45: 988-998.

Hooshidari F, Sefidkon F, Jamzad Z (2006). Identification and comparison of essential oil composition of *Salvia bracteata* Bank & Soland in two harvesting time. Iranian J. Medicinal and Arom. Plants, 22:3: 209-215.

Janda T, Szalai G, Tari I, Paldi E (1999). Hydroponics treatment with salicylic acid decreases the effects of chilling injury in Maize (*Zea mays* L.) plants . Planta , 208 :175 -180.

Javednia K,. Miri R, Jamalian A (2005). Composition oil of *Salvia macrosiphon* Boiss. From Iran. Flavour Fragr. J., 20: 542-543.

Kiddle GA, Doughty KG, Wallsgrove R M (1991). Acid -induced accumulation of glucosinolats in oilseed rape (*Brassica napus* L.) Leaves.J. Expre. Bot., 45: 1343-1346.

Lee H, Leon J, Raskin J (1995). Biosynthesis and metabolism of Salicylic acid. Proc Natl, Acad. Sic..S.A., 92: 4076-4079.

Matloubi-Moghaddam F, Amin G, Safavi-Poorsohi E (2000). Composition stembark essential oil from *Salvia macrosiphon* Boiss. Daru., 8: 28-29.

Lu Y, Foo LY (2002). Polyphenolics of *Salvia* sp . a review, Phytochemistry, 59 : 114–140

Mishina T, Lamb E, Zeier C (2007). Expression of a nitric oxide degrading enzyme induces a senescence programme in *Arabidopsis*. Plant Cell Environ., 30:39-52.

Mueller-Riebau FJ, Berger BM, Yegen O, Cakir C (1997). Seasonal variations in the chemical compositions of essential oils of selected aromatic plants growing wild in Turkey .Journal of Agricultural and Food Chemistry, 45: 4821–4825.

Munne -Bosch S, Penuclas J (2003) . Photo and antioxidative protection and a role for Salicylic acid during drought and recovery in field grown *Phillyrea angustifolia* plants. Planta, 217: 758-766.

Massada Y (1994). Analysis of essential oil by gas chromatography and spectrometry. New York: Wiley, 1976.

Pederson JA (2000). Distribution and taxonomic implications of some phenolics in the family Lamiaceae determined by ESR spectroscopy, Biochem. System. Ecol., 28: 229–253.

Ramaswami S, Briscese K, Gargiullo P, Vonngeldern RJ (1988). In Flavors and Fragrances—A World Perspective, Lawrence BM, Mookherjee BD, Willis BJ. Amsterdam:Elsevier:.37.

Sajjadi SE, Ghanadi A (2005). Essential oil of the Persian sage, *Salvia rhytidea* Benth. Acta Pharm., 55: 321–326.

Sivropoulou A, Nikolaou C, Papanikolaou E, Kokkini S, Lanars T, Arsenakis M (1997). Antimicrobial, cytotoxic and antiviral activity of *Salvia fructicosa* essential oil, J. Agric. Food Chem., 45: 3197–3201.

Tepe B, Donmez E, Unlu M, Candan F, Daferera D, Vardar-Unlu G, Polissiou M, Sokmen A (2004). Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* and *Salvia multicaulis*, Food Chem., 84 :519–525.

Van Den Dool H, Kratz PD (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr., 11: 463-471.

YanLi L, Craker LE, Potter T (1996). Effect of light level on essential oil production of Sage (*Salvia officinalis*) and Thyme (*Thymus vulgaris*). Acta Horticulturae. 426: 419–426.

Yoshida T, Sawasaki T (1978). On the variations of the percentage yield and the chemical composition of essential oil in clary Sage (*Salvia sclarea* L.). Japan. J. Trop. Agr., 21: 145– 149.