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The Effect Salicylic Acid and Jasmonic Acid Foliar Applications on Essence and Essential Oil of Salvia (*Salvia Officinalis* L.)

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ABSTRACT

In this work, the effects of two elicitors [jasmonic acid (JA) and salicylic acid (SA)] on accumulation of essential oils in shoot cultures of Sage (*Salvia officinalis* L.) were investigated. Of the two elicitors, JA was more effective in stimulating the accumulation of α-pinene, limonene, β-pinene, camphor, thymol, camphene, thujone-trans, thujone-cis, 1,8-cineole, borneol, borneol acetate, carvacrol, α-humulene and caryophyllene. At 50 and 100 µl, JA enhanced the production of camphor, thymol and carvacrol. Although 1 mM SA led to the same total production of many essential oils like β-pinene, camphor, thymol, thujone-cis, carvacrol, α-humulene and caryophyllene were less produced in JA (50 and 100 µl); only α-pinene, β-pinene, camphene, 1,8-cineole, veridiflorol were produced better in JA (50 and 100 µl). Manol was obtained only in SA (10 mM) and water treatments.

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INTRODUCTION

Salvia officinalis (Lamiaceae) is one of the most important medicinal and aromatic plants, with antioxidant, antimicrobial, spasmolytic, astringent, anti hidrotic and specific sensorial properties. Essential oils of the plant composed mainly of monoterpens. 1, 8-cineole, thujone and camphor are responsible for some of these effects. Essence extractions depend on genetic and environmental properties, essence process, and fresh weight of plant. Salicylic acid (SA), a naturally occurring plant hormone, acts as an important signaling molecule in plants, and has diverse effects on tolerance to abiotic stress. Exogenous application of SA may participate in the regulation of physiological processes in plants, such as stomata closure, ion uptake and transport, membrane permeability, photosynthesis and growth (Gunes *et al*., 2005). Its role in abiotic stress tolerance and osmotic stress has been reported (Van Breusegem and Vraneva, 2001; Raskin, 1992). Phenolic compound SA plays a vital role in the defense response against many pathogens and these, in turn, induce the expression of many defense related genes (Kachroo and Kachroo, 2007). SA expressed tolerance to various stress. There was an increase in the process of tolerance to different stress by SA (Senaratn *et al*., 2000). SA had the most effectiveness in many elicitors (Coste *et al*., 2011). At low levels of salinity, leads to decrease in germination and has no effect on high levels of salinity (Shahba *et al*., 2010). SA increases due to resistance to stresses such as salt stress. Foliar applications of SA resulted in greater root, shoot and total dry weight of calendula plants under salt stress (Bayat *et al*., 2012). SA decreased effects and damages of drought stresses on germination and seedlings growth (Baghizadeh and Hajmohammadrezaei, 2011). SA increased photosynthetic pigments, NPK, Fe, Zn, Mn, total carbohydrates and crude protein concentrations in leaves (Mady, 2009). SA reformed and detoxified super oxide radicals which made beneficial physiological and morphological effectiveness and then produced salt tolerant variety (Joseph *et al*., 2010). Nutrients increased number of umbel per Coriander (*Coriandrum sativum* L.) significantly but salicylic acid made reverse effectiveness (Rahimi *et al*., 2009). In pepper, SA increased growth and development (Mendoza *et al*., 2002). Under Cu-stressed, SA induced the synthesis of polypeptides and translocation of Cu and/or increased Cu-binding proteins (El-Tayeb *et al*., 2006). SA decreased polyphenol oxidase and peroxidase. It seems that SA can considerably alleviate oxidative damage that occurs under cold stress condition. SA resulted in decrease activity of antioxidant enzymes (Soltani *et al*., 2011). SA reduced disease severity and increased the amount of secondary compounds in infected and non infected daisy plants (Khavarinezhad and Asadi, 2006). SA failed to show any stimulatory effect on either cell growth or hypericin production (Walker *et al*., 2002). JA application to the stressed plants reduced the amount of lipid peroxidation and stimulated the synthesis of antioxidant enzymes, enhancing the content and yield of artemisinin as well (Aftab *et al*., 2011). JA elicitor (100 µM) enhanced total isoflavones production and stimulated accumulation of

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daidzein and genistein in *Pueraria lobata* (Thiem and Krawczyk, 2010). JA reduced transpiration and membrane-lipid peroxidation as expressed by malondialdehyde in strawberry plants (Wang, 1999).

1. Methodology:

Plant material and fertilizers:

Seeds of Sage (*Salvia officinalis* L.) were obtained from Iranian Seeds and Plant Improvement Institute. Seeds of sage were planted in field condition. Treatments that used after plants had 4 leaves were: T_1, water; T_2, acetone; T_3, JA 50 µl; T_4, JA 100 µl; T_5, JA 200 µl; T_6, JA 400 µl; T_7, SA 1 M; T_8, SA 10 M; T_9, SA 20 M; T_{10}, SA 40 M and control (without water, acetone, JA and SA).

Experimental conditions:

Field trials were established in 2012 and 2013 at Shahrekord (50°56/ E 32°18/ N) South Western Iran. The soil (Typic calci xerocrepts) physical and chemical properties are shown in Table 1. Topsoil of the experimental plot area was kept moist throughout the growing season when necessary. After soil test, the required nutrients were added to soil. Seeds were sown in pots in the farm condition on 22 May 2012 and 20 May 2013.

Table 1: Some physical and chemical properties of soil for experiment (0 -30) cm.

Year	Texture	E.C ($ds.m^{-1}$)	N_{total}	O.C	pH	K	P	Zn	Mn	Fe	Cu
			%			mg.kg^{-1}					
2012	Loam	8.1	0.11	0.2	8.2	770	45	0.97	11.2	8.1	1.3
2013	Loam	7.8	0.11	0.2	8.1	745	44	1	10.1	7.1	1.1

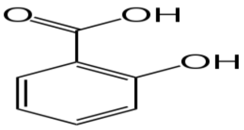

Fig. 1: Chemical structure of Salicylic Acid.

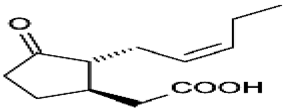

Fig. 2: Chemical structure of Jasmonic Acid.

Essential oil extraction:

At the beginning of the blooming stage, shoots of plants were harvested. 50 gr of shoot dry matter was added to 1000 cc of water and by Clevenger; essence extracted and then by GC/MS the chemical components of the essence was determined. The essential oils were analyzed by gas chromatography-mass spectrometry (GC/MS). Thermo Finnegan Trace 2000 GC/MS, made in the USA, was employed with a HP-5MS capillary column (30 m long and 0.25 mm wide, and a 0.25 µm of film thickness) at a 250°C of injector chamber. The initial column temperature was at 120°C for 5 min then raised to 280°C at the rate of 10°C/min. Helium was used as a carrier gas at a rate of 35 ml/min. MS parameters were as follows: ionization energy, 70eV; ion source temperature, 200°C; voltage, 3000 v; and mass range, 30 to 600. The compositions of the essential oil were identified by comparison of their retention indexes, retention times and mass spectra with those of authentic samples in Wiley library (Shibamoto, 1987; Adams, 2001).

Design and Statistical Analysis:

Experiments were arranged in a randomized complete block design and three replications. 100 plants were used for each trial. All data were subjected to analysis of variance (ANOVA) using the statistical computer package SAS and treatment means were separated using Duncan’s multiple range test at $P < 0.05$ level.

2. Results:

Our results showed that the best treatments were JA 50 µl, JA100 µl and SA 1mM. After injection of 0.1 µl of each essences in GC-MS, 27 essential oils were obtained of which the most of them were: α-pinene, limonene, β-pinene, camphor, thymol, camphene, thujone-trans, thujone-cis, 1,8-cineole, borneol, borneol acetate, manol, carvacrol, manool, α-humulene, caryophyllene (Tables 2 -4). Decrease in thujone-cis led to decrease in thujone-trans; and in the treatments, carvacrol was obtained, but viridiflorol was not obtained. In JA treatments, the maximum camphor and least carvacrol and thymol were in 50 µl; maximum carvacrol and thymol and least camphor, borneol, borneol acetate, α-humulene and caryophyllene were in 100 µl; the least camphene and maximum thujone-cis were in 200 µL; the least α-pinene, β-pinene, thujone-trans, thujone-cis, 1,8-cineole and maximum camphene, borneol, borneol acetate, α-humulene, caryophyllene were obtained in 400 µl. In SA treatments; the least borneol, camphor, thujone-trans, thujone-cis and maximum α-pinene, β-pinene, camphene and viridiflorol were in 1 mM, the least β-pinene, camphene, 1,8-cineole, α-humulene and caryophyllene and maximum thujone-trans, thujone-cis and manol were in 10 M; the least thymol and borneol acetate and most camphor were in 20 mM; the least α-pinene and maximum thymol, borneol and borneol acetate in 40 mM were detected. In all treatments, control treatment had the maximum borneol acetate and the least α-pinene, β-pinene and camphene. Acetone in all treatments had the maximum limonene, β-pinene and the least borneol acetate. The maximum α-pinene, manol, viridiflorol, α-humulene and caryophyllene and the least camphor, borneol, thujone-cis and 1,8-cineole in water treatment were obtained. Our results showed that by increasing carvacrol; borneol acetate, α-humulene and caryophyllene decreased; and on the other hand, by increasing camphor; β-pinene, borneol and viridiflorol decreased. There exists negative relationship among camphene, thujone-trans and thujone-cis too. Limonene was only detected in acetone and control treatments. Thymol was obtained in all of SA treatments but only detected in JA (100 µl). The maximum thujone-trans was in SA 1 mM and SA 10 mM; 1,8-cineole was most in SA 20 mM but least in water treatment. Manool was obtained only in SA 10mM and water. Carvacrol was detected only in JA treatments, but only viridiflorol was not obtained in these treatments. There were significant differences between treatments in two years (Table 4). Maximum quantity of essence was detected in JA 100 µl and SA 1 mM, in two years (Fig.3).

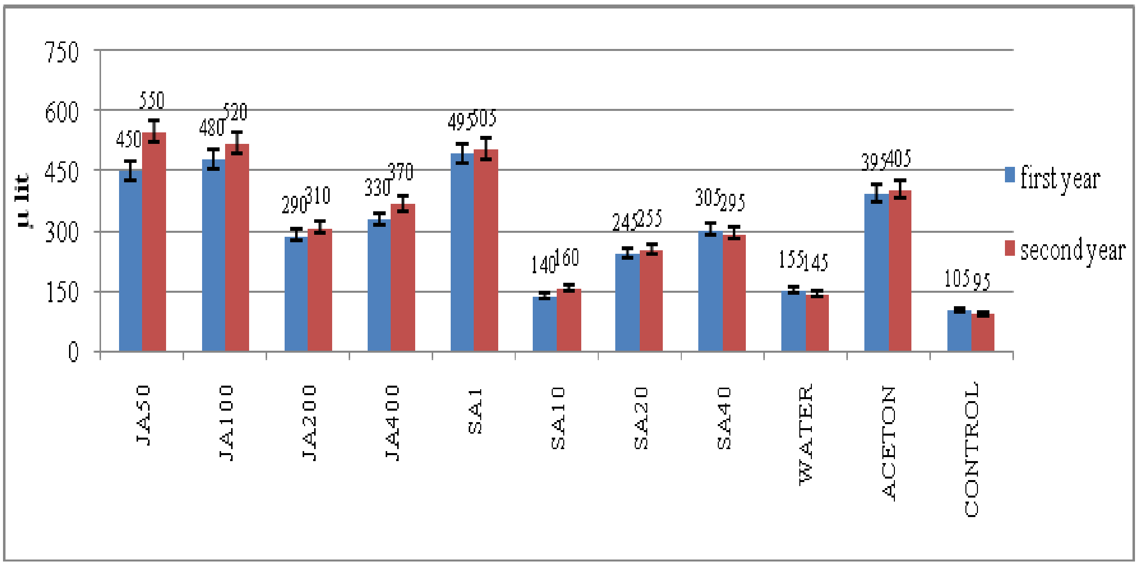

Fig. 3: Means of Essence concentration (JA (µl) and SA (mM)) in treatments in two years in 50 gr of shoot dry matter.

Table 2: Percentage of Essential oils in two elicitors [Jasmonic Acid (JA) and Salicylic Acid (SA)] in first year.

Treatment	α-Pinene	Camphene	β-Pinene	1,8-Cineole	Thujone-cis	Thujone-trans	Camphor	Borneol
JA (50 µL)	4.2±0.1	3.59±0.1	2.25±0.2	11.52±0.4	28.22±0.6	10.6±0.5	18.65±0.6	2.04±0.1
JA (100 µL)	4.08±0.2	3.6±0.2	2.38±0.1	11.23±0.5	25.73±0.8	9.89±0.2	15.87±0.5	1.59±0.2
JA (200 µL)	4.11±0.1	3.52±0.2	2,25±0.1	10.58±0.5	25.85±0.8	8.64±0.2	16.06±0.5	1.88±0.1
JA (400 µL)	3.87±0.2	5.25±0.1	2.08±0.2	9.45±0.2	24.03±0.6	8.43±0.5	16.45±0.4	1.93±0.1
SA (1 mM)	4.54±0.2	4.11±0.2	2.79±0.2	12.46±0.4	27.75±0.8	0.53±0.2	15.33±0.7	1.69±0.2
SA (10 mM)	3.27±0.1	3.01±0.1	1.79±0.2	10.8±0.3	32.14±0.8	12.85±0.6	16.55±0.8	1.79±0.1
SA (20 mM)	3.39±0.3	3.41±0.4	2.52±0.1	12.09±0.6	29.69±0.6	11.48±0.5	17.06±0.9	1.9±0.2
SA (40 mM)	3.06±0.2	3.41±0.1	2.1±0.1	12.05±0.6	28.6±0.4	10.6±0.5	16.4±0.5	3±0.1
Acetone	4.11±0.2	3.51±0.2	3.19±0.2	10.46±0.5	27.36±0.7	11±0.5	17.93±0.8	1.68±0.2
Water	5.32±0.2	3.27±0.1	2.33±0.1	4.8±0.7	21.78±0.6	6.61±0.5	9.67±0.1	0.74±0.1
Control	2.64±0.1	2.68±0.2	1.43±0.1	7.18±0.3	25.13±0.6	7.03±0.4	15.46±0.9	1.9±0.1
C.V	0.8	0.9	2.6	1	2.8	1.9	1.2	2.3

Table 2: Continue.

Treatment	Borneol acetate	Carvacrol	Caryophyllene	α-Humulene	Thymol	Viridiflorol	Manol	Limonene
JA (50 µL)	2.52±0.1	0.08±0.001	2.08±0.1	5.19±0.4	0±0	0±0	0±0	0±0
JA (100 µL)	2.23±0.2	0.48±0.001	1.76±0.2	3.09±0.1	3.35±0.3	0±0	0±0	0±0
JA (200 µL)	2.38±0.1	0.09±0.001	1.88±0.1	4.76±0.6	0±0	0±0	0±0	0±0
JA (400 µL)	2.52±0.1	0.08±0.001	2.58±0.1	5.89±0.1	0±0	0±0	0±0	0±0
SA (1 mM)	2.22±0.2	0±0	1.87±0.2	4.04±0.4	0.35±0.02	3.67±0.4	0±0	0±0
SA (10 mM)	2.18±0.1	0±0	0±0	2.14±0.1	0.35±0.05	2.21±0.4	0.53±0.01	0±0
SA (20 mM)	2.12±0.1	0±0	1.43±0.2	3.23±0.3	0.28±0.06	2.49±0.6	0±0	0±0
SA (40 mM)	2.32±0.2	0±0	1.43±0.2	4.2±0.3	0.4±0.1	2.22±0.2	0±0	0±0
Acetone	2.01±0.1	0±0	0±0	4.47±0.2	0±0	3.05±0.1	0±0	1.69±0.1
Water	0±0	0±0	4.06±0.7	11.49±0.9	0±0	10.16±0.5	5.36±0.3	0±0
Control	2.62±0.1	0±0	2.24±0.4	6.32±0.6	0±0	8±0.4	0±0	1.44±0.1
C.V	2.1	0.4	3.9	3.4	3.5	10.1	5.2	1.5

3. Discussion:

Various concentrations of elicitors gave different results. SA in low concentration did not inhibitory affect and made many components but in higher concentration reduced protein and essential oils (Canakci, 2008; Meher and Singh, 2011). 1mM concentration of SA had a stimulating effect while 10 and 40 mM concentration had varying degrees of inhibitive effects, therefore SA had a bidirectional physiological effect in a concentration-dependent manner (Canakci, 2011). The accumulation of phenolic compounds was stimulated with SA in low concentration. In *Salvia miltiorrhiza* (Dong *et al*., 2010), mung bean (Umair *et al*., 2012, Nazar *et al*., 2011), Caraway (Esfeiny farahani *et al*., 2011), cabbage, (Mba *et al*., 2007), *Ziziphus spina-christi* (Galal, 2012), Cucumber (Mardani *et al*., 2012), Calendula (Bayat *et al*., 2012), *Capsicum annuum* (Mahdavian *et al*., 2007), Basil and Majoram (Gharib, 2007), Pepper (Canakci, 2011) also showed decreasing effects of higher concentration of foliar application of SA and otherwise showed beneficial uses of it in low dosages. In this research we saw that although high concentration of SA in some essential oils made more than other treatments but generally 1mM of SA was the best of SA treatments. Many researchers showed that however SA have various effects on growth and development (Shakeel and Mansoor, 2012; Yu Ye *et al*., 2011, Nazar *et al*., 2011) but field applications of salicylic acid need optimum physiological concentration to increase nitrogen use efficiency particularly during germination and seedling growth (Kumar *et al*., 2010; Khan *et al*., 2010; Mahdavian *et al*., 2007). Increase in phenolic compounds and flavones after JA elicitation was observed in cells. In contrast, anthocyanins were in lower amounts in JA treatment (Gadzovska *et al*., 2007). Similar with SA, Jasmonic acid have various effects on components. In *Salvia miltiorrhiza (*Xiao *et al*., 2009*)*, *Brugmansia candida,* (Spollansky *et al*., 2000), *Hypericum perforatum* L. (Gadzovska *et al*., 2007), *Matricaria chamomilla* (Salimi *et al*., 2012), *Eleutherococcus senticosus* (Shohael *et al*., 2007), Sweet Basil (Kim *et al*., 2005), Kudzu (Thiem and Krawczyk, 2010), *Panax ginseng* (Babar Ali *et al*., 2007), *Glycyrrhiza glabra* (Shabani *et al*.,) showed in low and high concentration of JA, there was some of components were decreased or increased. JA under water stress increased total sugars, essential oil and major component and decreased total amino acids and proline concentration (Sheteawi, 2007, Sorial *et al*., 2010, Mardani *et al*., 2012). Many of our results of this research were accordance by Xiao *et al*., 2009 and Dong *et al*., 2010. Condition of research and many attributes for example edaphically properties affect on JA effectiveness.

Table 3: Percentage of Essential oils in two elicitors [Jasmonic Acid (JA) and Salicylic Acid (SA)] in second year.

Treatment	α-Pinene	Camphene	β-Pinene	1,8-Cineole	Thujone-cis	Thujone-trans	Camphor	Borneol
JA (50 µL)	4.4±0.1	3.79±0.1	2.65±0.2	11.82±0.4	28.02±0.6	11±0.5	18.85±0.6	2.44±0.1
JA (100 µL)	4.28±0.2	3.8±0.2	2.78±0.1	11.53±0.5	25.53±0.8	10.31±0.2	16.07±0.5	1.99±0.2
JA (200 µL)	4.31±0.1	3.82±0.2	2,65±0.1	10.88±0.5	25.65±0.8	9.06±0.2	16.26±0.5	2.22±0.1
JA (400 µL)	4.07±0.2	5.55±0.1	2.48±0.2	9.75±0.2	23.83±0.6	8.83±0.5	16.65±0.4	2.27±0.1
SA (1 mM)	4.74±0.2	4.41±0.2	3.2±0.2	12.76±0.4	27.55±0.8	0.93±0.2	15.53±0.7	2.1±0.2
SA (10 mM)	3.47±0.1	3.31±0.1	2.2±0.2	11.39±0.3	31.94±0.8	13.25±0.6	16.75±0.8	2.2±0.1
SA (20 mM)	3.59±0.3	3.71±0.4	2.52±0.1	12.69±0.6	29.49±0.6	11.88±0.5	17.26±0.9	2.3±0.2
SA (40 mM)	3.26±0.2	3.71±0.1	2.5±0.1	12.65±0.6	28.4±0.4	11±0.5	16.6±0.5	3.2±0.1
Acetone	4.31±0.2	3.81±0.2	3.59±0.2	11.06±0.5	27.16±0.7	11.4±0.5	18.17±0.8	1.88±0.2
Water	5.52±0.2	3.57±0.1	2.73±0.1	5.44±0.7	21.58±0.6	7±0.5	9.87±0.1	0.94±0.1
Control	2.84±0.1	2.98±0.2	1.83±0.1	7.78±0.3	24.93±0.6	7.43±0.4	15.66±0.9	2.11±0.1
C.V	0.7	1.6	2.2	1.2	3.1	2.1	1.4	2.6

Table 3: Continue

Treatment	Borneol acetate	Carvacrol	Caryophyllene	α-Humulene	Thymol	Viridiflorol	Manol	Limonene
JA (50 µL)	2.32±0.1	0.08±0.01	2.48±0.01	5.49±0.4	0±0	0±0	0±0	0±0
JA (100 µL)	2.03±0.2	0.28±0.01	2.2±0.02	3.29±0.1	3.75±0.3	0±0	0±0	0±0
JA (200 µL)	2.18±0.1	0.09±0.01	2,22±0.01	4.96±0.6	0±0	0±0	0±0	0±0
JA (400 µL)	2.32±0.1	0.08±0.01	2.98±0.01	6.11±0.1	0±0	0±0	0±0	0±0
SA (1 mM)	2.02±0.2	0±0	2.23±0.02	4.24±0.4	0.75±0.02	4.07±0.4	0±0	0±0
SA (10 mM)	1.92±0.1	0±0	0±0	2.34±0.1	0.75±0.05	2.61±0.4	0.33±0.01	0±0
SA (20 mM)	1.88±0.1	0±0	1.83±0.02	3.03±0.3	0.68±0.06	2.89±0.6	0±0	0±0
SA (40 mM)	2.12±0.2	0±0	1.83±0.02	4±0.3	0.8±0.01	2.62±0.2	0±0	0±0
Acetone	1.81±0.1	0±0	0±0	4.27±0.2	0±0	3.45±0.1	0±0	1.29±0.1
Water	0±0	0±0	4.46±0.07	11.29±0.9	0±0	10.56±0.5	5.16±0.3	0±0
Control	2.42±0.1	0±0	2.64±0.04	6.12±0.6	0±0	8.39±0.4	0±0	1.04±0.1
C.V	2.3	0.2	4.4	3.8	3.8	10.5	5.1	1.1

Table 4: Complex analysis of variance of main of essential oils in sage plants that are affected by JA, SA, Aseton and Water.

Source of variation	Degree of freedom	α-Pinene	Camphene	β-Pinene	1,8-Cineole	Thujone-cis	Thujone-trans	Camphor	Borneol
		Mean of Square							
Year(Y)	1	0.0001^{*}	0.0002^{*}	0.0004^{*}	0.0001^{*}	0.0001^{*}	0.0002^{*}	0.0013^{*}	0.0014^{*}
R/Y	4	0.00013^{*}	0.00012^{*}	0.00012^{*}	0.0012^{*}	0.00013^{*}	0.0012^{*}	0.0005^{*}	0.0007^{**}
T(JA,SA, water and Acetone)×Y	18	0.00007^{**}	0.00005^{*}	0.0012^{*}	0.0005^{*}	0.00077^{**}	0.00055^{*}	0.00017^{*}	0.00035^{*}
E	36	0.00005	0.00004	0.000032	0.000057	0.00009	0.00008	0.00007	0.00004
Coefficient of Variation		0.74	1	2.4	1	2.9	1.9	1.3	2.5

Table 4: Continue

Source of variation	Degree of freedom	Borneol acetate	α-Humulene	Viridiflorol	Limonene	Caryophyllene	Carvacrol	Thymol	Manol
		Mean of Square							
Year(Y)	1	0.0012^{*}	0.0002^{*}	0.001^{*}	0.0001^{*}	0.0002^{*}	0.0012^{*}	0.0001^{*}	0.0012^{*}
R/Y	4	0.0009^{**}	0.0007^{*}	0.00054^{*}	0.00012^{*}	0.00012^{*}	0.00022^{*}	0.00023^{*}	0.0029^{**}
T(JA,SA, water and Acetone)×Y	18	0.00037^{*}	0.00025^{*}	0.00047^{*}	0.00045^{*}	0.00035^{*}	0.00006^{*}	0.00017^{**}	0.00057^{*}
E	36	0.00005	0.000032	0.00001	0.00023	0.00078	0.00004	0.00005	0.00005
Coefficient of Variation		2.2	3.74	10.3	1.2	4.74	0.22	3.6	5.1

ns,* and **: Non significant, significant at the 5% and 1% levels of probability, respectively.

Conclusion:

This study shows the beneficial consumption of elicitors. JA and SA had increasing effect on essential oils. The best treatments were JA50 µl, JA100 µl and SA1 mM. 27 essential oils were obtained; the most of them were: α-pinene, limonene, β-pinene, camphor, thymol, camphene, thujone-trans, thujone-cis, 1,8-cineole, borneol, borneol acetate, carvacrol, α-humulene, caryophyllene. JA was more effective in stimulating the accumulation of α-pinene, limonene, β-pinene, camphor, thymol, camphene, thujone-trans, thujone-cis, 1,8-cineole, borneol, borneol acetate, carvacrol, α-humulene and caryophyllene. At 50 and 100 µl, JA enhanced the production of camphor, thymol and carvacrol. Although 1 mM SA led to the same total production of many essential oils like β-pinene, camphor, thymol, thujone-cis, carvacrol, manol, α-humulene and caryophyllene were less produced in JA (50 and 100 µl); only α-pinene, β-pinene, camphene, 1,8-cineole, veridiflorol were produced better in JA (50 and 100 µl). Although our results showed profit of JA (50 and 100 µl) and SA (1mM) than other treatments in two seasons but for determine the effects of climate and edaphic conditions on Sage, more researches must be performed, also different of species for treated by SA and JA were inevitable.

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