Effects of Exogenous Salicylic Acid Application on Growth, Metabolic Activities and Essential Oil Composition of *Satureja khuzistanica* Jamzad

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Abstract

Background: ***Satureja khuzistanica*** **Jamzad, with the common Persian name "marzeh khuzestani", is an endemic medicinal plant, distributed in the southwestern areas of Iran. Salicylic acid (SA) is a signaling molecule and a hormone-like substance that plays an important role in the plant physiological processes.**

Objective: This study was conducted to determine the influence of foliar SA application (0, 50, 100, 200 and 400 mg.L^{-1}) at two times including vegetative stage (VS) and both vegetative and reproductive stages (VS+RS) on growth parameters, enzymes activity including superoxide dismutase (SOD) and polyphenol oxidase (PPO), prptein content, essential oil percentage and composition of *S. khuzistanica* under field conditions.

Methods: The essential oils were isolated from aerial flowering parts of the plants by hydro-distillation method and then subjected to GC and GC-MS analyses to determine the oil constituents.

Results: Results showed that SA application at 100 and 200 mg.L^{-1} were the most effective treatments in growth characteristics, but the highest essential oil content and yield was obtained at 400 mg.L^{-1} SA treatment. In both spraying times, plants treated with 100 and 200 mg.L^{-1} SA concentration showed more PPO and SOD activity than control plants, respectively. Also, results showed that the 14 compositions were identified in essential oil of plants under all employed treatments. Carvacrol was the major component of oils, which is also showed more variability than that of other components.

Conclusion: It was concluded that foliar spray of SA at low concentration once at vegetative and second time at reproductive stage might be employed for enhancing both primary and secondary metabolites production of *S. khuzistanica* plants.

Keywords: ***Satureja khuzistanica,*** **Carvacrol, Essential oil, Growth, Salicylic acid**

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Introduction

Medicinal plants are an important resource in nature that can play critical role in human health, economy and income of countries, if they are well studied, cultivated and processed. The genus *Satureja*, belonging to the labiatae family and the Nepetoideae subfamily, is a high valuable medicinal plant that mainly distributed in the Mediterranean region, and 14 species are growing in northern, northwestern, western, southwestern, and central parts of Iran [1, 2]. The strongly scented plant *S. khuzistanica* is a popular herb for the local population, used as herbal tea and for its therapeutic virtues as an analgesic and antiseptic in the traditional medicine. Recently, antifungal, antibacterial, antinociceptive, antioxidant, antidiabetic, antihyperlipidemic, antibiofilm, and anti-inflammatory effects as well as the triglyceride-lowering potential of *S. khuzistanica* have also been documented [1, 3].

The production of essential oils not only depends upon the metabolic state and present developmental differentiation program of the synthesizing tissue, but also is highly integrated with the physiology of the whole plant. It has been demonstrated that signal molecules are very potential elicitors for induction of plant secondary metabolites [4]. Recent years, the applications of signal components as elicitors have evolved an effective strategy for the production of target secondary metabolites in plant cell cultures. However, it is still uncommon for commercial application [4]. It therefore, suggested that application of elicitors *in vivo* is an easy and direct channel to promote the yield of plant secondary metabolites in the field conditions. Salicylic acid (SA), a signal molecule has been found to generate metabolic and physiological responses. In addition to growth and development, the SA signaling pathway plays a key role in plant defense responses [5]. It has been used as a potent enhancer of some secondary metabolites in previous studies, for example, exogenous application of SA improves the production of alkaloids [6, 7], anthraquinones [8] and glucosinolates [9]. Recently, some studies indicate that SA signalling pathway is involved in biosynthesis of terpenoids including triterpenoids [10], diterpenoids [11], and sesquiterpenoids [12]. Review of literature revealed that there is no knowledge of the SA influence on *Satureja* species metabolic activities under field conditions. Therefore, the present investigation was undertaken to study the impact of different concentration and time of salicylic acid spraying on morphological, physiological and biochemical features of *Satureja khuzistanica* Jamzad under field conditions.

Material and Methods

Plant materials, growth conditions and salicylic acid application

The experiment was conducted at the research station of tropical and sub-tropical medicinal plants, Lorestan, Iran, during growing season of 2011. The geographic, edaphic and climatic characteristics of the location are given in tables 1 and 2. Trail field was plowed during the autumn and was disked before planting to provide a proper bed. The cuttings of *Satureja khuzistanica* (7 cm in height), which are prepared and rooted earlier in a controlled conditions, were planted as

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Table 1- Geographic and edaphic characteristics of the trail field

Climatic conditions	
Latitude (N)	33º 01´
Longitude (E)	32 º 45´
Altitude [m]	1100
Mean annual temperature [ºC]	16.5
Rainfall [mm/year]	475.98
Soil conditions	
Soil texture	Sandy loam
Sand [%]	58
Silt [%]	24
Clay [%]	18
Saturation percentage [%]	31
Electrical conductivity [dS/m]	2.6
pH	7.7
Total neutralizing value [%]	45
Organic carbon [%]	0.78
Total nitrogen [%]	0.08
Available phosphorus [ppm]	9.4
Available potassium [ppm]	140

Table 2- Mean rainfall and temperature of the trial location during the growing seasons.

	May	June	July	August	September	October	November
Rainfall [mm]	104	0	0	0	0	6	25
Temperature [ºC]	17.5	24.1	27.6	28.5	24.3	21.1	14.2

seedlings in early spring in the trail filed with the density of 25×55 cm (distance between plants × distance between rows). The experiment was carried out in a factorial based on completely randomized block design (CRBD). The experiment includes 3 blocks (replication) and each block is contained 10 plots. Each plot size was 4 × 2 m. Distance between blocks and plots were 2 and 1 m, respectively. SA was dissolved in absolute ethanol then added drop-wise to water (ethanol/water: 1/1000 v/v). SA treatment was applied on the foliage of *S. khuzistanica* plants at the five concentrations (0, 50, 100, 200 and 400 $mg.L^{-1}$) in two different plant growth stages, vegetative stage (VS) and vegetative+reproductive (VS+RS), with a hand sprayer. Control group of plants were sprayed with distilled water. A surfactant teepol (0.5%) was added with the control and SA treatment solutions. The volume of the spray was 50 ml per plant. Plant observations were recorded on randomly selected six uniform plants from each plot with considering border effect. At harvesting time (full flowering stage), samples were taken and plant height, canopy diameter, number of main stems, number of lateral branches, internodes length, plant fresh and dry weight, leaf weight, essential oil content and composition, biological yield, leaf yield, superoxid dismutase (SOD) and polyphenol oxidase (PPO) activity, total leaf protein content were assessed.

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Superoxide dismutase (SOD; E.C. 1.15.1.1) activity

A crude enzyme extract was prepared by homogenizing 0.5 gram of leaf tissue in extraction buffer containing 0.5% Triton X-100 and 1% polyvinyl pyrrolidone in 100 mM potassium phosphate buffer (pH 7.0) using a chilled mortar and pestle. The homogenate was centrifuged and the supernatant was used for the following enzyme assays. Total SOD activity was determined according to Beauchamp and Fridovich (1971). The reaction mixture contained 1.17×10^{-6} mol.L^{-1} riboflavin, 0.1 mol.L^{-1} methionine, 2×10^{-5} mol.L^{-1} KCN and 5.6×10^{-5} mol.L^{-1} nitroblue tetrazolium (NBT) salt dissolved in 3 ml of 0.05 mol.L^{-1} sodium phosphate buffer (pH 7.8). 3 ml of the reaction medium was added to 1 ml of enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes in a single row. The absorbance was read at 560 nm in the spectrophotometer against the blank. SOD activity is expressed in U mg^{-1} protein. (U = change in 0.1 absorbance h^{-1} mg^{-1} protein under assay conditions).

Polyphenol oxidase (PPO; E.C. 1.14.18.1) activity

Polyphenol oxidase activity was estimated following the method of Raymond *et al.* (1993) at 40 ºC. The reaction mixture contained 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.8), 0.2 ml pyrogallol 20 mM, and 0.03 ml enzyme extract. The increase in bsorbance was recorded at 430 nm. The PPO activity was defined as 1 lM of pyrogallol oxidized per min per mg protein [Unit mg^{-1} (protein)].

Leaf proteins analysis

Five hundred milligrams of leaf tissue were ground with liquid nitrogen, and then 0.3 mL of extraction buffer (0.1M pH 8.1, 5mM EDTA) were added and centrifuged for 10 min at 14,000 g at 4 ºC. Total protein was determined according to Bradford (1976).

Essential oil extraction

In order to extraction of essential oil, aerial parts of *S. khuzistanica* were collected at the full flowering stage. The essential oils were extracted by hydro-distillation of air-dried samples using a Clevenger-type apparatus in 3 hours. The samples were collected and their volumes measured before drying over anhydrous sodium sulphate. Essential oil yield was computed by multiplying the fresh shoot yield by the oil content.

Essential oil analysis procedure

GC-FID analysis was performed using a Thermoquest gas chromatograph, TRACE GC, with a flame ionization detector (FID). The analysis was carried out using fused silica capillary DB-5 column (60 m × 0.25 mm i.d.; film thickness 0.25 µm). The operating conditions were as follows: injector and detector temperatures, 250°C and 300°C, respectively; oven temperature, 60-250°C at the rate of 5°C/min and finally held isothermally for 10 min; carrier gas, N_2 at a flow rate of 1.0 ml/min; split ratio, 150. GC-MS analysis was performed using a Thermo Quest-Finnigan gas chromatograph equipped with above mentioned column and coupled with a TRACE mass quadrupole analyzer (Manchester, UK). Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 200°C

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and 250°C, respectively. Mass range was from m/z 43- 456. Gas chromatographic conditions were as given for GC-FID. The constituents of the essential oil were identified by calculating the retention indices under temperature-programmed conditions for n-alkanes (C6-C24) and the oil on a DB-5 column under the same chromatographic conditions. The identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparing their retention indices with authentic compounds or with those reported in the literature[1]. For quantification purpose, relative area percentages obtained by GC-FID were used without the use of correction factors.

Statistical analysis

Data were processed by the analysis of variance (ANOVA) in a 5 (SA concentration) × 2 (spraying time) factorial experiment with 3 replications on the basis of complete randomized block design (CRBD). The data were analyzed using computer SAS software (version 9.1; CoHort Software), and the means were compared by Duncan's multiple range test ($p< 0.05$). Because non-significant differences in interaction between SA spraying times × concentrations was found, results were presented in the form of separate factors, and not combination, except for SOD and PPO activity and protein content.

Results

Growth parameters

Data presented in table 3 show that the time of SA foliar application at VS and VS+ RS with 0, 50, 100, 200 and 400 $mg.L^{-1}$ significantly ($p< 0.05$) influenced the plant dry weight, essential oil percentage and oil yield. However, there was no significant difference between spraying times on other examined morphological characteristics including plant height, canopy diameter, number of main stems and lateral branches, internodes length, leaf weight and biological yield (table 3). SA application at VS was more pronounced on morphological features than biochemical characteristics such as essential oil values, enzymes activity and protein content. Results also indicated that the effect of SA at different concentrations were different on various plant traits. SA at 100 and 200 $mg.L^{-1}$ were the most effective treatments in improvement morphological characteristics when compared to corresponding untreated plants. Among the treatments, SA applied at 100 $mg.L^{-1}$ resulted in higher biological and leaf yield over other treatments.

Enzymes activity and protein content

SA application at VS+RS promoted enzymes (SOD and PPO) activity as compared to the one time spraying (VS). Different variations of SOD and PPO activity were observed between SA spraying times and concentration treatments (Fig. 1, 2). In both spraying times, specific activity of SOD increased continuously with increasing SA concentration up to 200 $mg.L^{-1}$, and thereafter declined. But the decrease of SOD activity under 400 $mg.L^{-1}$ was not significantly lower than that of control in both application times (Fig. 1). The PPO activity was noticed and pronounced at 100 $mg.L^{-1}$ SA concentration when applied in VS (Fig. 2). The protein content of *S. khuzistanica* plants also showed a varying pattern with different SA concentrations and spraying times (Fig. 3). It increased steadily with increasing

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Table 3- Effect of salicylic acid (SA) spraying times and concentrations on growth parameters and essential oil content and yield of *Satureja khuzistanica* JAMZAD

SA treatments	Plant height (cm)	Canopy diameter (cm)	No. of main stems	No. of lateral branches	Internodes length (cm)	Plant fresh weight (g)	Plant dry weight (g)	Leaf weight (g)	Leaf yield (g/ha)	Biological yield (kg/ha)	Essential oil content (%)	Essential oil yield (g/ha)
Spraying time												
VS	39.67±1.1^{a}	40.26±4.3^{a}	6±1.1^{a}	5.11±1^{a}	1.07±0.07^{a}	95.29±3.2^{a}	33.9±1.3^{a}	17. 8±0.9^{a}	1293.4±17^{a}	1740.7±11^{a}	1.22±0.04^{b}	890.76±24^{b}
VS+RS	39.54±1.2^{a}	38.38±3.5^{a}	5.6±0.5^{a}	5.02±0.7^{a}	1.05±0.05^{a}	91.94±2.9^{a}	23.6±2.2^{b}	17.7±1.6^{a}	1287.9±15^{a}	1717.9±14^{a}	1.40±0.02^{a}	1022.06±31^{a}
Concentration (mg.L^{-1})												
0	36.43±1.1^{c}	36.83±2.2^{c}	5.5±1.1^{a}	4.45±1.07^{a}	1.1±0.07^{b}	80.02±2.93^{d}	21.42±2.1^{b}	15.43±0.9^{c}	1140.6±17^{d}	1590.5±21^{c}	1.07±0.2^{c}	803.3±24^{e}
50	38.98±1.2^{b}	38.25±2.6^{b}	5.6±0.5^{a}	4.85±0.52^{a}	1.4±0.18^{a}	82.31±1.73^{d}	22.06±2.3^{ab}	16.38±0.6^{b}	1191.5±23^{c}	1604.4±37^{b}	1.18±0.1^{b}	861.6±23^{d}
100	40.55±1.9^{a}	41.25±2.3^{a}	5.9±0.7^{a}	5.07±0.7^{a}	1.5±0.01^{a}	88.97±3.77^{c}	22.69±1.5^{ab}	18.74±1.1^{a}	1324.4±38^{a}	1760.4±11^{a}	1.32±0.2^{ab}	962.9±36^{b}
200	40.65±1.4^{a}	41.58±2.7^{a}	5.9±1.1^{a}	4.95±1.05^{a}	1.5±0.03^{a}	103.54±5.9^{a}	24.2±2.2^{a}	18.21±2.3^{a}	1217.8±23^{b}	1650.8±34^{b}	1.21±0.1^{b}	885.8±25^{c}
400	39.41±1.6^{ab}	38.7±2.1^{b}	6.03±0.8^{a}	5.03±0.81^{a}	1.4±0.19^{a}	93.07±7.1^{b}	22.3±1.5^{ab}	16.96±1.1^{ab}	1278.9±41^{b}	1640.6±37^{b}	1.46±0.3^{a}	1068.2±38^{a}

VS: vegetative stage, RS: reproductive stage. The means for treatments indicating by the same letter do not differ significantly at α=0.05 according to Duncan test

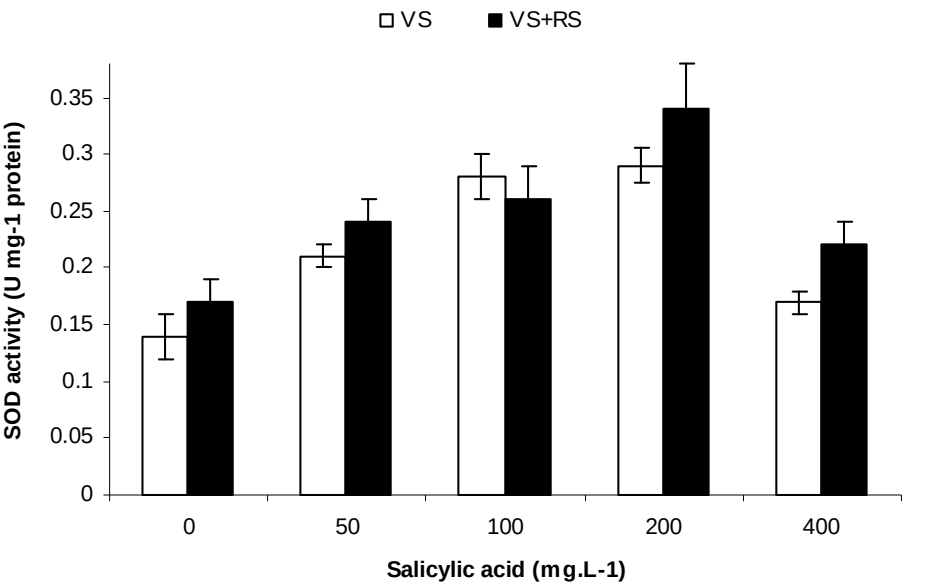


Fig. 1 - Variations of superoxide dismutase (SOD) activity in *Satureja khuzistanica* under different salicylic acid concentration and spraying time (VS: Vegetative stage, RS: Reproductive stage). The error bars represent the standard deviation (±SD) for replicates (n=3)

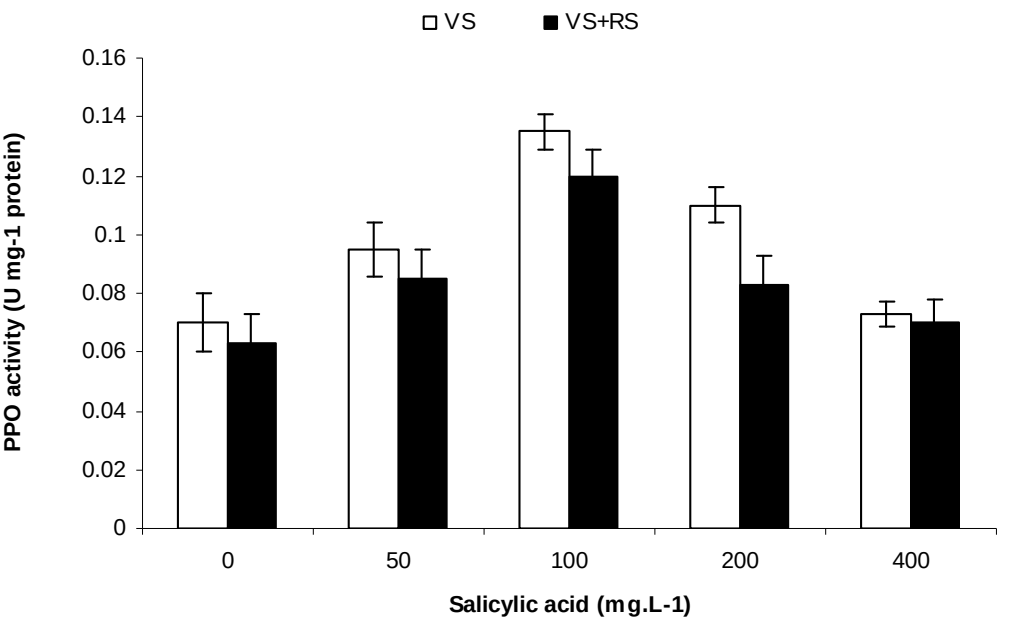


Fig. 2 - Variations of polyphenol oxidase (PPO) activity in *Satureja khuzistanica* under different salicylic acid concentration and spraying time (VS: Vegetative stage, RS: Reproductive stage). The error bars represent the standard deviation (±SD) for replicates (n=3)

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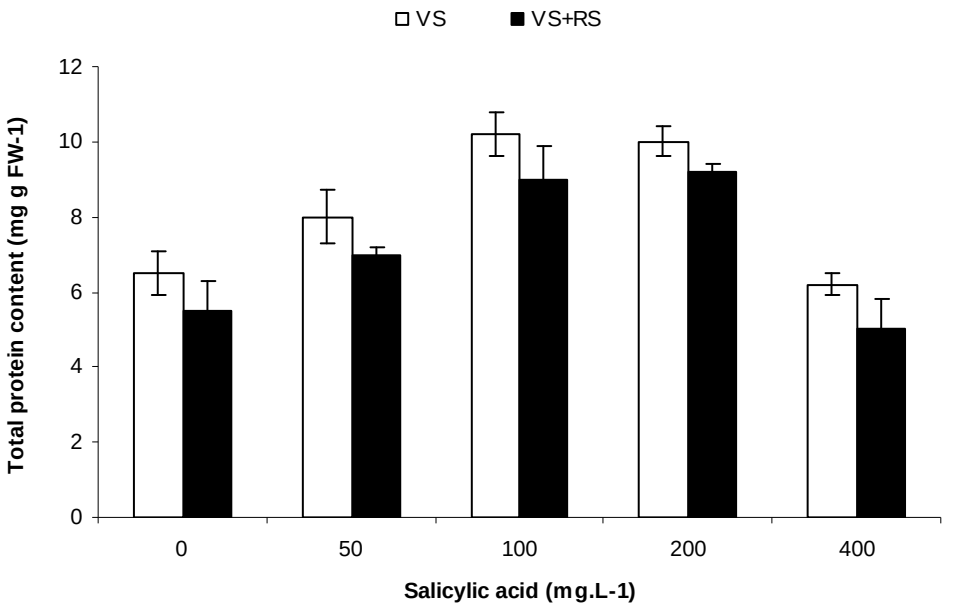


Fig. 3 - Variations of total leaf protein content in *Satureja khuzistanica* under different salicylic acid concentration and spraying time (VS: Vegetative stage, RS: Reproductive stage). The error bars represent the standard deviation (±SD) for replicates (n=3)

SA levels until 100 mg.L^{-1} and then followed a rapid decrease at 400 mg.L^{-1} in both application times. However, no significant variations were observed between 100 and 200 mg.L^{-1} SA concentrations under VS and VS+RS spraying times (Fig. 3).

Essential oil yield and composition

SA application significantly augmented the essential oil content and yield of *S. khuzistanica* plants compared to the control untreated plants (Table 3). The highest essential oil percentage (1.46%) and yield (1022 g/ha) were obtained at 400 mg.L^{-1} SA treatment when applied in two times (VS+RS). Essential oil composition of plants under different SA concentrations and application time are given in table 4. Generally, a total of 14 compounds, representing 88.6%-99.6% (at control and 100 mg.L^{-1} SA, respectively) of the total oil composition were identified in the employed treatments. Interestingly, carvacrol was the major component of oils as a very high percentage, which is also showed more variability than that of other components in two application times under employed treatments.

Discussion

Salicylic acid, a signal molecule has been found to generate metabolic and physiological responses especially in growth and development. In our current study, SA applied at low concentration resulted in higher biological and leaf yield over other treatments. Similarly, Kord and Hathout (1992) reported that foliar application of SA at low concentration (10^{-5} M) stimulated different morphological and growth parameters of tomato plants but reverse effects were

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Table 4- Essential oil composition of *Satureja khuzistanica* JAMZAD under different salicylic acid (SA) concentrations and application time

No	Essential oil compounds (%)	RI	SA application ($mg.L^{-1}$) in vegetative stage					SA application ($mg.L^{-1}$) in vegetative + reproductive stages				
			0	50	100	200	400	0	50	100	200	400
1	α-pinene	933	0.1±0.06	-	-	-	0.1±0.08	0.1±0.1	0.1±0.08	-	0.2±0.1	0.2±0.2
2	myrcene	981	0.7±0.3	-	0.1±0.2	0.8±0.3	0.5±0.2	0.6±0.3	0.3±0.2	0.5±0.2	0.5±0.3	0.6±0.2
3	α-terpinene	1013	1.2±0.8	0.1±0.2	0.1±0.1	0.2±0.1	0.2±0.3	0.4±0.2	-	0.1±0.1	0.1±0.1	0.2±0.1
4	*p*-cymene	1017	3.9±2.7	0.9±0.5	0.7±0.4	0.9±0.6	2.3±0.8	1.7±0.9	1.6±0.8	0.3±0.2	0.8±0.6	1.2±0.7
5	limonene	1026	-	0.4±0.2	0.2±0.1	0.8±0.5	0.8±0.5	0.2±0.1	0.5±0.2	0.4±0.3	0.1±0.1	-
6	Z-β-ocimene	1036	1.8±0.9	0.1±0.1	0.2±0.2	0.7±0.5	0.2±0.1	0.6±0.2	0.3±0.2	0.4±0.3	0.6±0.4	0.6±0.3
7	γ-terpinene	1053	4.3±2.8	0.2±0.1	0.3±0.2	2.1±2.0	1.1±0.7	0.5±0.2	0.7±0.3	0.3±0.1	0.3±0.2	0.3±0.1
8	α-terpinole	1175	1.1±0.9	0.2±0.1	0.2±0.2	-	0.8±0.4	0.7±0.3	0.4±0.1	0.3±0.2	0.5±0.2	0.4±0.3
9	thymol	1266	0.1±0.1	0.1±0.1	0.1±0.1	0.3±0.2	0.1±0.1	-	-	-	-	-
10	carvacrol	1282	83.6±3.84	96.1±3.11	96.3 ±2.76	90.1±2.21	91.4±2.14	92.8±1.54	94.5±2.34	96.5±1.22	95.5±1.82	94.7±2.71
11	thymyl acetate	1329	0.1±0.09	0.1±0.1	0.1±0.06	0.4±0.3	0.1±0.4	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1
12	β-caryophyllene	1425	0.3±0.06	0.2±0.1	0.2±0.1	-	0.2±0.4	0.3±0.2	0.1±0.1	-	0.2±0.1	0.3±0.1
13	α-humulene	1427	0.9±0.2	0.7±0.5	0.7±0.4	1.5±0.9	0.8±0.4	0.3±0.1	0.4±0.1	0.2±0.1	0.7±0.3	0.5±0.3
14	trans-β-bisabolene	1522	0.1±0.1	0.1±0.07	0.1±0.1	0.3±0.2	0.3±0.1	0.3±0.1	0.4±0.2	0.4±0.4	0.1±0.1	0.1±0.1
	Total identified (%)		98.2	99.2	99.3	98.2	98.9	98.6	99.4	99.5	99.6	99.2

Values obtained were expressed as mean ± SD (standard deviation) from three replications (n = 3) of each treatment

VS: Vegetative stage, RS: Reproductive stage. RI: Retention Index. Values obtained were expressed as mean ± SD (standard deviation) from three replications (n = 3) of each treatment

observed at high concentration (10^{-3} M) [16]. Our results in this research are in agreement with those of Gharib (2007) who reported that low concentration of SA increased photosynthetic activity in Basil and Marjoram, which enhanced their plant height, number of internodes, number of branches and leaves as well as fresh and dry weights [17]. Khandaker *et al.* (2011) stated that low concentration of SA as foliar application was more effective than higher concentration in improving growth characteristics and yield of red amaranth [18]. It is also reported that low concentration of SA promote and influence the growth, development, differentiation of cells, and tissues of flowering plants and enhanced the growth traits [19]. As reported by Senaratna *et al.* (2000) SA promotes some physiological processes and inhibiting others depending on its concentration, plant species, developmental stages and environmental conditions [20]. It is well known that SA prevent auxin oxidation, inhibit the ethylene biosynthesis and maintain IAA and gibberellins in plants [21], which could be the main reasons for SA-enhanced growth parameters. These findings are in accordance with those of obtained by Yildirim *et al.* 2008, who reported that the stimulation effect of SA on the biosynthesis of proteins was associated to an increase in photosynthetic pigments [22]. SA-enhanced growth *S. khuzistanica* plants in the present study might be associated with the regulatory effects of SA on cell division, plant growth and development and the changes in protein synthesis associated with plant growth and metabolism [23]. Likely, reported that red amaranth foliar application of SA enhanced accumulation of bioactive compounds, polyphenol, and antioxidant activity compared to the control plants [18]. It is reported that SA has a direct physiological effect through the alteration of antioxidant enzyme activities and SA can enhance enzymes activity and many metabolic substrates [24]. Our results are in a good line with those of obtained by Khan *et al.* (2010), who reported that these results may be due to the role of SA in enhancing physiological and biochemical aspects, or increasing N, P, K, and Ca content, activity in antioxidant enzymes and glutathione content [25].

In our current study, the precursors of carvacrol, *p*-cymene and *y*-terpinene, were present in low concentrations. Carvacrol is a monoterpenoid phenol biosynthesized via aromatization of gterpinene to *p*-cymene and subsequent hydroxylation of *p*-cymene. This phenol along with its two precursors *p*-cymene and *y*-terpinene appeared as the major components in numerous phenolic essential oils of the Lamiaceae family (e.g., in thyme, oregano, and savory oil) [26]. Yao and Tian (2005) demonstrated that SA stimulates phenolic compounds and the synthesis of new polyphenolic substances in sweet cherry fruit [27]. The enhancement occurred because SA it's self a plant-produced phenolic compound, also a growth regulator, which participates in the regulation of physiological processes such as secondary metabolites production in plants [28]. Corresponding to our results, many researchers have reported positive influence of different plant growth regulators (PGRs) on the essential oil yield and its main components regarding to different essential oil bearing plants [29]. Exogenous application of SA has earlier been reported to improve growth, yield and essential oil content in the case of basil and marjoram [17]. In context with lemongrass and other essential oil bearing plants, it was

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argued that SA-mediated increment in the volatile oil might be due to the SA-stimulated vegetative growth, population of leaf oil glands and carbohydrates content, and also due to the beneficial effect of SA on metabolism and enzymes activities responsible for mono or sesqueterpene-biosynthesis [30, 31]. The present results are in agreement with that obtained by Nadeem *et al.* (2012) who reported that exogenous applications of SA could have influenced the synthesis of major components of fennel essential oil [32]. In the present study, there was a significant and negative correlation ($r=0.46^{**}$) between essential oil percentage and yield with internodes length. Also, a significant and positive correlation ($r=0.71^{**}$) were observed between plant height and canopy diameter. Collectively, foliar application of SA depends on concentration, can regulate the plant growth parameters, and yield as well as chemical compounds in *S. khuzistanica* plants.

Conclusion

From the results of this study, it can be concluded that exogenous application of SA (100 $mg.L^{-1}$) as foliar spraying once at vegetative and second time at reproductive stage influences different morphological, physiological and biochemical aspects of *S. khuzistanica* plants, which could be used as a useful strategy in order to obtain more both essential oil (especially carvacrol) and biological yield.

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