JJBS

Volume 12, Number 5,December 2019
ISSN 1995-6673
Pages 659 - 662

Jordan Journal of Biological Sciences **Short Communication-2**

Salicylic Acid Modifies the Active Ingredients of Sour Orange

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Received March 13, 2019; Revised April 10, 2019; Accepted April 13, 2019

Abstract

The essential oils of sour orange have been used for different purposes especially for the food and pharmaceutical industries. Salicylic acid plays significant roles in essential oil metabolism. In the present work, the effects of salicylic acid on the essential oil composition are examined. Sour orange trees were exposed to foliar spray of salicylic acid (0, 20 and 40 mg/L), and the essential oil of each treatment was isolated from young leaves and peels using the hydro distillation method (HD); then it was analyzed by the GC and GC/MS equipment's. The obtained results indicate that the essential oil percentage and its characterized constituents are affected by the salicylic acid significantly. The major components of the peel and leaf oils were limonene and linalool. The highest percentage of the essential oil and its major constituents were recorded with salicylic acid at 40 mg/L. Oxygenated monoterpenes (OM) was the major chemical class of the leaf oil, while monoterpene hydrocarbons (MH) was formed as a major fraction of the peel oil. Sesquiterpene hydrocarbons (SH) and oxygenated sesquiterpenes (OS) were the minor classes. Salicylic acid resulted in different variations of all chemical classes, i.e., MH, OM, SH and OS.

Keyword: Salicylic acid, Sour orange, Essential oil, Limonene, Linalool

1. Introduction

Sour orange (*Citrus aurantium* L.) belongs to the Family Rutaceae which is grown in South and Central America as well as in the Mediterranean region (Ait *et al*., 2005). Sour orange trees resist the worst environmental conditions, and the most popular rootstock of various citrus trees in Egypt is that of the sour orange tree (Reams and Furr, 1972). The essential oils of the leaves and peels of sour orange have been used for various purposes including cosmetics, perfumes, and for food and pharmaceutical industries (Lawless, 1992; Lehrner *et al*., 200l; Lota e*t al*,. 2001).

The exogenous application of bio-stimulants such as salicylic acid has been a successful method in scientific research with the potential of improving essential oils' composition to be used as natural sources in food and drug industries (Bukar *et al.*, 2016). Salicylic acid caused significant increases in essential oils isolated from young shoots and the peels of grapefruit (Khalid *et al.*, 2018). The effects of salicylic acid on essential oils isolated from yarrow (*Achillea millefolium* Boiss) were investigated (Rowshan and Bahmanzadegan, 2013); results indicated that salicylic acid resulted in significant increments in essential oil yield, 1, 8-cineol and β-caryphyllene compared with the control. Summer savory (*Satureja hortensis* L.) plants exposed to 5 mg/L of salicylic acid (as foliar spray) produced higher values in carvacrol, γ-terpinene, and monoterpene hydrocarbons than in the control, 10 and 20 mg/L of salicylic acid (Pirbalouti *et al.*, 2016). Salicylic acid caused significant increases in carvacrol, α-thujene, α-pinene and p-cymene detected in the thyme (*Thymus daenensis* Celak.) essential oil (Pirbalouti *et al*., 2014). Peppermint (*Mentha piperita* L.) plants were treated with salicylic acid, and significant variations were detected due to different applications of salicylic acid (Saharkhiz and Goudarzi, 2014; Khanam and Mohammad, 2017). Responses of *Ammi visnaga*'s essential oil to salicylic acid (0, 5, 10 and 20 mg/L) were investigated by Talaat *et al.* (2014), and the data indicated that the treatment with 20 mg/L produced the greatest amounts of essential oil and its major constituents. The essential oil of tansy (*Tanacetum vulgare* L.) plants was significantly changed due to salicylic acid applications (Goudarzi *et al.*, 2016). Significant increases were recorded in essential oil yield, limonene, β-selinene, sedanolide, and sedanenolide of Celery (*Apium graveolens* L.) with salicylic acid treatments (Ahmed *et al.*, 2018).

Improving the productivity of essential oil-bearing plants was required to increase the natural recourses. Therefore, the aim of the present study is to evaluate essential oils isolated from young leaves and peels of sour orange trees treated with various doses of salicylic acid.

2. Materials and Methods

2.1. Location

The experiments were carried out at the citrus experimental farm of the Faculty of Agriculture, Cairo University during 2017-18. The sour orange trees were divided into three groups. The first and second groups were exposed to salicylic acid of 20 and 40 mg/L. The

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third group was subjected to distilled water (as control). The salicylic acid was applied as foliar spray to run-off to foliage at the end of January and the first week of February in both seasons. The analyses of soil used in this study are presented in Table 1.

Table 1. Soil analysis used in this study.

Physical properties		Ions (mg/100g soil)	
Sand	26.1%	P	18.7
Silt	33.5%	K	25.3
Clay	40.4%	Ca	75.7
Chemical properties		Na	37.9
pH	7.9 (1 : 2.5)	HCO_3	22.9
EC	1.7 (dS/m)	Cl	10.3
OM	0.9%	CO_3	12.8
$CaCO_3$	0.8%	SO_4	23.9
Total N	78.9%	NO_3	9.8

2.2. Harvesting

During the first week of April and last week of October (2017 and 2018), young leaves and fruits were collected respectively. The fresh young leaves and peels of fruit were divided into small pieces with a knife. Then, they were weighed to isolate the essential oil.

2.3. Essential oil Isolation

The fresh young leaves and peels were collected from each treatment, and then 250g from each replicate (3 replicates) of all treatments were subjected to hydro-distillation for three hours using a Clevenger-type apparatus (Clevenger, 1928). The essential yield was calculated as a relative percentage (v/w).

2.4. Essential Oil Analysis

2.4.1. GC and GC–MS Conditions

GC analyses were performed using a Shimadzu GC-9 gas chromatograph equipped with a DB-5 (dimethyl-siloxane, 5 % phenyl) fused silica column (J & W Scientific Corporation) (30 m X 0.25 mm i. d., film thickness 0.25µm). Oven temperature was held at 50 ºC for five minutes and then programmed to rise to 240 ºC at a rate of 3 ºC/ min. The flame ionization detector (FID) temperature was 265 ºC and the injector temperature was 250 ºC. Helium was used as carrier gas with a linear velocity of 32 cm/s. The percentages of compounds were calculated by the area normalization method, without considering response factors. GC–MS analyses were carried out in a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m X 0.25 mm i. d., film thickness 0.25 lm); oven temperature was 50–240 ºC at a rate of 4 ºC/min, transfer line temperature was 260 ºC, carrier gas was helium, with a linear velocity of 31.5 cm/s, split ratio was 1:60, ionization energy was 70 eV, scan time was 1s, and mass range was 40–300 amu.

2.4.2. Identification of Volatile Components

The components of the oils were identified through the comparison of their mass spectra with those of a computer library or with authentic compounds, and these were confirmed through comparing their retention indices (RI^L), either with those of authentic compounds or with data published in the literature (Adams, 1995). Mass spectra from the literature were also compared (Adams 1995). Further identification was made by comparison of their mass spectra on both columns with those stored in NIST-98 and Wiley-5 Libraries. The retention indices (RI^C) were calculated for all volatile constituents using a homologous series of n-alkanes.

2.5. Statistical Analysis

In this vary experiment, one factor was considered: salicylic acid (0, 20 and 40 mg/L). For each treatment there were three replicates, the experimental design followed a complete random block design (RCBD). The average data of essential oil contents of both seasons were statistically analyzed using 1-way analysis of variance (Snedecor and Cochran, 1990). The applications of that technique were according to the STAT-ITCF program version 7 (Foucart, 1982).

3. Results

3.1. Effect of Salicylic Acid on Essential Oil Contents

The effects of salicylic acid doses (0, 20 and 40 mg/L) on essential oils isolated from the leaves and peels of sour orange trees are presented in Table 2. In both seasons, it was clear that the values of the leaf and peel oils were significantly increased with various doses of salicylic acid compared with the control. The highest amounts of the leaf and peel oils were reported with the application of 40 mg/L of salicylic acid recording the values of 0.5, 0.6 % and 0.8, 0.9 % of the leaf and peel oils during the first and second seasons, respectively.

Table 2. Effect of Salicylic acid on essential oil contents.

Salicylic acid (mg/L)	Essential oil content (%)			
	Young leaves		Peels	
	Seasons			
	2017	2018	2017	2018
0	0.2	0.3	0.2	0.3
20	0.3	0.4	0.5	0.5
40	0.5	0.6	0.8	0.9
LSD: 0.05	0.1	0.1	0.2	0.2

3.2. Effect of Salicylic Acid on Essential Oil Constituents

The various components that are detected using the GC/MS analysis are presented in Table 3. Different variations were observed in all of the components with different doses of salicylic acid. The major components recorded under all treatments with the salicylic acid were limonene and linalool for the essential oils extracted from the peels and leaves respectively. Higher values of limonene were recorded in the peel oil than in the leaf oil, while the linalool component was produced in higher amounts in the leaf oil than in the peel oil. The greatest amounts of limonene and linalool were produced under the treatment of 40 mg/L of salicylic acid with the values of 75.3 % and 62.9 % of the peel and leaf oils respectively. All characterized constituents in this investigation were classified into four chemical groups as follows: 1) Monoterpene hydrocarbons (MH), 2) Oxygenated monoterpenes (OM), 3) Sesquiterpene hydrocarbons (SH) and 4) Oxygenated sesquiterpenes (OS). The MH was the major group in the peel oil while OM was the major one in

the leaf oil. The highest amounts of MH (81.5 %) and OM (67.5 %) were obtained from the treatments of control and salicylic acid at 40 mg/L of the peel and leaf oil, respectively. The greatest amounts of SH (2.3 %) and OS (1.6 %) resulted from the treatment of the leaf oil with the dose of 20 mg/L.

Table 3. Effect of Salicylic acid on essential oil components in young leaves and peels.

No	Components	Class	RI^C	RI^L	Salicylic acid treatments (mg/L)					
					Young leaves			Peels		
					0	20	40	0	20	40
1	α-Pinene	MH	949	939	0.5	0.6	0.6	1.1	0.4	0.5
2	Camphene	MH	955	953	0.9	1.3	1.1	-	-	-
3	Sabinene	MH	977	976	0.9	0.1	0.7	0.3	0.5	0.1
4	β- Pinene	MH	081	980	0.7	0.9	0.3	0.4	0.2	0.1
5	β-Myrcene	MH	991	991	1.8	1.5	1.6	0.1	0.3	0.3
6	Octanal	MH	1003	1001	-	-	-	0.9	0.7	0.4
7	α-Phellandrene	MH	1006	1005	1.9	1.7	1.3	0.4	0.5	0.5
8	ʒ-Carene	MH	1011	1011	0.7	0.5	0.7	0.4	0.5	0.6
9	Limonene	MH	1032	1031	18.8	19.9	20.5	74.7	74.9	75.3
10	*cis*-β-Ocimene	MH	1041	1040	0.6	0.9	0.8	0.4	0.8	0.9
11	*trans*-β-Ocimene	MH	1050	1050	0.4	0.5	0.9	1.2	0.9	0.7
12	α-Terpinene	MH	1063	1062	0.3	0.2	0.6	0.9	0.5	1.1
13	α-Terpinolene	MH	1089	1088	1.8	0.6	0.2	0.7	0.5	0.1
14	*trans*-Linalool oxide	OM	1077	1074	0.2	0.5	0.7	0.4	0.6	0.5
15	Linalool	OM	1099	1098	59.8	61.9	62.9	11.7	12.8	12.9
16	Terpinen-4-ol	OM	1190	1189	1.8	1.0	0.1	0.7	0.3	0.3
17	Decanal	OM	1206	1204	-	-	-	0.2	0.3	0.5
18	Nerol	OM	1230	1228	1.6	1.2	0.4	0.4	0.5	0.4
19	Citral	OM	1242	1240	0.8	0.5	0.9	0.2	0.3	0.4
20	Carvone	OM	1243	1242	-	-	-	0.3	0.1	0.1
21	Geraniol	OM	1256	1255	0.1	0.5	0.9	1.5	0.2	0.4
22	Neryl acetate	OM	1366	1365	0.6	0.2	0.7	0.2	0.3	0.2
23	Geranyl acetate	OM	1385	1383	1.7	0.7	0.9	0.4	0.5	0.5
24	β-Caryophyllene	SH	14120	1418	0.7	0.6	0.5	-	-	-
25	α-Caryophyllene	SH	1455	1454	-	-	-	0.1	0.7	0.4
26	E-β-Farnesene	SH	1459	1458	0.8	0.9	0.3	0.7	0.5	0.3
27	Germacrene D	SH	1482	1480	0.7	0.8	0.1	0.1	0.8	0.2
28	Nerolidol	OS	1566	1564	0.5	0.8	0.4	0.1	0.4	0.6
29	Caryophyllene oxide	OS	1582	1581	0.4	0.7	0.6	0.7	0.2	0.4
30	α-Bisabolol	OS	1685	1683	0.5	0.4	0.6	0.1	0.4	0.4
MH (Monoterpene Hydrocarbons).					29.3	28.7	29.3	81.5	80.7	80.6
OM (Oxygenated Monoterpenes).					66.6	66.5	67.5	16.0	15.9	16.2
SH (Sesquiterpene Hydrocarbons).					2.2	2.3	0.9	0.9	2.0	0.9
OS (Oxygenated Sesquiterpenes).					1.4	1.9	1.6	0.9	1.0	1.4
Total identified					99.5	99.4	99.3	99.3	99.6	99.1

4. Discussion

The results obtained in this study indicate that salicylic acid can cause various changes in peel and leaf oils extracted from sour orange. These results may be attributed to the fact that salicylic acid (phenol compounds) has been recognized as a regulator of plant physiological processes when applied exogenously to plants. The most investigated roles of salicylic acid are associated with its interference in plant resistance response to pathogen attacks and less than optimal biotic conditions (Jalal *et al.,* 2012). The stimulating effects of salicylic acid on essential oil composition may be attributed to the salicylic acid influences on nutrient uptake, cell elongation, cell division, cell differentiation, sink / source regulation, changes in the hormonal status, improvement of photosynthesis, transpiration and stomatal conductance that reflect various increases in essential oil composition (Blokhina *et al.,* 2003; Shakirova, 2007; El-Tayeb, 2005; Abreu and Munne-Bosch, 2009). The salicylic acid has improved the level of cell metabolism, a prerequisite for the synthesis of auxin and/or cytokinin that may cause an increase in essential oil composition (Metwally *et al.*, 2003; Gharib, 2006). Rowshan and Bahmanzadegan (2013) indicated that salicylic acid may increase the essential oil composition through increasing the numbers of oil glands and enzyme activities of mono and sesquiterpenes biosynthesis.

5. Conclusion

The effects of salicylic acid on sour-orange essential oils were observed in this trial. 40 mg/L of Salicylic acid resulted in highest values of essential oil content and major constituents of the leaves and peels. Different changes were found in the chemical classes (monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes) of both of the leaf and peel oils.

Acknowledgment

The authors extend their thanks to the National Research Centre for the encouragement and support in this research work through the internal project no. 11080301.

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