



## Effects of foliar spraying acetyl-coA on chemical compositions of rosemary (*Rosmarinus officinalis* L.)

Vahid Abdossi, Mahshad Hosseini and Mojtaba Khorrami\*

Department of Horticultural Sciences,

Science and Research Branch, Islamic Azad University, Tehran, Iran

(Corresponding author: Mojtaba Khorrami)

(Received 09 May, 2015, Accepted 15 July, 2015)

(Published by Research Trend, Website: [www.researchtrend.net](http://www.researchtrend.net))

**ABSTRACT:** Rosemary (*Rosmarinus officinalis* L.), belonging to the Lamiaceae family. Acetyl coenzyme A is an important molecule in metabolism playing role in many biochemical reactions. Its main function is to convey the carbon atoms within the acetyl group to the citric acid cycle (Krebs cycle) to be oxidized for energy production. The aim of this study was including two factors (acetyl-co A and the time of treatment application) on chemical compositions of (*Rosmarinus officinalis* L.). Acetyl-coA was used in 6 different concentrations (0, 25, 50, 100, 200, and 400 mM) which were applied to plants 1, 2, and 3 times with seven - day interval. The results obtained in our study indicated that the application of acetyl-CoA on first week, the highest limonene content (13.5%) obtained from control and the highest camphor content (10.3%) and borneol content (11.1%) obtained from foliar spraying acetyl-coA with concentrations (400 mM) and the highest verbenone content (11.6%) obtained from foliar spraying acetyl-coA with concentrations (50 mM). The results obtained on second week, the highest verbenone content (15.5%) and borneol content (11.7%) obtained from foliar spraying acetyl-coA with concentrations (400 mM) and the highest camphor content (15.1%) obtained from foliar spraying acetyl-coA with concentrations (200 mM) compared with the control. The results obtained on three week the highest camphor content (18.5%) and borneol content (14.6%) obtained from foliar spraying acetyl-coA with concentrations (400 mM) and the highest verbenone content (18.1%) obtained from foliar spraying acetyl-coA with concentrations (200 mM) compared with the control.

**Key words:** *Rosmarinus officinalis* L, acetyl-co A, Chemical compositions

### INTRODUCTION

Acetyl-CoA is an intermediate common to a variety of metabolic processes that are distributed across at least five different subcellular compartments. In plastids, acetyl-CoA is the precursor for de novo fatty acid biosynthesis (Nikolau *et al.*, 2003) and for the biosynthesis of glucosinylates. Mitochondrial acetyl-CoA is incorporated into the TCA cycle and is used for the generation of ATP and the synthesis of amino acid carbon skeletons (Falk *et al.*, 2004). Rosemary (*Rosmarinus officinalis* L.), belonging to the Lamiaceae family is an aromatic evergreen shrub plant and medicinal herb widely used around the world. Is a flowering plant that grows in Mediterranean countries, southern Europe and in the littoral region through Minor Asia areas wildly (Atti-Santos *et al.*, 2005). It is a well-known valuable medicinal herb that is widely used in pharmaceutical products and traditional medicine as a digestive, tonic, astringent, diuretic, diaphoretic and is useful for urinary ailments (Mahomoud *et al.*, 2005). Studies reported by Moreno *et al.* (2006) showed that rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative

bacteria. Activity of rosemary is mainly due to borneol and other phenolics in the terpene fraction. A group of terpenes (borneol, camphore, 1,8 cineole,  $\alpha$ -pinene, camphene, verbenone and bornyl acetate) in rosemary (Santoyo *et al.*, 2005).

Since, acetyl-coA as a chemical compound has not ever been reported to be applied on rosemary plant, this research was carried out to find out if it has any effect on this medicine plant. Jamshidi *et al.* (2009) reported that the major components of *R. officinalis* oil in Lalehzar origin were  $\alpha$ -pinene (43.9%), 1, 8-cineole (11.1%), camphene (8.6%),  $\beta$ -myrcene (3.9%), borneol (3.4%), camphor (2.4%) and Verbenol (2.3%). The major components of *R. officinalis* oil in Kerman origin were  $\alpha$ -pinene (46.1%), 1, 8-cineole (11.1%), camphene (9.6%), camphor (5.3%), sabinene (4.6%),  $\beta$ -myrcene (3.9), borneol (3.4%), bornyl acetates (2.8%), verbenone (2.3%) and linalool (2.1%). Studies reported by Verma and Rahman (2011) showed that the major components of *R. officinalis* oil were camphor (23.1-35.8%), 1,8-cineole (21.4-31.6%) and  $\alpha$ -pinene (6.7-15.6%). Aim of the present work was effects of foliar spraying acetyl-coA on chemical compositions of rosemary (*Rosmarinus officinalis* L.).

## MATERIALS AND METHODS

### A. Plant materials, chemicals and instruments

Rosemary plants, *Rosmarinus officinalis* L., were obtained from commercial growers. Firstly, the plants were planted pots filled with 30% coco peat and 70% perlite and then placed in a greenhouse. Foliar spraying of acetyl-coA was applied as the first factor in 6 different concentrations (0, 25, 50, 100, 200, and 400 mM) at 4 occasions (the second factor) when acetyl-coA was sprayed on plants 0, 1, 2 and 3 times with 7 days intervals.

### B. Essential oil extraction

Hundred gram powdered plant material was subjected to hydro-distillation (1000 ml distilled water) for 2 h using a Clevenger-type apparatus as recommended method in British pharmacopeia Samples were dried using anhydrous sodium sulfate (Merck Co. Germany) and then kept in amber vials at  $4 \pm 1^\circ\text{C}$  prior to use.

### C. GC/MS analysis

GC-MS analysis was performed using a Hewlett Packard 5973 with a fused silica capillary column 5% phenyl-poly-dimethyl-siloxane (DB-5MS 30 m x 0.25 mm i.d. and 0.25 $\mu\text{m}$  film thickness).  $\text{C}^\circ$  The column

temperature was programmed as follows: from 60 C for 5 $^\circ\text{C}$  and finally held at 220 $^\circ\text{C}/\text{min}$  to 220 $^\circ$  (3 min hold) then raised at 5 min. The carrier gas (helium) flow rate was 1mL/min. Identification of the essential oil components was accomplished based on comparison of retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILLEY/ChemStation data system) (Adams, 2007).

## RESULT AND DISCUSSION

The chemical constituents identified by GC-MS, are presented in Table 1,2,3. In the present work, the limonene, camphor, borneol and verbenone were the major components of *Rosmarinus officinalis* L. oil.

The results obtained in our study indicated that the application of acetyl-CoA on first week, the highest limonene content (13.5%) obtained from control and the lowest limonene content (11%) obtained from foliar spraying acetyl-coA with concentrations (200 mM). The highest verbenone content (11.6%) obtained from foliar spraying acetyl-coA with concentrations (50 mM). The highest camphor content (10.3%) and borneol content (11.1%) obtained from foliar spraying acetyl-coA with concentrations (400 mM) (Table 1).

**Table 1: Effects of foliar spraying acetyl-coA on chemical compositions of *R. officinalis* on first week.**

No	Compound	RI	0	25	50	100	200	400
1	Tricyclene	926	1.6	1.4	0.9	1.7	2	2.3
2	-Pinene	941	7.2	6.6	5.6	6.1	6.2	7.3
3	Camphene	953	4.8	3.9	3.7	4.2	3.7	4.4
4	-Pinene	975	3.5	4.1	4.3	3.8	4.2	3.6
5	3-octanone	984	1.4	1.7	2.1	2.6	2.6	2.7
6	-Myrcene	988	2.6	0.9	1.8	2.3	2.2	2.6
7	p-cymene	1021	4.8	3.5	3.7	4.4	4.1	4.9
8	Limonene	1030	13.5	12.1	13.1	12.5	11	12.2
9	1,8-Cineole	1035	1.7	2	1.6	2.1	1.6	1.9
10	-Terpinolene	1080	5	4.1	4.6	4.9	4.4	5.3
11	Linalool	1094	2.3	2.6	2	2.7	2.1	2.7
12	Cyranthenone	1120	1.1	0.9	1.4	1.6	1.3	0.9
13	Camphor	1136	8.8	8.1	8.3	7.7	9.6	10.3
14	Borneol	1170	10.4	9.6	10.7	9.3	10.1	11.1
15	Terpineol-4-ol	1179	2.6	2.1	2.3	1.8	3	1.9
16	Verbenone	1195	11.3	10.4	11.6	10.2	10.2	9.6
17	Borneolacetate	1281	6.1	5.2	6.2	6.3	5.5	5.9
18	Apigenin	1335	2.5	2.1	1.9	1.7	2.3	2.7
19	Geraniol acetate	1382	1	0.9	0.6	0.8	1.3	1.6
20	Trans-Caryophyllene	1425	1.2	0.9	1.6	1.3	2.4	1.8
21	Caryophyllene oxide	1583	3.5	2.6	2.9	3	3.1	2.7

RI (Retention Indices)

The results obtained in Table 2 indicated that the application of acetyl-CoA on second week, the highest limonene content (13.5%) obtained from control and the lowest limonene content (8.8%) obtained from foliar spraying acetyl-coA with concentrations (400 mM). The highest camphor content (15.1%) obtained from foliar spraying acetyl-coA with concentrations (200 mM) compared with the control. The highest verbenone content (15.5%) and borneol content (11.7%) obtained from foliar spraying acetyl-coA with concentrations (400 mM).

The results obtained in Table 3 indicated that the application of acetyl-CoA on three week, the highest limonene content (13.5%) obtained from control and the lowest limonene content (8.1%) obtained from foliar spraying acetyl-coA with concentrations (200 mM). The highest verbenone content (18.1%) obtained from foliar spraying acetyl-coA with concentrations (200 mM) compared with the control. The highest camphor content (18.5%) and borneol content (14.6%) obtained from foliar spraying acetyl-coA with concentrations (400 mM).

**Table 2: Effects of foliar spraying acetyl-coA on chemical compositions of *R. officinalis* on second week.**

No	Compound	RI	0	25	50	100	200	400
1	Tricyclene	926	1.6	3.8	2.4	2.7	2	2.4
2	-Pinene	941	7.2	5.1	5.8	6.2	5	6.6
3	Camphene	953	4.8	4.1	4	3.7	3.9	4.2
4	-Pinene	975	3.5	3.1	2.9	3.5	3.3	3.8
5	3-octanone	984	1.4	3.2	2.7	1	2.1	2.6
6	-Myrcene	988	2.6	4.8	1.7	2	2.6	2
7	p-cymene	1021	4.8	3.3	4.4	4.4	5.1	4.1
8	Limonene	1030	13.5	11.4	10.8	9.8	9	8.8
9	1,8-Cineole	1035	1.7	3.3	1.8	1.2	2.1	2.7
10	-Terpinolene	1080	5	4	3.6	4.4	4.2	4.8
11	Linalool	1094	2.3	2.6	2.1	1.5	3	2.6
12	Cyrsanthone	1120	1.1	2.7	3	2	1.3	0.8
13	Camphor	1136	8.8	8.1	10.6	10.9	15.1	14.8
14	Borneol	1170	10.4	9.7	11.4	9.5	11.2	11.7
15	Terpineol-4-ol	1179	2.6	3.1	3.3	3.6	2.3	2
16	Verbenone	1195	11.3	8.8	9.5	10.6	14.1	15.5
17	Borneolacetate	1281	2.5	1.8	2.9	3.3	2.2	2.6
18	Apigenin	1335	1	1.5	2.8	1.9	1.1	1.8
19	Geraniol acetate	1382	6.1	4.1	5.6	6.8	5	6.2
20	Trans-Caryophyllene	1425	1.2	2.9	3.1	3.6	2.3	1.8
21	Caryophyllene oxide	1583	3.5	4.7	3.7	3	2	3.8

RI (Retention Indices)

**Table 3: Effects of foliar spraying acetyl-coA on chemical compositions of *R. officinalis* on three week.**

No	Compound	RI	0	25	50	100	200	400
1	Tricyclene	926	1.6	1.6	2.7	3.1	1.1	1.7
2	-Pinene	941	7.2	7.3	6.9	7.4	6.1	4.9
3	Camphene	953	4.8	4.1	5	5.3	3.3	2.5
4	-Pinene	975	3.5	3.6	4.1	3.2	4.1	4.4
5	3-octanone	984	1.4	2	1.8	2.3	1.8	0.9
6	-Myrcene	988	2.6	1.9	1.7	2.1	2.4	3.1
7	p-cymene	1021	4.8	4.2	3.9	4.1	3.3	1
8	Limonene	1030	13.5	9.2	12.1	9.9	8.1	8.6
9	1,8-Cineole	1035	1.7	2.8	2.1	1	2.2	1.7
10	-Terpinolene	1080	5	6.1	4.6	5.3	4.5	4.9
11	Linalool	1094	2.3	3.2	2.6	2.6	1.8	2.5
12	Cyrsanthone	1120	1.1	1.1	1.8	1.1	1.2	1.7
13	Camphor	1136	8.8	9.9	8.1	7.9	17.7	18.5
14	Borneol	1170	10.4	10.2	11.2	12.6	13.9	14.6
15	Terpineol-4-ol	1179	2.6	2.3	1.9	2.8	1.3	1
16	Verbenone	1195	11.3	10.1	9.8	11.4	18.1	16
17	Borneolacetate	1281	2.5	2	3.1	2.5	2.4	1.7
18	Apigenin	1335	1	1.9	2.2	1.6	1	0.4
19	Geraniol acetate	1382	6.1	5.4	4.8	6.1	3.8	5.3
20	Trans-Caryophyllene	1425	1.2	1.9	2.5	1.7	2.2	1.3
21	Caryophyllene oxide	1583	3.5	3.1	2.7	3.3	3.1	1.6

RI (Retention Indices)

Acetyl CoA takes part in different aspects of metabolism including carbohydrates, lipids and terpenoids. Therefore, it seems that physiological changes induced by the application of acetyl CoA resulted from increased terpenoids especially GA. In microbodies, acetyl-CoA is generated during fatty acid b-oxidation. In the nucleus, acetyl-CoA is the substrate for the acetylation of proteins, such as histones and transcription factors, and regulates their function in maintaining or altering chromosome structure and/or gene transcription (Choi *et al.*, 2003; Sun and Spencer, 2003).

In the cytosol, acetyl-CoA is required for the biosynthesis of a plethora of phytochemicals, many of which are important for plant growth, development, and responses to environmental cues (Schmid and Doerner, 1990; Souter and Topping, 2002). Furthermore, acetyl CoA is a vital primary metabolite involved in different aspects of metabolism like respiration and lipid metabolism. Three molecules of acetyl CoA is required for producing of mevalonate which is converted into isopentenyl pyrophosphate, precursor of variety of isoprenoids and terpen derived compounds (Habibi and Heidari, 2011).

Thus acetyl CoA is implicated in metabolism of terpenoids and terpen-derived substances. In addition, acetyl CoA application, as a component of Krebs cycle, could change the status of ATP and reduce coenzymes in cells. GA-stimulated antioxidant enzymes result in decreased accumulation of active oxygen species and declined lipid peroxidation (Qing Zhu and Chao Han, 2011). Gibberellins could form sink via induction of especial physiological processes (Iqbal and Nazar, 2011). Additionally, acetyl-CoA, provides organisms with the chemical flexibility to biosynthesize a plethora of natural products that constitute much of the structural and functional diversity in nature. This is particularly exemplified in the plant kingdom where acetyl-CoA metabolism via carboxylation, condensation, or acetylation reactions is used for the production of many different classes of metabolites. By distributing this metabolism among separate cellular and sub-cellular compartments, plants have the potential of simplifying the regulatory processes that control this complex network (Miller and Bonner, 1954).

Ardalani *et al.* (2014) reported the effects of gibberellic acid and ethanol on flower yield and phenolic compositions of (*Callendula officinalis* L.) indicated that the level of gibberelin 500 ppm and 30% ethanol had the highest amount of phenolic compositions with the average of 0.92.

In the present work, the limonene, camphor, borneol and verbenone were the major components of *R. officinalis* oil. Tomei *et al.* (1995) investigated the essential oil from flowers and leaves of *R. officinalis* (collected from the wild in southern Spain) and found the main components to be camphor (32.33%), 1, 8-cineole (14.41%) and -pinene (11.56%). Studies reported by Viuda-Martos and Yolanda (2007) showed that the major components of *R. officinalis* leaves oil from Spain were -pinene (36.42%), camphor (15.65%), 1,8-cineole (12.02%) and camphene (11.08%). Angioni and Barra (2004) reported that the major components were -pinene, borneol, camphene, camphor, verbenone and bornyl-acetate, present in Sardinian *R. officinalis* L. oil. Studies reported by Tavassoli *et al.* (2011) showed that the major components of *R. officinalis* oil were 1,8-Cineole (23.14%), camphor (12.35%), -pinene (9.87%), -pinene (6.10%), borneol (5.61%), camphene (5.58%) and -terpineol (4.30%). In a comparative study, Chalchat and Carry (1993) investigated the comparison of Spain, Morocco and France rosemary oils. They concluded that, the main constituents of the essential oil from Spain were: -pinene (24.7%), 1,8-cineole (18.9%), camphor (18.9%), camphene (11.2%), myrcene (4.9%) and borneol (4.5%); that from Morocco were: 1,8-cineole (47.44%), -pinene (12.51%), camphor (7.9 %), -pinene (7.2%), and camphene (3.62%); whereas that from France were: -pinene (35.80%), bornyl acetate (14.30), camphene (8.3%), 1,8-cineole (5.3%), borneol (4.44%) and camphor (3.00%). Since morphological characteristics

can vary under different agroclimatic conditions, interactions between genotype and environment (Salehi *et al.*, 2014; Golparvar *et al.*, 2015).

## CONCLUSION

In conclusion, the results obtained in our study indicated that the application of acetyl-CoA on first week, the highest limonene content (13.5%) obtained from control and the highest camphor content (10.3%) and borneol content (11.1%) obtained from foliar spraying acetyl-coA with concentrations (400 mM) and the highest verbenone content (11.6%) obtained from foliar spraying acetyl-coA with concentrations (50 mM). The results obtained on second week, the highest verbenone content (15.5%) and borneol content (11.7%) obtained from foliar spraying acetyl-coA with concentrations (400 mM) and the highest camphor content (15.1%) obtained from foliar spraying acetyl-coA with concentrations (200 mM) compared with the control. The results obtained on threeweek the highest camphor content (18.5%) and borneol content (14.6%) obtained from foliar spraying acetyl-coA with concentrations (400 mM) and the highest verbenone content (18.1%) obtained from foliar spraying acetyl-coA with concentrations (200 mM) compared with the control.

## REFERENCES

- Adams RP. (2007). Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th edition (Allured Publishing Corporation, Carol Stream, IL) 456.
- Angioni A, Barra A. (2004). Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential of *Rosmarinus officinalis* L. *J. Agric. Food Chem*, **52**, 3530-3535.
- Ardalani HR, Hadipanah A, Pourrezvani SM. (2014). Foliar application gibberellic acid and ethanol on flower yield and phenolic compositions in marigold (*Callendula officinalis* L.). *Electronic Journal of Biology*, **10**(3), 73-78.
- Atti-Santos AC, Rossato M, Pauletti GF. (2005). Physicochemical evaluation of *Rosmarinus officinalis* L. essential oils, *Braz. Arch. Biol. Technol*, **48**, 1035-1039.
- Chalchat JC, Carry RP. (1993). Essential oils of Rosemary, the chemical composition between hydrodistillation and supercritical CO extraction. *J. Essent. Oil. Res*, **41**, 613.
- Choi CH, Hiromura M, Usheva A. (2003). Transcription factor IIB acetylates itself to regulate transcription. *Nature*, **424**, 965-969.
- Falk KL, Vogel C, Textor S. (2004). Glucosinolate biosynthesis: Demonstration and characterization of the condensing enzyme of the chain elongation cycle in *Eruca sativa*. *Phytochemistry*, **65**, 1073-1084.
- Golparvar AR, Hadipanah A, Mehrabi AM. (2015). Diversity in chemical composition from two ecotypes of *Mentha longifolia* L. and *Mentha spicata* L. (in Iran climatic conditions). *Journal of Biodiversity and Environmental Sciences*, **6**(4), 26-33.

- Habibi A, Heidari G. (2011). Influence of bio, organic and chemical fertilizers on medicinal pumpkin traits'. *J Med Plants Res*, **5**(23), 5590-5597.
- Iqbal N, Nazar R. (2011). Role of gibberellins in regulation of source-sink relations under optimal and limiting environmental conditions. *Curr. Sci*, **100** (7), 998-1007.
- Jamshidi R, Afzali Z, Afzali D. (2009). Chemical composition of hydrodistillation essential oil of Rosemary indifferent origins in Iran and comparison with other countries. *American-Eurasian J. Agric. & Environ. Sci.*, **5**(1), 78-81.
- Mahomoud AA, Al-Shihry SS, Son BW. (2005). Diterpenoid quinines from Rosemary (*Rosmarinus officinalis* L.). *J. Phytochemistry*, **66**, 1685-1690.
- Millerd A, Bonner J. (1954). Acetate activation and acetoacetate formation in plant systems. *Arch. Biochem. Biophys*, **49**, 343-355.
- Moreno S, Scheyer T, Romano CS, Vojnov AA. (2006). Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radical Res*, **40**, 223-231.
- Nikolau BJ, Ohlrogge JB, Wurtele ES. (2003). Plant biotin containing carboxylases. *Arch. Biochem. Biophys*, **414**, 211-222.
- Qing Zhu L, Chao Han L. (2011). Gibberellin A3 pretreatment increased antioxidative capacity of cucumber radicals and hypocotyls under suboptimal temperature. *Afr. J Agric Res*, **6**(17), 4091-4098.
- Salehi S, Golparvar AR, Hadipanah A. (2014). Effect of harvest time on yield and quality of *Thymus vulgaris* L. essential oil in Isfahan province, Iran. *Agriculturae Conspectus Scientificus*, **79**(2), 115-118.
- Santoyo S, Cavero S, Jaime L. (2005). Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. essential oil obtained via supercritical fluid extraction. *J. Food Protect*, **68**(4), 790-795.
- Schmid J, Doerner PW. (1990). Developmental and environmental regulation of a bean chalcone synthase promoter in transgenic tobacco. *Plant Cell*, **2**, 619-631.
- Souter M, Topping J. (2002). Hydra mutants of Arabidopsis are defective in sterol profiles and auxin and ethylene signaling. *Plant Cell*, **14**, 1017-1031.
- Sun JM, Spencer VA. (2003). Measurement of histone acetyltransferase and histone deacetylase activities and kinetics of histone acetylation. *Methods*, **31**, 12-23.
- Tavassoli S, Mousavi SM, Djomeh Z, Razavi SH. (2011). Comparative Study of the Antimicrobial Activity of *Rosmarinus officinalis* L. Essential Oil and Methanolic Extract. *Middle-East Journal of Scientific Research*, **9**(4), 467-471.
- Tomei PE, Cioni PL, Flamini G, Stefani A. (1995). Evaluation of the chemical composition of the essential oils of some Lamiaceae from Serrania de Ronda (Andalucia, Spain). *J. Essent. Oil Res*, **7**(3), 279-282.
- Verma RS, Rahman LU. (2011). Changes in essential oil content and composition of leaf and leaf powder of *Rosmarinus officinalis* cv. CIM-Hariyali during storage. *Maejo Int. J. Sci. Technol.*, **5**(2), 181-190.
- Viuda-Martos M, Yolanda R. (2007). Chemical Composition of the Essential Oils Obtained From Some Spices Widely Used in Mediterranean Region. *Acta Chim. Slov*, **54**, 921-926.