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NaCl salinity and Zn foliar application influence essential oil composition of basil (Ocimum basilicum L.)

Mohammad Bagher HASSANPOURAGHDAM[1*], Gholam Reza GOHARI[2], Seied Jalal TABATABAEI[2], Mohammad Reza DADPOUR[2], Mehdi SHIRDEL[2]

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ABSTRACT

Essential oils composition of hydroponically grown *Ocimum basilicum* L. plant was evaluated in response to salinity (control and 50 mM NaCl) and Zn foliar application (control, 100 and 200 mg l^{-1}). Essential oil constituents were quantified and identified by GC/EI-MS. In total, fifty seven components were identified in the six treatment combinations. Methyl chavicol (43.9–61.2 %) and linalool (11.4-16%) were the major components of all treatments. Salinity had deteriorative effect on methyl chavicol biosynthesis and accumulation. In contrast, integrated levels of salinity and 200 mgl^{-1} Zn had increment effects on linalool content. Germacrene D (2.2-3.9 %), 1,8-cineole (2.4-3.8 %), (Z)-α-bergamotene (0.1-2.6 %), (E)-β-farnesene (1.4-2.6 %), α-bulnesene (0.9-2.4 %), camphor (0.7-1.3 %) and (E)-β-ocimene (0.2-1.3 %) were the other main common constituents of oil. Considering the constant levels of zinc foliar application, salinity had raising effects on the contents of most above mentioned constituents. In conclusion, it seems that moderate salinity stress along with balanced levels of Zn foliar application changed the primary metabolites pathways in favor of major volatile oil components biosynthesis and that basil plant has the production potential under prevalent semi-saline conditions.

Key words: *Ocimum basilicum* L., essential oil, GC/MS, salinity, Zn foliar application, methyl chavicol, linalool

IZVLEČEK

KONCENTRACIJA NaCl V HIDROPONSKI RAZTOPINI IN FOLIARNI NANOS RAZTOPINE CINKA VPLIVAJO NA SESTAVO ETERIČNIH OLJ PRI BAZILIKI (*Ocimum basilicum* L.)

Raziskana je bila sestava eteričnih olj hidroponsko gojene bazilike (*Ocimum basilicum* L.), glede na vpliv slanosti (kontrola in 50 mM NaCl) ter foliarnega nanašanja cinka (Zn) (kontrola ter 100 oziroma 200 mg l^{-1}). Sestava eteričnih olj je bila ugotovljena z GC/EI-MS tehniko. Pri šestih kombinacija tretiranja je bilo ugotovljenih 57 sestavin. Metil kavikol (43,9–61,2 %) in linalool (11,4-16%) sta bili glavni sestavini pri vseh tretiranjih. Sol je negativno vplivala na sintezo in akumulacijo kavikola. Nasprotno, skupen vpliv solne raztopine in višje koncentracije cinka je povzročil povečanje vsebnosti linaloola. Germakren D (2,2-3,9 %), 1,8-kineol (2,4-3,8 %), (Z)-α-bergamoten (0,1-2,6 %), (E)-β-farnesen (1,4-2,6 %), α-bulnesen (0,9-2,4 %), kafra (0,7-1,3 %) in (E)-β-ocimen (0,2-1,3 %) so tudi bile glavne sestavine olja. Pri nespremenjeni koncentraciji cinka pri foliarni aplikaciji je sol vplivala na povečano koncetracijo omenjenih metabolitov. Zmerna slanost skupaj s foliarnim tretiranjem z raztopino cinka vpliva na spremenjeno sestavo metabolitov s tem da poveča biosintezo hlapnih sestavin olj; tako je ugotovljena prednost pridelovanja bazilike v razmerah zmerne slanosti.

Ključne besede: *Ocimum basilicum* L., bazilika, eterična olja, GC/MS, sol, Zn foliarno nanašanje, metil kavikol, linalool

[1] Department of Horticultural Sciences, Faculty of Agriculture, University of Maragheh, Maragheh 55181-83111, Iran

[2] Department of Horticultural Sciences, Faculty of Agriculture, University of Tabriz, Tabriz 51666, Iran

[*] Corresponding author: hassanpouraghdam@gmail.com , phone: +989144038472.

1 INTRODUCTION

The increasing importance of essential oils in various domains of human activities including pharmacy, perfumery, cosmetics, aromatherapy, food and drinks industry has opened a new era for exploitation of different intrinsic and extrinsic factors influencing their biosynthesis, accumulation and biological properties (Sonwa, 2000). Essential oils are very complex mixture of volatile compounds and their chemical composition and concentrations of individual components are strongly influenced by several integrated factors such as genetical and biochemical criteria (subspecies, natural hybridization and chemovariety) climatological and geographical conditions (light quality and quantity, soil characteristics, water availability and temperature), growing conditions (wild habitats, greenhouse production and different soilless culture systems) as well as agronomic parameters (fertilization, salinity level, macro and micronutrients availability and irrigation regime) (Hassanpouraghdam *et al*., 2009 & 2010).

High saline sodic condition has become a major restrain adversely affects plants physiological processes and limits field and horticultural crops performance particularly in arid and semi-arid regions of the world. High salt occurrence in the soil solution is linked to create the high osmotic pressure in the rhizosphere and ultimately reduced availability of water and nutrients to plant. Salinity conditions are known to affect plants physiological and biochemical potential, which in turn affect crops primary and secondary metabolism (Hebbara *et al*., 2003; Hendawy and Khalid, 2005).

Micronutrients greatly influence plants growth and development (Grejtovsky *et al.*, 2006; El-Tohamy *et al*., 2009; Akhtar *et al*., 2009). Among micronutrients, zinc is an essential one that plays role either as metal component of various enzymes or as a functional, structural or regulatory cofactor and is thus linked with photosynthesis and carbohydrate metabolism (Farahat *et al*., 2007). Zn has critical action on activity of carbon metabolism enzymes such as carbonic anhydrase, ribulose 1,5-bisphosphate carboxylase/oxygenase and fructose 1,6-bisphosphate (Misra *et al*., 2005). Misra *et al*. (2005) reported that essential oil biosynthesis in geranium was strongly influenced by Zn acquisition or deficiency. Overall, Zn is involved in carbon assimilation, saccharids accumulation, reactive oxygen radicals scavenging and finally carbon utilization in volatile oil biosynthesis (Misra *et al*., 2005; Nahed and Balbaa, 2007). Furthermore, Zn stimulate IAA production, starch formation and chlorophyll biosynthesis (Nahed and Balbaa, 2007; El-Wahab and Mohamad., 2008) and is necessary for DNA, RNA and protein synthesis (Nahed and Balbaa, 2007; El-Wahab and Mohamad., 2008).

Common basil (*Ocimum basilicum* L.) is a cosmopolitan herb and aromatic plant commonly growing in all parts of the world. Basil is a multipurpose plant with great applications in pharmaceutical, food and fragrance industries. Medicinally, this plant and its essential oil have long been used to treat nausea, dysentery, mental fatigue colds and rhinitis. The main volatile components of basil oil have been characterized as terpenoids and phenylpropane derivatives (Hassanpouraghdam *et al*., 2009 & 2010). In a series of studies conducted on basil we identified menthone (33.1 %) / estragol (21.5 %) and methyl chavicol (37.2-56.7 %) / linalool (13.1-21.1 %) as major essential oil components of field and hydroponically grown basil plants from Iran respectively (Hassanpouraghdam *et al*., 2009 & 2010).
The present experiment was conducted under the auspices of our previous works on the hydroponic management of high-value added crops production in order to finding an alternative production system for field grown *O. basilicum* plants encountered with progressive salinity conditions. Therefore, an attempt was made to evaluate the effects of salinity conditions and zinc foliar application on the essential oil composition of hydroponically grown *O. basilicum* L.

2 MATERIAL AND METHODS

This experiment was carried out at the Research Greenhouse of Department of Horticultural Sciences, University of Tabriz, Iran during spring-summer of 2009.

2.1 Plant material, growing conditions and treatments

Seeds of a native *O. basilicum* L. plants were directly sowed in 5L pots filled with medium sized perlite. During germination period and two first weeks of plantlets growth, they were irrigated with tap water. The quarter–strength modified Hoagland's nutrient solution was used for regular irrigation of plants for upcoming two weeks. Thenafter treatments were imposed. Treatments were factorial combinations of salinity [control (without salinity) and 50 mM NaCl] and Zn foliar applications [control (sprayed with distilled water) and 100 and 200 mg l^{-1} $ZnSO_4$, $7H_2O$] as randomized complete block design with 3 replications. For salinity study, 50 mM NaCl added to the half–strength solution was employed for the nourishment of related treatments.

$ZnSO_4$, $7H_2O$ solution was twice sprayed on plants. Once during early growth stage *i.e.* one month after sowing date followed by second application about 2 weeks later. The plants leaves were thoroughly wetted until solution drops.

pH and EC of Hoagland's nutrient solutions were adjusted at 6–6.5 and 2 dSm^{-1} by H_2SO_4 or KOH and water respectively. The experiment was carried out in an one–layer polyethylene covered greenhouse under natural sunlight. Temperature, humidity and PAR of greenhouse were 15-30 ºC, 40-50 % and 500 $\mu Molm^{-2}s^{-1}$ during growing period respectively. No pesticide or herbicide was applied on the plants. The aerial parts of plants were harvested at the full flowering stage, dried at room temperature and subjected to essential oil extraction.

2.2 Volatile oil extraction

Fifty grams of air dried powdered plant materials (aerial parts) were extracted by the hydrodistillation method during 3 hours in an all–glass Clevenger type apparatus. The extracted essential oils were dried over anhydrous Na_2SO_4 and stored in sealed glass vials, covered with aluminum foil to protect the contents from photo-conversion and kept under refrigeration until analysis. Pooled essential oil samples from three replicates were evaluated for its components by GC/EI-MS.

2.3 Instrumentation

A GC/MS instrument (Agilent 6890N GC and Agilent 5973 mass selective detector operating in the EI mode, USA) was employed for the compositional analysis of volatile oil. Ultra pure helium (99.99%, Air Products, UK) passed through a molecular sieve trap and oxygen trap (Chromatography Research Supplies, USA) was used as the carrier gas at a constant velocity of 1 ml/min. The injection port was held at 300ºC and used in the split mode; Split ratio 1:100, Volume injected: 5µl of the pure volatile oil. Detector temperature was 200ºC. Separation was carried out on an apolar HP5MS (5%-phenyl methyl poly siloxane; 30m × 0.25 mm i.d. and 0.25µm film thickness) capillary column (Hewllet-Packard, USA). The oven temperature was programmed as follows: 50ºC (held 2 min) raised to 110ºC at a rate of 10ºC/min, then heated to 200ºC at the 10ºC/min rate and finally increased to 280ºC at 20ºC/min, isothermal at the temperature for 2 min. The mass operating parameters were as follows: ionization potential: 70eV, interface temperature: 200ºC and acquisition mass range: 50-800.

2.4 Identification and quantification of volatile oil components

Relative percentage amounts of the volatile oil constituents were evaluated from the total peak area (TIC) by apparatus software. The components of the essential oil were identified by comparing their mass spectral fragmentation patterns with those of similar compounds from the database (NIST and WILEY library) as well as by comparing their Kovats gas chromatographic retention indices with those of the literature.

3 RESULTS AND DISSCUSION

The results obtained from the essential oil compositional analysis of *Ocimum basilicum* L. plants subjected to salinity and Zn foliar applications are presented in table 1. Salinity and Zn application combinations had appreciable quantitative but slight qualitative effects on essential oil constituents of basil. In total, fifty seven components were identified in the essential oil of six treatment combinations. Methyl chavicol – a phenylpropane derivative– was the most abundant components of all treatments with $NaCl_0$ Zn_{100} (61.2 %) and $NaCl_{50}$ Zn_{200} (43.9 %) treatment combinations had the highest and the least amount for this component respectively. It seems that salinity had negative effects on the biosynthesis and accumulation of this high–valued compound. Meanwhile, increasing Zn levels from 100 to 200 mg l^{-1} had no influential potential to compensate the deteriorative effects of salinity depression on this compound. Linalool – a fragrant oxygenated monoterpene – was the second major volatile oil component which $NaCl_{50}$ Zn_{200} (16 %) had the greatest amount for this compound. $NaCl_{50}$ Zn_0 (12 %) had the lowest sum for linalool. It is likely that salinity had promotive effects on this compound and the increasing levels of Zn foliar application had raising influence as well. Coolong *et al*. (2004) suggested that Zn fertility can influence changes in glucosinolates (GS) that may affect related plants flavor or medicinal attributes. In their study Zn linearly decreased gluconapin and sinalbin content of *Brassica rapa* plants. Gerjtovsky *et al*. (2006) reported that soil based Zn application only slightly affected the essential oil constituents, *i.e*. chamazulene and (E)-β-farnesene content of chamomile. Furthermore, an increased supply of Zn did not affect the content of flavone apigenin and coumarin herniarin in aforementioned study. Regarding the main components of essential oil it seems that there is some discrepancy and/or similarity between present study and reports of other scientists from elsewhere (cited in Hassanpouraghdam *et al*., 2009 & 2010). Germacrene D (2.2-3.9 %), 1,8-cineol (2.4-3.8 %), (Z)-α-bergamotene (0.1-2.6 %), (E)-β-farnesene (1.4–2.6%), α-bulnesene (0.9–2.4%), (E)-caryophyllene (1–2.1%), bicyclogermacrene (0.8–1.5 %), camphor (0.7–1.3 %) and (E)-β-ocimene (0.2–1.3 %) were the other common components of treatment combinations with amounts greater than one percent. Taking into account the constant levels of Zn foliar application, salinity had the elevating effects on the contents of 1,8-cineol, (Z)-α-bergamotene, (E)-caryophyllene, (E)-β-farnesene, germacrene D, bicyclogermacrene and α-bulnesene. It seems that salinity stress resulted growth reduction along with appropriate amounts of nutrient elements especially micronutrients changes the primary photosynthetic metabolic pool (sugars, amino acids and organic acid) in favor of secondary metabolites biosynthesis and accumulation. Like our results

Table1. Effects of salinity and zinc foliar application on essential oil composition of hydroponically grown *Ocimum basilicum* L.

No.	Compound	RI	%					
			$NaCl_oZn_o$	$NaCl_oZn_{100}$	$NaCl_oZn_{200}$	$NaCl_{50}Zn_0$	$NaCl_{50}Zn_{100}$	$NaCl_{50}Zn_{200}$
1	α-Pinene	0939	0.2	0.1	0.1	-	0.1	0.2
2	Camphene	0954	0.1	0.1	0.1	0.1	-	
3	Sabinene	0975	0.2	0.1	0.1	0.1	0.1	0.1
4	β-Pinene	0979	0.6	0.5	0.4	0.7	0.5	0.6
5	Myrcene	0991	0.7	0.4	0.4	0.5	0.3	0.5
6	α-Terpinene	1017	0.1	0.1	tr	-	-	0.1
7	(P)-Cymene	1025	-	-	tr	-	-	-
8	Limonene	1029	-	0.2	0.3	-	-	0.2
9	β –Phellandrene	1030	-	-	0.1	-	-	-
10	**1,8-Cineole**	**1031**	**3.5**	**2.4**	**2.3**	**3.8**	**3.1**	**2.8**
11	(Z)-β-Ocimene	1037	0.1	0.1	0.1	-	0.1	**1.5**
12	(E)-β-Ocimene	1050	**1.2**	**1.2**	**1**	**1.1**	**1.3**	0.2
13	γ-Terpinene	1060	0.1	0.1	0.1	0.1	0.1	0.1
14	(Z)-Sabinene hydrate	1070	0.1	-	-	-	-	-
15	Fenchone	1087	0.8	0.6	0.8	0.6	0.6	0.4
16	Terpinolene	1089	-	-	-	tr	tr	-
17	**Linalool**	**1097**	**13.1**	**13**	**15.4**	**11.4**	**12**	**16**
18	Camphor	1146	**1.1**	**1**	**1.3**	**1**	0.7	0.8
19	Borneol	1169	0.2	0.1	0.2	0.5	0.2	0.2
20	Terpinene-4-ol	1177	0.1	0.1	0.1	0.1	0.1	0.1
21	α-Terpineol	1189	-	0.3	-	-	-	0.3
22	**Methyl chavicol**	**1196**	**54.7**	**61.2**	**58.4**	**57.5**	**51.5**	**43.9**
23	Geraniol	1253	-	0.1	0.1	-	tr	-
24	α-Cubebene	1351	0.8	0.5	0.2	0.6	0.2	0.9
25	α-Longipinene	1353	-	-	-	-	0.2	-
26	Eugenol	1359	**1**	-	0.1	-	0.1	0.1
27	α-Ylangene	1375	-	-	-	0.1	-	-
28	α-Copaene	1377	0.2	0.2	0.6	0.4	0.4	0.4
29	β-Bourbonene	1388	0.1	0.1	0.1	0.1	0.2	0.2
30	β-Cubebene	1388	0.5	0.4	0.4	-	0.6	0.2
31	β-Elemene	1391	0.7	0.6	0.7	0.9	**1.1**	**1.8**
32	Methyl eugenol	1404	0.6	0.2	0.4	0.3	0.8	**1.5**
33	α-Cedrene	1412	-	0.1	-	-	-	0.2
34	**α-(Z)-ergamotene**	**1413**	**2.6**	**0.1**	**1.8**	**1.8**	**-**	**2.6**
35	**(E)-Caryophyllene**	**1419**	**1**	**1.3**	**1.3**	**1.3**	**1.1**	**2.1**
36	α-Guaiene	1440	0.4	0.5	0.5	0.7	0.8	**1.2**
37	Aromadendrene	1441	0.1	-	0.1	0.2	-	0.2
38	**(E)-β-Farnesene**	**1457**	**1.6**	**2.4**	**1.4**	**2**	**2.8**	**2.6**
39	**Germacrene D**	**1485**	**2.7**	**2.2**	**2.5**	**3.3**	**3.9**	**3.9**
40	**α-Amorphene**	**1485**	**-**	**-**	**1.6**	**1.7**	**-**	**-**
41	α-Zingiberene	1494	-	**1.9**	0.1	0.1	**2.2**	-
42	Bicyclogermacrene	1500	**1.3**	**1**	0.8	**1.1**	**1.3**	**1.5**
43	(E)-α-Farnesene	1506	-	-	-	0.4	-	-
44	α-Bulnesene	1510	**1.1**	0.9	**1.2**	**1.5**	**1.9**	**2.4**
45	γ-Cadinene	1514	**2**	**1.4**	-	0.1	**2.6**	**2.4**
46	Δ-Cadinene	1523	0.1	0.4	**2.2**	**2.8**	0.1	0.6
47	(Z)-Nerolidol	1533	0.1	-	0.1	0.1	0.1	0.2
48	α-Cadinene	1539	0.1	-	tr	0.1	0.1	-
49	(Z)-Calamenene	1540	0.4	-	0.3	-	0.5	-
50	Spathulenol	1578	-	-	-	0.3	0.3	0.5
51	Caryophyllene oxide	1583	0.1	0.1	0.1	0.2	0.3	0.4
52	Alloaromadendrene	1641	0.1	-	-	-	0.3	-
53	β-Eudesmol	1651	0.1	0.1	0.1	0.1	0.2	-
54	**α-Cadinol**	**1654**	**3**	**2**	**-**	**-**	**3.7**	**4.1**
55	α-Bisabolol	1686	0.1	-	0.1	0.1	0.2	-
56	β-Sinensal	1700	-	-	-	0.1	0.1	-
57	Phytol	1943	0.1	-	tr	0.1	0.1	-
	Total		97.8	98.1	98	98	96.9	98

Compounds are reported according to their elution order on non-polar column

Srivastava *et al.* (1997) noted that increased sugar content of leaves under Zn stress conditions positively influenced essential oil accumulation of peppermint plants. (Z)-β-Ocimene (1.5 %), β-elemene (1.8 %), methyl eugenol (1.5 %) and α-guaiene (1.2 %) were the constituents with their maximum amounts possessed by $NaCl_{50}$ Zn_{200}. Seemingly, moderate levels of salinity combined with raising levels of Zn foliar application had synergistic effects on production of some compounds. Misra and Sharma (1991) mentioned that Zn application stimulated the menthol concentration in Japanese mint. Furthermore, Chand *et al.* (2007) reported that integrated supply of vermicompost and zinc–enriched compost improved the percentage amount of geranium oil components such as cis-rose oxide, isomenthone and linalool. α-amorphone (1.6–1.7 %) and Δ-cadinene (0.1–2.8 %) were the other components with a scatter distribution between treatment combinations. α-Zingiberene (0.1–2.2 %) was a constituent with its greatest amounts under 100 mgl^{-1} Zn foliar application and only with minor impacts of salinity level. Srivastava *et al.* (2006) in their work on turmeric stated that there is a strong relationship between primary metabolic pathways and biosynthesis/accumulation of secondary metabolites. Those authors declared that proper correlation of carbon assimilation pathways and accumulation of secondary metabolic compound needs association of several intrinsic and extrinsic factors particularly optimum levels of micronutrients. α-Cadinol (2–4.1 %) and γ-cadinene (0.1–2.6 %) were two major sesquiterpene hydrocarbon constituents of studied oils with their maximum quantites belonged to $NaCl_{50}$ Zn_{200}. This means that combined controlled application of salinity and Zn had complement effects on major fifteen carbons sesquiterpenoidal components of essential oil. Similar to our results Hendawy and Khalid (2005) reported that zinc application under salinity levels had diverse increasing and/or decreasing effects on the volatile components of sage plants.

In general it appears that moderate salinity levels had promising effects on volatile oil profile of basil, and integrated foliar application of zinc ameliorated the presumable unhealthy effects of salinity in support of major volatile oil components. In summary, it seems that regarding volatile oil constituents, basil plant has the production potential under semi saline conditions. This production needs optimum growing conditions and balanced nutrient availability especially appropriate levels of micronutrients. However this claim needs detailed studies with other nutrient elements.

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