

EFFECTS OF MANGANESE AND COPPER ON ESSENTIAL OIL COMPOSITION OF LEMON BALM (*MELISSA OFFICINALIS* L.)

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Abstract

In this investigation the effects of foliar application Mn and Cu on essential oil composition of lemon balm were studied in two growth periods during 2012-2013. The essential oil obtained by hydro distillation and analyzed by GC and GC/MS showed that the main compounds in two cultivation seasons were geranial, neral and caryophyllene oxide. The highest amount of neral and geranial obtained from 150 ppm Cu foliar application in two cultivation seasons. Caryophyllene oxide content was highest at 150 ppm Cu × 300 ppm Mn foliar application.

Lemon balm (*Melissa officinalis* L.), a perennial medicinal herb belonging to *Lamiaceae* family, is native to south-central Europe, North Africa, the Mediterranean region and Central Asia. Pharmacological studies revealed that the most commonly known therapeutic properties of lemon balm extracts are carminative, sedative, anti-bacterial, antispasmodic, antiviral, anti-inflammatory, neuro-protective and antioxidant. The main constitute of balm oil were citronellal, geranial, neral, geranyl acetate, β-caryophyllene and Caryophyllene oxide (Bagdat and Cosge 2006, Manukyan 2011). The best defined function of manganese is in the photosynthetic reaction through which oxygen is produced from water (Marschner 1995). Copper is associated with enzymes involved in redox reactions being reversibly oxidized from Cu⁺ to Cu²⁺. Foliar application of mineral nutrients such as iron, manganese, and copper may be more efficient than application through the soil, where they are adsorbed on soil particles and hence are less available to the root system (Khalifa *et al.* 2009, Torun *et al.* 2001). The aim of this investigation was to determine the effect foliar application of Manganese and Boron on essential oil composition of lemon balm.

This experiment was carried out in Shahrekord (southwest of Iran) at an altitude 2202 m above sea level during the 2012 and 2013 growing seasons. The soil characteristics listed in Table 1. The Experiments design was a completely randomized blocks consisting of three replications and nine treatments (0, 150 ppm Mn, 300 ppm Mn, 150 ppm Cu, 300 ppm Cu, 150 ppm Cu×150 ppm Mn, 150 ppm Cu×300 ppm Mn, 300 ppm Cu×150 ppm Mn and 300 ppm Cu×300 ppm Mn) for two cultivation seasons. Two foliar fertilizers Librel Mn and Unibor were applied and all of them are mineral fertilizers. Librel Mn inclusive of 13% Mn chelated with EDTA and Unibor contains 15% B (obtained from The Chemical Company of England and Germany). These fertilizers were sprayed at three concentrations (Mn₁, Mn₂, Mn₃ were 0, 150 and 300 ppm of Mn, respectively and similar in B). The 50g air dried samples were subjected to water distillation for 3 h using a Clevenger type apparatus. The oil obtained was separated from water and dried over anhydrous sodium sulfate and stored at 4°C until the analysis. GC analysis was done on an Agilent Technologies 7890 GC equipped with FID and a HP-5MS 5% capillary column (30.00 m × 0.25 mm, 0.25 m film thicknesses) (Adams 2001).

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Table 2 lists the identified compounds in the essential oil obtained by hydro distillation of lemon balm. In both cultivation years 32 constituents were identified. The highest components of essential oils in two cultivation seasons were neral, geranial, caryophyllene oxide, E-caryophyllene and geranyl acetate. The highest amount of neral content obtained from 150 ppm Cu treatment in the second year (30.33%), whereas the lowest value of neral in all treatment observed in 300 ppm Mn treatment. Geranial is one of the principal components of lemon balm essential oil. Geranial ranged from 14.18 to 32.49% in different cultivation seasons and treatments (Table 2). 150 ppm Cu treatment in the first cultivation season recorded higher geranial than the control and other treatments. In two cultivation seasons, 150 ppm Cu application recorded lower geranial content in comparison to other treatments. Caryophyllene oxide ranged from 6.37 to 12.62 % in different seasons and treatments. In the first season cultivation Caryophyllene oxide declined significantly with the application of 150 ppm Cu × 150 ppm Mn than other treatments. 150 ppm Cu×300 ppm Mn registered the highest caryophyllene oxide content in the second years. E-caryophyllene and geranyl acetate concentrations were affected by Cu and Mn applications and therefore increased in various range compared with the control. The roles of micronutrients are crucial in crop nutrition and are important for achieving high yields. Six micronutrients including Mn, Fe, Cu, Zn, B and Mo are known to be required for all higher plants. The bioavailability of plant nutrients is strongly tied to the chemical property of soils (Marschner 1995). Foliar spray of different micronutrients has been reported to be equally or more effective as soil application by different researchers (Torun *et al.* 2001). Suggested that foliar spray could be used effectively to overcome the problem of micronutrients deficiency in subsoil.

Table 1. Some physico-chemical properties of experiment soil (0 -30 cm).

Year	Texture	E.C (ds/m)	N _{total} %	O.M	pH	mg/kg					
						K	P	Zn	Mn	Fe	Cu
2012	Loam	8.1	0.11	0.34	8.7	770	45	0.57	1.2	4.1	1.3
2013	Loam	7.8	0.11	0.34	8.5	745	44	0.45	1.1	3.1	1.1

Samia and Mahmoud (2009) on *Tritonia crocata*, Nasiri *et al.* (2010) on Chamomile, Khalifa *et al.* (2009) on *Iris*, Heidari *et al.* (2011) on sesame, Yarnia *et al.* (2012) on *Purple coneflower* and Younis *et al.* (2013) on *Rosa hybrida* reported an increase in essential oil content of plants as affected by spraying micronutrients. The main components of the oil were Caryophyllene oxide, E-Caryophyllene, Geranial, Geraniol, Chavicol and Neral for all treatments. Although Caryophyllene oxide, E-Caryophyllene, Geranial, Geraniol, Chavicol and Neral have been the major compositions of the essential oil in lemon balm, the quantity of these compounds has been significantly variable; the rate of neral and geranial were reported respectively as 15% and 14.5% by Hefendehl (1970), 19.6-36.1% and 25.3-47.5% by Tittel *et al.* (1982), 19.5% and 31.6% by Werker *et al.* (1985) and 30-40% and 50-60% by Ceylan *et al.* (1994). On the other hand, although the majority of literature shows neral and geranial as the main components of the essential oil of lemon balm, several studies presented citronellal, linalool and geranial as major chemical compositions (Bagdat and Cosge 2006). The main components of the essential oil were neral, geranial, geraniol, caryophyllene oxide, E-caryophyllene, Chavicol. This study provides some useful information about the efficacy of foliar application of micronutrients in soils with undesirable characteristics, and chemical properties in particular.

Table 2. Essential oil composition percentage of lemon balm during the 2012-2013 growing seasons.

Compound	RI	2012 (mg/g dry sample)						2013					
		Control	150 ppm Mn	300 ppm Mn	150 ppm Cu ^x 150 ppm Mn	150 ppm Cu ^x 300 ppm Mn	300 ppm Cu ^x 150 ppm Mn	150 ppm Cu ^x 150 ppm Mn	150 ppm Cu ^x 300 ppm Mn	300 ppm Cu ^x 150 ppm Mn	300 ppm Cu ^x 300 ppm Mn		
6-methyl-5-Hepten-2-ol	992	1.43	0.42	1.36	1.26	1.31	1.72	1.26	1.31	1.72	1.26		
Linalool	1097	0.45	0.44	0.49	0.47	0.56	0.25	0.47	0.56	0.25	0.48		
(Z-)Isocitral	1166	1.15	1.32	1.27	1.59	1.08	1.28	1.59	1.08	1.28	1.18		
Neral	1238	24.09	19.05	14.14	14.57	17.46	24.61	14.57	17.46	24.61	25.59		
Piperitone	1253	0.57	0.64	0.56	0.38	0.47	0.39	0.38	0.47	0.39	0.47		
Geraniol	1253	1.31	1.44	12.75	0.7	1.05	0.81	0.7	1.05	0.81	0.77		
Geranial	1267	28.90	20.81	18.58	32.36	27	19.19	32.36	27	19.19	30.82		
(E-) Caryophyllene	1419	7.54	7.17	7.36	6.37	7.38	6.09	6.37	7.38	6.09	7.35		
Caryophyllene oxide	1586	12.46	9.79	11.29	6.37	12.62	9.89	6.37	12.62	9.89	11.48		
(6-methyl-5-)Hepten-2-one	992	1	0.40	1.31	1.22	1.27	1.66	1.22	1.27	1.66	1.22		
Citronellal	1153	-	0.35	0.39	0.54	0.44	0.60	0.54	0.44	0.60	0.48		
(Z-)Isocitral	1166	1	1	1.25	1.71	1.16	1.38	1.71	1.16	1.38	1.27		
Neral	1238	22	20	13.99	15.73	17.28	24.36	15.73	17.28	24.36	25.33		
Geraniol	1253	1.32	1.67	12.62	0.75	1.03	0.80	0.75	1.03	0.80	0.76		
Geranial	1267	29.18	14.18	18.39	22.08	29.43	20.91	22.08	29.43	20.91	29.59		
(E-)Caryophyllene	1419	7.61	8.34	7.28	6.87	8.04	6.63	6.87	8.04	6.63	8.01		
Caryophyllene oxide	1586	12.58	11.39	11.17	6.87	13.75	10.78	6.87	13.75	10.78	10.51		
Abieta-8(14),13(15)-diene	2033	2.61	2.03	2.16	-	1.73	1.46	-	1.73	1.46	2.22		

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