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Rosmarinic acid and anthocyanin content improvement by foliar application of Fe and Zn fertilizer in Lemon balm (*Melissa officinalis* L.)

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Abstract

Melissa officinalis L. is a well-known medicinal plant from the family of lamiaceae. The plant is high in flavonoid. Flavonoids of lemon balm such as rosmarinic acid and anthocyanin have an antiviral and antioxidant effect. Micronutrients are the element that is necessary for the plant in low concentration. In this experiment the effect of micronutrient (Fe and Zn) was examined on the anthocyanin and rosmarinic acid yield. Results of analysis of variance showed that micronutrients significantly change the rosmarinic acid and anthocyanin content and maximum rate is seen in [Zn(0)+Fe(1g/l) and Zn(1g/l)+Fe(2g/l)] respectively. It can be concluded that these micronutrients positively change the rosmarinic acid and anthocyanin content.

***Key words*:** *Melissa officinalis*, anthocyanin, micronutrient, rosmarinic acid

Introduction

A natural product from medicinal plants includes components such as saponin, alkaloids, tannins, cardiac glycosides and anthraquinones. And these components synthized as defense components in plants (Bokaeian et al. 2014). Lemon balm (*Melissa officinalis*) is well-known lemon smelling medicinal plant that (Sari.2005) act as sedative, anti-inflammatory, antinociceptive and antioxidant (Kennedy et al.2002). The essential oils of this plant are used in therapy by means of its antiviral effect in curing herpes and Alzheimer's diseases (Perry et al.1999). Main components of lemon balm which have an antioxidant activity are flavonoid, phenolic acids, terpenes, rosmarinic acid and caffeic acids (Ribiero et al.2001). Rosmarinic is a cinnamic acid diversities and it is an ester of caffeic acid and 3,4 dihydroxyphenillactic acid. (Che and Ho 1997), (. Petersen and Simmonds 2003). One of the antioxidant of lemon balm is anthocyanin. Anthocyanin is a polyphenolic pigments. Blue and red colors in many plant is based on this pigment (Koes and Quattrocchino.1994). It is obvious that the qualitative and quantitative variation of secondary metabolites are influent by internal and external factors (Farahani et al.2009). One of these external factors is micronutrients. Zinc is an important essential micronutrient for plants. This micronutrient plays an important role in plant normal growth and also plays an important role in many enzymes of biochemical reactions in plants (Fox and Guerimot.1998), (Salehi et al., 2014). Fe is also an important micronutrient that has an important role in photosynthesis, respiration and electron transport

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chain (Theil.1987). There is no information about the effect of these micronutrients on the production of rosmarinic acid and anthocyanin. Thus, the aim of this study was to investigate the effect of Fe and Zn on rosmarinic acid and anthocyanin content in *Melissa officinalis* L.

Material and Method

The study was conducted in 2012 at Khorasgan (Isfahan) Branch, Islamic Azad University's experimental green house, center region of Iran.

Seeds of *M. officinalis* were supplied from the Stamix Company, Isfahan, Iran 2013. The seed sterilization was carried out according to the following scheme:

Plant materials

a) Washing with running tap water for 3 times.

b) Submersion in ethanol 70% during 60 seconds.

c) Mixing with NaClO 20% + Tween 20 0.05% during 10 min.

d) Rinsing 3 times with sterile distilled water.

For germination, the disinfected seeds were sown on solid 1/2MS Murashige and Skoog (1962) medium supplemented with 3% sucrose and without growth regulators under *in vitro* condition. All culture were kept at 25 ± 2ºC, photoperiod of 16 hrs light, with an intensity of 2400 lux provided by fluorescent tubes.

Acclimatization

In vitro cultured plantlets with well-developed root and shoot system were transferred into pots containing peat moss and pearlite (3:1). After 14 days, the plantlets were transplanted to soil and acclimated in a controlled greenhouse condition at 24 ºC under 16/8 hour (light/dark) photoperiod and irrigated regularly.

According to the experimental design, the plants were sprayed with combination of 0, 1, 2 and 3 g/l of Fe and Zn at 30 days of transplanting.

Data recorded

Anthocyanin was determined spectrophotometrically from lemon balm's samples using a method previously conducted by Wanger (1979). Anthocyanin extraction was done by 0.05 g of lemon balm fresh leaves. Samples were diluted in 5ml acidic methanol (10% HCl (v/v). anthocyanin concentration was measured at 550 nm against a reagent blank. In order to rosmarinic acid extraction 0.2 g of dry leaves were added to 190 ml of alcohol (50 percent v/v) and boiled in a water-bath under a reflux condenser. 2 ml of hydrochloric acid, 2 ml of a solution prepared by dissolving 10 g of sodium nitrite and 10 g of sodium molybdate in 100 ml of water and 2 ml of dilute sodium hydroxide solution was added to 1.0 ml of the extract solution and then diluted to 10.0 ml with water. The absorbance of the rosmarinic acid was measured at 505 nm by comparison with the blank solution.

Statistical analysis

Experiment was conducted as a completely randomized factorial design with 3 replications. Data were analyzed using the SAS version 9.1 statistical computer program. When the ANOVA indicated significant treatment effects (5 or 1%) based on the F-test, the Duncan's multiple range test ($P<0.5$) was used as a Post Hoc method to determine which treatments were significantly different from other treatments.

Results

Effect of Mn and Zn and their interaction on the anthocyanin of lemon balm

The result of analysis the variance (Table 1) showed significant changes in the effect of different concentrations of Fe and Zn in anthocyanin content (P< 0.05).

Table1: Mean squares (MS) of ANOVA on the basis of CRD anthocyanin contents by SAS v 9.1.

source	df	Mean of square anthocyanin
Zn	3	692.038*
Fe	3	4763.311*
Zn*Fe	9	1173.285*

*: significant at P≤0.05, ns: not statistically significant

The maximum anthocyanin content is reached in [Zn(0)+Fe(1g/l)] and is 2.51 times higher than sample control. Also in combination of these elements the best result is seen in [Zn(1g/l)+Fe(1g/l)]. (Fig 1)

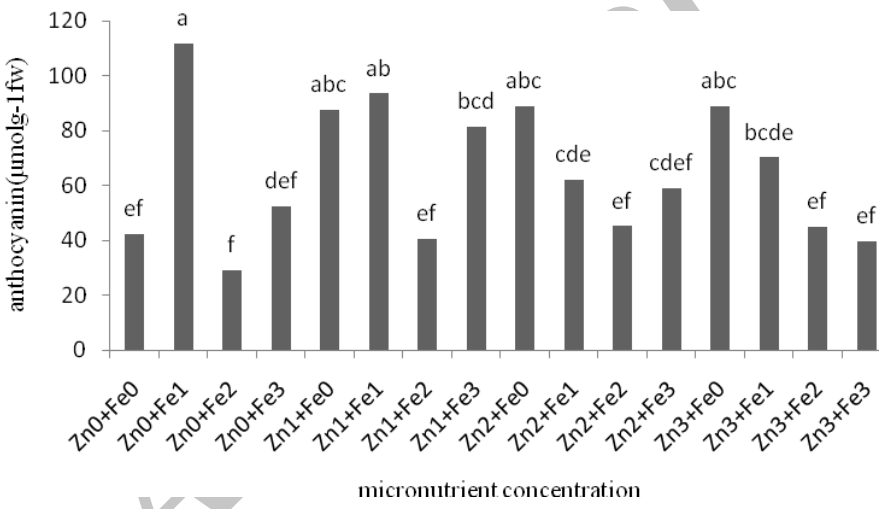

Figure 1: Effect of foliar application of micronutrients on anthocyanin

Effect of Mn and Zn and their interaction on the rosmarinic acid of lemon balm

The result of analysis the variance (Table 1) showed significant differences in the effect of different concentrations of Fe and Zn in rosmarinic acid content (P< 0.05).

Table2: Mean squares (MS) of ANOVA on the basis of CRD for rosmarinic acid contents by SAS v 9.1.

source	df	Mean of square Rosmarinic acid
Zn	3	0.011*
Fe	3	0.006*
Zn*Fe	9	0.007*

*: significant at P≤0.05, ns: not statistically significant

As can be shown in Fig. 2, the best result in rosmarinic acid content is observed in [Zn (1g/l) +Fe (2g/l)].

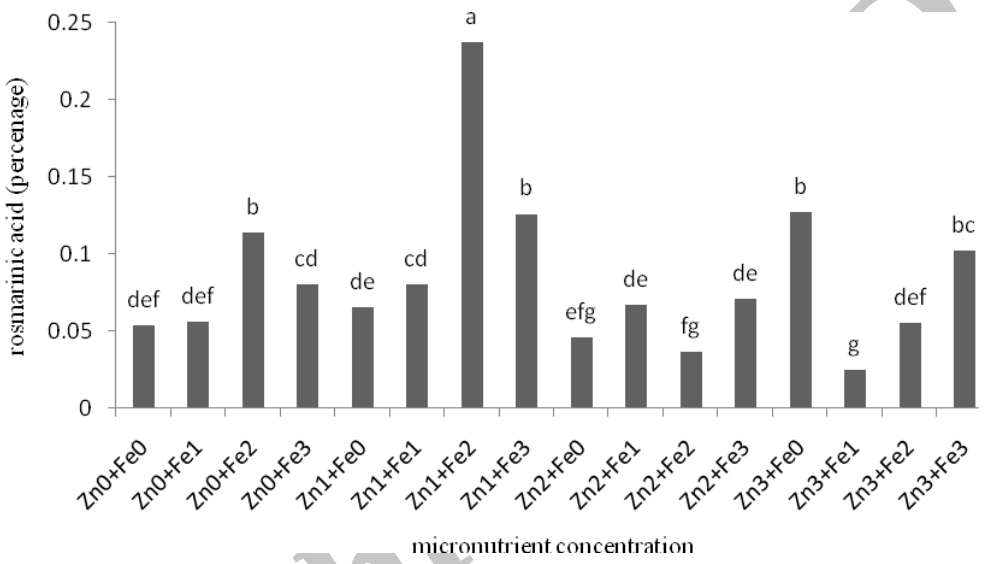

Figure 2: Effect of foliar application of micronutrients on rosmarinic acid

Discussion

Anthocyanins are one the most important anti-oxidant compounds that can scavenge the free radicals in plant. As it is mentioned in results the content of anthocyanin is significantly change under treatment with Zn and Fe. The results of this experiment are supported by previous study like Apati et al (2003) reported that in *solidaga canandeis* application of micronutrients have positive effects on anthocyanin synthesis. Also moor et al. (2009) stated that phosphorus increase the anthocyanin and ascorbic acid content in strawberry. Aziz et al (2007) reported that Ni and Co increases the flavonoid content in *hibiscus sabdariffa* L. Result show that Zn and Fe significantly change the rosmarinic acid content. Same result is achieved by other experiment. Bablar et al. reported that application of nitrogen increase the rosmarinic acid yield in Saturejah ortensis L. Nguyen and Niemeyer (2008) published that nitrogen increase the rosmarinic acid and caffeic acid in Ocimum basilicum L. Also Renata et al (2013) published that mineral fertilization increases the essential oils in medicinal plants. In other study Omidbeigi and Arjmandi(2002) showed that application of nitrogen and phosphorus increase the essential oils content in Thymus vulgaris. Alizadeh et al. (2010) reported that chemical fertilizer significantly changes the main component in Satureja hortensis L (Alizadeh et al. 2010).

Conclusion

From the above-mentioned results, it could be concluded that zinc and iron fertilizers have significant effect on both rosmarinic acid and anthocyanin. Since medicinal properties of lemon balm is based in its flavonoid application of Zn and Fe on these compounds have positive effects.

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