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Comparison of the efficiency of some different Zn sources on quantitative and qualitative yield of Lemon verbena

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Abstract

One of the most effective micronutrients in quantity and quality of medicinal herbs is zinc (Zn). The present study aimed to assess the impact of the foliar application of various Zn sources (0.2% w/v) including Zn chelated by amino acid (ZnAAC), Zn-sulfate and Zn-EDTA on qualitative and quantitative features of Lemon verbena *(Lippia citriodora* L.). The field experiment was performed in a randomized complete block design with three replications. Foliar application of treatments was conducted three times with one-month interval. Based on the findings, it was indicated that ZnAAC treatment has the highest effect on quantitative and qualitative characteristics of lemon verbena in comparison with other treatments. The maximum chlorophyll concentration, leaf area, leaf dry weight, essential oil content, and total antioxidant capacity (TAC) in both harvests were found in ZnAAC treatment. It was also found that Zn-sulfate was more effective compared to ZnEDTA in the studied characteristics. It was demonstrated that regardless of the source of applied Zn, all Zn application had a significant impact on all features in comparison with the control. The analysis indicated that citral (10.4-20.2), geranial (11.4-23.2), neral (7.93-18.6), truns-caryophyllene (2.34-3.22) and 1,8-cineol (1.01-1.35) were the predominant compounds in the essential oil in both harvests. The highest and lowest amounts of geranial, neral, and citral in both harvests were measured in ZnAAC (56.81%) and ZnEDTA (48.05%) treatments, respectively. Based on the findings, it seems that using ZnAAC can enhance the quantitative and qualitative characteristics of the lemon verbena.

Keywords: Amino acid, Essential oil, Foliar application, *Lippia citriodora* L.

Introduction

Lemon verbena (*Lippia citriodora* L.), belonging to Verbenaceae family, with a 1.5- 2 meters height is a shrub which is used in medical industry (Shahhoseini *et al.*, 2018; Tutin, 1998). Lemon verbena leaves contain alkaloids, flavonoids, mucilage, tannin, and acid phenols (Skaltsa and Shammas, 1988). The essential oil of this plant also has antimicrobial (Torrent Martia, 1976) and anti-fever effects (Nakamura *et al.*, 1997)."So, both leaves and essential oil are the most important parts of this plant (Shahhoseini *et al.*, 2013; Tabatabaie and Nazari, 2007).

Plant nutrition is one of the effective factors in improving plant growth (Marschner, 1995). Zinc (Zn) which is the essential micronutrients for plant nutrition is deficient in calcareous soils, which results in decreased plant growth (Khoshgoftarmanesh *et al.*, 2005). High Marschner soil pH, low organic matter, soil salinity and high amount of calcium carbonate effect on Zn bioavailability in calcareous soils (Alloway, 2008). Zinc deficit in plants is expressed as chlorosis of new leaves, stunting, and eventually decreasing plant growth (Marschner, 1995). Zeidan *et al*. (2010) showed positive effects of Zn on increasing plants yield and water use efficiency. It was demonstrated by Nasiri *et al*. (2010) that yield and essential oil content of chamomile increased by using Zn (*Matricaria chamomilla* L.) in calcareous soils. It was indicated by Misra *et al*. (2006) that using Iron (Fe) and Zn increased essential oil content in basil (*Ocimum basilicum* L.). Misra and Sharma (1991) have reported that dry weight, essential oil and menthol contents in Japanese mint (*Mentha arvensis* L.) were increased by using Zn. Also, in several reports on sweet basil (*Ocimum basilicum* L.) (Said-Al Ahl and Mahmoud, 2010), peppermint (*Mentha piperita* L.) (Akhtar *et al.*, 2009), and *Mentha piperita* (Peppermint) (Zehtab-Salmasi *et al.*, 2008) it was confirmed that Zn application incremented yield and essential oil content in medicine herbs.

Foliar application of Zn on leaves is an effective

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way of Zn supplying to plants by the elimination of Zn sorption on soil particles (Erdal *et al.,* 2004; Yassen *et al.,* 2010; Baloch *et al.,* 2008). In several studies on Lemongrass *(Cymbopogon flexuosus)* (Refaat *et al.,* 2001), *Mentha species* (Khalil *et al.,* 2005), and Rue (*Ruta graveolens* L.) (Naguib *et al.,* 2007), it was demonstrated that foliar applied of Zn results in yield improving. To increase plant yield in the farm, Zn sulfate and ZnEDTA are the two most common fertilizers of Zn (Alloway, 2008). Some reports have indicated that foliar usage of Zn fertilizers (sulfate and chelated) can improve qualitative and quantitative characteristics of medicine plants including basil (*Ocimum basilicum* L.) (Said-Al Ahl and Mahmoud, 2010), peppermint (*Mentha piperita* L.), rosemary (*Rosmarinus officinalis* Linn.) and marigli (*Salvia officinalis) (*Nahed and Balbaa, 2007).

Zn chelated by amino acid, which called Zn amino-chelate (ZnAAC), is another Zn fertilizer that has been recently used in plant nutrition. Amino acids identified as natural ligands are expected to bound metal cations by their carboxyl groups (Souri, 2016). In several studies, it was revealed that using amino acids on plants caused in increasing protein and yield. Das *et al*. (2002) reported that using foliar amino acid on Mulberry enhanced protein content. It was reported by Ghasemi *et al*. (2012) that foliar usage of Fe-amino acid chelated on tomato increased yield crop. Also, other studies with tea (*Thea sinensis* L. Sym Cammelia thea link) (Thomas *et al.,* 2009), chamomile (*Matricaria chamomilla* L.) (Golzadeh *et al.,* 2011, Karima *et al.,* 2005) and basil (*Ocimum basilicum* L.) (Saburi *et al.,* 2014), indicated that used of amino acids caused improve physiological characteristics and biochemical compounds.

Taking the important role of amino acids in the contribution of hormones and low-molecular nitrogen-based compounds synthesis, amino acid fertilizers may play a considerable effect on qualitative and quantitative properties of medicine herbs. Moreover, considering the key role of Zn on the improvement of medicine plants quality and quantity, this study aimed to compare the impact of various source of Zn on yield and essential content of Lemon verbena during two years.

Material and Methods

Field experiment: The study area is located in the agricultural research field of Lorestan University (33.4647° N, 48.3390° E). The mean monthly temperature at the site was 28ºC. Soil samples were taken before planting at 0 – 30 cm depth from the experimental site and were analyzed. Table 1 represents the physical and chemical features of the soils. Zn treatments included control (Without the use of Zn), Zn sulfate (Zn=22.7%), ZnEDTA (Zn=12%) (prepared from Merck company) and Zn-amino acid (Zn=8%). Based on the higher efficiency in Zn foliar application Lysine amino acid was selected as a complexing agent according to the procedure described by Ghasemi *et al*. (2013) and Rafie *et al*. (2017). Foliar-applied solutions contained 0.2 % of Zn (w/v) and were made by distilled water. The experiment was performed in a randomized complete block design with three replications. The cultivation was carried out manually. Twelve experimental plots were used with the size of 4.5 m^2 (length= 3 m; Width=1.5 m) and seedlings of lemon verbena were planted in the experimental plots. Plant spacing in each row was 70 cm and distance between two rows was 50 cm. Phosphorus and potassium fertilizers were added to the soil in the form of triple super phosphate (250 kg P ha^{-1}) and potassium sulfate (200 kg K ha^{-1}). Urea was applied at a rate of 150 kg N ha^{-1} at three steps. Iron- sulfate and manganese- sulfate were used 15 and 10 kg ha^{-1} pre-planting, respectively. Treatments of foliar applied were done three times in each harvest with one-month interval. Foliar-applied solutions contained 0.2 % of Zn (w/v) and were made by distilled water. To decrease the possible leaf damage caused by salts on sunlight or high temperature, the foliar application was made in early mornings. Samples were harvested two times during the growing seasons, (from March to November 2018). Harvest times were 145 (first harvest) and 110 (second harvest) days from transplanting to pre-flowering, respectively

Physiological and antioxidant assessments: Leaf chlorophyll content determined by the Arnon method (Arnon, 1949). Sampling was done one week after each harvest for measuring chlorophyll. 0.5 g of leaf material was collected from the plants and grinded in 10 ml of 80 % acetone. After filtration final volume was made up to 50 ml. The 5 ml of this solution was measured using a UV-visible spectrophotometer (Pharma Spec, UV-1700, Shimadzu, Japan). Using leaf area meter, model 3100 LI, LI-COR, NE, USA, the leaf area of plants was measured. In this regard, to obtain representative leaf area homogeneous samples leaves of the plant were collected (Tabatabaie and Nazari, 2007). Total antioxidant capacity was determined by the radical scavenging activity of sample (Blois, 1958).

Zn fractionation in leaf: Leaves cells were separated into two fractions: cell wall and an intracellular fraction. Based on the differential centrifuge technique, the cell wall of leaves was separated (Weigel and Jager, 1980). Frozen leaf tissues were milled in cold buffer solution containing 50 mM tris-HCl (pH=7.5), 250 mM sucrose and 1 mM dithioerythritol ($C_4H_{10}O_2S$) and centrifuged 15 mins. at 3000 r $mins.^{-1}$. Residual precipitation was considered as the cell wall. By adding concentrated nitric acid and per-chloric acid in the ratio of 1:3, cell wall fraction and whole leaf were digested and then using atomic absorption spectrophotometer, Zn concentrations in the digest solutions were measured (AAS) (Model 3400, Perkin Elmer, Wellesley, MA). The difference between Zn concentrations on the whole leaf from its concentration in the cell wall was considered as an intracellular fraction.

Extraction of essential oil: Utilizing a clevenger-type apparatus to extract essential oil content of plants,

Table 1- Characteristics of experimental soil

parameter	Mn	Fe	Mg	Zn	P	K	B	Cu	N	OC	$CaCO_3$	pH	EC	texture
	mg kg^{-1}								%				ds m^{-1}	
Value	5.2	3.1	330	0.4	7	210	0.5	0.6	0.13	0.5	26	7.62	2.1	loam

shade dried leaves (100 g) were exposed to hydro-distillation for 3 hrs. Essential oil samples were dried over anhydrous sodium sulfate and stored in dark glass bottle at low temperature (4 ^{o}C) until analysis.

Qualitative analysis of essential oil, gas chromatography analysis: Gas chromatography (GC) with a HP-5MS capillary column equipment was done by an Agient 789 N GC system. Dimensions of using column were 30 m × 0.25 m × 0.25 micrometer. The temperature was set at 40ºC for 5 mins. and after that reached to 250 ºC at a rate of 3 C/mins. The temperature of injector and detector (FID) was set at 260 ºC. Carrier gas was helium with a linear velocity of 1ml/min.

Gas chromatography-mass spectrometry analysis: Utilizing an Agilent 5975 C system equipped with the same column as mentioned before, gas chromatography-mass spectrometry analysis was conduced. The oven temperature was set at 40º C and then programmed to reach to 240º C at a rate of 4º C, transfer line temperature was 260º C, helium was selected as carrier gas with a linear velocity 1 ml/min, split ratio 1/60, ionized energy 70 eV; scan time 1sec; mass range 40-300 amu. A data bank mass spectrum was matched with recorded mass spectra for identification of components. Then Kovats retention indices relative to a series of n-alkanes (C7-C24) were compared with literature values. Component identification was performed through comparing their mass spectra with internal reference mass spectra library (NIST08 and Wiley 9.0)

Statistical Analysis: Statistical analyses were conducted utilizing SAS software (version 9.1) and considerable differences were identified between means utilizing LSD test at the 5% significance level.

Results and Discussion

Leaf area and Total chlorophyll: Zn foliar application, irrespective to applied source, significantly increased the leaf area and total chlorophyll compared with the control (Table 2). Also, based on the findings it was indicated that the effect of Zn sources on these characteristics was different. The maximum and minimum leaf area and total chlorophyll were detected in ZnAAC and Zn-EDTA treatments in two harvests, respectively.

Our results confirmed other findings regarding significant role of Zn (Christopher *et al.,* 2007) and amino acids (Foroutan nia *et al.,* 2015) on medicine herbs growth. It seems that increasing cell division, auxin content, enhancing contribution in RNA metabolism and higher water use efficiency in presence of Zn are reasons of leaf area enlargement and total chlorophyll intensification (El-Bassiouny *et al.,* 2008). Using ZnAAC treatment in comparison with other treatments, a significant increase in leaf area and total chlorophyll content was observed. One of the effective factors in the absorption and efficiency of elements in foliar application is size and type of accompanied ligand (Almendros *et al.,* 2015). Synthetic ligands such as EDTA (174 cm^3 $mole^{-1}$) have a larger size compared to ions (0.074 A°) and amino acids (1.5 A°), and subsequently it seems that it has less leaf absorption. It has been investigated that several plants may absorb amino acids directly for taking part in structural compositions and physiological processes (Nasholm *et al.,* 2009; Souri, 2016). Thon *et al*. (1981) demonstrated that amino acids increase leaf area and chlorophyll pigments by providing bioavailable nitrogen source to plant cells which uptakes faster than other nitrogen sources.

Lysine (applied amino acid in the present study) serves as a building block of proteins, and also is a precursor for glutamate, a key signaling amino acid which regulates plant growth and responses to the environment (Galili, 2002). Genetic, molecular, and biochemical evidence also indicates that during plant growth and development lysine synthesis and catabolism are regulated by intracellular mechanisms (Galili, 2002). Lysine is a precursor of glutamate in the plant that glutamic acid has a significant role in chlorophyll content enhancement (Glu → ALA → PBG → UroIII → ProtoIX→ Mg-ProtoIX → Pchl → Chla → Chlb) (Galili *et al.,* 2014). Therefore, it is expected that chlorophyll concentration will increase in the presence of lysine.

Leaf dry weight and essential oil content: Zn application, irrespective to the applied source, significantly increased both the essential oil content and leaf dry weight in comparison to the control in two harvests (Table 3). The findings have indicated that the effects of various sources of Zn on leaf dry weight and essential oil content were different. The maximum and minimum leaf dry weight and essential oil content (Mean of two harvest) were observed on ZnAAC and ZnEDTA treatments, respectively. The positive impact of Zn appears on carbohydrates metabolism, and its participation in structure of RNA-polymerase and carboxyl phosphate ribolose enzymes and consequently results in increasing sugar and starch concentrations in plant tissue and yield enhancement (Marschner, 1995). Zn is a key constituent of numerous enzymes related to chlorophyll synthesis and photosynthesis and also contributes crucially to a wide range of processes, such as growth hormone production like tryptophan, precursor of auxins (Alloway, 2008). Therefore, in plants, proper application of Zn affects positively the

Table 2- The effect of various Zn sources (ZnAAC, $ZnSO_4$ and ZnEDTA) on growth characteristics of Lemon verbena. Each value is the mean ± SE, n= 3. According to the results of the LSD test, treatments that showed significant differences with each other at P ≤ 0.05 are indicated by different letters.

Harvest	Treatments	LA (Cm^2)	Total chlorophyll (mg g^{-1} FW)
First Harvest	Control	4.2±0.5 [d]	0.9 ±0.1 [d]
	Zn-AAC	7.6±0.52 [a]	2.02±0.21 [a]
	Zn-EDTA	5.6±0.45 [c]	1.53±0.13 [c]
	$ZnSO_4$	6.5±0.71 [b]	1.85±0.19 [b]
Second Harvest	Control	4.0±0.3 [d]	0.5±0.08[c]
	Zn-AAC	7.4±0.35 [a]	1.75±0.12[a]
	Zn-EDTA	5.1±0.29 [c]	1.23±0.15 [b]
	$ZnSO_4$	6.3±0.38 [b]	1.19±0.12[b]

Table 3- The effect of various Zn sources (ZnAAC, $ZnSO_4$ and ZnEDTA) on leaves dry weight and yield essential oil of Lemon verbena. Each value is the mean ± SE, n= 3. According to the results of the LSD test, treatments that showed significant differences with each other at P ≤ 0.05 are indicated by different letters.

	Treatments	Leaf dry weight ($Kg\ ha^{-1}$)	Essential oil content ($g\ kg^{-1}$)
First Harvest	Control	680±35.7 [d]	8.85±0.41[c]
	Zn-AAC	1045±56.8[a]	14.00±0.82[a]
	Zn-EDTA	820±42.7 [c]	10.84±0.81[b]
	$ZnSO_4$	857±45.7 [b]	13.87±0.93[a]
Second Harvest	Control	520±36.9 [d]	10.88±0.6 [d]
	Zn-AAC	910±51.5 [a]	14.75±0.6[a]
	Zn-EDTA	780±62.7 [c]	12.8±0.73[c]
	$ZnSO_4$	800±41.8[b]	13.4±0.49[b]

leaves formation, leaf area and total photosynthesis leading to yield production and dry weight A significant increase in the leaf yield and essential oil content was observed in ZnAAC treatment among the used sources of Zn, compared to the other Zn sources. Some findings have indicated that onion bulb and wheat grain yield incremented by foliar application of amino acids (Amin *et al.*, 2011, Rafi *et al.*, 2017, and Ghasemi *et al.*, 2013). Jei *et al*. (2008) reported that increased yield of rice was higher in Zn and Fe chelated by amino acid (Zn-Fe-amino chelated) (23%-73%) compared to Zn and Fe sulfate (11%-35%) and Zn-Fe EDTA chelated (15%-63%).

The higher efficiency of ZnAAC is probably caused by the role of accompanied amino acid on various biological mechanisms such as cell division and growth (El-Bassiouny *et al*., 2008). Moreover, amino acids are the constituents of proteins that increased yield dry weight through playing important role in metabolism, protoplasm formation, cell division, and plant growth (Barker and Pilbeam, 2006). Also it seemed that some parts of increased yield in presence of ZnAAC caused by supplying some plant's nitrogen (even in small amount) and also having stimulator effects. Hildebrandt *et al*. (2015) reported that amino acids as nitrogen and carbon sources can release organic acids and ammonium in carbohydrate deficiency condition in plants and eventually enter to Krebs cycle to improvement yield crop. Talaat and Youssef (2002) indicated that amino acids influence on essential oil improvement in medicinal plants depends on applied amino acid concentration so that more concentrated amino acid results in essential oil decreasing. Regarding positive effect of amino acids on yield crop and essential oil content in lemongrass, our results agreed with the findings that were reported by Gamal El-Din *et al*. (1997).

Zn fractionation in leaf: According to the results, Zn application (Regardless of its source) increased Zn concentration in the leaves (Table 4). Compared to the Zn-EDTA treatment, Zn concentration in the leaves was higher in Zn-sulfate and ZnAAC treatments. The highest and lowest Zn concentrations in cell wall were found in Zn-sulfate and ZnAAC treatments, respectively.

Intracellular distribution of Zn contributes considerably to mobility of element in plants. Nishizono *et al*. (1987) indicated that 70- 90 percentage of Copper, Zn and Cd taken up by plants were distributed in cell wall. However, limited information exists regarding Zn intracellular distribution in leaves cells after spraying Zn fertilizers. It appears that in addition to genotype, type of Zn sources effects on efficiency of Zn foliar application. Wallinhan and Heymann-Herschberg (1956) have that Zn can be absorbed easily by citrus leaves while only 0.2 percentage of total applied Zn in walnut leaves was absorbed and translocated.

The results of this study indicated that in ZnAAC

Table 4- The effect of various Zn sources (ZnAAC, $ZnSO_4$ and ZnEDTA) on Zn distribution in Leaves of Lemon verbena. Each value is the mean ± SE, n= 3. According to the results of the LSD test, treatments that showed significant differences with each other at P ≤ 0.05 are indicated by different letters.

	Treatments	Zn concentration (mg kg^{-1})		
		Total	Cell wall	Intracellular
First Harvest	Control	2.1±0.19 ^{c}	1.4±0.17 ^{b}	0.7±0.1 ^{c}
	Zn-AAC	6.2±0.46 ^{a}	3±0.31 ^{b}	3.2±0.2 ^{a}
	Zn-EDTA	4.8±0.28 ^{b}	3.1±0.22 ^{ab}	1.7±0.15 ^{b}
	$ZnSO_4$	7.1±0.42 ^{a}	4.6±0.30 ^{a}	2.5±0.22 ^{a}
Second Harvest	Control	1.7±0.21 ^{c}	1.23±0.2 ^{c}	0.47±0.11 ^{d}
	Zn-AAC	5.1±0.46 ^{a}	2.4±0.25 ^{b}	2.7±0.21 ^{a}
	Zn-EDTA	3.6±0.31 ^{b}	2.7±0.15 ^{ab}	0.9±0.14 ^{c}
	$ZnSO_4$	6.6±0.35 ^{a}	4.1±0.36 ^{a}	2.5±0.12 ^{b}

Table 5- The effect of various Zn sources (ZnAAC, $ZnSO_4$ and ZnEDTA) on total antioxidant capacity (%) of Lemon verbena. Each value is the mean ± SE, n= 3. According to the results of the LSD test, treatments that showed significant differences with each other at P ≤ 0.05 are indicated by different letters.

	First Harvest	Second Harvest
Control	34.4±2.8^{d}	41.21±2.3^{d}
Zn-AAC	80.3±5.38 ^{a}	78.7±3.58^{a}
Zn-EDTA	51.5±4.75 ^{c}	54.7±4.75 ^{c}
$ZnSO_4$	72.2±3.98^{b}	68±3.98^{b}

treatment, intracellular Zn concentration of leaf increased higher compared to Zn-sulfate. It may be associated with charge reduction of Zn^{2+} by ZnAAC formation which limited Zn adsorption on negative charge of cell wall (Zhang and Patrick, 1999). Therefore, increase of Zn adsorption in ZnAAC treatment compared to Zn-sulfate can be clarified via facilitation of symplastic absorption of Zn through cell membrane because of binding Zn with carboxyl ($-COO^{-}$) and hydroxyl ($-OH^{-}$) groups of amino acids (Inouhe *et al*., 2012). On the other hand, amino acids have high-affinity transporters when taken up through leaf cell wall (Hirner *et al.,* 2006). Schonherr and Schreiber (2004) reported that by increasing molecular weight from 100 to 500 mole g^{-1}, crossing through cuticle layers of leaves is limited 13 times. It seems that higher molecular weight and larger size of EDTA (292.24 g mol^{-1}) is a crucial reason for lower Zn concentration in the cells in comparison with Zn-sulfate and ZnAAC treatments.

Total antioxidant capacity: Total antioxidant capacity (TAC) in all treatments, regardless of Zn sources, increased by 44% (mean of two harvests) in comparison with the control. The highest and lowest TAC in both harvests were measured in ZnAAC (78.7-80.3 %) and Zn-EDTA (51.5- 54.7%), respectively (Table 5). Zn is essential component of some substantial enzymes structure such as alcohol-dehydrogenase, superoxide dismutase, carbonic anhydrase and activates more than 70 enzymes in plants. It also has active contribution in other dehydrogenase (DH) enzymes like glutamic DH, lactic DH, malic DH, phosphate DH, aldolase, carboxy peptidase, alkaline phosphate, RNA-polymerase (protein constructor), carboxyl phosphate ribulose (starch constructor), and phospholipase (Alloway, 2008). Therefore it is expected that TAC in plants enhance in presence of Zn. It was also found that the highest TAC enhancement was related to ZnAAC treatment because of higher Zn absorption in presence of amino acids.

Predominant compounds of essential oil: The results showed that citral (10.4-20.2), geranial (11.4-23.2), neral (7.93-18.6), trans-caryophyllene (2.34-3.22), and 1,8-cineol (1.01-1.35) were chief mixtures in the essential oil in each harvest (Table 6 and 7). The results showed that different treatments effect component values and chemical profile of the essential oil. The highest and lowest contents of geranial, neral + citral in both harvests were observed in ZnAAC (56.81%) and ZnEDTA (48.05%) treatments, respectively. Also, limonene and 1, 8-cineol were maximum (4.24%) and minimum (3.89%) in ZnAAC and ZnEDTA treatments, respectively.

1,8-cineol, geranial, neral, and 6-methyl-5-heptene-2-one were measured as main compounds in essential oil of moroccan plant (Bellakhdar *et al*., 1993). Gomes *at al*. (2006) reported that geranial, neral and limonene were the most important components in essential oil which conpirms the present finding. There are several findings implied on the significant effect of applied fertilizers on essential oil quality of medicinal plants (Shahhoseini *et al.,* 2018).

Numerous researchers evaluated the effect of Zn usage on essential oil composition. Adams (2007)

Table 6- The effect of various Zn sources on essential oil compounds of *Lippia citriodora* L.in the first harvest

Compound	Rt	RI	Control (%)	ZnAAC (%)	ZnEDTA (%)	$ZnSO_4$ (%)
Limonene	17.65	1031	3.06	5.19	4.94	4.53
6-Methyl-5-hepten-2-one	15.77	988	0.65	1.01	1.07	0.80
1,8-cineol	17.80	1033	3.01	4.35	3.72	5.09
Citral	26.01	1240	14.4	16.12	16.04	15.1
Geranial	28.20	1270	24.8	29.2	27.4	26.8
Geranyl acetate	31.76	1383	1.64	1.08	2.05	1.83
trans-Caryophyllene	33.43	1444	2.34	1.22	1.38	2.78
Nerolidol	38.04	1564	1.43	1.84	1.72	1.59
spathulenol	39.03	1606	2.99	4.13	3.40	3.94
Caryophyllene oxide	39.16	1581	3.85	1.52	2.01	4.11
tau.-Cadinol	40.97	1620	0.86	0.35	0.95	0.68
beta.-Pinene	14.92	980	1.09	0.61	0.41	0.41
Decanal	25.33	1204	-	0.05	0.02	-
Alpha.-terpineol	25.15	1189	0.65	0.91	0.80	0.78
Ledene oxide	40.73	1682	0.93	1.01	0.92	0.31
1-octen-3-ol	15.59	978	0.75	0.31	0.25	0.20
Rosefuran	20.46	1104	0.10	0.07	0.10	0.11
Carvone	27.2	1240	0.22	0.11	0.15	0.15
gamma.-Muurolene	33.68	1477	0.35	0.21	0.17	0.25
Eremophila-1(10),11-diene	36.79	1514	0.86	0.65	0.32	0.11
(Z)-.beta.-Farnesene	33.96	1443	0.93	0.44	0.65	0.22
trans-.alpha.-Bergamotene	32.52	1436	0.12	0.04	0.09	0.14
beta.-Himachalene	35.08	1500	0.16	0.10	0.13	0.11
alpha.-Pinene	12.96	939	0.10	-	0.08	0.09
cis Limonene oxide	22.35	1134	0.11	0.06	0.09	0.08
Nonanal	21.13	1102	0.09	-	-	0.01
delta.-Cadinene	35.57	1524	0.48	0.43	0.23	0.25
Total (%)		-	-	-	-	-

Continue of Table 6-

Compound	Rt	RI	Control (%)	ZnAAC (%)	ZnEDTA (%)	$ZnSO_4$ (%)
Neral	26.98	1240	11.93	14.6	13.3	11.6
alpha.-Curcumene	35.47	1500	4.15	3.11	4.02	3.71
beta.-Myrcene	15.05	991	-	0.03	0.02	-
trans-Nerolidol	38.07	1564	1.52	0.75	0.90	1.67
alpha.-Terpineol	25.15	1189	0.66	1.01	0.96	0.82
Germacrene D	35.55	1490	0.73	0.41	0.98	0.80
gamma.-Eudesmol	41.61	1657	0.76	0.11	0.39	0.36
isospathulenol	40.71	1658	0.53	0.43	0.18	0.42
(Z,Z)-.alpha.-Farnesene	36.34	1502	0.91	0.42	0.52	0.37
Germacrene B	36.08	1556	0.41	0.47	0.40	0.29
alpha.-Selinene	34.82	1513	0.48	-	0.41	0.11
beta Bourbonene	32.05	1385	0.52	0.39	0.32	0.35
beta.-trans-Ocimene	18.31	1050	0.78	0.58	0.64	0.41
Rose furan epoxide	23.87	1172	-	0.48	0.52	-
cis-Geraniol	26.17	1255	0.18	0.09	0.16	0.53
Terpinolene	20.91	1088	0.45	0.28	0.39	0.21
Verbenol	21.95	1144	0.04	-	-	-
Piperitone	27.55	1277	0.19	0.07	0.09	0.12
Zingiberene	35.82	1499	0.42	-	0.22	0.27
alpha.-Humulene	34.67	1467	0.63	0.12	0.42	0.36
alpha.-Bisabolol	39.53	1686	0.13	0.10	-	0.11
Trans-limonene oxide	22.55	1139	0.46	0.18	0.15	0.20
Methyl eugenol	32.88	1410	-	0.12	0.09	0.03
Terpinene-4-ol	24.49	1177	0.09	-	0.04	0.09
Linalool	19.66	1098	0.52	0.31	0.44	0.38
Perillen	20.75	1099	0.31	-	0.11	0.14
trans-beta.-Ocimene	21.53	1050	0.73	0.88	0.83	0.76
Total (%)		-	93.50	95.95	95.59	94.33

Table 7- The effect of various Zn sources on essential oil compounds of *Lippia citriodora* L. in the second harvest

Compound	Rt	RI	Control (%)	ZnAAC (%)	ZnEDTA (%)	$ZnSO_4$ (%)
Limonene	17.65	1031	1.42	2.21	2.06	1.97
6-Methyl-5-hepten-2-one	15.77	988	0.65	0.95	0.74	0.72
1,8-cineol	17.80	1033	1.94	1.24	1.04	0.97
Citral	26.01	1240	12.2	16.4	16.4	14.7
Geranial	28.20	1270	18.01	21.1	19.7	18.2
Geranyl acetate	31.76	1383	1.17	1.93	1.86	1.66
trans-Caryophyllene	33.43	1444	2.85	2.64	2.76	2.72
Nerolidol	38.04	1564	1.93	2.93	2.75	2.42
spathulenol	39.03	1606	2.79	1.64	1.57	2.15
Caryophyllene oxide	39.16	1581	3.25	2.38	2.13	2.94
tau.-Cadinol	40.97	1620	2.92	3.02	2.82	2.54
beta.-Pinene	14.92	980	1.39	1.09	0.83	2.49
trans-beta.-Ocimene	23.76	1050	0.73	0.37	0.39	0.39
Alpha.-terpineol	25.11	1189	0.65	0.79	0.73	0.63
Ledene oxide	39.85	1682	0.93	1.21	0.98	1.14
1-octen-3-ol	15.59	978	0.75	0.31	0.27	0.63
Rosefuran	20.41	1104	0.86	0.47	0.56	0.71
Carvone	27.2	1240	1.07	0.71	0.92	0.95
gamma.-Muurolene	33.68	1477	0.65	0.21	0.37	0.61
Eremophila-1(10),11-diene	36.79	1514	0.86	0.25	0.38	0.92
(Z)-.beta.-Farnesene	34.23	1443	1.02	0.44	0.65	0.82
trans-.alpha.-Bergamotene	32.52	1436	0.59	0.04	0.09	0.34
beta.-Himachalene	35.08	1500	0.78	0.10	0.43	0.62
alpha.-Pinene	12.96	939	0.65	0.14	0.11	0.59
cis Limonene oxide	22.35	1134	0.92	0.35	0.72	0.54
Total (%)		-	-	-	-	-

Continue of Table 7-

Compound	Rt	RI	Control (%)	ZnAAC (%)	ZnEDTA (%)	$ZnSO_4$ (%)
Neral	26.98	1240	9.13	15.2	13.2	11.2
alpha.-Curcumene	35.47	1500	2.15	2.26	2.17	2.13
delta.-Cadinene	15.05	1524	1.08	1.12	1.02	1.13
trans-Nerolidol	38.07	1564	2.16	0.89	1.04	1.69
alpha.-Terpineol	25.15	1189	0.86	0.54	0.79	0.70
Germacrene D	35.55	1490	1.10	0.75	0.92	1.06
gamma.-Eudesmol	41.61	1657	0.66	0.57	0.71	0.41
isospathulenol	40.71	1658	0.53	0.65	0.31	0.29
(Z,Z)-.alpha.-Farnesene	36.34	1502	1.01	0.75	0.70	0.40
Germacrene B	36.08	1556	0.75	0.52	0.49	0.42
alpha.-Selinene	34.82	1513	0.52	0.43	0.40	0.34
beta Bourbonene	32.05	1385	1.40	1.02	1.29	1.27
beta.-trans-Ocimene	18.31	1050	1.08	0.81	0.96	1.02
Rose furan epoxide	23.87	1172	0.73	0.51	0.67	0.46
cis-Geraniol	26.17	1255	0.81	0.49	0.71	0.63
Terpinolene	20.91	1088	0.62	0.42	0.75	0.51
Perillen	21.95	1099	0.78	0.37	0.76	0.64
Piperitone	27.55	1277	0.82	0.27	0.49	0.77
Zingiberene	35.82	1499	0.67	0.23	0.43	0.57
alpha.-Humulene	34.67	1467	0.91	0.31	0.74	0.81
alpha.-Bisabolol	39.53	1686	0.85	0.18	0.65	0.75
Trans-limonene oxide	22.55	1139	0.77	0.31	0.50	0.66
Methyl eugenol	32.88	1410	0.76	0.51	0.72	0.82
Terpinene-4-ol	24.49	1177	0.21	0.31	0.45	0.25
Linalool	19.66	1098	0.88	0.63	0.75	0.64
Total (%)		-	92.22	92.97	92.88	91.94

observed that total content of monoterpenes of *Mentha spicata* essential oil increased with Zn application. It was indicated by Hassanpouraghdam *et al*. (2010) that methyl chavicol as main compound oil of basil

(*Ocimum basilicum* L.) incremented by Zn sulfate treatment. The same findings was reported on anise (*Pimpinella anisum* L.) (Pirzad *et al.*, 2013). These researchers expressed Zn effects on main metabolic pathways eventually leading to the biosynthesis of active components of essential oil. It was reported by Gerjtovsky *et al*. (2006) that soil application of Zn influenced chamazulene and (E)-β-farnesene of chamomile oil but Zn supply had no impact on flavones, apigenin, coumarin and herniarin. Misra and Sharma (1991) observed that Zn application stimulated the menthol concentration in *Mentha japonica*.

Misra *et al*. (2006) specified a strong association between primary metabolic pathways and biosynthesis/accumulation of secondary metabolites of *Pelargonium graveolens* L. Those authors confirmed that the appropriate relationship of carbon assimilation pathways and accumulation of secondary metabolic requires the relation of several internal and external factors, particularly optimum levels of micronutrients such as Zn. It was indicated by Cakmak *et al*. (2010) that increasing Zn concentration promoted protein content in wheat grain. Zn seems to increases essential oil content by increasing protein concentration.

Amino acids by participating in hormones biosynthesis are defined as precursor of various secondary compounds which effect plant defense system and photosynthesis efficiency. They also stimulate leaf cells to produce new cells and photoassimilates (Awad-El *et al.*, 2007). Yield crop and the essential oil content enhancement may be associated in increasing carbon and nitrogen due to amino acids application. It is also probable that amino acids increases protein concentration and essential oil content directly. Nagar and Sood (2006) mentioned that polyamines activates RNA and proteins synthesis. Nitrogen is basic element in proteins structure and its deficiency affects proteins quantity and quality. Nitrogen or amino acids are of great value in biological synthesis of pigments, vitamins, coenzymes, purines and pyrimidine's bases (Kamar and Omar, 1987). Other findings agreed with our results (Karima *et al.*, 2005; Talaat and Yousef, 2002).

Conclusion

Based on the results it was indicated that Zn application improves qualitative and quantitative features of the lemon verbena. The leaf dry weight and essential oil content increased in presence of Zn, regardless Zn sources, compared to the control by 45 and 34 %, respectively. It was also found that accompanied ligand binding Zn has tremendous value on Zn efficiency in the plant. As ZnAAC treatment had the maximum leaf dry weight, intracellular Zn, essential oil content, TAC and compound concentration in essential oil in comparison with two other treatments. However, it seems that more studies on medicine herbs are required based on plant type, climatic conditions and soil properties.

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