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Growth and essential oil composition affected by foliar nutrition application on lemon verbena plant

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Abstract
Lemon verbena considered as a new source of essential oil. Nutrient available in the nutritional environment of plants are capable of changing yield and essential oil. Losses of fertilization such as urea (CH_4N_2O) added to soils have been considered to be related to the loss of moisture from soil or high moisture status leading to water logging. The present work was carried out to illustrate the response of growth and essential oil to foliar nutrition. The application of foliar nutrition at the low level (2 ml L^{-1}) resulted in significant increase in the plant height, fresh and dry yield as well as dry matter content. The highest essential oil content resulted from the medium treatment of (3 ml L^{-1}) of foliar application. The highest amount of major compounds (D- limonene, 1, 8 cineol and citral) resulted from the highest level (4 ml L^{1}) of foliar application.

Key words: Lemon verbena, growth characters, essential oil.

1. Introduction
True verbena oil comes from *Lippia citriodora* Kunth, syn. *Verbena triphylla* L Herit., *Aloysia citriodora* Ort. (Family Verbenaceae), it is also called "lemon verbena". Verbena is a small shrub that grows to 1.5 m high. Although native to South America *Lippia citriodora* plant is available in Egypt [1], it is considered as a new source of essential oil with relatively high yield used in perfumery with its strong citral note. Lemon verbena essential oils have been studied by several researchers. It is a yellow-greenish liquid with a characteristic fresh, lemon like odor. It was found that the main constituents of *Lippia citriodora* oil under conditions of grass were geranial, neral and limonene constituting 66.3% of the total essential oil yield during May and increasing to 69% during September [2, 3].
Nutrient available in the nutritional environment of plants are capable of changing yield and essential oil [4-7]. Sharma [8] found that herbage and oil yields were further increased with the application of copper, zinc, iron, and boron over those received N, P and K application alone by about 32, 22, 27 and 20%, respectively. They added that NP and K application increased the menthol yield over the control by about 98% whereas the application of copper, iron and boron increased the menthol yield over N, P and K application. Singh [9] reported that applied foliar spray (Zn, Mn, Mg, Cu, B, Fe, Mo) had a little effect on *Mentha arvensis* essential oil but free menthol and menthone contents were varied. Khatab [10] found that spraying mint plant with "Foliatrin" as a foliar nutrient with the low level (0.2 ml L^{-1}) increased the fresh weight compared with the control treatment, also an increase in the oil production was observed due to application of Foliatrin as foliar spray. The highest basil essential oil yield was found at the highest NPK rate [4]. Nitrogen and phosphorus application contributes to growth and quantitative

changes in the essential oil of *Artemisia pallens* Wall but it does not modify its composition [11]. The application
of a higher NPK rate increased essential oil content in *Tagetes patula* L [12]. Increasing fertilization of summer savory plants with macro- and micro nutrient increased oil yield and also modifies its chemical composition [13]. Phosphorus application significantly increased growth and essential oil content of basil plant [14]. Phosphorus fertilization significantly increases oil yield in rose-scented geranium and also the content of citronellol and 10-epi-Û-eudesmol [15]. Iron application to growing thyme has a repressive effect on essential oil content and chemical composition of essential oil [16]. Differently, in basil grown under salt stress conditions, foliar application of zinc and iron increases the growth and linalool content of sweet basil essential oil [17]. Oregano yield and its essential oil were higher by 30 and 31% compared to the control under the influence of foliar application of calcium and magnesium [18]. At the same time, volatile oil content was not dependent on the application of Mg^{2+}; these differences resulted primarily from a significant increase in dry matter yield under the influence of plant feeding [18]. Mg has a positive effect on the quality and quantity of chamomile essential oil production [19]. El−Wahab [20] indicated that *Trachyspermum ammi* L. yield and its essential oil constituents were not largely affected by the applied 20 g L^{-1} of $MgSO_4$ but resulted in a significant increase in growth, essential oil content and main components of essential oil [21]. Application of NP with micronutrient increased the growth, yield and essential oil of some aromatic plants [22].
Losses of fertilizers [i.e. urea (CH_4N_2O), ammonium sulfate, [$(NH_4)_2SO_4$)], ammonium nitrate (NH_4NO_3)] added to soils have been considered to be related to the loss of moisture from soil, or high moisture status leading to water logging, so the present work was carried out to illustrate the response of growth characters and essential oil of lemon verbena to foliar nutrition.

2. Materials and methods

2.1. Experimental

Field experiments were performed during two successive seasons (2012 and 2013) at the farm of National Research Centre at Giza to study the effect of foliar nutrition on growth and essential oil productions of lemon verbena shrubs under the environmental conditions of Nubariya region, Egypt. Physical and chemical properties of soil used in this study were determined according to Jackson [23, 24] and are presented in Table 1. The plants were subjected to foliar nutrition by Foliatrian spraying (commercial name) with four concentrations (0, 2, 3 and 4 ml L^{-1}). The plants were sprayed twice; sprays were done during the third week of June and the last week of August of both seasons respectively. The experiment design was completely block randomized in three replicates. Each replicate was represented by 28 plants. All cultural practices were practiced according to the recommendations by the Egyptian Ministry of Agriculture.

Table 1. Mechanical and chemical analysis of the field soil

Components	Value
Sand %	48
Silt %	28
Clay %	24
PH	8
Total Nitrogen (ppm)	210
Available P (ppm)	90
Available K (ppm)	57
Electronic conductivity (dS m^{-1})	2

The plant materials used in both experiments were one year old during 2012 season and two year old during 2013 season which were grown on rows 60 cm. apart and the plants were spaced at 100 cm in between. The vegetative growth characters [plant height (cm), fresh and dry weight of leaves (g plant $^{-1}$ and ton ha^{-1}) & dry matter content (g plant $^{-1}$)] were recorded at the full blooming stage (during September) of both seasons. The analysis of foliatrian is presented in Table 2.

Table 2. Nutrient concentrations of foliatrian

Macro elements vales (%)		Micro elements vales (%)	
N	11.0	Fe	0.1
P	7.2	Zn	0.1
K	2.6	Mn	0.2
		Cu	0.1

2.2. Essential oil isolation

Fresh mass [divided into small pieces (0.5 - 1 cm)] was collected from each treatment, and then 500 g from each replicate of all treatments was subjected to hydro-distillation for 3 h using a Clevenger-type apparatus [25]. The essential oil contents were calculated as a relative percentage (v/w). In addition, total essential oils ml plant^{-1} and liter ha^{-1} were calculated by using the fresh mass. The samples of essential oils were dried over anhydrous sodium sulphate to identify the chemical constituents of the essential oil [25].

2.3. Gas chromatography

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a DB5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25µm). Oven temperature was held at 40°C for 5 min and then programmed until 250°C at a rate of 4°C/min. Injector and detector (FID) temperature were 260°C; helium was used as carrier gas with a linear velocity of 32 cm/s.

2.4. Gas chromatography-Mass spectrometry

GC-MS analyses were carried out on a Varian 3400 system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.); Oven temperature was 40 to 240°C at a rate of 4°C/min, transfer line temperature 260°C, injector temperature 250°C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, flow rate 1.1 ml/ min, Ionization energy 70 eV; scan time 1 s ; mass range 40-350 amu.

The components of the oils were identified by comparison of their mass-spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds. Kovat's indices [26] were determined by co-injection of the sample with a solution containing a homologous series of n-hydrocarbons, in a temperature run identical to that described above.

2.5. Statistical analysis

The averages of data were statistically analyzed using analysis of variance (ANOVA) and values of least significant difference (L.S.D) at 5% according to Snedecor and Cochran [27].

3. Results

3.1. Effect of foliatrian on plant growth characters

Data tabulated in Table (3) indicated that the application of foliar nutrition at the low level (2 ml L^{-1}) resulted in significant increase in the plant height, fresh and dry yield as well as dry matter content during both seasons while shortest plants and lowest weight of fresh and dry yield as well as dry matter were produced from the plants sprayed with the medium level (3 ml L^{-1}) of Foliatrin. ANOVA indicated that the changes in plant height, weight of fresh and dry yield & dry matter were significant.

3.2. Effect of foliatrian on essential oil content

The results given in Table (4) indicated that the application of foliar nutrition had a marked influence on the essential oil contents [%, (ml plant $^{-1}$) and (liter ha^{-1}). The highest essential oil content resulted from treating plants with 3 ml L^{-1} of Foliatrian with the

values of 0.7 and 0.6%; 0.9 and 0.7 ml plant $^{-1}$; 27.5 and 16.8 liter ha $^{-1}$ during the first and second seasons respectively. The lowest essential oil content resulted from the control treatment with the values of 0.5 and 0.4%; 0.8 and 0.5 ml plant $^{-1}$; 21.5 and 12.8 liter ha $^{-1}$ during the first and second seasons respectively. ANOVA indicated that the changes in essential oil contents were significant for Foliatrian treatments.

Table 3. Effect of foliatrian on the growth characters

Foliar Nutrition Treatments (ml L^{-1})	Plant Height (cm)		Fresh Yield (g plant $^{-1}$)		Fresh Yield (ton ha $^{-1}$)		Dry Yield (g plant $^{-1}$)		Dry Yield (ton ha $^{-1}$)		Dry Matter (%)	
	Season		Season		Season		Season		Season		Season	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	96.3	86.0	149.8	130.6	4.8	4.1	49.0	34.9	1.6	1.4	32.7	33.6
2 ml L^{-1}	106.4	94.5	161.5	142.6	6.3	4.5	60.1	49.3	1.9	1.6	37.4	34.6
3 ml L^{-1}	91.7	84.1	131.8	128.2	4.2	4.1	41.9	40.5	1.3	1.3	31.8	31.6
4 ml L^{-1}	96.1	87.6	146.2	133.3	4.6	4,2	49.4	45.3	1.5	1.4	31.7	34.0
LSD at:												
0.05	1.2	3.5	2.8	4.2	0.1	0.2	0.88	1.4	0.1	0.1	0.77	1.1
0.01	1.8	5.4	4.2	6.4	0.2	0.3	4.33	2.1	0.1	0.1	1.2	1.6

Table 4. Effect of foliatrian on essential oil content

Foliar Nutrition Treatments (ml L^{-1})	Essential oil					
	%		(ml plant $^{-1}$)		(liter ha $^{-1}$)	
	Season		Season		Season	
	1st	2nd	1st	2nd	1st	2nd
Control	0.5	0.4	0.8	0.5	21.5	12.8
2 ml L^{-1}	0.6	0.5	1.0	0.6	25.0	15.8
3 ml L^{-1}	0.7	0.6	0.9	0.7	27.5	16.8
4 ml L^{-1}	0.6	0.5	0.9	0.6	26.5	16.0
LSD at:						
0.05	0.02	0.01	0.01	0.01	0.3	0.5
0.01	0.03	0.02	0.02	0.02	0.5	1.0

3.3. Effect of foliatrian on the major constituents of essential oil

The major constituents of essential oil as detected by GC/MS (Table 5) were D- limonene (6.3 - 16.2%),), 1, 8 cineol (4.7% - 7.3%) and citral (19.9% - 28.8 %). The highest amount of major compounds resulted from the 4 ml L^{-1} treatment of Foliatrian with the values of 15.4 and 10.7% (limonene), 6.9 and 7.3% (1, 8 cineol) 28.8 and 23.5 (citral) during the first and second seasons respectively.

Table 5. Effect of foliatrian on the major constituents of essential oil

Foliar Nutrition Treatments (ml L^{-1})	Essential oil constituents (%)					
	D- Limonene		1,8 Cineol		Citral	
	Season		Season		Season	
	1st	2nd	1st	2nd	1st	2nd
Control	16.2	11.5	5.8	5.4	23.4	22.7
2 ml L^{-1}	8.9	6.6	5.9	4.7	23.9	19.9
3 ml L^{-1}	12.9	6.3	5.3	5.8	28.7	21.1
4 ml L^{-1}	15.4	10.7	6.9	7.3	28.8	23.5

4. Discussion

The effect of different treatments (Foliatrian treatments) on essential oil and its constituents may be due to its effect on the enzyme activity and metabolism of essential oil production [28]. Our results are in agreement with those obtained by Khatab [10] who found that significant increase in growth characters and essential oil of mint due to foliar spray with Foliatrian. Increasing fertilization of summer savory plants with macro- and micronutrient increased growth and oil yield but also modified its chemical composition [13]. Differently, in basil grown under salt stress conditions foliar application of zinc and iron increased the growth characters and linalool content in the oil [17]. Oregano essential oil yield is higher by 31% compared to the control under the influence of foliar application of calcium and magnesium [18]. Mg has a positive effect on the growth, yield, quality and quantity of chamomile essential oil production [19]. El−Wahab [20] indicated that

yield of *Trachysper-mum ammi* L. and essential oil constituents were not largely affected by the application of Mg. 20 g L^{-1} as $MgSO_4$ resulting in a significant increase in essential oil content, main components of essential oil [21]. Application of NP with micronutrient increased the growth, yield and essential oil of some aromatic plants [22]. Foliar application of zinc and iron increased the growth and linalool content in the oil [17].

Conclusion

The application of foliar nutrition (Foliatrin) at the low level (2 ml L-1) resulted in significant increase in the plant height, fresh and dry yield & dry matter content. The highest essential oil content resulted from treatment of 3 ml L-1 of foliar application. The highest amount of major compounds (D- limonene, 1, 8 cineol and citral) resulted from the 4 ml L-1 treatment of foliar application.

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