

## A Comparative Study on the Productivity and Chemical Constituents of Various Sources and Species of Calendula Plants as Affected by Two Foliar Fertilizers

<sup>1</sup>N.Y. Naguib, M.Y. Khalil and S.E. El Sherbeny

<sup>1</sup>Cultivation and Production of Medicinal and Aromatic Plants Department,  
National Research Centre, Giza, Egypt.

**Abstract:** Two field experiments were carried out during the two successive seasons of (2001-2002 and 2002-2003) in the Experimental Farm of Medicinal and Aromatic plants of N.R.C. Giza. The aim of this study was to investigate the characters of different species and sources of Calendula as well as study the effect of two foliar application at three rates of either Citren or Amco-combi on growth, yield of flowers, oil percent and yield as well as oil composition, carbohydrate percentages and total carotenoids content of three Calendula species. The studied species were *Calendula officinalis* L. (French source), *Calendula officinalis* L. (Slovakian source) and *Calendula stellata* L. (French source). The comparative study of various species and sources, showed that the maximum mean values of plant height, number of flowers, herb fresh and dry weight as well as essential oil content (%) were obtained as a result of *C. stellata* comparing with other species. On the other hand, *C. officinalis* Slovakian source gave the heaviest yield of flower heads with highest accumulation of total carbohydrate content and photosynthetic pigments. Otherwise, *C. officinalis* French source carrying largest number of branches. The growth parameters and flowers yield, in generally were significantly increased due to the application of either Citren or Amco-combi. The maximum values of these characters were obtained as a result of the highest rate of Amco-combi. The major constituents oil for *Calendula officinalis* introduced for French and Slovakian was Linalyl acetate, which reached to 36.50 and 38.64%, respectively. On the other hand linalool (34.40 %) was the major constituent in the essential oil of *Calendula stellata* L. The components of Calendula oil were influenced by different treatments. Thus the application of Citren and Amco-combi has increased linalyl acetate, linalool and limonene in the oil of Calendula. However, Citren was more effective than Amco-combi in this criteria. Furthermore; carbohydrates percentages, chlorophylls and total carotenoids showed positive response with increasing rates of foliar fertilizers. Meanwhile, the effect of Amco-combi was surpassed that of Citren fertilizer. Otherwise, *C. officinalis* Slovakian source was recommended to be cultivated for its production of heaviest flower yield and highest essential oil content comparing with other sources and species of Calendula under Egyptian conditions, also, for applying two foliar fertilizers at the highest concentration to enhance growth characters, essential oil content, as well as, its quality.

**Key words:** Calendula, Foliar fertilizers, Amco-combi, Citren, Essential oils

### INTRODUCTION

*Calendula* genus is native to the Mediterranean countries, it has been cultivated by the Egyptians, Greeks, Hindus and Arabs. *Calendula* grew in European gardens and has been used medicinally since the 12th century. Its name comes from the Latin word, calends, and belongs to Family Esteraceae. The petals have a pungent spicy flavor and the leaves have a bitter after taste. It is used more for medicinal than culinary purposes. Chevallier<sup>[1]</sup> reported that the yellow petals are an excellent remedy for red and inflamed skin, also, their antiseptic and healing

properties helping in preventing the spread of infection, and speed up the rate of repair. He added that *Calendula* cream is good for acne and diaper rash. An infusion is good for digestion and relieves colitis and symptoms of menopause. Blumenthal<sup>[2]</sup> stated that *Calendula* is a cleansing and detoxifying herb, and the infusion treat chronic infections. The flowers of *Calendula officinalis* were found to show a hypoglycemic effect, inhibitory activity of gastric emptying and gastro-protective effect Yoshikawa *et al*<sup>[3]</sup>. It reduce menstrual pain and regulate menstrual bleeding. The infusion makes an effective douche for yeast infections. Meanwhile, Ahmed and

**Corresponding Author:** M.Y. Khalil, Cultivation and Production of Medicinal and Aromatic Plants Department,  
National Research Centre, Giza, Egypt.

Qureshi<sup>[4]</sup> revealed that *Calendula* was taken internally to treat fevers promote menstruation and treat cancer. Also, Ostad *et al*<sup>[5]</sup>. reported that *Calendula officinalis*, is well known as anti-viral, anti-HIV, anti tumor, anti mutagenic and cytotoxic properties and possess estrogenic and anti estrogenic activities. Dried calendula petals are used in the spice trade as an inexpensive alternative to saffron as well as in many ointments to enhance their appearance by adding a gold color and also employed as an insect repellent.

Foliar fertilizer is particularly useful technique which can be designed to meet plants specific needs for one or more micro or macro nutrients especially trace minerals and enable to correct deficiencies, strengthen weak or damaged crops, speed growth and grow better and healthier plants. Micro and macro elements play an important role in promoting the growth and production of plants. Micro elements participate in most of the enzymatic reaction and they also play a role indirectly through the synthesis of several growth regulators.

It's known that most cultivated areas in Egypt appeared symptoms of deficiency of Mn, Zn, Fe, Cu and Mg<sup>[6]</sup>. Thus, the beneficial effects of macro and micro-nutrients foliar fertilizer upon growth and productivity of some medicinal and aromatic plants were reported by several authors, such as El-Kady<sup>[7]</sup>, spraying Zn, Mn, Cu and Fe on sunflower plants, Mostafa *et al*<sup>[8]</sup>, applying Fe and Zn on Chrysanthemum plants, Khalil *et al*<sup>[9]</sup>, using Cristalon, Amco-Better and Floactive on *Sinapis alba* and *Nigella Sativa*, Kandeel<sup>[10]</sup> working on *Ocimum basilicum* plants and employed (pholaz D) which contains Fe, Mn and Zn., El-Sherbeny *et al*<sup>[11]</sup>, spraying some microelements (B, Mo, Co and Pb) on *Cymbopogon citratus* L. Ezz El-Din *et al*<sup>[12]</sup>, used Ascobin and Super-grow on plantago, Aziz and El-Sherbeny<sup>[13]</sup>, applying Fe, Mn and Zn on *Sidiritis montana* L.

## MATERIALS AND METHODS

This investigation was conducted during two successive seasons of 2001-2002 and 2002-2003, at the Experimental Farm of the Medicinal and Aromatic Plants, Department, N.R.C. Giza, Egypt. Two source of seeds, the first one was (French), Conservatoire et Jardans Botanique de Nancy, France, *C. officinalis* L. and *C. stellata* L. The second source was Slovakia (Governmental Garden of Agricultural Ministry ) which exported (*C. officinalis*) seeds also. Seeds were planted on 15th October in at a distance of 30 cm between plants in plots of 4m<sup>2</sup> area (2x2m) with three rows, in complete randomized block design (18000 plants/fed.).

The experiment field was fertilized with 100 kg/fed. calcium supper phosphate, added within the soil preparation, 180 kg/fed. ammonium nitrate and 50 kg/fed. potassium sulphate as recommended doses. These quantities were divided into two portions, the first after one month of planting and the second one-month later.

The physical and chemical properties of the soil were determined according to Black<sup>[14]</sup> which recorded:

pH 8.1, EC 1.0 ds/m; 1.50% organic matter, 25.25% silt, 3.9% coarse sand, 29.70% fine sand 39.90% clay, i.e. the texture was a clay loam soil. The available nutrition elements 168ppm N, 31ppm P, 25ppm K, 1.28ppm Fe, 1.12 ppm Mn and 0.73ppm Zn.

Two foliar fertilizers Citren and Amco-Combi were applied. Citren is an organic nutrition contains 6% chelated iron, zinc and manganese and 15% citric acid. Citren was obtained from the ministry of Agriculture. While Amco-combi is a foliar fertilizer which contain 6% Mn, Mg and Cu, in chelated forms (obtained from Modern Company fir Fertilizer Production Lid.).

Citren fertilizer was sprayed at three concentrations 3.34, 6.66 and 13.3 cm/L, while Amco-combi levels were 1, 2 and 4 mg/5L beside the control (tap water) as seven treatments for each variety. Plants were sprayed twice, the first one month after cultivation while the second one month later. Flowers were harvested weekly and total seasonal yield gm/plant and Kg/fed. Had been calculated, and the following characters were estimated:

Vegetative growth and yield components included: - Plant height (cm), number of branches per plant, fresh and dry weight of the aerial parts gm/plant and calculated as ton/feddan, fresh and dry weight (gm/plant) of seasonal flower heads, and dry flower heads yield (ton/feddan). Chemical analysis: The following constituents were determined:

Essential oil percentages: which were determined in the air-dried flowers for each treatment according to Egyptian pharmacopoeia<sup>[15]</sup>. The essential oil yield (cc) per plant and L/fed. were calculated.

Oil constituents: The essential oil produced from various treatment at the second season was dehydrated over Anhydrous Sodium Sulphate, then subjected to GLC analysis with FID model<sup>[16]</sup>. Two relative percent of the different constituents was determined by Varian 4270 integrator, the identification of these compounds was achieved by matching their retention times with those of authentic samples injected in the same condition. More confirmation had been carried out by injection of the authentic samples with the oil samples.

Total carbohydrate percentages: estimated in dried leaves using the colorimetric method of Dubois *et al*<sup>[17]</sup>.

Photosynthetic pigments: Total Chlorophyll and Carotenoids contents were determined as described by AOAC<sup>[18]</sup>.

Statistical analysis: The data were statistically analyzed according to Gomez and Gomez<sup>[19]</sup>. The L.S.D. values were assessed whenever F. values at 0.05 levels were significant.

## RESULTS AND DISCUSSIONS

**Vegetative growth characters:** From data present in Table (1 and 2) it is clear in general that growth characters of different varieties and source of Calendula plants were significantly difference. Thus, the heighest plants and the maximum number of flowers were those obtained from *C. stellata*, while the shortest one with smallest number of heads of flower were the French source of *C. officinalis*. Meanwhile, *C. officinalis* (French source) carried the greatest number of branches compared with other two types. The fresh and dry weight of herb (gm/plant and ton/fed) of three types were arranged in descending order as *C. stellata*>, *C. officinalis* (French source) *C. officinalis* (Slovakian source).

On the other hand, the application treatment of Citren or Amco-combi were significantly improved the growth characters including plant height, number of branches, number of flowers, fresh and dry weight of herb (Table 1 and 2). However, the rates of improvement in growth varied between the species as well as the rates used of the two foliar fertilizers. Thus, the foliar application with various levels of Amco-combi caused the highest increments compared with Citren levels and control. Meanwhile, the highest dose was significantly effective in promoting and enhancing all the studied characters. Otherwise, the relative increased percent in comparison to control for *C. officinalis* (French source) were 34.0 and 36.4% for plant height 66.6 and 51.5 % for number of branches and 75.2 and 64.7% for number of flower heads, for first and second season, respectively. For the fresh and dry weight of herb, the highest significant pronounced increase were obtained by spraying plants with the highest rate of Amco-combi, which gave the heaviest plants weight. However the increased percent over control for these characters during first and second seasons reached to 71.8 and 71.4% for fresh weight gm/plant, 81.3 and 80.6% for dry weight (gm/plant), 80.9 and 94.3% for fresh weight (ton/fed), 159.4 and 94.1% for dry weight (ton/fed).

Concerning the combined effects, it is evident that the highest mean values of different growth characters were obtained with *C. stellata* plants treated with highest Amco-combi levels recorded 109.3 cm for plant height,

12.5 for number of branches/plant, 425.11 for number of flower head, 478.7 gm/plant for fresh herb weight, 109.18 gm/plant dry weight of herb, 6.9 ton/fed fresh herb and 1.950 dry herb weight .

**Yield of flower head:** As shown in Table (3), *C. officinalis* Slovakian source plants carried the heaviest yield of flower heads (gm/plant and ton/fed) followed by the second source of *C. officinalis* (French) then, *C. stellata*. Meanwhile, the difference between the three types for this character was significant. It is clear to notice that while *C. stellata* carrying the highest number of flower it having the lowest yield of flower head which meaning that these flower head has a very small size. However, *C. officinalis* Slovakian source, produced 14.10 and 15.37ton/fed. of flower, in first and second seasons, while *C. officinalis* (French) source produced 9.73 and 10.0 ton/fed and *C. stellata* giving 6.21 and 7.20 ton/fed for both successive seasons, respectively.

On the other hand, the flowers head yield of all varieties appeared positive effect by the foliar application of two foliar fertilizers. The application of Amco-combi at highest level (4 mg/5L) resulted in highest significant promotion in flower heads yield (gm/plant or ton/fed) at both seasons. It clearly noticed that increasing in Citren and Amco-combi levels progressively stimulated flower head yield. Meanwhile, Amco-combi was significantly favorable than Citren foliar fertilizer. Thus, the increase percentage in flower yield (gm/plant) reached to 49.4 and 50.0% and 47.42 and 40.67% (ton/fed) in comparison with control plant at first and second season, respectively.

It is obvious from tabulated data that using the combination between highest level of Citren or Amco-combi application to *C. officinalis* Slovakian source giving the highest flower heads yield (gm/plant and ton/fed) compared with other species and levels used. Moreover, Slovakian source of *C. officinalis* sprayed with 4mg/5L Amco-combi followed by 2 mg/5L was the superior treatment giving the highest flower head yield, throughout the two successive season.

The stimulatory effect of Amco-combi that contain Mn, Mg and Cu or Citren, that contain Zn, Mn, and Fe may be due to the fact that these micronutrients constitute an important part of several enzymes; thus they might play a key role in plant regeneration<sup>[20]</sup>. The effect may be explained by the synthesis of RNA and photosynthetic pigments which lead to accumulation of more metabolites, food reserves; as well as; the healthy good growth (Russell,<sup>[21]</sup>). The observed results were supported with those reported by Noggle and Fritz<sup>[22]</sup> who indicated that Mg, Fe, Cu and Mn are known to be essential for plant growth and very closely tied with

**Table 1:** Effect of Citren and Amco-combi fertilizers on plant height, number of branches and flower heads per plant of *Calendula* sp. During two successive seasons (2001-2002 and 2002-2003)

Characters/ Treatments	Plant height (cm)		No. of branches/plant		No. of flowers/plant	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
<i>Calendula officinalis</i> (French)						
Control	38.02	40	7.67	8.33	15.67	18.22
Citren 16.7 cm/5L.	40.53	42	9.33	10.27	17.67	19.54
Citren 33.3 cm/5L.	42.25	44.33	10.37	11.5	21.33	22.5
Citren 66.7 cm/5L.	44.36	46.67	11.67	12.33	22.33	27.18
Amco-combi 1mg/5L.	48.73	45	8.87	9.3	24.67	26.33
Amco-combi 2mg/5L.	50.4	48.67	10.37	11.15	26.18	30.11
Amco-combi 4mg/5L.	54.67	52	12	12.89	29.33	31.25
Average	45.57	45.52	10.04	10.82	22.45	25.02
<i>Calendula officinalis</i> (Slovakian)						
Control	56.67	58.3	6.67	7.6	36	38.07
Citren 16.7 cm/5L.	59	61.13	7.33	8.67	42.67	42.86
Citren 33.3 cm/5L.	61.33	65	8.17	9.33	44.5	49.5
Citren 66.7 cm/5L.	66.67	68.33	10.33	10.57	49	56.33
Amco-combi 1mg/5L.	63.55	64	9	10.25	54.33	60.54
Amco-combi 2mg/5L.	65	74.2	11	11.5	57.7	63.18
Amco-combi 4mg/5L.	69.33	81.04	11.67	12.01	61	68.33
Average	63.08	67.43	9.17	9.99	49.31	54.16
<i>Calendula stellata</i> (French)						
Control	75.58	79.33	6.67	7.6	216	262.21
Citren 16.7 cm/5L.	80.67	89.57	8.67	9.67	255.33	290.03
Citren 33.3 cm/5L.	88.65	95.67	11.33	12.09	290.62	327.33
Citren 66.7 cm/5L.	95.92	96.92	12.67	12.54	330.67	352.5
Amco-combi 1mg/5L.	94.67	98.67	8.33	10.5	290.89	380.77
Amco-combi 2mg/5L.	99.33	101.67	9.67	11.89	357.67	401.18
Amco-combi 4mg/5L.	104.21	109.33	11.67	12.54	375.5	425.11
Average	91.29	109.75	9.86	10.98	302.38	348.45
<b>Avarage</b>						
Control	56.76	59.21	7.07	8.24	89.22	106.17
Citren 16.7 cm/5L.	59.37	64.23	8.55	9.54	105.22	117.48
Citren 33.3 cm/5L.	64.08	68.33	9.96	14.08	118.82	133.11
Citren 66.7 cm/5L.	68.78	70.64	11.56	15.69	134	145.34
Amco-combi 1mg/5L.	68.99	69.22	8.73	10.5	123.3	155.88
Amco-combi 2mg/5L.	71.69	74.85	10.35	11.51	147.18	164.82

**Table 1:** (Continued)

Amco-combi 4mg/5L.	76.07	80.79	11.78	12.48	156.28	174.9
<b>LSD at 5% for</b>						
Variety (V)	0.16	1.26	0.57	0.16	0.66	0.54
Fertilizer (R)	0.16	0.88	0.81	0.19	0.82	0.75
V x F	0.1	1.53	0.9	0.32	1.41	1.46

**Table 2:** Effect of Citren and Amco-combi foliar fertilizers on fresh and dry weights of herb gm/ plant and ton/feddan of Calendula sp. during two successive seasons (2001-2002 and 2002-2003).

Characters/ Treatments	Herb fresh weight (g)		Herb dry weight (g)		Herb fresh weight ton/fed.*		Herb dry weight ton/fed.*	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
<i>Calendula officinalis</i> (French)								
Control	218.32	251.43	44.74	48.21	3.57	3.806	0.05	0.868
Citren 16.7 cm/5L.	240.37	280.72	54.81	63.09	4.327	5.046	0.987	1.136
Citren 33.3 cm/5L.	276.9	317.67	62.13	72.43	4.984	5.718	1.118	1.304
Citren 66.7 cm/5L.	318.33	361.33	72.58	82.38	5.73	6.504	1.304	1.483
Amco-combi 1mg/5L.	279.68	286.92	63.71	65.42	5.034	5.165	1.148	1.178
Amco-combi 2mg/5L.	369.5	373.23	84.85	85.1	6.651	6.718	1.517	1.532
Amco-combi 4mg/5L.	394.48	428.5	94.49	97.7	7.461	8.073	1.701	1.841
Average	299.65	328.54	68.19	73.48	5.4	5.86	1.12	1.33
<i>Calendula officinalis</i> (Slovakian)								
Control	123.33	172.64	28.12	39.36	2.22	3.108	0.506	0.709
Citren 16.7 cm/5L.	141.77	198.76	32.32	45.32	2.552	3.578	0.518	0.816
Citren 33.3 cm/5L.	152.43	204.68	34.77	46.63	2.744	3.682	0.626	0.839
Citren 66.7 cm/5L.	179.9	230.18	41.02	52.48	3.148	4.143	0.738	0.945
Amco-combi 1mg/5L.	189.67	229.87	43.24	53.42	3.418	4.138	0.778	0.962
Amco-combi 2mg/5L.	203.1	273.52	46.31	62.36	3.656	4.923	0.834	1.123
Amco-combi 4mg/5L.	235.4	309.87	53.79	70.65	4.243	5.938	0.968	1.354
Average	123.33	172.64	28.12	39.36	2.22	3.108	0.506	0.709
Control	175.09	231.36	39.94	52.89	3.15	4.22	0.71	0.96
<i>Calendula stellata</i> (French)								
Control	299.67	345.07	68.32	78.86	5.394	5.671	1.221	1.293
Citren 16.7 cm/5L.	335.14	419.47	76.42	95.64	6.033	7.55	1.376	1.722
Citren 33.3 cm/5L.	393.38	481.67	89.69	109.81	7.081	8.67	1.614	1.977
Citren 66.7 cm/5L.	440.32	514.7	100.39	117.35	7.926	9.265	1.808	2.112
Amco-combi 1mg/5L.	350.33	459.79	79.88	104.83	6.306	8.276	1.438	1.887
Amco-combi 2mg/5L.	404.69	550.8	92.26	125.63	7.284	9.914	1.661	2.261
Amco-combi 4mg/5L.	472.12	579.7	107.64	132.17	8.498	10.435	1.938	2.379
Average	385.09	478.74	87.8	109.18	6.93	8.54	1.58	1.95
<b>Average</b>								

**Table 2:** (Continued)

Control	213.77	256.38	47.06	55.47	3.721	4.195	0.592	0.957
Citren 16.7 cm/5L.	239.09	299.65	54.51	68.01	4.304	5.391	1.419	1.225
Citren 33.3 cm/5L.	274.23	334.67	62.15	76.29	4.936	6.0637	1.119	1.373
Citren 66.7 cm/5L.	312.85	368.73	71.33	84.07	5.601	6.637	1.283	1.198
Amco-combi 1mg/5L.	273.22	325.52	62.27	74.56	4.919	5.86	1.121	1.342
Amco-combi 2mg/5L.	325.76	399.18	79.47	91.03	5.862	7.185	1.337	1.639
Amco-combi 4mg/5L.	367.17	439.35	85.3	100.17	6.734	8.149	1.536	1.858
<b>LSD at 5% for For</b>								
Variety	2.76	2.16	0.14	0.12	0.051	0.014	0.021	0.075
Fertilizer	2.42	2.22	0.11	0.07	0.031	0.012	0.017	0.058
V x F	4.19	4.38	0.19	0.12	0.051	0.021	0.029	0.033

fed.\* = Feddan = 4200 m<sup>2</sup>

**Table 3:** Effect of Citren and Amco-combi foliar fertilizers on yield of flower heads ( gm/plant and ton/feddan) of *Calendula* sp. during two successive seasons (2001-2002 and 2002-2003)

Calendula varieties/ Treatments	<i>Calendula officinalis</i> (French)		<i>Calendula officinalis</i> (Slovakian)		<i>Calendula stellata</i> (French)		Average of fertilizers		LSD at 5%	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
<b>Seasons</b>										
<b>Yield of flower heads ton/fed.*</b>									<b>Characters</b>	
Control	346.5	430.75	563.67	626.7	223.1	230.8	377.75	429.42	Variety	52.33 40.30
Citren 16.7 cm/5L.	474.4	502.35	688.12	824.7	280.8	307.87	481.11	544.97		
Citren 33.3 cm/5L.	488.87	540.18	749.81	1071.3	318.33	350.67	519	654.05	Fertilizer	45.68 32.91
Citren 66.7 cm/5L.	530.8	578.1	962.11	1112.67	340.33	389.14	611.08	693.3		
Amco-combi 1mg/5L.	515.97	580.33	822.44	962.11	365.18	418.33	567.86	653.59	V x F	79.13 50.07
Amco-combi 2mg/5L.	580.33	618.17	1076.41	1126.33	376.12	465.11	677.62	736.53		
Amco-combi 4mg/5L.	609.67	652.5	1132.33	1250.17	402.76	492.67	714.92	798.44		
Average	506.65	557.48	856.41	996.28	329.52	379.23	564.19	644.33		
<b>Yield of flower heads ton/fed.*</b>										
Control	6.237	7.754	10.146	11.281	4.016	4.154	6.8	7.93		
Citren 16.7 cm/5L.	8.539	9.051	12.386	14.845	5.054	5.538	8.66	9.81		
Citren 33.3 cm/5L.	8.799	9.723	13.497	19.283	5.73	6.312	9.34	11.77	Variety	0.237 0.110
Citren 66.7 cm/5L.	9.554	10.406	17.32	12.028	6.126	7.001	11	9.81		
Amco-combi 1mg/5L.	9.287	10.447	14.804	17.318	6.573	7.53	10.22	11.77	Fertilizer	0.422 0.117
Amco-combi 2mg/5L.	10.446	11.128	19.375	20.274	6.77	8.372	12.2	13.26		
Amco-combi 4mg/5L.	10.974	11.745	20.382	22.503	7.25	8.868	12.87	14.37	V x F	0.732 0.125
Average	9.73	10	14.1	15.37	6.21	7.2	10.01	10.86		

fed.\* = Feddan = 4200 m<sup>2</sup>

**Table 4:** Effect of Citren and Amco-combi foliar fertilizers on oil %, oil yield ( cc/plant and L/feddan) of *Calendula* sp. during two successive seasons (2001-2002 and 2002-2003).

Characters/ Treatments	Oil %		Oil yield cc/plant		Oil yield L/fed.*	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
<b><i>Calendula officinalis</i> (French)</b>						
Control	0.062	0.069	0.215	0.297	3.87	5.366

**Table 4:** (Continued)

Citren 16.7 cm/5L.	0.065	0.074	0.318	0.471	5.724	8.478
Citren 33.3 cm/5L.	0.066	0.076	0.341	0.514	6.138	9.952
Citren 66.7 cm/5L.	0.068	0.076	0.415	0.595	7.47	10.71
Amco-combi 1mg/5L.	0.066	0.083	0.313	0.417	5.634	7.506
Amco-combi 2mg/5L.	0.07	0.084	0.372	0.476	6.696	8.568
Amco-combi 4mg/5L.	0.079	0.086	0.517	0.561	9.306	10.098
Average	0.068	0.078	0.356	0.476	5.691	8.668
<b><i>Calendula officinalis</i> (Slovakian)</b>						
Control	0.132	0.158	0.744	0.99	13.392	17.82
Citren 16.7 cm/5L.	0.155	0.163	1.067	1.344	19.207	24.192
Citren 33.3 cm/5L.	0.161	0.187	1.206	2.003	21.708	36.05
Citren 66.7 cm/5L.	0.168	0.19	1.616	2.114	29.088	38.052
Amco-combi 1 mg/5L.	0.169	0.164	1.39	1.519	25.02	27.342
Amco-combi 2mg/5L.	0.171	0.169	1.841	1.819	33.138	32.742
Amco-combi 4mg/5L.	0.176	0.174	1.993	1.97	35.874	35.546
Average	0.162	0.172	1.408	1.68	25.347	30.249
<b><i>Calendula stellata</i> (French)</b>						
Control	0.146	0.149	0.326	0.344	5.868	6.192
Citren 16.7 cm/5L.	0.151	0.157	0.424	0.483	7.632	8.694
Citren 33.3 cm/5L.	0.154	0.163	0.49	0.519	8.82	9.342
Citren 66.7 cm/5L.	0.158	0.185	0.538	0.63	9.684	11.34
Amco-combi 1mg/5L.	0.155	0.159	0.566	0.665	10.188	11.97
Amco-combi 2mg/5L.	0.178	0.164	0.669	0.763	12.042	13.734
Amco-combi 4mg/5L.	0.194	0.189	0.781	0.931	14.058	16.758
Average	0.166	0.167	0.542	0.619	9.756	11.147
<b>Average</b>						
Control	0.113	0.125	0.428	0.544	7.71	9.792
Citren 16.7 cm/5L.	0.124	0.131	0.603	0.766	10.854	13.788
Citren 33.3 cm/5L.	0.127	0.142	0.679	1.012	12.222	18.448
Citren 66.7 cm/5L.	0.131	0.15	0.856	1.113	15.414	20.034
Amco-combi 1 mg/5L.	0.13	0.135	0.756	0.867	13.188	15.606
Amco-combi 2 mg/5L.	0.14	0.139	0.961	1.019	16.392	18.348
Amco-combi 5 mg/5L.	0.15	0.15	1.097	1.154	19.746	20.801
<b>LSD at 5% for</b>						
Variety (V)	0.008	0.009	0.022	0.018	0.102	0.105
Fertilizer (R)	0.009	0.01	0.018	0.026	0.111	0.114
V x F	0.012	0.012	0.031	0.045	0.12	0.123

fed.\* = Feddan = 5200 m<sup>2</sup>

reactions of photosynthetic activity in plants.

The promotive effect of foliar fertilizers were in agreement with those obtained by El-Leithy<sup>[23]</sup> and Youssef<sup>[24]</sup> they reported that foliar spraying with Fe, Zn, and Mn, had stimulatory responses in growth characters of *Nigella sativa*. Similarly, Khalil *et al.*<sup>[9]</sup> on *Sinapis alba* and *Nigella sativa* using Cristalon, Amco-Better and Floactive, Refaat and Balbaa<sup>[25]</sup> applying mixed fertilizer contains Zn, Fe and Mn to lemongrass, Aziz and El-Sherbeny<sup>[13]</sup> with *Sideritis montana* L., reported that vegetative growth parameters and yield of different plants recorded significant increase with the application of various foliar fertilizer.

**Chemical constituents:** - Essential oil percentage and yield: The data presented in Table (4) showed the essential oil content of various species and sources of *Calendula* and their response to foliar fertilizer with different levels of Citren and Amco-combi.

The results revealed that, in general, the Slovakian source of *Calendula officinalis* contain the highest percentage of essential oil percentages, and gave the highest yield of essential oil, followed by *C. stellata* and *C. officinalis* French source which recorded lowest values for these characters.

Regarding the effect of Citren and Amco-combi on volatile oil formation of various *Calendula* types, it is noticed that all foliar fertilizers levels significantly increased the volatile oil percentage and its absolute yield in both seasons. Generally, Amco-combi and Citren at highest levels had more significant enhancement effect on volatile oil percent and yield in comparison to lower levels or control. The superior effect of Amco-combi in stimulation oil yield accumulation, could be attributed to its superior effect on promotion and increasing the number of flower heads as well as yield of these plants comparing with Citren foliar fertilizer.

Concerning the combined effects, it was evident that the highest oil yield percentage value was recorded with (French source) of *C. officinalis* treated with highest level of Amco-combi which recorded (0.194 and 0.189%) for first and second seasons, while the highest yield (gm/plant and ton/fed) were obtained with Slovakian source of *C. officinalis* using also Amco-combi at highest level (1.99-1.97 gm/plant and (35.874 - 35.546 ton/fed) for two successive seasons, respectively.

The increment in the produced essential oil yield per plant and feddan as a result of using Citren or Amco-combi, could be explained through their effective role in raising flower number and weight. Otherwise, the essential oil biosynthesis in plant is the result of integration of several metabolic pathways which require linking of

several steps such as continuous production of precursors, their transport and trans-location to the active site of synthesis, and finally upon normal functioning of associated metabolic pathways such as carbon fixation, respiration, and isoprenoid pathway. Under nutrient deficiency, there might be an alteration in the availability, utilization of precursors, as well as, the content of photosynthesis and accumulation<sup>[26]</sup>. The above mentioned results could be supported by the finding of El-Kholy *et al.*<sup>[27]</sup> on *Ocimum basilicum* treated by fertex, Khalil *et al.*<sup>[9]</sup> on *Sinapis alba* and *Nigella sativa* applying three foliar fertilizers, Cristalon, Amco-Better and Floactive, Kandeel,<sup>[10]</sup> spraying some micro-nutrients on *Ocimum basilicum*, Aziz<sup>[28]</sup> applying magnesium and copper to *Calendula* plants. Aziz and El-Sherbeny<sup>[13]</sup> treating *Sideritis montana* with Fe, Mn and Zn.

Oil composition: The qualitative and quantitative analyses of the essential oil of *calendula spp.* were performed by G.L.C. and shown in Tables (5, 6 and 7). The identified constituents of calendula oil extracted reached to 92.58, 98.76, and 92.94 % for *Calendula officinalis* L. (French variety), *Calendula officinalis* L. (Slovakian variety) and *Calendula stellata* L respectively; the complementing percents were for unknown components of the oil. Meanwhile, the total hydrocarbons constituted 28.09, 26.72, and 28.61 %, while the corresponding percents of oxygenated compounds were 64.49, 72.04, and 64.33 % for the three varieties of *calendula* in same order.

The major oil component of French and Slovakian source of *Calendula officinalis* L. was linalyl acetate considered as an oxygenated compound and reached to 36.50 and 38.64% respectively. The second main oxygenated compound was linalool that represented at 20.88 and 19.61%. On the other hand, linalool take place as the major one 34.40% for *Calendula stellata* L., while linalyl acetate and Limonene was 21.63%. and showed superiority over other hydrocarbon compounds, the least compound it constituted about 19.16, 18.28, and 21.63 % in the three traits respectively.

The total hydrocarbons percentage of *Calendula* oil responded in a decreasing manner for the increasing the doses of the applied two fertilizers. On the contrary; the oxygenated compounds were gradually increasing in response to the increasing doses of the two foliar fertilizers applied to *Calendula officinalis* L. (French source), and *Calendula stellata* L. while a reversed tendency was noticed for *Calendula officinalis* L. (Slovakian source).

The application of Citren and Amco-combi resulting in increasing linalyl acetate in *C. officinalis* of French and Slovakian (source) as well as linalool for *C. stellata*. In addition, limonene content promoted and rose for various

**Table 5:** Effect of Citren and Amco combi foliar application on the essential oil constituents in flower heads of *Calendula officinalis* (French).

Treatments/ Constituents	Control	Citren 16.7 cm/5L	Citren 33.3 cm/5L	Citren 66.7 cm/5L	Amco-combi 1mg/5L	Amco-combi 2mg/5L	Amco-combi 4mg/5L
"-Pinene	0.45	0.44	0.4	0.38	0.46	0.43	0.22
\$\$-Pinene	2.47	1.96	2.25	1.42	0.33	2.07	2.12
Myrcene	0.19	1.16	0.57	0.46	0.35	0.14	0.45
Phellandrene	1.12	0.98	0.27	0.77	2.16	1.16	0.17
P-cymene	2.76	2.07	0.18	0.19	0.25	0.23	0.45
Limonene	19.16	21.13	21.97	19.84	23.9	22.5	19.77
Terpinene	0.98	0.77	0.65	0.98	0.1	1.11	0.98
Caryophyllene	0.96	1.11	0.94	1.11	0.49	0.88	0.88
<b>Total hydrocarbons</b>	<b>28.09</b>	<b>29.62</b>	<b>27.23</b>	<b>25.15</b>	<b>28.04</b>	<b>28.52</b>	<b>25.04</b>
1,8 Cineol	0.47	0.52	2.27	0.39	2.36	1.33	1.34
Linalool	20.88	22.12	20.5	20.03	18.19	18.06	19.66
Linalyl acetate	36.5	41.33	45.79	48.11	42.03	46.5	47.48
Camphor	1.98	0.77	0.3	0.04	1.04	0.96	0.23
Borneol	0.96	0.84	0.56	0.53	1.09	1	0.15
Carvone	0.43	0.41	0.56	0.86	0.18	0.08	0.56
Geraneol	2.66	2.18	0.15	0.12	2.66	2	1.17
Geranyl acetate	0.17	0.25	0.03	2.1	1.08	0.54	0.43
Caryophyllene oxide	0.44	0.47	0.32	0.16	0.38	0.17	0.09
Total oxygenated	64.49	68.89	70.48	72.34	69.01	70.64	71.11
Un-identified	7.42	1.53	2.29	2.51	2.95	0.84	3.85

**Table 6:** Effect of Citren and Amco combi foliar application on the essential oil constituents in flower heads of *Calendula officinalis* (Slovakian).

Treatments/ Constituents	Control	Citren 16.7 cm/5L	Citren 33.3 cm/5L	Citren 66.7 cm/5L	Amco-combi 1mg/5L	Amco-combi 2mg/5L	Amco-combi 4mg/5L
"-Pinene	0.38	0.52	0.47	0.45	0.46	0.4	0.33
\$\$-Pinene	2.18	2.09	1.96	1.33	0.77	1.78	2.02
Myrcene	0.29	1.06	0.88	0.39	0.36	0.44	0.46
Phellandrene	0.74	0.87	0.75	0.64	2.09	1.33	0.98
P-cymene	2.22	2	0.09	0.19	0.88	0.62	0.45
Limonene	18.28	21.54	19.33	19	23.6	21.55	20.33
Terpinene	1.57	0.89	0.77	0.56	0.44	1.33	0.99
Caryophyllene	1.06	1.18	0.87	1.25	51	0.97	1.13
<b>Total hydrocarbons</b>	<b>26.72</b>	<b>30.15</b>	<b>25.12</b>	<b>23.81</b>	<b>27.52</b>	<b>27.42</b>	<b>26.69</b>
1,8 Cineol	0.66	0.98	2.21	0.55	2.54	1.99	1.47
Linalool	19.61	24.15	21.5	18.48	16.18	19.5	18.66

**Table 6:** (Continued)

Linalyl acetate	38.64	39.27	43.17	45.67	42.18	44.77	44.98
Camphor	5.63	0.65	0.4	0.11	0.98	0.76	0.18
Borneol	1.84	0.84	0.56	0.53	1.09	1	0.15
Carvone	2.54	0.51	0.53	0.77	0.28	0.08	0.56
Geraneol	1.9	2.18	0.15	1.02	2.16	1.96	0.22
Geranyl acetate	0.17	0.25	0.13	1.99	1.08	0.36	0.55
Caryophyllene oxide	1.05	0.47	0.35	0.26	0.52	0.22	0.13
Total oxygenated	72.04	69.3	69	69.38	67.01	70.64	66.9
Un-identified	1.24	0.55	5.88	6.81	5.47	1.94	6.01

**Table 7:** Effect of Citren and Amco-combi foliar application on the essential oil constituents in flower heads of *Calendula stellata* L.

Treatments/ Constituents	Control	Citren 16.7 cm/5L	Citren 33.3 cm/5L	Citren 66.7 cm/5L	Amco-combi 1mg/5L	Amco-combi 2mg/5L	Amco-combi 4mg/5L
"-Pinene	0.44	0.41	0.29	0.26	0.21	0.07	0.04
\$-Pinene	3.18	4.07	3.26	2.84	5.9	5.66	5.2
Myrcene	0.99	1.35	1.42	1.02	0.68	0.69	0.59
Phellandrene	0.25	0.43	0.33	0.41	0.29	0.12	0.22
P-Cymene	0.01	0.04	0.19	0.85	0.5	0.49	0.31
Limonene	21.63	17.57	17.48	18.65	21.1	21.37	22.24
Terpinene	0.99	1.15	1.42	0.9	1.02	0.68	0.63
Caryophyllene	1.12	0.98	0.77	1.12	1.18	1.07	0.99
<b>Total hydrocarbons</b>	<b>28.61</b>	<b>26</b>	<b>25.16</b>	<b>26.05</b>	<b>31.88</b>	<b>30.15</b>	<b>25.02</b>
1,8 Cineol	0.56	0.44	0.64	1.12	0.68	0.18	1.18
Linalool	34.4	42.37	45.71	53.78	35.84	38.18	40.31
Linalyl acetate	21.63	18.65	16.57	12.48	21.8	22.24	24.1
Camphor	0.81	0.87	0.65	0.47	1.21	1.02	0.81
Borneol	0.6	0.48	0.67	0.07	0.54	0.72	1
Carvone	3.18	2.84	3.26	4.07	2.41	1.85	4.18
Geraneol	2.76	1.57	2.42	0.73	1.11	2.17	2.75
Geranyl acetate	0.3	0.19	0.22	0.16	0.54	0.38	0.22
Caryophyllene oxide	0.09	0.12	0.31	0.18	0.01	0.22	0.18
Total oxygenated	64.33	67.53	70.45	73.06	64.14	66.96	74.73
Un-identified	7.96	6.47	4.39	0.89	3.98	0.89	0.25

*Calendula* spp. Citren foliar fertilizer; however; was more effective on the constituents of the oil than Amco-combi. The application of Citren at the high level gave the most pronounced effect with *Calendula stellata* as it led

to the highest percent of linalool reached to 53.78 %, while it was 34.40% in untreated control plants.

The constituents of the volatile oil of *Calendula* spp. had been previously identified by Megahid<sup>[29]</sup> and

**Table 8:** Effect of Citren and Amco-combi foliar fertilizers on total carbohydrate percentages, total chlorophyll content mg/g in herb and total carotenoids contents (mg-1) in flower heads of *Calendula sp.* during two successive seasons (2001-2002 and 2002-2003).

Characters/ Treatments	Total carbohydrates (%)		Total chlorophyll (mgG <sup>1</sup> )		Total carotenoids (mgG <sup>1</sup> )	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
<i>Calendula officinalis</i> (French)						
Control	27.33	28	0.61	0.62	0.81	0.83
Citren 16.7 cm/5L.	29.12	29.33	0.66	0.69	0.88	0.89
Citren 33.3 cm/5L.	31.5	33.4	0.71	0.76	0.91	0.93
Citren 66.7 cm/5L.	39.22	35.67	0.75	0.77	0.94	0.94
Amco-combi 1mg/5L.	29.49	30.02	0.67	0.82	0.87	0.83
Amco-combi 2mg/5L.	32.57	30.67	0.74	0.85	0.91	0.93
Amco-combi 4mg/5L.	33.64	32.54	0.76	0.9	0.94	0.95
Average	36.09	33.68	0.8	0.99	0.97	0.96
<i>Calendula officinalis</i> (Slovakian)						
Control	26.44	25.17	0.69	0.68	0.88	0.87
Citren 16.7 cm/5L.	29.67	29	0.85	0.88	0.84	0.95
Citren 33.3 cm/5L.	31	29.67	0.89	0.92	0.98	0.98
Citren 66.7 cm/5L.	33.54	31.24	0.96	0.94	1.01	1.05
Amco-combi 1mg/5L.	30.13	32.11	0.87	0.98	0.88	0.97
Amco-combi 2mg/5L.	32.09	34	0.92	1.06	0.94	1.05
Amco-combi 4mg/5L.	34.11	35	0.98	1.29	1.09	1.11
Average	35.82	38.37	1.06	1.37	1.08	1.16
<i>Calendula stellata</i> (French)						
Control	24.5	25.68	0.65	0.67	0.7	0.71
Citren 16.7 cm/5L.	24.67	29.02	0.71	0.7	0.74	0.77
Citren 33.3 cm/5L.	28.13	28.67	0.75	0.75	0.78	0.81
Citren 66.7 cm/5L.	30.18	30.54	0.8	0.79	0.81	0.85
Amco-combi 1mg/5L.	25.37	29.33	0.75	0.76	0.81	0.84
Amco-combi 2mg/5L.	32.67	34.5	0.87	0.89	0.89	0.91
Amco-combi 4mg/5L.	36.59	37	0.97	0.95	0.94	0.98
Average	28.87	30.68	0.78	0.79	0.81	0.84
<b>Average</b>						
Control	26.09	26.28	0.65	0.68	0.79	0.8
Citren 16.7 cm/5L.	27.82	29.12	0.74	0.76	0.82	0.87
Citren 33.3 cm/5L.	30.21	30.58	0.78	0.81	0.89	0.91
Citren 66.7 cm/5L.	34.31	32.48	0.84	0.83	0.92	0.95
Amco-combi 1mg/5L.	28.33	30.48	0.76	0.85	0.85	0.88
Amco-combi 2mg/5L.	32.44	33.05	0.84	0.93	0.91	0.96
Amco-combi 4mg/5L.	34.78	34.84	0.9	1.05	0.99	1.01

**Table 8:** (Continued)

Average	33.59	34.24	0.88	1.05	0.95	0.99
<b>LSD at 5% level</b>						
Variety (V)	1.27	0.93	0.07	0.06	0.03	0.06
Fertilizer (F)	1.45	1.11	0.02	0.03	0.06	0.04
V x F	1.57	1.14	0.01	0.07	0.09	0.08

El-Sahhar *et al*<sup>[30]</sup> they mentioned that linalool where the major constituent in flower heads of *Calendula officinalis* L. Aziz and El-Sherbeny<sup>[13]</sup> on *Calendula officinalis* found that linalyl acetate surpassed linalool compound, and appeared to be the major one for *C. officinalis*.

**Total carbohydrate percentages:** The total carbohydrate content in the herb of *Calendula officinalis* (French source) recorded the highest percentage values comparing with the other two calendula-studied plants (Table 8). Thus, these percentages reached to 36.09 and 33.68% in first and second seasons, respectively, while the Slovakian source appeared 35.82 and 38.37% for the two successive seasons, and the lower content for *C. stellata* was 28.87 and 30.68%.

Foliar fertilizer application of either Citren or Amco-combi at various levels resulted in significant promotion in the accumulation of total carbohydrate for different sources and species. The highest carbohydrate percentages has been detected in first season with the application of the highest level of Citren or Amco-combi which recorded 34.31 and 32.48% for Citren and 34.84% for Amco-combi for first and second seasons, respectively.

Concerning the effect of the interaction between various levels of fertilizer and different *Calendula species*, it is evident that combined treatment of highest citren levels applied to *C. officinalis* French source resulted to highest carbohydrate accumulation that that reached to 29.49 and 30.02% for first and second seasons, respectively. These results reflects the role of applying the two foliar fertilizer in improving the total carbohydrate content in different *Calendula species* plants, which considered as a main source for the synthesis of essential oil as a secondary plant product.

The promotive effects of both foliar fertilizers on carbohydrate content were reported with several foliar nutrient applied to various medicinal and aromatic plants such as El-Kholy *et al*<sup>[27]</sup> and Kandeel<sup>[10]</sup> on *Ocimum basilicum*, Refaat and Balbaa<sup>[25]</sup> on lemongrass; Khalil *et al*<sup>[9]</sup> with *Sinapis alba* and *Nigella sativa*.

**Photosynthetic pigments:** Data presented in Table (8) revealed that *C. officinalis* (Slovakian source) contained

the highest chlorophyll and carotenoid contents comparing with the other two studied *Calendula*. However, Slovakian source replaced the second order while the third order was *C. stellata*.

The promotive effect for synthesis and accumulation of photosynthetic pigments has been recorded with various levels of Citren and Amco-combi applied to different *Calendula* plants. It was clear from the presented data that the highest levels of the two foliar fertilizers were more effective than lower levels, and Amco-combi fertilizer was superior than Citren. However, the highest photosynthetic pigments were found with *C. officinalis* (Slovakian source) treated with highest levels of Amco-combi which reached 0.98 and 1.29 mg<sup>-1</sup> for total chlorophyll and 1.09 and 1.11 mg<sup>-1</sup> for total carotenoids, for the two successive seasons, respectively. The corresponding values for untreated plants were 0.69 and 0.68 mg<sup>-1</sup> for total chlorophyll, 0.88 and 0.87 mg<sup>-1</sup> for total carotenoids for untreated plants.

The increase in the accumulation of photosynthesis pigments by foliar fertilizer may be due to the role of the micro-nutrients in chlorophyll synthesis. It has been well established that copper is directly involved in the photosynthetic electron transport chain as a constituent of plastocyanin. Plastocyanin operates as an electron carrier between the two photochemical systems of photosynthesis. However, the promotive effect for photosynthetic content could be supported with those reported by Mohamed and Wahba<sup>[31]</sup>, on *Tagetes erecta* and Aziz<sup>[28]</sup> on *Calendula officinalis*.

**Conclusion:** Generally, the obtained results revealed that under Egyptian conditions, *C. officinalis* Slovakian source was the most promising than the other two types, French source of *C. officinalis* and *C. stellata*, concerning vegetative growth and chemical constituents. Moreover, applying foliar fertilizer as Citren or Amco-combi, at highest level resulted in highest improvement of growth character, yield and chemical constituents.

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