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Supplying Calendula Plants with Some Micronutrients as Foliar Spray under Egyptian Soils Features

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Abstract: For the sake of studying the effect of foliar spray of Zn and Mn as sole or combined treatments on the growth, flowering and some chemical constituents of Calendula plantsthe present work was carried out. It had been deduced that foliar application of Zn and Mn alone or together gave significant increased on all Vegetative growth and flowers parameters under study as compared with control plants in both seasons.Data also, showed that the highest increase in growth,and flowering were especially presented when equal concentrations from Zn and Mn (0.30%) were added together as compared with other treatments. The interaction effects between different Zn and Mn foliar spray significantly promoted chemical constituents (head flower essential oil plant^{-1}, beta carotene, chlorophylla and b, total carotenoids, protein, xanthophylls, total flavonoides, total carotenoids in dry ray flowers, total carbohydrate percentage in stems and leaves N, P and K %.Zn, Mn and Fe (ppm) in herb and seed in Calendula plants.
Keywords: zinc (Zn) and manganese (Mn) foliar spray- vegetative growth - pigments, chemical constituents - Calendula plants.

Introduction

*Calendula officinalis*L. (Pot Marigold) is a plant in the genus Calendula (marigolds), in the family Asteraceae. It is probably native to southern Europe through its long history of cultivation makes its precise origin unknown, and maybe of garden origin. It is also widely naturalized further north in Europe (north to southern England) and elsewhere in warm temperate regions of the world. Many previous studies revealed that significant increase *Calendula Officinalis L.* is used for treatment of skin disorders and pain, and as a bactericide, antiseptic and anti-inflammatory[1,2,3,4] immunomodulatory[5].The petals andpollen grains of pot marigold contain, triterpenoid esters (ananti-inflammatory);carotenoids, flavaxanthin; auroxanthin; antioxidants, and compounds that are used in industrial paints and industrial nylon [6, 7 ,8, 9, 10] .

Foliar feeding is a way to reduce the use of chemical fertilizers and reduce environmental risks, especially nowadays to optimize fertilizer use in the world is a main policy. Using foliar fertilization is considered an effective preventive and curative measure to compensate their deficiency practice for the application of some micronutrients. Applications of micronutrients are more suitable than the soil application, Due to the rapid overcoming on deficient, easy to use, reduce the toxicity caused by accumulation and prevent of elements stabilization in the soil and avoiding losses through fixation. Micronutrients are essential for normal growth of plants, and involved in all metabolic and cellular function. Plants differ in their need for micronutrients such as manganese, zinc and iron [11]. Zinc (Zn) and Manganese (Mn) are well known essential micronutrients, which play important role in vegetative and reproductive cycle of plants[12,13,14].Several of these elements are redox-active that makes them essential as catalytically active cofactors in enzymes, others have

enzyme-activating functions and growth and development would stop if specific enzymes were not present in plant tissue and yet others fulfill a structural role in stabilizing protein[15,16,17]indicated that the application of zinc increases quality and quantity of yield, nitrogen efficiency, phosphor, carotenoid, and increases essential oil in *Calendula Officinalis*[18]. However, the morphological and physiological processes in plants are affected by several treatments of micronutrients that results to better yield in plants as in borago and calendula plants [17, 18]

The purpose of this research work was to study the effect of foliar spray of Zn and Mn as sole or combined treatments on the growth, flowering and some chemical constituents of Calendula plants.

Materials and Methods

A field experiment was conducted during two successive seasons (2014/2015 and 2015/2016) at the Experimental farm in Faculty of Agriculture, Fayoum University, Egypt to study the effect of foliar spray of Zn and Mn as sole or combined treatments on the growth, flowering and some chemical constituents of Calendula plants. Prior to any practices, a composite soil sample was taken from the soil surface (0-30 cm) of the experimental site.

Table 1. Some characteristics of the experimental site

Years	Mechanical analysis				Hydraulic conductivity (cm3/hr)						
	Sand %	Silt %	Clay %	Texture class							
2014	31.22	31.27	35.82	Sandy clay	0.029						
2015	32.87	31.99	37.67	Sandy clay	0.027						
Years	Chemical properties										
	N	P	K	Fe	Zn	Mg	Mn	EC dSm^{-1}	pH	CaCo3 %	O. M %
2014	17.55	23.77	96.67	3.77	0.82	0.33	8.77	2.91	7.51	4.73	1.27
2015	18.67	22.87	97.61	3.67	0.87	0.37	8.78	2.73	7.37	4.96	1.28

The seeds of Calendula plants were kindly obtainedprovided by the Department of Medicinal and AromaticPlants, Ministry of Agriculture, Egypt and sown in nursery on 15thAugust 2014 and 2015 seasons.Uniformseedlings were transplanted after 45 days from sowing date on 1st Oct. All Agricultural practices necessary for seedlings production were achieved. The experiment was laid out as a factorial design on the base of completely randomized blocks with three replications. The plot area was (6 × 1.80) = 10.8 m^2 and included three ridges; each ridge was 60 cm apart and 6m in length. The seedlings of Calendula were transplanted with the distance between seedlings was 30 cm.

Aqueous solution of $ZnSO_4 \cdot H_2O$ (Zn 35%, S 12%) or $MnSO_4 \cdot 3H_2O$ (Mn 26%, S 15%) were applied at the rate of 0, 0.15, 0.30 and 0.60 percent. The treatments were applied two times on the plants; the first time at 30 days after transplanting and the second is one month after the first application. Few drops of Triton B were added to the spray solution to serve as a wetting agent. The plants were sprayed twice at stem elongation and flowering stages. Sprays were applied in the morning (8-10 a.m.) using a hand pressure sprayer. The control plants were sprayed with distilled water. The volume of the spraying solution was maintained just to cover completely the plant foliage until drip. All the plants received normal agriculture practices whenever they needed. In addition, they received uniform treatments of manure 15 m^3/feddan., irrigation and fertilization at the field according to common practices i.e. Calendula plants fertilized with 300 kg/feddan ammonium sulphate (20.5%N), 300 kg/feddan calcium super phosphate (18% P_2O_2) and 100 kg/ feddan potassium sulphate (48%K_2O). Half of the N. and K. rates were added after 30 days from transplanting and the second application was done after 30 days from the first application. Application of calcium super phosphate was done as one dose during the preparation of the soil. All agriculture practices operations other than experimental treatments necessary for growth and development as cultivation, irrigation and pest control were followed whenever it was necessary and were done according to the recommendations of Ministry of Agriculture, Egypt.

Data recorded:

Studied Characters: after 75 days from transplanting, at full blooming, nine plants were chosen from each treatment to determine the studied characters:

Morphological Characters; plant height (cm), number of branches plant^{-1}, fresh and dry matter of leaves plant^{-1}(g), number of leaves plant^{-1}and fresh and dry matter plant^{-1} (g) of herb, flowers number plant^{-1}, inflorescences diameter plant^{-1}, inflorescences fresh and dry matter plant^{-1}, fresh and dry matter of ray flowers plant^{-1} and seed mass plant^{-1}.

Chemical constituents;

1. Extraction of essential oil:Dry whole plant and flower head at flowering stage (50g) were subjected to Hydro distillation for 3h using a Clevenger type Hydro distillation for 3h using a Clevenger type [21].
2. Chlorophylla, b and total carotenoids in leaves were determined using the method described by[22].
3. Pigments in dry flowers (Beta-carotene and xanthophylls) mg g^{-1}: the pigments were determined at last collection according to method described by [23].
4. Xanthophyll content was determined at wavelength of 470 and 485 nm according to [24]
5. Total flavonoids (mg g^{-1} D. M.): were determined in dried flower-head according to [25]
6. Total carotenoids in dry ray flowers (mg g^{-1} D. M.): were determined in dried flower head according to [26].
7. Total carbohydrate percentage in stems and leaves (%): dry matter of herb of each treatment was used for determination total carbohydrates% were colorimetrically determined using phenol-sulphoric acid reagent method as outlined by [27]
8. The percentage of total free amino acids (%):dry matter of herb were extracted by using ethanol 80% then, determined using ninhydrin reagent method as outlined by [28]
9. Total N, P and K,in herb and seeds were determined according to the methods of the [29.] Zinc, manganese, iron content were determined using atomic absorption spectrophotometer to method described by [29]

Statistical analysis; the means of data recorded in the two successive seasons were subjected to the analysis of variance according to [30] The Least Significant Differences (LSD) at *P*=0.05 level was used to verify the differences between means of the

Results and Discussion

Effect of foliar application of Zinc, Manganese and their interaction on Vegetative growth and flowers parameters in Calendula plants:

From the data in Tables (2&3) it is obvious that foliar application of zinc and manganese alone or together gave significant increased on all vegetative growth and flowering parameters under study as compared with control plants in both seasons This is in agreement with[31, 32]. They revealed that micronutrients such as iron, manganese and zinc have important roles in plant growth and yield of aromatic and medicinal plants. Increased in growth parameter with Mn spray might be due to the fact that Mn activate IAA oxides, which oxidize IAA in plants. The plant treated with Zn shows increase plant height due to its role in synthesis of tryptophan, which is a precursor of auxin (IAA) and is essential in nitrogen metabolism, which stimulates growth. However, zinc and manganese at a rate of 0.30% were dominant in having the outmost increase for all previously mentioned characters in both seasons. While that increase started to abate with the highest concentration (0.60%). In interaction, all treatments led to obvious increases in this respect. The most significant increases were obtained by using Zn+ Mn at rate of 0.30% for each as compared with other treatments. It was noticed also that with the addition of Manganese at a high rate (0.60%) with Zinc at less concentration (0.15 %), there was a reduction but the increase is resumed with increasing the amount of Zinc until it reaches its optimum record with applying both elements at 0.30% together. That increase turns to be reduced again with Zinc + Manganese at 0.30 + 0.60% respectively. The increase continued in reduction with the rest treatments until it was restored with adding Zinc + Manganese at 0.60 + 0.30% respectively which show reduction again with Zinc added with Manganese at 0.60% for both. This was apparent in all vegetative growth attributes except with plant height plant^{-1} (in the first season) and number of branches plant^{-1} and dry matter of leaves plant^{-1} (in the second season). Likewise, flowers number plant^{-1}, inflorescences diameter plant^{-1}, inflorescences fresh and dry weight plant^{-1}, fresh and dry weight of ray flowers plant^{-1} and seed weight plant^{-1} (Table 3) positively responded to Zinc and Manganese foliar spray as individual treatments in both seasons especially when plants sprayed with 0.30% from each element compared to the control. The addition of Zinc with Manganese as a duel interaction had significant increments in all studied flowering characters in both seasons.[33,34,35,36]confirm that foliar application of micronutrients improve morphological and physiological process in calendula plants and yield[37].

Generally, the highest increase was especially presented when equal concentrations from Zinc and Manganese (0.30%) were added together as compared with other treatments.

Table 2. Effect of foliar application of (Zn) or\ and (Mn) as well as their interaction on their interaction on the vegetative growth characters of *Calendula officinalis* L. plant at two successive seasons:

Zn / Mn	1st season					2nd season				
	Plant height plant^{-1} (cm)									
	0%	**0.15%**	**0.30%**	**0.60%**	**Mean**	**0%**	**0.15%**	**0.30%**	**0.60%**	**Mean**
0%	65.10	67.70	69.50	70.60	68.23	73.70	74.70	76.80	75.00	75.05
0.15%	68.70	70.50	70.40	71.80	70.35	77.10	78.70	77.40	77.30	77.63
0.30%	69.80	76.60	76.70	72.70	73.95	76.10	80.40	83.20	83.10	80.70
0.60%	69.00	69.70	72.50	73.00	71.05	77.40	78.50	82.40	77.10	78.85
Mean	68.15	71.13	72.28	72.03		76.08	78.08	79.95	78.13	
L.S.D 5%	(a)= 2.07		(b) = 3.01		(axb)=5.01	(a)= 2.11		(b) = 2.98		(axb)=4.89
Number of branches plant^{-1}										
0%	3.49	3.67	5.49	4.52	4.29	1.99	4.44	3.47	3.37	3.32
0.15%	4.33	4.67	5.67	5.33	5.00	3.00	5.01	8.08	5.39	5.37
0.30%	5.67	5.67	6.00	5.67	5.75	4.89	6.97	8.27	7.05	6.80
0.60%	4.67	4.49	5.66	5.31	5.03	2.41	5.21	6.25	8.01	5.47
Mean	4.54	4.63	5.71	5.21		3.07	5.41	6.52	5.96	
L.S.D 5%	(a)= 0.09		(b) = 0.11		(axb)=0.21	(a)= 0.12		(b) = 0.13		(axb)=0.26
Fresh matter of leaves plant^{-1} (gm)										
0%	42.30	45.80	74.90	41.70	51.18	54.80	59.80	80.10	52.90	61.90
0.15%	49.20	56.30	88.00	44.30	59.45	54.40	67.90	90.90	87.40	75.15
0.30%	68.10	88.30	93.80	90.90	85.28	60.40	73.00	99.09	98.50	82.75
0.60%	61.00	49.10	90.50	63.00	65.90	58.50	75.00	97.90	90.30	80.43
Mean	55.15	59.88	86.80	59.98		57.03	68.93	92.00	82.28	
L.S.D 5%	(a)= 3.01		(b)= 3.02		(axb)=8.07	(a)= 3.01		(b)= 3.02		(axb)=8.07
Dry matter of leaves plant^{-1} (gm)										
0%	6.30	7.30	10.10	9.10	8.20	9.20	11.00	11.80	10.80	10.70
0.15%	7.60	8.10	12.50	8.90	9.28	10.60	12.70	12.60	14.30	12.55
0.30%	10.20	10.70	14.20	12.20	11.83	10.30	13.60	15.40	14.00	13.33
0.60%	7.50	7.90	12.70	11.20	9.83	10.50	13.10	14.10	14.60	13.08
Mean	7.90	8.50	12.38	10.35		10.15	12.60	13.48	13.43	
L.S.D 5%	(a)= 0.31		(b)= 0.32		(axb)= 0.71	(a)= 1.02		(b)= 1.01		(axb)= 1.73
Number of leaves plant^{-1}										
0%	79.00	108.00	118.00	111.00	104.00	98.99	120.00	140.00	135.00	123.50
0.15%	82.00	115.00	133.00	126.00	114.00	104.00	137.00	155.00	149.00	136.25
0.30%	96.00	134.00	160.00	148.00	134.50	141.00	148.00	172.00	162.00	155.75
0.60%	119.00	110.00	150.00	126.00	126.25	115.00	133.00	169.00	156.00	143.25
Mean	94.00	116.75	140.25	127.75		114.75	134.50	159.00	150.50	
L.S.D 5%	(a)= 4.07		(b)=4.02		(axb)=8.04	(a)= 3.08		(b)= 3.01		(axb)=6.02
Shoots fresh matter plant^{-1} (gm)										
0%	56.30	67.40	75.60	67.10	66.60	74.60	76.80	95.60	86.80	83.45
0.15%	64.80	63.90	83.50	82.90	73.78	75.30	84.80	102.70	93.30	89.03
0.30%	66.60	73.00	107.30	104.90	87.95	86.20	92.90	129.30	124.90	108.33
0.60%	64.70	65.10	85.10	74.60	72.38	75.30	102.20	83.20	102.40	90.78
Mean	63.10	67.35	87.88	82.38		77.85	89.18	102.70	101.85	
L.S.D 5%	(a)= 8.11		(b)= 6.23		(axb)= 16.17	(a)= 8.33		(b)= 5.33		(axb)= 16.65
Shoots dry matter plant^{-1} (gm)										
0%	8.99	9.45	10.56	9.93	9.73	6.12	6.23	7.77	7.15	6.82
0.15%	10.36	10.69	12.34	10.7	11.02	7.57	7.17	7.91	9.78	8.11
0.30%	10.96	10.98	14.24	12.03	12.05	7.91	9.24	15.62	15.4	12.04
0.60%	10.77	9.99	11.7	11.69	11.04	7.71	7.16	9.54	8.19	8.15
Mean	10.27	10.28	12.21	11.09		7.33	7.45	10.21	10.13	
L.S.D 5%	(a)=1.20		(b)=1.21		(axb)=2.49	(a)=1.22		(b)=1.24		(axb)=2.48

Table 3.Effect of foliar application of (Zn) or\ and (Mn) as well as their interaction on their interaction on the flowering traits characters of *Calendula officinalis* L. plant at two successive seasons.

Zn	1st season					2nd season				
	Flowers Number of plant^{-1}									
Mn	0%	0.15%	0.30%	0.60%	Mean	0%	0.15%	0.30%	0.60%	Mean
0%	17.33	25.33	20.00	18.00	20.17	20.00	22.67	20.67	27.33	22.67
0.15%	21.67	23.67	19.33	24.33	22.25	23.33	24.00	28.33	28.00	25.92
0.30%	20.33	26.00	35.33	28.33	27.50	28.00	28.00	34.00	30.00	30.00
0.60%	20.00	21.33	32.67	26.00	25.00	24.00	23.33	33.33	30.00	27.67
Mean	19.83	24.08	26.83	24.17		23.83	24.50	29.08	28.83	
L.S.D 5%	(a)=3.11	(b) =3.11	(axb)=6.23			(a)=2.80	(b) =2.18	(axb)=5.59		
inflorescences dry meter plant^{-1} (cm)										
0%	5.07	5.50	5.52	5.90	5.50	6.97	7.38	7.40	7.80	7.39
0.15%	6.12	5.66	7.47	5.94	6.30	9.09	9.46	9.47	9.28	9.33
0.30%	7.41	7.29	7.80	7.66	7.54	9.33	9.10	9.68	9.54	9.41
0.60%	7.24	7.56	7.50	7.41	7.43	8.00	7.55	9.40	7.85	8.20
Mean	6.46	6.50	7.07	6.73		8.35	8.37	8.99	8.62	
L.S.D 5%	(a)=0.35	(b) =0.35	(axb)=0.71			(a)=0.35	(b) =0.35	(axb)=0.70		
inflorescences fresh matter plant^{-1} (gm)										
0%	45.90	70.40	72.80	63.20	63.08	45.70	67.90	73.20	67.50	63.58
0.15%	43.80	71.90	54.00	86.50	64.05	44.30	67.80	59.70	85.20	64.25
0.30%	61.10	61.80	125.60	68.00	79.13	60.30	59.70	112.50	71.70	76.05
0.60%	61.80	81.90	80.10	76.20	75.00	63.30	83.30	86.00	70.00	75.65
Mean	53.15	71.50	83.13	73.48		53.40	69.68	82.85	73.60	
L.S.D 5%	(a)=2.61	(b)=2.43	(axb)=4.72			(a)=3.31	(b)=4.71	(axb)=9.43		
Dry matter of inflorescences plant^{-1} (gm)										
0%	13.30	15.40	14.80	14.30	14.45	16.30	16.50	18.70	16.70	17.05
0.15%	13.90	16.10	16.60	14.40	15.25	15.70	18.70	18.90	16.70	17.50
0.30%	15.50	16.80	18.80	18.10	17.30	17.80	19.10	21.20	19.60	19.43
0.60%	14.00	14.20	16.40	17.30	15.48	16.30	17.60	17.30	20.10	17.83
Mean	14.18	15.63	16.65	16.03		16.53	17.98	19.03	18.28	
L.S.D 5%	(a)=0.90	(b)=0.80	(axb)=1. 60			(a)=0.81	(b)=0.82	(axb)=1.63		
Fresh matter of ray flowers plant^{-1} (gm)										
0%	57.40	63.30	61.90	62.30	61.23	65.10	69.40	76.20	70.40	70.28
0.15%	76.70	57.90	76.20	75.10	71.48	84.10	65.70	84.20	82.40	79.10
0.30%	68.40	78.60	82.30	79.30	77.15	71.40	86.40	90.30	87.10	83.80
0.60%	66.00	74.70	77.20	74.30	73.05	74.00	82.30	85.20	82.20	80.93
Mean	67.13	68.63	74.40	72.75		73.65	75.95	83.98	80.53	
L.S.D 5%	(a)=2.71	(b)=2.73	(axb)=5.44			(a)=2.70	(b)=2.72	(axb)=5.41		
Dry matter of ray flowers plant^{-1} (gm)										
0%	5.62	5.82	8.96	7.45	6.96	6.83	8.15	8.70	8.83	8.13
0.15%	7.38	6.55	7.86	7.45	7.31	7.06	9.15	10.42	8.91	8.89
0.30%	8.86	7.95	9.84	9.06	8.93	10.10	10.36	10.77	10.70	10.48
0.60%	8.66	6.90	8.82	7.60	8.00	8.87	9.18	10.03	8.88	9.24
Mean	7.63	6.81	8.87	7.89		8.22	9.21	9.98	9.33	
L.S.D 5%	(a)=0.29	(b)=0.22	(axb)=0.45			(a)=0.50	(b)=0.32	(axb)=0.65		
Seed mass of plant^{-1} (gm)										
0%	8.10	9.10	13.80	8.70	9.93	11.10	11.50	12.40	12.30	11.83
0.15%	8.20	9.80	11.70	11.30	10.25	11.50	11.70	13.40	12.30	12.23
0.30%	11.40	13.10	14.70	11.60	12.70	12.20	12.30	16.10	14.10	13.68
0.60%	11.80	9.20	12.70	14.10	11.95	12.10	13.20	13.20	14.80	13.33
Mean	9.88	10.30	13.23	11.43		11.73	12.18	13.78	13.38	
L.S.D 5%	(a)= 1.37	(b) = 1.22	(axb)=3.82			(a)= 1.17	(b) =1.13	(axb)=2.61		

Chemical constituents:

The obtained results of both seasons in Table (4, 5, 6 and 7) revealed that spraying Calendula plants with either Zinc or manganese individually and in combination gave high significantly increased on contents of chemical constituents as compared to the untreated treatment. These results are in a good harmony with [38on]*Rutagraveolens*and[39] on chamomile, they found that foliar application with Fe, Zn or Mn individual or in mixtures affected positively on the above-mentioned records of plant chemical composition. Using Zn or Mn concentration at a rate of 0.30%, as sole and their interaction at rate of 0.30 + 0.15%, respectively were was more effective than the other treatmentsfor head flower essential oil plant^{-1}hasbeen supported by [40]on chamomile and[35, 36.] .Moreover adding Zn or Mn at 0.60% for both individually and 0.60 + 0.60% as duel interaction gave the highest records of chlorophylla and b, total carotenoids (mg g^{-1} F. M.) and xanthophylls content in dry ray flowers in the second season[33].

The collected results revealed that the best treatments which led to the highest xanthophylls content in dry ray flowers in the first season, total flavonoides, total carotenoids in dry ray flowers, total carbohydrate percentage in stems, Beta carotene in dry ray flowers (mg g^{-1} D. M .)and K % in seedswere the addition of zinc at 0.30% , Manganese at 0.30% and zinc + Manganese at 0.30 + 0.30%.The highest total carbohydrate percentage in leaves and Manganese content in seeds and in herb was given by spraying Zn at 0.30% or Mn at 0.60% or both together at 0.30 + 0.60%, respectively. The percentage of total free amino acids, protein, N and zinc in seeds and in herb was highly raised with spraying Zinc at 0.60%, Manganese at 0.30% and their interaction at 0.60 + 0.30%, respectively. As for P %, K % in herb and Fe in seeds and in herb, the highest values were obtained from the moderate concentration of zinc (0.30%), the lowest one from Manganese (0.15%) and their interaction at 0.30 + 0.15% respectively.These results are owing to the use of micronutrients that play an important role in the representation of critical auxins that increase cell division and increase the content chlorophyll in the leaf , Also, due to that zinc helps in building the chlorophyll through its direct impact in the composition of amino acids and carbohydrates and energy compounds used in the construction chlorophyll As well as, it's importance in building the necessary RNA in protein synthesis and stimulates the enzymes that participate in biological processes for the formation of chlorophyll. [41]

Conclusion

In brief, either using Zinc or Manganese individually or in combination prove high effects on Calendula plants especially with regard to vegetative attributes, floral characters, some chemical analysis such as (Chlorophyll a, b, cartenoids, protein, xanthophylls, total flavonoides, total carbohydrate percentage in stems and leaves, percentage of total free amino acids) and macro and microelements (N, P, K, Zn, Fe and Mn) which in turn enhance the plant quality and hence preserving humans lives from their deficiency that causes harmful impacts. Therefore, foliar spray was applied instead of soil application to substitute the plant with its requirements which could be lost, especially in alkaline soils of Egypt.

Table 4.Effect of foliar application of (Zn) or (Mn) as well as their interaction on their interaction on the Head flower essential oil, Chlorophylla, b and total carotenoids, beta-carotene, xanthophyll and total flavonoides in dry ray flowers of *Calendula officinalis*L. plant at two successive seasons.

Zn / Mn	1st season					2nd season				
	Head flower essential oil plant^{-1} (ml)									
	0%	0.15%	0.30%	0.60%	Mean	0%	0.15%	0.30%	0.60%	Mean
0%	0.051	0.058	0.071	0.084	0.066	0.048	0.055	0.068	0.082	0.063
0.15%	0.066	0.058	0.100	0.072	0.074	0.067	0.058	0.098	0.069	0.073
0.30%	0.093	0.081	0.080	0.074	0.082	0.091	0.075	0.075	0.068	0.077
0.60%	0.075	0.076	0.066	0.058	0.069	0.073	0.076	0.066	0.058	0.068
Mean	0.071	0.068	0.079	0.072		0.070	0.066	0.077	0.069	
L.S.D 5%	(a)=0.002		(b)=0.003		(axb)=0.006	(a)=0.002		(b)=0.003		(axb)=0.005
Chlorophylla (mg g^{-1} F.M.)										
0%	0.81	0.86	0.88	0.90	0.86	0.77	0.73	0.78	0.80	0.77
0.15%	0.97	0.99	1.00	1.07	1.01	0.78	0.80	0.88	0.90	0.84
0.30%	0.88	0.88	0.93	0.99	0.92	0.88	0.87	0.78	0.88	0.85
0.60%	1.00	1.02	1.00	1.09	1.03	0.91	0.99	1.00	1.03	0.98
Mean	0.92	0.94	0.95	1.01		0.84	0.85	0.86	0.90	
L.S.D 5%	(a)=0.03		(b)=0.02		(axb)=0.05	(a)=0.05		(b)=0.04		(axb)=0.07
Chlorophyllb (mg g^{-1} F. M.)										
0%	0.55	0.63	0.63	0.64	0.61	0.51	0.53	0.57	0.59	0.55
0.15%	0.56	0.63	0.64	0.64	0.62	0.56	0.57	0.58	0.62	0.58
0.30%	0.65	0.67	0.7	0.74	0.69	0.61	0.62	0.7	0.73	0.67
0.60%	0.66	0.73	0.76	0.77	0.73	0.62	0.63	0.7	0.79	0.69
Mean	0.61	0.67	0.68	0.70		0.58	0.59	0.64	0.68	
L.S.D 5%	(a)=0.04		(b)=0.04		(axb)= 0.08	(a)=0.03		(b)=0.03		(axb)=0.07
Total carotenoids (mg g^{-1} F. M.)										
0%	0.39	0.41	0.43	0.45	0.42	0.36	0.4	0.44	0.45	0.41
0.15%	0.44	0.45	0.45	0.49	0.46	0.38	0.44	0.46	0.48	0.44
0.30%	0.47	0.48	0.5	0.57	0.51	0.43	0.44	0.47	0.54	0.47
0.60%	0.48	0.48	0.52	0.58	0.52	0.47	0.48	0.52	0.57	0.51
Mean	0.45	0.46	0.48	0.52		0.41	0.44	0.47	0.51	
L.S.D 5%	(a)=0.04		(b)=0.03		(axb)= n.s.	(a)=0.04		(b)=0.03		(axb)= n.s.
Beta-carotene in dry ray flowers (mg g^{-1} D. M.)										
0%	1.14	1.13	1.66	1.56	1.37	1.65	1.72	1.96	1.92	1.81
0.15%	1.48	1.49	1.75	1.74	1.62	1.67	1.96	1.99	1.89	1.88
0.30%	1.76	1.83	1.96	1.78	1.83	2.07	2.06	2.17	2.10	2.10
0.60%	1.54	1.62	1.72	1.66	1.64	1.99	1.99	1.88	1.99	1.96
Mean	1.48	1.52	1.77	1.69		1.85	1.93	2.00	1.98	
L.S.D 5%	(a)=0.14		(b)= 0.12		(axb)= 0.23	(a)=0.11		(b)= 0.09		(axb)= 0.21
Xanthophyll in dry ray flowers (mg g^{-1} D. M.)										
0%	1.07	1.03	1.50	1.71	1.33	1.39	1.36	1.97	1.78	1.63
0.15%	1.24	1.67	1.30	1.20	1.35	1.57	1.97	1.50	1.60	1.66
0.30%	1.47	1.35	1.77	1.43	1.51	1.68	1.87	1.89	1.84	1.82
0.60%	1.50	1.36	1.53	1.57	1.49	1.77	1.67	1.76	2.08	1.82
Mean	1.32	1.35	1.53	1.48		1.60	1.72	1.78	1.83	
L.S.D 5%	(a)=0.08		(b)= 0.07		(axb)= 0.13	(a)=0.05		(b)= 0.06		(axb)= 0.12
Total flavonoides in dry ray flowers (mg g^{-1} D. M.)										
0%	10.70	12.80	13.50	14.70	12.93	13.70	15.00	17.50	16.10	15.58
0.15%	13.20	14.20	15.00	14.70	14.28	14.90	14.90	18.10	17.40	16.33
0.30%	14.40	17.60	20.00	19.90	17.98	17.10	18.10	18.60	17.80	17.90
0.60%	14.40	14.10	19.40	16.10	16.00	17.70	15.40	17.60	16.20	16.73
Mean	13.18	14.68	16.98	16.35		15.85	15.85	17.95	16.88	
L.S.D 5%	(a)= 1.13		(b) = 1.09		(axb)= 3.11	(a)= 1.11		(b) = 1.04		(axb)= 3.01

Table 5. Effect of foliar application of (Zn) or (Mn) as well as their interaction on their interaction on total carotenoids in dry ray flowers, total carbohydrate percentage in stems and leaves (%), protein (%) in herb, total free amino acids (%) in herb of *Calendula officinalis*L. plant at two successive seasons.

Zn / Mn	1st season					2nd season				
	Total crotenoides in dry ray flowers (mg g^{-1} D. M.)									
	0%	**0.15%**	**0.30%**	**0.60%**	**Mean**	**0%**	**0.15%**	**0.30%**	**0.60%**	**Mean**
0%	1.82	1.87	2.17	2.08	1.99	2.00	2.08	2.11	2.10	2.07
0.15%	1.84	1.97	2.12	2.03	1.99	2.03	2.10	2.13	2.12	2.10
0.30%	2.09	2.1	2.21	2.11	2.13	2.10	2.13	2.16	2.15	2.14
0.60%	1.82	1.99	2.18	2.14	2.03	2.04	2.12	2.14	2.12	2.11
Mean	1.89	1.98	2.17	2.09		2.04	2.11	2.14	2.12	
L.S.D 5%	(a)= 0.04		(b) = 0.03		(axb)= 0.07	(a)= 0.04		(b) = 0.04		(axb)= 0.08
Total carbohydrate percentage in stems (%)										
0%	18.89	21.07	26.73	22.73	22.36	20.82	23.55	26.87	24.5	23.94
0.15%	20.91	24.9	28.27	26.82	25.23	21.01	25.67	29.73	25.82	25.56
0.30%	23.93	26.67	30.22	28.13	27.24	25.22	28.37	31.17	30.78	28.89
0.60%	19.82	25.48	29.48	27.91	25.67	22.48	26.55	29.55	27.44	26.51
Mean	20.89	24.53	28.68	26.40		22.38	26.04	29.33	27.14	
L.S.D 5%	(a)=0.64		(b)=0.63		(axb)=1.22	(a)= 0.71		(b)=0.63		(axb)=1.34
Total carbohydrate percentage in leaves (%)										
0%	13.00	13.70	19.60	16.60	15.73	15.70	17.30	18.60	19.80	17.85
0.15%	14.60	17.00	20.80	20.20	18.15	16.80	23.80	22.30	20.60	20.88
0.30%	15.60	18.20	22.50	21.70	19.50	18.70	18.60	25.30	24.40	22.77
0.60%	15.90	17.80	23.80	22.40	19.98	21.10	23.10	26.70	24.60	23.88
Mean	14.78	16.68	21.68	20.23		17.87	20.70	23.23	22.35	
L.S.D 5%	(a)= 0.95		(b) = 0.93		(axb)= 1.91	(a)= 0.91		(b) = 0.82		(axb)= 1.73
Protein (%) Herb										
0%	8.06	12.50	10.19	11.63	10.63	5.94	9.75	10.69	9.94	9.06
0.15%	11.19	10.69	9.19	11.56	10.69	8.81	7.06	10.56	12.00	9.63
0.30%	12.38	11.81	12.00	12.56	12.19	11.06	10.44	11.69	12.06	11.31
0.60%	10.19	7.31	12.25	11.19	10.25	11.19	10.81	8.75	7.75	9.63
Mean	10.44	10.56	10.94	11.75		9.25	9.50	10.44	10.44	
L.S.D 5%	(a)= 1.82		(b) = 1.15		(axb)= 2.34	(a)= 1.44		(b) = 1.22		(axb)= 2.19
Total free amino acids (%) Herb										
0%	0.28	0.28	0.27	0.28	0.28	0.26	0.26	0.27	0.27	0.27
0.15%	0.25	0.28	0.35	0.34	0.31	0.23	0.29	0.28	0.28	0.27
0.30%	0.35	0.29	0.34	0.38	0.34	0.32	0.32	0.32	0.35	0.33
0.60%	0.32	0.34	0.34	0.37	0.34	0.26	0.31	0.32	0.33	0.31
Mean	0.30	0.30	0.33	0.34		0.27	0.30	0.30	0.31	
L.S.D 5%	(a)= 0.03		(b) = 0.03		(axb)= n.s.	(a)= 0.03		(b) = n.s.		(axb)= n.s.

Table 6. Effect of foliar application of (Zn) or (Mn) as well as their interaction on their interaction on nitrogen, phosphorus and potassium in herb and Seeds % of *Calendula officinalis* L. plant at two successive seasons

Zn / Mn	1st season					2nd season				
	N % Herb									
	0%	**0.15%**	**0.30%**	**0.60%**	**Mean**	**0%**	**0.15%**	**0.30%**	**0.60%**	**Mean**
0%	1.29	2.00	1.63	1.86	1.70	0.95	1.56	1.71	1.59	1.45
0.15%	1.79	1.71	1.47	1.85	1.71	1.41	1.13	1.69	1.92	1.54
0.30%	1.98	1.89	1.92	2.01	1.95	1.77	1.67	1.87	1.93	1.81
0.60%	1.63	1.17	1.96	1.79	1.64	1.79	1.73	1.40	1.24	1.54
Mean	1.67	1.69	1.75	1.88		1.48	1.52	1.67	1.67	
L.S.D 5%	(a)=0.10		(b)=0.08	(axb)=0.16		(a)=0.12		(b)=0.08	(axb)=0.17	
N % Seeds										
0%	1.62	1.58	2.61	2.49	2.08	1.91	1.68	2.42	2.40	2.10
0.15%	3.09	3.21	3.02	2.68	3.00	2.42	2.98	2.63	2.51	2.64
0.30%	2.43	2.33	2.28	2.20	2.31	2.50	2.50	2.65	2.51	2.54
0.60%	2.47	2.70	2.65	2.58	2.60	1.61	2.63	2.57	2.51	2.33
Mean	2.40	2.46	2.64	2.49		2.11	2.45	2.57	2.48	
L.S.D 5%	(a)=0.24		(b)= 0-22	(axb)=0.47		(a)=0.21		(b)= 0-18	(axb)=0.41	
P % Herb										
0%	0.25	0.38	0.39	0.39	0.35	0.31	0.37	0.39	0.39	0.37
0.15%	0.42	0.43	0.44	0.41	0.43	0.40	0.39	0.43	0.39	0.40
0.30%	0.38	0.35	0.43	0.37	0.38	0.31	0.32	0.42	0.39	0.36
0.60%	0.39	0.38	0.33	0.34	0.36	0.33	0.34	0.42	0.40	0.37
Mean	0.36	0.39	0.40	0.38		0.34	0.36	0.42	0.39	
L.S.D 5%	(a)=0.03		(b)=0.02	(axb)=0.03		(a)=0.02		(b)=0.02	(axb)=0.04	
P % Seeds										
0%	0.46	0.52	0.51	0.52	0.50	0.47	0.48	0.48	0.53	0.49
0.15%	0.52	0.46	0.63	0.64	0.57	0.46	0.45	0.56	0.64	0.53
0.30%	0.59	0.59	0.47	0.53	0.55	0.56	0.55	0.44	0.53	0.52
0.60%	0.55	0.51	0.53	0.49	0.52	0.53	0.47	0.48	0.46	0.48
Mean	0.53	0.52	0.54	0.55		0.51	0.49	0.51	0.52	
L.S.D 5%	(a)=0.02		(b)= 0.02	(axb)=0.05		(a)=0.03		(b)= 0.01	(axb)=0.03	
K % Herb										
0%	1.99	2.29	2.38	2.32	2.24	2.29	2.41	2.51	2.43	2.41
0.15%	2.49	2.43	2.69	2.61	2.56	2.61	2.56	2.65	2.55	2.59
0.30%	2.20	2.68	2.59	2.39	2.46	2.39	2.56	2.53	2.50	2.50
0.60%	2.50	1.70	2.27	2.58	2.27	1.93	2.34	2.34	2.46	2.27
Mean	2.29	2.28	2.48	2.48		2.31	2.47	2.51	2.49	
L.S.D 5%	(a)=0.20		(b) = 0.16	(axb)= 0.32		(a)= 0.12		(b) = 0.13	(axb)= 0.22	
K % Seeds										
0%	1.40	1.53	1.54	1.42	1.47	1.25	1.44	1.48	1.43	1.40
0.15%	1.45	1.58	1.85	1.90	1.69	1.38	1.49	1.68	1.56	1.53
0.30%	1.76	1.67	2.19	1.52	1.78	1.63	1.68	1.75	1.52	1.64
0.60%	1.71	1.67	1.92	1.68	1.75	1.48	1.44	1.62	1.69	1.56
Mean	1.58	1.61	1.88	1.63		1.44	1.51	1.63	1.55	
L.S.D 5%	(a)= 0.17		(b) = 0.06	(axb)=0.13		(a)= 0.16		(b) = 0.11	(axb)= 0.15	

Table 7. Effect of foliar application of (Zn) or (Mn) as well as their interaction on their interaction on Zn in seeds and herb (ppm), Mn in seeds and herb (ppm) and Fe in seeds and herb (ppm), of *Calendula officinalis* L. plant at two successive seasons.

Zn / Mn	1st season					2nd season				
	Zn (ppm) Seeds									
	0%	0.15%	0.30%	0.60%	Mean	0%	0.15%	0.30%	0.60%	Mean
0%	48.10	51.70	51.20	56.00	51.75	55.40	54.50	53.80	58.50	55.55
0.15%	51.60	52.00	61.80	61.10	56.63	54.50	54.80	64.60	63.90	59.45
0.30%	55.90	57.70	56.80	68.80	59.80	58.70	59.20	57.90	70.00	61.45
0.60%	51.70	60.40	55.70	67.20	58.75	54.60	63.00	57.60	69.10	61.08
Mean	51.83	55.45	56.38	63.28		55.80	57.88	58.48	65.38	
L.S.D 5%	(a)=1.91		(b)=1.63	(axb)=3.14		(a)=1.07		(b)=1.2	(axb)=2.4	
Zn (ppm) Herb										
0%	40.90	47.80	40.50	55.90	46.28	49.90	47.30	48.10	57.60	50.73
0.15%	45.90	45.20	53.70	48.00	48.20	46.70	47.10	57.40	58.80	52.50
0.30%	47.30	47.10	51.40	56.70	50.63	47.60	56.80	56.60	59.50	55.13
0.60%	47.90	46.90	47.90	52.10	48.70	47.70	51.60	56.40	57.50	53.30
Mean	45.50	46.75	48.38	53.18		47.98	50.70	54.63	58.35	
L.S.D 5%	(a)= 1.33		(b)= 2.27	(axb)=6.13		(a)= 2.82		(b)=3.80	(axb)= 6.77	
Mn (ppm) Seeds										
0%	88.20	96.00	96.50	96.50	94.30	89.50	98.10	93.90	98.00	94.88
0.15%	92.10	87.60	96.10	105.80	95.40	90.40	92.00	97.50	103.50	95.85
0.30%	93.30	94.80	118.50	102.80	102.35	90.90	96.50	121.30	105.70	103.60
0.60%	92.00	97.50	129.20	101.00	104.93	94.10	100.30	131.90	107.20	108.38
Mean	91.40	93.98	110.08	101.53		91.23	96.73	111.15	103.60	
L.S.D 5%	(a)=6.03		(b)=3.82	(axb)=7.63		(a)=4.32		(b)=3.62	(axb)=7.34	
Mn (ppm) Herb										
0%	48.80	54.30	59.97	56.40	54.87	48.90	53.00	56.48	56.10	53.62
0.15%	56.30	54.19	59.48	55.00	56.24	54.20	55.50	56.57	54.20	55.12
0.30%	55.40	57.50	59.70	59.50	58.03	54.40	56.20	62.99	63.20	59.20
0.60%	65.90	56.00	60.59	58.60	60.27	56.10	66.00	67.09	63.30	63.12
Mean	56.60	55.50	59.94	57.38		53.40	57.68	60.78	59.20	
L.S.D 5%	(a)=1.89		(b)=1.66	(axb)=3.61		(a)=1.07		(b)=1.22	(axb)=2.43	
Fe (ppm) Seeds										
0%	495.00	545.00	462.00	446.00	487.00	414.00	413.00	585.00	483.00	473.75
0.15%	574.00	572.00	694.00	592.00	608.00	498.00	573.00	682.00	586.00	584.75
0.30%	507.00	510.00	514.00	488.00	504.75	504.00	518.00	499.00	499.00	505.00
0.60%	410.00	405.00	492.00	588.00	473.75	473.00	576.00	500.00	486.00	508.75
Mean	496.50	508.00	540.50	528.50		472.25	520.00	566.50	513.50	
L.S.D 5%	(a)= 33.00		(b) = 33.00	(axb)= 65.00		(a)= 27.00		(b) = 35.00	(axb)= 70.00	
Fe (ppm) Herb										
0%	207.00	294.00	292.00	295.00	272.00	199.00	210.00	259.00	220.00	222.00
0.15%	256.00	297.00	349.00	311.00	303.25	255.00	227.00	363.00	349.00	298.50
0.30%	295.00	280.00	291.00	288.00	288.50	281.00	277.00	263.00	264.00	271.25
0.60%	212.00	239.00	273.00	206.00	232.50	222.00	252.00	271.00	280.00	256.25
Mean	242.50	277.50	301.25	275.00		239.25	241.50	289.00	278.25	
L.S.D 5%	(a)= 2.00		(b) = 23.00	(axb)= 46.00		(a)= 9.00		(b) = 9.00	(axb)= 17.00	

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