

MORPHOLOGICAL AND CHEMICAL INVESTIGATIONS OF MARIGOLD GROWN IN SANDY AND CALCAREOUS SOILS

BY

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ABSTRACT

An experiment was carried out on *Calendula officinalis* L. plants at Maryout (calcareous soil) and El – Sheikh Zowaid (sandy soil) locations during (2002/2003 and 2003/2004) to study the effect of plant spacing (100 and 50 cm) and nitrogen levels (0, 60, 80 and 100 kg N / fed) on the morphological characteristics of the plants and the physical chemical properties of the inflorescences extracts using four organic solvents (petroleum ether, n-hexane, 96 % and 80 % ethyl alcohol). The results show that, the maximum values of number of branches, number of inflorescences, inflorescences diameter (cm), fresh and dry weight (g) per plant were obtained from the interaction between (100 cm plant spacing and 100 kg N level). Meanwhile, non significant difference was observed between 80 and 100 kg N / levels in both locations. The maximum plant height (cm) and inflorescences yield (kg) per fed. were produced from (50 cm plant spacing and 100 kg N level) treatment.

The highest essential oil, nitrogen and protein percentages in *Calendula* inflorescences in both locations were resulted from (100 cm plant spacing and 100 kg N level) treatment. The maximum percentages of *Calendula* inflorescences extracts using petroleum ether and ethyl alcohol 96 % were obtained from (100 cm plant spacing and 100 kg N level) treatment. While n – hexane gave the highest percentage of inflorescences extracts in the treatment of (50 cm plant spacing and 100 kg N level) in both locations.

The physical properties and chemical constants in different extracts of *Calendula* inflorescences (specific gravity, refractive index, melting point, congealing point, color, taste, odor and solubility in different solvents) and (acid value, ester value, saponification value) were determined.

Different components of *Calendula* inflorescences extracts were identified quantitatively using gas liquids chromatography technique.

Key words: *Calendula officinalis* L., plant spacing, nitrogen levels and volatile oil.

INTRODUCTION

Calendula officinalis L. plant belongs to family Asteraceae (Compositae). Lockie, 2000 indicated that, *Calendula* is native to Southern Europe, but now found throughout the world. Grieve, 1994 reported that, only the common deep orange – flowers of *C. officinalis* L. is of medicinal value.

The antiseptic and anti-inflammatory properties of *Calendula* have made it one of the longest established popular herbal remedies, and is usually given to heal and soothe cuts and broken skin. It may prevent the development of disfiguring scars from torn and jagged wounds. In addition, non – alcohol – based preparations of the remedy may soothe eczema.

Calendula is prescribed internally and externally for leg and varicose ulcers, post – operative wounds and ruptured muscles or tendons. It may be used to treat torn perneal tissues following childbirth, joint wounds where there is loss of synovial fluid and bleeding in the gums after a tooth extraction .

Bown, 1995 mentioned that, parts used in *C. officinalis* are flower petals. The properties are a bitter sweet, salty herb that stimulates the liver, gall bladder and uterus, soothes the digestive system, supports the heart and clears infections. It benefits the skin especially, reducing inflammation, controlling bleeding and healing damaged or irritated tissues.

Lawless, 1997 reported that, *Calendula officinalis* L. was used as an ancient medical reputed, for skin complaints, menstrual irregularities, varicose veins, hemorrhoids, conjunctivitis and poor eyesight. The flowers, specific for enlarged or inflamed lymph nodes, sebaceous cysts, duodenal ulcer and inflammatory skin lesions. The infused oil is used for a wide range of skin problems including cracked and rough skin, disappear rash, grazes, cracked nipples, varicose veins and inflammations.

The coloration of food stuffs improve their appearance and makes them more attractive to the consumer. Thus it is necessary to investigate all nature substances that are suitable for coloration to avoid synthetic colors which may be dangerous for the public health.

Bown, 1995 reported that, uses of the herb as culinary , petals are used as a substitute for saffron in rice and soup, and infused to give color to cheese, butter, milk desserts and cakes, also added fresh to salads.

Polunin and Robbins, 1994 found that, *C. officinalis* L. contains triterpenes; carotenoids; saponins; flavonoids including quercetin; rutin; volatile oil; resin and chlorogenic acid. Vidal - Ollivier *et al.*, 1989 stated that, six saponins were isolated from the flowers of *C. officinalis* L. Oleanolic acid 3 - O - β - D - glucuronide is a basic unit of all these compounds.

Many investigations were carried out to study the effect of plant spacing and nitrogen fertilization on growth parameters, yield and active constituents in many medicinal and aromatic plants i.e Afify *et al.*, 1993 on *Salvia officinalis* L., Omer, 1998 on *Origanum syriacum*, Badawi, 2000 on *Hibiscus sabdariffa* L. and Atakisi *et al.*, 2001 on *Salvia officinalis* L.

This study aimed to investigate the effect of plant spacing and nitrogen fertilization levels on the morphological and chemical properties of pot marigold plants at Maryout and El - Sheikh Zowaid locations.

MATERIAL AND METHODS

Two field experiments were carried out during two successive seasons of 2002/2003-2003/2004 at Maryout Research Station, Alexandria Governorate, represents the calcareous soil (39.10 % calcium carbonate) and El-Sheikh Zowaid Research Station, North Sinai Governorate represents the sandy soil (98.25 % sand). The experiments were irrigated with 2697 ppm for Maryout location and 3792 ppm for El-Sheikh Zowaid location.

The study aimed to investigate the interaction between four levels of nitrogen fertilization 0, 60, 80 and 100 kg N per feddan using ammonium sulfate 20.5 % N and two plant spacing 50 and 100 cm on ridges. Planting was achieved according to split plot design with three replicates. Plot dimensions were 3m and 5m with three ridges 0.60 m apart. Plants were disseminated in hills on the first of November, one plant per hill. Culture practices were done as recommended.

I-Morphological characteristics were recorded after three weeks from the beginning of flowering as follow :

- 1 - Plant height (cm).
- 2 - Number of branches per plant.
- 3 - Number of inflorescence per plant.
- 4 - Inflorescence diameter (cm).
- 5 - Fresh and dry weights of inflorescence per plant(g).
- 6 - Estimated dry weight of inflorescence per fed (kg).

Successive harvests of pot marigold inflorescence were performed weekly from the beginning of flowering on the first of January till the last week of April.

II-Chemical analysis of *Calendula officinalis* L inflorescence :

1-Nitrogen and protein percentage were determined according to James, 1995 and Ranganna, 1978, respectively.

2-Volatile oil percentage was carried out according to Güenther, 1972 and carotenoids percentage were carried out according to Fadeel, 1962 and Cherry, 1973.

3-The physical properties in different extracts (specific gravity, refractive index, melting point and congealing point and solubility in different solvents) were carried out according to A. O. A. C. (1990).

4-The chemical constants in different extracts (acid value, ester value and saponification value) were determined according to Farag, 1996.

5- Gas liquid chromatography of the extracts with different organic solvents were determined according to Nelson *et al.*, 1969.

The data statistical analyzed by computer SAS program. LSD test was used to compare the average means of treatments.

Meteorological data of Maryout and El – Sheikh Zowaid locations during 2003 – 2004 were as follow :

First season						
Locations	Maryout location			El – Sheikh Zowaid location		
Months	Air temp. (°C)	RH %	Rain (mm)	Air temp. (°C)	RH %	Rain (mm)
Nov.2002	19.4	71.43	2.03	19.56	70.91	2.79
Dec. 2002	15.31	75.69	69.60	14.84	83.75	75.44
Jan.2003	14.82	74.97	38.10	14.13	82.43	37.08
Feb.2003	12.92	70.33	83.80	13.16	73.51	15.48
March2003	13.93	74.01	40.64	14.23	81.41	41.15
April 2003	18.41	71.28	0.25	18.50	78.05	13.21
Second season						
Nov.2003	19.75	74.98	4.52	18.90	87.31	2.29
Dec. 2003	14.87	74.06	41.91	14.49	81.64	23.62
Jan.2004	12.84	75.34	70.86	13.06	79.91	33.02
Feb.2004	13.35	78.66	56.64	13.37	88.64	29.21
March2004	16.06	71.04	2.03	16.60	81.52	19.05
April 2004	18.60	70.76	0.25	17.73	85.41	0.25

Source : Appli. Meteorological Lab. Desert Research Center

RESULTS AND DISCUSSION

I – Morphological characters of *Calendula officinalis* L . as affected by plant spacing and nitrogen levels in Maryout and El – Shiekh Zowaid locations .

1 – Plant height : -

It is clear from Table (1) that in Maryout location (calcareous soil) the tallest plants were obtained from the interaction between 50 cm planting distance and 100 kg N / feddan which were 67.00 and 68.00 cm in the first and second seasons, respectively. While the shortest plants were resulted from the interaction between 100 cm planting distance and zero nitrogen per feddan level. On the other hand in El – Sheikh Zowaid location (sandy soil),the tallest plants in the first and second seasons 65.00 and 67.00 cm were obtained from the same former interaction.

It could be noticed that the *Calendula officinalis* L. plants at Maryout is taller than those in El – Sheikh Zowaid location. These results are in agreement with those obtained by Omer, 1998 on *Origanum syriacum*, Afify, et al. , 1993 and Atakisi et al, 2001 on *Salvia officinalis* L.

2 – Number of branches : -

The effect of plant spacing and nitrogen levels on number of branches per plant are recorded in Table (2) . It shows that, there is a significant decrease in number of branches due to the decrease of plant spacing and the nitrogen levels in both seasons at Maryout and El – Sheikh Zowaid locations. In Maryout the highest values of number of branches were 11.00 and 11.33 obtained from 100 cm plant spacing interacted with 100 kg N / fed. for both seasons, respectively. While the lowest values of number of branches were obtained from 50 cm spacing and zero nitrogen level. At El –

Sheikh Zowaid location the same trend was observed. These results are in agreement with those obtained by Afify *et al.*, 1993 on *Salvia officinalis* L.

Table (1) Effect of plant spacing and nitrogen levels on plant height (cm) of *Calendula officinalis* L. plants.

Treat Space N Level	Plant height									
	Maryout location									
	1 st Season					2 nd Season				
	0	60	80	100	Mean	0	60	80	100	Mean
100 cm	45.50	51.50	55.50	56.00	52.13	47.00	53.30	59.00	60.50	55.00
50 cm	51.00	60.50	65.50	67.00	61.25	53.00	61.50	68.00	70.00	63.13
Mean	48.25	56.00	60.50	62.00	---	50.00	57.50	63.50	65.25	---
L. S. D. 0.05 * 7.78					L. S. D. 0.05 * 4.69					
** 4.16					** 2.12					
*** 5.89					*** 2.99					
El - Sheikh Zowaid Location										
100 cm	44.00	49.00	54.00	55.00	50.50	45.00	52.00	54.33	57.00	52.08
50 cm	48.00	56.00	63.00	65.00	58.00	50.00	58.00	64.00	67.00	59.75
Mean	46.00	52.50	58.50	60.00	---	47.50	55.00	59.17	62.00	---
L. S. D. 0.05 * 4.05					L. S. D. 0.05 * 3.15					
** 4.33					** 2.86					
*** 5.98					*** 4.04					

*Plant spacing **Nitrogen level ***Plant spacing x nitrogen level.

Table (2) Effect of plant spacing and nitrogen levels on number of branches *Calendula officinalis* L. plants.

Treat Space N Level	Number of branches per plant									
	Maryout location									
	1 st Season					2 nd Season				
	0	60	80	100	Mean	0	60	80	100	Mean
100 cm	8.00	9.33	10.67	11.00	9.75	8.33	10.00	11.00	11.00	10.17
50 cm	5.67	7.00	8.00	8.33	7.25	6.00	6.67	8.33	8.67	7.42
Mean	6.83	8.17	9.33	9.67	---	7.17	8.33	9.67	10.00	---
L. S. D. 0.05 * 1.64					L. S. D. 0.05 * 0.62					
** 1.14					** 1.18					
*** 2.00					*** 1.66					
El - Sheikh Zowaid Location										
100 cm	7.00	8.67	10.00	10.67	9.08	7.33	8.33	10.07	11.00	9.33
50 cm	5.00	6.00	7.33	8.00	6.58	5.33	6.33	7.33	7.67	6.67
Mean	6.00	7.33	8.67	9.33	---	6.33	7.33	9.00	9.33	---
L. S. D. 0.05 * 1.64					L. S. D. 0.05 * 0.95					
** 0.10					** 0.66					
*** 1.42					*** 0.94					

*Plant spacing **Nitrogen level ***Plant spacing x nitrogen level.

3 – Number of inflorescences : -

Recorded data in Table (3) show that, in Maryout location, the number of inflorescences per plant was significantly affected with plant spacing and nitrogen levels, in addition with the interaction between the two treatments. The maximum number of inflorescences per plant were 440.00 in the first season and 453.00 in the second season, obtained from the interaction between 100 cm plant spacing and 100 kg nitrogen level. While the minimum number of inflorescences per plant were 245.00 and 252.00 in both seasons obtained from 50 cm plant spacing and zero kg nitrogen level. In El – Sheikh Zowaid location the same trend was observed.

Table (3) Effect of plant spacing and nitrogen levels on number of inflorescences per plant in *Calendula officinalis* L. plants.

Treat	number of inflorescences per plant										
Space	Maryout location										
	1 st Season					2 nd Season					
	0	60	80	100	Mean	0	60	80	100	Mean	
Leve											
100 cm	58.13	395.00	430.00	440.00	380.75	264.00	398.00	445.00	453.00	90.00	
50 cm	45.00	320.11	345.00	351.33	315.25	252.00	330.00	357.00	365.00	26.00	
Mean	51.48	357.39	387.00	394.50	—	258.00	364.00	401.00	409.00	—	
L. S. D. 0.05		*	18.63				L. S. D. 0.05	*	5.59		
		**	9.49					**	8.88		
		***	13.42					***	12.55		
El – Sheikh Zowaid Location											
100 cm	97.00	330.00	369.00	373.33	317.33	202.00	340.33	379.00	382.33	25.92	
50 cm	30.33	208.67	249.00	244.67	208.17	138.00	230.00	254.67	269.33	20.75	
Mean	63.67	269.33	309.00	316.00	—	170.00	285.17	319.83	324.33	—	
L. S. D. 0.05		*	11.54				L. S. D. 0.05	*	13.68		
		**	9.86					**	9.32		
		***	13.95					***	13.17		

*Plant spacing **Nitrogen level ***Plant spacing x nitrogen level.

4 – Inflorescence diameter (cm) : -

It could be noticed from data in Table (4) that, inflorescence diameter was affected significantly with plant spacing in both seasons at Maryout and El- Zowaid locations. Meanwhile the inflorescence diameter was influenced significantly with all nitrogen levels except the treatments of 80 and 100 kg nitrogen levels which had insignificant difference between them in both locations and both seasons.

The maximum inflorescence diameter in Maryout location were 8.80 and 9.70 cm in the first and second seasons, respectively. Meanwhile it was 8.50 cm in El – Sheikh Zowaid location in both seasons resulted from the interaction between 100 cm plant spacing and 100 kg nitrogen level. On the other hand the minimum inflorescence diameter were 4.80 and 4.90 cm in Maryout and 4.50 and 4.60 cm in El-Sheikh Zowaid in both seasons, respectively

obtained from 50 cm plant spacing and receiving zero nitrogen level.

Table (4) Effect of plant spacing and nitrogen levels on inflorescences diameter (cm) of *Calendula officinalis* L. plants.

Treat		Inflorescences diameter (cm)									
Space	N Level	Maryout location									
		1 st Season					2 nd Season				
		0	60	80	100	Mean	0	60	80	100	Mean
100 cm		6.30	7.50	8.60	8.80	7.80	6.50	7.70	8.90	9.71	8.03
50 cm		4.80	6.10	7.30	7.30	6.40	4.90	6.30	7.50	7.60	6.58
Mean		5.60	6.80	7.95	8.05	---	5.70	7.00	8.20	8.30	---
L . S . D .		0.05	*	0.93			L . S . D .	0.05	*	0.56	
			**	0.36				**	0.35		
			***	0.50				***	0.49		
EI - Sheikh Zowaid Location											
100 cm		6.20	7.20	8.40	8.50	7.58	6.40	7.47	8.50	8.50	7.72
50 cm		4.50	5.70	7.00	7.10	6.10	4.60	5.80	7.20	7.30	6.23
Mean		5.40	6.45	7.70	7.80	---	5.50	6.63	7.85	7.90	---
L . S . D .		0.05	*	0.25			L . S . D .	0.05	*	0.90	
			**	0.33				**	0.31		
			***	0.47				***	0.44		

*Plant spacing **Nitrogen level ***Plant spacing x nitrogen level.

5 - Inflorescences fresh weight per plant :-

Data in Table (5) show inflorescences fresh weight per plant as affected with plant spacing and nitrogen levels. A significant difference in fresh weight per plant could be observed between the plant spacing 100 and 50 cm treatments, and no significant difference could be noticed between 80 kg and 100 kg nitrogen levels. Meanwhile the interaction between 100 cm plant spacing and 100 kg nitrogen level gave the heaviest inflorescences fresh weight per plant, which were 414.85 and 437.62 g at Maryout location and 369.63 and 375.08 g at EI - Sheikh Zowaid location for both seasons, respectively. The lightest inflorescences fresh weight per plant was obtained from the planting on 50 cm and receiving zero nitrogen level for both locations.

6 - Inflorescences dry weight per plant :-

Data in Table (6) show that, inflorescences dry weight per plant took the same trend of fresh weight of Inflorescences per plant as affected with plant spacing and nitrogen levels. at Maryout location the heaviest inflorescences dry weight per plant were 67.62 and 70.56 g in the two seasons, respectively obtained from the interaction between 100 cm plant spacing and 100 kg nitrogen level, while it were 63.21 and 64.89 g for both seasons in EI - Sheikh Zowaid location. On the other hand the lightest inflorescences dry weight per plant in both locations and both seasons were produced from using 50 cm plant spacing and receiving zero nitrogen level.

Table (5) Effect of plant spacing and nitrogen levels on inflorescences fresh weight (g / plant) of *Calendula officinalis* L. plants.

Treat Space	Inflorescences fresh weight (g / plant)										
	Maryout location										
	1 st Season					2 nd Season					
Level	0	60	80	100	Mean	0	60	80	100	Mean	
100 cm	97.19	365.88	395.03	414.85	343.24	199.63	377.38	413.36	437.62	54.50	
50 cm	29.24	210.81	233.85	238.78	203.17	124.03	218.71	243.22	246.44	08.10	
Mean	63.21	288.35	314.44	326.82	---	161.83	298.05	328.29	337.03	---	
L . S . D . 0.05		*	13.48	L . S . D . 0.05			*	14.48			
		**	10.70				**	14.43			
		***	15.13				***	20.41			
El - Sheikh Zowaid Location											
100 cm	77.50	310.06	357.83	369.63	303.75	181.99	323.29	374.82	375.08	13.80	
50 cm	17.35	198.67	241.76	242.29	199.97	124.29	218.13	250.90	251.87	11.30	
Mean	47.42	254.27	299.80	305.96	---	153.14	270.71	312.86	313.48	---	
L . S . D . 0.05		*	11.35	L . S . D . 0.05			*	11.81			
		**	8.77				**	8.76			
		***	12.40				***	12.39			

*Plant spacing **Nitrogen level ***Plant spacing x nitrogen level.

Table (6) Effect of plant spacing and nitrogen levels on inflorescences dry weight (g / plant) of *Calendula officinalis* L. plants.

Treat Space	Inflorescences dry weight (g / plant)										
	Maryout location										
	N Level	1 st Season					2 nd Season				
	0	60	80	100	Mean	0	60	80	100	Mean	
100 cm	31.55	58.54	65.18	67.62	55.72	31.94	60.38	68.20	70.56	57.77	
50 cm	20.27	36.26	40.92	41.31	34.69	21.09	37.18	42.56	43.13	35.99	
Mean	25.91	47.40	53.05	54.47	---	26.52	48.78	55.38	56.84	---	
L . S . D . 0.05		*	0.51	L . S . D . 0.05			*	2.33			
		**	1.55				**	2.40			
		***	2.20				***	3.39			
El - Sheikh Zowaid Location											
100 cm	30.18	52.71	60.93	63.21	51.76	30.94	54.97	63.72	64.89	53.63	
50 cm	18.78	31.76	38.68	39.32	32.13	19.89	34.90	40.14	40.80	33.93	
Mean	24.48	42.23	49.81	51.26	---	25.42	44.94	51.93	52.85	---	
L . S . D . 0.05		*	1.95	L . S . D . 0.05			*	1.90			
		**	1.48				**	1.449			
		***	2.09				***	2.11			

*Plant spacing **Nitrogen level ***Plant spacing x nitrogen level.

7 - Inflorescences dry weight per feddan :-

The estimated inflorescences dry weight per feddan (kg) in *Calendula officinalis* L. as affected by plant spacing and nitrogen levels was tabulated in Table (7). The total dry weight of inflorescences per plant in all treatments reflect the estimated yield but it could be noticed that the narrow plant spacing 50 cm receiving 80 or 100 kg nitrogen per feddan gave the maximum yield, while it decreased significantly when the planting achieved on 100 cm with any nitrogen level. As for Maryout location the maximum estimated yield were produced from the interaction between 50 cm plant spacing and 100 kg nitrogen per feddan. On the other hand the lowest estimated yield resulted from 100 cm plant spacing and zero kg nitrogen per feddan. The same trend was observed in El - Sheikh Zowaid location.

As morphological characters are described before, it could be noticed that the cultivation of *Calendula officinalis* L. plants at Maryout location is performed best than El - Sheikh Zowaid location, and this may be attributed to the environmental factors. More over no difference between the yield using 80 kg nitrogen per feddan and using 100 kg nitrogen per feddan in the two locations. Same results were confirmed by Omer,1998 on *Origanum syriacum* and Atakisi et al,2001 on *Salvia officinalis* L.

II- Chemical analysis of *Calendula officinalis* L. inflorescences as affected by plant spacing and nitrogen levels in Maryout and El - Sheikh Zowaid locations :-

Table (7) Effect of plant spacing and nitrogen levels on inflorescences dry weight (kg / fed.) of *Calendula officinalis* L. plants.

Treat Spacing	Inflorescences dry weight (kg / fed.)									
	Maryout location									
	1 st Season					2 nd Season				
Leve	0	60	80	100	Mean	0	60	80	100	Mean
100 cm	10.31	390.23	434.50	450.76	371.45	212.92	402.50	454.65	470.33	85.10
50 cm	70.26	483.46	545.59	550.79	462.53	281.12	495.73	567.50	575.00	79.84
Mean	40.29	436.85	490.05	500.78	---	247.02	449.12	511.08	522.67	---
L. S. D. 0.05 *		9.11			L. S. D. 0.05 *		15.53			
		** 14.12					** 20.12			
		*** 19.97					*** 28.45			
El - Sheikh Zowaid Location										
100 cm	01.15	351.37	405.50	421.33	344.84	206.22	366.36	424.75	432.55	57.47
50 cm	50.33	423.38	515.75	523.34	428.20	265.15	465.33	535.57	544.04	52.52
Mean	25.74	387.38	460.63	472.34	---	235.69	415.85	480.16	488.30	---
L. S. D. 0.05 *		15.53			L. S. D. 0.05 *		25.47			
		** 12.59					** 10.05			
		*** 17.81					*** 14.21			

*Plant spacing **Nitrogen level ***Plant spacing x nitrogen level.

1 - Total nitrogen and protein percentage :

Data in Table (8) show that, the maximum percentage of nitrogen in *Calendula officinalis* L. inflorescences from Maryout location and El - Sheikh Zowaid resulted from 100 cm plant spacing acted with 100 kg nitrogen level. While the lowest percentage were obtained from 50 cm plant spacing and zero nitrogen level. From results obtained in Table (8) nitrogen percentage in all treatments reflects the protein percentage in *Calendula officinalis* L. inflorescences, which estimated from the assumption that nitrogen percentage equal 16 % from protein content according to James, 1995 and Ranganna , 1978.

Table (8) Total nitrogen and protein percentage in *Calendula officinalis* L. inflorescences as affected by plant spacing and nitrogen levels.

Maryout location								
Space N Level	Nitrogen %				Protein content			
	0	60	80	100	0	60	80	100
100cm	2.05	3.01	3.92	4.13	12.81	18.81	24.50	25.81
50cm	2.03	2.90	3.52	3.85	12.69	18.13	22.00	24.06
El- Sheikh Zowaid location								
100cm	2.03	2.35	3.22	3.16	12.69	14.69	20.13	22.56
50 cm	2.02	2.11	2.24	2.95	12.63	13.19	14.00	18.44

2 - Volatile oil (V / W) and carotenides (V / W) percentages .

As for volatile oil percentage as affected by plant spacing and different nitrogen levels in *Calendula officinalis* L. inflorescences. Data recorded in Table (9) show that, the highest volatile oil percentage in Maryout location obtained from the interaction between 100 cm plant spacing with 80 kg nitrogen or 100 kg nitrogen level, respectively. Meanwhile in El - Sheikh Zowaid location the percentage of volatile oil reached 0.31 % produced from the interaction between 100 cm or 50 cm plant spacing and 100 kg nitrogen level.

As for carotenoides content in *Calendula officinalis* L. inflorescences as affected by plant spacing and nitrogen levels. Data in Table (9) show that, at Maryout location, the treatment of 100 cm plant spacing with 100 nitrogen level gave the highest percentage of carotenoides which reached 8.97 mg / 100 g in the first season and 9.21 mg /100g in the second season. While the maximum percentage of it, reached 9.05 and 9.42 mg / 100 g obtained from plant spacing 50 cm and 100 nitrogen level in the first and second seasons, respectively at El - Sheikh Zowaid location. These results are in agreement with those obtained by Karawya, et al., 1975 and Megahed, 1985 on *Calendula officinalis* L. inflorescences.

Table (9) Volatile oil (ml /100 g) and carotenoids (mg / 100 g) percentage in *Calendula officinalis* L . inflorescences as affected by plant spacing and nitrogen Levels.

Maryout location								
Space N Level	Volatile oil % (ml / 100 g)				Carotenoids (mg / 100 g)			
	1 st Season				1 st Season			
	0	60	80	100	0	60	80	100
100cm	0.29	0.30	0.32	0.31	5.47	5.59	6.20	8.97
50cm	0.28	0.28	0.30	0.30	5.44	6.94	7.25	8.19
2 nd Season				2 nd Season				
100cm	0.28	0.30	0.31	0.31	5.22	6.01	6.34	9.21
50cm	0.28	0.29	0.29	0.30	5.19	6.83	7.00	8.22
El- Sheikh Zowaid location								
1 st Season				1 sr Season				
100cm	0.28	0.29	0.29	0.30	5.07	5.71	6.07	7.02
50cm	0.28	0.28	0.29	0.30	5.11	5.92	7.94	9.05
2 nd Season				2 nd Season				
100cm	0.29	0.29	0.30	0.31	5.26	5.55	6.00	6.80
50cm	0.29	0.29	0.30	0.31	5.36	6.10	8.11	9.42

3 -Physical properties of *Calendula officinalis* L . inflorescences extracts in Maryout and El – Sheikh Zowaid locations .

Data recorded in Table (10 &11) show the effect of different organic solvents on physical properties of *Calendula officinalis* L . inflorescences extracts at Maryout and El – Sheikh Zowaid locations . At Maryout location , specific gravity at (55 / 55 °C) , refractive index at 55 °C , melting range and congealing range were 0.916 , 1.486 , 56 – 58 °C and 54 – 56 °C found in *Calendula officinalis* L . inflorescences extract with ethyl alcohol 96 % , respectively .While the minimum former values were obtained from petroleum ether extract. The Physical properties of *Calendula officinalis* L . inflorescences extracts with different organic solvents at El – Sheikh Zowaid locations took the same trend. The color, taste and odor for all extracts in both locations are mentioned in table (10 &11).

It could be concluded that all extracts obtained from each of *Calendula officinalis* L . with the applied solvents (n – hexane , petroleum ether , ethyl alcohol 96 % and 80 %) were in solid state at room temperature.

For studying the solubility of different extracts of *Calendula officinalis* L . inflorescences , data in table (10 &11) show that all extracts with n – hexane, petroleum ether , ethyl alcohol 96 % and 80 % were insoluble in water at Maryout and El – Sheikh Zowaid samples and mostly soluble in ether , petroleum ether , acetone , chloroform and benzene .

Table (10) Physical properties of *Calendula officinalis* L . inflorescences extracts obtained by different organic solvents :

Property	Maryout samples			
	Petroleum ether	n - hexane	Ethyl alcohol 96 %	Ethyl alcohol 80 %
Specific gravity at 55 / 55 °C	0.873	0.899	0.916	0.903
Refractive index at 55 °C	1.460	1.467	1.486	1.473
Melting range	50 - 52 °C	50 - 52 °C	56 - 58 °C	56 - 58 °C
Congeeing range	48 - 50 °C	48 - 50 °C	54 - 56 °C	54 - 56 °C
Color	Yellowish	Orange yellow	Brownish yellow	Brownish orange
Taste	Less aromatic	Aromatic	Aromatic	Aromatic
Odor	Bland to slightly acid	Bland to slightly acid	Bland to slightly acid	Bland to slightly acid
Solubility :				
Ether	Soluble	Soluble	Slightly soluble	Slightly soluble
Petroleum ether	Soluble	Soluble	Soluble	Soluble
Acetone	Soluble	Soluble	Soluble	Soluble
Chloroform	Soluble	Soluble	Partially soluble	Partially soluble
Benzene	Soluble	Soluble	Soluble	Soluble
Water	Insoluble	Insoluble	Insoluble	Insoluble

Table (11) Physical properties of *Calendula officinalis* L. inflorescences extracts obtained by different organic solvents :

Property	EI - Sheikh Zowaid samples			
	Petroleum ether	n - hexane	Ethyl alcohol 96 %	Ethyl alcohol 80 %
Specific gravity at 55 / 55 °C	0.868	0.893	0.910	0.903
Refractive index at 55°C	1.492	1.469	1.483	1.473
Melting range	49 - 51 °C	49 - 51 °C	55 - 57 °C	55 - 57 °C
Congeeing range	47 - 49 °C	48 - 50 °C	53 - 55 °C	53 - 55 °C
Color	Orange to red	Orange to red	Brownish yellow	Brownish orange
Taste	Less aromatic	Aromatic	Strong aromatic	Aromatic
Odor	Less acid	Bland to slightly acid	Bland to slightly acid	Bland to slightly acid
Solubility :				
Ether	Soluble	Soluble	Slightly soluble	Slightly soluble
Petroleum ether	Soluble	Soluble	Soluble	Soluble
Acetone	Soluble	Soluble	Soluble	Soluble
Chloroform	Soluble	Soluble	Partially soluble	Partially soluble
Benzene	Soluble	Soluble	Soluble	Soluble
Water	Insoluble	Insoluble	Insoluble	Insoluble

4 -Chemical properties of *Calendula officinalis* L .

inflorescences extracts obtained by different organic solvents :

Data recorded in Table (12) show the effect of different organic solvents on chemical properties of *Calendula officinalis* L . inflorescences extracts from Maryout and EI - Sheikh Zowaid locations . The highest acid values were 11.30 and 10.93 in Maryout and EI - Sheikh Zowaid samples ,respectively resulted from the extraction with ethyl alcohol 96 % , while the lowest acid values were obtained from the extracts with petroleum ether for both locations , respectively . Meanwhile the highest ester values were 107.35 in Maryout sample and 112.40 in EI - Sheikh Zowaid sample resulted from n - hexane extraction and the lowest ester values were obtained from ethyl alcohol 80 % extracts . As for the saponification value ,it could be noticed that n - hexane extracts had the highest saponification value which were 116.65 and 122.12 in Maryout and EI - Sheikh Zowaid samples ,respectively . While

ethyl alcohol 80 % extracts had the lowest saponification values in both locations , respectively .

Table (12) Chemical constants of the extracts of *Calendula officinalis* L . inflorescences obtained by different organic solvents :

Properties	Maryout location			
	Petroleum ether	n - hexane	Ethyl alcohol 96 %	Ethyl alcohol 80 %
Acid value	7.65	9.30	11.30	10.76
Ester value	102.60	107.35	98.30	96.32
Saponificatio value	110.25	116.65	109.60	107.08
El - Sheikh Zowaid samples				
Acid value	7.20	9.72	10.93	10.20
Ester value	104.35	11.40	102.30	95.30
Saponificatio value	111.55	122.12	113.23	105.50

5 - Volatile oil components of *Calendula officinalis* L . inflorescences as affected with different organic solvents in Maryout and El - Sheikh Zowaid locations .

Table (13) show the main components of *Calendula officinalis* L . inflorescences in the extracts of selective organic solvents using gas liquid chromatographic techniques . Data revealed that the number of separated components and its percentage in every extract depending upon the retention time of each component .

With using petroleum ether, n - hexane, ethyl alcohol 96 % and ethyl alcohol 80 % extracts in Maryout samples , there were 12 , 12 , 15 and 19 peaks detected , respectively . While there were 15 , 15 , 22 and 29 different peaks detected from El - Sheikh Zowaid samples using the same mentioned organic solvent extracts , respectively .

From the above results , we can concluded that El - Sheikh Zowaid samples contain higher number of identified peaks than the other habitat samples .

The maximum percentage of the main component isolated from petroleum ether extract was α - terpene (57.32 %) in Maryout sample. Mean while it was cyclohexanone , 2 - methylene - 5 - 1 methyl ethyl (21.37 %) in El - Sheikh Zowaid sample. As for n - hexane extract , the highest percentage were α - cadinene (18.55 %) and thymol (30.98 %) obtained from Maryout and El - Sheikh Zowaid samples, respectively. Thujone with (30.00 %) and α - cadinene with (20.88%) were separated in ethyl alcohol (96 %) extracts in Maryout and El - Sheikh Zowaid samples, respectively. While the main components in ethyl alcohol (80 %) extracts were α - cadinene (27.11 %) and thujone (25.31 %) for both locations , respectively.

Table(13) Gas liquid chromatography of the extracts of *C. officinals* L.

Components	Maryout samples %				El-sheikh Zowaid samples %			
	Pet. ether	n-hexane	E.alcohol 96%	E. alcohol 80 %	Pet . ether	n-hexane	E.alcohol 96%	E. alcohol 80 %
A -Terpene	7.323	-	-	-	-	-	-	0.125
B- Pinene	-	-	-	-	-	0.067	3.507	1.343
Cineol	-	-	-	-	-	-	4.037	-
Ocemene	-	-	-	0.028	-	-	0.124	0.285
Camphene	-	-	-	-	-	-	-	0.959
Unknown	-	-	-	-	-	-	-	0.924
Menthol	-	-	-	-	-	-	2.795	0.205
Me - chavicol	-	-	0.540	-	-	-	-	0.556
Geranyl acetate	-	-	0.907	-	1.318	-	0.283	0.123
Me - acetate	-	-	1.081	-	-	-	-	-
Carvacrol	-	-	-	0.767	-	-	0.881	0.144
Caryophyllene	-	-	2.213	-	-	-	0.792	2.111
Eugenol	-	-	1.654	1.120	-	-	-	-
Carvone	-	-	-	-	0.534	0.233	-	1.981
Phellendrene	-	9.923	2.741	13.948	6.902	9.014	6.580	17.301
Menthone	-	-	4.241	1.255	9.098	5.786	-	5.134
Thymol	1.134	-	0.459	1.109	-	0.981	0.642	-
β- Bourbonene	-	-	0.890	0.504	-	0.954	0.788	1.317
Limonene	-	0.759	2.809	3.557	3.091	9.317	1.998	2.174
Linalool	4.328	3.996	7.181	5.935	4.627	3.980	8.406	11.751
Linalyl acetate	3.299	9.861	-	1.107	4.614	4.082	1.475	10.521
Cyclohexanone,2-methylene 5 -(1-methyl ethyl)	5.661	8.795	-	7.323	1.377	7.494	2.167	0.437
E - 2 - decanol	2.264	2.962	1.637	6.744	-	1.767	2.839	3.245
α -cadinene	2.374	8.559	-	27.111	7.358	-	0.879	-
γ - muurolene	1.503	2.850	-	3.695	-	-	1.824	1.427
Thujone	-	4.529	0.004	-	4.744	-	-	25.313
B- damascenone	0.573	3.180	1.831	9.408	2.344	-	1.779	9.689
α -amorphene	-	0.544	1.812	12.308	1.747	1.557	3.663	-
Me - eugenol	1.047	4.042	-	1.081	0.217	0.821	1.997	0.545
Unknown	0.220	-	-	-	0.874	-	-	-
Unknown	0.274	-	-	-	-	-	-	-
Unknown	-	-	-	1.545	1.156	1.695	-	1.432
β - oplopenone acid	-	-	-	1.456	-	-	1.659	0.179
β- Gurjunene	-	-	-	-	-	-	-	0.072
α -Cadinol	-	-	-	-	-	-	-	0.405
Unknown	-	-	-	-	-	-	-	0.016
Dedecanoic acid	-	-	-	-	-	2.253	0.885	0.285

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دراسات مورفولوجية وكيميائية على الأقحوان المنزرع في الأراضي الرملية والجيرية

الهام محمد عطية - زهيرة توفيق زكي إبراهيم - فاطمة على احمد
مركز بحوث الصحراء - المطرية - القاهرة

يتبع نبات الأقحوان العائلة المركبة ويتميز بأهمية الطبية حيث انه يدخل في مضادات الالتهابات وكضمامات للجروح والبثرات ومنتشط للكبد والمرارة ويساعد في منع النزيف ويقلل من التهابات العقد الليمفاوية علاوة على استخدام مستخلص البتلات كملونات طبيعية لبعض المنتجات الغذائية بديلا عن الملونات الصناعية.

وقد تم إجراء التجربة على نبات الأقحوان في كل من محطة بحوث مريوط (أرض جيرية) ومحطة بحوث الشيخ زايد (أرض رملية) والتابعتين لمركز بحوث الصحراء في موسمي ٢٠٠٣ و ٢٠٠٤ م وذلك لدراسة تأثير كل من مسافات الزراعة (١٠٠ - ٥٠ سم) وأربع مستويات من التسميد النيتروجيني (٦٠ و ٨٠ و ١٠٠ كيلو جرام نتروجين للفدان) على الصفات المورفولوجية للنبات والصفات الفيزيائية والكيميائية لمستخلصات الأزهار بواسطة أربع مذيبات عضوية وهي (الإثير البترولي والهكسان والكحول الإيثيلي ٩٦% و ٨٠%).

وقد أظهرت النتائج الآتي :

- ١- أن الزراعة على مسافة ١٠٠ سم ومستوى تسميد ١٠٠ كم نتروجين للفدان قد أعطى أكبر عدد من الأفرع والأزهار وكذلك أكبر قيمة لأقطار الأزهار والوزن الطازج والجاف للأزهار لكل نبات وكانت هناك فروق معنوية بين الصفات السابقة لكل من مسافتي الزراعة (١٠٠ و ٥٠ سم) بينما لم تظهر النتائج فروق معنوية عند استخدام معدل التسميد (١٠٠ و ٨٠ كجم نتروجين للفدان لكل من منطقتي مريوط والشيخ زايد).
- ٢- أن الزراعة على مسافة ٥٠ سم في كلا المنطقتين وباستخدام معدل ١٠٠ كجم نتروجين للفدان قد أعطى أعلى ارتفاع للنبات وكذلك أعلى محصول للأزهار الجافة للفدان.
- ٣- أعطت الزراعة على مسافة ١٠٠ سم مع التسميد النيتروجيني بمعدل ١٠٠ كجم نتروجين للفدان أعلى نسبة من النتروجين والبروتين الكلي في الأزهار في كلا المنطقتين.
- ٤- أن أعلى نسبة منوية للزيت العطري في منطقة مريوط كانت عند الزراعة على مسافة ١٠٠ سم ومعدل تسميد ٨٠ كجم نتروجين للفدان بينما

في منطقة الشيخ زويد كانت أعلى نسبة للزيت العطري عند الزراعة على مسافتي ١٠٠ و ٥٠ سم مع معدل التسميد (١٠٠ كجم نتروجين للفدان) لكل منهما. وأوضحت النتائج أن أعلى نسبة من الكاروتينات في منطقة مريوط كانت عند الزراعة على مسافة ١٠٠ سم مع التسميد بمعدل ١٠٠ كجم نتروجين بينما كانت في الشيخ زويد عند الزراعة على مسافة ٥٠ سم مع معدل التسميد ١٠٠ كجم نتروجين للفدان.

٥ - تم دراسة الصفات الطبيعية للمستخلصات والتي تتمثل في (الكثافة النوعية - معامل الانكسار - نقطة الذوبان - نقطة التجلط - اللون - المذاق - الرائحة والذائبية في الأثير والأثير البترولي والاسيتون والكلورفورم والبنزين والماء). في كلا منطقتي الدراسة.

٦ - تم دراسة الخواص الكيميائية للمستخلصات في كل من مريوط والشيخ زويد والتي تتمثل في (رقم الحموضة - رقم الأستر - رقم التصبن).

٧ - تم تحديد المكونات المختلفة في مستخلصات الأزهار باستخدام جهاز كروماتجرافيا السائل الغازي في كلا المنطقتين لتحديد المكونات الكمية والنوعية لكل مستخلص.