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EFFECT OF NITROGEN ON YIELD AND ACTIVE CONSTITUENTS OF *CALENDULA OFFICINALIS* L.

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The field experiment was carried out during two successive seasons (2001 – 2002) at Maryout Res. Station to study the effect of ammonium sulfate (as a source of N fertilization) with two levels (40 and 60 Kg N/fed.) and its method of application i.e. all amount added at once or divided into two or three equal portions after30, 60 and 90 days from transplanting on yield, volatile oil, carotenoids and minerals content of *Calendula officianlis* L. flowers. Yield characters i.e. number of flowers/plant as well as flowers fresh and dry weights were affected with nitrogen levels and its application. The maximum yield of flowers was obtained as a result of 60 kg N/fed especially when this amount was divided into two portions. Concerning the effect of N application on carotenoids content and volatile oil percentage, it can be noticed that these constituents reached its maximum values also as a result of using 60 kg N/fed divided into two portions. Minerals status was affected by N fertilization. Rising N level increased N percentage in flowers. On the other hand, increasing N fertilization decreased P and K percentage in *Calendula officianlis* L. flowers.

Keywords: nitrogen fertilization, carotenoids, volatile oil, asteraceae, *Calendula officianlis* L.

One of the most important ornamental plants is *Calendula officianlis* L., (potmarigold), belongs to family Asteraceae (compositae) which is one of largest families of plant kingdom. It includes about 25.000 species in over 15.000 genera. *Calendula officianlis* L. known as ornamental plant for planting in beds and borders, besides its uses as aromatic, drug and colour containing plants. The petals of pot marigold flowers contain yellow pigments formed of carotenoids, carotene, lycopin and calendulin in addition to volatile oil (Hussein, 1985). The flowers are used in case of dysmenorrhea and for the production of calendulin which is used in colouring food products as jellies and jams.

Bailey (1947) mentioned that heads of potmarigold are used sometimes for flavouring in cookary. Grieve (1994) mentioned that only the common deep orange flowers of potmarigold had a medicinal value. The florets are used in medicine as vulnerary and anti-emetic. The flowering plants were formerly used for removing warts and the petals are used to adultrate safron flowers which are very expensive (Hussein, 1985).

Wallis (1960) stated that potmarigold contains traces of volatile oil, a bitter principle, and calendulin, the latter being a tasteless substance swelling in water and used chiefly in the form of the tincture diluted with water as an application to bruises to promote the absorption of effused blood. Many investigations were carried out dealing with N fertilization and its effect on growth and yield of medicinal and aromatic plants, by Jacoub (1995), Hammam (1996), Bhasker *et al.* (1998), Ibrahem (2000) and Abd El-Wahab (2002).

The present work aimed to study the influence of ammonium sulphate as a source of nitrogen fertilizer as well as the split application of nitrogen fertilization on flowers yield and active constituents mainly, volatile oil and carotnoids of *Calendula officianlis* L.

MATERIALS AND METHODS

The present investigation was carried out during two successive seasons of 2001 and 2002 at Maryout Experimental Station, Alexandria Governorate, Egypt.

Soil of the experimental area was characterized by loamy sand texture, with pH 8.4 and about 36.7% $CaCO_3$. Meteorololgical data of Maryout Res. Station during (2000- 2001 & 2001- 2002) in table (1).

Table (1). Meterological data recovered at the experimental site during 2000-2002.

Month	Air temp (°C)	Relative humid %	Total rain (mm)	Month	Air temp (°C)	Relative humid %	Total rain (mm)
	2000 – 2001				2001 – 2002		
Sept. 2000	25.45	73.47	5.59	Sept. 2001	26.54	71.03	0
Oct. 2000	22.08	71.70	59.94	Oct. 2001	22.90	72.5	21.37
Nov. 2000	19.08	77.59	6.35	Nov. 2001	19.82	67.34	3.81
Dec. 2000	19.23	77.97	5.32	Dec. 2001	-	-	-
Jan. 2001	14.10	77.38	28.70	Jan. 2002	13.35	74.55	82.55
Feb. 2001	13.85	66.88	8.38	Feb. 2002	13.37	69.81	22.35
March 2001	17.42	74.89	0.76	March 2002	14.11	75.66	3.81
April 2001	19.41	66.85	-	April 2002	21.01	68.15	4.6
May 2001	22.05	67.54	0.59	May 2002	23.28	68.01	0.58

Source : Appli. Meteorolgical Lab., Desert Research Center, El-Matareya, Cairo, Egypt.

Seeds of *Calendula officianlis* L. were sown in seed beds on 15th Sept. for both seasons. Uniform seedlings at 45 days old, about 10 cm. in height were individually transplanted. All agriculture practices were applied (watering as necessary by surface irrigation)

A split plot design with three replicates was used. The plot area was six square meters, each plot contained (30 plants) as a replicate. The study included the following treatments:

1. Control (Zero N).
2. 60 Kg. N/fed. was applied in three sub treatments:
   A) Added at once after 30 days from transplanting.
   B) Divided into two equal portions, added after 30 and 60 days from transplanting, respectively.
   C) Divided into three equal portions, added after 30, 60 and 90 days from transplanting, respectively.
3. 40 kg.N/fed. was applied in three sub treatments:
   A) Added at once after 30 days from transplanting.
   B) Divided into two equal portions, added after 30 and 60 days from transplanting, respectively.
   C) Divided into three equal portions, added after 30, 60 and 90 days from transplanting, respectively.

Successive harvests of potmarigold flowers were preformed from mid Feb. till mid May at weekly intervals every season (2001 and 2002). Data of flowering characters i.e. number of flowers per plant, fresh weight of flowers per plant and air dried weight of flowers per plant and per feddan were recorded.

The percentage of volatile oil in air dried flowers (V/W) was determined according to Guenther (1961). Satisfactory results were obtained by distilling 100 gm. dried flowers for 2.0 – 2.5 hours using steam distillation method, till no further increase in the oil was observed.

The carotenoids content was extracted by pure acetone according to Fadeel (1962). The concentration of carotenoids were expressed in (mg./gm.) of fresh weight of flowers:

Carotenoids = (4.695 x E 440) – (0.268 x Chlorophyll A+B) = mg/L.

Mg/gm = (mg/L x dilution) / weight x 100

where E. = optical density at given wave length, according to Cherry, (1973).

N,P and K elements were determined in the acid digested solution, using a mixture of hydrogen peroxide and sulfuric acid (4:10). Element extraction was made on 0.2 gm.

Nitrogen content in flowers was determined by modified Microkjeldahl method as described by James (1995).

Protein percentage was estimated by multiplying nitrogen percentage by 6.25. This was based on the assumption that the protein contains 16% nitrogen, according to Ranganna (1978).

Phosphorus determination was carried out using ammonium molybdate method according to Snell and Snell (1949). The method is based on the determination of the blue colour of molybdenum resulted from the addition of three reagents (ammonium molybdate, hydroquinone and sodium sulfite). The absorbance was read at 660 nm using spectronic 601. The results were calculated from a standard curve of potassium dihydrogen phosphate. While potassium was estimated using flame photometer method according to Chapman and Pratt (1961).

The data was statistically analyzed by computer programme (SAS Institute, 1994). L.S.D. test was used to compare the average means of treatments.

RESULTS AND DISCUSSION

Flowers yield and its components

Data recorded in table (2) and fig. (1) indicate that flowers number/plant as well as fresh and dry weight of flowers were significantly affected by the interaction between nitrogen levels and its application method. The highest values of number of flowers/plant tabulated in table (1-A) and fig. (1-A) were 316.00 and 342.67 obtained from 60 kg N/fed divided into two portions compared with 273.0 and 291.67produced from adding 40 kg N/fed. divided into two portions in the first and second seasons, respectively.

The maximum values of flowers fresh weight (gm./plant) and flowers dry weight (gm/plant or kg/fed) were obtained as a result of using 60 kgN/fed added in two portions. Data in table (2-B) and fig. (1-B) showed that the highest values of flowers fresh weight/plant were 299.53 gm. and 331.85 gm. in the first and second seasons, respectively. Meanwhile dividing the N fertilizer amount into two equal portions had a pronounced effect compared with those fertilized with the same amount added at once or divided into three portions.

Concerning the effect of nitrogen levels and its application methods on flowers dry weight, data recorded in tables (2-C and 1-D) and figs. (1-C and 1-D) showed the same trend which was 28.23 gm. per plant in the first season and 29.95 gm per plant in the second season.

The highest yield of flowers dry weight per fed. were 564.60 kg and 599.00 kg per fed. in the first and second seasons, respectively, as a result of the interaction between 60 kg N/fed. and dividing it into two portions.

However, it can be concluded that dividing the amount of 60 kgN/fed or 40 kg N/fed into two portions had a significant increase in

flowers number and yield of flowers compared with other application methods.

The increase of flowers yield due to nitrogenous fertilization might be attributed to the increase in growth leading to more yield in number and weight of flowers.

These results were in agreement with those obtained on several medicinal plants by several investigators (Jacoub, 1995; Hammam 1996; Bhasker *et al.*, 1998; Attia 2000; Badawi, 2000; Ibrahem, 2000; and Abd-El Wahab, 2002).

TABLE (2). Effect of Nitrogen levels and application methods on *Calendula officianlis* L. during 2001-2002.

N level(*) / Appli. methods(**)	(A) Number of flowers/plant						(B) Flowers fresh wt. (gm/plant)					
	1st Season (2001)			2nd Season (2002)			1st Season (2001)			2nd Season (2002)		
	40 Kg N/fed.	60 Kg N/fed.	Mean	40 Kg N/fed.	60 Kg N/fed.	Mean	40 Kg N/fed.	60 Kg N/fed.	Mean	40 Kg N/fed.	60 Kg N/fed.	Mean
Control	218.67	218.67	218.67	240.33	240.35	240.33	204.87	204.87	204.87	221.78	221.78	221.78
Once	265.67	286.67	276.17	281.00	303.00	292.00	242.90	267.12	255.01	269.00	285.19	277.10
Two portions	273.00	316.00	294.50	291.67	342.67	317.17	252.35	299.53	275.94	277.15	331.85	304.50
Three portions	270.67	300.33	285.50	279.00	31933	299.17	245.90	281.25	263.58	260.00	302.50	281.25
Mean	257.00	280.42		273.00	301.33		236.59	263.19		256.98	285.35	
L.S.D. 0.05	*	11.74		*	8.39		*	24.37		*	9.84	
	**	14.63		**	12.07		**	8.54		**	10.67	
	***	20.69		***	17.07		***	12.08		***	15.09	

N level(*) / Appli. methods(**)	(C) Flowers dry wt. (gm./plant)						(D) Flowers dry wt. (kg/fed.)					
	1st Season (2001)			2nd Season (2002)			1st Season (2001)			2nd Season (2002)		
	40 Kg N/fed.	60 Kg N/fed.	Mean	40 Kg N/fed.	60 Kg N/fed.	Mean	40 Kg N/fed.	60 Kg N/fed	Mean	40 Kg N/fed.	60 Kg N/fed.	Mean
Control	13.90	13.90	13.90	14.29	14.29	14.29	278.00	278.00	278.00	285.80	285.80	285.80
Once	16.15	20.92	18.54	17.02	21.59	19.31	323.00	418.40	370.70	340.40	431.80	386.10
Two portions	18.50	28.23	23.37	19.09	29.95	24.52	370.00	564.60	467.30	381.80	599.00	490.40
Three portions	14.12	22.53	18.33	14.05	22.91	17.98	287.40	450.60	366.50	281.00	458.20	359.60
Mean	15.67	21.40		15.86	22.19		313.35	427.90		317.25	443.70	
L.S.D. 0.05	*	0.13		*	1.72		*	25.67		*	34.45	
	**	1.16		**	1.42		**	23.27		**	28.35	
	***	7.02		***	5.49		***	140.45		***	109.74	

* N Level ** Application *** N level x Application

Effect of nitrogen levels and its applications on volatile oil percentage and cartenoids content in flowers

Volatile oil percentage

The relation between nitrogen treatments and volatile oil percentage is shown in table (3). It can be noticed that the increase of volatile oil percentage was parallel to the increase of nitrogen fertilization level. Concerning the effect of dividing the fertilizer amount, it can be mentioned that dividing it into two portions gave the highest values in both seasons. The maximum values of volatile oil percentage was obtained as a result of dividing 60 kg. N/fed . into two portions which were 0.27% and 0.29% in the first and second seasons, respectively. Thus, nitrogen fertilization might

enhance volatile oil biosynthesis, through playing indirect role in the volatile oil biosynthesis process. In fact, nitrogen might participate in plant metabolism resulting in more plant metabolites.

These results were in agreement with those obtained on several medicinal plants by Zheljazkov and Margina (1996), Youssef *et al.*(1998), Ibrahem (2000) and Abd El-Wahab (2002).

Carotenoids contents

The data on cartenoids content in *Calendula officianlis* L. fresh flowers as affected by nitrogen levels (60 kg N/fed and 40 kg N/fed) and its application method are shown in table (4). It was noticed that the greatest carotenoids content were 8.90 mg/gm and 9.95 mg/gm. fresh flowers, obtained from using the high level of nitrogen fertilizer (60 kg N/fed) when divided into two portions in the first and second seasons, respectively.

However, the higher nitrogen level, the higher carotenoids content and vice versa.

These results concerning the increment of photosynthetic pigments content may be explained by more availability of mineral to plants due to more amount of nitrogen enhancing their uptake by the plants.

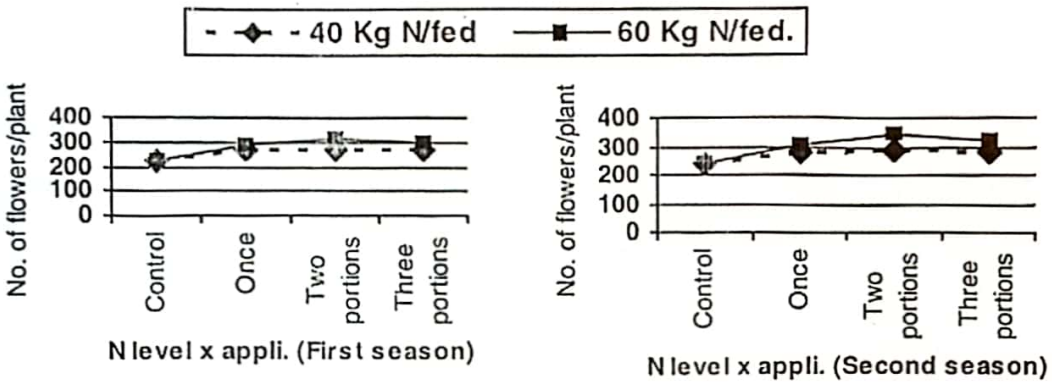
Mineral status and crude protein percentage in flowers as affected by N fertilization and its application methods

Average values of N, P, K and crude protein percentages of potmarigold flowers as affected by different treatments (40 Kg N or 60 kg N) added once, twice or three portions are presented in table (4). It could be appeared that nitrogen percentage in flowers increased with the increment of nitrogen fertilization level. Moreover, dividing the N fertilizer amount into two portions produced the highest values of nitrogen percentage among all application methods. The highest N percentages were 3.39% and 4.00% obtained from 60 kg N/fed divided into two portions in the first and second seasons, respectively.

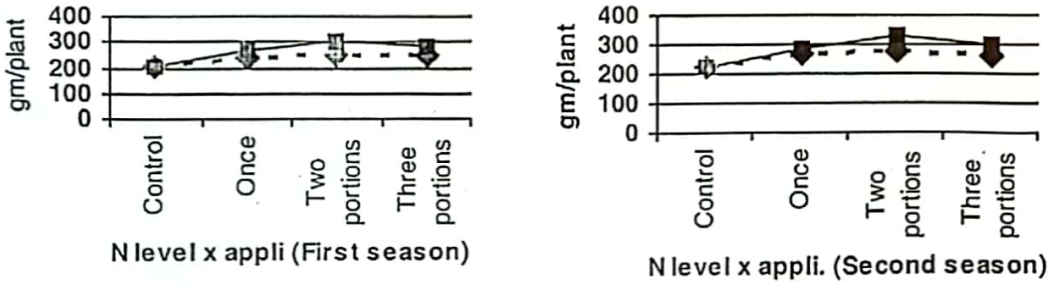
On the other hand, high nitrogen fertilization level (60 kg N/fed) decreased P% and K% in both seasons.

Total crude protein percentage in *Calendula officianlis* L. dried flowers as affected by different N fertilization treatments are shown in table (4). Generally, the increase of nitrogen percentage in flowers produced increment of crude protein. The highest crude protein percentages were 21.19% and 25.13% produced from using 60 kg N/fed dividied into two portions in the first and second seasons, respectively. The lowest percentages of crude protein in pot marigold flowers were 7.13% and 7.44% obtained from control treatments in both seasons.

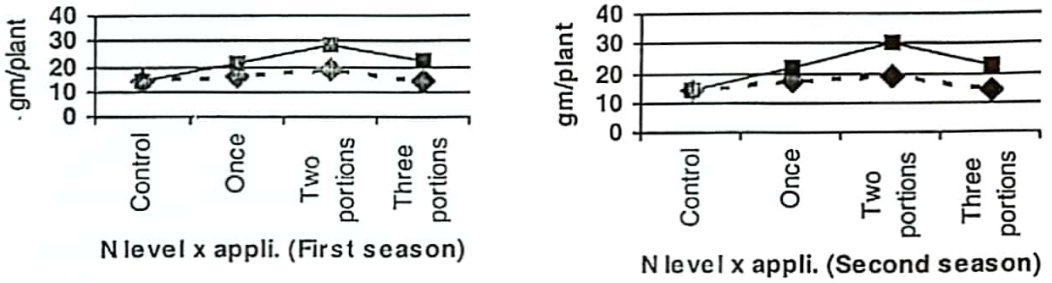
Abdalla *et al.* (1991) stated that N fertilization on *Calendula officianlis* L. increased the N content and decreased the levels of P and K in treated plants.



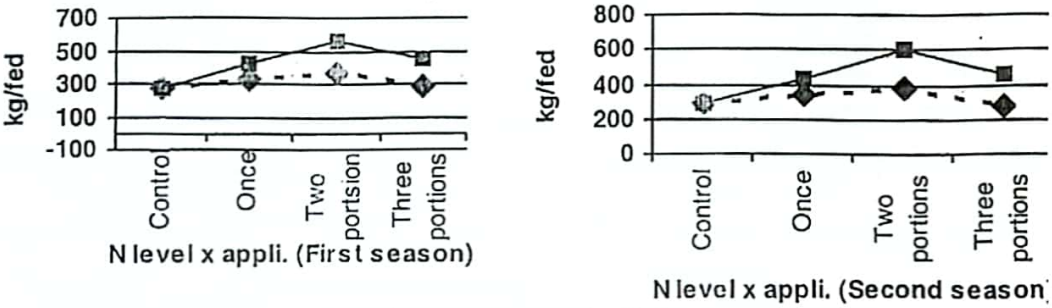
(A) Number of flowers/plant



(B) Flowers fresh wt. (gm/plant)



(C) Flowers dry wt. (gm/plant)



(D) Flowers dry wt. (kg/fed.)

Fig. (1). Effect of Nitrogen levels and application methods on *Calendula officianlis* L. during 2001-2002.

TABLE (3). Effect of N fertilization and its application on volatile oil percentage and carotenoids content (mg/g) in *Calendula officinalis* L. flowers during 2001 and 2002 seasons.

Contents	Control	1st season 2001					
		40 kg/ N/fed.			60 kg N/fed.		
		One portion	Two portions	Three portions	One portion	Two portions	Three portions
Volatile oil % (ml./100 gm)	0.12	0.18	0.24	0.20	0.20	0.27	0.26
Carotenoids content (mg/g.)	5.50	7.01	7.68	6.59	7.72	8.90	6.50
2nd season 2002							
Volatile oil % (ml./100 gm)	0.16	0.19	0.26	0.19	0.21	0.29	0.24
Carotenoids content (mg/g.)	5.62	7.20	7.95	6.61	7.83	9.95	7.14

TABLE (4). Effect of N fertilization and its applications on nitrogen, phosphorus, potassium and crude protein percentage of *Calendula officianlis* L. flowers during the 2001-2002.

First season (2001)							
Element	Control	40 kg N/fed.			60 kg N/fed		
		One portion	Two portions	Three portions	One portion	Two portions	Three portions
N	1.14	2.26	3.33	2.25	2.29	3.39	2.31
P	0.29	0.37	0.44	0.49	0.32	0.39	0.40
K	1.17	1.19	1.10	1.15	1.12	0.99	1.05
Crude protein	7.13	14.13	20.81	14.06	14.31	21.19	14.44
Second season (2002)							
N	1.19	2.27	3.11	2.34	2.68	4.00	2.85
P	0.36	0.48	0.40	0.43	0.46	0.39	0.40
K	1.09	1.29	1.18	1.23	1.20	1.05	1.09
Crude protein	7.44	14.19	19.44	14.63	16.75	25.13	17.13

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تاثير التسميد بالأزوت على المحصول والمكونات الفعالة لنباتات الأقحوان

زهيرة توفيق زكى ابراهيم ، فاطمة على احمد ، الهام محمد عطية
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تم إجراء تجارب حقلية خلال موسمين متتالين ٢٠٠١ – ٢٠٠٢ بمحطة بحوث مريوط لدراسة تاثير التسميد النتروجينى بسماد سلفات النشادر (بواقع ٦٠ كجم نتروجين للفدان ، ٤٠ كجم نتروجين للفدان) مع إضافة السماد فى كل معاملة على دفعة واحدة أو مقسما على دفعتين أو ثلاث دفعات متساوية على المحصول والزيت الطيار والكاروتينات والمحتوى من عناصر النتروجين والفوسفور والبوتاسيوم لأزهار الأقحوان .

وقد أوضحت النتائج أن مكونات المحصول مثل عدد الأزهار لكل نبات ، وكذلك الوزن الرطب والجاف للأزهار تأثر باضافة النتروجين – وقد تم الحصول على أعلى محصول نتيجة التسميد بـــ ٦٠ كجم نتروجين للفدان وخاصة عند تقسيم هذه الكمية إلى جزئين.

وبالنسبة لتأثير النتروجين على الكاروتينات والزيت الطيار فقد وجد أن أعلى قيمة تم الحصول عليها نتيجة لإضافة ٦٠ كجم نتروجين على دفعتين أيضا ، وقد تأثر المحتوى الكيماوى من النتروجين والفوسفور والبوتاسيوم فى الأزهار بزيادة معدل التسميد النتروجينى.