

**EFFECT OF NITROGEN FERTILIZER AND  
GIBBERELIC ACID ON GROWTH, FLOWERING AND  
CHEMICAL COMPOSITION OF (*CALENDULA  
OFFICINALIS* L.) PLANT.**

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**ABSTRACT**

The impacts of three different N fertilizer levels (1, 2 and 4 g urea/ pot) and four concentrations of gibberellic acid (GA3), 0.0, 100, 200 and 300 ppm, on vegetative growth, flowering and chemical composition of *Calendula officinalis* L. plants were assessed during the two successive seasons, 2000/ 2001 and 2001/ 2002 at the experimental area, Faculty of Agriculture, Cairo university, Fayoum branch.

The obtained results clearly showed that increasing N applied rate from 1 to 2 g urea/ pot significantly, increased plant height, number of branches/ plant, number of flowers/ plant and flower diameter. However, further increase of N application to 4 g urea/ pot did not reflect any valuable effect of the aforementioned vegetative or flowering traits. The relationship between N applied and flower N percent was positive, but was negative with leaf carotein content, while the intermediate N level gave better results on leaf carbohydrate contents than the other used N levels. Foliar application of GA3 at 200 – 300 ppm appeared to reflect desirable effects on all the studied vegetative and flowering traits. Pronounced enhancing influences on leaf chlorophyll, carotenoids and carbohydrate contents were obvious as a consequence of spraying GA3 at 100 ppm in 2000/ 2001 and at 200 ppm 2001/ 2002, but flower N content positively respond to GA3 application at 100 ppm in one out of the two experimental seasons. The interaction of N level and GA3 concentration, seemed to reflect some positive significant effects on the vegetative, flowering and chemical

**composition. Generally, addition of 2 or 4 g urea/ pot together with spraying GA3 at 200 or 300 ppm was remarked in this respect.**

## INTRODUCTION

Fertilization in general and with nitrogen in particular has been known as a vital step in stepping up the growth and flowering of many ornamental plants. For instance, Yadar and Singh (1997), Dahiya *et al.* (1998), Chadha *et al.* (1999), Hameed and Sekar (1999), Ram *et al.* (1999) and Broschat and Moore (2001) working on marigold plants and El-gendy *et al.* (2001), working on *Ocimum basilicum* declared that

increasing N application, up to a particular level, consistently increased vegetative growth parameters and flowering traits as well as the total contents of carbohydrates and nitrogen.

The effectiveness of utilization growth regulators as GA3 on improving and promoting the vegetative growth, flowering and chemical composition of different ornamental plant species has been recognized by many investigators (Girwany *et al.* (1990) and Singh *et al.* (1991) on marigold, Maximoos (1993) on gerbera and El-Keltawy *et al.* (1997), Haggag (1997), Talukdar and Paswan (1998) on chrysanthemum and Devendra *et al.* (1999) on Tuberosa. The presence of nitrogen together with GA3 resulted in the best results on the growth of *Calendula officinalis* (Nasr, 1994) and on basil (Santos *et al.*, 1998).

Therefore, this investigation aimed to study the combination effect of nitrogen and GA3 on vegetative growth, flowering and chemical composition of (*Calendula officinalis* L.) plant.

## MATERIALS AND METHODS

Two pot experiments were carried out at the experimental area, Faculty of Agriculture, Cairo University in Fayoum, during the successive seasons of 2000/ 2001 and 2001/ 2002, to investigate the effect of N fertilization and gibberellic acid (GA3) application on (*Calendula officinalis* L.) plants.

The seeds were supplied by the Research Center of Medicinal and Aromatic Plants, Giza, Egypt and were sown in the nursery on 15<sup>th</sup> of September in 2000 and 2001 seasons. All agro-managements required for seedlings production were followed. Uniform seedlings of 45 days old were transplanted into clay pots of 30 cm diameter. Each pot was filled with air dried soil (1 clay : 1 sand) and contained one seedling .

Treatments comprised three different N rates, 1, 2 and 4 g urea/ pot and four various concentrations of GA3, 0, 100, 200 and 300 ppm. Such quantities of urea were divided into three equal portions, for each pot. Addition of these portions began at the fifth week of transplanting, then at one week intervals. Foliar application of GA3 treatments were performed three times (at the 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks after transplanting).

The experimental layout was a factorial experiment in a randomized complete block design with five replicates. The following measurements were determined at the end of each season:

1-Vegetative growth characters in terms of plant height and number of branches/ plant (main and secondary shoots).

2- Flowering traits expressed as, inflorescence diameter, total number of inflorescences/ plant and fresh weight of inflorescence.

3- Chemical composition: concentrations of total chlorophylls and carotenoids, in the fresh leaves, at the beginning of flowering, were determined according to the methods described by Welburn and Lichtenthaler (1984). Total carbohydrates in the inflorescences was colorimetrically estimated as outlined by Herbert *et al.*(1971). Total N in the dry flowers was determined using orange G dye colorimetric method according to Hafez and Hikkelsem (1981) .

All obtained data were statistically analyzed according to the design used and comparisons among means of the different treatments were achieved using Least Significant Difference test (L.S.D.) at  $p = 0.05$  (Snedecor and Cochran, 1980 ).

## RESULTS AND DISCUSSION

### Vegetative Growth Characters

Data arranged in Tables (1&2) exhibited that N application, to the growing *Calendula officinalis* plants at the intermediate level of 2g urea/ pot was remarkable and associated, significantly, with higher mean magnitudes for plant height and number of branches/ plant than those of the lower or higher N level i.e.1 or 4 g urea/ pot. However, plant height and number of branches/ plant were similar whether N application was 1 or 4g urea/ pot. The obtained results matched well with those reported by Yadar and Singh (1997), Dahiya *et al.* (1998), Chadha *et al.* (1999), Hameed and Sekar (1999), Ram *et al.* (1999) and Broschat and Moore (2001) working on marigold plants. They concluded that, the response of vegetative growth to N application was positive at the intermediate level, but above or lower which the response, often, became negative.

In both seasons, spraying the foliage of *Calendula officinalis* plants with GA3 at 300 ppm concentration, significantly resulted in taller plants than those of the lower concentrations Tables (1&2). The same effect on number of branches/ plant was true when the plants were sprayed with 200 or 300 ppm GA3, however, the difference between them, was not significant. The favourable effect of spraying GA3 on plant height and number of branches/ plant might be attributed to the role of GA3 on cell division in the sub-apical meristeme and or cell expansion (Greulach and Haesloop (1985). The obtained results are supported by the findings of Singh *et al.* (1991) and Nasr (1994), who retrieved that increasing GA3 concentration increased all studied growth parameters of marigold plants. Likely, El-Keltawy *et al.* (1997) emphasized that spraying GA3 at 100 ppm increased stature of marigold plant.

Concerning the interaction between the different amounts of urea fertilizer and the various concentrations of GA3 on plant height and number of branches/ plant varied between the two seasons; the significant response was obvious in one out the two experimental

seasons Tables (1&2). Comparisons among the twelve interactive treatments clearly indicated that the treatment combination of 2 g urea/ pot + 200 or 300 ppm GA3 recorded the best mean values of plant height and number of branches/ plant. The stimulative effects of N by GA3 interaction are in agreement with those of Nasr (1994) on *Calendula officinalis* and Santos *et al.* (1998) on basil, who mentioned that the combination of N with GA3 increased the vegetative growth of plants.

**Flowering Traits :**

The results listed in Tables (1&2) clarified that, increasing the amount of N fertilizer from 1 to 2 g urea/ pot significantly promoted the number of inflorescences/ plant and diameter and fresh weight/ inflorescences to go forward, in 2000/ 2001 and 2001/ 2002 seasons. The exception was in 2000/ 2001 season as the inflorescence fresh weight was similar with the applications of 1 and 2 g urea/ pot. However, increasing N applied rate from 2 to 4 g urea/ pot did not reflect any pronounced effect on the aforementioned flowering traits except that of inflorescence fresh weight in 2000/ 2001 season. The obtained results are in harmony with those reported by Yadar and Singh (1997), Dahiya *et al.* (1998), and Chadha *et al.* (1999), Hameed and Sekar (1999), Ram *et al.* (1999) and Broschat and Moor (2001), on marigold plants. They concluded that nitrogen fertilization increased flowering traits.

Increasing the concentration of GA3 from 0.0 to 100 ppm and furtherly to 200 and 300 ppm was accompanied by progressive significant increase in number of flowers/ plant and the trend was exactly the same in both seasons (Tables 1&2). Inflorescences diameter and fresh weight, also, significantly responded to the foliar application of GA3, but the response was not often similar, in both seasons. Generally, spraying GA3 at 200 and 300 ppm were adequate to produce larger inflorescences diameter and 100 and 200 ppm were favourable to attain the highest inflorescence fresh weight. The desirable influences of GA3 on the studied flowering traits might be attributed to the role of GA3 on stimulating branching and cell elongation (Gulati *et al.*, 1979). Haggag (1997) mentioned that spraying the foliage of chrysanthemum with GA3 at 100 ppm increased the diameter of inflorescence.

The interaction effects of N levels by GA3 concentrations on the number of inflorescence/ plant and fresh weight/ inflorescences were significant in 2000/ 2001 and 2001/ 2002 seasons, but diameter/ inflorescences significantly affected in one out of the two experimental seasons Tables (1&2). The varying statistical comparisons among the different combination treatments showed obviously that number of inflorescences/ plant was the greatest with the treatment combination of 2 or 4 g urea/ pot + 300 ppm GA3, and fresh weight/ inflorescence was the heaviest with 2g urea/ pot + 200 or

**Table (1):** Effects of nitrogen fertilizer and GA<sub>3</sub> on vegetative growth and flowering traits of *Calendula officinalis* in the first season, 2000/2001.

Urea gm	Plant height (cm)				Number of branches/ plant				Number of flowers/ plant				Flower diameter (cm)				Flower fresh weight (gm)			
	1	2	4	Mean	1	2	4	Mean	1	2	4	M	1	2	4	Mean	1	2	4	Mean
GA3 ppm																				
0.00	36.07 cd	37.46 cd	31.67 e	35.04 C	84.12 a	86.67 a	82.00 a	84.26 C	84.80 j	116.7 f	88.67 ij	96.71 D	4.75 a	4.77 a	4.67 a	4.73 B	0.84 ef	0.80 fg	0.88 de	0.84 D
100	37.11 cd	37.87 bcd	35.23 d	36.74 C	86.09 a	93.00 a	83.33 a	87.47 B	94.67 hi	154.0 c	89.67 hij	112.8 C	4.97 a	5.13 a	5.15 a	5.08 A	0.88 de	0.79 g	1.02 ab	0.90 B
200	38.97 bc	43.73 a	36.60 cd	39.77 B	89.01 a	95.67 a	92.00 a	92.23 A	108.4 g	171.3 b	125.7 e	135.1 B	4.94 a	5.27 a	4.93 a	5.05 A	0.93 c	1.05 a	0.98 b	0.98 A
300	41.25 ab	44.50 a	44.70 a	43.48 A	89.95 a	97.67 a	93.67 a	93.76 A	117.6 f	192.3 a	140.3 d	150.1 A	4.77 a	4.86 a	4.66 a	4.76 B	0.86 e	0.84 ef	0.92 cd	0.87 C
Mean	38.35 B	40.88 A	37.05 B		87.29 B	93.25 A	87.75 B		101.4 C	158.6 A	111.1 B		4.86 B	5.01 A	4.85 B		0.88 B	0.87 B	0.95 A	

Values marked with the same letter (s), within the main effects and interactions, are not significantly Different at  $p = 0.05$  using LSD test.

**Table (2):** Effects of nitrogen fertilizer and GA<sub>3</sub> on vegetative growth and flowering traits of *Calendula officinalis* in the second season, 2001/2002

Urea gm GA3 ppm	Plant height (cm)				Number of branches/ plant				Number of flowers/ plant				Flower diameter (cm)				Flower fresh weight (gm)			
	1	2	4	Mean	1	2	4	Mean	1	2	4	Mean	1	2	4	Mean	1	2	4	Mean
0.00	25.05 a	28.63 a	24.27 a	25.98 C	139.8 e	176.7 c	139.7 e	152.1 B	175.4 g	199.7 ef	196.0 ef	190.3 D	4.64 fg	4.73 def	4.53 g	4.63 C	1.22 cd	1.27 cd	1.24 cd	1.24 B
100	26.59 a	34.53 a	24.33 a	28.49 B	138.9 e	165.0 cd	147.7 de	150.5 B	192.9 f	243.7 c	220.0 d	218.8 C	4.70 efg	5.20 a	4.32 h	4.74 B	1.13 de	1.17 d	0.98 e	1.09 C
200	27.70 a	34.70 a	28.47 a	30.29 B	169.7 c	260.7 b	172.3 c	200.9 A	205.1 def	259.0 abc	252.3 bc	238.8 B	4.85 cde	5.10 ab	5.00 bc	4.98 A	1.27 de	1.48 b	1.22 cd	1.32 B
300	29.28 a	36.97 a	32.33 a	32.86 A	181.8 c	297.7 a	181.7 c	220.4 A	213.6 de	267.7 ab	276.3 a	252.5 A	4.83 cdef	4.90 cd	5.12 ab	4.95 A	1.34 bc	1.64 a	1.33 bc	1.43 A
Mean	27.16 B	33.71 A	27.35 B		157.6 B	225.0 A	160.3 B		196.7 B	242.5 A	236.2 A		4.75 B	4.98 A	4.74 B		1.24 B	1.39 A	1.19 B	

Values marked with the same letter (s), within the main effects and interactions, are not significantly Different at  $p = 0.05$  using LSD test.



300 ppm GA3, but inflorescence diameter was the greatest with the treatment combination of 2 g urea/ pot 100 ppm GA3.

#### **Chemical composition**

As seen in Tables (3&4), the effect of N application rate on total carotenoids content in leaves and N content in flowers was significant and the trend was exactly similar in both seasons. Increasing N application up to the highest rate correspondingly increased flower N content, but reversely decreased leaf carotenoid content. The influence of N applied rate on total leaf chlorophyll content varied between the two seasons. In the first season, the lowest N rate (1 g urea/ pot) significantly achieved the highest concentration of N, whereas in the second season, the reverse was true. Regarding carbohydrate contents in inflorescences, the intermediate N rate (2 g urea/ pot) significantly resulted in higher content than lower N level (1 g urea/ pot). However, increasing N application from 2 to 4 g urea/ pot did not, significantly, exceed inflorescences carbohydrate contents.

In this respect, Yadar, *et al* (1999) reported that on African marigold total leaf chlorophyll and nitrogen content increased consistently with nitrogen application up to 180 ppm.

Tables (3 & 4) clarified that total chlorophylls and carotenoids contents in leaves were significantly the best as the foliar application of GA3 was at 100 ppm in 2000/ 2001 season and at 200 ppm in 2001/ 2002 season. Likely value of total carbohydrate contents in inflorescences was significantly the highest when the plants were treated with GA3 at 100 ppm in both seasons. Unlikely application GA3, irrespective of the used concentration, did not appear to have any valuable effect on the percentage of N in flowers, In 2000/ 2001 season. However, in 2001/ 2002 season spraying GA3 at 100 ppm was favourable and significant attained the highest mean value of N percent in flowers.

These results are on the line with Haggag (1997) on chrysanthemum plants, retrieved that GA3 at 200 ppm, total carbohydrates content decreased, while chlorophyll content was increased.

Concerning interactions of the fertilizer and the growth regulator(GA3), recorded determinations fluctuated significantly, however, observation of the highest ones differed as the season and parameter. The highest values of chlorophyll and carotenoids were

**Table (3):** Effects of nitrogen fertilizer and GA<sub>3</sub> on chemical composition of *Calendula officinalis* in the first season, 2000/ 2001

Urea gm	Chlorophyll (A + B) (mg/ g)				Total carotenoids (mg/ g)				Carbohydrates %				Nitrogen %			
	1	2	4	Mean	1	2	4	Mean	1	2	4	Mean	1	2	4	Mean
0.0	2.04 b	1.68 f	1.81 e	1.84 D	0.72 c	0.28 j	0.30 h	0.43 C	18.3 4	34.6 8	34.4 8	29.1 7	1.59 c	1.97 c	2.0 2	1.86 A
100	2.17 a	2.08 b	1.93 cd	2.06 A	0.77 a	0.45 e	0.33 g	0.52 A	18.7 6	35.6 8	35.0 9	29.8 6	1.54 de	1.41 fg	2.3 5	1.77 B
200	2.07 b	1.94 cd	1.64 f	1.88 C	0.71 d	0.26 l	0.29 I	0.42 D	18.9 3	35.5 3	35.8 8	30.1 1	1.50 def	2.52 a	1.1 0	1.71 B
300	2.15 a	1.91 d	1.97 c	2.01 B	0.75 b	0.27 k	0.41 f	0.48 B	17.5 8	34.7 4	31.4 0	27.9 1	1.45 efg	1.35 g	2.0 6	1.62 C
Mean	2.11 A	1.90 B	1.84 C		0.74 A	0.32 C	0.33 B		18.4 0	35.1 6	34.2 1		1.52 C	1.81 B	1.8 8	

Values marked with the same letter (s), within the main effects and interactions, are not significantly

Different at  $p = 0.05$  using LSD test.

**Table (4):** Effects of nitrogen fertilizer and GA<sub>3</sub> on chemical composition of *Calendula officinalis* in the second season, 2001/ 2002

Urea gm GA <sub>3</sub> ppm	Chlorophyll (A + B)				Total Carotenoids				Carbohydrates %				Nitrogen%			
	1	2	4	Mean	1	2	4	Mean	1	2	4	Mean	1	2	4	Mean
0.0	2.19 e	1.65 f	2.46 d	2.10 D	0.77 c	0.39 I	0.40 h	0.52 C	19.29 a	36.29 a	36.42 a	30.67 B	1.69 e	2.05 c	2.32 b	2.02 B
100	2.44 d	1.60 f	2.48 d	2.51 C	0.77 c	0.41 g	0.38 j	0.52 C	19.53 a	36.87 a	36.75 a	31.05 A	1.84 d	2.36 b	2.57 a	2.26 A
200	2.71 c	3.00 ab	3.10 a	2.93 A	0.82 a	0.47 e	0.49 d	0.59 A	19.33 a	36.28 a	36.58 a	30.74 AB	1.53 f	1.16 g	2.56 a	1.75 C
300	2.66 c	2.88 b	3.00 ab	2.85 B	0.81 b	0.45 f	0.47 e	0.58 B	19.13 a	35.62 a	36.46 a	30.40 B	1.71 e	2.29 b	2.15 c	2.05 B
Mean	2.51 B	2.53 B	2.76 A		0.79 A	0.43 B	0.44 B		19.32 B	36.27 A	36.55 A		1.69 C	1.97 B	2.40 A	

Values marked with the same letter (s), within the main effects and interactions, are not significantly Different at  $p = 0.05$  using LSD test.

observed, generally, in association with either 2 g urea and 100 ppm of GA3, or 4 g urea combined with 200 ppm in the first season. However in the second season, the highest percentages of carbohydrates and nitrogen were obtained at the highest rate of urea (4 g/ pot) with the median GA3 concentration (200 ppm).

In this regard, Santos, *et al* (1998) reported that the best growth of basil plants was observed in the presence of nitrogen at 0.4 g/ litre in association with GA3 at 100 ppm concentration.

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## الملخص العربى

### تأثير استخدام التسميد النيتروجينى والجبريلين على النمو الخضرى والإزهار والتركيب الكيماوى لنبات الأقحوان *Calendula officinalis*

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أجريت هذه الدراسة فى محطة التجارب بكلية زراعة الفيوم، جامعة القاهرة لدراسة تأثير مستويات مختلفة من التسميد النيتروجينى 1 ، 2 ، 4 جرام من اليوريا / أصيص مع الجبريلين (بتركيزات صفر ، 100 ، 200 ، 300 جزء فى المليون) ، على النمو الخضرى والإزهار والتركيب الكيماوى لنبات الأقحوان *Calendula officinalis* خلال موسمين متتاليين 2001/2000 ، 2002/2001 . وقد تم التوصل إلى النتائج الآتية :

**باستبعاد تأثير الجبريلين** ، ففى كلا الموسمين كانت أعلا القياسات (بفروق معنوية) للنمو الخضرى والزهرى مرتبطة، بصفة عامة، بالتركيز المتوسط لليوريا ( 2 جرام/إصيص)، فيما عدا بالنسبة للوزن الطازج للأزهار فقد سجل عند أعلا تركيز لليوريا (4 جرام/إصيص). وبالنسبة للتركيب الكيماوى، فقد أظهرت اليوريا تأثيرا معنويا فى كلا الموسمين، فقد سجلت أعلا التقديرات للكلوروفيل (أ + ب) والكاروتين عند أقل تركيز لليوريا (1 جرام/إصيص)، أما إصيص).

**وباستبعاد تأثير اليوريا** ، كان تأثير الجبريلين، بصفة عامة، إيجابيا على النمو الخضرى والإزهار حيث زادت قيمها تدريجيا مع زيادة التركيز، فيما عدا أنه قد لوحظ فى الموسم الأول أن أعلا قياسات لقطر الأزهار ووزنها الجاف سجل عند 100 ، 200 جزء فى المليون على الترتيب. أما بالنسبة للتركيب الكيماوى، فقد اختلف تأثير الجبريلين فى كلا الموسمين. ففى الموسم الأول سجلت أعلا القياسات للكلوروفيل (أ + ب)، الكاروتين الكلى، والنسب المئوية للكربوهيدرات والنيتروجين عند أعلا تركيز ( 300 جزء فى المليون)، انخفضت هذه القيم تدريجيا عند التركيزات 100 ، 200، صفر جزء فى المليون على الترتيب. وفى الموسم الثانى، سجلت أعلا التقديرات للكلوروفيل والكاروتين عند 200 جزء فى المليون، بينما كانت أعلا النسب المئوية للكربوهيدرات والنيتروجين عند 100 جزء فى المليون.

- بالنسبة للكربوهيدرات والنيتروجين فقد لوحظت أعلا النسب عند أعلا تركيز لليوريا (جرام/ أصيص) .

**وبالنسبة للتداخل بين الجبريلين والنيتروجين** ، فقد أوضحت النتائج أن أعلا قياسات للنمو الخضرى سجلت (بصفة عامة) خلال الموسمين عند استخدام اليوريا بتركيز 2 جم/ إصيص مرتبعا مع أعلا تركيز من الجبريلين ( 300 جزء فى المليون)، فيما عدا قطر الأزهار ووزنها الطازج خلال الموسم الأول لوحظت أعلا القيم عند 200 جزء فى



المليون من الجبريلين، وقطر الأزهار في الموسم الثاني كانت عند 100 جزء في المليون. بينما لوحظت أقل القيم (بصفة عامة) عند أعلا تركيز من كل من اليوريا والجبريلين. أما بالنسبة للتركيب الكيماوى فقد لوحظت أعلا القيم (بصفة عامة) للكلورفيل والكاروتين، خلال الموسمين، كانت عند استخدام اليوريا بتركيز 1 جم/ إصيص مرتبطا مع 200 جزء في المليون من الجبريلين، أما النسب المئوية للكربوهيدرات والنيتروجين فقد لوحظت أعلا نسب في الموسم الأول عند 200 جزء في المليون من الجبريلين، مرتبطا مع 4، 2 جم يوريا/ إصيص على الترتيب، أما في الموسم الثاني فكانت عند 100 جزء في المليون من الجبريلين، مرتبطا مع 2، 4 جم يوريا/ إصيص على الترتيب. أما أقل القياسات المسجلة خلال الموسمين لكل المواد الكيماوية المذكورة، بصفة عامة، (فيما عدا الكربوهيدرات) كانت عند 200 جزء في المليون من الجبريلين، فيما عدا قيم الكلوروفيل والكاروتين في الموسم الثاني، فقد كانت هذه القيم عند 100 جزء في المليون من الجبريلين. أما الكربوهيدرات، فقد سجلت أعلا نسب عند 300 جزء في المليون من الجبريلين مع 1 جم يوريا/ إصيص). أما بالنسبة لتأثير النيتروجين، فقد اختلفت الكمية المستخدمة التي أعطت أقل القياسات حسب المادة الكيماوية المقدره.