

Effects of Different Nitrogen Sources and Doses of Application on Growth and Active Constituents of *Cynara cardunculus* L. plants.

¹Atef Z. Sarhan, ²Hend E. Wahba, ¹Amal A. Nasr, ²Adel B. Salama, ²Heba M. Gad

¹Ornamental Horticulture Dept., Faculty of Agriculture, Cairo University, Giza, Egypt.

²Medicinal and Aromatic Plants Research Dept., Pharmaceutical and Drug Industries Division, National Research Centre, Dokki, Egypt.

ABSTRACT

Cynara cardunculus L. have been known for long time as a medicinal plant for treatments of many diseases but its agricultural studies and chemical composition did not have enough researches in Egypt. Two field experiments were carried out at the Experimental Farm of Agriculture Faculty, Cairo University during two successive seasons 2009/2010 and 2010/2011, to study the effect of nitrogen dressing application (Urea, ammonium nitrate and ammonium sulphate) at different doses (0,40, 60 and 80 N unit/fed) on growth, yield parameters and chemical composition plants. The results showed that application of all nitrogen forms and doses and their interaction significantly increased the growth characteristics and chemical composition of *Cynara cardunculus* as compared to untreated plants. The best treatment was ammonium nitrate at 60 N unit/fed which increased quantitative and qualitative plant production.

Key words: *Cynarac cardunculus*, Polyphenols, Flavonoids, chlorogenic acid, Nitrogen, lipids, Fertilization.

Introduction

Cynara cardunculus L. is a cross-pollinated and highly heterozygous plant belonging to the family Asteraceae (Compositae), is an herbaceous perennial crop commonly named cardoon.

This plant is used worldwide and represents a notable ingredient of the Mediterranean diet (Fратиanni *et al.*, 2007). In fact, this multipurpose plant is used in several dishes as soups and/or salads. *Cynara cardunculus*, flowers are traditionally used for cheese preparation, while leaves are particularly known in folklore for their therapeutic potential as diuretic, choleric, cholagogue, antidiabetic and antimicrobial, (Fратиanni, *et al.*, 2007 and Krizkova *et al.*, 2004).

Also, this plant has been growing in recent years with good and stable yield. The stem as a source of lignocellulosic biomass for energy (Piscioneri *et al.*, 2000; and Raccuia and Melilli, 2007). The seeds used as a source of protein and edible oil, as well as a source of oil for producing biodiesel (Encinar *et al.*, 2002). After oil extraction from seeds, the residual flour could be used for animal feed, both for the quantity and quality of its proteins (Fernandez and Manzanares, 1990; Foti *et al.*, 1999 and Maccarone *et al.*, 1999).

The cultivated cardoon has been cultivated as a vegetable (the commercial product is the enlarged bleached petiole) since ancient times, but now a days the land area devoted to this crops (2000-3000 ha) is mainly localized in Spain, Italy, France and Greece (portis *et al.*, 2005b). The wild cardoon is a robust thistle with large spiny leaves, branched flowering stem and purple flowers (portis *et al.*, 2005a)

Previous chemical investigations had shown the presence of saponins, sesquiterpene, lactones, flavones, sterols, coumarins and lignans in leaves and seeds (Koubaa and Damak, 2003; Pinelli *et al.*, 2007; Sevcikova *et al.*, 2002 and Valentao *et al.*, 2002). Moreover, the extraction of pharmacological active compounds from cardoon plant is also a potential arise, inulin compound in the roots of cardoon plant (Raccuia and Melilli, 2004a and b) and cynarin and silymarin (Curt *et al.*, 2002). which are considered as bitter tasting compounds. Cynarin and silymarin are found in the leaves and improve liver and gall bladder function, and stimulate the secretion of digestive juices especially bile and lowers blood cholesterol levels (Grammelis *et al.*, 2008).

In traditional European medicine leaves, rich in polyphenols. Which are had different purposes pharmacological properties according to their constituents and extracts (Clifford, 1992; Gebhardt, 1997; Perez-Garcia *et al.*, 2000 and Jimenez-Escrig *et al.*, 2003). Recently, there has been an increase in the use of these poly phenolic compounds in cosmetics, (Lupo, 2001) and Peschel *et al.*, 2006).

Due to the increasing importance of such plant and the data concerning its growth and chemical composition is limited under Egyptian conditions. Therefore, it seemed of great importance to find out the most successful practices to enhance their vegetative and flowering characteristics and increase the

active ingredients. One of these factors is the effect of fertilization, particularly nitrogen. Nitrogen is a main component for increasing the growth and active ingredients. Nitrogen plays the most recognized role in the plant for its presence in the structure of the protein molecule. In addition, nitrogen is found in such important molecules as purines, pyrimidines, porphyrines and coenzymes. Purines and Pyrimidines are found in the nucleic acids RNA and DNA which essential for protein synthesis. The porphyrin structure is found in metabolically important compounds such as chlorophyll pigments and the cytochromes which are essential in photosynthesis and respiration. In the recent years many researchers applied nitrogen and proved a remarkable enhancement in plant growth and chemical composition on some medicinal plant i.e., Eich *et al.* (2005) on *Cynara cardunculus*, Paradiso *et al.* (2007) on Roman artichoke. Shaheen *et al.* (2007), Santamaria *et al.* (2007), Doltra *et al.* (2007) and Leskovar *et al.* (2012) on *Cynara scolymus*.

Materials And Methods

This work was carried out during two successive seasons 2009/2010 and 2010/2011 at the Experimental Farm of Agriculture Faculty, Cairo University, Giza .The seeds of *Cynara cardunculus* were obtained from Germany.

1. Nature of soil:

Samples of soil were taken before cultivation and subjected for physical and chemical analysis in Soil Science Department, National Research Center according to method of Jackson (1973) and the results were as follow:

The physical properties:

clay 22%, silt 51%, sand 26.3%, soil texture (sandy loam).

Chemical properties:

pH 8.0, E.C (dsm⁻¹) 1.15, organic matter 0.60%. available N 1.4%, available P 0.83%, available K 0.27%.

Cations:

(Milliequivalent/L) Ca⁺⁺ 12.2, Mg⁺² 3.7, Na⁺¹ 0.27, K⁺¹ 0.27.

Anions:

(Milliequivalent/L) CO₃⁻ 0.0, HCO₃⁻ 1.1, Cl⁻¹ 1.4, SO₄⁻ 13.5.

2. Soil preparation for Cultivation:

During both seasons, the soil was mechanically ploughed and planked twice for cultivation. Calcium superphosphate (15.5% P₂O₅) at the rate of 200 kg/fed was added.

3. Cultivation procedures and maintenance:

For propagation, the seeds were sown in plastic bags of 23X18 cm in a medium of clay (1 sand: 1clay) under the sun screen. The uniform healthy cardoon seedlings (60 day old) were transplanted into the field on the first week of November in both seasons, at distance 100 cm apart between plants into plots 4m² (2 X 2). Each plot contained 2 rows, with 6 plants. All other horticultural practices were done as needed. The experiment was conducted to clear the role of the effect of nitrogen fertilization on growth yield and chemical composition of cardoon plants

4. Nitrogen fertilizer:

This experiment was carried out to study the effect of different nitrogen sources and doses on growth, yield and chemical composition of *Cynara cardunculus*. Three sources of nitrogen were applied at the rate of 0, 40.60 and 80 N unit/fed and the nitrogen sources were urea (46%) ammonium nitrate (33%) and ammonium sulphate (21%) which were applied as three separate side dressing. The first addition was after 7 weeks from transplanting and the second was 6 weeks later, while the third was

after 6 weeks from second addition. Potassium sulphate (48% K₂O) at the rate of 150Kg/fed was added to the experimental plots into two equal amounts where, the first dose was added with the second dose of nitrogen and second was added during the third dose of nitrogen.

Data recorded:

The parameters for this experiment were recorded as follow: Plant height in cm, Number of leaves/plant . Fresh and dry weight of aerial parts (g/plant).were recorded (before the start of flowering stage). At the end of May. Number of head / plant and dry weight of head (g/plant) were recorded. The yield of seeds (g/plant and g/plot) were recorded at the end of the experiment. (at the first of August)

Chemical analysis:

Preparation of sample:

Fresh weight was recorded immediately after harvesting and the dry weight of leaves and heads were determined after an initial drying in oven at 50°C to constant weight. Sample were ground in a mill to fine powder and kept in plastic bags in a desiccators over CaCl₂ till chemical investigation except in case of seeds which immediately powdered before chemical investigation.

Determination of total carbohydrate:

Total carbohydrates in the dried leaves were determined according to Dubois *et al.* (1956). Calculated by using standard curve of glucose.

Determination of total flavonoids:

Determination of total flavonoids content in the dry leaves were determination by pectrophotometer according to Kosalec *et al.* (2004).

Determination of total phenols:

Phenols content was determined in the dry aerial parts by spectrophotometer according to Falleh *et al.* (2008)

Determination of Chlorogenic acid content:

Chlorogenic acid content was determined in the dry leaves using HPL Caccording to the method reported by Sharaf-Eldin *et al.* (2007).

The used HPLC equipment and conditions:

The HPLC system was a HP 1100 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an auto-sampler, quaternary pump and a diode array detector were used. The quantization was integrated by Chemstation chromatographic software interfaced to a personal computer.

The analytical column was ZORBAX Eclipse XDB C18 column (15 cm x 4.6 mm I.D., 5 µm, USA). Operative conditions were: mobile phase A, methanol; mobile phase B, 2% acetic acid; flow rate, 1mL min; fixed wavelength, 325 nm; injected quantity, 20 µL; elution program, A (%)/B (%): 0 min 5/95; 10 min 25/75; 20 min 50/50; 30 min 100/0; 40 min 5/95. Identification of phenolic compounds was performed by comparison with the retention times of standard substances

Determination of fixed oil percentage:

Fixed oil percentage in seeds for each treatment was determined according to the method of A.O.C.S (1964).

Fatty acid methyl esters of control plant was analyzed by gas chromatography (GC).

Saponification of lipoidal matter:

Saponification of lipoidal matter was carried out according to Tsuda*et al.* (1960)

Conditions of GC Analysis:

Type of GC: Perkin Elmer Auto System XL Equipped with flame ionization detector (FID). Fused silica capillary column ZB-Wax (60 m x 0.32 mm i.d). Oven temperature was maintained initially at 50°C for 2 min and programmed from 50 to 220°C at rate 4°C/min., held at Injector temp. 230°C. Detector temp. 250°C. Carrier gas: Helium, flow rate 1 ml/min.

Determination of Minerals(%):

Nitrogen, phosphorus, and potassium content (%), in the plant samples were determined according to Cottenie *et al.* (1982).

Statistical analysis:

Treatments were arranged in complete randomized block design for this experiment. Each treatment includes three replicates. Data of each season was statistically analyzed applying the ANOVA test (MS DOS/ Costat Exe Program) according to Gomez and Gomez (1984).

Results And Discussion

1- Growth characters of *Cynara cardunculus* plants:

1.1. Plant height (cm):

Data presented in Table (1) indicated clearly that, all nitrogen sources and/or different doses had significant effect on plant height of *Cynara cardunculus* in both seasons as compared to untreated plants. The maximum mean values of plant height were resulted from ammonium nitrate (113.22 and 118.00 cm/plant) followed by ammonium sulphate treatment (112.89 and 114.67 cm/plant) then urea treatment (105.22 and 111.78cm/plant) during the two seasons respectively. The differences between the sources of nitrogen were significant. This trend was observed in both seasons.

The effect of nitrogen doses on plant height, in the same table showed that the medium dose (60 unit) of nitrogen was the best treatment in this concern which produced the tallest plants (112.67cm and 117.55cm) in the first and second seasons, respectively. On the other hand, the differences between the medium (60 unit) and high dose (80 unit) of nitrogen were insignificant.

The effect of interaction between the sources and doses of nitrogen was significant for ammonium nitrate and ammonium sulphate under 60 units application of nitrogen.

Our results are in harmony with those reported Shaheen *et al.* (2007) who showed that treating *Cynara scolymus* with 100 – 120 kg N/fed as ammonium sulphate gained the best values of plant height.

1.2. Number of leaves / plant:

Also data presented in Table (1) indicate that, the number of leaves of *Cynara cardunculus* plant insignificantly responded to the different sources of nitrogen as compared to control plants but the differences between nitrogen doses were significant. The plants fertilized with ammonium nitrate or ammonium sulphate were superior than those fertilized with urea in both seasons.

For the effect of different nitrogen doses, data showed that, the doses had significant effect on number of leaves in both seasons. The nitrogen application at three doses were effective in promoting the number of leaves / plant. This trend was true in the two seasons.

The effect of interaction between nitrogen sources and doses, cleared that the highest number of leaves (18.67 and 19.33) was observed with plants fertilized with ammonium nitrate at 80 unit/fed for the first and second season, respectively.

The trend of results were in accordance with those of Eich *et al.* (2005) on *Cynara cardunculus* who found that, the leaf yield improved with increasing N fertilizer rates. EI-Gengaihi *et al.* (2000) studied the response of *Boragoofficinalis* to different nitrogen sources. The results obtained in this experiment concluded that plant height and number of branches were greatly increased by urea.

1.3. Fresh and dry weight of leaves:

Data tabulated in Table (1) showed clearly that, all different nitrogen sources and doses significantly increased the fresh weight of leaves g/plant as compared to untreated plants during the two experimental seasons.

Ammonium nitrate significantly increased fresh weight of leaves comparing to ammonium sulphate and urea in both seasons. The heaviest mean values obtained from ammonium nitrate were (3301.11 and 3496.22 g/plant) the lowest mean values were obtained from urea treatment (2669.50 and 2752.22 g/plant) while in the first and second seasons, respectively.

Concerning the effect of applied doses on the fresh weight of leaves, results in the same Table showed significant differences among all doses of application as compared to untreated plant in both seasons. The total fresh weight of leaves g/plant was increased gradually by increasing the dose of nitrogen. The differences between low dose (40 unit/fed) and the other doses (60 and 80 unit/fed) were significant, while the differences between medium (60 unit/fed) and high dose (80 unit/fed) were insignificant

Concerning the interaction effect between sources and doses of nitrogen, the heaviest leaves fresh weights (3524.33 and 3783.33 g/plant) were produced from plants fertilized by ammonium nitrate at 60 unit/fedin the first and second season respectively.

The dry weight of leaves (g/plant) had similar trend of results of mentioned with the fresh weights where, the treatments which encouraged the fresh weight were the same which produced the high values of dry herb (Table,1)

Biesiada *et al.* (2008) on *Lavandula angustifolia*, who indicated that, supplying 100kg N/ha of ammonium nitrate appeared to be the most suitable for the fresh and dry inflorescence yield. Omer *et al.* (2008) on *Ocimum americanum* found that, the application of ammonium sulfate at the rate of 60kg N/fed at two equal portions in each cut could be recommended for maximizing fresh and dry herb yield.

Table 1: Effect of different nitrogen sources and/or doses on plants height, Number of leaves/plant, fresh and dry weight of *Cynara cardunculus* plant during 2009/2010 and 2010/2011 seasons.

Treatments	Growth characters								
	plant height (cm)		Number of leaves /plant		Fresh weight of herb (g/plant)		dry weight of herb (g/plant)		
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	
Urea	Cont.	94.67	96.33	14.33	15.00	1754.00	1850.00	302.79	312.93
	40	101.67	106.33	16.00	17.00	2444.17	2352.67	445.96	417.73
	60	105.67	113.00	17.00	17.67	2681.67	2625.00	466.11	442.49
	80	108.33	116.00	17.67	18.33	2882.67	3279.00	509.03	562.51
Mean		105.22	111.78	16.89	17.67	2669.50	2752.22	473.70	474.24
Ammonium nitrate	40	110.67	114.00	17.00	17.33	3086.67	3050.00	542.58	509.49
	60	116.67	121.33	18.33	18.33	3524.33	3783.33	630.17	665.17
	80	112.33	118.67	18.67	19.33	3292.33	3655.33	567.37	640.79
	Mean		113.22	118.00	18.00	18.33	3301.11	3496.22	580.04
Ammonium sulphate	40	109.67	111.67	16.33	17.67	2603.23	2763.33	462.30	489.40
	60	115.67	118.33	17.33	18.67	3083.33	3096.00	527.75	519.52
	80	113.33	114.00	18.00	18.00	3198.33	3051.00	553.30	531.69
	Mean		112.89	114.67	17.22	18.11	2961.63	2970.11	514.45
Mean of doses	Cont.	94.67	96.33	14.33	15.00	1754.00	1850.00	302.79	312.93
	40	107.34	110.67	16.44	17.33	2711.36	2722.00	483.61	472.21
	60	112.67	117.55	17.55	18.22	3096.44	3168.11	541.34	542.39
	80	111.33	116.22	18.11	18.55	3124.44	3328.44	543.23	578.33
LSD at (0.05)									
N.S.		1.99	1.62	ns	ns	177.37	152.71	32.89	35.25
N.D.		2.30	1.87	1.50	1.15	204.81	176.34	37.98	40.70
N.S. x N.D.		3.29	1.56	ns	ns	293.69	252.87	54.46	58.36
1 st : first season		2 nd : second season							
N: Nitrogen		40, 60 and 80: N unit/fed.							
N.S.: Nitrogen sources		N.D.: Nitrogen Doses							

1.4. Number of head / plant:

Mean values of the number of head as affected by different nitrogen sources and/or doses are tabulated in Table (2). showed that, for the sources of nitrogen, ammonium nitrate has more promoting in increment the number of head of *Cynara cardunculus* plants. On the other hand, urea and ammonium sulphate showed an equal effect in increasing the number of head per plant. In other words, the mean values of the number of head of ammonium nitrate treatment were (13.00 and 12.44) head/plant against (11.89 and 11.56) for urea treatment as well as (11.11 and 11.00) for ammonium sulphate treatment during the first and second season, respectively.

For different doses of nitrogen, it is clear as shown in the same Table, that the application of nitrogen at 40 unit/fed nitrogen produced the least number of head/plant. While the dose of 60 and 80 unit/fed produced the same values in this concern. Therefore the differences between them were insignificant. This trend has been noticed in both seasons.

The effect of interaction between nitrogen sources and doses of application was insignificant during the two seasons. The highest number of heads/plant (13.67 and 14.00) was observed with plants fertilized with ammonium nitrate at 60 unit/fed

Our results are in agreement with the findings of Paradiso *et al.* (2007) they evaluated that, Nitrogen fertilization promoted the earliness and increased the yield, of globe artichoke for spring production. With a remarkable effect on the weight of the main heads as well as the number and the weight of the secondary heads.

Table 2: Effect of different nitrogen sources and doses on number of head/plant, weight of head /plant and weight of seed g/plant of *Cynara cardunculus* plant during 2009/2010 and 2010/2011 seasons.

Treatments	Growth characters								
	Number of head /plant		Weight of head (g/plant)		Weight of seed (g/plant)		Weight of seed (g/plot)		
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	
Control	9.00	9.67	142.46	131.73	30.20	32.81	181.18	196.86	
Urea	40	11.00	10.00	162.23	151.00	47.23	58.56	283.40	351.36
	60	11.33	12.67	185.80	179.60	50.99	64.84	305.94	389.02
	80	13.33	12.00	206.20	212.24	53.55	61.88	321.30	371.30
Mean		11.89	11.56	184.74	180.95	50.59	61.76	303.55	370.56
Ammonium nitrate	40	12.33	11.00	210.72	207.21	55.58	69.42	333.48	416.52
	60	13.67	14.00	242.03	251.87	58.89	70.10	353.36	420.58
	80	13.00	12.33	265.37	275.39	61.25	74.33	367.52	446.00
Mean		13.00	12.44	239.37	244.82	58.58	71.28	351.45	427.70
Ammonium sulphate	40	11.33	10.00	194.35	184.67	43.86	51.55	263.18	309.28
	60	11.67	10.00	218.35	198.14	45.81	57.11	274.84	342.68
	80	10.33	13.00	198.1	212.33	55.44	60.98	332.62	365.88
Mean		11.11	11.00	203.6	198.38	48.37	56.55	290.21	339.28
Mean of doses	Control	9.00	9.67	142.46	131.73	32.20	32.81	193.18	196.86
	40	11.55	10.33	189.10	180.96	48.89	59.84	293.35	359.05
	60	12.22	12.22	215.39	209.87	51.90	64.02	311.38	384.09
	80	12.22	12.44	223.22	233.32	56.75	65.73	340.48	394.39
LSD at (0.05)									
N.S.		1.06	ns	3.61	3.83	2.34	1.25	13.49	7.51
N.D.		1.22	1.37	4.17	4.43	2.70	1.44	15.57	8.67
N.S. x N.D.		ns	ns	5.97	6.46	3.88	2.07	22.33	12.42
1 st : first season		2 nd : second season							
N: Nitrogen		40, 60 and 80: N unit/fed							
N.S.: Nitrogen sources		N.D.: Nitrogen Doses							

1.5. Weight of head g/plant:

Data presented in Table, (2) revealed that, the sources and doses of nitrogen and their interaction, significantly increased the dry weight of head as compared to untreated plants. Ammonium nitrate was superior as compared to urea or ammonium sulphate in enhancing dry weight of head in both seasons. The heaviest dry weight of head (239.37 and 244.82 g/plant) was recorded with ammonium nitrate treatment, while ammonium sulphate came in the second rank (203.60 and 198.38 g/plant) followed by urea which gave the lowest values in this concern, (184.74 and 180.95 g/plant) in the first and second seasons, respectively.

The effect of the application of different nitrogen doses on dry weight of head was clearly observed through the results from the same Table (2) which indicate that, dry weight increased by using the different doses of nitrogen. These increments were increased gradually by increasing the dose up to 80 unit/fed. The dose of nitrogen at 80 unit/fed increased the dry weight of head by 13.90% and 18.04% in the first season, while these increments were 15.97% and 28.93% in the second season in comparison to 40 and 60 unit/fed, respectively. The differences between the three doses were significant in both seasons. For the interaction effect between nitrogen sources and doses. The data in the same table showed that the dry weight of head increased by increasing the doses for both urea and ammonium nitrate up to 80 unit/fed. The heaviest dry weight (265.37 and 275.39 g/plant) was recorded with ammonium nitrate at 80 unit/fed in the first and second season, respectively.

The above results of dry weight of head are in accordance with Aziz (2004) who supplied *Achillea millefolium* plants with different levels of ammonium sulphate, stated that 50 kg N/fed was the best treatment for increasing flower heads production. Biesiada *et al.* (2008) on *Lavandula angustifolia*, indicated that 100 kg N/ha of ammonium nitrate appeared to be the most suitable for dry inflorescence yield.

1.6. The seed yield:

Also, the data tabulated in Table (2) cleared that, seed yield as g/plant and kg/fed was significantly increased with different sources and doses of nitrogen fertilizer.

Nitrogen as ammonium nitrate was superior comparing to the other two nitrogen sources (urea and ammonium sulphate) in seed production. Ammonium nitrate treatment produced the highest mean values of seed, followed by urea, then ammonium sulphate during the two seasons. In other words, the highest mean values in this concern were (58.58 and 71.28g/plant) for ammonium nitrate treatment comparing to (50.59 and 61.76g/plant) for urea as well as (48.37 and 56.55 g/plant) for ammonium sulphate treatment in the first and second seasons, respectively. These differences in the mean values of seed yield for urea, ammonium nitrate and ammonium sulphate were significant, these results behaved similarly in both seasons.

Concerning the effect of applied doses on seed yield, data in Table (2) showed that, the high doses of nitrogen (80 unit/fed) gave the highest values of seed yield g/plant (56.75 and 65.73g/plant) for first and second seasons, respectively. On the other hand, the 60 unit/fed plant gave a higher yield (51.90 and 64.02 g/plant) than 40 unit/fed (48.89 and 59.84 g/plant) but this increase was insignificant, this trend was observed in the two seasons.

The interaction between nitrogen sources and doses of application showed that ammonium nitrate at 80 unit/fed produced the maximum values of seed yield g/plant, this trend was clear in both seasons.

The results of seed yield g/plot showed similar trend of those seed yield g/plant.

Elia *et al.* (1991) on globe artichoke cultivar "Talpiot" found that, the application of 150 kg N/ha increased the seed yield by 3 t/ha. No further increase was observed with 300 kg N/ha. The mentioned results are in harmony with Refaat *et al.* (1999) on *Borago officinalis* who concluded that, increasing the nitrogen levels as ammonium nitrate and ammonium sulphate increased seed yield/plot and /ha but increasing urea levels decreased the yield of seed.

2. Chemical constituents of *Cynara cardunculus* plants:

2.1. Carbohydrate content in leaves %:

Data in Table (3) demonstrated that, carbohydrate percentage was affected by different nitrogen sources. The three sources of nitrogen significantly increased carbohydrate in the herb of *Cynara cardunculus* plant as compared to untreated plants. In both seasons, ammonium sulphate gave the highest response of accumulation of carbohydrate in leaves, this effect is similar during the two seasons.

For the doses of nitrogen, all used doses increased the carbohydrate percentage in the leaves of *Cynara cardunculus* plant. The dose of nitrogen at 80 unit /fed produced the maximum value of the carbohydrate percentage (12.16% and 12.15%), followed by the dose at 60 unit/ fed which produced 11.14% and 10.99%, then the low level (40 unit/fed) gave (10.57% and 10.46%) for the first and second seasons, respectively.

The effect of interaction between sources and doses of nitrogen cleared that either sources or doses increased carbohydrate in the two seasons compared to untreated plants but these increments were insignificant (Table, 3). The application of high dose (80 unit/fed) of ammonium sulphate and urea treatments gave the maximum values of carbohydrate followed by application of ammonium nitrate.

Similar trend of results was reported by Jacoub, (1995) on *Ocimum basilicum* and Salama *et al.* (2003) on *Verbascum thapsus*.

2.2. Total flavonoid in leaves mg/g:

Under the different nitrogen sources and doses the total flavonoid in leaves of *C. cardunculus* plant significantly increased in both seasons as compared to control plants.

As the source of nitrogen, ammonium nitrate has more promoting effects in increasing the total flavonoid in the leaves. On the contrary urea treatment in the first season showed the least value of total flavonoid, while ammonium sulphate gave the least value in this concern in the second season.

Comparing the effects between the different doses of nitrogen, the data in the same table showed that, maximum values of flavonoids were with the high dose of nitrogen (80 unit/fed). This trend was true in both seasons.

The interaction effect of different sources and doses of nitrogen on the total flavonoid indicated that ammonium nitrate at moderate dose (60 unit/fed) gave the maximum value compared to the other treatments in the first season while in the second season the ammonium sulphate at the high dose 80 N unit/fed gave the maximum value.

These results are in accordance with Borella *et al.* (2001) conducted to evaluate the effect of varying NPK treatments on the flavonoid content of *Baccharis trimera* plants. They reported that mineral fertilization (N-P-K) exhibited enhanced flavonoid contents. Barbara (2002) on goldenrod noticed an increase in flavonoid accumulation with increasing nitrogen level.

Table 3: Effect of different nitrogen sources and/or doses on carbohydrate, flavonoid, polyphenol and chlorogenic acid content of *Cynara cardunculus* leaves during 2009/2010 and 2010/2011 seasons.

Treatment		Chemical constituents								
		Carbohydrate %		Flavonoid mg/g		Polyphenol mg/g		Chlorogenic acid mg/100g	Oil %	
		1 st	2 nd	1 st	2 nd	1 st	2 nd	2 nd	1 st	2 nd
Urea	Control	9.20	9.50	3.53	3.46	8.20	8.80	464.55	24.33	23.13
	40	10.08	10.03	4.36	5.71	11.07	10.63	642.40	26.83	26.50
	60	11.29	10.62	4.54	6.30	11.24	11.92	799.22	27.50	27.25
	80	12.60	12.21	5.04	5.69	12.08	11.00	942.58	28.17	28.50
Mean		11.32	10.95	4.65	5.90	11.46	11.18	763.36	27.50	27.42
Ammonium nitrate	40	10.35	10.24	5.05	5.89	11.72	10.93	639.48	27.17	26.75
	60	10.72	11.33	6.12	6.26	11.81	11.75	693.04	28.33	28.83
	80	11.58	11.78	5.08	6.44	12.24	14.00	853.07	27.33	27.50
	Mean		10.89	11.12	5.42	6.20	11.92	12.23	688.04	27.61
Ammonium sulphate	40	11.27	11.11	4.04	4.83	10.44	10.48	548.26	27.33	27.00
	60	11.41	11.02	4.69	5.03	10.96	10.62	591.53	28.17	28.25
	80	12.31	12.47	5.82	6.48	11.42	11.49	633.79	28.00	27.67
	Mean		11.67	11.53	4.85	5.45	10.94	10.86	561.78	27.83
Mean of doses	Control	9.20	9.50	3.53	3.46	8.20	8.80	464.55	24.33	23.13
	40	10.57	10.46	4.48	5.59	11.08	10.68	610.05	27.11	26.75
	60	11.14	10.99	5.12	6.31	11.34	11.43	694.60	28.00	28.11
	80	12.16	12.15	5.31	6.31	11.91	12.16	809.81	27.83	27.89
LSD at (0.05),										
N.S.		0.45	0.48	0.35	0.17	0.39	0.32	-----	ns	ns
N.D.		0.52	0.55	0.40	0.20	0.45	0.37	-----	1.29	0.86
N.S. x N.D.		ns	ns	0.57	0.28	ns	0.53	-----	ns	ns
1 st : first season		2 nd : second season								
N: Nitrogen		40, 60 and 80: N unit/fed.								
N.S.: Nitrogen sources		N.D.: Nitrogen Doses								

2.3. Total polyphenol in leaves mg/g:

The mean values of total polyphenol in leaves of *Cynara cardunculus* plant showed significant response to the different sources of nitrogen as compared to untreated plants (Table,3). The maximum values of total polyphenol in leaves were recorded with ammonium nitrate treatment which gave highest response of accumulation of polyphenol, then ammonium sulphate and followed by urea treatment.

For different doses of nitrogen, it was clear as shown in the same Table that the application of nitrogen at the three doses significantly increased polyphenol comparing to untreated plants. These increments were gradually with increasing the dose of nitrogen, therefore the high level of nitrogen (80 unit/fed) was the best one in this regard. The differences between the three doses of nitrogen were significant in both seasons.

The interaction between sources and doses of nitrogen showed that the most promising effect on the accumulation of polyphenol was ammonium nitrate the at highest level (80 unit/fed).

Our results are in harmony with the obtained results agreed with findings of (Falleh *et al.*, 2008) All organs exhibited high polyphenol content, comprised between 7.5 and 15.0 mg/g DW.

4- Chlorogenic acid in leaves mg/100g:

Also, the results in (Table, 3) clear the effect of nitrogen sources and/or doses on the chlorogenic acid in the leaves. It can be noticed that, there was an increment in the total chlorogenic acid as compared to untreated plants. Fertilized the plants with urea as a source of nitrogen was more effective on the accumulation of the chlorogenic acid in the leaves as compared to ammonium nitrate and ammonium sulphate. On the other hand, nitrogen as ammonium nitrate was superior than ammonium sulphate treatments.

As for the doses of nitrogen, it is obvious from the same data that all doses of nitrogen increased the content of chlorogenic acid in the leaves of *Cynara cardunculus* plant as compared to untreated plants, these increments were gradually with increasing the dose of nitrogen, therefore the high level of nitrogen (80 unit/fed) was the best one in this regard.

For the effect of interaction between sources and doses of nitrogen on the total chlorogenic acid in leaves, the data in the same table indicated that all sources of nitrogen at high dose (80 unit/fed) produced the maximum values of chlorogenic acid as compared to the two other doses (40 and 60 unit/fed). The source of nitrogen urea at 80 unit/fed had more promoting effects in increasing chlorogenic acid in herb of *Cynara cardunculus* (942.58 mg/100g), followed by ammonium nitrate at the same dose (80 unit/fed) which produced 853.07mg/100g, then by ammonium sulphate which gave (633.79 mg /100g). On the other hand, the three sources of nitrogen with low dose (40 unit/fed) showed the lowest effect in increasing chlorogenic acid in leaves.

2.5. Total lipid content:

The oil, extracted from the seeds of cardoon, called artichoke oil, is similar to safflower and sunflower oil in composition and use. The oil of seeds of *Cynara cardunculus* L. contains high levels of α -tocopherol, which offers stability against oxidation (Maccarone *et al.*, 1999). These characteristics make *Cynara* oil suitable for human consumption.

The lipid content in the herb of *Cynara cardunculus* plant was determined, the obtained results are presented in (Table, 3). It is evident that the lipid is the lowest in the seed of control plants (24.33% and 23.13%) for the first and second season.

It could be seen from the table that all the used sources and doses of nitrogen significantly increased the lipid content in the seed as compared to untreated plant.

Application of different nitrogen sources (urea, ammonium nitrate and ammonium sulphate) gave about the same value, therefore the difference between the different sources of nitrogen were insignificant during the two seasons. The mean values of the different treatments of sources were ranged between (27.50% to 27.83%) and (27.42% to 27.69%) in the first and second season, respectively.

Studying the effects between the different doses of nitrogen, the data showed that, the medium dose (60 unit/fed) was the most effective in increasing the lipid in seed, while the low dose (40 unit/fed) gave the lowest value in this concern. The differences between, the medium and high dose of nitrogen were insignificant. These results were clear in the data of the two seasons.

In both seasons the interaction between sources and doses of nitrogen showed that the maximum values of total lipid in the leaves were resulted from ammonium nitrate at the same dose (28.33% and 28.83%), then urea at 80 unit/fed (28.17% and 28.50%) for the first and second season, respectively. Generally, the differences between these values were insignificant in both seasons.

Many investigators such as (Curt *et al.*, 2002) reported that, the range of values of seed oil content in *Cynara cardunculus* was greater for the multilocal experiment (20.0–31.6%) than for the experiment on populations (22.0–28.8%), but the effect of the agricultural year was noticed in both experiments.

2.6. Fatty acids:

Fixed oil GC analysis of methylated fatty acids of *C. cardunculus* seed (Table,4) indicated that linoleic acid was the main fatty acid and accounted for 58.91% and followed by oleic acid 26.23% then palmitic acid 13.85%, linolenic acid reached 0.17%. The total saturated fatty acid accounted for 14.41%, while unsaturated ones reached 85.56% from total fatty acids.

These results are in accordance with Curt, *et al.* (2002), who stated that *Cynara* oil profile was characterised in terms of major fatty acids as: 10.7% palmitic, 3.7% stearic, 25.0% oleic and 59.7% linoleic.

Table 4: Fatty acid of *Cynara cardunculus* fixed oil:-

Peak No.	Rt.	Scientific name	Carbon number	Fatty acid %
1	32.437	Myristic	C14:0	0.13
2	36.971	Palmitic	C16:0	13.85
3	38.740	Margaric	C17:0	0.06
4	39.042	Stearic	C18:0	0.05
5	41.615	Oleic	C18:1	26.23
6	42.622	Linoleic	C18:2	58.91
7	43.783	Linolenic	C18:3	0.17
8	44.946	Arachidic	C20:0	0.32
9	46.069	Paullinic	C20:1	0.25
Total saturated fatty acids				14.41
Total unsaturated fatty acids				85.56
Total				99.97

2.7. Mineral content:

a. Nitrogen percentage:

Data presented in Table (5) showed that, nitrogen percentage in the leaves of *Cynara cardunculus* plants was influenced by different nitrogen sources and doses in both seasons as compared to untreated plants. As for sources of nitrogen, the nitrogen percentage in the leaves was higher with urea treatment than those fertilized either with ammonium nitrate or ammonium sulphate. These differences in the nitrogen percentage due to nitrogen sources were insignificant in the first season and significant in the second season. On other words, urea treatment was the most effective in increasing nitrogen percent which gave the highest mean value (3.04% and 2.95%) whereas ammonium sulphate gave the least mean value (2.86% and 2.60%). for the first and second season, respectively.

Concerning the effect of adding nitrogen at different doses, it is clear that, addition the medium dose (60 units/fed) recorded the maximum values of nitrogen percentage in the leaves as compared to 40 unit/fed and 80 units/fed. The differences due to doses of nitrogen were significant in both seasons.

Nitrogen percentage in leaves of *C. cardunculus* showed no significant response to the interaction between nitrogen sources and doses of application in both seasons. In the first season the highest value of nitrogen in the leaves was with both urea at 60 units/fed and ammonium nitrate at 80 unit/fed. While, in the second season was with both urea and ammonium nitrate at 60 units/fed. Generally, no clear trend for the effect of interaction between sources and doses of nitrogen on the nitrogen percentage in leaves.

These finding were confirmed by the previous works of Shaheen *et al.* (2007) on *Cynara scolymus* who showed that applying ammonium sulphate at 120 kg N/fed gained the best values of N content.

Table 5: Effect of different nitrogen sources and doses on N, p, k content of *Cynara cardunculus* plant during 2009/2010 and 2010/2011 seasons.

Treatments		Mineral content					
		N%		P%		K%	
		1 st	2 nd	1 st	2 nd	1 st	2 nd
Urea	Control	2.18	2.09	0.32	0.29	1.63	1.59
	40	2.99	2.90	0.48	0.39	1.84	1.87
	60	3.09	2.96	0.44	0.39	2.06	1.92
	80	3.04	2.98	0.39	0.44	2.02	1.89
Mean		3.04	2.95	0.44	0.41	1.97	1.89
Ammonium nitrate	40	2.70	2.94	0.42	0.36	1.9	1.67
	60	2.89	2.96	0.39	0.38	1.94	1.70
	80	3.09	2.81	0.43	0.44	1.92	1.73
Mean		2.89	2.90	0.41	0.39	1.92	1.70
Ammonium sulphate	40	2.74	2.55	0.39	0.38	2.21	2.13
	60	3.04	2.64	0.38	0.36	2.07	2.02
	80	2.79	2.61	0.33	0.35	1.99	1.77
Mean		2.86	2.6	0.37	0.36	2.09	1.97
Mean of doses	Control	2.18	2.09	0.32	0.29	1.63	1.59
	40	2.81	2.80	0.43	0.38	1.98	1.89
	60	3.01	2.85	0.40	0.38	2.02	1.88
	80	2.97	2.80	0.38	0.41	1.98	1.80
LSD at (0.05)							
N.S.		ns	0.17	0.04	0.03	0.06	0.05
N.D.		0.28	0.20	ns	0.03	0.07	0.06
N.S. x N.D.		ns	ns	ns	ns	0.03	0.03
1 st : first season	2 nd : second season						
N: Nitrogen	40, 60 and 80: N unit / fed.						
N.S.: Nitrogen sources	N.D.: Nitrogen Doses						

b. Phosphorus percentage:

Regarding the application of nitrogen the results in Table (5) showed that the lowest percentage of phosphorus resulted by the control plants which recorded 0.32% and 0.29% during the first and second season respectively. On other word, it could be seen that all the used sources and doses of nitrogen increased the phosphorus percentage in the leaves of *C. cardunculus* during the two seasons. Application of urea as a source of nitrogen showed the highest effect on phosphorus percentage (0.44% and 0.41%) in leaves, followed by ammonium nitrate treatment that recorded (0.41% and 0.39%), then ammonium sulphate which gave (0.37% and 0.36%) for the first and second respectively. The differences between these treatments were significant in the two seasons.

All application doses of nitrogen increased the phosphorus percentage in the leaves during the two seasons compared to unfertilized treatment. The three doses of nitrogen gave about the same values in this concern, therefore the differences between all doses were insignificant.

Data percent in the same table indicated that, phosphorus percentage was insignificantly influenced by interaction between different nitrogen sources and doses in both seasons. Also from these data it can be noticed that no clear trend was obtained. In the first season urea at 40 unit/fed gave the maximum value of phosphorus percentage, while in the second season, both urea and ammonium nitrate at high doses (80 unit/fed) gave the maximum value in this concern.

The above mentioned results were agreed with those obtained by Abd El-Azim (2003) who found that, fertilizing *Salvia officinalis* plant with 150 kg ammonium sulphate, caused a significant increase in P content in the plant.

C. Potassium percentage:

Table (5) exhibited data on the potassium percentage of *C. cardunculus* plant as influenced by different sources of nitrogen as well as doses and their interactions during 2009/2010-2010/2011 seasons.

All the applied nitrogen sources significantly increased the potassium percentage in the leaves in the two seasons. Ammonium sulphate gave the highest value (2.09% and 1.97%) as compared with (1.97% and 1.89%) for urea treatment, and (1.92% and 1.70%) for ammonium nitrate in the first and second seasons, respectively.

As for effect of applied doses, the data in the same table showed that potassium percentage responded significantly to all doses of nitrogen in both seasons as compared to untreated plants. The three levels of nitrogen gave about the same values in these concerns during in the two seasons. Therefore, the differences between them were insignificant.

On the other hand, the potassium content showed significant response to the interaction between nitrogen sources and doses of application in both seasons. The addition of ammonium sulphate at low dose (40 unit/fed) produced the maximum value of potassium percentage in leaves of *C. cardunculus* (2.21% and 2.13%) for first and second seasons, respectively.

The above mentioned results agreed with those of Abd El-Azim (2003) who found that, fertilizing *Salvia officinalis* plant with 150 kg ammonium sulphate, caused a significant increase in K contents in the plant.

References

- A.O.C.S., 1964. Official and Tentative Methods of American Oil Chemists Society. Ed. by the American Oil Chemists Society, Chicago, Illinois, USA.
- Abd El-Azim, W.M., 2003. Production of *Salvia officinalis* L. plant under Sinai conditions. Ph.D. Thesis, Fac. Agric., Cairo Univ., Egypt.
- Aziz, E.E., 2004. Comparative study on the effect of ammonium nitrate and ammonium sulphate through the application of poultry manure on the productivity of *Achillea millefolium*, L. plants. Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 12(1): 371-389.
- Barbara, K., 2002. The effect of soil material and nitrogen fertilization on growth and development of goldenrod (*Solidago virga-aurea*, L.). Folia Hort., 14(1): 187-193.
- Biesiada, A., A. Sokół-Łętowska and A. Kucharska, 2008. The effect of nitrogen fertilization on yielding and antioxidant activity of lavender (*Lavandula angustifolia* Mill.). Acta Sci. Pol., Hortorum Cultus, 7(2): 33-40.
- Borella, J.C., A. Fontoura, J.A. Menezes and S.C. Franca, 2001. Effect of mineral fertilization (N-P-K) and seasonality in yield and total flavonoids in male individuals of *Baccharis trimera* Less. (Asteraceae) Carqueja. Revista Brasileira, de Plantas Medicinai, 4(1): 99-102.
- Clifford, M.N., 1992. Sensory and dietary properties of phenols. Bull. Liaison, Groupe polyphenols., 16: 19-31.
- Cottenie, A., M. Verloo, L. Kiekens, G. Velghe and R. Camerlynck, 1982. Chemical analysis of plants and soils. Laboratory of Analytical and Agrochemistry State University Ghent-Belgium, p: 61.
- Curt, M.D., G. Sanchez, J. Fernandez, 2002. The potential of *Cynara cardunculus* L. for seed oil production in a perennial cultivation system. Biomass and Bioenergy, 23: 33-46.
- Doltra, J., J.M. Carpintero, F. Berbegal, S. Khayyo and C. Ramos, 2007. Use of a crop model to study the response of artichoke to different levels of soil mineral nitrogen. Acta Horticulturae, 730: 257-264.
- Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colourimetric method for determination of sugars and related substance. Analytical Chem., 28(3): 350-356.
- Eich, J., C. Baier, M. Grun, D. Wagenbreth and R. Zimmermann, 2005. Artichoke leaves used for herbal drug production: influence of nitrogen fertilization on yield and on pharmaceutical quality. Acta Horticulturae; 681: 545-551.
- El-Gengaihi, S.E., H.E. Wahba and A.M. Reffaat, 2000. The response of borage plants (*Borago officinalis*, L.) to different nitrogen source and rates. Egypt J. Hort., 27(3): 409-423.
- Elia, A., F. Paolicelli and V.V. Bianco, 1991. Effect of sowing date, plant density and nitrogen fertilizer on artichoke (*Cynara scolymus* L.): preliminary results. Advances in Horticultural Science, 5(3): 119-122.
- Encinar, J.M., J.F. Gonzalez, J.J. Rodriguez and A. Tejedor, 2002. Biodiesel fuels from vegetable oils: transesterification of *Cynara cardunculus* L. oils with ethanol. Energy Fuels, 16: 443-450.
- Falleh, H., R. Ksouri, K. Chaieb, N. Karray-Bouraoui, N. Trabelsi, M. Boulaaba and C. Abdelly, 2008. Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. Comptes Rendus Biologies, 331: 372-379.
- Fernandez, J., and P. Manzanares, 1990. Production and utilization of *Cynara cardunculus* L.: biomass for energy, paper-pulp and food chemistry. In: Grassi, G., Gossi, G., dos Santos, G. (Eds.), Biomass for Energy and Industry. Elsevier Applied Science Publishing, New York, pp: 1189-1984.

- Foti, S., G. Mauromicale, S.A. Raccuia, B. Fallico, F. Fanella and E. Maccarone, 1999. Possible alternative utilization of *Cynara* spp. I. Biomass, grain yield and chemical composition of grain. *Industrial Crops and Products*, 10 (3): 219-228.
- Fратиани, F., M. Tucci, M. De Palma, R. Pepe and F. Nazzaro, 2007. Polyphenolic composition in different parts of some cultivars of globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori). *Food Chemistry*, 104: 1282-1286.
- Gebhardt, R., 1997. Antioxidative and protective properties of extracts from leaves of the artichoke *Cynara scolymus* L. against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. *Toxicology and Applied Pharmacology*, 144: 279-286.
- Gomez, K.A. and A.A. Gomez, 1984. *Statistical Procedures for Agriculture Research* 2nd Ed. pp.180 John Wiley and sons, New York U.S.A.
- Grammelis, P., A. Malliopolou, P. Basinas and N.G. Danalatos, 2008. Cultivation and Characterization of *Cynara cardunculus* for Solid Biofuels Production in the Mediterranean Region *Int. J. Mol. Sci.*, 9: 1241-1258.
- Jackson, M.L., 1973. "Soil Analysis". Constable Co. Ltd., London, pp: 1-15.
- Jacob, W.R., 1995. Effect of chemical fertilization on growth and oil yield of sweet basil (*Ocimum basilicum*, L.) Plants. M.Sc. Thesis, Fac. Agric. Cairo Univ., Egypt.
- Jimenez-Escrig, A., L.O. Dragsted, B. Daneshvar, R. Pulido, F. Saura-Calixto, 2003. In vitro antioxidant activities of edible artichoke (*Cynara scolymus* L.) and effect on biomarkers of antioxidants in rats. *J. Agric. Food Chem.*, 51: 5540-5545.
- Kosalec, I., M. Bakmaz, S. Pepeljnjak and S. Vladimir-Knezevic, 2004. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *Acta Pharm.*, 54: 65-71.
- Koubaa, I., and M. Damak, 2003. A new dilignan from *Cynara cardunculus*. *Fitoterapia*, 74: 18-22.
- Krizkova, L., P. Mucaji, M. Nagy and J. Krajcovic, 2004. Triterpenoid cynara saponins from *Cynara cardunculus* L. reduce chemically induced mutagenesis in vitro. *Phytomedicine*, 11: 673-678.
- Leskovaar, D.I., T. Shinohara, S. Agehara and B. Patil, 2012. Integrated approaches for annual artichoke production in southwest Texas. *Acta Horticulturae*, 942: 235-238.
- Lupo, M.P., 2001. Antioxidant and vitamins in cosmetics. *Clin. Dermatol.*, 19: 467-473.
- Maccarone, E., B. Fallico, F. Fanella, G. Mauromicale, S.A. Raccuia and S. Foti, 1999. Possible alternative utilization of *Cynara* spp. Part II. Chemical characterisation of their grain oil. *Ind. Crop Prod.*, 10: 229-237.
- Omer, E.A., A.A. Elsayed, A. El-Lathy, M.E. Khattab and A.S. Sabra, 2008. Effect of the nitrogen fertilizer forms and time of their application on the yield of herb and essential oil of *Ocimum americanum*, L. *Herba Polonica*, 54(1): 34-46.
- Paradiso, R., B. Cuocolo and S. Pascale, de. 2007. Gibberellic acid and nitrogen rate affect yield and quality of artichoke. *Acta Horticulturae*, 730: 211-216.
- Perez-Garcia, F., T. Adzet and S. Canigual, 2000. Activity of artichoke leaf extract on reactive oxygen species in human leukocytes. *Free Rad. Res.*, 33: 661-665.
- Peschel, W., F. Sanchez-Rabaneda, W. Diekmann, A. Plescher, I. Gartzia, D. Jimenez, R. Lamuela-Raventos, S. Buxaderas and C. Codina, 2006. An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chem.*, 97: 137-150.
- Pinelli, P., F. Agostini, C. Comino, S. Lanteri, E. Portis and A. Romani, 2007. Simultaneous quantification of caffeoyl esters and flavonoids in wild and cultivated cardoon leaves. *Food Chemistry*, 105(4): 1695-1701.
- Piscioneri, I., N. Sharma, G. Baviello and S. Orlandini, 2000. Promising industrial energy crop, *Cynara cardunculus*: a potential source for biomass production and alternative energy. *Energy Convers. Manage*, 41: 1091-1105.
- Portis, E., A. Asquadro, C. Comino, G. Mauromicale, E. Saba and S. Lanteri, 2005a. Genetic structure of island populations of wild cardoon [*Cynara cardunculus* L. var. *sylvestris* (Lamk) Fiori] detected by AFLPs and SSRs. *Plant Science*, 169(1): 199-210.
- Portis, E., L. Barchil, A. Acquadro, Macuai and S. Lanteri, 2005b. Genetic diversity assessment in cultivated cardoon by AFLP (amplified fragment length polymorphism) and microsatellite markers. *Plant Breed.*, 124(3): 299-304.
- Raccuia, S.A. and M.G. Melilli, 2004a. *Cynara cardunculus* L., a potential source of inulin in Mediterranean environment: screening of genetic variability. *Austr. J. Agri. Res.*, 55: 693-698.
- Raccuia, S.A. and M.G. Melilli, 2004b. Plant Architecture And Biomass Partitioning Variation As Affected By Plant Density In *Cynara cardunculus* L. Var. *Sylvestris* Lam. In ISHS Acta Horticulturae 730: VI International Symposium on Artichoke, Cardoon and Their Wild Relatives; ISHS: Leuven, Belgium.
- Raccuia, S.A. and M.G. Melilli, 2007. Biomass and grain oil yields in *Cynara cardunculus* L. genotypes grown in a Mediterranean environment. *Field Crops Res.*, 101(2): 187-197.

- Refaat, A.M., H.E. Wahba and S. El-Gengaihi, 1999. The effect of foliar nutrient with urea and methods of planting on Borage (*Borago officinalis*) productivity Egyptian Journal of Horticulture, 27(4): 425-438.
- Salama, A.B., S.M. Hassan, S.E. El-Gengaihi and N.S. Abu-Taleb, 2003. Comparative studies of mineral and organic fertilization on *Verbascum thapsus* plant. Egypt. J. Hort., 30(1-2): 111-124.
- Santamaria, P., O. Ayala, D. Buttarro, A. Parente and B. Kirkayak, 2007. N-rate and N-source for producing artichoke cv. Concerto transplants in float bed under green house conditions. Acta Horticulturae., 730: 315-319.
- Sevcikova, P., Z. Glatz and J. Slanina, 2002. Analysis of artichoke extracts (*Cynara cardunculus* L.) by means of micellarelectrokinetics capillary chromatography. Electrophoresis, 23: 249-252.
- Shaheen, A.M., Fatma, A. Rizk, A.M. Elbassiony and Z.S.A. El-Shal, 2007. Effect of ammonium sulphate and agricultural sulphur on the artichoke plant growth, heads yield and its some physical and chemical properties. Research Journal of Agriculture and Biological Sciences, 3(2): 82-90.
- Sharaf-Eldin, M.A., W.H. Schnitzler, G. Nitz, A.M. Razin, I.I. El-Oksh, 2007. The effect of gibberellic acid (GA3) on some phenolic substances in globe artichoke (*Cynara cardunculus* var. *scolymus* (L.) Fiori) Scientia Horticulturae, 111: 326-329.
- Tsuda, K., K. Sakai and Y. Kishida, 1960. Gas chromatography of fatty acid from *Borago officinalis* Journal American Chemical Society, 82: 1442.
- Valentao, P., E. Fernandes, F. Carvalho, P.B. Andrade, R.M. Seabra and M.L. Bastos, 2002. Antioxidative Properties of Cardoon (*Cynara cardunculus* L.) Infusion against superoxide radical, hydroxyl radical, and hypochlorous acid. J. Agric. Food Chem., 50: 4989-4993.