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Effect of Seasonal Variation on the Growth and Chemical Composition of *Cynara Cardunculus* L. Plants

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Abstract

Cynara cardunculus L. (Family Asteraceae), is a highly nutritious plant contains medically active compounds such as flavonoid, polyphenol, caffeoylquinic acids. During the few decades seasonal changes affect the production of crop worldwide. In this study, we studied the changes in the vegetative growth and chemical composition during the growing season from the first of February to the first of June. The samples were harvested monthly from the three replicates during 2009/2010 and 2010/2011 seasons. The results obtained in this study showed changes in physiological factors (plant height, number of leaves) and leaves biomass (fresh and dry weight) increased progressively with age of plant from February to June. But these increments were reached beyond in May at a significant level. The active ingredient, carbohydrate, flavonoid and polyphenol increased gradually with age up to May which gave the maximum value. While, caffeic acid, vanillic acid and chlorogenic acid reached the maximum value during April month.

1. Introduction

Cynara cardunculus L. (Family Asteraceae) commonly known as cardoon, is an herbaceous perennial plant originated from Mediterranean countries. This plant has many therapeutic potential as antidiabetic, antimicrobial, cholagogue, choleretic and diuretic [1,2]. Two active compounds, chlorogenic acid and cynarin (phenolic in nature) are found in the cardoon plant belongs to derivatives of caffeic acid. The leaves were also used as a stimulant in liver cell regeneration, choleretic lipid-lowering and hepatoprotective [3-7]. The importance of *C. cardunculus* is mainly referred to its content of the flavonoids and polyphenol. The metabolic processes leading to accumulation of these active constituents in the plant are basically controlled by the physiological age of the plant and the surrounding environmental conditions, as well as, the genetic factors. Therefore, it is of great importance from the production point of view, to follow up the growth parameters and chemical composition of the plant throughout the growing season. Hammouda *et al.* [8], found that the flavonoid and polyphenol contents in *C. scolymus* (Romanian strain) cultivated in Egypt varied with plant age, and plants 9- to 10-months-old were most suitable for pharmaceutical preparations. During seasonal changes, many investigators reported that the content of phenolics and flavonoids varied with the developmental phase of the plants. Djurdjevic *et al.* [9] observed that the maximum content of total phenolics was found in *Conyza Canadensis* L. plants during the flowering and fruiting time in August rather than phenolics peaked during elongation and excessive plant growth in May and June or in September. Phenolic acid (P-coumaric, ferulic, p-hydroxybenzoic, vanillic and syringic) revelled from the stem have a maximum twice reached maximum in May and in August. In a study on the effects of seasonal variation of the major secondary metabolites present in the extract of *Eremanthus mattogrossensis* less (Asteraceae) leaves. The main compounds were identified and quantified, and the metabolites were grouped by chemical class (caffeoylquinic acids, flavonoids, and sesquiterpene lactone). Statistical analysis indicated a straight correlation between the quantity of metabolites and seasonality, suggesting that environmental properties elicit important metabolic responses [10]. Also,

Thomsen *et al.* [11] observed that, the major alkamides in the roots of *Echinacea purpurea* were recorded its lowest concentration in the mid of autumn and early winter while the total concentration of lipophilic compounds in *Echinacea pallida* showed the same pattern. It is also noted that, all of the major active phenolic acids in *E. purpurea* were maximum concentrations in spring. Keeping in view, the objective of this study was designed and investigates the variation in the yield and chemical composition of *C. cardunculus* during the vegetative cycle of the plant which determine the optimum time for its harvesting yield and quality ingredients.

2. **Materials and Methods**

The seeds of *Cynara cardunculus* were obtained from Dr. Helmut Junge, ABiTEP GmbH, Berlin, Germany, providing the plant material, via Jelitto GmbH, Germany. This work was carried out during two successive seasons 2009/2010 and 2010/2011 at the Experimental Farm of Agriculture Faculty, Cairo University, Giza. This experiment was done to follow up the changes in the vegetative growth and chemical composition during the growing season from the first of February to the first of June where samples were taken monthly from the three replicates during 2009/2010 and 2010/2011 seasons.

2.1. Nature of soil

Samples from the soil were taken before cultivation, and were subjected for physical and chemical analysis in Soil Science Department, National Research Centre according to method of Jackson [12]. Physical properties: clay 22.0% (dry matter), silt 51.0%, sand 26.4%, organic matter 0.6%, soil texture (sandy loam). Chemical properties: pH 8.0, E.C. (dS/m^{-1}) 1.15, available N 1.40% (dry matter), available P 0.83%, available K 0.27%. Cations: (Milliequivalent/L) Ca^{2+} 12.2, Mg^{2+} 3.7, Na^{+} 0.27, K^{+} 0.27. Anions: (Milliequivalent/L) CO_3^{2-} 0.0, HCO_3^{-} 1.1, Cl^{-} 1.4, SO_4^{2-} 13.5.

2.2. Soil preparation for cultivation

During both seasons, the soil was mechanically ploughed and planked twice. During preparation of the soil for cultivation mixture of calcium superphosphate (15.5% P_2O_5) at the rate of 475 kg ha^{-1} as a source of phosphorus was added and mixed well manually with the soil.

2.3. Cultivation procedures and maintenance

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 a distance 100 cm apart between plants into plots 6 m^2 (3 X 2). Each plot contained 2 rows, with 6 plants. All other horticultural practices were done as needed for the whole period of the growing season. The plants were fertilized with 590 Kg ha^{-1} ammonium sulphate (20.5% N) as a source of nitrogen. The nitrogen was applied as a side dressing at three equal additions. The first addition was after 8 weeks of transplanting and the second was 6 weeks later, while the third was added after 6 weeks from the second addition. Potassium sulphate (48% K_2O) as a source of potassium at the rate of 355 Kg $ha^{-1}$was added at two equal amounts, the first dose was added with the second dose of nitrogen and a second was added with the third dose of nitrogen.

2.4. Data recorded

On the basis of phenology of cardoon we have analysed the following parameters in term of plant height, number of leaves and biomass of leaves (fresh and dry weight) per plant.

2.5. Chemical analysis

2.5.1. Estimation of total carbohydrate

Total carbohydrates in the dried leaves were determined according to Dubois *et al.* (1956) [13]. Total carbohydrates were calculated by using standard curve of glucose.

2.5.2. Estimation of total flavonoids

Determination of total flavonoids content in the dry leaves of *C. cardunculus* was determined by spectrophotometer according to Kosalec *et al.* [14].

2.5.3. Estimation of total phenols

Phenols content was determined in the dry aerial parts by spectrophotometer according to Falleh *et al*. [15].

2.5.4. Estimation of chlorogenic acid, caffeic acid, and vanillic acid content in leaves of cardoon through High Performance Liquid Chromatography (HPLC)

Using HPLC (Agilent Technologies, Palo Alto, CA, USA) chlorogenic acid, caffeic acid, and vanillic acid content was determined in the aerial parts (dry matter) according to the method described by Sharaf-Eldin *et al.* [16] and according to the modification reported by Wahba *et al.* [17].

2.6. Statistical analysis

This study was performed in a completely randomized manner with different treatment with three replicates. Data of each season were statistically analysed by ANOVA [18]. The LSD (least significant difference) level at 5% was used to compare the means value according to Snedecor and Cochran [19].

3. **Results and discussion**

3.1. Growth parameters

3.1.1. Plant height cm plant^{-1}

Table (1) compiles the data of growth parameters taken through the first and second seasons (2009/2010 and 2010/2011) from the first of February to the first of June. The results show that the plant height increased gradually from February and attain the highest height (cm plant^{-1}) in start of June, but the differences between May and June were insignificant. On the other hand the differences between other months were significant. The least value in this concern was obtained in February (88.33 and 93.33 cm plant^{-1}) against the highest values (108.33 and 113.33 cm plant^{-1}) in June for the first and second seasons, respectively.

3.1.2. Number of leaves plant^{-1}

Table (1) showed the same trend occurred in the case of plant height. The number of leaves increased progressively with age of the plant from February (8.33 and 9.67) till June (21.0 and 20.33) for the first and second seasons, respectively. Generally, it is noticed that the values of leaves number plant^{-1} in May and June were insignificant.

Table 1: Effect of age and seasonal variation in plant height (cm) and number of leaves plant^{-1} of *Cynara cardunculus* during 2009/2010 and 2010/2011 seasons.

Months	**Growth characters**			
	Plant height (cm)		**Number of leaves plant^{-1}**	
	1st season	**2nd season**	**1st season**	**2nd season**
February	88.33	93.33	9.33	9.67
March	91.67	95.00	10.33	11.33
April	96.67	101.67	17.00	16.67
May	106.67	111.67	20.33	21.33
June	108.33	113.33	21.00	20.33
LSD$_{(0.05)}$	**7.79**	**8.47**	**1.63**	**1.56**

3.1.3. Fresh and dry weight of leaves g plant^{-1} and ton ha^{-1}

From the data illustrated in Figure (1), the results reveal that the production of herb increased progressively and gradually from February till June. The differences for fresh weight between February and other months were significant, but the increased in the fresh weight through April and May months were insignificant as compared to fresh weight of herb through June month. This trend was true during both seasons. In the same Figure (1), the dry weight of herb shows a similar trend such as fresh weight. The fresh and dry weight of herb (leaves) ton ha^{-1} had similar of results of fresh and dry weight g plant^{-1}. The treatments which encouraged the fresh weights were the same which produced the high values of fresh and dry weight of leaves g plant^{-1}.

3.2. Chemical composition

3.2.1. Total carbohydrate (%) in leaves

The results in Figure (2) show that the percentage of total carbohydrate increased gradually from February to May, and attain the highest value in May where reached to 13.68% and 13.95% against 7.70% and 7.96% in February for the first and second seasons, respectively. On the other words, the total carbohydrate content

increased with age from February till May, while the percentage of total carbohydrate in June decreased as compared to May. The differences between the values of total carbohydrate % in February, March, April and May were significant, but the difference between May and June was insignificant.

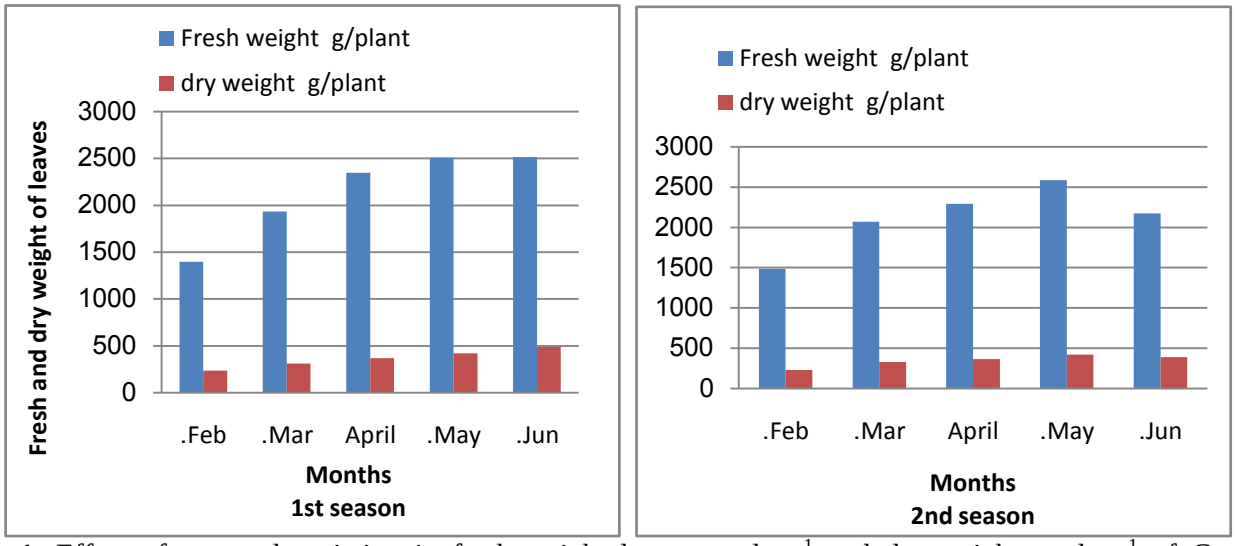

Fig. 1. Effect of seasonal variation in fresh weight leaves g plant^{-1} and dry weight g plant^{-1} of C*ynara cardunculus* during 2009/2010 and 2010/2011 seasons.

3.2.2. Total flavonoid in leaves mg g^{-1}

The total flavonoid in leaves of *C. cardunculus* plant ranged between 2.93 to 7.81 mg g^{-1} in the 1st season and 3.56 to 7.96 mg g^{-1} in the 2nd season during the growth season (Figure 2). The results clear that, the total flavonoids decreased progressively from February to June. The maximum value in this concern (7.81 and 7.96 mg g^{-1}) was resulted from the leaves which harvested at the start of growth in February against the lowest value in June (2.93 and 3.56 mg g^{-1}) for the first and second seasons, respectively.

3.2.3. Total polyphenol in leaves mg g^{-1}

Data in Figure (2) indicate that leaves of *C. cardunculus* were collected monthly from February till June. The range of values of polyphenol content was (4.86 to 7.86 mg g^{-1}) in the 1st season and (5.26 to 7.91 mg g^{-1}) in the 2nd one. The maximum value of polyphenol in herb was in May (7.86 and 7.91 mg g^{-1}), follow by (6.24 and 6.48 mg g^{-1}) in April, then (6.17 and 6.42 mg g^{-1}) in June. On other hand, the lowest value was obtained from the sample of February. The differences between these values were significant in both seasons, except the values between the samples of April and June during the second season.

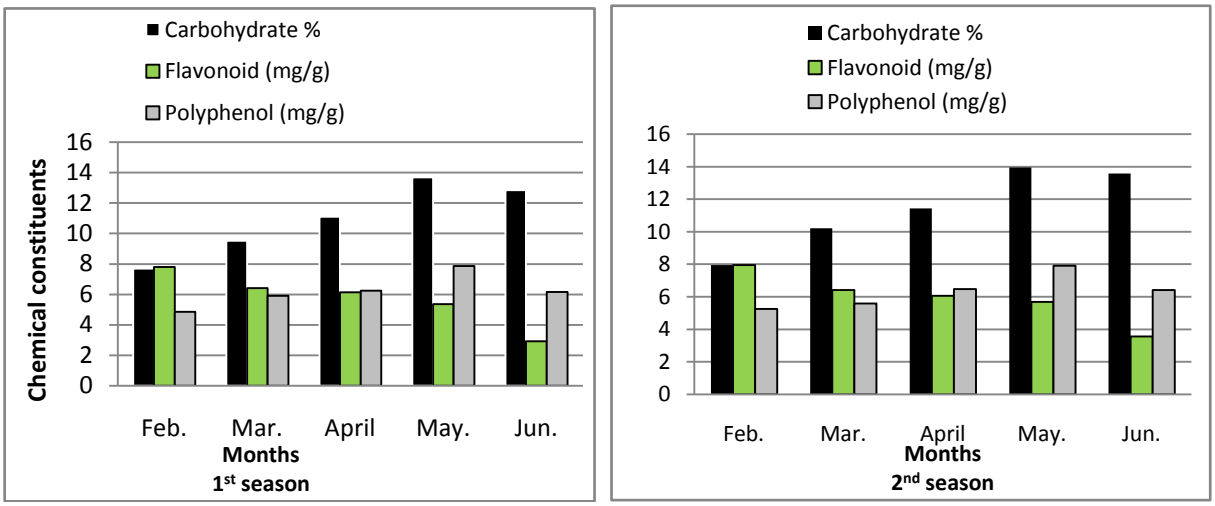

Fig. 2. Effect of seasonal variation in percentage of the chemical content of *Cynara cardunculus* leaves during 2009/2010 and 2010/2011 seasons.

3.2.4. Total caffeic acid, vanillic acid and chlorogenic acid in dry leaves

The data of the second season only presented in Figure (3) is evident that production of leaves for both caffeic acid, vanillic acid and chlorogenic acid were suitable in April, where gave the highest values for these compounds; on the contrary the month of Feb. gave the lowest amount of these compounds. On the other words, the total caffeic acid, vanillic acid and chlorogenic acid increased gradually from Feb. to April which reaching 28.19, 125.44, 535.68 mg $100g^{-1}$, respectively. On the contrary, the values of these compounds decreased during May and Jun months. Our results are in agreement with the finding of Djurdjevic *et al.* [9] on *Conyza Canadensis* L. plant, Gouvea *et al.* [10] on *Eremanthus mattogrossensis* and Thomsen *et al.* [11] on *Echinacea purpurea*. *Conyza Canadensis* L. is also used in Moroccan Rif for its phyto-therapeutic actions against liver inflammation [20].

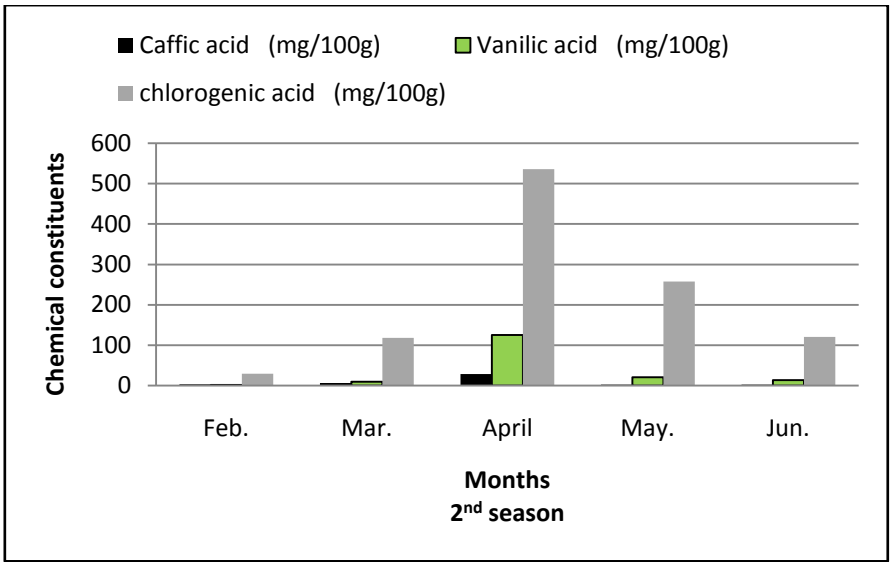

Fig. 3. Effect of seasonal variation of total caffeic acid, vanillic acid and chlorogenic acid content of *Cynara cardunculus* leaves during 2010/2011 season.

Conclusions

According to our results, we recommend to the cardoon's growers aiming to obtain the highest values of fresh and dry weight of leaves along with the high values of chemical constituents, to harvest the cardoon leaves during May under Giza's, Egypt climatic conditions, and/or in general before flowering stage. While, aiming to obtain high values of carbohydrate and polyphenol, should harvest cardoon leaves during May, but for flavonoid content, harvesting should be during February. If aiming to obtain high values of caffeic acid, vanillic acid and chlorogenic acid, should harvest cardoon leaves during April.

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