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Original Article

Yield and Antioxidant Activity of Artichoke Leaves (*Cynara scolymus* L.) Affected by some Agronomical Factors in Golestan Province of Iran

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Abstract

Artichoke (*Cynara scolymus* L) belongs to Asteraceae. A factorial experiment based on the randomized complete block design with three replications was carried using depths of root available water (RAW) and sowing time (ST) as treatments. Leaf length and width, number of leaves per plant and the biomass yield were recorded before laboratory analysis. In laboratory total phenols, total flavonoids, antioxidant activity and the content of chlorogenic and caffeic acids of leaf extracts were measured. The contents of caffeic and chlorogenic acid and the antioxidant activity varied based on the sowing times. A significant interaction effect of the treatments was observed on the content of chlorogenic and caffeic acid as well as on the antioxidant activity (IC_{50}) of the leaf extract. Plants which were grown at minimum RAW (33%) produced 189 mg/g more chlorogenic acid than the plants grown at maximum RAW (100%). It seems that under the conditions of the province Golestan, a delay in planting time led to a decrease of leaf yield both quantitatively and qualitatively. Based on the obtained results it can be concluded that under moderate water stress, the highest metabolite accumulation could be expected.

Key words: Antioxidant activity, artichoke, caffeic acid, chlorogenic acid, root available water

Introduction

Artichoke (*Cynara scolymus* L.) is one of the oldest medicinal plants [1] known since ancient time and had been used as a food digestive aid in ancient Greece and Roma. Artichoke belongs to Asteraceae. It has strong, prickly, deeply-cut leaves and large terminal heads that resemble single flowers [2-4]. Artichoke is known as a nutritional and pharmaceutical plant [4,5]. The pharmaceutical value of the artichoke leaves has been widely studied and the results confirmed the leaves as a rich source of polyphenols [6,7]. Wang *et al.* [7] showed that the young leaves and receptacle have higher polyphenol compounds than older ones. The roots of this plant are a rich source of the polysaccharide inulin in which is important in human health. It seems that the antioxidant properties of the plant refer to the ratio of mono- and dicaffeoylquinic acids and flavonoids [8-10]. The role of artichoke industrial byproducts as a source of antioxidant phenolics in the fictionalization of foodstuffs for decreasing lipid peroxidation and increasing health promoting properties have been studied by Lorach *et al.* [11]. Hydroxycinamic acids such as chlorogenic acid, dicaffeoylquinic acids, caffeic acid and ferulic acid, and flavonoids such as luteolin and apigenin glycoside are the main compounds of artichoke leaves extract. The physiological response of artichoke to the agronomical factors in aspect of secondary metabolite accumulations was not intensively investigated. Most of studies focused on the head production as vegetable. In present study, the effect of sowing time and the amount of root available water (RAW) on artichoke grown under

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the ecological conditions of the province Golestan was studied.

Material and Methods

The present experiment was carried out as factorial based on a randomized complete block design with three levels of root water available (RAW), two sowing times and three replications in one growing season in 2009. Seeds were planted in small pots and the 4-6 leaves plantlets were planted in the field on the 5th and 20th of May (15 days interval). Every 10 days the water demand of plants was calculated and the plants were irrigated with three different levels of water based on the depth of irrigation. Based on the depth of root activity (60 cm) of artichoke in a 10 days period of irrigation the plants were irrigated with 100, 66 and 33% of root available water. The depth of irrigation (Ig) in 100% of water demand in centimeter was calculated according to the formula: $Ig= (FC-\theta x) \times \rho a \times Rz \times 1/0.8$.

Where: FC and θx meaning the percentage of weight humidity in field capacity and the weight humidity of the soil according to the maximum depth of root development in centimeter, respectively. The value 0.8 means irrigation efficiency of 80%. The soil specific gravity (pa) in g/cm^3 was obtained by field sampling. The depth of irrigation in 66% and 33% RAW was obtained by multiplication of the formula above with 0.66 and 0.33, respectively. The depth of irrigation in the control treatment was zero and the water supplementation in this treatment was only obtained from rainfall. By measuring the water content of the soil before irrigation, the amount of rainfall was automatically included into the water balance. To calculate the amount of water for each irrigation period, one day before irrigation, the soil samples were randomly taken from the three depths of 0-20cm, 20-40 cm and 40-60 cm and then the samples of every replication were combined. After calculating the wet weight of the soil, the dry weight of the samples was measured after drying at 105 °C for 3 hours. The difference between the wet and dry weight was assumed as soil humidity and the amount of water was calculated based on the soil humidity. More information about the weather and soil conditions is presented in Tab.1 andTab.2.

Plants were harvested when the length of outer leaves reached 100-120cm (18/08/2009 and 06/10/2009, first and second harvests, respectively). The harvested leaves were dried under room conditions (21-24 °C temperature and 40-50% relative humidity). Yield components like plant hight and the biomass yield were recorded at the field in both harvests.

Contrary to calculate the amount of phenol, flavonoid, antioxidant activity as well as the content of caffeic and chlorogenic acids; only the leaf samples of first harvest were used. For measuring the content of caffeic acid and chlorogenic acid of leaf extract using HPLC, the leaf samples were dried at 38 °C for 48 hours and then ground using a grinder. 50 milligram of finely ground samples were added to a 50 ml Erlenmeyer flask containing 12.5 ml of a methanol-water mixture in a ratio of 20:80 (v/v): the containing mixture was ultrasound-treated for 30 min. Then double-distilled water was added to the sample solution at a ratio of 1:1 resulting in a methanol: water ratio of 40:60. A part of the sample was then added to a 10 ml test tube and centrifuged in 5000 rpm for 15 min and the supernatant was collected in HPLC vials and kept at -18 °C till analysis. For preparing a methanolic leaf extract to measure the content of phenol and flavonoids and the antioxidant activity, a certain amount of ground leaf (2 gr) was mixed with methanol and filtered after 24 hours.

Table 1 Metrological data of research station (2009)

	April	May	June	July	August	September	October	November	December
Tem	12.6	18.8	24.3	29.1	27.1	25.3	21.4	17	10
RF	63.2	29.8	13.1	5.9	41.2	82.6	86.1	70.5	73
SD	130.8	177.9	196.8	276.2	130.6	222.9	130.3	149.7	124.4
RH	75	72	65	59	71	67	66	66	76

Tem: Temperature (°C), RF: rainfall (mm), SD: Sunny days (h), RH: relative humidity

Table 2 characters of the soil of research station

EC	Organic matter	Soil texture	pH	Water at FC 0-20cm (g)*	Water at FC 20-40cm(g)	Water at FC 40-60 cm(g)*
1.72 ds/m	5%	Silty clay	7.85	34.4	34.6	30

*****FC: field capacity; water content of different soil depths in gram

The extraction was repeated three times for each sample and the methanol was evaporated using a rotary evaporator and freeze dryer. Radical scavenging potency of samples (antioxidant activity) was measured using diphenylpicrilhydrazyl (DPPH). 4 ml extract were mixed with 1 ml DPPH (10µM) and incubated in the dark for 30 min. The absorption of samples and blank (including all solutions except the sample) was read at 517 nm. A lower IC_{50} (minimum concentration of extract needed to neutralizes 50% of free radicals of sample) means high antioxidant activity and vice versa [12].

The flavonoid content of the samples was measured using aluminum chloride as indicator and spectrophotometery 420 nm using quercetin reference substance. The results are reported as equivalent quercetin per gram dry extract. Total phenol content was measured using Folin-ciocalteau reagent. 0.5 ml of extract was mixed with 0.5 ml of Folin-Ciocalteau and 0.05 ml of sodium carbonate (10%) and the absorption recorded at 760 nm after blending for one hour. Gallic acid was used as standard for the calibration curve and the total phenol was reported as equivalent gallic acid in one gram dry extract. The obtained data was analyzed using SPSS software and the mean values were compared according to the least significant difference ($LSD_{5\%}$) method.

Results and Discussion

Water is one of the most important agents in plant development and growth. Based on the obtained results most of the measured parameters significantly varied under different treatments. However, the interaction of sowing time and root available water (RAW) was significant only in some cases. Plant growth and yield were influenced by the sowing time. Plants of the first sowing dates were taller (57.01 cm) than those (37.3 cm) planted 15 days later. The height of the plants showed similar differences in the second harvest as well. In the second harvest, the plants of the first sowing date were taller than those planted with 15 days delay; however, the difference in plant height was lower than in the first harvest (74.8 cm and 70.6 cm respectively in the first and second harvest). An appropriate sowing time can provide a longer growth period and a more effective use of the environmental conditions to complete physiological and biochemical processes in the plants. In addition, sowing time is important to meet the ecological needs of the plants in some special period of time. For instance, delayed sowing time of biennial plants at early spring, remains plant at rosette stage till next season. Finding the correct sowing time of a plant leads to the maximum use of the environmental conditions. There are few studies about the effect of sowing time on artichoke, which cover only head production, but not the leaf quality as a pharmaceutical material [9,13].

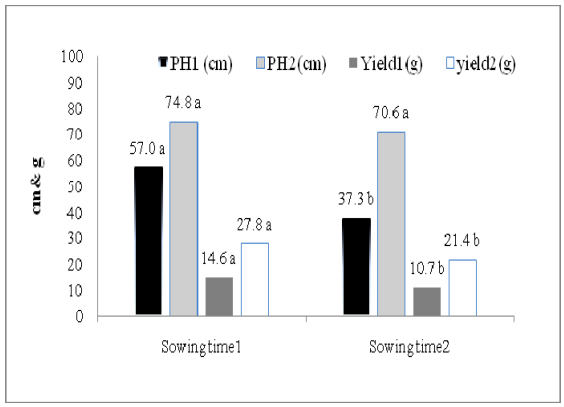

Fig. 1 Effect of sowing time on leaf yield (g/plant) and plant height (pH, cm) of artichoke at two harvest times in 2009, Different letters a, b, mean significant difference

As shown in Fig. 1, the artichoke leaf yield on the two planting dates was significantly different. The results showed that in the first harvest, the leaf yield of the plants planted earlier was higher than the one of the second planting date (14.6 g and 10.7 g for first date and second date respectively). In both sowing times, the leaf yield of plants of the second harvest significantly increased, and this increase was relatively two times more than the first harvest (Fig.1).

Plant height as an important factor in biomass production of artichoke, were significantly affected by RAW at the time of the first harvest (Fig. 2). Differences in plant height were observed under different irrigation regimes: plants receiving 100% of the required water were higher (58.3 cm) than those of other treatments. Contrary to that, the lowest plant height (30.3 cm) was recorded at a RAW of33% of the plant water demand. In plants grown at a RAW of 66%, an average height of 49.7 cm was recorded. In the first harvest, also leaf yield per plant was strongly influenced by root water availability. A maximum leaf yield of 17.4 g per plant was observed in plants with 100% RAW with the respective value amounting to 13.4 g in plants grown at a RAW of 66% and 6.6 g/plant in plants grown at a RAW of 33%. Pellicciari and Sismondo

[14] showed that when they used different amounts of water with different evaporation rate, a little effect on the cumulative yield was observed. Results of other researchers about the effect of irrigation on the vegetative body of the artichoke are consisted with the results obtained in this study. For example, Husain and Stewart [13] reported that, 25 mm of water via drip irrigation, increased vegetative growth and flower bud number of Green Globe artichoke.

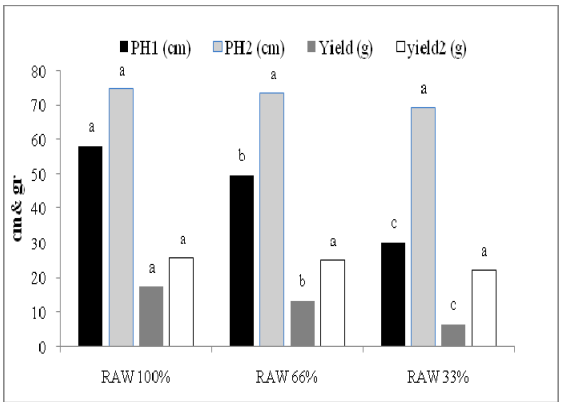

Fig. 2 Effect of depth of water available to roots on plant height (cm) and yield (g), year 2009.Different letters a, b .., mean significant difference

Boari *et al.* [15] showed that, the flower buds yield of artichoke (var. 044) influenced by low temperature and water availability. Foti and *et al.* [16] showed that there was little difference between 50 and 100% offset evaporation. The results of Macua *et al.* [17] on the effect of irrigation on Blanca Ciudadela artichoke cultivars using sprinkler irrigation method showed that, the maximum TSS (total soluble solid) was recorded in the plants which were irrigated by minimum amount of irrigation. Also Tarantino and *et al* [18] studied two volumes of different irrigation water based on evapotranspiration rates of 1 and 1.5with variety of artichokes Violeta de Provenza and results showed that the number of buds was increased by high irrigation level. Also some other studies on artichoke irrigation demonstrate the importance of water on artichoke biomass production [11,14,15].

Unlike the first harvest, in the second harvest, no decrease in the leaf yield with a decreasing amount of RAW was observed. Even between 100% and 66% RAW, the difference was not significant. In both sowing times, a dry hot weather was recorded from sowing time to the first harvest. Opposite to that duration between first and second harvests encountered to a wet and relatively moderate weather. With regard to the ecological needs of artichoke as a Mediterranean plant, a better growth in the second harvest is justified. Therefore, lacking differences in biomass and yield components due to irrigation can be attributed to a more rainfall and cooler weather after the first harvest which reduced the water demand and stress of plants.

Data analysis of variance showed that, some of the leaf quality parameters of artichoke were influenced by sowing time and water regime. Sowing time significantly affected caffeic acid and antioxidant capacity of the leaf extract. In addition the total phenol content with 5% possibility was influenced by sowing time. The effect of RAW on chlorogenic acid and the antioxidant capacity of leaves were significant. Evaluation of the interaction effect on measured parameters showed that the amount of chlorogenic acid, caffeic acid and antioxidant activity was significantly different. Results showed that the early planted plants had 11.5 mg caffeic acid more than plant of the second date (Fig. 3). Similar trends were observed in leaf extract antioxidant activity. As is shown in Fig. 3, The IC50 (minimum concentration of extract at which 50% of free radicals can be neutral) of leaf extract of plants of first sowing time was significantly more than that of plants of the second sowing time (15.7 and 8.2 in first and second sowing, respectively). The effect of sowing time on the accumulation of flavonoid compounds did not show significant difference (Fig. 3). In contrast, the content of caffeic acid, phenol and antioxidant activity of the leaf extract (IC50), were significantly affected sowing time.

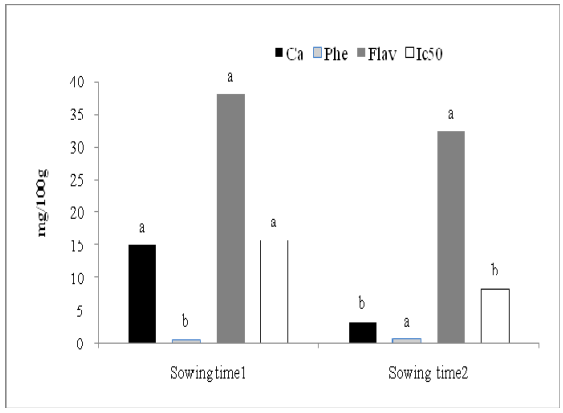

Fig. 3 Effect of sowing time (ST) on the content of caffeic acid (Ca mg/100g), phenol (mg gallic acid/ml), flavonoid (mg quercetin/ml) and onIC50 (mg/ml) of artichoke leaves.

From Fig. 4 it can be found that by reducing the rate of RAW, the concentration of flavonoids

declined. Contrast to that, caffeic acid increased when water supply decreased from 100 to 66%. No significant difference in the content of total phenol was observed under different RAW levels. The IC50 of leaf extract and the concentration of chlorogenic acid (see Fig. 5) were influenced by depth of root available water. By reducing water from 100% to 66%, the amount of IC50 increased. However, the reduction from 66% to 33% RAW was accompanied by a reduction of 7.6 mg per 100 ml (Fig. 4).

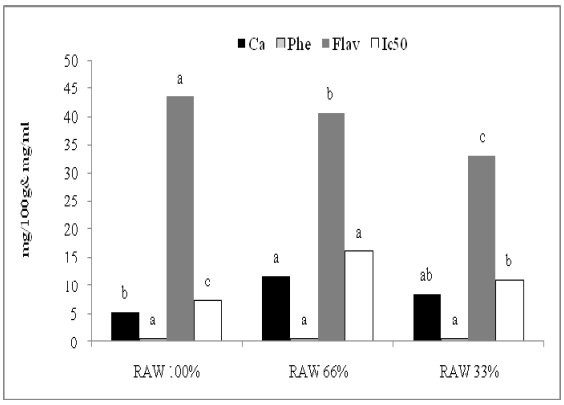

Fig. 4 Effect of depth of available root water on caffeic acid (Ca, mg/100g), phenol (phe, mg gallic acid/ml), flavonoid (flav, mg quercetin/ ml) and IC50 (mg/ml) Artichoke leaf.

Generally it can be concluded that, the reduction in water availability from 100% to 66% induced water stress in plants and stress caused an increase in antioxidant activity of the compounds. Excessive water stress (33%) caused changes in the physiological structure of the plants and reduced the chemical reactions of the plant tissue which lead to a reduction in IC50. By reducing the available water of root, chlorogenic acid accumulation was increased. So that, compared with 100% RAW (494.4 mg/100g), the accumulation of chlorogenic acid of plants that were under 33% RAW treatments, increased by 189 mg in 100 gram.

Interaction effect of sowing time and water regime showed that caffeic acid accumulation was extremely influenced by sowing time and RAW.As shown in Fig. 6, in plants of the first sowing time reducing the water supply from 100% to 66%, the amount of caffeic acid increased from 10.7 to 23.3(mg/100g). In contrast, by reducing water from 66% to 33% RAW a reduction in caffeic acid production was observed. In the plants of second sowing time, the caffeic acid accumulation in leaves was quite different.

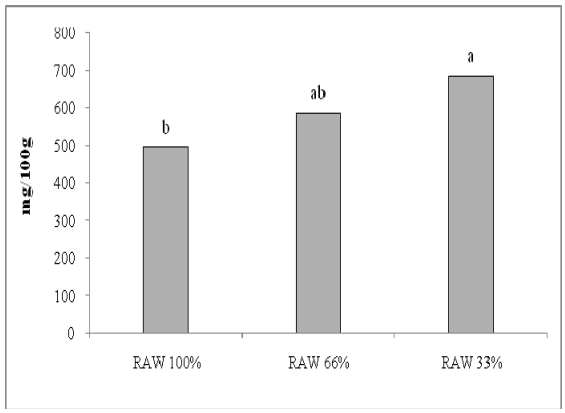

Fig. 5 Effect of depth of root available water (RAW) on the amount of chlorogenic acid (mg/100g) of artichoke leaves.

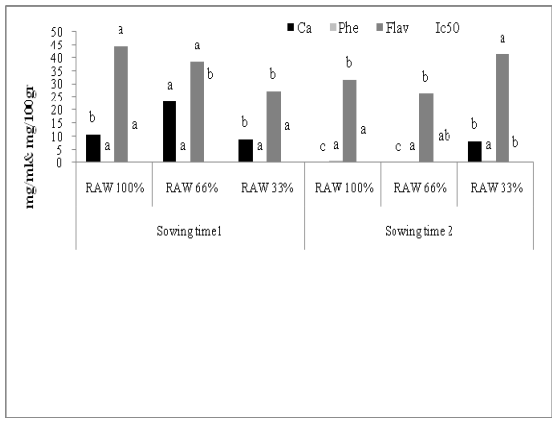

Fig. 6 Interaction effect of sowing time and depth of root available water on the concentration of caffeic acid (Ca, mg/100g), phenol (phe, mg gallic acid/ml), flavonoid (Flav, mg quercetin/ ml) and onIC_{50} (mg/ml) of artichoke leaves.

Fig. 6 shows that, in the first planting date the highest rate of caffeic acid was obtained from the 66% RAW, while in the second planting date with the same water supplementation, the lowest caffeic acid concentrations were recorded. In contrast, plants that were irrigated with the minimum RAW had the highest caffeic acid accumulation. The total phenol of all samples was not influenced by the interaction of treatments. As is shown in Fig. 7, chlorogenic acid content of leaves was significantly influenced by interaction between water regime and sowing time. Maximum amount of chlorogenic acid was recorded in plants of early sowing time which were under 66% water regimes. In contrast, no significant difference was observed between 100 and 33% RAWs.

Changes in the amount of chlorogenic acid of leaves of late sowing time which was affected by irrigation regime were quite different with plants of early sowing time.

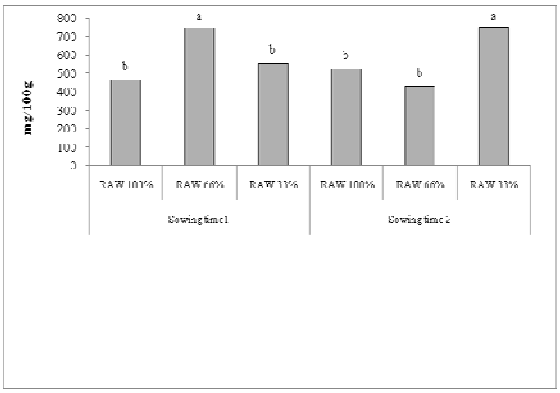

Fig. 7 Interaction effect of sowing time and depth of root available water on the content of chlorogenic acid (mg/100g)

Unlike the first planting date, at the second time, maximum chlorogenic acid was observed under 33% RAW regime (Fig. 7). Results showed that the highest IC_{50} (three times more than that of other regimes) was observed in the samples of early sowing time which were treated with 66% RAW.

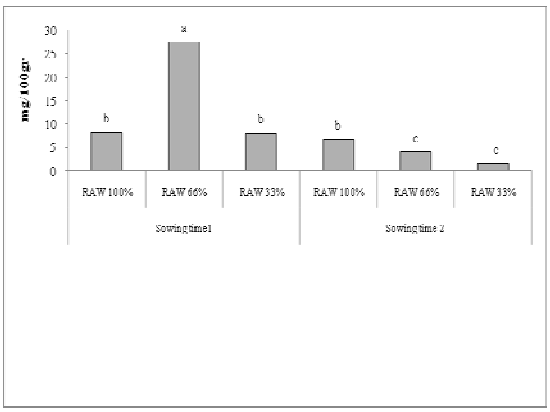

Fig. 8 Effect of interaction between sowing time and depth of root available water on IC50

Unlike the first sowing date, at the second time, the highest IC_{50}antioxidant capacities of samples increased at lower RAW. So that, IC_{50} of leaf samples of 33% RAW was three folds more than that 100% RAW. There is little information about water stress on the accumulation of phenolic compounds. Chung *et al*. [20] showed that water stress in *Rehmannia glutinosa* Steud. led to extreme reduction in chlorogenic acid and salicylic acid compared with control. Also his results showed that the total phenol of plant was reduced nearly 50%compared with control plants (62.27 and113.4 µg/g respectively in control and stressed plant). Paul *et al*., [21] showed that amount of proline and the alkaloid compounds of *Rauvolfia serpentina* Baill. leaves grown in dry condition were significantly higher than irrigated plants. Based on the studies of others water stress increase the accumulation of active compounds, and so the results obtained in this study are consistent with findings of other researchers.

Conclusion

Regarding to the obtained results, it can be concluded that artichoke normally growing in weather conditions of the Golestan Province of Iran shows good vegetative growth. It must be mentioned that more number of harvesting time and subsequently biomass yield led to high and affordable economical yield. Artichoke is relatively tolerant to hostile environmental conditions and is able to attain reasonable yield under water stress conditions. However, similar to other plants of the Asteraceae, artichoke seems to be more potent under the temperate and Mediterranean climate conditions. Study of planting date and depth of root water availability on secondary metabolites accumulation is in the beginning of way. However, result of the present study showed that the plant secondary metabolites, especially chlorogenic acid, caffeic acid and antioxidant agents are influenced by applied agricultural treatments. It seems that delayed plant sowing, leads to yield reduction. However, the accumulation of secondary metabolites directly or indirectly, is affected by planting time. Therefore, it is recommended that Artichoke should be planted in the climate condition of Golestan province early in spring. Finally, to achieve more secondary metabolites and antioxidant potential of leaves, moderate water stress in a part of plant growing phase is advisable.

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