

# Yield and quality responses of drip-irrigated spinach to different irrigation quantities in a semi-arid region with a high altitude

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## Abstract

The effect of different irrigation quantities on the growth, marketable yield, crop quality (antioxidant activity, mineral and total phenolic content) and irrigation water use efficiency (IWUE) of spinach grown in a semi-arid region of high altitude (1795 m a.s.l.) was determined. Plants were irrigated with three different irrigation quantities which determined considering 100 (I1), 85 (I2) and 70% (I3) of the evaporation obtained from a Class A pan. Irrigation quantities in the I1, I2 and I3 treatments as two years average were 290, 264.3 and 238.6 mm, respectively. The I1 treatment provided the highest growth, marketable yield (28.06 t\*ha<sup>-1</sup>) and IWUE (9.7 kg\*m<sup>-3</sup>). However, mineral and total phenolic content and antioxidant activity in the leaves of spinach were significantly higher under lower water application conditions. The I2 treatment resulted with the highest antioxidant activity and content of N, K, Mg, Na, Fe, Cu and total phenolics. As a result of the study, I1 treatment in spinach production could be suitable for water sufficient regions due to higher yield and IWUE. However, I2 treatment may be more appropriate for water scarce semi-arid regions of high altitude for obtaining higher minerals and antioxidant activity.

**Keywords:** antioxidant activity, irrigation water use efficiency, marketable yield, mineral and total phenolic content

## Introduction

Fresh water resources inadequate for the growing population in world increase the stress on the agricultural water use because agricultural irrigation is the largest consumer of the available freshwater. A part of 70% of all water withdrawn for agricultural, municipal and industrial use is used in agriculture (UNESCO, 2012).

High crop water productivity values are preferred in water-scarce arid and semi-arid regions (Geerts and Raes, 2009). Therefore, determining of the optimal water amounts applied to plants for obtaining high water productivity is necessary. Effective

water use in agriculture requires current and also practical irrigation scheduling. Evaporation pans providing practical data are preferred mostly for creating irrigation schedules. Also, pan evaporation values are valuable because they reflect all meteorological effects in the region placed. Class A pan is the most common pan type used for preparing the irrigation scheduling of many plants (Imtiyaz et al., 2000).

Spinach is a high nutritive vegetable crop due to content of many vitamins and minerals (Nishihara et al., 2001). Also spinach is a valuable vegetable in terms of antioxidant activity and total phenolics (Chu et al., 2002; Ismail et al., 2004; Song et al., 2010). Therefore, it is used extensively for human nutrition in the world. Also in Turkey, spinach is a widely produced and consumed vegetable. The production area and amount for 2012 of Turkey were 18489.9 ha and 222225 t, respectively (TSI, 2014).

Vegetation period of spinach lasts 60 (70) and 100 days in Mediterranean and arid regions, respectively (Allen et al., 1998). Generally, period from sowing to harvest varies between 6 to 10 weeks depending on the agronomic conditions (air temperature, soil moisture and fertility). Short vegetation period leads to low seasonal water consumption. Therefore, many studies indicated that seasonal water consumption values of spinach was low (Leskovar and Piccinni, 2005; Leskovar et al., 2008; Nishihara et al., 2001; Piccinni et al., 2009). Moreover, the shallow rooting depth of 30 cm of spinach requires sufficient and regular moisture in soil for obtaining high yield.

The crop production losses are inevitable in water-scarce dry regions. Irrigation water use efficiency (IWUE) defined as the ratio of crop yield to the applied water is a good indicator to evaluate effectiveness of irrigation water (Fairweather et al., 2003). Therefore, water productivity can be increased with lower water applications from optimal level without causing a significant reduction in yield. Many researchers found that water productivity of spinach increased under lower water applied conditions although decreasing yield (Leskovar and Piccinni, 2005; Leskovar et al., 2005; Nishihara et al., 2001). Similarly, high IWUE values under less irrigation conditions in a semi-arid region of high altitude were determined in cucumber by Sahin et al. (2015b), in radish by Sahin et al. (2015c) and in lettuce Sahin et al. (2015a). On the contrary, Kuslu et al. (2014) found high IWUE values in squash irrigated with higher water amounts in a semi-arid region with a high altitude.

Water-scarce arid and semi-arid regions will face more water stresses than others in the future. Therefore, these regions need to be explored for effective water-using ways in agriculture. Also, air temperature is another significant agronomic factor affecting agricultural production. The phenological development of plants is affected at an important level in cold regions and high altitudes (White and Howden, 2010). Low environmental temperatures reduce nutrient uptake and conduction by the roots in some plants (Hasanuzzaman et al., 2013). However, light influences the nutrient uptake by photosynthetic plants positively (Crawford, 2014). Therefore, a two-year research was conducted with the aim to determine the growth, marketable yield, antioxidant activity, content of minerals and total phenolics and irrigation water use efficiency of spinach under different irrigation levels in a semi-arid region of high altitude.

## Materials and methods

### Experimental site

Spinach field experiments were conducted during summer 2010 and 2011 at the Agricultural Research Station of Agricultural Faculty of Ataturk University, Erzurum, Turkey (39.933° N and 41.237° E, altitude of 1795 m above sea level). According to the mean annual precipitation (1971-2013) the climate is semi-arid. During the growing seasons of the research (17 June-29 July in 2010 and 15 June-29 July in 2011), the mean monthly temperature, relative humidity, wind speed and daily sunshine values taken from Erzurum Meteorological Station (39.95° N, 41.17° E, 1757 m a.s.l., 5 km from experimental area) are given in Table 1. The total evaporation and precipitation values respectively measured by a Class A pan and a pluviometer placed in the experimental area are also given in Table 1.

The soil layer of 30 cm in the experimental field was clay loam texture (29.5% clay, 35.4% silt and 35.1% sand) with low organic C (1.65 g\*kg<sup>-1</sup>) and CaCO<sub>3</sub> (2.24%) contents. The soil moisture contents at field capacity (-0.03 MPa) and wilting point (-1.5 MPa) were respectively 30.1 and 18.6 as percent of the weight. The bulk density, electrical conductivity and pH values of the soil was 1.31 Mg\*m<sup>-3</sup>, 1.38 dS\*m<sup>-1</sup> and 7.58, respectively. These analyses were made considering standard procedures described by Blake and Hartge (1986), Gee and Bauder (1986), Klute (1986), Mc Lean (1982), Nelson (1982), Nelson and Sommers (1982) and Rhoades (1982).

Table 1. Climatic data of the experimental area in the growing periods in trial years

Parameters	2010		Average or total	2011		Average or total
	June <sup>1</sup>	July <sup>2</sup>		June <sup>3</sup>	July <sup>2</sup>	
Mean temperature (°C)	14.9	19.5	18.0	15.8	19.4	18.1
Relative humidity (%)	65.1	57.0	59.6	57.4	54.4	55.5
Wind speed (m*s <sup>-1</sup> )	2.5	3.3	3.0	2.8	4.1	3.6
Daily sunshine (h)	8.2	9.8	9.3	11.3	8.3	9.4
Precipitation (mm)	22	59	81	5	15	20
Evaporation (mm)	85	203	288	96	216	312

<sup>1</sup>Calculated from daily data between 17-30 June.

<sup>2</sup>Calculated from daily data between 1-29 July.

<sup>3</sup>Calculated from daily data between 15-30 June.

### Planting and irrigation

The experiment was designed as completely randomized block design with three replicates. Matador cultivar of spinach (*Spinacia oleracea* L.) seeds wetted with tap water for one day were sown to each plot with 16.5 cm between plants and 20 cm between rows on June 17 in 2010 and on June 15 in 2011. Each plot area with 8 m length and three rows was 4.8 m<sup>2</sup>. Before planting in 2010, aged cattle manure of 30 t\*ha<sup>-1</sup> was added to the experimental field. No pesticides were applied during experiments and weeds were removed manually.

The experiment consisted of three treatments. Irrigation treatments with three different regimes (I1, I2 and I3) were adjusted using evaporation values from a Class

A pan in the experimental site. Irrigations were initiated with seed sowing. During the first 3 weeks, the spinach plants in all plots were irrigated equally. Irrigation quantities in this period were determined considering 100% of evaporated water from the Class A pan. Scheduled irrigations were initiated on July 9 in 2010 and on July 7 in 2011 and continued to the harvest. During this period, irrigation levels were selected as 100% for the I1 treatment, 85% for the I2 treatment and 70% for the I3 treatment of evaporated water from the Class A pan. Irrigations were made average three days intervals throughout growing period in both years. The highest irrigated treatment was considered control treatment. The irrigation quantities were calculated using the below equation (1):

$$I = E_{\text{pan}} \times IR \times WR \quad (1)$$

where, I is the irrigation quantity (mm),  $E_{\text{pan}}$  is the cumulative evaporation amount between the two irrigations (mm), IR is the irrigation ratio and WR is the wetting ratio (1.0). Irrigation ratios were 1.0, 0.85 and 0.70 for I1, I2 and I3 treatments, respectively. These ratios were determined considering the plant coefficients ( $k_c$ ) proposed for spinach (Allen et al., 1998; Piccinni et al., 2009).

The irrigation water (groundwater) with pH of 7.40, electrical conductivity of  $0.295 \text{ dS} \cdot \text{m}^{-1}$  and sodium adsorption ratio of 0.45 was applied to the plants by drip irrigation system. The system was set up from a centrifugal pump, control unit (a screen filter, manometers, valves and a water meter) and pipe lines (main pipe, manifolds and driplines). Driplines which had in-line type emitters with a distance of 0.33 m were placed to each row in the plots. The emitter flow rate was  $4 \text{ L} \cdot \text{h}^{-1}$  under an operating pressure of 0.1 MPa. The irrigations were manually controlled by a valve connected to the manifold of each plot.

Water productivity was evaluated with irrigation water use efficiency (IWUE) which indicates the yield per unit irrigation (Howell, 2001). The IWUE was calculated using the following equation (2):

$$IWUE = \frac{Y}{I} \quad (2)$$

Where, IWUE is the irrigation water use efficiency ( $\text{kg} \cdot \text{m}^{-3}$ ), Y is the total marketable yield ( $\text{kg} \cdot \text{decare}^{-1}$ ) and I is the amount of seasonal irrigation quantity (mm).

### Harvesting, measurements and analysis

Fifteen spinach plants in the middle of the central row of the plots were harvested by hand on July 29 in 2010 and 2011. During the harvest, marketable parts of the spinach plants were separated from their roots by cutting with a knife and each part were separately weighted as plant and root weights, respectively. Numbers of leaves were determined. Additionally, root diameter was measured by a caliper at the cutting point and root and plant lengths were measured by a ruler during the harvest. Marketable yield ( $\text{t} \cdot \text{ha}^{-1}$ ) was calculated by multiplying the average plant weight with the plant number in an area of 1 ha.

Spinach leaves cleaned with distilled water for the analysis of mineral content (N, P, K, Ca, Mg, S, Na, Fe, Cu, Mn, Zn and B) were dried for 48 h at a constant heat of  $68^\circ\text{C}$  in an oven and then they were powdered. Nitrogen content was determined by

the Micro-Kjeldahl method (Bremner and Mulvaney, 1982). Other observed minerals were determined by wet digestion method using a HNO<sub>3</sub>-H<sub>2</sub>O<sub>2</sub> acid mixture (2:3 v/v) in a microwave unit (Speedwave MWS-2 Berghof products + Instruments Harresstr.1. 72800 Enien Germany) with three different steps [first step: 145 °C, 75% radio-frequency power (RF), 5 min; second step: 180 °C, 90% RF, 10 min; and third step: 100 °C, 40% RF, 10 min] as described by Mertens (2005a). The minerals were analysed by using an ICP-OES spectrophotometer (Inductively Couple Plasma spectrophotometer Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) according to the suggested approaches by Mertens (2005b).

The antioxidant activity and phenolic compounds were analyzed in the extracts of spinach leaves. Firstly, the spinach pulp of 10 g was mixed with 10 ml ethanol and stirred for 6 hours with a magnetic stirrer, and then suspension was filtered through a Whatman No. 1 filter paper (Sengul et al., 2011). Prepared extracts were kept in a freezer at a constant heat of -20 °C until the analysis. The determination of the total phenolics content in the extracts was made using the Folin–Ciocalteu colorimetric method (Gulcin et al., 2002) with analytical grade gallic acid as a standard. Antioxidant activity was determined by the β-carotene bleaching method (Kaur and Kapoor, 2002) with some modifications (Sengul et al., 2011).

### Statistical analysis of data

The measured data were evaluated statistically by the analysis of variance (ANOVA) in the MINITAB software (Minitab Inc., 1996). The parameter means were separated by Duncan's multiple range test.

## Results and discussion

### Irrigation quantity

Irrigation was conducted 13 and 14 times throughout 2010 and 2011 growing periods, respectively. As shown in Figure 1, total irrigation quantities applied to the highly irrigated I1 treatment were 278 mm in 2010 and 302 mm in 2011. While the I2 and I3 treatments were achieved with lower irrigation water of 8.5 and 17.1%, respectively in 2010 compared to the I1 treatment, these treatments were also achieved lower water of 9.2 and 18.4% in 2011. The average daily irrigation water amounts applied in 2010 were 6.32, 5.78 and 5.24 mm in the I1, I2 and I3 treatments, respectively. These values were 6.56 mm in the I1 treatment, 5.96 mm in the I2 treatment and 5.36 mm in the I3 treatment also in 2011. Considering the climatic parameters in Table 1, 2011 growing period was more arid according to 2010 growing period. Therefore, more irrigation water in 2011 was applied to spinach plots compared to 2010. However, high irrigation quantities were applied during 2010 and 2011 growing periods because high evaporation values (Table 1). Therefore, considering short growing period, irrigation quantities applied in this study were higher compared to many study (Leskovar and Piccinni, 2005; Leskovar et al., 2008; Nishihara et al., 2001; Piccinni et al., 2009).

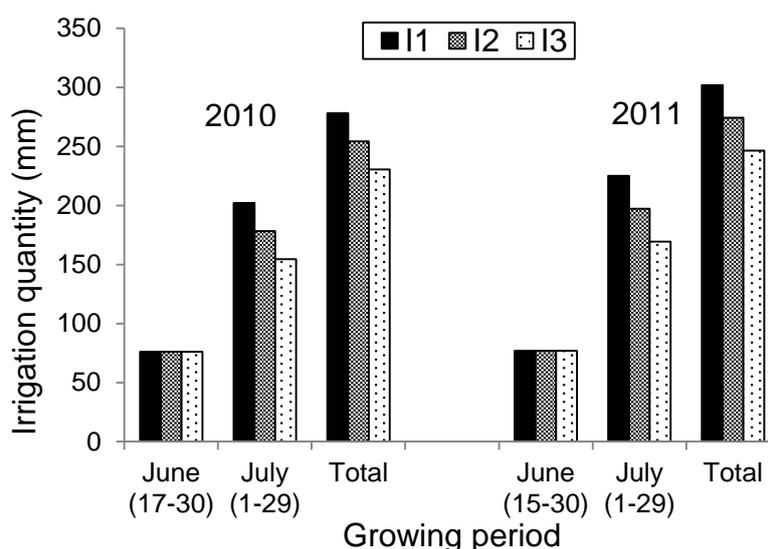
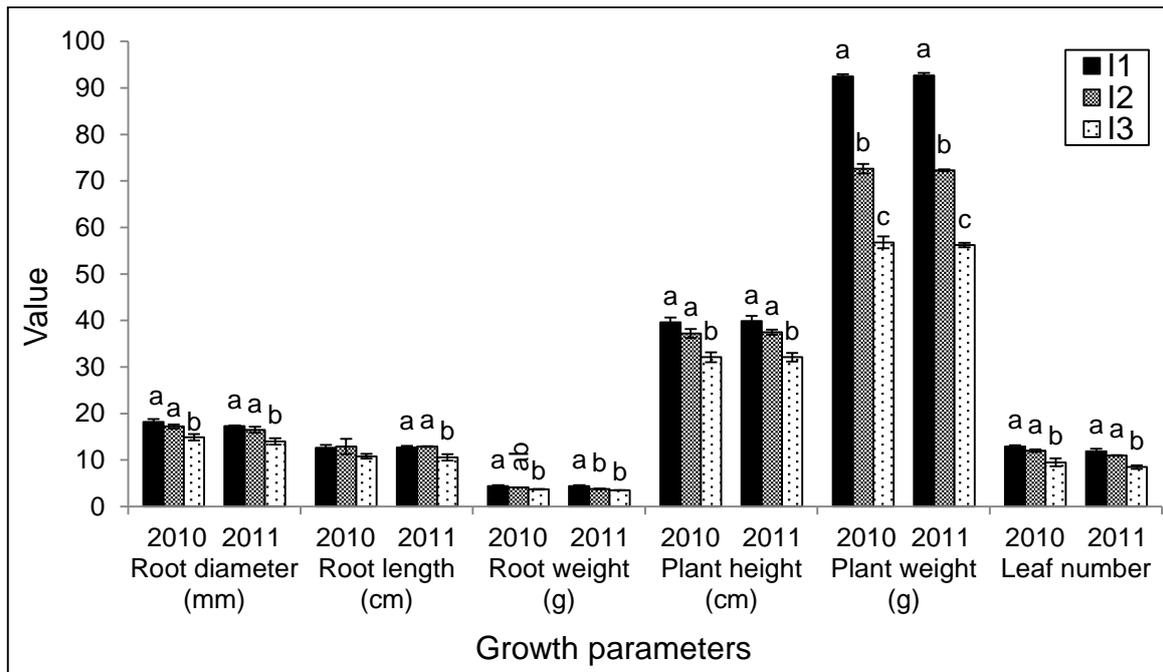


Figure 1. Monthly and seasonally irrigation quantities applied to spinach under different irrigation levels (I1, I2 and I3) in trial years

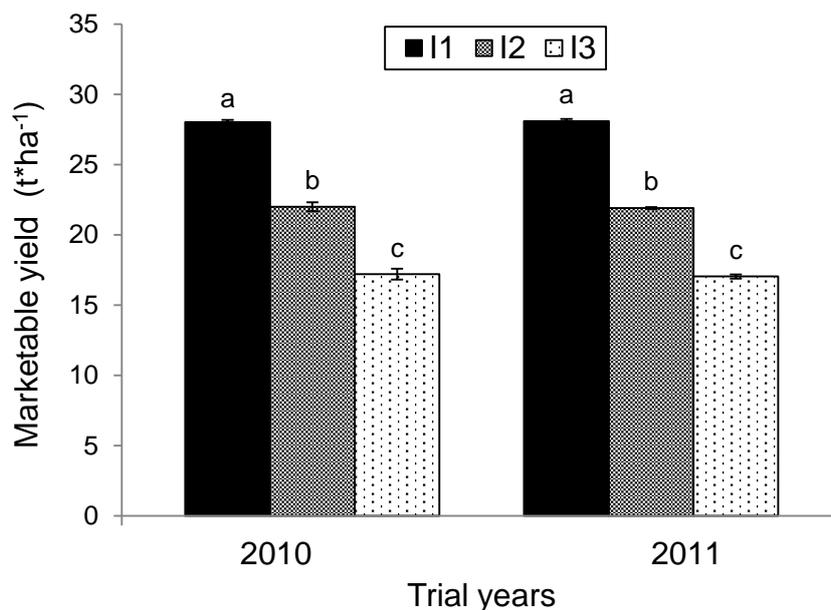
### Growth, marketable yield and irrigation water use efficiency (IWUE)

During harvest morphologic parameters of spinach were measured: weight and number of leaves, plant height, diameter, length and weight of root. Spinach responded to irrigation quantity deficit with less growth. Therefore, the highest growth values were obtained under more irrigated conditions (Figure 2). While spinach root and plant growth properties under the I1 and I2 treatment were statistically similar in trial years, the I3 treatment provided the lowest root and plant growth. However, plant weight in the I1 treatment was statistically higher than the treatments of I2 and I3. Decreasing the irrigation quantities reduced the plant weight at an important level (Figure 2). Therefore, marketable yield of the spinach was the highest in the I1 treatment ( $28.03 \text{ t} \cdot \text{ha}^{-1}$  in 2010 and  $28.10 \text{ t} \cdot \text{ha}^{-1}$  in 2011) (Figure 3). As shown in Figure 3, the marketable yield values in 2010 and 2011 were similar. Considering the two years average values, the marketable yield of the I2 and I3 treatments were 21.8 and 39.0% lower than the yield value of the I1 treatment, respectively. The results of this study indicate that spinach should be irrigated with higher water amounts for obtaining higher yield. The change between the marketable yields with applied seasonal irrigation water amounts was significantly linear (Figure 4). Many researchers also found that spinach yield was reduced significantly with the decreased irrigation quantities (Imtiyaz et al., 2000; Leskovar and Piccinni, 2005; Leskovar et al., 2005, 2008).



Means marked with the same lowercase in each trial year for each parameter do not differ significantly ( $P < 0.05$ ).

Figure 2. Growth parameter values of spinach under different irrigation levels (I1, I2 and I3) in trial years



Means marked with the same lowercase in each trial year do not differ significantly ( $P < 0.01$ ).

Figure 3. Marketable yield of spinach under different irrigation levels (I1, I2 and I3) in trial years

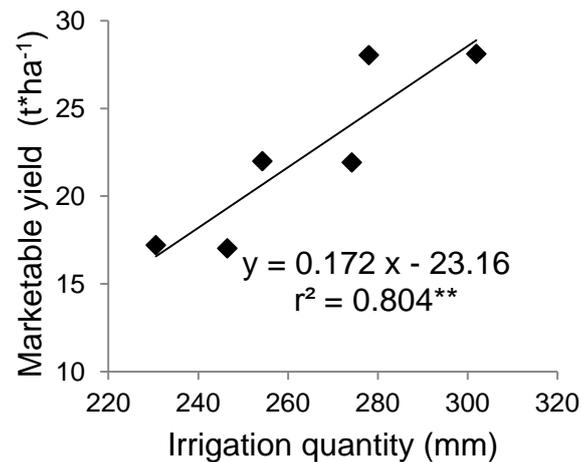
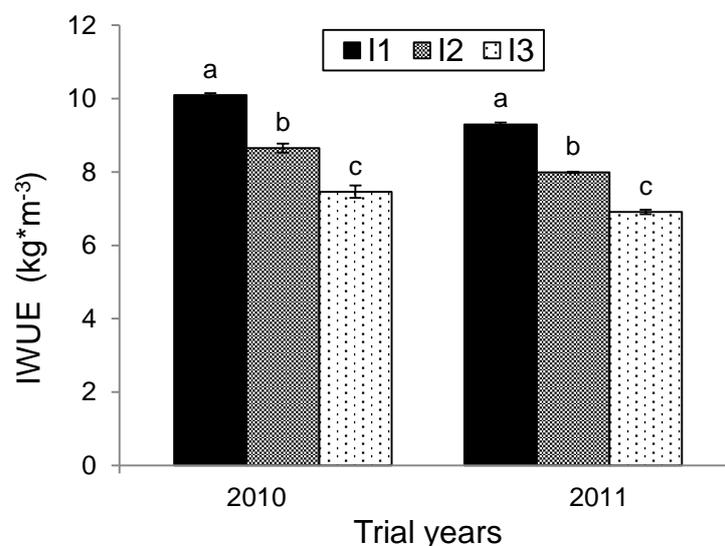


Figure 4. The relationship between the seasonal irrigation quantity and marketable yield of spinach

Irrigation water use efficiency (IWUE) of spinach was reduced significantly with the decreasing irrigation quantities in both years (Figure 5). The IWUE values in I1 treatment were 10.1 kg\*m<sup>-3</sup> in 2010 and 9.3 kg\*m<sup>-3</sup> in 2011. Considering the two years average values, the IWUE values in I2 and I3 treatments were 14.2 and 25.9% lower than the value of I1 treatment. IWUE was decreased linearly with the decrease of marketable yield (Figure 6). The determination coefficient of the linear relationship equation between marketable yield and IWUE was significantly high ( $r^2 = 0.905$ ). However, the determination coefficient of the linear relationship equation between the irrigation quantity and IWUE was low ( $r^2 = 0.517$ ) (Figure 6). Therefore, it could be



Means marked with the same lowercase in each trial year do not differ significantly ( $P < 0.01$ ).

Figure 5. Irrigation water use efficiency (IWUE) of spinach under different irrigation levels (I1, I2 and I3) in trial years

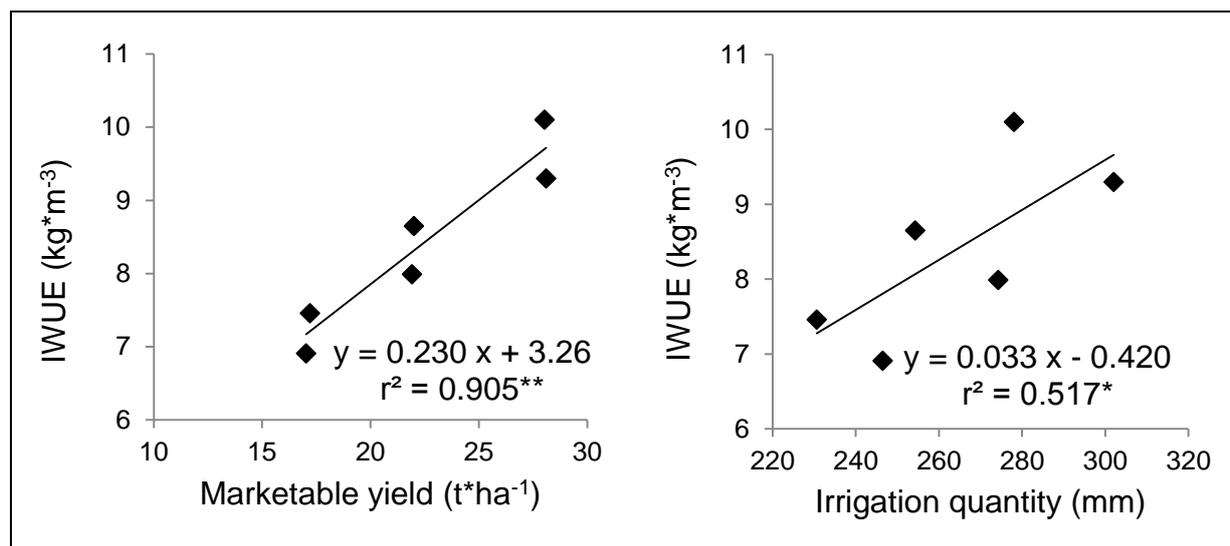


Figure 6. The relationships between the irrigation water use efficiency (IWUE) with spinach marketable yield and seasonal irrigation quantity

concluded that IWUE was more affected by the decrease in the yield compared to the decrease in the irrigation quantity. Similar to results of this study, Imtiyaz et al. (2000) determined that lesser marketable yields of spinach under lower seasonal water application caused lesser irrigation production efficiency. Also, Leskovar and Piccinni (2005) observed that decreasing water amounts decreased the water use efficiency in some commercial spinach cultivars due to the increased yield loss.

### Minerals, total phenolic contents and antioxidant activity

The content of minerals (macrominerals: N, P, K, Ca, Mg, S, and Na, and microminerals: Fe, Cu, Mn, Zn, and B) in the leaves of spinach, expressed on a dry matter basis, is shown in Table 2. Potassium was the most abundant macromineral in the leaves. Iron content was determined as the highest when considering the microminerals.

The results of this study indicated that mineral content was significantly improved under lower water application conditions. While the highest N, K, Mg, Na, Fe and Cu contents were determined in the plants under the I2 treatment in 2010 and 2011, the I3 treatment also provided the highest P, Ca, S, Zn and B contents. Considering the two years average values, N, P, K, Ca, Mg, S, Na, Fe, Cu, Zn and B contents were significantly higher by 5.4%, 17.9%, 2.9%, 6.6%, 7.0%, 5.9%, 3.6%, 19.5%, 8.5%, 22.5% and 19.8% compared to the I1 treatment, respectively. Many study results pointed out that the mineral content in some vegetables fruits (squash, cucumber, radish) increased under less irrigation conditions (Kuslu et al., 2014; Sahin et al., 2015b,c). High mineral content under lower water application conditions determined in this study are valuable for human nutrition because minerals play important roles for the occurrence of certain physicochemical processes required for a healthy life and for controlling the disease states of humans (Soetan et al., 2010). High mineral content under lower soil moisture conditions in this study may be due to the change of soil solution chemistry. Increasing solution chemistry due to the decreased water

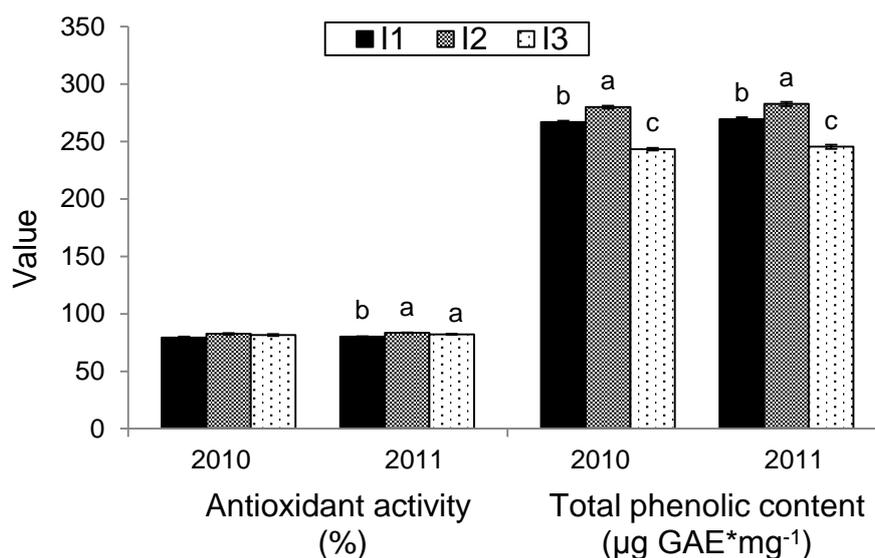
content in soil may affect the nutrient uptake by plants. Similarly, Misra and Tyler (1999) indicated that the solution chemistry of soil and nutrient uptake by plants are affected at important level with soil water content. However, the effect of the changes in soil moisture on the plant nutrient uptake may be more complex. Also, changes in soil moisture content influence the physiology and morphology of the plant roots (Ehlken and Kirchner, 2002).

Table 2. Mineral content (mean± the standard error of the mean) in the leaves of spinach under different irrigation levels (I1, I2 and I3) in trial years

Years	Parameters	I1	I2	I3	P value
2010	Macrominerals (%)				
	N	2.57±0.049 b	2.72±0.012 a	2.56±0.015 b	0.017
	P	0.28±0.002 b	0.29±0.002 b	0.33±0.002 a	0.000
	K	3.77±0.016 b	3.88±0.040 a	3.71±0.026 b	0.016
	Ca	1.52±0.004 b	1.54±0.006 b	1.62±0.010 a	0.000
	Mg	0.57±0.002 b	0.61±0.002 a	0.60±0.005 a	0.000
	S	0.17±0.002 b	0.17±0.002 b	0.18±0.001 a	0.002
	Na	0.28±0.001 b	0.29±0.002 a	0.26±0.001 c	0.000
	Microminerals (mg*kg <sup>-1</sup> DM)				
	Fe	342.0±5.29 b	409.0±7.0 a	388.0±8.89 a	0.002
	Cu	38.76±0.61 b	42.10±0.88 a	36.75±0.19 b	0.003
	Mn	75.03±1.27 a	70.11±0.31 ab	65.49±1.31 b	0.002
	Zn	43.15±1.82 b	50.23±0.66 a	52.88±0.56 a	0.003
	B	20.11±0.56 b	23.07±0.39 ab	24.09±0.83 a	0.010
2011	Macrominerals (%)				
	N	2.59±0.038 b	2.72±0.018 a	2.57±0.018 b	0.011
	P	0.28±0.002 b	0.29±0.002 b	0.33±0.003 a	0.000
	K	3.77±0.017 b	3.88±0.035 a	3.72±0.021 b	0.011
	Ca	1.53±0.004 b	1.55±0.006 b	1.63±0.009 a	0.000
	Mg	0.57±0.003 b	0.61±0.002 a	0.60±0.004 a	0.000
	S	0.17±0.002 b	0.17±0.002 b	0.18±0.001 a	0.001
	Na	0.28±0.001 b	0.29±0.002 a	0.27±0.002 c	0.000
	Microminerals (mg*kg <sup>-1</sup> DM)				
	Fe	342.7±4.91 b	409.3±6.49 a	389.0±7.77 a	0.001
	Cu	38.85±0.56 b	42.14±0.82 a	36.84±0.21 b	0.002
	Mn	75.20±1.18 a	70.18±0.45 ab	65.64±1.14 b	0.001
	Zn	43.25±1.78 b	50.28±0.76 a	53.00±0.44 a	0.002
	B	20.15±0.54 b	23.09±0.44 ab	24.14±0.76 a	0.008

Means marked with the same lowercase in each row of each experimental year do not differ significantly ( $P < 0.01$  or  $P < 0.05$ ).

The values of total phenolic content and antioxidant activity of spinach leaves are given in Figure 7. In both years, antioxidant activity was the highest in the I2 treatment (82.57% in 2010 and 83.38% in 2011). The values of the I2 treatment were statistically similar with the antioxidant activity values of the I3 treatment. Also, I2 treatment had the highest total phenolic contents (279.9  $\mu\text{g GAE}\cdot\text{mg}^{-1}$  in 2010 and 282.7  $\mu\text{g GAE}\cdot\text{mg}^{-1}$  in 2011) and it was followed by I1 treatment (266.9  $\mu\text{g GAE}\cdot\text{mg}^{-1}$  in 2010 and 269.6  $\mu\text{g GAE}\cdot\text{mg}^{-1}$  in 2011). Considering the two years average values, I2 treatment provided higher antioxidant activity of 4.1% and total phenolic content of 4.9% compared to the I1 treatment. The results of this study were similar



Means marked with the same lowercase in each trial year do not differ significantly ( $P < 0.01$ ).

Figure 7. Antioxidant activity and total phenolic content in the leaves of spinach under different irrigation levels (I1, I2 and I3) in trial years

to the results of studies conducted by other researchers on different vegetables. Oh et al. (2010) indicated that mild water stress before harvest increased total phenolic concentration and antioxidant capacity in lettuce. Different lettuce cultivars and varieties grown under water-deficit conditions revealed higher contents of phenolic compounds (Eichholz et al., 2014). Antioxidant activity in cucumber fruits was higher in less irrigation conditions compared to higher irrigation conditions (Sahin et al., 2015b). Kuslu et al. (2014) found that the antioxidant activity in squash fruits were higher under lower irrigation conditions. High antioxidant activity and total phenolic content was obtained also for less irrigated radish (Sahin et al., 2015c). Phytochemicals (antioxidants) in fruits and vegetables are important for decreasing or preventing the risk of most chronic diseases (Chu et al., 2002; Song et al., 2010). Therefore, it could be said that relatively low water application may be preferred for human nutrition.

## Conclusions

Irrigation with water amount equal to 100% of the evaporation from a Class A pan resulted in the highest marketable yield and IWUE. However, mineral and total phenolic content and antioxidant activity of the spinach leaves were the highest under lower water application conditions. As a conclusion, it could be said that full irrigation (the irrigation equal to 100% of the Class A pan evaporation) may be preferred for obtaining a high yield and IWUE values in the water sufficient regions. However, the nutrients and phenolic compounds is vital to support human life and health. Therefore, lower irrigation (the irrigation equal to 85% of the Class A pan evaporation) may be more appropriate for obtaining a spinach yield with high quality in especially water scarce semi-arid regions with a high altitude.

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