Erdoğan Bayram S (2022)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 50, Issue 4, Article number 12894
DOI:10.15835/nbha50412894
Research Article

Effects of prolonged water stress on biomass yield and nutrient uptake by aerial parts of mint (*Mentha* × *piperita* L.)

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Abstract

Mint (*Mentha* × *piperita* L.) is a medicinal and aromatic plant known for its high-water requirement. However, water stress is an important factor limiting vegetative development of this plant. Studying the growth, productivity, and nutrient uptake of this plant under various water stress levels is necessary to optimize its fertilization programs. In this study, the effects of different water stress levels and cutting times on the fresh biomass yields and the amounts of nutrient uptake of peppermint by different aerial parts were examined in two consecutive years. The field experiment was arranged as five irrigation treatments (125, 100, 75, 50 and 25% of full irrigation), during the season of maximum water demand (June-September), applied by drip irrigation. Results showed that at all cutting times in both years, the amounts of nutrient elements taken up by the different parts generally increased in parallel with increasing water stress, while fresh biomass yields decreased. However, this increase observed in nutrient element uptake was an increase relative to the dramatic decrease in the number of plants in severe stress conditions. The effect of long-term water stress on the nutrients taken up by different parts of the plant was mostly observed in Ca, Mg, N, Fe and Cu. As a result, in mint growing under Mediterranean conditions, despite the non-significant differences, in terms of the amounts of nutrient elements taken up by aerial parts, T75 treatment which provided 25% water saving compared to T100 could be recommended for one cut and one year cultivation in practice. Severe stress levels are not appropriate in mint growing was concluded.

Keywords: aerial part; fresh herba; harvest; irrigation; macro-micro elements; mint

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Introduction

Medicinal and aromatic plants have received much attention in several fields such as the agro-alimentary, perfume and pharmaceutical industries and in natural cosmetic products (Baatour *et al*., 2010). The Lamiaceae family is considered one of the most highly valued in terms of cosmetic and medicinal value. Peppermint (*Mentha* × *piperita* L.) is a member of the Lamiaceae family, and is the commercially most important mint species (Kumar *et al*., 2012). It is a perennial shrub (50-90 cm high) with branched stems which are often purplish or tinged violet, dark or light green, and short-petioled leaves, and the flowers are purple or pinkish. It is frequently used as a medicinal and aromatic plant and is also extensively used in the pharmaceutical and food industries (Singh *et al*., 2015). Peppermint thrives well to a wide range of soil and climatic conditions, although

Received: 31 Aug 2022. Received in revised form: 06 Dec 2022. Accepted: 08 Dec 2022. Published online: 12 Dec 2022.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

it adapts best to a fairly cool, preferably moist climate, and with deep well drained soils rich in organic matter (Pushpangadan and Tewari, 2006).

Water stress is considered as one of the most limiting factors in plant growth and yield in many areas of the world with agricultural production (Kostrzewska, 2017). Water stress results in negative water potentials in the root zone, and thus a reduction of water availability, which affects the plant growth and development (Chai *et al.*, 2016). Also, the effects of water stress are highly dependent on the stage of plant growth. Water stress during the vegetative growth reduces growth parameters and yield (Alva, 2008). Water stress also plays a crucial role, as the soil water availability is directly concerned with nutrient element uptake by plants (Stagnari *et al.*, 2016). Abiotic environmental stresses, particularly salinity and drought, have the greatest effect on medicinal plants (Heydari *et al.,* 2008).

In peppermint, water stress had a negative effect on most of the growth parameters. As irrigation level increased from 100-45% FC, fresh and dry shoot weights, root dry weight, internode length, shoot to root ratio and biomass reduced (Khorasaninejad *et al.*, 2011). Similarly, Keshavarz Mirzamohammadi *et al.* (2021) reported that deficit irrigation led to a remarkable reduction in LAI, dry matter weight and essential oil content in the same species. Moreover, water stress (70% FC) reduced the plant fresh weight and water content, but it did not affect the dry weight in peppermint (García-Caparrós *et al.*, 2019). On the other hand, water stress in Japanese mint reduced the plant height, fresh and dry weight as compared with unstressed plants (Misra and Srivastava, 2000).

The effect of water deficit on mineral content and nutritional balance in mint (*Menth*a x *piperita*), grown under Mediterranean climate conditions is still relatively poorly documented. Water stress impacts plant nutritional status, inducing increases or decreases of ion concentrations in plant tissues (Corell *et al.,* 2012). Fe, Mn and Zn concentrations in Japanese mint (*Mentha arvensis*) shoots generally increased as the level of water stress increased. However, no relationship between water stress level and Cu uptake was observed (Misra and Srivastava, 2000). In terms of macro elements, leaf N and K concentrations did not change in *M. piperita* under water stress, but the P concentration decreased (García-Caparrós *et al.*, 2019). On the other hand, Hassan *et al.* (2013) reported that, in two different cuts in rosemary (*Rosmarinus officinalis* L.), the percentages of N, P and K decreased as the irrigation level decreased from 100 to 60% FC. Corell *et al.* (2012) reported that the mean N content among three different cuts of sage (*Salvia officinalis* L.) significantly increased as the level of water deficit increased, while the values of K, Mg, and P decreased in the 1st cut (September). In the second cut (April), the higher mean values of N and K and the lower mean values of Mg and P were measured under different water stress levels. Nevertheless, the effect of environmental abiotic stress factors on the mineral concentration of plants still needs to be investigated (Stagnari *et al.*, 2016).

A better understanding of the effects of water stress on plant nutrition is important for developing strategies to minimize the damage caused by water stress and its possible effects on nutrient deficiencies. There are few studies related with the effect of long-term water stress on the amounts of nutrient elements taken up by the mint plant. Therefore, the aim of this study was to determine the effects of long-term water stress on the yield and the amounts of nutrient elements taken up by the stem and leaves of the mint plant, and thereby to serve as a guide to levels of fertilization and irrigation for growing mint in the ecological conditions in which the study was conducted.

Materials and Methods

Experimental site and growth conditions

This study was conducted in the research area of the Department of Horticulture of the Faculty of Agriculture of Ege University, İzmir, Turkey (38°27'25.09"N; 27°13'20.59"E; 31 m a.s.l.) in the 2018 and 2019 growth seasons. The research area has typical Mediterranean climatic conditions, in which most of the precipitation falls during the winter months. There was no rain during the growing period in the summers of either year. The monthly total rainfall and mean temperature of the experiment area in 2018 and 2019 are given in Figure 1.

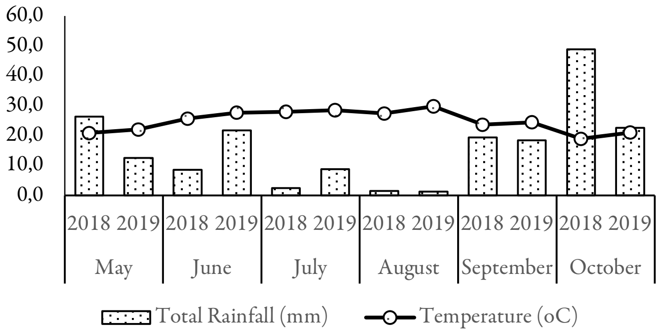

Figure 1. Monthly total rainfall and mean temperature (TSMS 2020)

Soil analysis

Soil samples were taken each year in October at a depth of 0–30 cm. Air-dried samples were passed through a 2 mm sieve and prepared for analysis. Soil sand, clay and silt contents were determined by the hydrometric method; pH and total water-soluble salt were determined in a paste of soil saturated with water with a pH meter with a glass electrode and a conductometer. Lime contents were determined with a Scheibler calcimeter, and the amounts of organic matter were determined by wet digestion with $K_2Cr_2O_7$ and H_2SO_4. With regard to plant nutrient elements in the soil, total nitrogen was determined by the modified Kjeldahl method, and available phosphorus was determined colorimetrically after extraction with sodium bicarbonate. Available K^+, Ca^{++}, Na^+ and Mg^{++} contents were determined by ICP-OES after extraction with 1 N NH_4OAc. Fe, Mn, Zn and Cu were also determined by ICP-OES after extraction with 0.05 M DTPA+TEA. Some physical and chemical properties of the soil are shown in Table 1.

Table 1. Soil physical and chemical properties of the research area

Years	pH	EC µS.cm^{-1}	%						mg kg^{-1}								
			Organic matter	Lime	Sand	Clay	Silt	Total N	P	K	Mg	Ca	Na	Fe	Cu	Zn	Mn
2018	7.6	643	1.5	2.3	64	17	19	0.05	14	349	304	4067	19	10	8	5	34
					Sandy Loam												
2019	7.6	643	1.5	2.3	64	17	19	0.12	7	402	414	4059	29	5.6	6.5	10	8

Plant material and agronomic details

The plant material used was peppermint (*Mentha* x *piperita* L.), which is a natural hybrid of spearmint (*M. spicata* L.) and water mint (*M. aquatica* L.). This plant distributes widely in temperate and sub-temperate climatic regions of the world. The selected clonal material was provided by the Aegean Agricultural Research Institute in İzmir, Turkey. Shoot cuttings were used as propagation material in the study. Cuttings were collected in November 2017 and divided into parts 3-5 cm in length. These were planted at intervals of 20 cm, with 40 cm between rows, at a depth of 5 cm, with about 60 cuttings in each plot. Then, the plots were irrigated carefully with refreshing water. According to the results of soil analysis, 7 kg N, 5 kg P_2O_5 and 7 kg K_2O daa^{-1} were applied to each plot. Half of the nitrogen and all of the phosphorous were given in April, and the rest of the nitrogen was applied after the 1st cut in July. Weed management was done mechanically by hand.

Peppermint has abundant hairy roots, and the main roots have a rhizome structure. Most of the roots are found at a depth of 20-30 cm in the soil. Therefore, the effective root depth of 30 cm was considered as the depth to be irrigated.

Experimental design, irrigation treatments

The field experiment was designed in a randomized block design with three replications of five irrigation levels and conducted on a total of 15 plots. Each experimental plot size was 4.8 m^2 (1.6 x 3 m), and the distance between the plots was 2 m. Five irrigation treatments were applied. Three of these were deficit (T25, T50, T75), one was excess water (T125) and one was full irrigation (T100). In T100, the amount of water decreased has been completed to the field capacity every five days. T25, T50, T75 and T125 were given 25%, 50%, 75% and 125% of the water given to T100 respectively. Irrigation was performed by surface drip irrigation. The amount of irrigation water to be applied in order to replenish the soil water deficit to field capacity was calculated by Eq. 1.

$$I = \frac{FC - AW}{100} * As * D * A \quad \text{(Eq.1)}$$

In the equation, I is the amount of irrigation water (L); FC is the soil water content on the basis of weight at field capacity (%); AW is the soil moisture before irrigation on the basis of weight (%); As is the soil bulk density (g cm^{-3}); D is the efficient root depth (mm), and A is the plot area (m^2). The volume of irrigation water applied to each experimental plot was measured by flow meters installed in the nozzle of the hose used for irrigation.

Total fresh biomass yield

The plants were harvested by hand with a saw knife, 5 cm above the surface, and immediately weighed to obtain plot yields. The plot yield was then converted to kg ha^{-1}.

Plant nutrient analysis

Plant nutrient analysis was carried out each year after two cuts. Plant samples were collected at the initial stage of blooming in each cut after the elimination of side effects. Approximately one hundred completely matured fourth or fifth terminal leaves with stems were collected from each plot. The plant samples were dried in a drying oven at 65±5 °C until a stable weight was reached. Then they were separated into stems and leaves and the amount of dry material was determined and prepared for analysis by grinding. The plant samples were reduced to ash at a temperature of 550 °C and dissolved in 3N HCl, and the amounts of K, Ca, Mg, Fe, Cu, Zn and Mn in the extracts obtained were determined by ICP-OES (Vandecasteele *et al*., 2018). P was determined by spectrophotometer by the vanadomolybdophosporic yellow colour method, and the total amount of N of each plant part was determined by the modified Kjeldahl method. The amounts of all nutrient elements taken up by the leaves and stems were expressed on the basis of fresh weight (FW) not basis of dry weight (Mohammad *et al.*, 1998; Gupta *et al*., 2005; Zhang *et al.,* 2018). Because, in general, the total amount of plant nutrients required to grow a plant depends on the total weight of it, as described by grams of nutrient per fresh weight of plant (g kg^{-1}) (Zhang *et al.*, 2018).

Data analysis

SPSS 25.0 software was used for all statistical analysis. Differences between factors were determined by univariate analysis and differences between groups were determined by Duncan's multiple range test. According to irrigation treatments, differences between the mean amounts of taken up by different parts in each cut and the amounts taken up by the same parts belonging to the cuts of the same year were determined by the independent t test. All statistical analyses were evaluated at the 5% probability level.

Results and Discussion

Total fresh biomass yield

The biomass yield increased in parallel with the increasing irrigation treatments in all cuts of both years. The highest total biomass yield among the cuts was measured in the 1st cut of 2018 in the T100 control group, while the lowest value was observed in the 2nd cut of 2019 in the T100 control group. The highest total biomass yields were obtained in the 1st cuts of the T100 control groups in both years. Total biomass yields were higher in the 1st cuts than in the 2nd cuts in both experimental years (Table 2). This is attributable to the deficit irrigation treatments which give rise to decreases in harvestable material (Fereres and Soriano, 2007; Shormin *et al.*, 2009; Meskelu *et al.*, 2014; Abdi *et al.*, 2019) in plots. At the same time, the effects of the cut periods and cut x treatment interaction on the total biomass yield were found to be significant. In other words, total biomass yield decreased from one cut to the next. Said-Ahl *et al.* (2019) reported that cutting time can cause a reduction in the biomass yield of aromatic plants. Only in the 2nd cut of the two consecutive years did additional water application (T125) significantly increase the total biomass yield compared to the control group (T100). These results showed that biomass yields were sensitive to irrigation levels and cut periods. In fact, Mitchell and Yang (1998) reported that peppermint yield was sensitive to irrigation with both deficit and excess irrigation compared with the optimum treatment.

Table 2. Total biomass yield with different cuts and water stress levels $kg\ ha^{-1}$

Irrigation treatment	1st Year		2nd Year	
	1st Cut	2nd Cut	1st Cut	2nd Cut
T25	15465 b	1861 c	729 b	493 b
T50	20053 b	6375 b	2042 b	984 b
T75	24778 a	11882 a	5094 b	1184 b
T100	26438 a	13153 a	14281 a	410 b
T125	24709 a	13905 a	13011 a	2537 a
Treatment mean	22288	9435	7031	1121
Cut mean	15862 A		4076 B	
Cut	*		*	
Treatment	*		*	
Cut x treatment	*		*	

In the same column, different lowercase letters indicate statistically significant differences between treatments. In the same line, different capital letters indicate statistically significant differences between different years' cuts ($p \leq 0.05$). (T25: 25%; T50: 50%; T75: 75%; T125: 125% of T100. T100: full irrigation)

Uptake of macro elements

The results of mean amounts of macro nutrients taken up by different plant parts subjected to different water stress levels for each cut in the two consecutive years are given in Figures 2, 3, 4, 5 and 6 as $kg\ 100\ kg\ FW^{-1}$.

Nitrogen (N)

The highest amounts of N taken up by both parts were in the 2nd cut of the 1st year, while the lowest amounts were taken up in the 1st cut of the 2nd year. The highest values were found in the leaves in T25 (0.18 $kg\ 100\ kg\ FW^{-1}$) and in the stems in T50 (0.32 $kg\ 100\ kg\ FW^{-1}$), while the lowest amounts found were 0.03 $kg\ 100\ kg\ FW^{-1}$ in the leaves and 0.01 $kg\ 100\ kg\ FW^{-1}$ in the stems in T75, T100 and T125. Comparing the 1st year cuts, the differences between T25 treatments in the amounts taken up by the leaves and the differences between T25 and T50 in the amounts taken up by stems were found to be significant. When the differences of cuts in the 2nd year were compared, only the amounts taken up by the stems showed a significant difference in T125 treatments. Significant differences were found in the N uptake of the various parts in T25, T50 and T75 in the 1st cut and in T25 and T50 in the 2nd cut in the 1st year. In the 1st cut of the 2nd year in all treatments apart from T25, and in the 2nd cut in T75 and T100 only, the differences in the amount of N taken up by the different parts were found to be significant at the 5% level (Figure 2). In general, it was found that the highest amounts of N were taken up by the various parts in treatments where the water stress was highest (Figure 2).

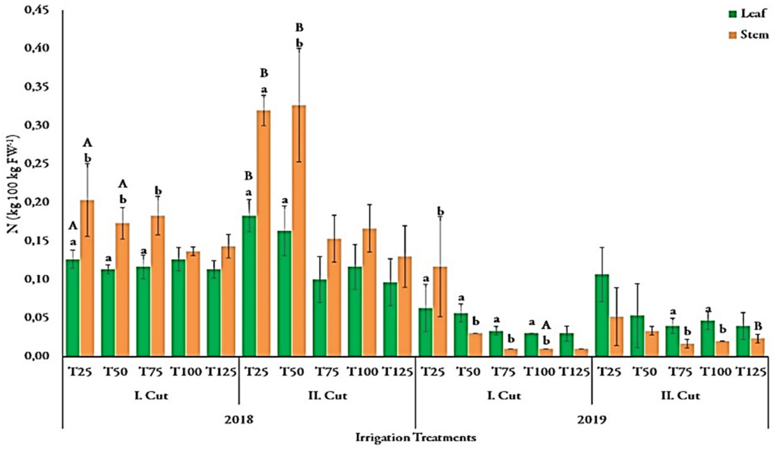

Figure 2. N uptake of leaves and stems under different cut periods and water stress levels
Different lowercase letters indicate statistically significant differences between the mean amounts of nutrients taken-up by different plant parts in the same treatment ($p \leq 0.05$). Different capital letters indicate statistical significance between the mean amounts of nutrients taken-up by the same plant parts in the same treatments in different cuts of the same year ($p \leq 0.05$). (T25: 25%; T50: 50%; T75: 75%; T125: 125% of T100 respectively, T100: full irrigation)

It was thought that the increase in the nitrogen concentration of the aerial parts of the plants might be proportionately higher in relation to a reduction in the number of plants under severe stress conditions. Similarly, Corell *et al.* (2012), in a study over three cut periods in which five different stress levels (0, 25, 50, 75 and 100% of ET0) were applied, reported that increasing stress levels increased the nitrogen content of sage (*Salvia officinalis*) plants, and that the difference in mean nitrogen contents between cut periods was statistically significant. Hassan *et al.* (2013), reported that the nitrogen content of the leaves of *Rosmarinus officinalis* plants varied by 2.18-2.47 kg 100 kg FW^{-1} in relation to an increase in water stress treatments (100-80-60% FC), and that it was reduced in cuts carried out in the same season. On the contrary, in studies conducted in other ecologies, it has been reported that the leaf nitrogen contents of different plant species of the Lamiaceae family fell or did not change significantly in relation to stress conditions (Khalid, 2006; Garcia-Caparrós *et al.*, 2019).

Phosphorus (P)

When all cuts were considered, the highest amounts (0.15 kg 100 kg FW^{-1}) of P in the leaves and stems were observed in T50 in the 1st cut of the 2nd year. The least amounts were found to be 0.06 kg 100 kg FW^{-1} in the leaves in T50 in the 1st cut of the 1st year and in the stems in T125 in the 1st cut and T75 in the 2nd cut of the 2nd year. When the cuts of both years were compared, only the amounts of P taken up by stems, statistically significant differences at 5% level were seen at T25 treatments. Also, no significant difference between stress levels was found in any cut regarding the amounts of P taken up by the various parts (Figure 3).

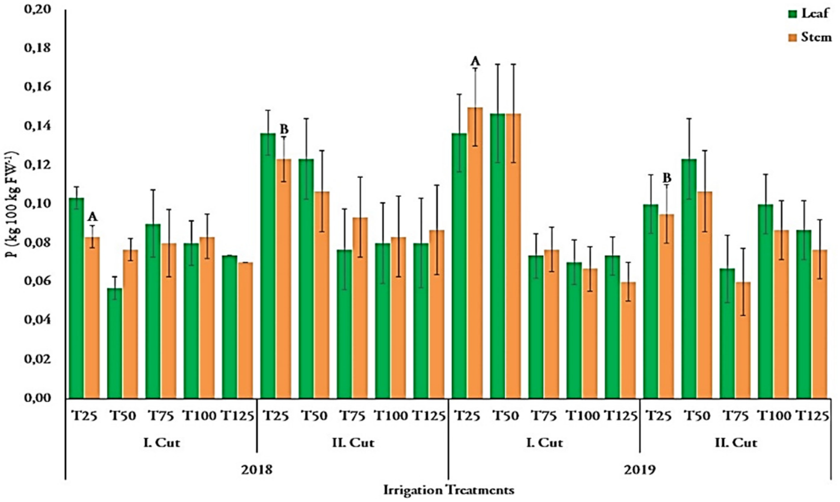

Figure 3. P uptake of leaves and stems under different cut periods and water stress levels
Different lowercase letters indicate statistically significant differences between the mean amounts of nutrients taken-up by different plant parts in the same treatment ($p \leq 0.05$). Different capital letters indicate statistical significance between the mean amounts of nutrients taken-up by the same plant parts in the same treatments in different cuts of the same year ($p \leq 0.05$). (T25: 25%; T50: 50%; T75: 75%; T125: 125% of T100 respectively, T100: full irrigation)

Hassan *et al.* (2013) reported that the P contents of the leaves of *Rosmarinus officinalis*, a medicinal-aromatic plant, were reduced in all cuts by increasing water stress from 100% FC to 60% FC, and that the P contents of the leaves increased from 0.24 kg 100 kg FW^{-1} to 0.33 kg 100 kg FW^{-1}. The values formerly reported are similar to our findings concerning the amounts taken up by aerial parts. Similar to the results obtained from treatment T75 (25% deficit irrigation) in our study, Garcia-Caparos *et al.* (2019) reported that there was no statistical difference between the P content of the plant *Salvia sclarea* under 30% deficit irrigation conditions and the control treatment.

Potassium (K)

The highest amount of K taken up by the leaves in all cuts was 0.79 kg 100 kg FW^{-1} in T25 in the 1st cut of the 1st year. As for the stems, it was 0.99 kg 100 kg FW^{-1} in T50 in the 2nd cut of the 1st year. The lowest amount was found to be 0.26 kg 100 kg FW^{-1} in the 2nd cut of the 2nd year in T75 for leaves, and 0.39 kg 100 kg FW^{-1} in the 1st cut of the 2nd year in T125 for stems. Comparing the 1st year cuts, the amounts of K taken up by the leaves showed significant differences between T50, T100 and T125, but the amounts taken up by the stems did not show significant differences between the treatments. Comparing 2nd year cuts, no difference was found between treatments in the amounts of K taken up by the leaves, but significant differences were found between T25, T100 and T125 in terms of the amounts taken up by the stems. No significant difference was seen between treatments in terms of the amounts of K taken up by the different parts in the 1st cut of the 1st year, and in the 2nd cut, only the differences between treatments T25, T50 and T75 were found to be statistically significant. In terms of the amounts of K taken up by leaves and stems, the differences between treatments T25, T75 and T100 in both cuts of the 2nd year, and in the 2nd cut, T125 were also found to be significant additionally (Figure 4). In general, the highest amounts of K taken up by both parts were found in severe water stress treatments.

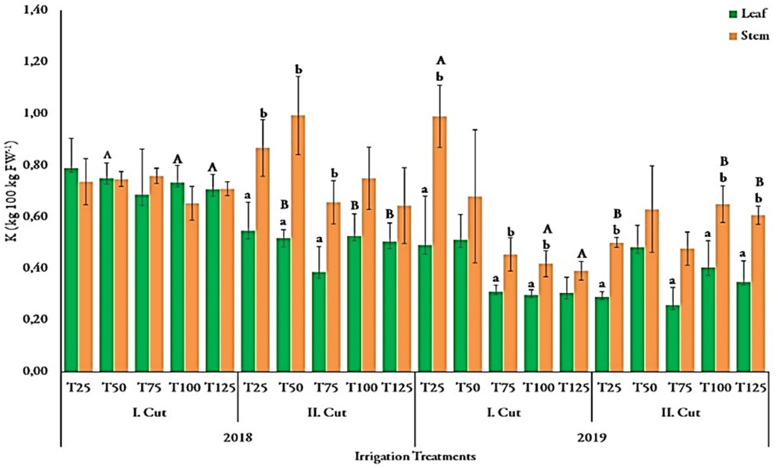

Figure 4. K uptake of leaves and stems under different cut periods and water stress levels
Different lowercase letters indicate statistically significant differences between the mean amounts of nutrients taken-up by different plant parts in the same treatment ($p \leq 0.05$). Different capital letters indicate statistical significance between the mean amounts of nutrients taken-up by the same plant parts in the same treatments in different cuts of the same year ($p \leq 0.05$). (T25: 25%; T50: 50%; T75: 75%; T125: 125% of T100 respectively, T100: full irrigation)

In fact, increasing water stress increases the absorption of K and its movement to aerial parts, and it was reported that this plays an important role in plants in regulating osmotic pressure and the opening and closing of stomata (Ashraf *et al*., 2002; Alizadeh and Nadian, 2010). Sarhadi *et al.* (2016) reported in a water stress study in which 50% and 75% of field capacity was applied to henna (*Lawsonia inermise* L.) plants, that the highest leaf K content, 1.47%, was found in treatment T50. They also reported that water stress had a significant effect on the amounts of K in the leaves. Our findings showed that K% amounts obtained in the aerial parts in T25 (leaves) and T50 (stems), which totaled 1.78 kg 100 kg FW^{-1}, are similar to the value reported above. Similarly, in studies conducted in different ecologies, it was reported that water stress increases the K contents of the aerial parts of such plants as *Ocimum gratissimum* (Osuagwu *et al*., 2010) and *Medicago sativa* (Akhondi *et al*., 2006). Moreover, leaf K contents increased in all cuts of *Rosmarinus officinalis* (from 2.23% to 2.37%) (Hassan *et al*., 2013) and *Ocimum basilicum* and *O. americanum* plants in parallel with increasing water stress levels (Khalid, 2006). In addition, no significant difference was found between water stress levels in terms of leaf potassium contents in six different species of the Lamiaceae family (Garcia-Caparros *et al*., 2019) or in *Thymus vulgaris* (Sarajuoghi *et al*., 2014) plants.

Calcium (Ca)

The highest amounts of Ca uptake in both parts in all cuts were found in T25. This was 0.64 kg 100 kg FW^{-1} in the leaves in the 2nd cut of the 1st year and 0.36 kg 100 kg FW^{-1} in the stems in the 1st cut of the 2nd year. The lowest amounts were found in the 2nd cut of the 2nd year as 0.18 kg 100 kg FW^{-1} in leaves in T75 and T125, and as 0.09 kg 100 kg FW^{-1} in stems in T100 in the 1st cut of the 2nd year (Figure 5). When the different cuts of two consecutive years were compared, significant differences were found in Ca uptake in the leaves only in T25, and in the stems in T25 and T50 in 2018. No statistically significant difference was observed between the treatments in terms of Ca uptake by the leaves, and a significant difference was found in the amounts of Ca taken up by the stems only in T25 in 2019. Significant differences were determined between all treatments in terms of the amounts of Ca taken up by stems and leaves in the 1st cut of the 1st year, and in the 2nd cut between treatments T25, T100, and T125. In the 1st cut of 2019, significant differences were found between T50, T75, T100 and T125 in terms of the amounts of Ca taken up by the different parts, and in the 2nd cut between T25, T50 and T125 (Figure 5).

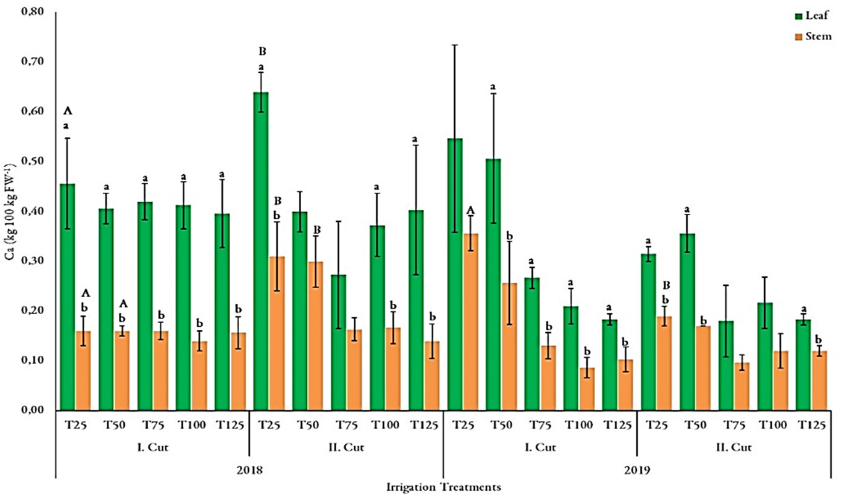

Figure 5. Ca uptake of leaves and stems under different cut periods and water stress levels
Different lowercase letters indicate statistically significant differences between the mean amounts of nutrients taken-up by different plant parts in the same treatment ($p \leq 0.05$). Different capital letters indicate statistical significance between the mean amounts of nutrients taken-up by the same plant parts in the same treatments in different cuts of the same year ($p \leq 0.05$). (T25: 25%; T50: 50%; T75: 75%; T125: 125% of T100 respectively, T100: full irrigation)

Our results showed that the highest amounts of Ca taken up by leaves and stems in both cuts of both years were in T25 and T50. The data on the effect of water stress on plant Ca content is particularly uncertain, with a general increase being reported in some studies (De Carvalho, 2005), and a reduction being reported in others (Kaya *et al*., 2006; Yu *et al*., 2007). In a study researching the physiological and morphological responses of *Thymus vulgaris* plants to five different irrigation levels, it was reported that the Ca content of the aerial parts varied between 0.8% and 1.25%, with the greatest amount of Ca found in the 80% FC treatment. At the same time, it was reported that leaf Ca contents did not show a statistically significant difference at different stress levels (Sarajuoghi *et al*., 2014). It was reported that at three different water stress levels applied to henna plants (T50, 75 and 100), the highest Ca in leaves was found to be 0.84% in T50, and that water stress significantly affected amounts of Ca in leaves (Sarhadi *et al*., 2016). The values reported above in accordance with the highest total amount (1%) taken up by the aerial parts (leaves and stems) in our research. Sarhadi *et al*. (2016) emphasized that plants exposed to water stress showed drought resistance by increasing the accumulation of Ca as well as K ions. Similarly, it has been reported that water stress increased the percentage of Ca in the aerial parts of various medicinal aromatic plants (Sardans and Peñuelas, 2008; Afsharmanes *et al.,* 2009; Hussein *et al*., 2013).

Magnesium (Mg)

The highest amounts of Mg taken up in the leaves in the course of the two growing years were determined to be 0.16 kg 100 kg FW^{-1} in T75 in the 1st cut of the 1st year, and in the 2nd cut in T25 and T50. In the stems, it was 0.26 kg 100 kg FW^{-1} in the 1st cut of the 2nd year in T50. The lowest amounts taken up by the leaves were found to be 0.06 kg 100 kg FW^{-1} in the 1st cut of the 2nd year in T100 and T125, and in the 2nd cut of the same year in T75. The lowest amounts of Mg taken up by the stems were found to be 0.04 kg 100 kg FW^{-1} in the 1st cut of the 1st year in T100. No statistically significant difference between the cuts of the 1st year was detected in the treatments in terms of the amounts of Mg taken up by both parts. When the 2nd year cuts were compared, Mg uptake by leaves showed non-significant differences between the treatments. Statistically significant differences were found between treatments T25, T50 and T125 in terms of the amounts taken up by the stems. Statistically significant differences were found between all irrigation treatments regarding the amounts of Mg taken up by the different parts in the 1st cut of the 1st year, and in the 2nd cut between all treatments except for T75. Statistically significant interactions were found regarding the amounts of Mg taken up by the different parts in the 1st cut of the 2nd year only in T50, and in the 2nd cut between T25 and T125 (Figure 6).

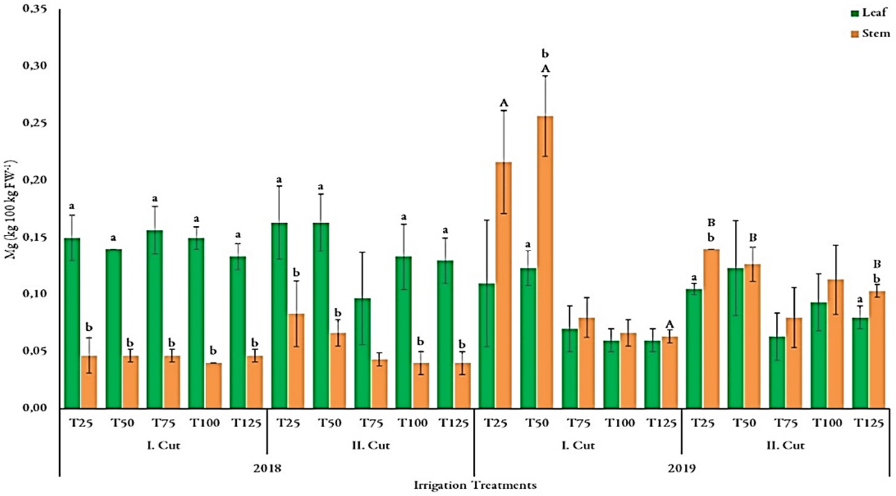

Figure 6. Mg uptake of leaves and stems under different cut periods and water stress levels
Different lowercase letters indicate statistically significant differences between the mean amounts of nutrients taken-up by different plant parts in the same treatment ($p \leq 0.05$). Different capital letters indicate statistical significance between the mean amounts of nutrients taken-up by the same plant parts in the same treatments in different cuts of the same year ($p \leq 0.05$). (T25: 25%; T50: 50%; T75: 75%; T125: 125% of T100 respectively, T100: full irrigation)

It was found that in general, the greatest amounts of Mg taken up by both parts were at the level of T50. As with calcium, information on the effect of water stress on the magnesium content of plants is unclear and complex. The findings of some studies indicate that water stress increases the amount of Mg (De Carvalho, 2005), while some claim that it causes a reduction (Kaya *et al.*, 2006; Yu *et al.,* 2007). Sarajuoghi *et al.* (2014) reported that water stress significantly affected Mg content in *Thymus vulgaris* plants, and that the greatest Mg contents obtained were 0.74% in the 80% FC treatment. Our findings relating to the highest total amounts of magnesium taken up by the aerial parts (0.42%) are that they are somewhat below the amounts reported. It is thought that this difference may arise from differences in the characteristics of the plant species and ecological conditions.

Uptake of micro elements

The results of mean values of micro nutrients taken up by different plant parts subjected to different water stress levels for each cut in two consecutive years are given in Figures 7, 8, 9 and 10 as g 100 kg FW^{-1}.

Iron (Fe)

It was determined that the highest amount of Fe taken up by the leaves at all cuts was 4.95 g 100 kg FW^{-1} in the 2nd cut of the 1st year, and for the stems it was 6.49 g 100 kg FW^{-1} in the 2nd cut of the 2nd year in T25. The lowest amount taken up by the leaves was 1.93 g 100 kg FW^{-1} in the 2nd cut of the 1st year in T75. In the stems it was found to be 1.27 g 100 kg FW^{-1} in the T100 in the 1st cut of 2nd year. As compared the 1st year's cuts, significant differences were found in Fe amounts taken up by both parts between T25 and T50, and in the 2nd year's cuts, Fe amounts taken up only by stems, significant differences were predicted between T25 and T100. In relation with the amounts of Fe taken up by different parts, in the 1st cut of 2018 in all irrigation treatments, as in the 2nd cut only T100; in the 1st cut of 2019 in T100 and T125, as in the 2nd cut significant differences were predicted between T25 and T100 treatments respectively (Figure 7).

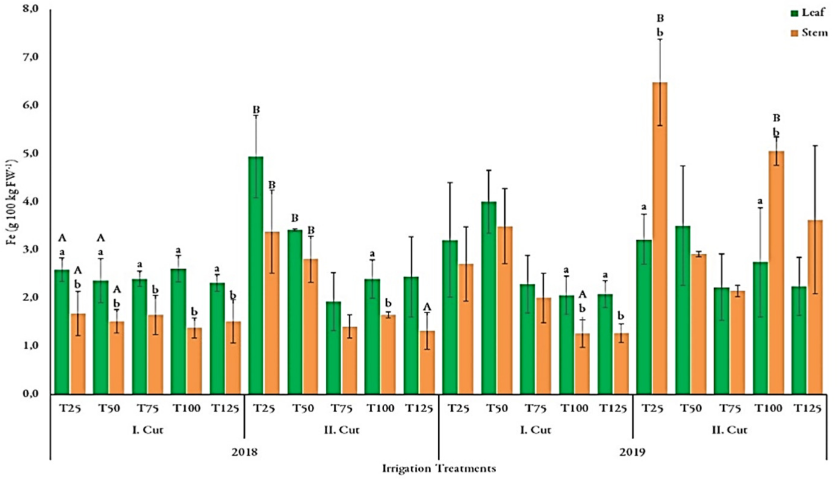

Figure 7. Fe uptake of leaves and stems under different cut periods and water stress levels
Different lowercase letters indicate statistically significant differences between the mean amounts of nutrients taken-up by different plant parts in the same treatment ($p \leq 0.05$). Different capital letters indicate statistical significance between the mean amounts of nutrients taken-up by the same plant parts in the same treatments in different cuts of the same year ($p \leq 0.05$). (T25: 25%; T50: 50%; T75: 75%; T125: 125% of T100 respectively, T100: full irrigation)

Copper (Cu)

The highest amounts of Cu taken up over the two vegetation years were found to be 0.89 g 100 kg FW^{-1} in the 1stcut of the 1st year in the leaves in T75, and 0.82 g 100 kg FW^{-1} in the stems in T50 in the 1st cut of the 2nd year. The lowest amounts were determined to be 0.23 g 100 kg FW^{-1} in T100 in the 1st cut of the 2nd year in the leaves, and 0.28 g 100 kg FW^{-1} in the 2nd cut of the 1st year in the stems. Comparing the cuts of the 1st year, only the amounts of Cu taken up by the leaves showed a difference between T25 treatments. In the cuts of the second year, no significant differences were found between the amounts of Cu taken up by the leaves. The amounts taken up by stems showed a significant difference only between T100 treatments at the 5% level. In both cuts of the 1st year, significant differences were found between all irrigation treatments with regard to amounts of Cu taken up by the different parts. Regarding to the amounts of Cu taken up by the different parts in the 1st cut of the 2nd year, the differences between treatments T50, T75, T100 and T125 and in the 2nd cut the differences between T25 and T50 were found to be significant (Figure 8).

Zinc (Zn)

The highest amount of Zn taken up by both parts of the plants at all cuts was found in the 1st cut of the 2nd year in T25, as 1.96 g 100 kg FW^{-1} in the leaves and 2.45 g 100 kg FW^{-1} in the stems. As for the lowest amounts, this was in the 1st cut of the 2nd year in T100 (0.84 g 100 kg FW^{-1}) in the leaves, and, in the 1st cut of the 1st year in T100 and in the 2nd cut in T125 (0.37 g 100 kg FW^{-1}) treatments in the stems. Comparing the 1st year's cuts, statistically significant differences were shown in the amount of Zn taken up by the leaves only in T25 and T100, and in the 2nd year's cuts, only in T25. The amounts of Zn taken up by the stems in T25 and T125 were significant in the 2nd year. In both cuts in 2018, significant differences were found between all irrigation treatments in terms of the amounts of zinc taken up by the different parts. Significant differences were found in amounts of zinc taken up by leaves and stems only in T25 and T100 in the 1st cut and in the 2nd cut only in T125 in 2019 (Figure 9).

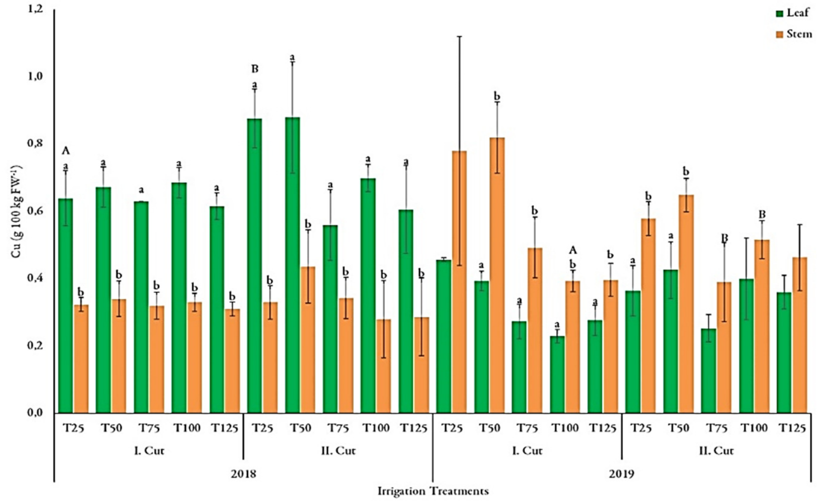

Figure 8. Cu uptake of leaves and stems under different cut periods and water stress levels
Different lowercase letters indicate statistically significant differences between the mean amounts of nutrients taken-up by different plant parts in the same treatment ($p \leq 0.05$). Different capital letters indicate statistical significance between the mean amounts of nutrients taken-up by the same plant parts in the same treatments in different cuts of the same year ($p \leq 0.05$). (T25: 25%; T50: 50%; T75: 75%; T125: 125% of T100 respectively, T100: full irrigation).

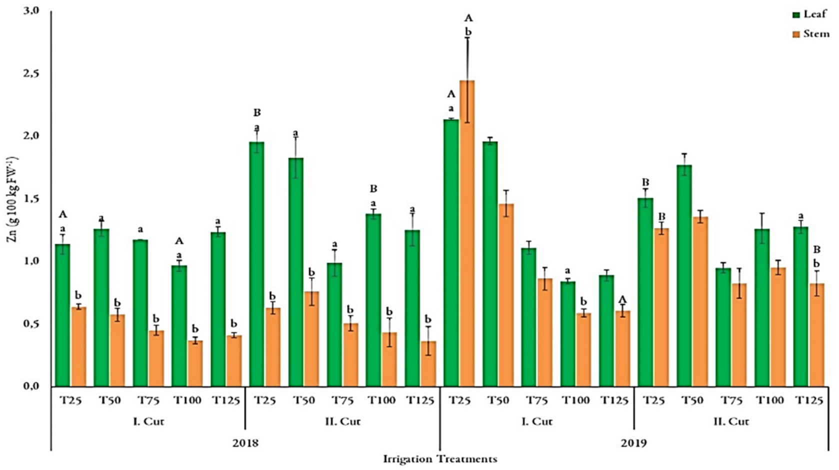

Figure 9. Zn uptake of leaves and stems under different cut periods and water stress levels
Different lowercase letters indicate statistically significant differences between the mean amounts of nutrients taken-up by different plant parts in the same treatment ($p \leq 0.05$). Different capital letters indicate statistical significance between the mean amounts of nutrients taken-up by the same plant parts in the same treatments in different cuts of the same year ($p \leq 0.05$). (T25: 25%; T50: 50%; T75: 75%; T125: 125% of T100 respectively, T100: full irrigation).

Manganese (Mn)

The highest amount of Mn taken up in all cuts was found to be 2.29 g 100 kg FW^{-1} in the 1st cut of the 1st year in T25 in the leaves, while 1.92 g 100 kg FW^{-1} in T50 for stems, in the 1st cut of the 2nd year. The lowest amounts were found to be 0.87 g 100 kg FW^{-1} in the leaves and 0.84 g 100 kg FW^{-1} for stems in T75 in the 2nd cut of the 2nd year. A statistically significant difference at the 5% level was found between the cuts of the 1st year only in T75 regarding the amounts of Mn taken up by the stems, and between the cuts of the 2nd year only in T25 regarding the amounts of Mn taken up by the leaves. Regarding the amounts of Mn taken up by the different parts, statistically significant differences were found at the 5% level between all irrigation treatments in the 1st cut of the 1st year. In the 1st cut of the 2nd year, the difference was found to be significant in T25 only (Figure 10).

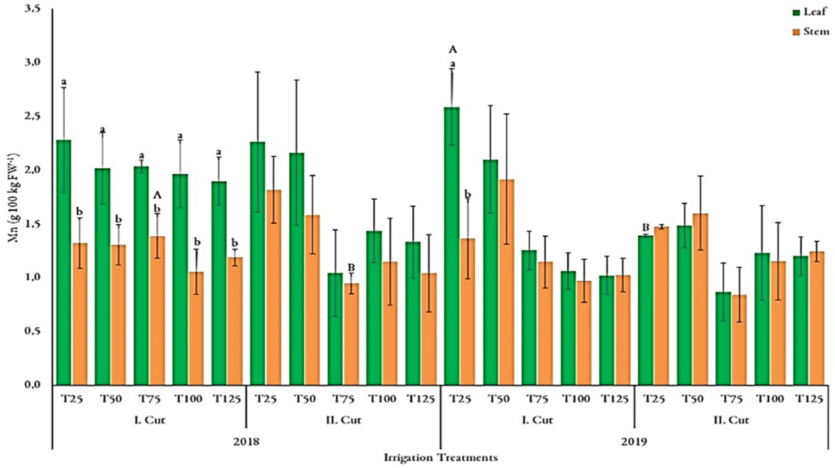

Figure 10. Mn uptake of leaves and stems under different cut periods and water stress levels
Different lowercase letters indicate statistically significant differences between the mean amounts of nutrients taken-up by different plant parts in the same treatment ($p \leq 0.05$). Different capital letters indicate statistical significance between the mean amounts of nutrients taken-up by the same plant parts in the same treatments in different cuts of the same year ($p \leq 0.05$). (T25: 25%; T50: 50%; T75: 75%; T125: 125% of T100 respectively, T100: full irrigation).

Low concentrations of Fe, Zn and Mn observed in plants exposed to water stress lead to a reduction in chlorophyll content and leaf area in mint, and significantly affect the accumulation of oil. This has been reported by many researchers (Croteau *et al.*, 1981; Srivastava and Luthra, 1991). If an assessment is made by considering all micro elements, it can be said that the highest amounts of micro elements taken up by leaves and stems, as with macro elements, are in treatments T25 and T50 at severe water stress levels. Similarly, Misra and Srivastava (2000) reported that the amount of Fe, Zn and Mn in Japanese mint leaves increased due to water stress. Sarajuoghı *et al.* (2014) observed the highest Fe content of *T. vulgaris* under stress applied at 80% of the field capacity. However, Pirzad *et al.* (2012) reported that water stress levels of 95%, 80%, 65% and 50% did not have a statistically significant effect on the amounts of Fe and Zn taken up by *Matricaria chamomilla* L. plants, but that severe stress conditions reduced the amounts of Fe and Zn taken up. On the contrary, it has been reported that in *Hypnum cupressiforme* (hypnum moss) (Sardans and Peñuelas, 2008) and maize (İbrahim *et al.*, 2020), water stress significantly supressed Fe take-up, and thus reduced the plants' Fe contents.

Conclusions

In this study, the average yield obtained in the 1st year was higher than in the 2nd year, and also the yields from the 1st cuts were found to be higher than the 2nd cuts in both years. In general, it was determined that the greatest amounts of all macro and micro elements taken up by both parts of the plant were in treatments of severe and medium stress (T25 and T50), and the lowest amounts were in treatments of slight or no stress (T75, T100 and T125). Due to prolonged exposure of the plants to severe water stress, the number of plants (biomass yield) in the T25 and T50 plots was reduced. Depending on this decrease, the amount of nutrients taken up by both parts seems to have increased relatively in these plots. However, these stress levels cannot be recommended in mint production in similar ecologies as the biomass yield is significantly reduced. In treatment T75, the amounts of nutrients taken up by the aerial parts were found to be higher than the control (T100), in terms of yield, T75 and T100 were in the same group statistically, in other words, there was no statistical difference between them. Therefore, under Mediterranean semi-arid conditions, the T75 irrigation level can be recommended for one-year cultivation and single cut in mint. In this way, a 25% water saving will be achieved in the amount of irrigation water used. At the recommended deficit irrigation level (T75), the leaves took up 0.12, 0.09, 0.69, 0.42 and 0.16 kg 100 kg FW^{-1} and the stems took up 0.18, 0.08, 0.76, 0.16 and 0.05 kg 100 kg FW^{-1} of N, P, K, Ca and Mg respectively. Fe, Cu, Zn and Mn were taken up at 3.88, 0.89, 1.17 and 2.04 g 100 kg FW^{-1} respectively by the leaves, and at 1.65, 0.32, 0.45 and 1.39 g 100 kg FW^{-1} by the stems.

Authors' Contributions

The author read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This work is part of the project grant number 2018-ZRF-024, financially supported by the Ege University Scientific Research Project Commission, Turkey.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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