

Growth and Quality of Sage (*Salvia officinalis*), Parsley (*Petroselinum crispum*) and Nasturtium (*Tropaeolum majus*) as Affected by Water Deficit

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ABSTRACT

A pot experiment was conducted to assess the growth and quality traits of sage (*Salvia officinalis*), parsley (*Petroselinum crispum*) and nasturtium (*Tropaeolum majus*) to water deficit. Plants were grown in pots, and the treatments were: 100 % of field capacity (well-watered), continuous moderate water deficit (80% of field capacity), only two weeks before harvest moderate water deficit was adjusted (80% of field capacity). The results showed that continuous moderate water deficit (80 % of field capacity) decreased plant height, plant fresh weight but increased dry matter % of the three plants under study. Essential oil percentage was increased under water deficit conditions in sage and parsley and the highest mean values of essential oil (%) were achieved under water deficit of 80 % of field capacity. Water deficit had relatively little effect on the essential oil composition of sage and parsley. The greater the water deficit the higher values of the flavone content of parsley and the glucotropaeolin content of nasturtium. Water deficit at 80 % of field capacity for two weeks produced considerable amounts of flavone and terpenes in parsley, terpenes in sage and glucotropaeolin in nasturtium. At the same time it did not have a negative remarkable effect on growth traits in the three plants.

Key words: Sage, Parsley, Nasturtium, Water deficit, Essential oil, Flavones, polyphenols, glucotropaeolin.

Introduction

Water deficiency is one of the most important environmental stresses influencing agricultural productivity around the world and particularly in Egypt (Mediterranean region) where the climate described as hot, dry summers, and mild, wet winter (Mooney, 1987). In this way, plants in Egypt are frequently exposed to water deficit especially in summer, i.e. a combination of high temperatures, high light. Since water works as a reagent in most metabolic processes, such as gas exchange, carbohydrate metabolism, amino acids and other organic compounds (Krieg, 1993). The stresses may lead to a considerable yield reductions, damage in the cell membranes that are at specific danger, prompting a conceivable loss of cell structure and even plant death (Halliwell and Gutteridge, 1989; Fuchs *et al.*, 1997).

Producing medicinal and aromatic plants with the most elevated yield and quality by optimization of irrigation is crucial because water is a noteworthy a major component of the fresh plant material, and essentially influences both weight and quality (Jones and Tardieu, 1998).

In spite of the fact that water deficit is generally considered as a negative factor in agriculture, being responsible for severe yield losses, medicinal plants developed under semi-arid conditions generally deliver higher concentrations of active substances than same species grown under moderate climates (Kleinwächter and Selmar, 2015).

Sage is a valuable medicinal and aromatic plant and its essential oil mainly used in medications and hygienic products industries (Bruneton, 2001). Determination of *Salvia officinalis* growth under water stresses or water deficit has been studied by few researchers and could be a well guidance for cultivation of resistant plants in dry regions (Hosseini and Abadie, 2004). The author revealed that *Salvia officinalis* had good growth in all water stresses treatments and conserved their freshness under severe water stress. He reported that this species could establish as a resistant medicinal plant in dry region or water deficit. Overall content as well as the concentration of total monoterpenes of sage plants cultivated under moderate water deficit were markedly higher than in the corresponding, well-watered control plants (Selmar and Kleinwachter, 2013). In contrast, sage production is limited by availability of water when studying the growth, productivity, and nutrient absorption of *Salvia officinalis* L. under five irrigation regimes (100, 75, 50, 25, and 0 % of ET₀) (Corell *et al.*, 2012). Results showed that reduced irrigation (from 100% to 0% of ET₀) caused a decrease of the dry weight of the plant, essential oil production, and an increment of the nitrogen (N) content together with a decrease of the levels of

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phosphorus (P), potassium (K), and magnesium (Mg). Also, water deficit reduced stem length and root length of *Salvia splendens* (Stephanie *et al.*, 2005; Asadi *et al.*, 2012).

Parsley (*Petroselinum crispum*) is a common herb native to the Mediterranean region, and widely used as a herb or spice in Europe, America, and Middle Eastern countries. It is growing for consumption at fresh or dried forms and as a source of essential oils. Fresh, dried and dehydrated leaves used as a flavoring, garnishing ingredient and condiment. Parsley had a high concentration of vitamin C, antioxidants. Moreover, parsley contains mineral elements such as iron (Kmieciak, 1999). Parsley leaf oil is used primarily in flavoring meat, sauces, canned food, seasonings, etc., a lesser extent in perfumery (Shaath *et al.*, 1986) and in the pharmaceutical industries (Sellar, 2001; Wander and Bouwmesster, 1998).

The effect of water stress on parsley has been studied by many researchers were they mentioned that the effect of water stress depends on the stress conditions (timing and intensity) of regions, the parsley variety and growth stage (Petropoulos *et al.*, 2006). When water stress occur at vegetative growth (before flowering stage) that lead to decrease plant height, number of leaves, leaf area, dry matter production (Sharifi *et al.*, 2005; Abbaszadeh *et al.*, 2008; Taheri *et al.*, 2008; Aliabadi *et al.*, 2009). Meanwhile, water stress during the reproduction stage may cause reduction the period between a seed formation and fullness (Mohamed *et al.*, 2002). Generally, water stress causes reduction on plant fresh and dry weight (Gamalei, 2002; Sarker *et al.*, 2005).

To increase the production and the quality of the parsley, which is mainly sensitive to water stress, providing a permanent source of water especially in summer should be insured. The influence of water stress on the production of parsley essential oil were also studied. Plant growth (foliage and root weight, leaf number) was significantly reduced by water stress, even at 30–45% deficit but water stress increased the yield of essential oil (on a fresh weight basis) from the leaves curly-leaved parsley (Petropoulos *et al.*, 2008). However, on a m² basis foliage oil yield increased significantly. Moreover, water stress improved quality but decreased yield parameters of curly leaved parsley (Najla *et al.*, 2012).

Nasturtium (*Tropaeolum majus*) which is a herb native to South America and it is employed as an herbal medicine against urinary tract diseases in Germany. Various pharmacological studies have confirmed that the leaves of nasturtium were the primary site of benzylglucosinolate synthesis (Lykkesfeldt and Moller, 1993) and it contains high amounts of the glucosinolate glucotropaeolin (Kleinwächter *et al.*, 1993) that possess wide pharmacological properties such as antibacterial, antifungal, antiseptic, aperient, depurative, expectorant, purgative, vulnerary, antineoplastic, demulcent, laxative and stimulant activities. Additionally, extracts and preparations have natriuretic and diuretic (Gasparotto *et al.*, 2012), hepatoprotective (Koriem *et al.*, 2010), anti-inflammatory activities (Butnariu and Bostan, 2011). Nasturtium is regarded as an invasive species in many countries and it is known as a medicinal crop.

Since the effect of water deficit on nasturtium growth has not been previously reported the objective of this research were to study the impact of water deficit on the productivity and the quality of sage, parsley and nasturtium, throughout some morphological and biochemical criteria.

Materials and Methods

To investigate the influence of water deficit on growth and the concentrations of secondary compounds of parsley (essential oil, flavones, terpenes), sage (essential oil, terpenes) and nasturtium (glucosinolates). Seeds of the three experimental plants were directly cultivated at the middle of October 2012 and 2013 seasons in pots.

Measuring the evapotranspiration and inducing the water deficit: 30 pots filled with 3 kg soil were used for each of the three plant species to determine the evapotranspiration. During the course of the experiment, the pots had to be weighed daily in the morning to adjust the following field capacity treatments:

1. 100 % field capacity (well-watered).
2. Continuous moderate water deficit (80% field capacity).
3. Moderate water deficit two weeks before harvest (80% field capacity).

Representing samples of fresh herbs of each replicate were subjected to hydro-distillation for 3 hours using Clevenger apparatus to extract and to determine essential oil percentage according to Egyptian Pharmacopoeia and recorded on the basis of oil volume to herb fresh weight (ml/100g fresh herb). The essential oil was separately dehydrated over anhydrous sodium sulphate and kept in silica vials and stored at 2°C till GLC analysis. Samples of each treatment were subsequently analyzed using GLC (Perkin Elmer Autosampler 2000)

The extracted oil has been diluted with n-hexane, injected into a GLC using an auto-sampler and the different compounds have been separated on a HP-INNOWAX (60 x 0.25 x 0.25 μm) capillary column. Helium was used as carrier gas (flow rate 1.5 ml/min). The temperature programme was; 35 °C to 230 °C (2.5 °C/min) in course of time (92 min). Injector and flame ionization detector temperature were 250 °C and 300 °C, respectively. Area percentages were obtained using a PC programme (Maestro chromatography data system). For identification of single compounds internal and external standard substances have been used.

HPLC analysis of glucotropaeolin was performed using a RP 18 column (250 x 4 mm) (Matallana *et al.*, 2006). Based on the peak areas of glucotropaeolin and the internal standard, the amounts of glucotropaeolin were calculated. Total flavone concentration was also determined (Kleinwächter *et al.*, 2015).

The collected data were subjected to the analysis of variance in Randomized Complete Block Design (RCBD) arrangement (Snedecor and Cochran, (1990) using MSTAT-C V.2.1 software package (Steel *et al.*, 1997). Differences among means were compared for each trait by Duncan multiple range test (DNMRT) (Duncan *et al.*, 1995).

Results and Discussion

Effect of water deficit on sage:

Results in Table 1 demonstrated the impact of water deficit on the growth of the sage plant. Continuous exposition of sage (*Salvia officinalis*) to moderate water deficit 80 % of field capacity (FC) significantly decreased the plant height, plant fresh weight but increased the essential oil content in the leaves compared to the well-watered plants (100 % FC).

Table 1: Effect of water deficit on the growth of sage

Water deficit Treatments	Plant height (cm)	Essential oil (%)	Fresh weight g plant ⁻¹	Dry weight g plant ⁻¹	Dry matter (%)
1 st season					
100% FC	29.00 a	0.36 c	29.80 a	8.69 a	29.18 b
80% FC	25.67 b	0.50 a	19.40 b	7.16 a	34.38 a
80% FC two weeks	28.50 a	0.45 b	23.05 b	7.96 a	36.380 a
2 nd season					
100% FC	20.33 a	0.73 a	24.23 a	7.24 a	25.23 b
80% FC	16.00 b	0.70 a	15.90 b	6.78 a	34.45 a
80% FC two weeks	17.00 b	0.65 b	20.62 ab	6.52 a	33.89 a

Different small letters on the same column indicate significant difference ($p \leq 0.05$).

Concerning the plant height, it was significantly higher by 11.5 and 21.3% in 100% of FC as compared to continuous 80 % of FC in the first and second seasons, respectively. With respect to plant fresh weight, it was significantly higher as a result of 100% of FC as compared to 80 % of FC by 35.0 and 34.0 % in first and second seasons, respectively. No significant differences in the average of plant dry weight were observed with water deficit treatments in both seasons. On the contrary, essential oil percent (%) and dry matter (%) were expanded (Table 1) with water deficit from 100% of FC to 80 % of FC in both seasons. The essential oil percent at 80 % of FC (0.50 %) was significantly higher by 39 % than 100% of FC in the first season, yet in the second season the essential oil percent at continuous 80 % of FC (0.70 %) was significantly higher by 8 % than 80% of FC for two weeks. There is no doubt that the water deficit has led to an increase in the dry matter % either in continuous water deficit or for just two weeks in both seasons which reflected on plant dry weight and make the differences insignificant.

Regarding terpene content in sage plant no significant difference was observed with water deficit treatments (Fig.1).

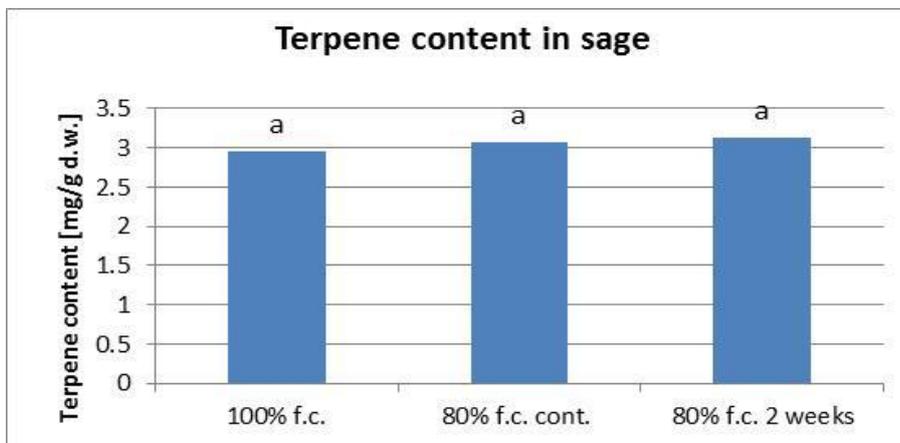


Fig. 1: Effect of water deficit on terpene content of sage.

Results of the present study revealed that water deficit decrease the morphological characteristics; meanwhile, increased essential oil % and dry matter %. These outcomes showed that plant has different mechanisms and strategies to adapt to the dry conditions. Water deficit causes a reduction in number and size of plant leaves to diminish the loss of water through evaporation. On the other hand, this reduction of number and size leaf means the reduction of photosynthesis process and consequently low yield. The impact of three water stress levels as 75% (severe), 50% (moderate) and 0% (control) in sage plant (*Salvia officinalis*) were studied. Reduction of biomass (4.96%) and height (14.15 cm) under severe water stress condition (12.63%, 19.78 cm) for moderate water stress, and (19.53%, 26.50 cm) for control, respectively, i.e. greater the water stress the slower the plant development (Bettaieb *et al.*, 2009).

Additionally, results of this experiment are in concurrence with those of Rizopoulous, and Diamantoglon, (1991); Munné Bosch, (2001); Singh *et al.*, (2000). The effect of water stress on *Dracocephalum moldavica* was studied. Water stress (40% of FC) reduced plant height, leaf width and length, internodes length and shoot yield. However, 40% of FC stress level increased essential oil % (Safikhani *et al.*, 2007).

The GLC analysis of sage essential oil in Table (5) showed that eleven hydrocarbon compounds were identified, representing about 97 % of the total oil.

The main components of the oil were α -thujone (28.45 %) followed by camphor (24.92 %), β -thujone (10.05 %) and 1,8-cineol (9.61 %). Viridiflorol, α -humulene, β -pinene, camphene, α -Pinene, β -caryophyllene and manool were present in smaller amounts and other components were less than 1%. Results of this study indicated that water deficit had relatively slight effect on the composition of the essential oil of the curly parsley. A little increase in α -thujone percentage was observed with water deficit coinciding with a small decline in the percentage of β -thujone.

These results are probably due to that the variation in the volatile oils pattern is more related to the genetic background of each individual (intrinsic factors) rather than to environment (extrinsic factors).

Table 2: Effect of water deficit on chemical composition of essential oil of sage

Compound	100% FC	80% FC for 2 weeks	80% FC cont.
α -Pinene	3.530	3.735	3.750
Camphene	4.650	4.770	5.080
β -Pinene	2.555	2.815	2.885
1,8-Cineol	9.610	9.465	10.060
α -Thujone	28.445	31.515	31.000
β -Thujone	10.045	8.865	7.870
Camphor	24.915	22.430	23.720
β -Caryophyllene	1.355	1.490	1.295
α -Humulene	2.985	3.025	2.865
Viridiflorol	3.770	3.845	3.740
Manool	0.220	1.460	0.835

Effect of water deficit on parsley

Results in Table 3 showed the effect of water deficit on the growth of parsley plant. Results observed that different water deficit conditions significantly influenced the development of parsley plants. Continuous water deficit (80 % of FC) presented lower values of plant height (20.33 and 22.00 cm, for first and second season, respectively) when compared to well-watered plants (100% of FC). Also, the same trend was observed with plant dry weight (1.19 and 2.01 g, for first and second season, respectively) and plant fresh weight (7.23 in 2nd seasons).

Table 3: Effect of water deficit on the growth of parsley

Treatments	Water deficit	Plant height (cm)	Essential oil (%)	Fresh weight g plant ⁻¹	Dry weight g plant ⁻¹	Dry matter (%)
1st season						
100% FC		24.66 a	0.153 c	8.77 a	1.47 b	20.25 b
80% FC		20.33 b	0.200 a	7.58 a	1.79 b	28.06 a
80% FC for two weeks		24.33 a	0.183 b	7.23 a	1.98 a	28.59 a
2nd season						
100% FC		26.44 a	0.083 c	8.90 ab	1.50 b	18.81 c
80% FC		22.00 b	0.107 b	7.23 b	2.01 ab	26.77 a
80% FC for two weeks		23.78 b	0.132 a	11.07 a	2.99 a	24.20 b

Different small letters on the same column indicate significant difference ($p \leq 0.05$).

On the contrary, water deficit at 80% of F.C. presented higher values of dry matter % (28.06 and 26.77 %, for first and second season, respectively) and essential oil % (0.20% in the 1st season) when compared to 100% of F.C. However, essential oil % had the maximum value (0.132%) at 80 % of FC. in the 2nd season.

The effect of water deficit on flavone contents of parsley is presented in Fig. 2, where water deficit at 80 % of FC presented higher values of flavone contents when compared to the lower value at 100% of FC.

Data in Fig 3 showed the effect of water deficit on total content of terpenes and phenylpropanoids of parsley. Water deficit at 80% FC for 2 weeks presented higher values of terpenes and phenylpropanoids contents when compared to the lower value at 100% FC. i.e. the greater the water deficit the higher values of flavone, terpenes and phenylpropanoids contents of parsley plant.

No significant difference in plant fresh weight was observed in the 1st season (Table 3). The lower values of biomass may be due to the low water levels that caused stomata closing, decreasing photosynthesis rate and consequently, plant biomass (Tardieu *et al.*, 1992). Meanwhile, the results of a study examined the effect of four water deficit levels (20, 40, 60 and 80%) on *Melissa officinalis* plant, revealed that the maximum essential oil yield was observed at 40 % water stress (Farahani *et al.*, 2009). Also, greater essential oil yields of *Pimpinella anisum*, were recorded when the plant was submitted to a water deficit below 20% (Zehtab-Salmasi *et al.*, 2001).

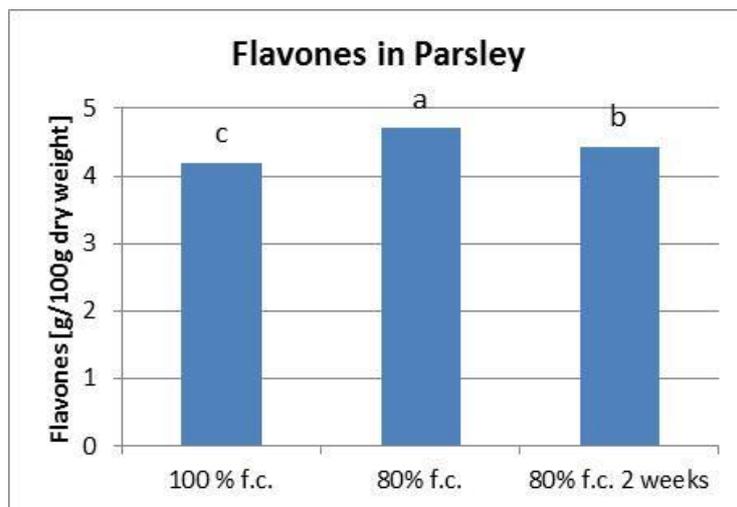


Fig 2: Effect of water deficit on flavone content of parsley

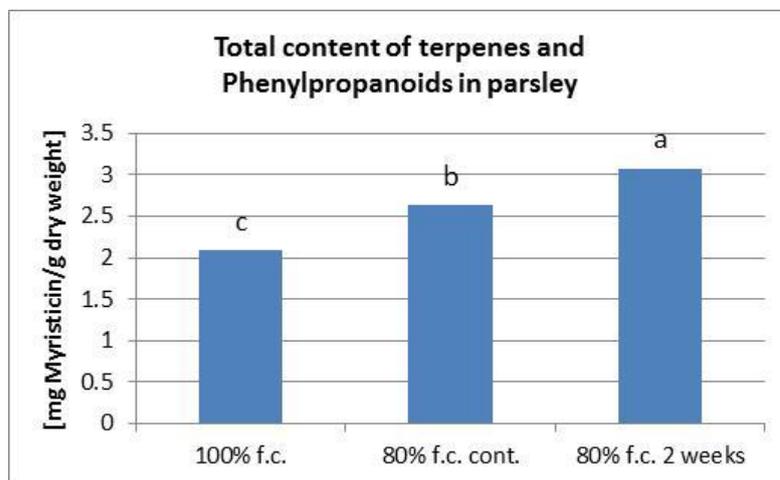


Fig 3: Effect of water deficit on total content of terpenes and phenylpropanoids of parsley

Effect of water deficit on the chemical composition of parsley essential oil

The GLC analysis of parsley essential oil in Table (4) showed that nine hydrocarbon compounds were identified, representing about 97-98% of the total oil. The major constituents of the oil were myristicin (52.84 %) followed by β -phellandrene (20.19 %), myrcene (4.44 %) and 1,3 Menthatriene (4.24 %). Terpinolene,

cymene, limonene, β -elemene and sesquiphellandrene were present in smaller amounts and other components were less than 1%.

Water deficit did not appear to affect the relative composition of the curly parsley essential oil except for a slight decrease in the myristicine content while the amount of the rest of components remained unchangeable.

Table 4: Effect of water deficit on the chemical composition of the essential oil of parsley

Compound	100% FC	80% FC for 2 weeks	80% FC cont.
Myrcene	4.440	5.525	6.425
Limonene	1.350	1.585	1.815
β -Phellandrene	20.195	20.58	22.95
Terpinolene	3.165	2.960	3.170
Cymene	2.740	2.785	2.650
1,3 Menthatriene	4.245	6.735	3.725
β -Elemene	3.385	2.875	3.240
Sesquiphellandrene	2.870	2.345	2.680
Myristicine	52.84	47.105	48.195

Studies on essential oil composition in response to water stress or water deficit have come up with different results. While water deficit treatments slightly affected essential oil composition in our study. In contrast, the concentrations of individual volatile compounds of parsley essential oil were clearly affected by the different irrigation doses used in another study which seem to indicate that the lowest irrigation dose ID1 (860 m³ha⁻¹) increased the concentrations of most of the key and main components of the essential oil of parsley shoots, (El-Zaiedia *et al.*, 2016). Also, Petropoulos *et al.*, (2008) concluded that water stress caused changes in the relative contribution of certain aroma constituents of the essential oils (principally 1,3,8-p-menthatriene, myristicin, terpinolene + p-cymenene). These results indicated that the plants to respond to water stress and change the chemical composition of the essential oil they need more greater water deficit (severe stress) (Borges *et al.*, 2016).

Effect of water deficit on nasturtium

Exposure of nasturtium plants to different levels of water deficit caused a progressive decrease in the morphological traits (Table 5). Plant height of nasturtium plants decreased when plants were subjected to 80 % of FC for two weeks compared to 100% of FC, but no significant differences were observed in plant height for the same respective treatments for plant in the 1st season.

Water deficit at continuous 80% of F.C. decreased leaves number plant⁻¹ (14 and 12 leaves plant⁻¹ compared to well-watered plants in the first and second seasons, respectively) meanwhile, 80% of F.C. for two weeks had no significant effect on leaves number in both seasons.

Concerning plant fresh weight, both 80% of FC and 80 % of FC for two weeks decreased plant fresh weight. Despite the water deficit at 80 % of FC that declined leaves number in both seasons, the difference in plant fresh weight failed to be significant in the second season

At the same time, water deficit had insignificant effect on dry weight plant⁻¹ in both seasons (Table 5).

On the contrary, dry matter % was higher when nasturtium plants subjected to water deficit at 80% of FC (17.42 and 16.58 in the first and second season , respectively), but there were insignificant effect of 100 % of FC and 80 % of FC for 2 weeks in both seasons. Also, glucotropaeolin content (Fig. 4) increased with water deficit at 80% of FC and 80% for 2 weeks compared to 100% of FC.

Table 5: Effect of water deficit on the growth of nasturtium (*Tropaeolum majus*)

Treatments	Plant height (cm)	Leaves number	Fresh weight g plant-1	Dry weight g plant-1	Dry matter (%)
1 st season					
100% FC	33.90 a	17 a	55.57 a	5.42 a	15.98 b
80% FC	29.17 a	14 b	41.46 b	4.47 a	17.42 a
80% FC for two weeks	29.94 a	16 a	43.47 b	4.85 a	16.43 ab
2 nd season					
100% FC	34.66 a	15 a	34.97 a	4.08 a	13.56 b
80% FC	29.33 ab	12 b	27.03 a	3.86 a	16.58 a
80% FC for two weeks	25.33 b	14 a	32.36 a	4.42 a	14.90 b

Different small letters on the same column indicate significant difference ($p \leq 0.05$).

From the results in Table 5 it is clear stress causes a decreasing in plant height, fresh weight and leaves number plant⁻¹ and probably these results due to disruption of photosynthesis, transpiration and other metabolic

processes (Jones and Tardieu, 1998; Sarker *et al.*, 2005). The dry matter% increased significantly under water deficit conditions.

The plants under water deficit showed only a slightly increased concentration of glucotropaeolin content (about 10 %; Fig. 4), where there is no difference between plants with continuous water deficit and the plants that exposed to water deficit only for two weeks

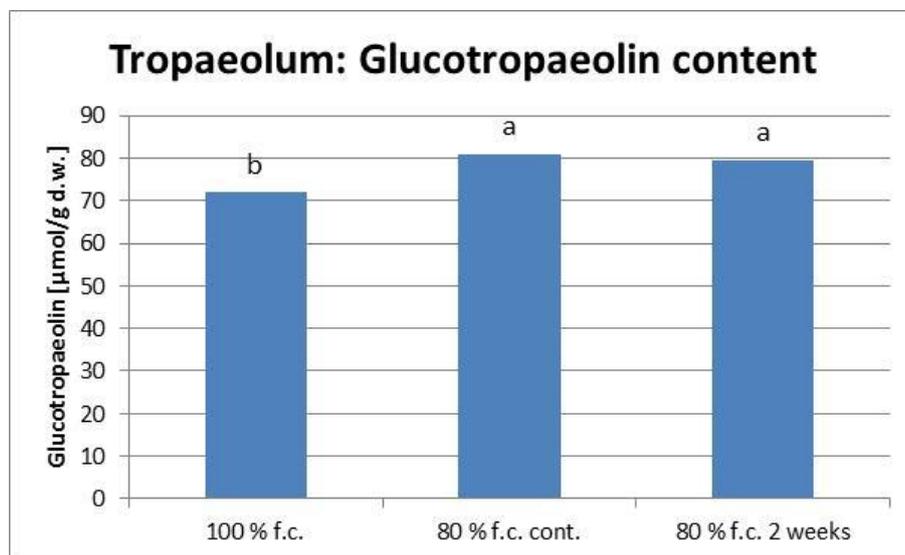


Fig. 4: Effect of water deficit on glucotropaeolin content of nasturtium

Conclusions

The present study shows that water deficit decrease the growth characteristics of sage, parsley and nasturtium meanwhile, increased essential oil percent % (sage and parsley) and dry matter % of the three plants under study. The concentrations of individual volatile compounds of parsley and sage essential oils were slightly affected by the different water deficient levels. Water deficit increase the glucotropaeolin content by about 10 %.

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