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Effect of Deficit Irrigation on Growth, Yield and Volatile Oil Contenton *Rosmarinus officinalis* L. Plant

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The influence of deficit irrigation on the growth, yield and essential oil content of (*Rosmarinus officinalis*L.) was investigated. Three irrigation treatments were applied in this experiment. The first treatment was 100% of the field capacity as a control. The second and third treatments were received 80% and 60% of the field capacity, respectively as deficit irrigation treatments. Deficit irrigation significantly reduced growth parameters and relative water content of rosemary plants compared to the control. The volatile oil percentage was increased; however yield was decreased by applying deficit irrigation treatments. The GC results showed that the main components were α-pinene, 1,8 cineol, linalool, camphor and borneol and were affected by deficit irrigation. Chlorophyll content was gradually increased with increasing irrigation frequency however, carbohydrate percentage increased by deficit irrigation treatments. Deficit irrigation, as a possible technique for saving water, can be used to control growth and save water in rosemary cultivation.

Keyword:Rosemary, Irrigation, Field Capacity, Chlorophyll, Volatile Oil, Carbohydrates

1. Introduction

Rosemary, *Rosmarinus officinalis* L. (Lamiaceae), is an evergreen plant typical of the Mediterranean region. Rosemary has long been considered an important plant for its essential oil used in perfumes and medicine (Miguel et al., 2007). The plant was reported to possess several medicinal properties like carminative, stomachic, nervine spasmodic, stimulant. The leaves were also reported to possess anti-oxidant properties and used for culinary purposes (Singh and Guleria, 2013). The volatile oil of rosemary reaches to 1.43 % (Zaouali et al., 2013) with the main component of 1.8-Cineole (35.8%) exhibit some medicinal purposes such as anti-inflammatory, antiseptic, antispasmodic and anti-diabetic (Juhas et al., 2009; Abu-Al-Basal, 2010; Beninca et al., 2011). There is limited published research on rosemary growing criteria despite its popularity and its several uses.

Water is one of the important factors affecting plant growth and yield. In addition, water resources need to be used efficiently because of the increasing competition of the limited water resources between domestic, industrial and agricultural consumptions. Increasing plant production per unit of water is one of the greatest challenges facing the researchers especially in arid and semi-arid areas, which have limited water resources and in tropic and sub-tropics, characterized by hot dry weather.

Exposing rosemary plant to water stress led to a decrease in growth parameters at different cuts while the volatile oil percentage improved by water stress. The oil constituents were also affected by deficit irrigation (Leithy et al., 2006). Water stress negatively affected the plant height and the yields of basil plant. On the contrary, the essential oil ratio of the plant increased as the applied amount of irrigation water decreased. Water stress has a positive effect on essential oil composition of the plant (Omidbaigi et al., 2003; Moeini Alishah et al., 2006; Bettaieb et al., 2009; Ekren et al., 2012). In addition, water deficit decreased oil yield of rosemary as previously reported by different authors (Khalid, 2006; Bettaieb et al., 2009; Ekren et al., 2012). Under water stress, fresh and dry weights of the herbs were significantly influenced and the essential oil percentage, the main constituents of the essential oil, proline and total carbohydrate content increased, and the N, P, K, and protein contents decreased (Khalid, 2006). The chlorophyll content was decreased with decreasing the irrigation water and this decrease was correlated with relative water content in leaves (Munne-Bosch and Alegre, 2000). Although studies in relation to cultivation techniques in rosemary have been realized, the agronomic and physiological responses to irrigation are scarce (Nicola´s et al., 2008). There have been few studies performed on rosemary in Taif region. Therefore, the aim of this study was to investigate the effect of different water regimes on the growth, yield and volatile oil of *Rosmarinus officinalis* L. plant under Taif region conditions.

2. Materials and Methods

2.1. Plant Materials

This study was conducted at the greenhouse of Biology Dep., Faculty of Science, TaifUniversity during 2011 and 2012 season. Rooted cuttings of rosemary (*Rosmarinus officinalis,* L.) were cultivated in plastic pots 30 cm filled with sandy soil. The physical properties of soil used were (sand, 82.40%, silt 7.10% and clay 10.50%) and chemical properties were (pH, 8.37, Ec, 2.33 dsm^{-1}, OM, 0.11%, Total Ca CO_3, 0.98%, Total N, P, K were 0.17,0.036 and 0.043%, respectively). The recommended dose of NPK chemical fertilizers were applied for all pots in 6 equal doses. The first three doses were added during the growing period till the first cut and the other three ones were added during the growing period till the second cut. All other agriculture practices needed during rosemary growth were done when required.

2.2. Irrigation Treatments

Three irrigation treatments were applied in this experiment. The first treatment was 100% of the field capacity as a control. The second and third treatments were received 80 and 60% of the field capacity, respectively as deficit irrigation treatments. The deficit irrigation treatments were applied after 3 weeks from cultivation in the pots and continued till the end of the experiment. During the experiment, all of the pots were weighed daily, and the amount of water lost was replaced to maintain the soil water content. Treatments were arranged in a complete randomized block design with three replicates.

2.3. Growth Characters

The growth parameters studied in this experiment were plant height (cm), number of branches/plant, both fresh and dry weights and total herb yield/plant (g). The growth parameters were measured at the fist cut and repeated again at the second one.

2.4. Relative Water Content (RWC)

Leaf RWC was determined and calculated from the following relationship:

$(W_{fresh} - W_{dry}) / (W_{turgid} - W_{dry}) \times 100$, where W_{fresh} is the sample fresh weight, W_{turgid} is the sample turgid weight after saturating with distilled water for 24 h at 4 °C, and W_{dry} is the oven-dry (70 °C for 48 h) weight of the sample (Weatherley, 1950).

2.5. Volatile oil content

2.5.1. Volatile oil determination

The volatile oil percentages in rosemary leaves obtained from each replicate of every treatment were determined by a water distillation method described in British Pharmacopea (1963), using the following equation:

Volatile oil percentage = oil volume in the graduated tube / fresh weight of sample x 100. Then, the oil yield /plant was calculated.

2.5.2. Volatile oil composition

The obtained volatile oil from second cut was dehydrated over anhydrous sodium sulphate and stored in refrigerator until GC-MS analysis. Essential oil samples were performed using GC-HP 5890 Colum HP 130 meter Internal Diameter 0.25 millimeter equipped with flame ionization detector (FID) and a carbon wax fused silica column (50 m x 0.25 mm. i. d., film thickness 0.1 µm). Helium gas was used. The oven temperature was programmed from 60 to 240^{o} C at 3^{o} C per minute.

2.6. Chemical constituents

2.6.1. Chlorophyll determination

Samples of fresh leaves were taken for chlorophyll determination. Extraction in acetone was repeated until all pigments were extracted. Chlorophyll content was determined in samples of fresh leaves according to Sadasivam and Manickam (1992). The absorbance of extracts was determined by a spectrophotometer (type Pharmacia, LKB-Novaspec II). The chlorophyll content was calculated as mg g^{-1} fresh weight.

2.6.2. Carbohydrates and Nutrient Percentages

Leaf samples were air dried at 70 °C for 24 h, then ground to fine powder and was taken for determination of the following chemical analyses:

- Total carbohydrate percentages were determined as previously described by **H**erbert *et al.* (1971).
- Nitogen percentages were determined by micro-Kjeldahl method, phosphorus was spectrophotometrically determined and potassium was determined by flame photometer as described by A.O.A.C. (1995).

2.7. Statistical Analysis of Results

The obtained data were subjected to statistical analysis using MSTAT program, USA. The analysis of variance (ANOVA) was performed to compare means. Means were separated using LSD testorDuncan multiple range test when required at significance level of 0.05.

3. Results

3.1. Growth Parameters

Data presented in Table (1) clearly show that the plant height of rosemary plants was significantly affected by irrigation treatments. The plant height was significantly decreased with decreasing irrigation levels in the first and second cut. The tallest plants were obtained by applying 100 % FC (26.58 and 39.50 cm) while the treatment of 60% FC recorded the shortest plants (21.08 and 32.33 cm) in both cuts, respectively. The number of branches per plant was significantly increased with increasing irrigation levels in the first and second cut. The treatment of 60% FC gave (6.17 and 8.08) compared to (8.08 and 11.67) which obtained by 100% FC in both cuts (Table 1).

Table 1: Effect of deficit irrigation treatments on plant height and number of branches of rosemary plant.

Treatments	Plant height (cm)		No. of branches/plant	
Irrigation (Field capacity FC) %	1st cut	2nd cut	1st cut	2nd cut
Control 100	26.58	39.50	8.08	11.67
80	23.17	35.92	7.67	9.92
60	21.08	32.33	6.17	8.08
LSD 5%	0.55	0.62	0.34	0.36

3.2. Relative Water Content (RWC %)

Data presented in Fig. (1) clearly indicate that the RWC of rosemary leaves was increased as the irrigation level increased and this increment was significant among the irrigation treatments. The highest irrigation level (100 % FC) resulted in the highest RWC in both cuts.

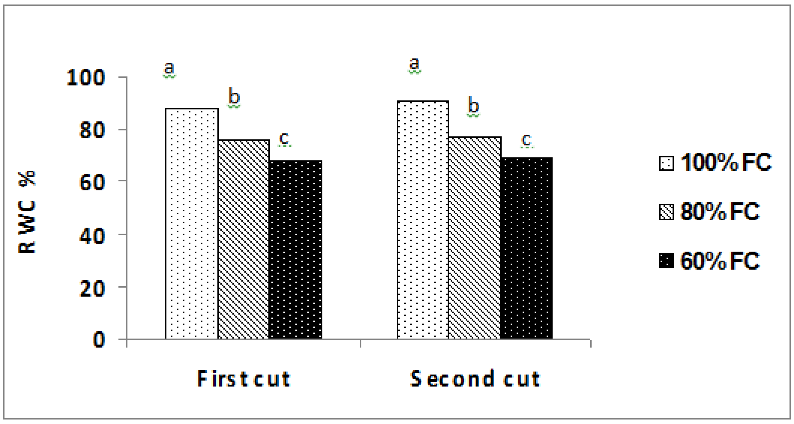

Fig 1: Effect of deficit irrigation treatments on relative water content (RWC %) of rosemary plant in the first and second cuts. Bars had different letters are significantly differ for each other according to Duncan multiple range test at $P = 0.05$.

3.3. Herb yield

The deficit irrigation negatively affected the fresh and dry weights of herb and hence the total herb yield for both cuts. The fresh and dry weights of herb per plant significantly decreased with decreasing FC level and reached its minimum values by applying 60% FC treatment in both cuts (Table 2).

The total herb yield as affected by irrigation levels was presented in Fig. (2). It followed the same trend of the fresh and dry weight. If we considered the treatment of the highest FC level equal 100% the reduction percentage in herb fresh weight was 12.54 and 23.51 % for the treatments of 80 and 60 % FC, respectively.

Table 2: Effect of deficit irrigation treatments on fresh and dry weights per rosemary plant.

Treatments	Fresh weight (g)		Dry weight (g)	
Irrigation (Field capacity FC) %	1st cut	2nd cut	1st cut	2nd cut
Control 100	57.5	105.83	11.47	19.32
80	50.67	92.17	10.12	18.63
60	42.42	82.5	9.38	16.75
LSD 5%	1.08	1.37	0.05	0.12

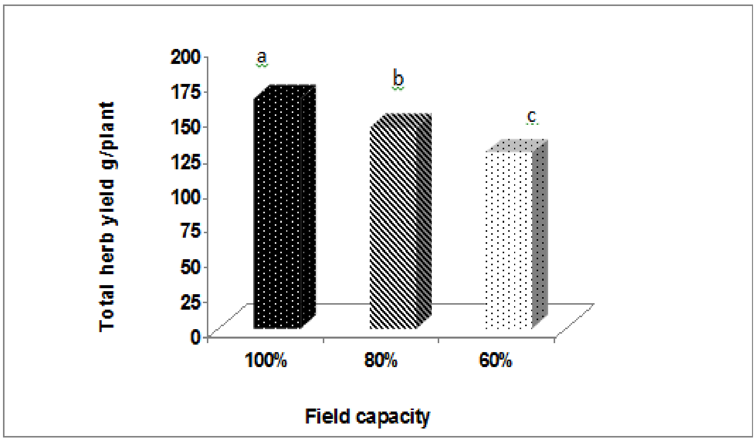

Fig 2: Effect of deficit irrigation treatments on total herb yield of rosemary plant (Total of fresh herb yield of two cuts). Bars had different letters are significantly differ for each other according to Duncan multiple range test at $P = 0.05$

3.4. Volatile Oil Content

As shown in Table (3), the average volatile oil percentage of rosemary plant was significantly affected by irrigation levels at 0.05 significant level. Deficit irrigation improved the volatile oil percentage since it was increased by decreasing the irrigation level. The highest percentage was obtained by irrigation at 60% FC however; the treatment of 100% FC gave the lowest value in this concern. In the second cut the volatile oil percentage was higher than in the first one. An opposite trend was observed for volatile oil yield per plant in both cuts (Table 3) and hence for total oil yields (Fig. 3) since they increased with increasing the irrigation level. Considering that obtaining oil yield by 100 % FC treatment equal 100 %, the reduction in total oil yield per plant was 10.18 and 15.31 % for the treatments of 80 and 60 % FC, respectively.

Table 3: Effect of deficit irrigation treatments on volatile oil percentage and yield per rosemary plant.

Treatments	Volatile oil (%)		Volatile oil yield ml/plant	
Irrigation (Field capacity FC) %	1st cut	2nd cut	1st cut	2nd cut
Control 100	0.98	1.01	0.56	1.07
80	1.02	1.03	0.52	0.95
60	1.1	1.11	0.47	0.92
LSD 5%	0.004	0.01	0.012	0.02

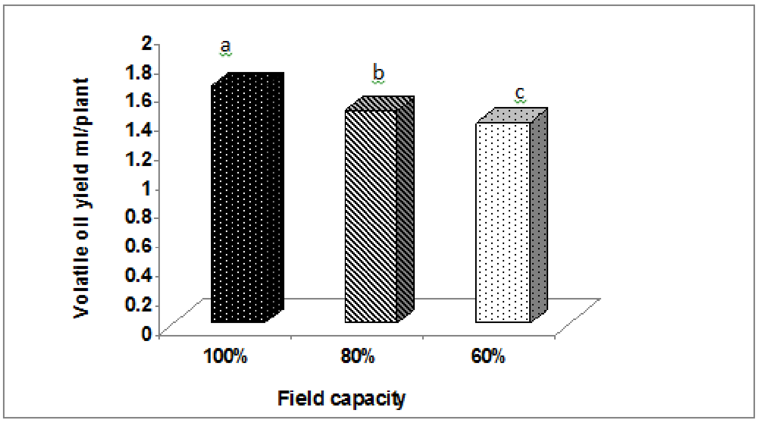

Fig 3: Effect of deficit irrigation treatments on volatile oil yield of rosemary plant (Total of oil yield of two cuts). Bars had different letters are significantly differ for each other according to Duncan multiple range test at *P = 0.05*.

3.5. Volatile Oil Composition

Data presented in Table (4) indicate that the volatile oil composition of rosemary was affected by irrigation levels. The main components of volatile oil were α-pinene, 1, 8 cineol, linalool, camphor and borneol. Deficit irrigation increased α-pinene, 1, 8 cineol and borneol especially when 60% of FC was applied. On the other hand, linalool and camphor were decreased by deficit irrigation

Table 4: Effect of deficit irrigation treatments on volatile oil composition of rosemary plant.

Treatments	Volatile oil composition %				
Irrigation (Field capacity FC) %	α-pinene	1,8 Cineol	Linalool	Camphor	Borneol
Control 100	9.04	11.37	5.62	14.03	8.33
80	11.36	10.55	4.21	14.13	6.85
60	21.59	19.53	3.10	3.32	10.90

3.6. Total Carbohydrate Percentage

The total carbohydrate percentage was significantly increased by deficit irrigation and the highest percentage was recorded by lowest irrigation level however, frequent irrigation decreased the carbohydrate percentage in both cuts (Table 5).

Table 5: Effect of deficit irrigation treatments on carbohydrate and chlorophyll content of rosemary plant.

Treatments	Carbohydrate (%)		Total chlorophyll (mg g^{-1} FW)	
Irrigation (Field capacity FC) %	1st cut	2nd cut	1st cut	2nd cut
Control 100	13.79	15.53	1.38	1.40
80	15.20	16.96	1.34	1.34
60	16.18	18.21	1.06	1.07
LSD 5%	0.17	0.16	0.01	0.02

3.7. Total Chlorophyll Content

Data presented in Table (5) clearly show that decreasing the irrigation level from 100 to 60 % FC significantly decreased the total chlorophyll content of rosemary leaves. The highest values in this respect (1.38 and 1.40 mg g-1 FW) were obtained by the highest irrigation level (100 % FC) in both cuts, respectively.

3.8. N, P and K Percentages in Leaves

Based on our experimental data, the percentage of N, P and K were decreased by decreasing the irrigation level from 100 to 60 % FC in both cuts. The highest values in this respect were recorded by applying 100% FC treatment however the lowest irrigation level (60% FC) gave the lowest N, P and K percentages (Table 6).

Table 6: Effect of deficit irrigation treatments on NPK percentages of rosemary plant.

Treatments	N%		P%		K%	
Irrigation (Field capacity FC) %	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control 100	2.45	2.47	0.31	0.33	2.30	2.37
80	2.36	2.37	0.29	0.31	2.25	2.30
60	2.18	2.20	0.24	0.25	2.23	2.27
LSD 5%	0.01	0.02	0.01	0.01	0.01	0.01

4. Discussion

The obtained results show that the irrigation levels significantly affected the growth, yield, volatile oil content and chemical constituents of rosemary plant. In the present study, water deficit decreased plant height, branch number and both fresh and dry weights compared to control which applied of 100% FC. These morphological changes in growth can be considered as a morphological adaptation of the plant to water and environmental stresses to reduce transpiration and to induce a lower consumption of water (Banon et al., 2003; Stanhill and Albers, 1974). One of the first signs of water shortage is the decrease in turgor which causes a decrease in both growth and cell development, especially in the stem and leaves. The growth of cells is the most important process that is affected by water stress and the decrease in the growth of cells leads to decrease the plant height. Otherwise, Deficit irrigation had altered the morphology of rosemary plants, reducing plant height and shoot growth (Nicola´s et al., 2008). Growth reduction as a result of water deficit has been widely reported (Sa´nchez-Blanco, et al., 2004; Leithy et al., 2006; Bettaieb et al., 2009; Ekren et al., 2012).

Moreover, reducing both stomatal conductance and biomass from aerial parts could be involved in the ability of plant to resist drought conditions (Díaz-Lópeza et al., 2012). In the case of deficit irrigation, plants have mechanisms for preventing turgor loss such as stomata closure and osmotic adjustment accompanied by decreases in elasticity (A´ lvarez et al., 2009). Decreasing the dry weight under water deficit could be a result of a reduction in the chlorophyll content as our data indicated, and consequently, photosynthesis efficiency, as reported by Khalid (2006). On the other hand, deficit irrigation increased the volatile oil percentage of rosemary however oil yield was negatively affected. The increase in the essential oil ratio and the decrease in oil yield with the increase in water stress have also been documented by Omidbaigi et al. (2003), Khalid (2006) and Moeini Alishah et al. (2006).

It has been suggested that under stress a higher density of oil glands due to the reduction in leaf area results in an elevated amount of volatile oil accumulation (Simon et al., 1992). In addition, the stimulation of essential oil production under water stress may be due to the fact that plants produce high terpene concentrations under environmental stress conditions because of a low allocation of carbon to the growth, suggesting a trade-off between growth and defense (Turtola et al., 2003). Increasing volatile oil ratio with water deficit may be also due to the increment in total carbohydrates as our data indicated since volatile oils are formed as secondary metabolites. Not only oil ratio but also oil composition was affected since some components were increased and other components were decreased. Khalid (2006) and Ekren et al., (2012) found that the essential oil content and composition were affected by different water treatments.

In our experiment, chlorophyll loss is a negative consequence of water stress; however, it has been considered as an adaptive feature in plants grown under water deficit (Munne-Bosch and Alegre, 1999). In addition, the negative effect of deficit irrigation was reflected in decreasing the chlorophyll content of rosemary leaves. On the other hand, some authors found an opposite trend since chlorophyll increased by deficit irrigation. Khayatnezhad et al., (2011) and Alaei (2011) reported that drought stress condition increased the leaf chlorophyll content in wheat genotypes. This is because the exact effect of deficit irrigation may vary according to the intensity of the water stress imposed (Cameron et al., 1999).

Deficit irrigation had a negative effect on NPK of rosemary plant. As a result of vegetative growth reduction, the absorption of nutrient elements could be decreased (Pascale et al., 2001). These results support the growth reduction obtained in our study at deficit irrigation because that effect may be resulted from deficiency of nutrients, as our results shown, and that high irrigation level could compensate for nutrient deficiency (Silber et al., 2003). So, our results obtained here explain each other. The carbohydrate percentage was positively correlated with deficit irrigation and this may be the reason of improving the volatile oil percentage of rosemary as our data showed. These results support the findings of Diaz-Lopez et al., (2012) on *Jatropha curcas* seedlings. Carbohydrates as the main organic solutes involved in plant osmotic adjustment may leads to a decrease in leaf osmotic potential to maintain turgor and this is also an important adaptive mechanism in plants subjected to deficit irrigation (Hessine et al., 2012). Finally, it could be concluded that deficit irrigation, as a possible technique for saving water, may improve water relations by reducing water consumption and can be used to control growth, increase volatile oil ratio and save water in rosemary cultivation.

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