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A Time-Course Study on Essential Oil of Rosemary (*Rosmarinus officinalis*) Under Drought Stress

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Abstract

Along with the present study, the changes in essential oil profile of rosemary (*Rosmarinus officinalis*) under drought stress were investigated. The leaf samples of rosemary were collected on three consecutive days and then the drought stressed groups were irrigated as recovery stage. Accordingly, 26 compounds were identified using gas-chromatography coupled with headspace system. Of the compounds, α-pinene, camphene, β-pinene, β-myrcene, p-cymene, D-limonene, eucalyptol, and camphor are of the major compounds, representing the 84.874 % of the identified compounds. Of those compounds, α-pinene, β-myrcene, and camphor percentage increased with the drought but the percentage of β-pinene decreased. Moreover, the changes in lipid, amide and carbohydrate regions for the samples were examined using Attenuated Total Reflectance Fourier Transform Infrared spectroscopy. The intensities: 2920 to 2852, 1727 to 1687 and 1452 to 1035 cm^{-1} bands corresponding to the lipids, amides, and carbohydrates, respectively were higher in CRD_1, CRD_2, CRD_3, CD_3, SD_3, SRD_1. Considered all experimental groups, the intensities were partially higher in control group. For the discrimination of the experimental groups, variance analysis, clustering analysis, and principal component analysis were performed. Drought and well-watered (control) groups were clearly discriminated and confirmed using differential statistical

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tools, suggesting the plausible role of metabolites in response to the changing environmental conditions.

Keywords: ATR-FTIR, Drought, Essential oil, GC-MS Headspace, Rosemary, *Rosmarinus officinalis*.

Abbreviations: **ATR-FTIR:** Attenuated Total Reflectance Fourier Transform Infrared spectroscopy; **D_0:** The first sampling before drought treatment; **CD_1:** First day after first sampling; **CD_2:** Second day after first sampling; **CD_3:** Third day after first sampling; **CDR_1:** Fourth day after first sampling; **CDR_2:** Fifth day after first day sampling; **CDR_3:** Sixth day after first day sampling; **SD_1:** First stressed day after first sampling; **SD_2:** Second stressed day after first sampling; **SD_3:** Third stressed day after first sampling; **SRD_1:** First recovery day after three stressed days; **SRD_2:** Second recovery day after three stressed days; **SRD_3:** Third recovery day after three stressed days.

Kuraklık Stresi Altındaki Biberiyenin (*Rosmarinus officinalis*) Uçucu Yağ Bileşenlerinin Zamana Bağlı Olarak Değişimi Üzerine Bir Çalışma

Özet

Bu çalışma ile birlikte, kuraklık stresi altındaki biberiyenin (*Rosmarinus officinalis*) uçucu yağ profilinde meydana gelen değişimler araştırılmıştır. Biberiye yaprağı örnekleri üç ardışık gün içerisinde toplanmış ve daha sonra kuraklık stresine maruz bırakılan bitkiler iyileştirme amaçlı sulanmıştır. Sonuç olarak GC-MS Headspace sistemi ile yapılan analize göre 26 bileşen belirlenmiştir. a-pinen, kamfen, β-pinen, β-myrcene, p-cymene, D-limonen, okaliptol ve kafur, tanımlanan bileşiklerin % 84, 874'ünü temsil eden ana bileşenler olarak belirlenmiştir. Bu bileşiklerin a-pinen, β-myrcene ve kafur yüzdesi kuraklıkla artmış, ancak β-pinen yüzdesi azalmıştır. Ayrıca, Zayıflatılmış Toplam Yansıma Fourier Dönüşümü Kızılötesi spektroskopisi kullanılarak biberiye yapraklarındaki lipit, amit ve karbonhidrat bölgelerinde meydana gelen değişimler de incelenmiştir. Lipitlere, amitlere ve karbonhidratlara karşılık gelen 2920 ila 2852, 1727 ila 1687 ve 1452 ila 1035 cm^{-1} bant yoğunluklarının sırasıyla CRD1, CRD2, CRD3, CD3, SD3, SRD1'de daha yüksek olduğu belirlenmiştir. Tüm deney

grupları dikkate alındığında, kontrol grubunda lipit, amit ve karbonhidrat bant yoğunluklarının kısmen daha yüksek olduğu gözlenmiştir. Deney gruplarının ayrımı için varyans analizi, kümeleme analizi ve temel bileşen analizi yapılmıştır. Kuraklık ve sulanan (kontrol grubu) gruplar, ayırt edici istatistiksel araçlar kullanılarak doğru bir şekilde ayırt edilmiş ve doğrulanmıştır. Ayrıca değişen çevresel koşullara yanıt olarak metabolitlerin olası rolü ortaya konulmuştur.

Anahtar Kelimeler: ATR-FTIR, Kuraklık, Uçucu yağ, GC-MS Headspace, Biberiye, *Rosmarinus officinalis*.

1. Introduction

In agricultural production, abiotic and biotic stress factors cause significant changes in quality and quantity properties of the plants. Of those abiotic stress factors, drought stress is considered and classified as of the most devastating stress factors limiting plant growth and shifting the plant metabolism. However, plants might exhibit positive behaviors concerned with secondary metabolite synthesis, production, secretion, and storage in response to the drought circumstances [1-3]. Shifting secondary metabolism as a response to the drought was considered as a strategy for sustaining life of the plant itself. The reaction of plants to drought stress induced impairments has been thoroughly discussed for several species, postulating and confirming that the shift in plant metabolism due to the stress factors. It has been proposed and considered as an advantageous phenomenon favouring for the cultivation of medicinal and aromatic plants [4-5].

It is worthy to note that today's agricultural trend and approaches are not only addressed on agricultural production and trade significance but also quantity and quality of the metabolites of the plants since the efficacy of the plants for their aromatic, medicinal, pharmaceutical, therapeutic, cosmetic and industrial values are coupled with the secondary metabolite content. Of the great diversity of secondary metabolites, essential oil is of the most important secondary metabolites. In this context, numerous researches on various plants for essential oil content and composition under water-limited stress conditions have been performed [6-17].

Of the medicinal and aromatic plants, rosemary (*Rosmarinus officinalis*) belonging to the Lamiaceae is an evergreen plant species distributed under a wide range of climates but mainly in the Mediterranean region. Rosemary is an aromatic plant and considered as an important source for its essential uses in perfume and medicinal purposes [18]. The essential oil content of rosemary was reported to range from 0.86 % [19] to 1.43 % [20]. Of the essential oil composition of rosemary, camphor, α-pinene, β-pinene, 1,8cineole, bornyl acetate, and borneol were predominant compounds [21-22] but the percentage of the compounds might change in response to environmental conditions.

Fourier transform infrared (FTIR) spectroscopy is a method measuring the vibrations of molecular bonds and generating a spectrum that corresponds to the metabolic fingerprint of a sample [23-25]. In this context, many reports in various fields have been documented using FTIR techniques [26-31]. Herewith, FTIR was considered as a fast tool and probe for rapid measure and estimation of the molecular changes [23].

Along with the present study, the effects of drought stress on essential oil composition of the rosemary leaves were investigated through water-holding for three days and then recovery for three days. Furthermore, changes in amide, lipid and carbohydrate regions of the leaf samples were screened using FTIR.

2. Material and Methods

2.1 Plant Material

Uniform transplants of rosemary were kindly provided from Agricultural Application and Research Center, Kilis 7 Aralik University, Kilis, Turkey.

2.2 Time Course Experiments

Experimental design scheme of the present study is given in Figure 1. Briefly, a pot experiment was conducted at greenhouses of the Agricultural Application and Research Center. The pots were arranged in complete randomized block designs with the three replicates corresponding to ten seedlings. Rosemary transplants were grown

under control and drought conditions. After adaptation of the transplants to greenhouse conditions, the plants were subjected to the drought stress for three days and leaf samples were collected on successive days for three days at the same time for each day. After three-day stress, the seedlings were irrigated with their 100% field capacity for three days. The leaves were sampled on successive days for three days at the same time for each day, as well. Experiments were conducted in a greenhouse with a 14 h photoperiod. Mean temperature and relative humidity were 26-30 ºC during day and 16-20 ºC at night, 60 % respectively. After harvest, leaf samples were left for drying under shadow in laboratory conditions for chromatographic analysis.

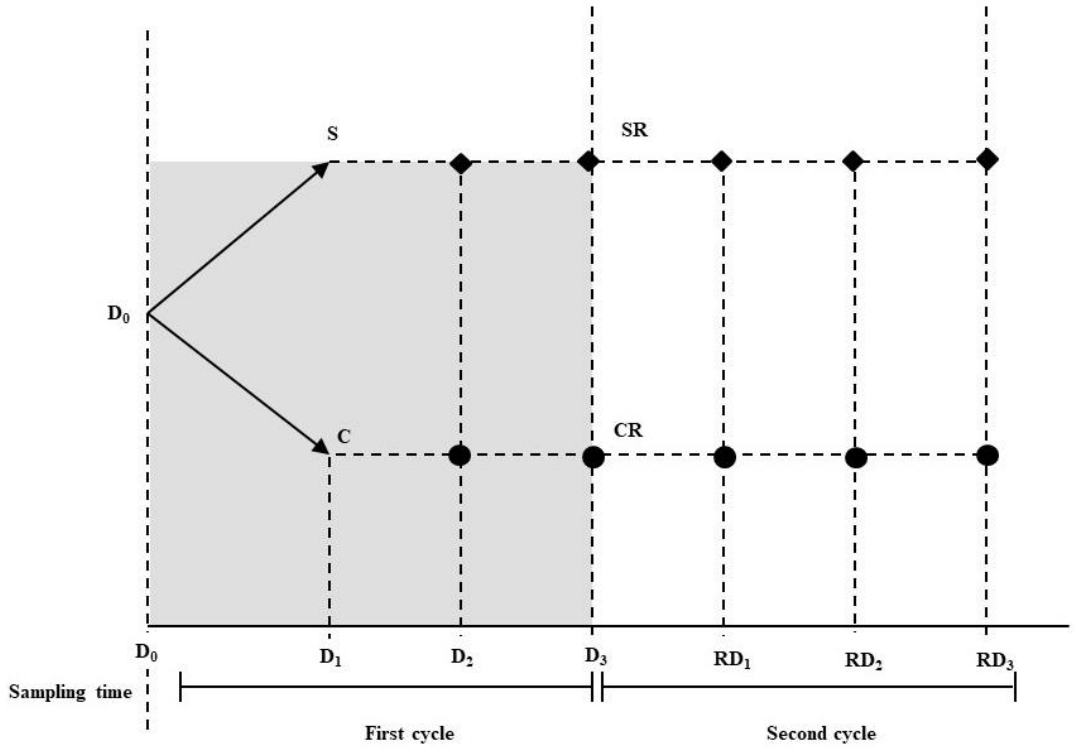

Figure 1. Experimental design scheme of the present study

2.3 Gas Chromatography Headspace Analysis

Headspace conditions were as follows: GC Cycle Time (min): 63; Sample Volume (mL): 2.5; Incubation Time (min):30; Incubation Temperature (°C): 70; Syringe Temperature (°C): 70. After optimization of running conditions, essential oil analysis was performed by GC equipped with HP-5 MS capillary column (30 m x 0.25 µm x 250 µm) and 5977 (Agilent Technologies) with mass selective detector 7890B

(Agilent Technologies) model GC-MS. An electron ionization system with ionization energy of 70 eV was used for GC-MS detection. Helium was a carrier gas at a flow rate of 1 mL/min. Injector and MS transfer line temperature were set at 250°C. Column temperature was initially kept at 50°C for 2 min, then gradually increased to 200°C at 6°C/min and finally raised to 250°C at 10°C/min. Samples were injected automatically with split ratio 10:1. Individual components were identified by electronic libraries (W10N14 and NIST14).

2.4 Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy

The ATR-FTIR spectra of 10 mg of the dried leaf samples of rosemary were recorded in Agilent Cary 600 Series FTIR. The cumulative scanning frequency was 32 min^{-1}. The scanned region was set to be 4000 to 400 cm^{-1}, with a spectral resolution of 4 cm^{-1}.

2.5 Statistical Analysis

XLSTAT statistical program was used to determine statistical significance levels by employing the independent one-way ANOVA followed by Duncan multiple range test and the differences between individual means were considered to be statistically important at $p<0.05$. Also, cluster analysis of the results was performed using XLSTAT. Moreover, the discrimination for experimental groups was done with the principal component analysis using PAST software.

3. Results and Discussion

3.1 Changes in essential oil compounds under experimental conditions

Essential oil compounds identified in rosemary leaves are listed in Table 1-3. following their elution order on the HP-5 column. Also, the changes in percentage of the compounds were visualized using Heat map (Table 4), indicating the increase from lowest (blue) to highest (red) colour scale for essential percentage for each compound obtained from different treatments. The variance analysis showed that all identified compounds were significantly affected by drought stress, highlighting that essential oil compounds were sensitive to drought due to the plausible induction or suppression of

specific enzymes involved in biosynthesis of the compounds. In leaf samples of the D_0 group corresponding to the first sampling before treatments, 26 compounds were identified. Of the compounds, α-pinene (42.920 %), camphene (9.460 %), β-pinene (1.683 %), β-myrcene (3.657 %), p-cymene (2.317 %), D-limonene (7.487 %), eucalyptol (12.993 %), and camphor (4.357 %) are of the major compounds, representing the 84.874 % of the identified compounds (Table 1). Those compounds are common to essential composition of rosemary leaves but the percentage and content of the compounds vary with the season, geographic origin, environmental factors, extraction methods and plant organs [32-33].

Herein, the study was addressed on two main objectives. The first one was to determine the essential oil compound changes by the sampling on successive days (D_0, CD_1, CD_2, CD_3, CDR_1, CDR_2, CDR_3 for control group). Along with the results obtained after six day-samplings, the relationship between six-day-development of the plant and its essential oil composition was issued. For the later one (D_0, SD_1, SD_2, SD_3, SRD_1, SRD_2, SRD_3 for stress group), the drought stress specific or stress-induced compounds were identified after three-successive samplings under water-held conditions. Moreover, three-day stressed groups were irrigated as a recovery group in order to determine or improve the essential compounds.

Of the major compounds identified herein, percentage of α-pinene was more pronounced under drought stress conditions and the biosynthesis of α-pinene was positively induced by the first day of drought (SD_1) and then decreased for the next two days (SD_2, SD_3) but the percentage was higher than the control group. However, the percentage of the compound reached the highest value during the first day of the recovery (SRD_1) and then decreased during the following two days (SD_2, SD_3). Similarly, drought [22, 34] and salt stress [35-36] increased α-pinene in rosemary. Also, severe water stress increased α-pinene in different aromatic plant species such as *Salvia officinalis* [37], *Petroselinum crispum* [38], *Foeniculum vulgare* [39] and *Satureja hortensis* L. [40].

However, water stress-induced reduction in α-pinene percentage was observed in *Ocimum basilicum* and *Ocimum americanum* [41], *Carum copticum* L. [42 and (*Carum carvi* L.) [2]. Furthermore, the α-pinene percentage in regularly irrigated group (control group) decreased steadily from D_0 to CRD_1 but then increased for the next two experimental days (CRD_1 and CRD_2). It is worthy to note that quality and quantities of the essential oil composition depend on the environmental, ontogenetic, annual and diurnal variations. The components which are not present at the current stage of the plant may emerge in the following developmental stages for sustainable developments of the plant itself [43-44].

Even though the effect of different harvesting time and drought stress affected significantly the percentage of camphene ($p<0.001$), there were no clear and obvious differences in quantity of the compound. However, the highest value was obtained in the first day of recovery on stress group. Decrease in camphene in the percentage was reported in *R. officinalis* under mild and severe water stress [22] but increased in *S. officinalis* [37] and *F. vulgare* [39].

With drought stress, the percentage of β-pinene decreased. However, recovery did not affect very much the percentage of the compound in drought stressed group. Noteworthy that β-pinene increased under irrigated conditions (control group) for six days. However, mild and severe water stress increased the percentage of β-pinene in *R. officinalis* [22], *S. hortensis* L. [40], *S. officinalis* [37], *O. basilicum* L. [41], *F. vulgare* [39], and *P. crispum* [38].

β-myrcene percentage differed among experimental groups ($p< 0.0001$) and increased with the drought. Moreover, there were no clear differences in percentages on the consecutive samplings for both groups but the changes were significant for days in each experimental group ($p< 0.0001$). Increases in β-myrcene percentages were also reported under severe drought stress [22, 39, 40].

p-cymene percentage was maintained with the drought but decreased by the time in the control group. The percentage of the compound under drought exhibited a decline after second day of the recovery stage. The changes in the percentages were significant ($p< 0.0001$). For the consecutive days in control group, a decline in the percentage was

recorded except CRD1 corresponding to the fourth day of the experiments. Zali et al. (2018) [39] reported an increase in p-cymene in *Foeniculum vulgare* under drought. In *Satureja hortensis* L. the content of the compound peaked under severe water stress treatment from near to full flowering stage [40].

D-limonene percentage decreased under both group but it was higher under drought conditions ($p < 0.0001$), suggesting that the decline in the percentage might be contributed to the normal developmental stage but the decline might be slightly improved with water holding. A slight increase but maintained changes for limonene content in *Satureja hortensis* L were also reported [40]. A strong rise in content was documented for *Foeniculum vulgare* under drought [39].

Eucalyptol percentage was not very much affected with the drought stress but the percentage increased during recovery period. Moreover, the percentage in samples under continuously and regularly irrigation increased. There were statistically significant differences for consecutively harvest for regularly irrigated samples, stressed and recovered samples ($p<0.0001$) (Table 1-3). However, the percentage significantly augmented with the moderate and severe stress in rosemary [22].

With drought, the percentage of camphor increased and peaked at the third day of stress (SD_3) but there were slight decreases after recovery ($p=0.02$). For the regularly irrigated plants, there were decreases in the percentage of the compound by the time ($p < 0.0001$). Similar stress induced increases were also documented in *Foeniculum vulgare* [39].

The changes in secondary metabolites have been explained in different way by different authors but the most common idea is about the accumulation of the metabolites is about the decline in uptake of CO_2 due to stomatal closure driven by water stress conditions. Subsequently, the consumption of reduction equivalents (NADPH+H+) for the CO_2-fixation via Calvin cycle decreases substantially, causing surplus of NADPH+H+. It is worthy to note that enhancement or decline is considered as a consequence of adaptive strategies of the plant. Since plant system is complex, dynamic and variable within the plant species, genotypes, varieties, chemotypes, and ecotypes, a common, uniform and universal explanation cannot be easily illustrated for metabolite

accumulation. Moreover, the severity, duration or combination with another simultaneous stress factors may bring about the compositional and content changes [44-46].

Table 1. Essential oil compounds identified in rosemary leaves

			Control			Drought			Control- Recovery Stage			Drought Recovery Stage			Sig.
Components	**RT**	**D_0**	**CD_1**	**CD_2**	**CD_3**	**SD_1**	**SD_2**	**SD_3**	**CRD_1**	**CRD_2**	**CRD_3**	**SRD_1**	**SRD_2**	**SRD_3**	***p***
3-Thujene	8.490	0.133 **bc**	0.030 **bc**	0.207 **ab**	0.313 **a**	0.103 **bc**	0.000 **c**	0.127 **bc**	0.000 **c**	0.113 **bc**	0.337 **a**	0.000 **c**	0.123 **bc**	0.125 **bc**	0.002
α-pinene	8.705	42.920 **c**	43.587 **c**	38.153 **d**	37.323 **d**	46.197 **a**	44.433 **bc**	43.577 **c**	35.040 **e**	38.670 **d**	38.737 **d**	47.010 **a**	46.363 **a**	46.140 **ab**	< 0.0001
Camphene	9.064	9.460 **de**	9.063 **f**	9.957 **ab**	9.987 **ab**	9.290 **ef**	9.817 **b**	9.720 **bc**	9.540 **cd**	9.820 **b**	9.947 **ab**	10.153 **a**	9.943 **ab**	9.800 **bc**	< 0.0001
Verbenene	9.192	1.213 **e**	1.073 **f**	0.723 **g**	0.687 **g**	1.333 **c**	1.390 **b**	1.247 **de**	1.470 **a**	0.683 **g**	1.037 **f**	1.413 **b**	1.267 **d**	1.205 **e**	< 0.0001
o-Cymene	9.602	0.180 **bc**	0.133 **d**	0.077 **e**	0.707 **a**	0.203 **b**	0.180 **bc**	0.160 **cd**	0.170 **c**	0.090 **e**	0.137 **d**	0.173 **bc**	0.157 **cd**	0.155 **cd**	< 0.0001
β-pinene	9.769	1.683 **fg**	2.890 **c**	2.883 **c**	5.037 **b**	1.830 **ef**	1.837 **ef**	2.090 **d**	5.500 **a**	5.010 **b**	4.957 **b**	1.543 **g**	2.010 **d**	1.970 **de**	< 0.0001
3-Octanone	9.939	0.280 **a**	0.197 **def**	0.197 **def**	0.210 **d**	0.250 **b**	0.233 **bc**	0.210 **d**	0.183 **f**	0.213 **cd**	0.213 **cd**	0.187 **ef**	0.207 **de**	0.220 **cd**	< 0.0001
β-myrcene	10.058	3.657 **a**	2.490 **f**	2.383 **g**	2.657 **e**	3.040 **c**	2.907 **d**	2.853 **d**	2.660 **e**	2.543 **f**	2.470 **fg**	2.943 **d**	3.117 **bc**	3.215 **b**	< 0.0001
α-phellandrene	10.445	0.647 **a**	0.453 **e**	0.417 **g**	0.440 **ef**	0.543 **b**	0.510 **c**	0.510 **cd**	0.433 **fg**	0.390 **h**	0.396 **h**	0.490 **d**	0.507 **cd**	0.525 **bc**	< 0.0001
3-Carene	10.601	1.710 **bcd**	1.363 **e**	0.923 **fg**	0.967 **f**	1.673 **bcd**	1.773 **bc**	1.600 **cde**	2.060 **a**	0.687 **g**	0.980 **f**	1.887 **ab**	1.450 **de**	1.410 **de**	< 0.0001
α -terpinene	10.757	0.990 **a**	0.737 **f**	0.817 **bc**	0.823 **b**	0.797 **cd**	0.747 **ef**	0.787 **d**	0.607 **h**	0.740 **f**	0.727 **fg**	0.707 **g**	0.770 **de**	0.790 **cd**	< 0.0001
p-Cymene	10.962	2.317 **b**	1.827 **ef**	1.637 **g**	1.483 **h**	2.033 **d**	2.213 **bc**	2.180 **c**	2.653 **a**	1.797 **f**	1.760 **fg**	2.550 **a**	1.940 **de**	1.785 **f**	< 0.0001
D-Limonene	11.081	7.487 **a**	5.153 **e**	5.027 **e**	4.550 **f**	6.693 **c**	6.573 **c**	6.670 **c**	6.247 **d**	4.943 **e**	4.053 **g**	7.097 **b**	6.853 **bc**	6.705 **c**	< 0.0001
Eucalyptol	11.171	12.993 **f**	14.553 **e**	19.837 **d**	24.420 **b**	12.610 **f**	11.840 **g**	11.330 **g**	22.090 **c**	24.407 **b**	25.077 **a**	11.477 **g**	12.757 **f**	13.115 **f**	< 0.0001
γ-terpinene	11.826	0.903 **a**	0.667 **e**	0.830 **b**	0.910 **a**	0.710 **d**	0.653 **e**	0.703 **d**	0.407 **g**	0.783 **c**	0.853 **b**	0.530 **f**	0.713 **d**	0.685 **de**	< 0.0001
α- Terpinolene	12.586	1.070 **a**	0.713 **e**	0.720 **e**	0.703 **e**	0.923 **bc**	0.887 **d**	0.943 **b**	0.627 **g**	0.660 **f**	0.570 **h**	0.733 **e**	0.893 **cd**	0.925 **bc**	< 0.0001
Filifolone	12.989	0.097 **bc**	0.087 **cde**	0.107 **abc**	0.110 **abc**	0.127 **ab**	0.120 **abc**	0.140 **a**	0.110 **abc**	0.053 **e**	0.057 **de**	0.100 **bc**	0.090 **bcd**	0.105 **abc**	0.0000
Camphor	14.060	4.357 **c**	4.437 **c**	3.320 **d**	3.260 **d**	5.609 **a**	5.577 **a**	5.813 **a**	4.326 **c**	3.227 **d**	3.190 **d**	5.040 **b**	5.550 **a**	5.625 **a**	< 0.0001
β-pinanone	14.440	0.137 **b**	0.130 **b**	0.080 **cd**	0.000 **e**	0.140 **ab**	0.150 **ab**	0.137 **b**	0.140 **ab**	0.080 **cd**	0.053 **d**	0.167 **a**	0.097 **c**	0.080 **cd**	< 0.0001
Pinocarvone	14.491	0.063 **b**	0.043 **d**	0.000 **e**	0.000 **e**	0.053 **c**	0.000 **e**	0.000 **e**	0.130 **a**	0.000 **e**	0.000 **e**	0.050 **cd**	0.000 **e**	0.000 **e**	< 0.0001
endo-Borneol	14.563	0.740 **f**	0.970 **c**	1.170 **a**	1.107 **b**	0.903 **d**	1.033 **c**	1.030 **c**	0.847 **de**	1.100 **b**	0.997 **c**	0.867 **d**	0.787 **ef**	0.720 **f**	< 0.0001
3-Pinanone	14.785	0.493 **cd**	0.480 **d**	0.410 **e**	0.400 **e**	0.580 **b**	0.610 **a**	0.580 **b**	0.427 **e**	0.370 **f**	0.347 **f**	0.497 **cd**	0.510 **c**	0.485 **cd**	< 0.0001
D-Verbenone	15.624	0.853 **a**	0.270 **d**	0.327 **c**	0.373 **b**	0.207 **e**	0.210 **e**	0.220 **e**	0.180 **ef**	0.277 **d**	0.263 **d**	0.180 **ef**	0.187 **ef**	0.150 **f**	< 0.0001
Bornyl acetate	17.362	0.263 **f**	0.200 **g**	0.410 **c**	0.630 **a**	0.263 **f**	0.320 **de**	0.453 **b**	0.093 **h**	0.413 **c**	0.393 **c**	0.183 **g**	0.300 **e**	0.330 **d**	< 0.0001
Caryophyllene	20.401	0.177 **ef**	0.237 **d**	0.387 **a**	0.363 **a**	0.170 **ef**	0.183 e	0.183 **e**	0.137 **gh**	0.293 c	0.320 **b**	0.130 **h**	0.155 **fgh**	0.165 **efg**	< 0.0001

Means in the same row by the same letter are not significantly different to the test of Duncan (α=0.05)

Table 2. Essential oil compounds identified in rosemary grown under continuous irrigation for six days

			Control			Control Recovery Stage				
Components	**RT**	**D0**	**CD1**	**CD2**	**CD3**	**CRD1**	**CRD2**	**CRD3**	**p**	**Sig.**
3-Thujene	8.490	0.133 **ab**	0.030 **b**	0.207 **ab**	0.313 **a**	0.000 **b**	0.113 **ab**	0.337 **a**	0.038	Yes
α-pinene	**8.705**	**42.920 a**	**43.587 a**	**38.153 b**	**37.323 b**	**35.040 c**	**38.670 b**	**38.737 b**	**< 0.0001**	**Yes**
Camphene	**9.064**	**9.460 b**	**9.063 c**	**9.957 a**	**9.987 a**	**9.540 b**	**9.820 a**	**9.947 a**	**< 0.0001**	**Yes**
Verbenene	9.192	1.213 **b**	1.073 **c**	0.723 **d**	0.687 **d**	1.470 **a**	0.683 **d**	1.037 **c**	< 0.0001	Yes
o-Cymene	9.602	0.180 **b**	0.133 **c**	0.077 **d**	0.707 **a**	0.170 **b**	0.090 **d**	0.137 **c**	< 0.0001	Yes
β-pinene	**9.769**	**1.683 d**	**2.890 c**	**2.883 c**	**5.037 b**	**5.500 a**	**5.010 b**	**4.957 b**	**< 0.0001**	**Yes**
3-Octanone	9.939	0.280 **a**	0.197 **bc**	0.197 **bc**	0.210 **b**	0.183 **c**	0.213 **b**	0.213 **b**	< 0.0001	Yes
β-myrcene	**10.058**	**3.657 a**	**2.490 c**	**2.383 d**	**2.657 b**	**2.660 b**	**2.543 c**	**2.470 cd**	**< 0.0001**	**Yes**
α-phellandrene	10.445	0.647 **a**	0.453 **b**	0.417 **d**	0.440 **bc**	0.433 **cd**	0.390 **e**	0.396 **e**	< 0.0001	Yes
3-Carene	10.601	1.710 **b**	1.363 **c**	0.923 **d**	0.967 **d**	2.060 **a**	0.687 **d**	0.980 **d**	< 0.0001	Yes
α -terpinene	10.757	0.990 **a**	0.737 **c**	0.817 **b**	0.823 **b**	0.607 **d**	0.740 **c**	0.727 **c**	< 0.0001	Yes
p-Cymene	**10.962**	**2.317 b**	**1.827 c**	**1.637 de**	**1.483 e**	**2.653 a**	**1.797 cd**	**1.760 cd**	**< 0.0001**	**Yes**
D-Limonene	**11.081**	**7.487 a**	**5.153 c**	**5.027 c**	**4.550 d**	**6.247 b**	**4.943 c**	**4.053 e**	**< 0.0001**	**Yes**
Eucalyptol	**11.171**	**12.993 e**	**14.553 d**	**19.837 c**	**24.420 a**	**22.090 b**	**24.407 a**	**25.077 a**	**< 0.0001**	**Yes**
γ-terpinene	11.826	0.903 **a**	0.667 **d**	0.830 **b**	0.910 **a**	0.407 **e**	0.783 **c**	0.853 **b**	< 0.0001	Yes
α- Terpinolene	12.586	1.070 **a**	0.713 **b**	0.720 **b**	0.703 **b**	0.627 **d**	0.660 **c**	0.570 **e**	< 0.0001	Yes
Filifolone	12.989	0.097 **a**	0.087 **ab**	0.107 **a**	0.110 **a**	0.110 **a**	0.053 **c**	0.057 **bc**	0.004	Yes
Camphor	**14.060**	**4.357 ab**	**4.437 a**	**3.320 c**	**3.260 cd**	**4.326 b**	**3.227 cd**	**3.190 d**	**< 0.0001**	**Yes**
β-pinanone	14.440	0.137 **a**	0.130 **a**	0.080 **b**	0.000 **c**	0.140 **a**	0.080 **b**	0.053 **b**	< 0.0001	Yes
Pinocarvone	14.491	0.063 **b**	0.043 **c**	0.000 **d**	0.000 **d**	0.130 **a**	0.000 **d**	0.000 **d**	< 0.0001	Yes
endo-Borneol	14.563	0.740 **e**	0.970 **c**	1.170 **a**	1.107 **b**	0.847 **d**	1.100 **b**	0.997 **c**	< 0.0001	Yes
3-Pinanone	14.785	0.493 **a**	0.480 **a**	0.410 **b**	0.400 **b**	0.427 **b**	0.370 **c**	0.347 **c**	< 0.0001	Yes
D-Verbenone	15.624	0.853 **a**	0.270 **d**	0.327 **bc**	0.373 **b**	0.180 **e**	0.277 **cd**	0.263 **d**	< 0.0001	Yes
Bornyl acetate	17.362	0.263 **d**	0.200 **e**	0.410 **b**	0.630 **a**	0.093 **f**	0.413 **b**	0.393 **c**	< 0.0001	Yes
Caryophyllene	20.401	0.177 **d**	0.237 **c**	0.387 **a**	0.363 **a**	0.137 **e**	0.293 **b**	0.320 **b**	< 0.0001	Yes

Means in the same row by the same letter are not significantly different to the test of Duncan (α=0.05)

Table 3. Essential oil compounds identified in rosemary grown under drought conditions for three days and then under regular irrigation (recovery) for three days

			Drought Stage			Drought Recovery Stage				
Components	**RT**	**D_0**	**SD_1**	**SD_2**	**SD_3**	**SRD_1**	**SRD_2**	**SRD_3**	**p**	**Sig.**
3-Thujene	8.490	0.133 **a**	0.103 **b**	0.000 **c**	0.127 **a**	0.000 **c**	0.123 **a**	0.125 **a**	< 0.0001	Yes
α-pinene	8.705	42.920 **c**	46.197 **a**	44.433 **b**	43.577 **bc**	47.010 **a**	46.363 **a**	46.140 **a**	< 0.0001	Yes
Camphene	9.064	9.460 **cd**	9.290 **d**	9.817 **b**	9.720 **bc**	10.153 **a**	9.943 **ab**	9.800 **b**	0.000	Yes
Verbenene	9.192	1.213 **c**	1.333 **b**	1.390 **ab**	1.247 **c**	1.413 **a**	1.267 **c**	1.205 **c**	< 0.0001	Yes
o-Cymene	9.602	0.180 **ab**	0.203 **a**	0.180 ab	0.160 **b**	0.173 **ab**	0.157 **b**	0.155 **b**	0.119	No
β-pinene	9.769	1.683 d	1.830 **c**	1.837 **c**	2.090 **a**	1.543 **e**	2.010 **b**	1.970 **b**	< 0.0001	Yes
3-Octanone	9.939	0.280 **a**	0.250 **b**	0.233 **bc**	0.210 **d**	0.187 **e**	0.207 **de**	0.220 **cd**	< 0.0001	Yes
β-myrcene	10.058	3.657 **a**	3.040 **cd**	2.907 **e**	2.853 **e**	2.943 **de**	3.117 **bc**	3.215 **b**	< 0.0001	Yes
α-phellandrene	10.445	0.647 **a**	0.543 **b**	0.510 **cd**	0.510 **cd**	0.490 **d**	0.507 **cd**	0.525 **bc**	< 0.0001	Yes
3-Carene	10.601	1.710 **c**	1.673 **c**	1.773 **b**	1.600 **d**	1.887 **a**	1.450 **e**	1.410 **f**	< 0.0001	Yes
α -terpinene	10.757	0.990 **a**	0.797 **b**	0.747 **d**	0.787 **bc**	0.707 **e**	0.770 **c**	0.790 **bc**	< 0.0001	Yes
p-Cymene	10.962	2.317 **b**	2.033 **d**	2.213 **c**	2.180 **c**	2.550 **a**	1.940 **e**	1.785 **f**	< 0.0001	Yes
D-Limonene	11.081	7.487 **a**	6.693 **cd**	6.573 **d**	6.670 **cd**	7.097 **b**	6.853 **bc**	6.705 **cd**	0.001	Yes
Eucalyptol	11.171	12.993 **ab**	12.610 **b**	11.840 **c**	11.330 **d**	11.477 **cd**	12.757 **ab**	13.115 **a**	< 0.0001	Yes
γ-terpinene	11.826	0.903 **a**	0.710 **b**	0.653 **c**	0.703 **b**	0.530 **d**	0.713 **b**	0.685 **b**	< 0.0001	Yes
α- Terpinolene	12.586	1.070 **a**	0.923 **bc**	0.887 **c**	0.943 **b**	0.733 **d**	0.893 **c**	0.925 **bc**	< 0.0001	Yes
Filifolone	12.989	0.097 **b**	0.127 **ab**	0.120 **ab**	0.140 **a**	0.100 **b**	0.090 **b**	0.105 **ab**	0.065	No
Camphor	14.060	4.357 **c**	5.609 **ab**	5.577 **ab**	5.813 **a**	5.040 **bc**	5.550 **ab**	5.625 **ab**	0.02	Yes
β-pinanone	14.440	0.137 **b**	0.140 **ab**	0.150 **ab**	0.137 **b**	0.167 **ab**	0.097 **c**	0.080 **c**	0.000	Yes
Pinocarvone	14.491	0.063 **a**	0.053 **a**	0.000 **b**	0.000 **b**	0.050 **a**	0.000 **b**	0.000 **b**	< 0.0001	Yes
endo-Borneol	14.563	0.740 **cd**	0.903 **b**	1.033 **a**	1.030 **a**	0.867 **bc**	0.787 **cd**	0.720 **d**	< 0.0001	Yes
3-Pinanone	14.785	0.493 **cd**	0.580 **ab**	0.610 **a**	0.580 **b**	0.497 **c**	0.510 **c**	0.485 **c**	< 0.0001	Yes
D-Verbenone	15.624	0.853 **a**	0.207 **b**	0.210 **b**	0.220 **b**	0.180 **c**	0.187 **c**	0.150 **d**	< 0.0001	Yes
Bornyl acetate	17.362	0.263 **cd**	0.263 **d**	0.320 **bc**	0.453 **a**	0.183 **e**	0.300 **bcd**	0.330 **b**	< 0.0001	Yes
Caryophyllene	20.401	0.177 **a**	0.170 **b**	0.183 **a**	0.183 **a**	0.130 **d**	0.155 **c**	0.165 **b**	< 0.0001	Yes

Means in the same row by the same letter are not significantly different to the test of Duncan (α=0.05)

Table 4. Heat map of the essential oil profile under irrigated and drought conditions

	Initial stage	Control			Control-Recovery Stage			Drought			Drought -Recovery Stage		
Components	**D_0**	**CD_1**	**CD_2**	**CD_3**	**CRD_1**	**CRD_2**	**CRD_3**	**SD_1**	**SD_2**	**SD_3**	**SRD_1**	**SRD_2**	**SRD_3**
3-Thujene	0.133	0.03	0.207	0.313	0	0.113	0.337	0.103	0	0.127	0	0.123	0.125
α-pinene	**42.92**	**43.587**	**38.153**	**37.323**	**35.04**	**38.67**	**38.737**	**46.197**	**44.433**	**43.577**	**47.01**	**46.363**	**46.14**
Camphene	**9.46**	**9.063**	**9.957**	**9.987**	**9.54**	**9.82**	**9.947**	**9.29**	**9.817**	**9.72**	**10.153**	**9.943**	**9.8**
Verbenene	1.213	1.073	0.723	0.687	1.47	0.683	1.037	1.333	1.39	1.247	1.413	1.267	1.205
o-Cymene	0.18	0.133	0.077	0.707	0.17	0.09	0.137	0.203	0.18	0.16	0.173	0.157	0.155
β-pinene	**1.683**	**2.89**	**2.883**	**5.037**	**5.5**	**5.01**	**4.957**	**1.83**	**1.837**	**2.09**	**1.543**	**2.01**	**1.97**
3-Octanone	0.28	0.197	0.197	0.21	0.183	0.213	0.213	0.25	0.233	0.21	0.187	0.207	0.22
β-myrcene	3.657	2.49	2.383	2.657	2.66	2.543	2.47	3.04	2.907	2.853	2.943	3.117	3.215
α-phellandrene	0.647	0.453	0.417	0.44	0.433	0.39	0.396	0.543	0.51	0.51	0.49	0.507	0.525
3-Carene	1.71	1.363	0.923	0.967	2.06	0.687	0.98	1.673	1.773	1.6	1.887	1.45	1.41
α -terpinene	0.99	0.737	0.817	0.823	0.607	0.74	0.727	0.797	0.747	0.787	0.707	0.77	0.79
p-Cymene	**2.317**	**1.827**	**1.637**	**1.483**	**2.653**	**1.797**	**1.76**	**2.033**	**2.213**	**2.18**	**2.55**	**1.94**	**1.785**
D-Limonene	**7.487**	**5.153**	**5.027**	**4.55**	**6.247**	**4.943**	**4.053**	**6.693**	**6.573**	**6.67**	**7.097**	**6.853**	**6.705**
Eucalyptol	**12.993**	**14.553**	**19.837**	**24.42**	**22.09**	**24.407**	**25.077**	**12.61**	**11.84**	**11.33**	**11.477**	**12.757**	**13.115**
γ-terpinene	0.903	0.667	0.83	0.91	0.407	0.783	0.853	0.71	0.653	0.703	0.53	0.713	0.685
α- Terpinolene	1.07	0.713	0.72	0.703	0.627	0.66	0.57	0.923	0.887	0.943	0.733	0.893	0.925
Filifolone	0.097	0.087	0.107	0.11	0.11	0.053	0.057	0.127	0.12	0.14	0.1	0.09	0.105
Camphor	**4.357**	**4.437**	**3.32**	**3.26**	**4.326**	**3.227**	**3.19**	**5.609**	**5.577**	**5.813**	**5.04**	**5.55**	**5.625**
β-pinanone	0.137	0.13	0.08	0	0.14	0.08	0.053	0.14	0.15	0.137	0.167	0.097	0.08
Pinocarvone	0.063	0.043	0	0	0.13	0	0	0.053	0	0	0.05	0	0
endo-Borneol	0.74	0.97	1.17	1.107	0.847	1.1	0.997	0.903	1.033	1.03	0.867	0.787	0.72
3-Pinanone	0.493	0.48	0.41	0.4	0.427	0.37	0.347	0.58	0.61	0.58	0.497	0.51	0.485
D-Verbenone	0.853	0.27	0.327	0.373	0.18	0.277	0.263	0.207	0.21	0.22	0.18	0.187	0.15
Bornyl acetate	0.263	0.2	0.41	0.63	0.093	0.413	0.393	0.263	0.32	0.453	0.183	0.3	0.33
Caryophyllene	0.177	0.237	0.387	0.363	0.137	0.293	0.32	0.17	0.183	0.183	0.13	0.155	0.165

3.2 Correlation between major essential oil compounds

Herewith correlation analysis, it was aimed to determine whether the coefficient and directions of correlation vary with the drought or not. Hence, correlation analysis for both drought and control group were separately performed. Accordingly, correlation coefficients between major essential compounds of rosemary leaves under irrigated and drought conditions were given in Table 5-6. According to the correlation matrix of the major compounds in leaves, under regular irrigated conditions (control), there were positive correlations between α-pinene and β-myrcene (r=0,428), α-pinene and D-limonene (r=0,285), α-pinene and camphor (r=0,435), camphene and eucalyptol (r=0,774), β-myrcene and p-cymene (r=0,497), β-myrcene and D-limonene (r=0,844), β-myrcene and camphor (r=0,495), p-cymene and D-limonene (r=0,780), p-cymene and D-camphor (r=0,732) and D-limonene and camphor (r=0,754). The remained correlations were negative (Table 5).

For drought, there were positive correlations between α-pinene and camphene (r=0,439), α-pinene and eucalyptol (r=0,066), α-pinene and camphor (r=0,319), camphene and p-cymene (r=0,252), camphene and camphor (r=0,113), β-myrcene and D-limonene (r=0,769), β-myrcene and eucalyptol (r=0,762), p-cymene and D-limonene (r=0,520) and D-limonene and eucalyptol (r=0,263) (Table 6).

Based on the correlation analysis, changes in coefficient and directions of correlation were observed with drought. Correlation coefficient between α-pinene and camphene, α-pinene and β-myrcene, α-pinene and D-limonene, α-pinene and eucalyptol, camphene and p-cymene, camphene and D-limonene, camphene and eucalyptol, camphene and camphor, β-myrcene and p-cymene, β-myrcene and eucalyptol, β-myrcene and camphor, p-cymene and camphor, D-limonene and eucalyptol, D-limonene and camphor. Those results suggest that the coefficient and directions of correlations are not constant but dynamic in response to the varying growth conditions since drought caused metabolic perturbations through inducing or inhibiting biosynthesis pathway of the metabolites, highlighting the responsive structure of the plants against environmental conditions.

Table 5. Correlation matrix (Pearson (n) of essential oil compounds under control

Variables	α--pinene	Camphene	β-myrcene	p-cymene	D-limonene	Eucalyptol	Camphor
α--pinene	**1**	-0.638	0.428	-0.097	0.285	-0.793	0.435
Camphene	-0.638	**1**	-0.288	-0.477	-0.509	0.774	-0.908
β-myrcene	0.428	-0.288	**1**	0.497	0.844	-0.590	0.495
p-cymene	-0.097	-0.477	0.497	**1**	0.780	-0.328	0.732
D-limonene	0.285	-0.509	0.844	0.780	**1**	-0.698	0.754
Eucalyptol	-0.793	0.774	-0.590	-0.328	-0.698	**1**	-0.782
Camphor	0.435	-0.908	0.495	0.732	0.754	-0.782	**1**

Table 6. Correlation matrix (Pearson (n) of essential oil compounds under drought conditions

Variables	α-pinene	Camphene	β-myrcene	p-cymene	D-limonene	Eucalyptol	Camphor
α-pinene	**1**	0.439	-0.362	-0.191	-0.261	0.066	0.319
Camphene	0.439	**1**	-0.407	0.252	-0.057	-0.427	0.113
β-myrcene	-0.362	-0.407	**1**	-0.067	0.769	0.762	-0.776
p-cymene	-0.191	0.252	-0.067	**1**	0.520	-0.625	-0.566
D-limonene	-0.261	-0.057	0.769	0.520	**1**	0.263	-0.964
Eucalyptol	0.066	-0.427	0.762	-0.625	0.263	**1**	-0.264
Camphor	0.319	0.113	-0.776	-0.566	-0.964	-0.264	**1**

3.3 Principal component analysis (PCA) and Agglomerative hierarchical clustering (AHC)

The discrimination can be evaluated from the principal component analysis scores plot between experimental groups using identified essential oil compounds as shown in Table 7 and Figure 2,4,6. This pair of graphs is a biplot, i.e., essential oil components were more expressed in rosemary leaf samples in the same area of the graph. The experimental groups (D_0, SD_1, SD_2, SD_3, CD_1, CD_2, CD_3, SRD_1, SRD_2, SRD_3, CRD_1, CRD_2, CRD_3) in each group represent a similar essential oil composition, illustrating that there are significant differences between groups on the level of essential oil components. Along with the present study, we discriminated the groups using the major essential components (α-pinene, camphene, β-myrcene, p-cymene, D-limonene, eucalyptol, camphor) identified herein. Moreover, three principal component analysis were performed to visualize each experimental group (drought and control) and combined group including both groups. In this context, it was aimed to determine whether the compositional changes are stress and developmental dependent or not. For control group, a better discrimination was revealed on the 2-D visualization of the plotted scores, where the two principal components accounted for 84,21 % of total variance. As shown in Table 7 and Figure 2, the first axis and second axis explained 64,195 % and 20,015 % of total variance. As previously stated, that for control group, plants were regularly irrigated for six days. CD_2, CD_3, CRD_2, and CRD_3 were clearly differed from the first day of the treatments (D_0 and D_1). For stress group, the similar but clearer discrimination was observed, accounting the 76.561 % of total variance (first axis:46. 491 % and second axis: 30.07 %). Considered all experimental groups, results obtained from the principal component analysis showed the presence of the well-discriminated and defined groups for drought and control groups, suggesting that leaf essential oils of rosemary significantly respond to the environmental conditions through not only biomass production but also secondary metabolites. The results were supported and coupled with cluster analysis, as well (Table 8 and Figure 3, 5, 7). For three analysed groups (drought, control and drought + control), three clusters were composed. Of those groups, three classes were observed through cluster analysis for Control + Drought, Class 1 included D_0, SD_1, SD_3, SD_2, SRD_2, SRD_3, SRD, CD_1; Class 2 involved CD_3, CRD_3, CRD_2 and Class 3 included CD_2 and CRD_1 (Table 8). Considered

all groups, D_0 and CD_1 were similar to drought groups, indicating that responses against drought emerged after second day of the treatment (CD_2). Furthermore, recovery did not affect significantly the essential oil composition of drought for clustering discrimination.

Table 7. Principal components (PC) with their eigenvalue and % variance

	Control		Drought		Control + Drought	
PC	**Eigenvalue**	**% variance**	**Eigenvalue**	**% variance**	**Eigenvalue**	**% variance**
1	4.49362*	64.195	3.25437	46.491	4.37187	62.455
2	1.40106*	20.015	2.10492	30.07	0.981412	14.02
3	0.860912	12.299	1.17926	16.847	0.913603	13.051
4	0.151363	2.1623	0.409436	5.8491	0.494968	7.071
5	0.066729	0.95327	0.049061	0.70088	0.144715	2.0674
6	0.026314	0.37591	0.002962	0.042313	0.072528	1.0361

*In factor analysis, factors with an eigenvalue greater than or equal to 1 are generally considered to be significant.

Table 8. Classes by clustering analysis for experimental groups

	Control			Drought			Control + Drought		
Class	1	2	3	1	2	3	1	2	3
Objects	2	3	2	1	4	2	8	3	2
Sum of weights	2	3	2	1	4	2	8	3	2
Within-class variance	5.046	1.029	9.275	0	1.032	0.536	5.013	1.275	13.927
Minimum distance to centroid	1.588	0.664	2.153	0	0.355	0.518	1.094	0.758	2.639
Average distance to centroid	1.588	0.819	2.153	0	0.79	0.518	1.963	0.912	2.639
Maximum distance to centroid	1.588	0.974	2.153	0	1.405	0.518	3.254	1.086	2.639
	D0	CD3	CD2	D0	SD1	SD3	D0	CD3	CD2
	CD1	CRD3	CRD1		SRD2	SD2	SD1	CRD3	CRD1
		CRD2			SRD3		SD3	CRD2	
					SRD1		SD2		
							SRD2		
							SRD3		
							SRD1		
							CD1		

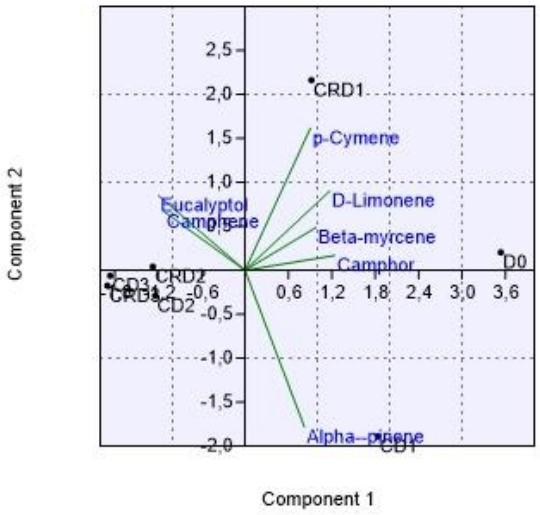

Figure 2. PCA for control

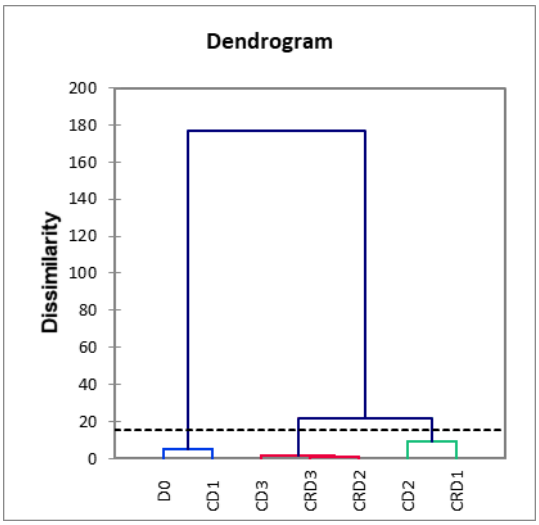

Figure 3. AHC for control

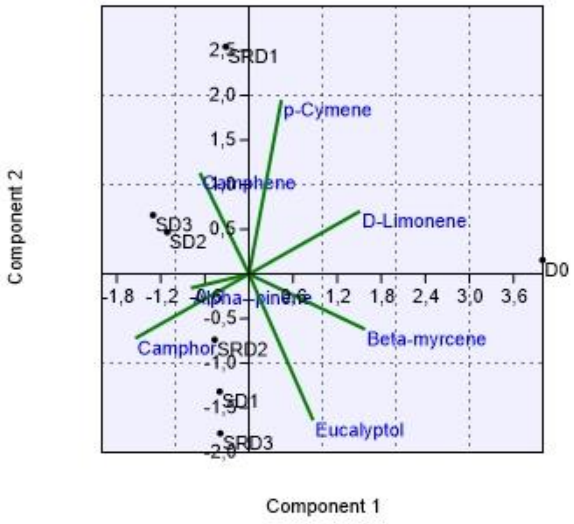

Figure 4. PCA for drought stress

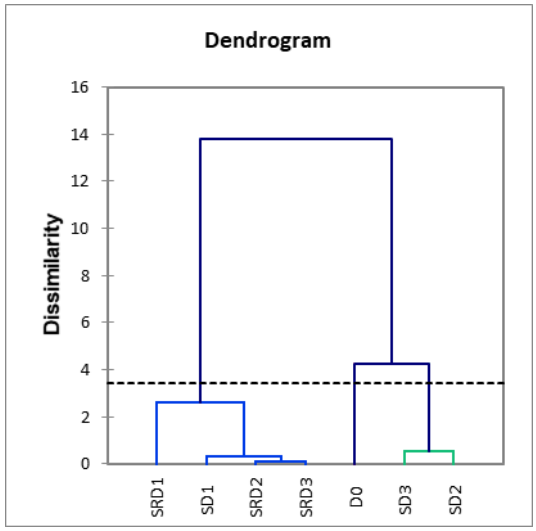

Figure 5. AHC for drought stress

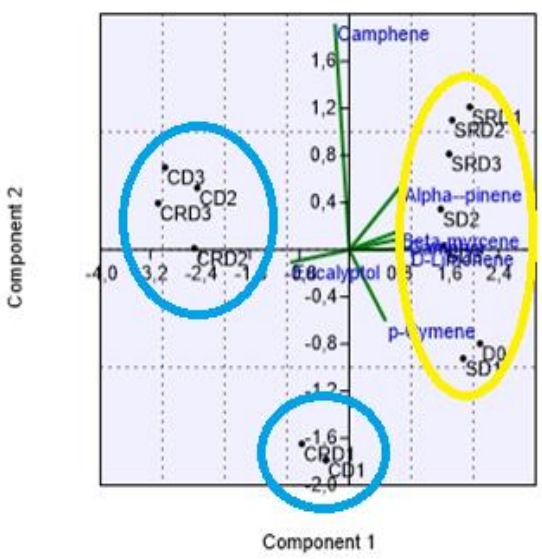

Figure 6. PCA for control and drought

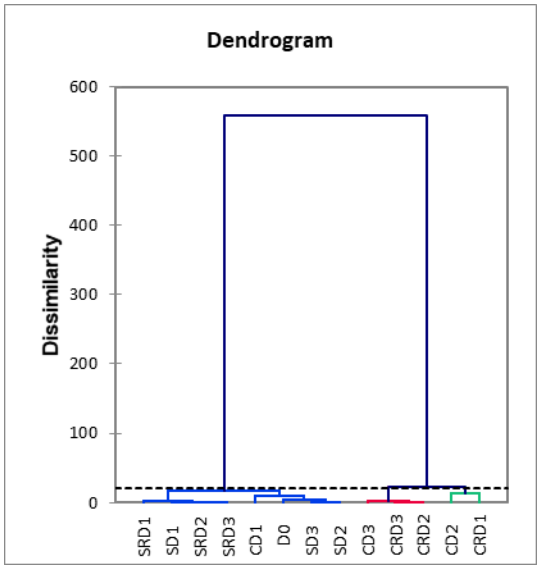

Figure 7. AHC for control and drought

3.4 ATR-FTIR analysis of the samples

As reported in many and various studies, it has been emphasized on the possible and plausible uses of FTIR spectroscopy regarding providing quick and high throughput data on drought stress [23, 47]. After normalization of FTIR spectra, the changes in lipids, amides, and carbohydrates in the control and drought stressed plants were determined. The results concerned with the intensities of the experimental groups were given in Table 9, Figure 8, 9, 10. The intensities: 2920 to 2852, 1727 to 1687 and 1452 to 1035 cm^{-1} bands corresponding to the lipids, amides, and carbohydrates, respectively were higher in CRD_1, CRD_2, CRD_3, CD_3, SD_3, SRD_1. Considered all experimental groups, the intensities were partially higher in control group. The similar results were reported in *Zea mays* exposed to progressive drought [23].

Herewith, the lowest bands were seen at SRD_1 for lipid, amide and carbohydrate regions (except at band 1035). However, there were differential responses in the intensities at lipid, amide and carbohydrate regions for other drought stressed and recovered stress groups. Furthermore, we should note that the highest bands were observed at SRD_2 out of stressed groups and lipid, amide and carbohydrate regions for control group- in general- increased by the time. For forthcoming studies,

developmental stage and stress dependent protein, lipid and carbohydrate profiles can be revealed.

Table 9. Changes in lipid, amide and carbohydrate regions

	Lipid		Amide		Carbohydrate	
Treatments	**2.920 (%)**	**2.852 (%)**	**1.727 (%)**	**1.687 (%)**	**1.452 (%)**	**1.035 (%)**
D_0	90.458	93.550	96.369	95.388	95.015	92.299
CD_1	91.783	94.485	97.303	96.5167	95.848	93.677
CD_2	92.216	94.666	97.345	96.797	96.382	94.743
CD_3	94.176	96.154	98.032	97.510	97.286	96.188
CRD_1	93.730	95.727	97.885	97.442	97.332	96.267
CRD_2	94.712	96.474	98.282	97.892	97.877	97.440
CRD_3	95.692	97.136	98.572	98.259	98.153	97.556
SD_1	92.233	94.804	97.286	96.632	96.490	94.999
SD_2	91.367	94.099	97.152	96.452	96.456	95.465
SD_3	93.668	95.781	97.845	97.376	97.174	96.205
SRD_1	90.222	93.275	96.807	96.155	96.282	95.233
SRD_2	95.628	97.141	98.532	98.114	97.970	97.319
SRD_3	91.359	94.089	97.194	96.665	96.810	95.635

The values are of the means of three replicates

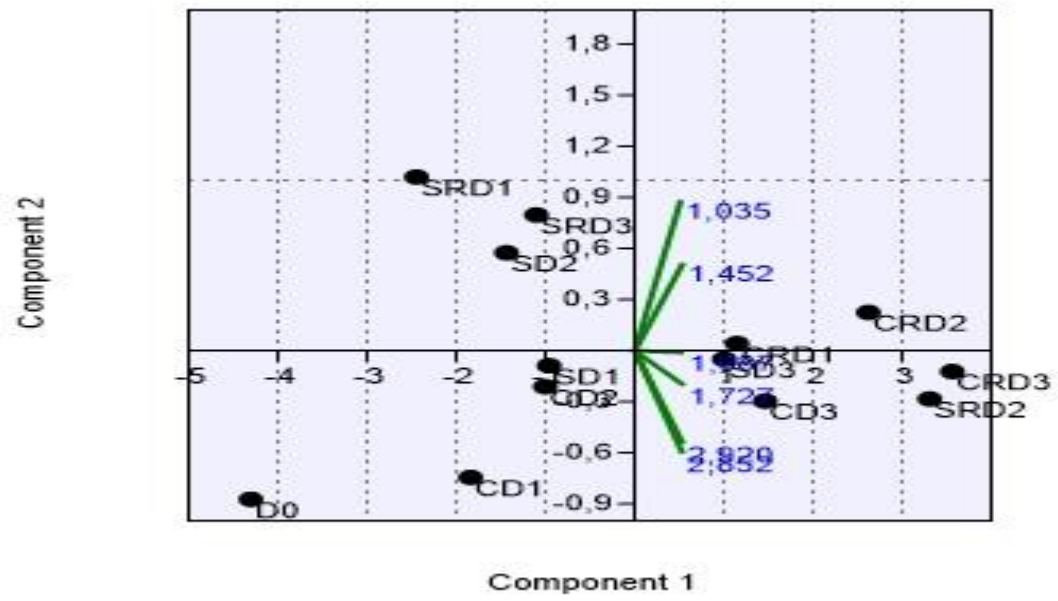

Figure 8. PCA for FTIR results of the experimental groups

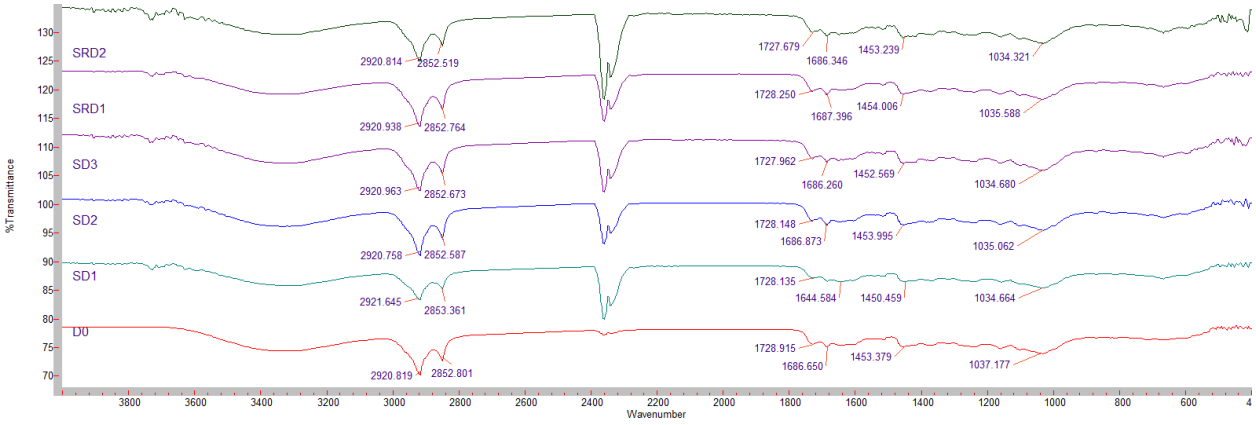

Figure 9. FTIR results for the drought stressed groups

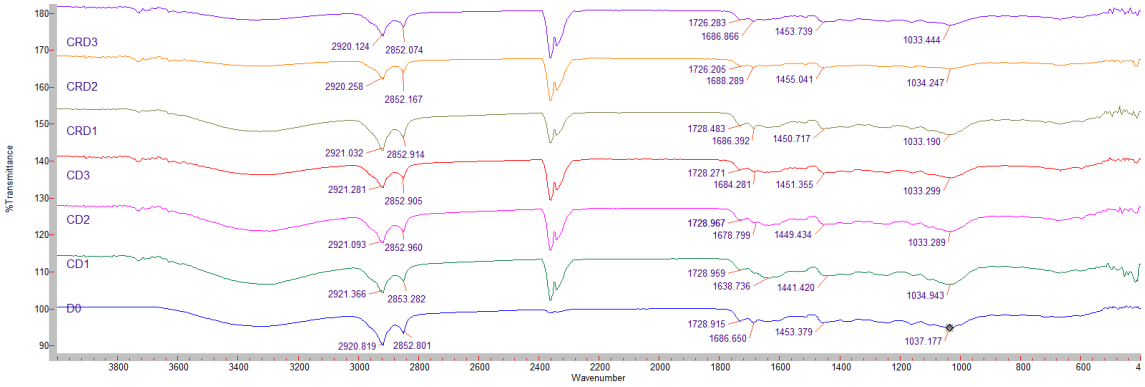

Figure 10. FTIR results for control groups

3.5 Highlights and Limitations of the study& Future outlook

Up to best knowledge, this is the first study concerned with essential oil changes on successive days under irrigated and non-irrigated conditions. Along with the study, the essential oil components in leaves of rosemary were monitored for six days for both experimental groups but the essential oil yield was not reported. In the first forthcoming studies, the essential oil yield coupled with components might be examined since the quality and bio-efficacy or other uses of essential oil are dependent on yield and its components.

4. Conclusion

Herewith, the possible influence of the drought stress on rosemary leaf essential oil profiles was monitored. In this context, leaf samples were harvested on consecutive days in order to screen the changes by the time and severity caused by drought.

Accordingly, the expected major compounds were determined but in different percentage, which was hypothesized as consequences of stress and developmental stages. Along with the current study, α-pinene, camphene, β-pinene, β-myrcene, p-cymene, D-limonene, eucalyptol, and camphor are of the major compounds, representing the 84.874 % of the identified compounds. Of those compounds, α-pinene, β-myrcene, and camphor percentage increased with the drought but the percentage of β-pinene decreased. Moreover, the changes in lipid, amide, and carbohydrate regions for the samples were monitored and considered all experimental groups, the intensities of the leaves of groups were partially higher in control group, suggesting that major metabolites might be consumed or allocated into different parts of the plant. Finally, the experimental groups were clearly discriminated and confirmed using differential statistical tools, suggesting the plausible role of metabolites in response to the changing environmental conditions.

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