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EXOGENOUSLY APPLIED 24-EPIBRASSINOLIDE MODULATES PHYSIOLOGICAL AND BIOCHEMICAL CONSTITUENTS IN LAVENDER (*Lavandula angustifolia*) PLANTS UNDER DROUGHT STRESS CONDITIONS

SUMMARY

Drought is one of the most important environmental constraints that negatively affect the growth and yield of plants worldwide. Application of plant growth regulators such as brassinosteroids is a practical and alternative approach to ameliorate the drought-induced damages in plants. In the present research, the effect of exogenously applied EBL and various drought stress intensities on lavender plants were studied in a factorial field experiment at two locations in northeast of Iran. The results revealed that drought stress had negative impacts on shoot dry matter, and pigments content of lavender plants in both regions, however, EBL application led to improve in plant characteristics under drought stress. The greatest essential oils content and free proline content were observed in plants sprayed with EBL under drought stress conditions. Drought stress resulted in a significant increase in H_2O_2 and MDA content in plants, but the content of these oxidative stress markers was lower in plants sprayed with EBL compared to the non-sprayed plants. Drought stress increased the total phenol in plants. The major compounds of lavender EOs were α-thujene, γ-terpinene, linalool oxide, α-terpinolene, α-thujone, camphor and α- humulene. The foliar EBL application partially ameliorated the negative impacts of drought stress and improved the growth and morpho-physiological attributes of lavender plants along with their essential oil content and major constituents.

Keywords: Brassinolide, Drought stress, Essential oil, *Lavandula angustifolia*.

INTRODUCTION

Lavender (*Lavandula angustifolia* Mill., Lamiaceae) is an aromatic medicinal plant recommended in traditional medicine for pregnancy, relief of

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convulsion, insomnia, anxiety, skin issues and treatment of several neurological disorders (Babar *et al*., 2015). The origin of Lavender is believed to be from the Mediterranean, Middle East and India. The vegetative body of *Lavandula angustifolia* has a pleasant odor that is caused by its essential oils (EOs), which synthesized and stored in lavender leaves and flowers and special pores (Haig *et al*., 2009). Lavender EOs are widely used in perfume and cosmetics production, hygiene, medicine, aromatherapy, food industry, beverages, and eco-friendly pesticides and herbicides, etc. (Tonutti and Liddle, 2010). The genus Lavandula is exclusively distributed across the Mediterranean areas and encompasses about twenty species of small evergreen shrubs with aromatic foliage and flowers. Linalool, linalyl acetate, camphor, terpinen-4-ol, β-o-cymene and 1, 8-cineole were identified as the major components of lavender essential oils (EOs) (Price, 1993; Koulivand *et al*., 2013; Brailko *et al*., 2017).

Drought is a major threat to crop yields in arid and semi-arid areas of the world, limiting plant growth, development and productivity (Hussain *et al*., 2018). Drought stress leads to lower plant yield through disrupting various plant metabolic/physiological processes such as turgor maintenance, carbon fixation rate, CO_2 exchange, ultimately leading to increased oxidative damage (Farooq *et al*., 2009; Hussain *et al*., 2018). In other words, when plants are under stress, different types of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$), and singlet oxygen ($^1O_2$) are generated, especially within the chloroplasts. ROS reacts with proteins and lipids, and cause damage to cellular structures and metabolism, especially those related to photosynthesis (Lawlor and Tezara, 2009). Plants counter drought stress by adopting various strategies including activating antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase, and peroxidase) and non-enzymatic antioxidant systems (*e.g.* ascorbic acid, glutathione, carotenoid, and *a*-tocopherol) for ROS scavenging. In response to drought, the osmotic adjustment occurs through the accumulation of compatible solutes such as soluble sugars, organic acids, proline, amino acids, alcoholsugars, and ions, leading to the maintenance of higher turgor potential (Chaves *et al*., 2009; Zhang *et al*., 1999).

Biological, chemical and agronomical methods have been applied to decrease the negative effects of drought stress in plants. Among these, the use of plant hormones is a promising and practical strategy to promote crop yield under drought stress (Tanveer *et al.*, 2019, Mohammadi *et al*., 2020). Exogenous application of growth regulators has been shown to improve plant tolerance to various abiotic stresses such as drought, heavy metal, and salinity stresses (Shahzad *et al*., 2018; Tanveer *et al*., 2019; Mohammadi and Moradi, 2013). Brasinosteroids (BLs) constitute a class of phytohormones that play several roles in the growth of plants. 24-Epibrassinolide (EBL) is an active byproduct of biosynthesis of brassinolide that is able to stimulate various plant metabolic processes including photosynthesis, and biosynthesis of proteins and nucleic acids.

Environmental perturbations, particularly drought, are the main obstacles to agricultural and horticultural production in many regions of the world, especially in arid and semi-arid regions such as Iran. Hence, it is central to develop drought-tolerant cultivars and explore the potential of diverse tolerance-inducing materials to reduce the adverse effects of drought stress in order to achieve acceptable economic threshold yields for crop and medicinal plants. In this regard, application of plant growth regulators such as brasinosteroids is a potentially useful tool in alleviating the detrimental effects of drought in plants, as well as enhancing the yield of medicinally important secondary metabolites. There is currently no research on the effects of drought stress and EBL on the growth and EOs yield in lavender. Given the pharmacological importance of the plant, the present study was conducted to identify the effect of EBL on growth, and EOs yield of lavender plants under different drought stress conditions.

MATERIAL AND METHODS

Plant materials and experimental set up

Field experiments were conducted to investigate the effects of EBL (the plant metabolic inducer and activator) and various drought levels (irrigation regimes corresponding to FC, 100, 60, and 30% FC) on growth, physiological parameters, and essential oil production of *Lavandula angustifolia* plants, during 2018 (mid-April to late-October) in two fields, Ahar and Kaleybar, located in East Azarbaijan province, Iran. The climatic data of both regions in the period of field trials are presented in Figure 1. The chemical and physical characteristics of the soils in both regions are given in Table 1.

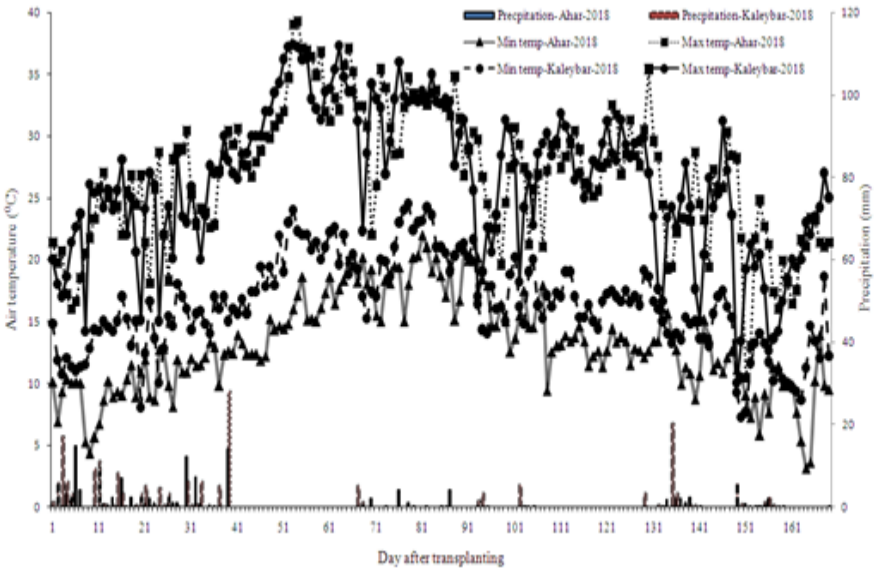


Figure 1: Air temperature (°C) and precipitation (mm) of the cultivated locations (Kaleybar and Ahar) during the plant growth period.

Table 1. The physical and chemical characteristics of soils of the cultivation regions.

Region	Texture	Sand (%)	Silt (%)	Clay (%)	K (mg/kg)	P (mg/kg)	N (%)	EC (ms)	pH
Ahar	Clay loam	30.15	25	35.84	392	10.16	0.23	1.05	7.53
Kaleybar	Clay	29.15	17.5	53.34	280	7.75	0.14	2.28	7.5

This experiment was carried out in a factorial arrangement in two regions namely Ahar and Kaleybar located at East Azarbaijan province of Iran with three replications. The first factor was spraying of EBL at two levels (0, 100 mg L^{-1}) and the second factor was different irrigation regimes (corresponding to FC, 100, 60, and 30% FC) on lavender plants. The treatments (irrigation regimes, and EBL application) were applied three weeks before flowering. TDR was used to determine the stress level.

The EBL (24-Epibrassinolide) was obtained from Sigma-Aldrich (St. Louis, USA). The EBL powder was completely dissolved in deionized water and dispensed into flask. Thereafter, the volume was made up with distilled water and mixed thoroughly to achieve a homogeneous solution. Then, the volumetric flask was completely wrapped with aluminum freezer foil to keep out light, and kept at 6 °C for at least 24 hours. We chose foliar spraying due to the absorption capacity of the solutions by leaves and aerial organs of the plants and ease of application, and the produced concentrations (0 and 100 mg L^{-1}) were sprayed on the canopy.

Lavender plants were harvested at the full flowering stage. A subsample of the plants was immediately put into the liquid nitrogen container, and then placed in an ice box containing dry ice with a lid and transferred to the laboratory and stored in -20 °C freezer. The remaining samples were dried in clean facilities in the shade for a week.

Photosynthetic Pigment Measurements

The fresh plant leaf (0.1 g each) samples were ground in a mortar with 20 mL of distilled acetone. The extract was centrifuged at 2800 g for 10 minutes. The clear supernatant was made up to 10 mL with 80% acetone. The absorbance of the extract was read at 470 (carotenoids)), 645 (chlorophyll a), and 663 (chlorophyll b) nm for measuring total chlorophyll and carotenoids (Lichtenthaler and Wellburn, 1983). Photosynthetic contents were estimated according to the following equations and expressed as mg g^{-1} fresh weight (fw).

Chl *a* (mg g^{-1} fw^{-1}) = $12.25 \times A_{663.2} - 2.79 \times A_{646.8}$

Chl *b* (mg g^{-1} fw^{-1}) = $21.50 \times A_{646.8} - 5.10 \times A_{663.2}$

Cl *a* + *b* (mg g^{-1} fw^{-1}) = $7.15 \times A_{663.2} + 18.71 \times A_{646.8}$

Carotenoids (mg g^{-1} fw^{-1}) = $(1000 \times A_{470} - 1.82 \times$ Chl *a* $- 85.02 \times$ Chl *b*)/198

Determination of H_2O_2, MDA and Proline Content

The levels of H_2O_2 in leaves of lavender plants were determined by following the method of Velikova *et al*. (2000), using an extinction coefficient of

0.28 μM^{-1} cm^{-1}. The samples absorbance was noted at 390 nm, and the H_2O_2 content was expressed as μmol g^{-1} FW, based on the standard calibration curve.

The lipid peroxidation status of leaf samples was analyzed through quantifying their malondialdehyde (MDA) content by the thiobarbituric acid (TBA) reaction. The MDA content was measured in lavender leaves according to the method of Heath and Packer (1968), using an extinction coefficient of 155 mM^{-1} cm^{-1}. Free proline was extracted from lavender leaf tissues using aqueous sulphosalicylic acid and quantified using the ninhydrin method (Bates *et al*., 1973).

Total Phenols Estimation

The content of total phenols in lavender plant extracts was determined using Folin-Ciocalteu test. In brief, to every, 25 μl of the sample, 125 μl of Folin–Ciocalteu's reagent (10 % v/v in distilled water), 100 μl Na_2CO_3 (7.5% w/v) were pipetted. The amount of reaction absorbance was noted at 765 nm against a blank sample, after 90 min. Measurements were compared with a standard curve made from gallic acid solutions (10, 50, 100, 250, 500, and 1000 mg L^{-1}) and expressed as a microgram of gallic acid equivalents per mL of sample (McDonald *et al*., 2001). The measurements were repeated three times (n=3).

Extraction of Essential Oil (EOs)

The shade-dried lavender leaf samples (40 g) were grounded and hydrodistilled for 3 h using a British Pharmacopoeia model Clevenger-type apparatus (British Pharmacopoeia, 1988). Anhydrous sodium sulfate (Na_2SO_4) was added to each extracted essential oil for removing possible water drops, and then kept at 4℃ before GC and GC/MS analyses. The essential oils content expressed on a dry weight basis (w/w %), and were calculated according to following equation: EOs content (%) = (distilled EOs (g)/ 40 g) ×100

Identification of Essential Oils Constituents

EOs samples were analyzed using gas chromatograph (GC) instrument. GC device of Agilent Technologies model A7890 gas chromatograph was used for the analysis. The device was equipped with a flame ionization detector, and the quantification of materials was performed on Euro Chrom 2000 (KNAUER) using the area normalization technique while dismissing the response factors. The analysis was performed using a HP-5 fused silica capillary column (30 m × 0.32 mm id., film thickness 0.25 μm, J and W Scientific Inc., Rancho Cordova, CA). The operating conditions were designed in a manner that the injector and detector temperatures were 280 °C and 260 °C, respectively. Helium was used as the gas carrier with a linear velocity of 22.73 cm/s. The oven temperature was programmed to increase from 60 °C to 210 °C at a rate of 3 °C/min.

The GC/MS analysis was performed on a Hewlett Packard (HP) 6890 GC/MS system equipped with a HP-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was programmed to increase from 60 °C to 220 °C at a rate of 6°C /min. The transfer line temperature was 280 °C and the injector temperature was 250 °C. The carrier gas was Helium, and the flow rate was 1 ml/min. The ionization energy of MS was 70 eV. The components of

the oils were identified by comparison of their mass spectra with those 130 of a computer libraries (Wiley 275 database) or with authentic compounds. These were confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature. The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes. The components were identified by comparing their retention indices (RI, HP-5) with those reported in the literature and also by comparing their mass spectra with the Wiley GC/MS Library, Adams Library, Mass Finder Library data and the existing mass spectra data (Adams, 2007; Davies, 1990).

Statistical Analysis

The SAS statistical software was used to perform the analysis of variance (ANOVA) and statistical analysis of the dataset. The hierarchical cluster analysis (HCA) and heatmap analyses were conducted using MetaboAnalyst platform (http://www.metaboanalyst.ca). Duncan's Multiple Range Test (DMRT) with the probability level of 0.01 ($P \leq 0.01$) was used to statistically compare the means.

RESULTS AND DISCUSSION

Morphological parameters

Combined ANOVA indicated that the interactions of cultivation regime, foliar EBL application, and drought stress on dry matter of lavender plants were significant at $p \leq 0.01$ (Table 2). The maximum plant dry matter was obtained in the Ahar region under 60% FC with foliar EBL application, and the lowest dry matter was observed under 30% FC (Table 2).

Drought stress adversely affects plant cell turgor, stomatal conductance, photosynthesis, respiration and transpiration, and interferes with other metabolic processes that directly depend on the presence of water. Crop growth and fitness decline occurs under drought conditions due to limited photosynthesis efficiency (Taiz and Zeiger, 2006). In our study, the maximum plant dry weight of lavender plants belonged to the EBL application under 60% FC level in Ahar region; and the lowest dry weights were observed for the untreated plants (sprayed with water) under 30% FC level in both studied regions (Table 2). Brasinosteroids can absorb water and maintain cell turgor by stimulating the biosynthesis of osmotic adjustments, causing an increased stomatal conductance and greater intake of CO_2 into plants (Pustovoitova *et al*., 2001). Exogenous application of brasinosteroids was shown to reduce the detrimental effects of mild drought stress in sugar beet (*Beta vulgaris*) plants (Schilling *et al*., 1991). The decreased growth of plants could be due to the reduction of turgor pressure and consequently decrease in cell division under drought stress conditions (Cabuslay *et al*., 2002, Mohammadi and Abdollahi, 2016). Greater growth attributes of plants in the Ahar region compared to the Kaleybar region under all three irrigation regimes can be partially attributed to the superior nutrient status of the soil in the Ahar region (*e.g.* higher soil content of N, P, and K; Table 1). Previous studies indicated that the water potential was maintained in plants under stress with

external application of brasinosteroids (Shahid *et al*., 2015) and it was also observed in our results (data not shown). Therefore, brasinosteroids could help to improve the plant's water relations under stress conditions.

Table 2. Analysis of mean (mean comparison) for some studied traits in lavender (Lavandula angustifolia) plants with or without 24-epibrasinolide foliar spray under different drought stress conditions and both regions.

Region	24-epibrasinolide application	Drought stress level	Total dry matter (g)	Total chlorophyll (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)
Ahar	Control	Well-watered	21.70±1.91 cd	0.776±0.08 abc	0.802±0.17 a
		60 % FC	24.70±2.78 c	0.830±0.03 abc	0.838±0.025 a
		30 % FC	9.38±1.78 gh	0.541±0.05 de	0.568±0.01 bcd
	100 mg L^{-1}	Well-watered	28.03±2.94 b	0.795±0.22 abc	0.560±0.19 bcd
		60 % FC	38.29±0.56 a	0.852±0.19 a	0.599±0.22 bcd
		30 % FC	12.79±3.02 ef	0.657±0.03 bcd	0.482±0.17 cd
Kaleybar	Control	Well-watered	15.06±1.14 e	0.513±0.04 de	0.555±0.07 bcd
		60 % FC	20.82±1.21 d	0.609±0.08 de	0.710±0.10 ab
		30 % FC	7.16±0.62 h	0.323±0.02 f	0.382±0.02 d
	100 mg L^{-1}	Well-watered	18.49±0.13 d	0.521±0.02 de	0.463±0.02 cd
		60 % FC	24.21±0.75 c	0.628±0.03 cd	0.597±0.13 bc
		30 % FC	10.71±0.92 fg	0.418±0.01 ef	0.390±0.02 d

*: Means followed by the same letter(s) in each column are not significantly different based on Duncan's Multiple Range Test (n=3).

Physiological parameters

The main effects of cultivation region, drought stress and EBL foliar application on chlorophyll content were significant at $p \leq 0.05$ (Table 2). The highest total chlorophyll contents belonged to the EBL application treatment without drought stress or with moderate drought stress (60% FC) in lavender in Ahar (Table 2). The cultivation region had significant effects on chlorophyll content, so that its highest amount was found in the Ahar region; and moderate drought stress (60% FC) also had an additive effect on this trait (Table 2). Drought stress also showed significant effect on carotenoid content (Table 2).

Exogenous application of EBL and cultivation region had significant effects on carotenoid value of lavender plants at $p \leq 0.01$ (Table 2).

Drought stress and cultivation region had significant effects on total chlorophyll content of plants (Table 2). Photosynthetic pigments can be damaged in plants under intense stress conditions that consequently results in the production of ROS (Slama *et al*., 2007). The highest total chlorophyll content belonged to the moderate drought stress (60% FC) treatment in Ahar; while severe drought (30% FC) caused a significant decrease in the chlorophyll content of plants (Table 2). Compared to the severe drought stress (30% FC), the high content of total chlorophyll under moderate drought stress conditions may be due to the preservation of high RWC in leaves, leading to the maintenance of necessary factors for the synthesis of photosynthetic pigments.

Beside their role as adjuvant pigments, carotenoids play antioxidant and free radical scavenging roles in plants exposed to the moderate stress conditions

(Egert and Tevini, 2002). Carotenoids are also likely to decrease under severe stress conditions (*e.g*. 30% FC). Lavender plants grown in the Ahar region had a significantly higher carotenoid content than those of Keleybar (Table 2), because of Ahar (a cold semi-arid) region compared to Kaleybar (a cold semi-humid) site. The lowest carotenoid content was observed under 30% FC (*i.e*., severe drought stress) (Table 2).

The main effect of the cultivation region and the interaction between drought stress and EBL foliar application on lipid peroxidation (MDA content) of plants was significant (Table 3). Interaction of cultivation region, drought stress and foliar EBL application was significant on H_2O_2 content of lavender plants (Table 3). The interaction of cultivation region and drought stress, and foliar EBL application was significant on proline content of plants (Table 3). However, the highest proline content was observed in lavender plants with EBL application under severe drought stress in Ahar (Table 3); and the lowest proline content was obtained in well-watered (100% FC) plants treated with EBL in Kaleybar region (Table 3).

The maximum MDA content was obtained under no EBL application and severe drought stress (30% FC) conditions, whereas non-stress conditions and EBL application significantly reduced the MDA content of plants exposed to moderate and severe drought stress intensities (Table 3). The high MDA content of plants under severe drought conditions could be attributed to increases in ROS, membrane peroxidation, and the leakage of ions from the membrane, eventually leading to cell damage and reduced plant growth (Foyer *et al*., 1994). Here, foliar EBL application led to the improved performance (growth and physiological attributes) of lavender plants under drought stress conditions, which is in line with known capacity of brassinolide to protect plants under stress conditions possibly through changes in membrane characteristics, increase in membrane stability, and the reduced leakage of solutes/ions from membrane (Khripach *et al*., 1998).

The maximum H_2O_2 content belonged to untreated plants under severe drought conditions in Ahar, while the lowest content was obtained in plants undergone foliar EBL application and non-stress conditions in Kaleybar region (Table 3). Drought stress negatively affects plant growth through increasing the generation of ROS such as H_2O_2, reducing photosynthesis and disturbing the water balance, which ultimately lead to declined growth and productivity of plants (Talaat *et al*., 2015). Brasinosteroids have the potential to significantly reduce the ROS in plants by improving their antioxidant system and increasing the overall plant tolerance to stress conditions (Hemmati *et al*., 2018).

Pytochemical parameters

The interaction of cultivation region, drought stress and foliar EBL application was significant on the EOs yield of lavender plants (Table 4). EBL application and severe drought stress in Kaleybar region resulted in the highest EOs yield in Lavender, whereas the lowest EOs yield was observed in untreated

plants under severe drought stress (Table 4). In a study on thyme, drought stress with (70%FC and 90% FC) led to increased and decreased EOs yield, respectively (Mohammadpour Vashvaei *et al.*, 2015). In the present study, the boosted EOs yield could be due to the prevention of intracellular oxidation events under drought stress conditions.

EBL application had a significant impact on total phenol content of the lavender plants; and the interaction between cultivation region and drought stress was statistically significant. The highest total phenol content was obtained with EBL application under severe drought stress (Figure 2). Furthermore, the highest phenol content was obtained under mild drought stress in both regions, and severe drought stress was in Ahar; and the lowest phenol content was seen in Kaleybar region (Figure 2).

Table 3. Analysis of mean (mean comparison) for some studied traits in lavender (*Lavandula angustifolia*) plants with or without 24-epibrasinolide foliar spray under different drought stress conditions and both regions.

*: Means followed by the same letter(s) in each column are not significantly different based on Duncan's Multiple Range Test (n=3)

Region	24-epibrasinolide application	Drought stress level	H_2O_2 content (µmol g^{-1}FW)	MDA content (nmol g^{-1} FW)	Proline ontent (µmol g^{-1}FW)
Ahar	Control	Well-watered	0.27±0.01 cd	0.884±0.11 g	5.700±0.12 ef
		60 % FC	0.39±0.01 b	4.032±0.67 c	8.023±0.35 c
		30 % FC	0.58±0.05 a	6.194±0.77 a	7.071±0.45 d
	100 mg L^{-1}	Well-watered	0.21±0.01 e	0.748±0.16 g	6.205±0.12 de
		60 % FC	0.28±0.03 c	2.116±0.16 ef	9.170±0.96 b
		30 % FC	0.30±0.03 c	3.271±0.15 d	12.294±0.64 a
Kaleybar	Control	Well-watered	0.23±0.02 de	0.716±0.05 g	4.838±0.50 fg
		60 % FC	0.30±0.02 c	3.697±0.21 cd	6.078±0.62 de
		30 % FC	0.37±0.02 b	5.348±0.47 b	4.297±0.71 g
	100 mg L^{-1}	Well-watered	0.17±0.01 f	0.381±0.08 g	4.545±0.10 g
		60 % FC	0.22±0.03 e	1.768±0.26 f	6.854±0.59 d
		30 % FC	0.23±0.02 de	2.380±0.35 e	8.189±0.22 c

The results of the EOs analysis indicated that there were 28 compounds in lavender plants following employed treatments in both locations (Tables 5, 6). The major compounds of lavender EOs were α-thujene, γ-terpinene, linalool oxide, α-terpinolene, α-thujone, camphor and α- humulene. Interactions of cultivation region, drought stress, and EBL application on linalool oxide, β-thujone, lavandulol, terpinene-4-ol, and lavandulyl acetate compounds were significant (Table 5, 6).

Table 4. Analysis of mean (mean comparison) for essential oil contents in lavender (Lavandula angustifolia) plants with or without 24-epibrasinolide foliar spray under different drought stress conditions and both regions.

Region	24-epibrasinolide application	Drought stress level	Essential oil content (w/w) %
Ahar	Control	Well-watered	0.87±0.18 def
		60 % FC	0.70±0.12 efg
		30 % FC	0.46±0.03 g
	100 mg L^{-1}	Well-watered	1.24±0.02 bc
		60 % FC	1.42±0.03 ab
		30 % FC	0.85±0.03 def
Kaleybar	Control	Well-watered	0.64±0.04 fg
		60 % FC	0.93±0.20 def
		30 % FC	0.49±0.09 g
	100 mg L^{-1}	Well-watered	0.96±0.14 cde
		60 % FC	1.12±0.09 cd
		30 % FC	1.57±0.19 a

*: Means followed by the same letter(s) in each column are not significantly different based on Duncan's Multiple Range Test (n=3).

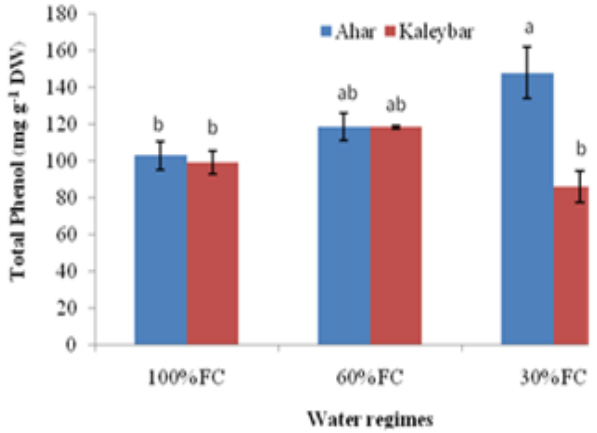


Figure 2: Effect of drought stress on total phenol contents of lavender (Lavandula angustifolia) plants in Ahar and Kaleybar locations.

The results of the EOs analysis indicated that there were 28 compounds in lavender plants following employed treatments in both locations (Tables 5, 6). The major compounds of lavender EOs were α-thujene, γ-terpinene, linalool oxide, α-terpinolene, α-thujone, camphor and α- humulene. Interactions of cultivation region, drought stress, and EBL application on linalool oxide, β-

thujone, lavandulol, terpinene-4-ol, and lavandulyl acetate compounds were significant (Table 5, 6).

The results also indicated that the highest amounts of α-thujene, γ-terpinene, α- terpinolene, α- thujone, camphor, lavandulol, and α-humulene were obtained in no-stressed plants. The highest amounts of α-thujene, linalool oxide, α- thujone, and α- humulene were obtained in the foliar EBL treated plants. Furthermore, the highest amounts of α-terpinolene, sabinol, lavandulol and camphor were observed in the Kaleybar region (Table 5, 6). The correlation matrix among pairs of the 28 terpenoid constituents exist in the EOs of lavender plants upon experimental treatments is represented in Figure 3A. This information could increase our knowledge about changing patterns in the EOs compositions as well as the correlation among these constituents in response to the employed treatments. The hierarchical cluster analysis divided the EOs constituents based on correlation coefficients into two main categories/clusters, each of them grouped into several sub-clusters. According to the correlation matrix analysis (Figure 3A), the highest correlations were found among (+)-4-carene, linalool oxide, α-thujene, trans-caryophyllene, viridiflorene and α-humulene under the experimental treatments. Correlation analysis of the EOs components revealed that there were positive correlations among the number of terpenoids (monoterpenes and sesquiterpenes) constituents and grouped together, however, the other compounds with lower correlations were grouped in another cluster. Therefore, EOs constituents that are classified in the same group/cluster responded similarly to the employed treatments. The obtained correlations may be due to the similar impacts of EBL-mediated regulation of the expression of genes (Sharma *et al*., 2017), post-transcriptional regulation and redox homeostasis (Zhao *et al*., 2017), activities of various enzymatic antioxidants (Bajguz, 2000), signaling compounds (Xi and Yu, 2010), and other factors affecting biosynthesis, regulation and accumulation of secondary metabolites.

The correlation among pairs of the studied traits (*i.e*., plant dry weight, RWC, plant height, chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, MDA, H_2O_2, essential oils content, proline, and total phenolics) in response to the EBL application and drought stress levels is shown in Figure 3B. The results obtained by hierarchical cluster analysis technique could be visualized using a color-coded heatmap and dendrograms based on the Pearson correlation coefficient of each trait with other traits, resulting in two main clusters. The various clusters exhibit different response patterns of the studied traits to the reference treatments. Furthermore, our findings suggest that the EBL and drought stress treatments caused to a co-induction of plant height and dry weight, RWC, chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, and essential oil content of lavender plants, however, other traits such as MDA, H_2O_2, proline and total phenols have lower correlations with application of EBL and increasing drought stress levels. Heatmap based on the relative levels of studied traits in

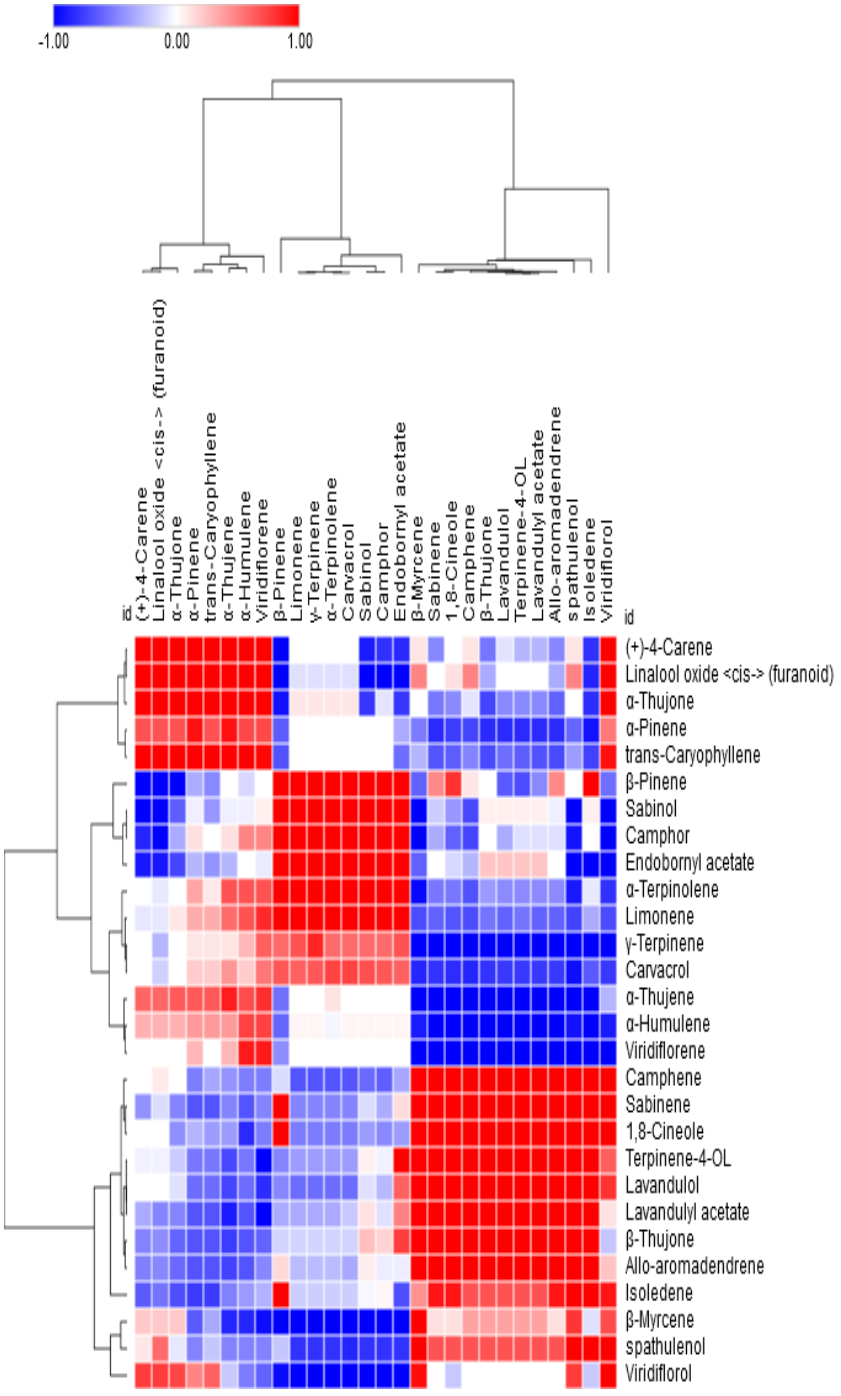
lavender plants in response to the different employed treatments is summarized in Figure 3C.

Table 5. Essential oils constituents in lavender (*Lavandula angustifolia*) plants with or without 24-epibrasinolide foliar spray under different drought stress conditions in Ahar region.

Compounds	RI	LRI	Control			100 mg L^{-1}		
			100% FC	60% FC	30% FC	100% FC	60% FC	30% FC
α-Thujene	925	924	9.23±0.45	6.21±0.54	10.48±0.98	5.337±0.11	5.781±0.16	8.231±0.97
α-Pinene	931	932	4.12±0.11	4.341±0.21	3.999±0.11	2.586±0.07	2.678±0.07	3.894±0.11
Camphene	945	946	1.687±0.04	1.632±0.04	1.612±0.04	1.725±0.06	1.651±0.04	1.712±0.03
Sabinene	971	969	1.121±0.03	0.09±0.02	1.085±0.03	0.073±0.01	0.11±0.01	1.061±0.01
β-Pinene	974	974	2.101±0.08	1.45±0.03	1.945±0.08	1.365±0.06	1.389±0.06	1.73±0.03
β-Myrcene	989	988	1.43±0.05	0.431±0.04	1.62±0.04	0.383±0.04	0.421±0.08	1.54±0.04
(+)-4-Carene	1015	1008	0.823±0.02	6.23±0.26	0.887±0.06	5.196±0.15	5.551±0.14	0.992±0.08
Limonene	1027	1024	0.69±0.01	2.31±0.04	0.65±0.08	2.225±0.09	2.761±0.09	0.61±0.06
1,8-Cineole	1029	1026	1.342±0.07	3.431±0.11	1.373±0.07	2.784±0.14	2.541±0.08	1.421±0.07
γ-Terpinene	1057	1054	7.012±0.1	1.32±0.05	6.809±0.87	1.264±0.07	1.331±0.04	6.912±0.74
Linalool oxide	1065	1067	2.871±0.08	35.651±2.54	2.632±0.14	39.897±2.07	41.12±2.04	2.541±0.06
α-Terpinolene	1087	1086	13.023±1.01	4.651±0.12	12.589±1.04	3.397±0.11	3.441±0.06	12.867±1.03
α-Thujone	1105	1101	10.121±0.81	11.112±0.87	9.538±0.41	12.398±1.11	13.011±1.18	9.876±0.87
β-Thujone	1115	1112	1.321±0.04	0.551±0.04	1.406±0.06	0.699±0.04	0.54±0.08	1.411±0.04
Sabinol	1139	1137	3.87±0.21	0.241±0.02	3.422±0.08	0.256±0.02	0.243±0.01	3.651±0.04
Camphor	1144	1141	16.02±1.21	0.832±0.06	15.008±1.21	0.961±0.08	0.922±0.07	15.78±1.74
Lavandulol	1165	1165	1.672±0.06	0.321±0.04	5.499±0.17	0.338±0.06	0.311±0.03	5.567±0.84
Terpinene-4-ol	1176	1174	0.432±0.03	0.112±0.01	0.502±0.08	0.062±0.01	0.11±0.02	0.511±0.05
Endobornyl acetate	1286	1284	0.421±0.02	0.143±0.02	0.455±0.03	0.124±0.02	0.132±0.01	0.471±0.08
Lavandulyl acetate	1290	1288	0.451±0.04	0.122±0.01	0.479±0.04	0.131±0.03	0.125±0.02	0.311±0.07
Carvacrol	1300	1298	0.911±0.07	1.711±0.08	0.881±0.07	1.62±0.07	1.76±0.04	0.811±0.06
trans-Caryophyllene	1422	1417	1.432±0.06	3.811±0.19	1.388±0.06	3.479±0.14	3.54±0.09	1.365±0.10
Isoledene	1442	1374	4.023±0.15	0.32±0.04	3.976±0.10	0.4±0.07	0.387±0.04	3.211±0.14
α-Humulene	1457	1452	6.981±0.24	4.532±0.14	6.251±0.45	5.818±0.19	5.67±0.12	7.125±0.64
Viridiflorene	1498	1496	0.612±0.07	0.342±0.06	0.506±0.07	0.447±0.07	0.387±0.07	0.451±0.07
Spathulenol	1582	1577	0.476±0.03	0.111±0.02	0.457±0.05	0.01±0.01	0.11±0.02	0.461±0.09
Allo-aromadendrene	1588	1458	0.231±0.02	0.082±0.01	0.199±0.02	0.055±0.02	0.08±0.01	0.176±0.02
Viridiflorol	1597	1592	0.321±0.04	3.421±0.12	0.309±0.06	3.297±0.10	3.421±0.13	0.311±0.03
Total of compounds identified (%)			94.748	95.511	95.957	96.327	99.524	95
Classes of constituents								
Monoterpene hydrocarbons			39.659	35.677	40.031	33.251	35.224	37.969
Oxygenated monoterpenes			41.013	47.215	42.84	49.57	50.705	43.931
Sesquiterpene hydrocarbons			13.279	9.087	12.32	10.199	10.064	12.328
Oxygenated Sesquiterpene			0.797	3.532	0.766	3.307	3.531	0.772

Table 6. Composition of essential oils in lavender (*Lavandula angustifolia*) plants with or without 24-epibrasinolide foliar spray under different drought stress conditions in Kaleybar region.

Compounds	RI	LRI	Control			100 mg L^{-1}		
			100% FC	60% FC	30% FC	100% FC	60% FC	30% FC
α-Thujene	925	924	3.869±0.45	4.12±0.23	3.34±0.69	2.236±0.11	1.346±0.12	1.453±0.23
α-Pinene	931	932	1.029±0.04	1.098±0.08	1.11±0.01	0.121±0.03	0.084±0.02	0.098±0.02
Camphene	945	946	0.667±0.03	0.543±0.03	0.543±0.03	7.321±0.85	6.384±0.41	6.76±0.81
Sabinene	971	969	0.057±0.01	0.032±0.01	0.211±0.01	2.453±0.11	1.566±0.14	1.65±0.08
β-Pinene	974	974	1.784±0.11	1.65±0.04	1.762±0.12	2.231±0.14	1.084±0.08	1.097±0.06
β-Myrcene	989	988	0.076±0.01	0.08±0.02	0.211±0.03	0.543±0.03	0.515±0.07	0.511±0.07
(+)-4-Carene	1015	1008	0.131±0.02	0.143±0.03	0.265±0.02	0.432±0.01	0.364±0.03	0.321±0.05
Limonene	1027	1024	6.513±0.74	4.74±0.21	6.871±0.74	0.267±0.02	0.294±0.05	0.276±0.04
1,8-Cineole	1029	1026	2.851±0.12	2.761±0.41	2.98±0.22	5.45±0.32	4.229±0.19	4.11±0.13
γ-Terpinene	1057	1054	2.674±0.06	1.65±0.08	3.21±0.14	0.211±0.02	0.187±0.03	0.197±0.02
Linalool oxide	1065	1067	3.354±0.08	3.421±0.21	3.87±0.21	8.23±0.89	5.99±0.24	5.12±0.14
α-Terpinolene	1087	1086	12.43±1.1	14.12±1.25	10.21±1.02	1.87±0.07	1.448±0.11	0.55±0.08
α-Thujone	1105	1101	0.558±0.07	0.551±0.04	0.66±0.03	0.321±0.03	0.314±0.03	0.321±0.04
β-Thujone	1115	1112	1.698±0.11	0.654±0.03	2.675±0.11	12.54±1.64	13.657±1.28	14.21±1.27
Sabinol	1139	1137	41.099±2.31	43.78±2.74	37.51±1.79	0.211±0.06	0.179±0.03	0.121±0.07
Camphor	1144	1141	8.126±0.45	9.345±0.68	9.342±0.84	0.211±0.04	0.248±0.04	0.112±0.06
Lavandulol	1165	1165	0.088±0.01	0.098±0.02	0.211±0.03	20.123±2.31	23.536±2.47	26.122±2.64
Terpinene-4-ol	1176	1174	0.158±0.02	0.165±0.01	0.187±0.01	11.098±1.09	13.387±1.28	15.23±1.84
Endobornyl acetate	1286	1284	0.219±0.04	0.211±0.03	0.276±0.03	0.121±0.02	0.118±0.02	0.211±0.03
Lavandulyl acetate	1290	1288	0.362±0.06	0.367±0.04	0.431±0.02	14.54±1.01	16.059±1.05	18.121±1.27
Carvacrol	1300	1298	3.908±0.21	3.76±0.10	3.267±0.41	0.186±0.02	0.193±0.03	0.091±0.02
trans-Caryophyllene	1422	1417	0.556±0.05	0.655±0.03	0.633±0.02	0.121±0.02	0.1±0.02	0.021±0.01
Isoledene	1442	1374	0.353±0.07	0.358±0.04	0.441±0.03	0.765±0.07	0.89±0.04	0.11±0.03
α-Humulene	1457	1452	2.184±0.05	2.32±0.1	4.32±0.11	0.231±0.03	0.391±0.06	0.102±0.02
Viridiflorene	1498	1496	0.263±0.03	0.276±0.03	0.341±0.05	0.111±0.01	0.127±0.02	0.05±0.01
spathulenol	1582	1577	0.05±0.01	0.07±0.01	0.11±0.01	0.167±0.02	0.194±0.01	0.078±0.02
Allo-aromadendrene	1588	1458	0.105±0.04	0.121±0.02	0.211±0.03	1.54±0.11	1.487±0.03	1.21±0.11
Viridiflorol	1597	1592	0.062±0.01	0.076±0.01	0.098±0.02	2.67±0.21	2.338±0.11	0.98±0.14
Total of compounds identified (%)			95.224	97.165	95.296	96.321	96.709	99.233
Classes of constituents								
Monoterpene hydrocarbons			19.056	15.261	20.858	28.676	25.795	26.894
Oxygenated monoterpenes			72.595	78.028	68.284	62.04	65.387	69.788
Sesquiterpene hydrocarbons			3.461	3.73	5.946	2.768	2.995	1.493
Oxygenated Sesquiterpene			0.112	0.146	0.208	2.837	2.532	1.058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trans-Caryophyllene
α-Thujene
α-Humulene
Viridiflorene
β-Pinene
Limonene
γ-Terpinene
α-Terpinolene
Carvacrol
Sabinol
Camphor
Endobornyl acetate
β-Myrcene
Sabinene
1,8-Cineole
Camphene
β-Thujone
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Lavandulyl acetate
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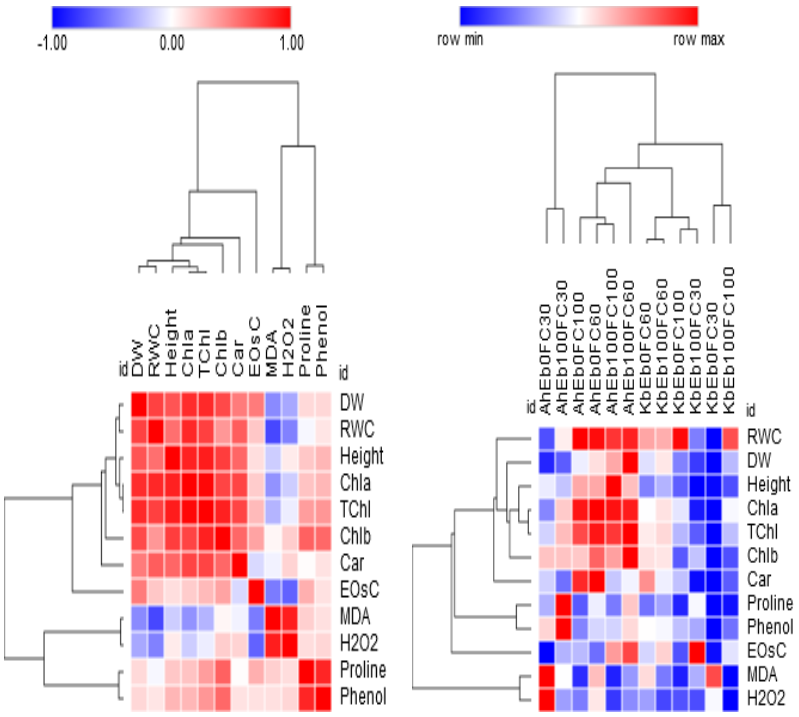

(B) (C)

Figure 3: (A) Classification of the terpenoid compounds [(oxygenated sesquiterpene (OS), sesquiterpene hydrocarbons (SH), oxygenated monoterpenes (OM), monoterpene hydrocarbons (MH)] exist in the essential oils of lavender (Lavandula angustifolia) plants upon exogenous application of 24-epibrassinolide (Eb, 0 and 100 mg/L) under drought stress levels (100, 60, and 30% FC) at two cultivated sites, Ahar (Ah) and Kaleybar (Kb). Hierarchical cluster analysis (HCA) with Pearson correlation on 28 essential oil constituents identified. The colors of the matrix boxes represent the magnitude and direction of the association: intense red and blue indicate strong positive and negative correlations, respectively. (B) The HCA with Pearson correlation coefficient among various examined traits [(plant dry weight (DW), leaf relative water content (RWC), plant height, chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (TChl), carotenoids (Car), essential oils content (EOsC), malondialdehyde (MDA), hydrogen peroxide (H2O2), free proline and total phenolics content in the lavender plants foliar sprayed with 24-epibrassinolide under drought stress conditions. (C) HCA with heatmap based on the different morpho-physiological traits measured in lavender plants upon experimental treatments, showing the treatment-variable relationships. Two main clusters were identified at the variable level.

CONCLUSIONS

Drought, in the changing climatic conditions of the world, is a major environmental constraint for plant production and productivity. However, 24-epibrassinolide, a plant hormone and an active by-product produced during brassinolide biosynthesis, has been recognized as an effective drought stress ameliorating approach. In the present study, drought stress adversely affected lavender (*Lavandula angustifolia*) plants performance via interference in primary- and secondary metabolism due to enhanced levels of cellular injury indices such as hydrogen peroxide, malondialdehyde, and decreased levels of plant biomass, photosynthetic pigments (chlorophylls and carotenoids). Moreover, foliar application of 24-epibrassinolide was not only able to mitigate the negative impacts of water deficit stress, but also enhanced dry weight, essential oils production, free proline as well as total phenolics content.

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