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Research Article

24-epibrassinolide improves the growth and essential oil of coriander leaves (*Coriandrumsativum* L.) under drought stress

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Abstract

Coriander (*Coriandrum sativum* L.) is used as an aromatic and medicinal vegetable. Plant growth regulators improve plant performance under unfavorable conditions. In this study, the interaction effects of 24-epibrassinolide (0, 0.5 and 1 µM) and three levels of drought stress (-2, -3.5 and -4.5 bars) were investigated on growth parameters and coriander leaves essential oil. The highest main stem length, diameter, shoot fresh weight, and shoot dry weight were obtained by 0.5 µM of 24-epibrassinolide (EBR) under -3.5 bar. Both 0.5 and 1 µM concentrations of 24-epibrassinolide protected leaves of chlorophyll *a* and *b* against the effects of water deficit. The plants under -3.5 and -4.5 bar water deficits showed more leaf electrolyte leakage compared to the control (-2 bar). The highest percentage of all six measured essential oil components (linalool, (e)-2-decenal, 2-hexa-decenal, carvone, α-pinene and (E)-2-dodecenal) were obtained under moderate and severe levels of regulated water deficit. Foliar spray of EBR 0.5µM under severe drought stress with -4.5 bar of matric potential increased the main essential oil compositions such as linalool, (E)-2-decenal and (E)-2-hexa-decenal.

Keywords: 24-epibrassinolide, Coriander essential oils, Linalool, drought stress

Introduction

Coriander (*Coriandrum sativum* L.), from the Apiaceous family and native to the Mediterranean region, is an important leafy vegetable as well as a spice crop grown in many countries for its tender green leaves and aromatic fruits (Lal and Ravindra, 2016). The main constituents of coriander essential oil are: linalool, α-pinene, limonene, camphor, (E)-2-decenal, dodecanal, (E)-2-tridecenal, dodecanal and carvone which have been reported to possess strong antioxidant activities (Shibamoto and Wei, 2007; Zekovica *et al.*, 2016).

In coriander, growth and essential oil production are influenced by various environmental factors, for example, maturation stage (Msaada *et al*, 2007; Msaada *et al.*, 2009a), regions (Msaada *et al.*, 2009b) and abiotic stress such as salinity (Neffati and Marzouk, 2008; Neffati *et al*., 2011). There is a strong correlation between temperature, radiance during fruit development, the water supply of coriander, and its essential oil content (Carrubba *et al*., 2006). Limiting water availability is a serious problem for plants because, physiologically, during water limitation, a chemical signal is transmitted from roots to leaves through the xylem, stimulating partial stomatal closure, which results in a drastic decrease in the level of intracellular CO_2 and plant growth characteristics (Cheng *et al*., 2018). Drought, a main abiotic stress, affects the physiological and biochemical processes of plants, especially the synthesis and accumulation of secondary metabolites such as essential oils (Bukhari *et al*., 2020).

It was observed that in coriander, for instance, the amount of thymol decreased as a result of drought stress. This can be explained by the fact that transpiration leads to increased monoterpene emissions, with elevated levels expected to be observed at the beginning of the drought period (Mirniyam *et al*., 2022). It is claimed that moderate drought, moderate nutrient limitation, and low temperatures might lead to an increased carbon pool available for the synthesis of secondary metabolites (Sellami *et al*., 2011). In light of numerous reports in the past two decades, the complex BR signaling under different stress conditions (drought, salinity, extreme temperatures and heavy metals/metalloids) that drastically hinders the normal metabolism of plants is gradually being untangled and revealed. (Chaudhuri *et al*., 2022). Brassinosteroids are a group of naturally occurring plant steroidal compounds with multiple biological activities (Cao *et al*., 2005; Ozdemir *et al*., 2004). They can offer the possibility of increasing crop yields through both

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changing plant metabolism and protecting plants from environmental stresses (Cao *et al*., 2005).

Under normal conditions, the activity of ascorbate peroxidase, catalase, and superoxide dismutase enzymes, as well as the synthesis of guaiacol peroxidase, carotenoids and phenolic compounds, maintains this balance. But under drought stress, this balance is disturbed, the production of free radicals increases, and oxidative stress is created (Khanna Chopra and Selote, 2007). Antioxidants are molecules that block the release of free radicals and prevent cell destruction. These substances can give free radicals electrons to convert them to their stable form and prevent their destructive effects. The degree of antioxidant activity and the rate of increase of antioxidants in plants depend on the plant species, developmental stage, metabolic conditions, duration and intensity of stress. Under drought stress conditions, the activity of antioxidant enzymes in tolerant plants is greater than that of sensitive plants, so it seems that antioxidant enzymes play an important role in increasing plant tolerance to drought stress.

The aim of present study was to investigate the effect of 24-epi-brassinolide (EBR) on coriander plants growth parameters under mild and severe drought stress and to determine the best combination of EBR concentration and drought stress on coriander essential oil contents and yield.

Materials and methods

Plant material: Coriander seeds were bought from PakanBazr®, Iran, disinfected with 0.2% benomile, and rinsed thoroughly with sterile distilled water. The seeds were sown on 0.5×20 mas furrows and ridges, respectively, in an individual research farm in Jiroft (Southeast of Kerman province) in 2020.

Irrigation and 24-epibrassinolide treatments: Three levels of drought stress (-2 (control),- 3.5 and -4.5 bar of soil matric potentials as the first factor and three concentrations of plant hormone soluble in ethanol (0, 0.5 and 1 µM EBR) Purchased from Sigma-Aldrich® as the second factor by foliar spray were used as treatments in this study. Initially, all seedlings were regularly irrigated once a week, but when the second leaf emerged, we applied drought stress levels. Common cultural practices such as weeding, pest and disease control, and fertilization were done normally.

Growth parameters: Two months after the commencement of drought stress, plant growth parameters such as: main stem length and diameter, shoot fresh and dry weight, leaves chlorophyll *a* and *b*, leaves relative water content, leaves electrolyte leakage and essential oil yield and composition were measured by the following methods:

Chlorophyll *a* and *b* measurement: Leaf samples (0.25 g) were homogenized in acetone (80%). Extract was centrifuged at 3,000×g, and absorbance was recorded at 646.8 nm and 663.2 nm for the chlorophyll assay and 470 nm for the carotenoids assay by a UV-Visible spectrophotometer (Cary50, Germany). Photosynthetic pigments were calculated according to the following formula (Lichtenthalerand Wellburn, 1983):

Chl *a* = (12.25 A663.2– 2.79 A 646.8)

Chl *b* = (21.21A 646.8 – 5.1 A 663.2)

Car = (1000 × A470 – 3.27 × chl a – 104 × chl b) / 227

Relative water content (RWC): Leaves RWC were determined and calculated by:

(FW- DW) / TW- DW) × 100

where FW is the sample fresh weight, TW is the sample turgid weight after saturating with distilled water for 24 h at 4 °C, and DW is the oven-dry (70 °C for 48 h) weight of the sample (Weatherley, 1950).

Leaf electrolyte leakage: One cm^2 piece of leaf without midribs or major veins was placed in test tubes containing 10 mL of distilled water, and the EC was recorded at room temperature. The same was kept at 40 °C for half an hour and recorded after cooling, and then boiled at 100 °C for another half an hour. EL was determined by the formula (Lutts *et al*., 1996).

Electrolyte Leakage (%) = (EC at 40 °C – EC at room temperature) / (EC at 100 °C) × 100.

Extraction of essential oil: The essential oil of leaf samples was extracted through hydro distillation by Clevenger apparatus. Fresh leaves from each treatment were collected during the full flowering stage, air-dried in shade, and weighed before the extraction of essential oil. 100 g of leaves were put in the flask of Clevenger apparatus and 1 L of distilled water was added. The samples were heated continuously for 4 h at 120 °C. Since the extraction rate of volatiles from coriander leaves was very low (0.1%), it was difficult to collect the essential oil directly from the receiving tube of the Clevenger. Therefore, the distillates containing essential oil from the receiving tube were collected in a glass bottle, and we put them in a liquid extractor containing dichloromethane as the solvent and run it continuously for 7 h. After that, the solvents were evaporated via rotary evaporator and concentrated to 1 mL, and the pure essential oils were weighted to calculate their percentage yields (w/w). The essential oils were stored in sealed tubes at -20°C until further analysis. All experiments were repeated twice (Shahwar *et al*., 2012).

Identification of essential oil composition: The essential oil composition of coriander leaves was identified by comparison with the gas chromatographic retention index. An Agilent model 6890 (Agilent Technologies, Santa Clara, CA) gas chromatograph equipped with a 60 m × 0.32 mm i.d. (df) (0.25 µm) bonded phase DB-1 fused silica capillary column (Bellefonte, PA, USA) and a flame ionization detector were used to obtain the Kovats gas chromatographic retention indices. Then the results are compared to reference standards. The oven temperature was programmed from 70 to 250 °C at 3 °C/min and held for 40 min. The linear helium carrier gas flow rate was 29 cm/s. The injector temperature and detector temperature

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were 200 and 250°C, respectively (Shahwar *et al*., 2012).

Vegetative yield: The yield of vegetative organs including leaves and tender stems of plants was calculated during three harvest at 35, 45 and 55 days after planting in autumn.

Oxidative enzymes: To measure the polyphenol oxidase activity, the method of Raymond *et al*. 1993 was used. Also, the activity of catalase was measured by the method of Dehindsa *et al*. 1981, and finally, the guayacolperoxidaseenzyme activity was measured using the method of Mohseni *et al*., 2019.

The field experiments were carried out as a split plot based on a complete block design with three replications and 9 treatments. The data were subjected to statistical analysis using MSTAT software. Analysis of variance (ANOVA) and Duncan's multiple range tests at 5% of the probability level were performed to determine significant differences among the treatments.

Results

Growth parameters: The main stem length (cm) and diameter (mm) were significantly decreased under drought stress (P≤5%). Exogenous application of 0.5 µM 24-epibrassinolide (EBR) improved the growth of the coriander control plants (Table 3). The interaction between treatments (drought stress levels and 24-epi-brassinolide concentrations) was significant, and the highest values of stem length and diameter were obtained by foliar spraying of 0.5µM of EBR (35.4 cm and 2.3 mm, respectively). It seems that the treatment of EBR with 0.5 µM compared with a concentration of 1 µM showed better results to mitigate the effects of water deficit on the length and diameter of the main stem as two important shoot growth parameters.

The interaction of drought and EBR concentrations was significant on Shoot fresh and dry weights (Table 1). It was observed that the shoot fresh and dry weights of coriander were greater in 0.5 and 1µM of EBR compared to control under all water deficit irrigation. In other words, application of 24-epibrassinolide can alleviate the negative effects of the water deficit on coriander fresh and dry weight, especially at 1 µM (Table 3).

There were statistically significant differences in the effect of both EBR and drought on coriander leaf photosynthetic pigments (Table 2). The leaves chlorophyll *a* and *b* contents decreased under the second (-3.5 bar) and third (-4.5 bar) levels of water deficit. Both 0.5 and 1µM concentrations of EBR were able to protect the leaf chlorophyll *a* and *b* against the damaging effects of water deficit. The foliar application of EBR on control plants (-2 bar) had no significant effect on the pigment contents. However, spraying of 1 µM EBR under moderate (-3.5 bar) and severe drought levels (-4.5 bar) resulted in significant rises of chlorophyll *a* and *b* (P<0.05) (Table 4).

Leaf relative water content (RWC) and electrolyte leakage: The highest values of relative water content and the least of electrolyte leakage of coriander leaves were obtained at -2 bar (control). The electrolyte leakage (EL) is considered an indicator of membrane permeability, and it was measured in the leaves. During the drought period, electrolyte leakage increased markedly (Table 4). Plants affected by the second and third levels of drought showed higher membrane permeability than the control (-2 bar). Application of EBR at 1 µM could help to maintain the leaf relative water content of coriander under moderate and severe stress levels and decrease the leaf electrolyte leakage.

Leaf essential oils: Essential oil was extracted from coriander leaves. Generally, increase in essential oil yield was observed under -3.5 (moderate stress) and -4.5 bar (severe stress). While the highest percentage of all six essential oil (linalool, (E)-2-decenal, (E)-2-hexa-decenal, carvone, α-pinene and (E)-2-dodecenal) were obtained under severe levels of drought stress (Figure 1). It seems that foliar spray of EBR 0.5µMunder severe drought increased the main essential oil composition such as: linalool (Figure 1A), (E)-2-decenal (Figure 1B), (E)-2-hexa-decenal (Figure 1C). The lowest essential oil yield showed in control plants. Notably, severe drought (-4.5 bars) had significant and strong effects on production of α-pinene in coriander leaves (Figure 1C).

Oxidative enzymes: The highest activities of polyphenol oxidase and catalase were obtained in severe drought stress conditions (-4.5 bar) and related to coriander plants treated with brassinolide 1 µM. By comparing irrigation treatments, it is clear that reducing the amount of irrigation water (up to the more severe stress level of this experiment, -4.5 bar) caused a significant increase in the activity of this enzyme. In such a way that plants treated with matric potential -4.5 times and -2 times had the highest and lowest enzyme activity, respectively (Table 4).

Vegetative yield: According to the results of the table 1, the mutual effects of drought stress and foliar spraying with brassinolide were significant at the 1% probability level for the yield trait. At the medium stress level, irrigation at a matric potential of -3.5 bar, the use of 0.5 µM concentration of brassinolide compared to 1 µM and zero concentration (without the use of brassinolide) was able to maintain the vegetative yield of coriander. It seems that the concentration of 0.5 µM brassinolide preserves plant yield better than the concentration of 1 micromolar in drought stress conditions, so this concentration can be introduced as the sample concentration of this compound in maintaining the yield of coriander under the conditions of this experiment (Table 4).

Discussion

A decrease in plant growth parameters was observed under drought stress (Tables 1 and 2). The reduction of main stem length and diameter and fresh and dry weights was more pronounced compared to control

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Table 1. The ANOVA analysis of the interaction between drought and EBR on some characteristics of Coriander

S.O.V	df	Relative water content	Electrolyte leakage	catalase	Polyphenol oxidase	Guaiacol peroxidase	Vegetative yield	stem length	stem diameter	Shoot fresh weight
Block	2	48.358^{ns}	0.001^{ns}	0.00007^{ns}	0.00001^{ns}	0.0003^{ns}	103427.5^{ns}	1.543^{ns}	61.34^{ns}	2.45^{ns}
Drought (A)	2	1678.2^{**}	0.242^{**}	0.123^{**}	0.009^{**}	0.281^{**}	18320.3^{*}	0.0312^{*}	678.61^{*}	22.36^{*}
EBR (B)	2	63.51^{ns}	0.028^{**}	0.02^{**}	0.00002^{**}	0.012^{ns}	3427.5^{**}	0.18^{*}	165.72^{*}	1.76^{*}
(A) ×(B)	4	22.56^{ns}	0.007^{ns}	0.001^{**}	0.00005^{*}	0.002^{ns}	38460.7^{**}	0.045^{*}	51.44^{*}	2.09^{*}
Error	-	33.57	0.003	0.0002	0.00001	0.005	7.23	0.0415	2.03	11.9
C.V	-	7.97	9.45	5.42	5.56	14.15	20.8	11.38	5.5	3.45

***, ** and ns are significant at 5%, 1% and non-significant levels, respectively**

Table 2. The ANOVA analysis of the interaction between drought and EBR on some characteristics of Coriander

S.O.V	df	Shoot dry weight	Chl *a*	Chl *b*	Linalool	E-2-decenal	E-2_ hexadecenal	carvone	α-pinene	E-2-Do decenal
Block	2	0.151^{ns}	0.016^{ns}	0.003^{ns}	0.890^{ns}	103427.5^{ns}	0.009^{*}	0.18^{ns}	0.83^{ns}	0.66^{ns}
Drought (A)	2	12.44^{**}	0.838^{*}	0.638^{*}	59.81^{**}	18320.3^{*}	32.41^{**}	3.31^{**}	70.56^{**}	468.8^{**}
EBR (B)	2	0.215^{ns}	0.049^{*}	0.048^{*}	0.846^{**}	3427.51^{**}	0.39^{**}	0.526^{**}	0.71^{*}	75.18^{**}
(A) ×(B)	4	0.056^{*}	0.024^{*}	0.015^{*}	0.713^{**}	38460.7^{**}	0.087^{**}	0.270^{**}	0.457^{*}	26.31^{*}
Error	-	0.15	0.012	0.001	0.219	7.23	0.006	0.182	0.339	6.42
C.V	-	7.71	6.55	5.24	5.29	20.8	1.52	23.33	3.45	11.28

***, ** and ns are significant at 5%, 1% and non-significant levels, respectively**

Table 3. Means comparison for morphological and physiological characteristics of coriander under different levels of drought and EBR

Drought deficit levels (bar)	24-epibrassinolide (Mμ)	Shoot fresh weight (g)	Main stem diameter (mm)	Main stem length (cm)	Chlorophyll b (mg g^{-1}FW)	Chlorophyll a (mg g^{-1}FW)	Shoot dry weight (g)
-2	0	67.4 ± 0.23^{b}	2.1 ± 0.24^{a}	34.3 ± 1.04^{a}	1.15 ± 0.05^{a}	2.09 ± 0.03^{a}	10.24 ± 0.02^{a}
	0.5	67.8 ± 0.37^{b}	2.3 ± 0.29^{a}	35.4 ± 1.11^{a}	1.1 ± 0.03^{a}	2.03 ± 0.05^{ab}	10.21 ± 0.01^{a}
	1	71.3 ± 0.16^{a}	2.2 ± 0.41^{a}	33.6 ± 1.08^{a}	1.12 ± 0.03^{a}	2.05 ± 0.23^{a}	10.22 ± 0.02^{a}
-3.5	0	61.8 ± 0.41^{c}	1.8 ± 0.18^{b}	29.7 ± 0.94^{b}	0.73 ± 0.1^{c}	1.57 ± 0.13^{c}	8.85 ± 0.03^{b}
	0.5	65.4 ± 0.33^{bc}	2.2 ± 0.31^{a}	$31.\pm1.01^{b}$	0.72 ± 0.02^{c}	1.55 ± 0.09^{c}	8.80 ± 0.02^{b}
	1	66.9 ± 0.35^{bc}	1.7 ± 0.16^{c}	28.5 ± 0.94^{b}	0.96 ± 0.03^{b}	1.86 ± 0.11^{b}	9.27 ± 0.02^{ab}
-4.5	0	$54.\pm0.51^{d}$	1.6 ± 0.19^{c}	25.1 ± 1.48^{c}	0.54 ± 0.01^{d}	1.41 ± 0.13^{c}	7.77 ± 0.03^{c}
	0.5	56.1 ± 0.47^{d}	2.1 ± 0.34^{b}	29.1 ± 1.24^{b}	0.56 ± 0.02^{d}	1.45 ± 0.10^{c}	7.75 ± 0.04^{c}
	1	68.4 ± 0.24^{b}	1.5 ± 0.14^{c}	24.2 ± 1.63^{c}	0.69 ± 0.01^{c}	1.61 ± 0.08^{bc}	8.11 ± 0.03^{bc}

Means with the same letter in the same column do not significantly differ at P ≤%5

Table 4. Means comparison of the morphological and physiological characteristics of coriander under different levels of drought and EBR

Drought levels (bar)	EBR (μM)	Catalase (unit/mg Protein/min)	Electrolyte leakage (mm)	Relative water content(%)	Vegetative yield (kg/ha)	Guaiacol peroxidase	Polyphenol oxidase
						(unit/μmol Protein/min)	
-2	0	0.113 ± 0.05^{e}	0.48 ± 0.03^{d}	87 ± 2.68^{a}	5360 ± 24^{a}	0.317 ± 0.03^{e}	0.025 ± 0.007^{e}
	0.5	0.119 ± 0.05^{e}	0.46 ± 0.07^{d}	87 ± 1.52^{a}	5500 ± 25^{a}	0.321 ± 0.03^{e}	0.028 ± 0.007^{e}
	1	0.115 ± 0.05^{e}	0.47 ± 0.04^{d}	86 ± 2.68^{a}	5545 ± 25^{a}	0.342 ± 0.04^{de}	0.027 ± 0.007^{e}
-3.5	0	0.288 ± 0.07^{b}	0.72 ± 0.1^{c}	67 ± 1.29^{b}	4750 ± 23^{b}	0.454 ± 0.04^{cd}	0.054 ± 0.009^{d}
	0.5	0.273 ± 0.06^{b}	0.73 ± 0.02^{c}	67 ± 1.3^{bc}	5240 ± 24^{a}	0.462 ± 0.04^{c}	0.057 ± 0.009^{d}
	1	0.383 ± 0.07^{b}	0.59 ± 0.12^{c}	74 ± 2.08^{b}	5047 ± 23^{b}	0.579 ± 0.06^{bc}	0.067 ± 0.01^{c}
-4.5	0	0.397 ± 0.07^{a}	0.84 ± 0.02^{a}	58 ± 2.31^{c}	2878 ± 19^{d}	0.661 ± 0.06^{ab}	0.085 ± 0.01^{b}
	0.5	0.393 ± 0.07^{a}	0.85 ± 0.04^{a}	58 ± 1.70^{c}	3412 ± 21^{c}	0.665 ± 0.06^{ab}	$0.0860.01^{b}$
	1	0.411 ± 0.09^{a}	0.69 ± 0.06^{bc}	65 ± 1.98^{bc}	2986 ± 22^{d}	0.714 ± 0.06^{a}	$0.0990.01^{a}$

Means with the same letter in the same column do not significantly differ at P ≤%5

plants. However, EBR, to some extent, reduced the deleterious effects of water stress. Various studies have also reported that BR applications increase water stress tolerance in plants (Coll *et al.*, 2015; Yadava *et al.*, 2016).

The application of brassinosteroid analogues was efficient, especially when applied under drought stress the appearance, it was the treatment where the greatest number of oil and a substantial increase in estimated yield were observed. The use of this growth regulator in

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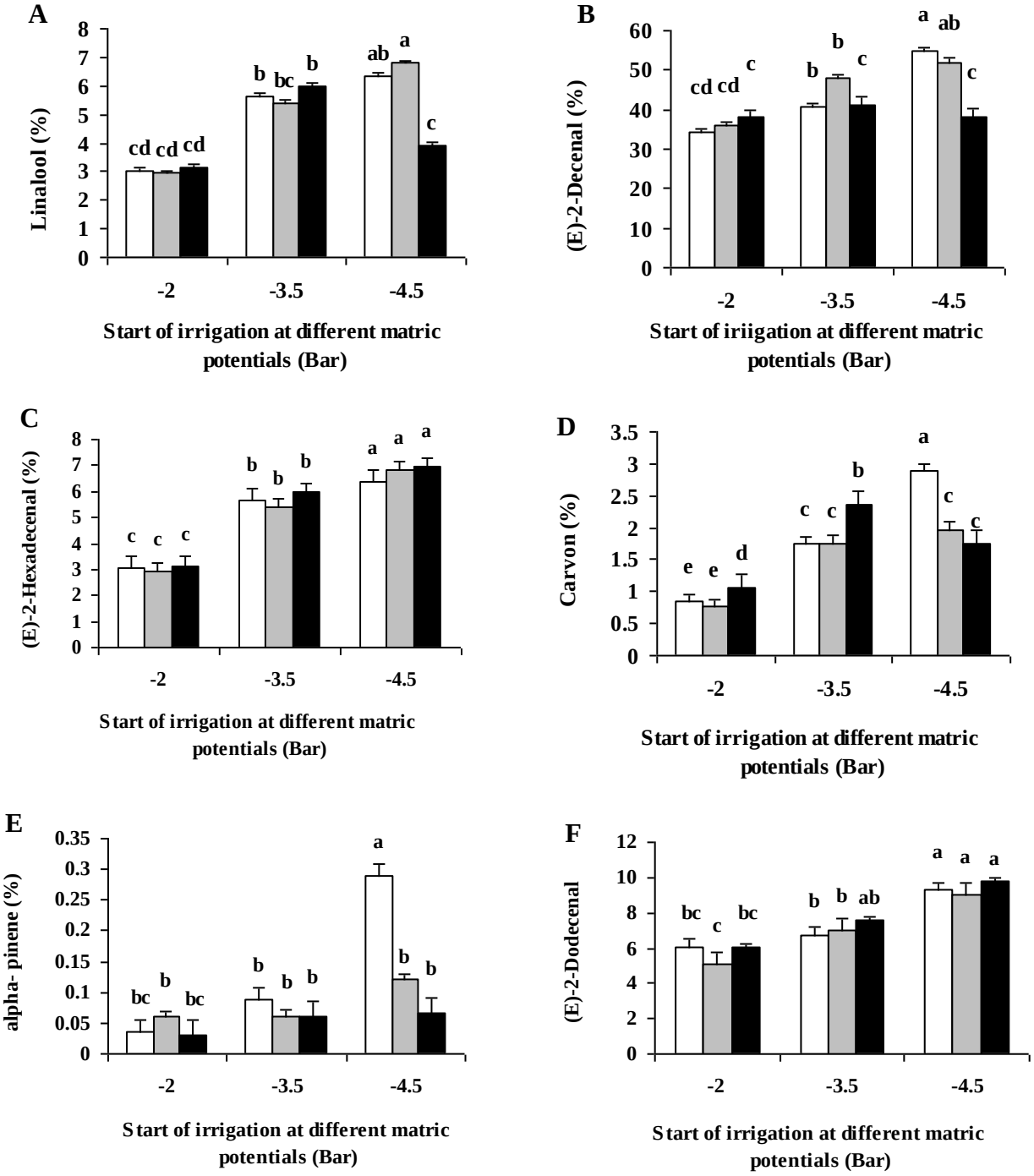

Figure 1. Interaction effects between different water deficit levels and 24-epi-brassinolide on some essential oils (%) of coriander leaves. (white columns: 0, gray columns: 0.5 µM of brassinolide and black columns: 1 µM of brassinolide) (A: Linalool, B: (E)-2-decenal, C:E-2-hexadecenal, D:Carvone, E:α-pinene, F:E-2-dodecenal)

several fruits, such as passion fruit, indicated an increase in the number of fruits in the BR-3 treatment was Brassinosteroids, especially 24-EBR, caused an increase in plant biomass (Li *et al*., 2008).

Application of BRs could partially alleviate the detrimental effect of water stress on the growth of soybeans by improving the antioxidant system and promoting dry weight accumulation (Zhang *et al*., 2008). BRs participate in the processes of gene expression, transcription and translation in normal and stressed plants (Zhang *et al*., 2008). Brassins were reported to cause elongation of all parts of the bean plant, *viz*., length of stem, shoots, roots, weight of pods and quality of buds (Katsumi, 1985; Choi *et al.*, 1986). However, an increase was also observed in the leaf biomass and leaf area of *Cucumis sativus* by exogenous application of brassinosteroids (Yu *et al*., 2004).

Water stress in our study also reduced chlorophyll

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contents. In this study, Chl *b* was more sensitive to water stress than Chl *a* (Li *et al*., 2008). Although the application of EBR significantly increased the chlorophyll content. This effect was more noticeable at 1 µM EBR. Foliar sprays of BR to salt stressed seedlings ameliorated the negative effects of salt by increasing chlorophyll content (Anuradha and Rao, 2003). Our result may suggest that EBR protects photosynthetic pigments from water induced oxidative stress. This view is further supported by the fact that chloroplasts are a major source of reactive oxygen species production (ROS) in plants (Ormaetxe *et al*., 1998). The protective effect of exogenous BRs against oxidative stress generated by several environmentally unfavorable conditions such as drought (Behnamnia *et al.*, 2009), salinity (Nunez *et al*., 2003), and metals (Hayat and Ahmad, 2003; Ali *et al*., 2008). Brassinosteroids remove the inhibitory effects of salt and drought stress on pigment levels, and this could be one of the reasons for the noticed growth stimulation induced by brassinosteroids under saline and drought conditions (Anuradha and Rao, 2003).

The extent of membrane damage by salinity was assessed by an indirect measurement of solute leakage. Water deficit stress induced significant increases in electrolyte leakage compared to the control, as shown in Figure 1. This phenomenon has already been observed in various crops (Lutts *et al.*, 1996; Ghoulam *et al*., 2002), which could be associated to chain reactions initialized by free radicals (Cowan *et al.*, 1992). It seems that BRs may help membrane integrity by enhancing the level of the antioxidant enzymes that protect plants from oxidative damage (Ali *et al*., 2008). Leaf relative water content (LRWC) is an important physiological factor, which determines the tolerance of plants to drought stress (Sanchez-Blanco *et al*., 2002). In this experiment relative water content notably decreased under water stress, and electrolyte leakage increased significantly. Foliar spray of EBR reverse the negative effects of water stress on coriander plants and maintain the integrity of membrane cells. BR application resulted in increases in relative water content (RWC), nitrate reductase activity, chlorophyll content and photosynthesis under water stress conditions (Sairam *et al.*, 2005). Brassinolide (BL) application was shown to modulate stomatal aperture by promoting stomatal closure and inhibiting stomatal opening, which is an important mechanism for preventing water loss and maintaining the leaf RWC (Haubrick and Assmann, 2006). The coriander essential oil has many promising antimicrobial and antioxydative activities that can be used in the food, pharmaceutical and cosmetic industries. The essential oil can be extracted from all parts of plants. In this study, essential oil was extracted from leaves at the beginning of flowering stage. The essential oil yield was reduced under water deficit stress, but the essential oil percentage was increased under water deficit stress. EBR enhanced the production of all six major components of EO under moderate (-0.35 bar) and severe (-0.45 bar) water stress. Drought stress increases the essential oil percentage of many medicinal and aromatic plants. Drought stress had a significant effect on oil yield and oil percentage of calendula. The results showed that the highest oil yield was achieved under non-drought conditions, and the highest oil percentage was achieved under drought condition (Rahmani *et al*., 2008). The effect of water deficit on fatty acids and essential oil yield and composition of *Salvia officinalis*aerial parts was investigated (Bettaieb *et al.*, 2008).

Drought significantly decreased the foliar fatty acid content and the double bond index (DBI) degree. Mainly by a strong reduction of the linolenic acid proportion and the disappearance of the palmitoleic acid. Moderate water deficit increased the essential oil yield (expressed as g/100 g on the basis of dry weight). The main essential oil constituents were camphor, thujone and 1,8-cineole. Regulated low-water stress enhances the accumulation of bioactive substances in some crops and medicinal plants (Hodges and Toivonen, 2008). Under water-deficit conditions, assimilation of CO_2 decreased. Consequently, an oversupply of the reducing equivalent ($NADPH_2$) occurs in the plant. This, in turn, pushes metabolic processes towards the synthesis of highly reduced compounds, such as isoprenoids, phenols, essential oils or alkaloids (Kleinwachter and Selmar, 2015). Catabolism of carbohydrates, fatty acids, and amino acids provides precursors required for the biosynthesis of compounds which support flavor and aroma development in fruits and vegetables (Schwab *et al*., 2008). Furthermore, waterdeficit stress also affects reactive oxygen metabolism in *Catharanthus roseus*. Jaleel *et al*. (2008) analyzed the changes in the reactive oxygen metabolism of *Catharanthus roseus* (L.) plants for H_2O_2 content, lipid peroxidation, and the free radical quenching systems (nonenzymatic and enzymatic antioxidants) under water (drought) stress and concluded that the water-deficit areas may be used for the cultivation of medicinal plants like *C. roseus*, and the economically important alkaloid production can be enhanced. The important thing is that, according to some studies it has been found that the protective effect of volatile isoprenoids can be particularly relevant under drought when stomata close, resulted in elevated leaf temperatures due to reduced transpiratory cooling of leaves. These conditions lead to a major buildup of volatiles inside the leaves (Sharkey and Singsaas, 1995). The effects of salinity stress on the essential oil of coriander leaves showed a significant increase in essential oil yield. The composition of EO was vary among the different part of plant. Linalool was the main compound, followed by linoleic acid (Neffatiand Marzouk, 2008).

Polyphenol oxidase enzyme is one of the important enzymes in the plant's defense system, which plays a role in defense against biological and environmental

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stresses. Changing the activity of this enzyme during environmental stress affects the amount of phenolic compounds in the cell (Saiedian *et al*., 2007). On the other hand, the application of brassinosteroid can increase the activity of catalase and also increase the activity of peroxidase in radish seeds. Water deficit stress and treatment of the tomato plant with brassinosteroid increased the activity of the antioxidant enzymes, including catalase, superoxide dismutase and ascorbic peroxidase, and the amount of antioxidant compounds, including ascorbate, carotenoids and proline. The activity of antioxidant enzymes usually increases in conditions of abiotic stress, and this increase in activity is related to the increase in cell protection. During periods of drought stress, water communication plays a key role in regulating the activity of antioxidant defense mechanisms in onion leaves (Behnamnia *et al*., 2009).

Conclusion

It can be concluded that EBR could mitigate the deleterious effects of water stress on some of the morphological and biochemical traits of coriander. Further experiments will be carried out to determine the mechanism of the effect of EBR on coriander fruit yield. Considering the results of this study, the application of EBR to coriander leaves under water deficit irrigation can be a potential new source of greater, cheaper and faster essential oil production.

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