ORIGINAL ARTICLE

Application of zeolite and bacterial fertilizers modulates physiological performance and essential oil production in dragonhead under different irrigation regimes

Khalil Karimzadeh Asl[1] · Mehrnaz Hatami[2]

Abstract

Organic farming and low-input production agroecosystems are the major components of sustainable agriculture; therefore, application of biofertilizers containing beneficial soil microorganisms to reduce environmental difficulties related to the use of chemical and manufactured fertilizers is the first step towards sustainability. Considering the importance of the production of medicinal and aromatic plant materials in sustainable ways, a 2-year field research was done as a split factorial experiment based on a randomized complete block design with three replicates. Three factors were investigated: (1) water-deficit stress in three levels comprising irrigation after 30%, 60%, and 90% depletion of available soil water (ASW) as main factor, (2) zeolite application (0 and 8 t ha^{-1}), and (3) seed inoculation with biofertilizers including nitroxin, phosphate barvar-2, and mixture of nitroxin × phosphate barvar-2, and the control (non-inoculation) as subplots. Results showed that the examined traits such as leaf relative water content, chlorophyll a and b, and total chlorophyll contents, leaf soluble sugars and proline contents, flower yield, essential oil percentage and yield, and irrigation water use efficiencies for flower yield (IWUE$_{FY}$) and for essential oil yield (IWUE$_{EOY}$) were significantly ($p < 0.05$) influenced by experimental treatments in dragonhead for both years. According to second-order interaction among treatment groups, the highest IWUE$_{EOY}$ (3.117 ml m^{-3}) was obtained in nitroxin × phosphate barvar-2 and zeolite treatments under 60% depletion of ASW. A positive and highly significant ($r_{0.01} = 0.85$ and 0.98) correlation was observed between essential oil content and essential oil yield and IWUE$_{EOY}$, respectively. Regarding significant interaction between biofertilizers and zeolite, the treatment combinations of zeolite and nitroxin × phosphate barvar-2 could improve physiological functions and essential oil yield of dragonhead plants following irrigation after 60% depletion of ASW.

Keywords *Dracocephalum moldavica* L. · Biostimulants · Flower yield · Essential oil production · Deficit water stress · Photosynthetic pigments

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Introduction

Dracocephalum moldavica L. (known as Moldavian dragonhead) is an annual herb and aromatic medicinal plant belonging to the mint (Lamiaceae/Labiatae) family originated/naturalized from around southern Siberia and the Himalaya and typically cultivated in temperate areas of Eastern Europe and central Asia (Omidbaigi 2005). It includes approximately 45 species in the world, and has received much attention for use in several fields such as flavour and fragrance, food and pharmaceutical industries (Omidbeigi 1997).

Biosynthesis of secondary bioactive compounds/metabolites in the aromatic medicinal herbs is differently impacted by both genetics and environmental agents (Gholizade et al. 2010b). On the other hand, the phytochemical quality and

quantity of plants are affected by both special biotic and abiotic environmental agents called elicitors (Clark and Menary 1980). However, the full control of environmental factors is not possible, but can be managed with specific procedures, in which plants under such adverse conditions seem to maximize their potential to provide optimum yield. To enhance plant productivity under environmental perturbation conditions, good agricultural practices to adequately address soil and water conservation objectives such as effective management of inputs in time, maintaining soil structure and organic content, scheduled irrigation based on plant requirements and soil characteristics, avoiding soil salinization through limiting water input to the plant requirements, preventing drainage and fertilizer run-off, understanding important physiological and biochemical pathways and various mechanisms of defense to reduce stress are mainly required (Hatami and Ghorbanpour 2014; Ibanez et al. 2010). The global demand for agricultural crops and products is increasing and is anticipated to be at least twofold as the world's population reaches approximately 9.1 billion people by the year 2050. Both greater quantity and higher quality of food in response to increasing demand will need a greater emphasize on the agricultural section. Therefore, good agricultural operations along with effective inputs and technology use are one of the best tools to improve plant performance.

Organic production and low off-farm input farming systems are the major aims of sustainable agriculture; therefore, using biological fertilizers to diminish environmental concerns associated with the use of synthetic fertilizers is a main step towards sustainability. Moreover, to be environmentally friendly, the application of biofertilizers such as nitroxin (with free-living and or containing symbiotic and non-symbiotic nitrogen fixing bacteria) as well as phosphate-solubilizing soil microorganisms improves the quality of the soil physical, chemical and biological characteristics and enhances soil fertility and plant productivity without adverse impacts on the environment (Tahami et al. 2017).

Drought is one of the most significant environmental stressors/perturbations influencing growth, development and production of plants, especially in the arid and semi-arid areas of the world (Baiazidi-Aghdam et al. 2016). Water-deficit stress significantly impacts a broad spectra of plant developmental, physiological and biochemical processes (Geerts and Reas 2009) and productivity. Therefore, watering treatments must be applied efficiently to improve the sustainability of cultivation in agricultural ecosystems (Geerts and Reas 2009).

In countries where water shortage is the major restricting factor for cultivation and agriculture, researchers and farmers use some efficient ways to decrease adverse impacts of water deficit stress; one possible way to mitigating the influence of drought stress on plant performance is through the addition of natural zeolite to the soil environment

(Manivannan et al. 2007). Zeolites may have great potential to be used as valuable materials in reclamation and restoration of soil and storing available soil water for plant growth and production (Zhang et al. 2007).

With respect to the importance of water in agricultural sector and the spread of water shortage by population growth especially in arid and semi-arid areas of the world, cultivation of water-efficient medicinal plants such as dragonhead in a sustainable manner seems advantageous for these regions. Little is known regarding the use of biofertilizers and zeolite under different watering conditions and their double and triple interactions on physiological functions and efficiency of irrigation for flowering parts and essential oil yield in field-grown *D. moldavica* L. plants. Thus, the present work was aimed to investigate the impacts of zeolite and bacterial biofertilizer application on physiological characteristics, essential oil content and yield of *D. moldavica* L.under deficit water stress conditions in two continuous years.

Materials and methods

Plant materials, experimental setup and treatments

A 2-year, 2014 and 2015, field research was performed at the experimental area of the Seed and Plant Improvement Institute in Karaj, Iran (latitude 35°48′N, longitude 51°26′E, altitude 1321 m above mean sea level) with relatively temperate and arid climate, and mean annual precipitation and temperature of 250 mm and 14.2 °C, respectively. The daily precipitation and air temperature during the experiment in both 2014 and 2015 years are given in Fig. 1. The soil samples were taken from 0 to 30 cm depth approximately 2 months before sowing, and the related physical and chemical properties of the tested soil are presented in Table 1. According to the soil chemical analysis, macronutrients except nitrogen were at sufficient levels; therefore, only a nitrogen-containing fertilizer; urea (46% N), was applied at a rate of 140 kg ha^{-1} basis to the soil before commencing irrigation treatment.

This study was performed as a split factorial experiment based on a randomized complete block design (RCBD) with three replicates. Three factors were investigated: (1) drought stress in three irrigation regimes [(consisting irrigation after 30% (I1), 60% (I2), and 90% (I3) depletion of available soil water)] as main factor, (2) zeolite application (0 and 8 t ha^{-1}), and (3) seed inoculation with biofertilizers including nitroxin, phosphate barvar-2, and a mixture of nitroxin × phosphate barvar-2, and the control (non-inoculation with biofertilizers) as subplots. Nitroxin (containing 10^7 CFU/ml free-living nitrogen-fixing bacteria including *Azotobacter* sp., *Azospirillum* sp.,

Fig. 1 Daily maximum and
minimum precipitation (mm)
and air temperature (°C) during
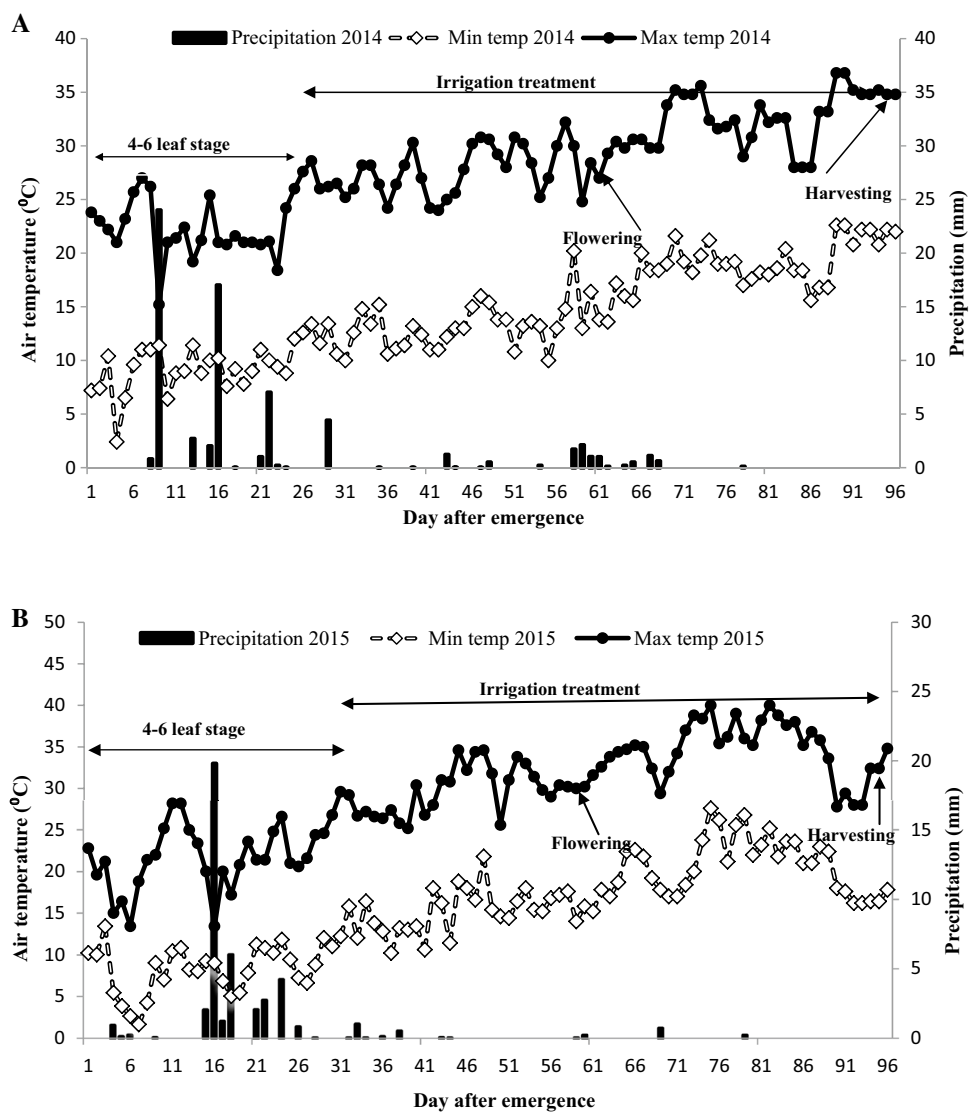the growing seasons in 2014 (**a**)
and 2015 (**b**)

Table 1 Selected
physicochemical properties
of soil and zeolite used in the
study

Soil (clay-loam)		Zeolite	
Characteristics	Quantity	Characteristics	Quantity
Bulk density	1.4 g cm^{-3}	P_2O_5	0.1 g kg^{-1}
Sand	31%	K_2O	24 g kg^{-1}
Silt	29%	CaO	16 g kg^{-1}
Clay	40%	MgO	1 g kg^{-1}
Total N	0.08 mg kg^{-1}	Fe_2O_3	12 g kg^{-1}
Available P	13.5 mg kg^{-1}	Al_2O_3	85.5 g kg^{-1}
Available K	180 mg kg^{-1}	TiO_2	0.1 g kg^{-1}
Organic C	0.69%	MnO	0.3 g kg^{-1}
EC	1.1 ds m^{-1}	SiO_2	720 g kg^{-1}
pH	7.8	Na_2O	12.3 g kg^{-1}
Field capacity (FC, % by volume)	18.72	CEC	200 meq 100 g^{-1}
Water-holding capacity at FC	242 g kg^{-1}	Cl	–
Wilting point	8.67	SO_3	–

and phosphate-solubilizing bacteria from *Pseudomonas* genus) is the traditional name of biological nitrogen. Also, phosphate barvar-2 (phosphate-solubilizing bacteria) containing *Pantoea agglomerans* (p5) and *Pseudomonas putida* (p13) (each mL contains 10^8 microorganisms). The field was deeply (35–40 cm) ploughed in the late autumn after being fallowed, and then was ploughed superficially (10–12 cm) in the early spring and finally levelled by hand trowels. Experimental plots consisted of six rows that were 3 m long and 0.75 m apart (each plot size: 2.25 m^2), which were prepared after soil arrangement. There were 4 m gaps between the blocks, and a 1.5 m alley was considered between the plots to avoid lateral water movement and other interferences.

The seeds of *D. moldavica* L. were provided from Pakan Bazr Co. Esfahan, Iran. The seed viability was further examined before starting research and was determined to be 98% on average.

The seeds were planted in the end of April in soil at a uniform depth of 1 cm, and seedling emergence subsequently occurred about 3 weeks after planting (mid/late May).Thinning operations were done at the end of the second week of emergence, so that the distance between plants on the lines and plant spacing among lines was 12.5 and 40 cm, respectively. Weeding operations were carried out manually without using any chemical herbicides. No pesticides and fungicides were used as well during both years of the experiment. Flowering process initiated approximately 60 days after emergence (i.e. mid/late July). The plants were finally harvested at 60–70% flowering stage (i.e. about 95 days after emergence or late August) in the both experiment years.

The zeolite physicochemical characteristics are also given in Table 1. A random sample was taken from ten plants per each plot area after leaving out 1 m from the edge of the field (i.e. beginning and end of each experimental plot), where plants grow differently due to the margin/and or border effect.

Irrigation treatment

In all experimental plots, dragonhead plants were watered in a uniform manner when 30% of soil moisture, namely available soil water (ASW), was drained or depleted for 25 days (i.e. 4–6 leaf growth stage). Then when the plants were approximately 15 cm tall (i.e. 45 days after sowing or mid June), watering regimes (irrigation treatments) were applied and continued until plants reached 60–70% flowering stage (i.e. 115 days after sowing). All experimental operations such as soil ploughing, seed preparation, cultivation, treatments, etc. were repeated in the following year (2015). Similarly, for 2015 the plots containing plants were irrigated two times in May–June (4–6 leaf growth stage) and the irrigation treatments were initiated in mid-June and continued until early August 2015.

The volume of water accumulated in rhizosphere (the root zone of the soil) between field capacity (FC) and the permanent wilting point (PWP) is described as ASW, which can be easily used by plants. ASW was calculated based on the following equation:

$$ASW = \left(W_{FC} - W_{PWP} \right) \times Bd \times V, \tag{1}$$

where W_{FC} and W_{PWP} are the gravimetric soil–water content (%) at FC and PWP, respectively, Bd refers to the value of soil bulk density (g cm^{-3}) and V indicates the soil layer volume (m^3) at the depth of the root zone in *D. moldavica* L., which was determined to be different three times (i.e. June, July and August) during both the 2014 and 2015 growing season and approximately 20, 35 and 55 cm, respectively.

Readily available soil water (RAW) refers to the fraction of ASW that a plant can readily uptake from the entire root zone without enduring the consequences of drought stress. It was measured according to the procedure of Allen et al. (1998) as follows:

$$RAW = p \times ASW. \tag{2}$$

The value of p normally differs for various plants ranging from 0.3 to 0.7 based on root depth; however, the minimum p value is considered for shallow-rooted plants at maximum rates of evapotranspiration (ETc) (> 8 mm day^{-1}) and the maximum p value is designated for deep-rooted plants at minimum rates of ETc (< 3 mm day^{-1}). The p factor was applied to measure the required time of watering to avoid drought stress. A value of 0.50 for p is commonly used for many plants. Therefore, in this study the value 0.50 was used for *D. moldavica* L. The fraction p is specified as evaporation power of air according to the following equation:

$$p = p_{rec} + 0.04 \left(5 - ET_c \right), \tag{3}$$

where p_{rec} is the suggested factor for many field crops and p is the fitted factor for atmospheric evaporative requirement. Subsequently, the watering treatments/regimes were planned according to the procedure of Kramer and Boyer (1995) based on maximum allowable depletion (MAD) percentage of ASW in the root zone and then irrigation treatments were applied as follows:

[I1: irrigation after applying 30% of ASW (i.e., well watered), I2: irrigation after applying 60% of ASW (i.e., moderate water-deficit stress), I3: irrigation after applying 90% of ASW (i.e., severe water-deficit stress)] from the root zone. A TDR prob was used 2 days after irrigation to determine the soil water content and 1 day before the next irrigation.

The ASW depletion value related to the soil water potential (Ψ) was calculated via a soil water characteristic curve. The irrigation volume (V_{irrig}) required for increasing the root zone soil water to FC was determined by the following equation as described by Tafteh and Sepaskhah (2012):

$$V_{\text{irrig}} = \frac{\text{ASW} \times f}{\text{Ea}}. \qquad (4)$$

Here, f is the depletion fraction for soil water (30%, 60%, and 90%) from the root depth, and Ea is the efficiency of irrigation (%), which was supposed to be approximately 70% for the entire growing period. The water for irrigation treatments was distributed by a system of pipes and the value was determined by a flow meter. Furthermore, the cumulative values of soil water applied (mm) for different irrigation regime treatments (I1, I2 and I3) over the growing seasons in 2014 and 2015 are shown in Table 2.

Measurements

Determination of leaf relative water content (RWC)

At 60–70% flowering stage, leaf relative water content, soluble sugars and free amino acid proline contents, chlorophyll a, b and total chlorophyll, flower yield, essential oil percentage and yield, and $IWUE_{EOY}$ of three plants ($n = 3$) per experimental unit were determined. RWC was determined on leaf tissues obtained from the fourth fully developed leaf at the top of the plant. The fresh weights (FW) of leaves were measured immediately after sampling. Then, they were submerged in double distilled water in test containers at room temperature (22 °C) for 6 h and the turgid weight (TW) was estimated. Dry weight (DW) was measured after drying the samples in an electrical oven at 70 °C for 48 h. Finally, RWC was measured according to the following equation (Levitt 1980):

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100. \qquad (5)$$

Determination of plastid pigments

For extraction of chlorophyll (Chl), fresh leaf tissue (0.5 g) was blended with acetone (10 ml 80% V/V). The absorption of mixture was spectrophotometerically recorded at 645 and 663 for Chl a and Chl b, respectively. Plastid

pigment contents were calculated as previously described by Lichtenthaler (1987) as follows:

$$\text{Chl}\,a = \left(12.25 \times A_{663}\right) - \left(2.79 \times A_{647}\right) (\text{mg g}^{-1}\,\text{fw}^{-1}), \qquad (6)$$

$$\text{Chl}\,b = \left(21.5 \times A_{647}\right) - \left(5.1 \times A_{663}\right)(\text{mg g}^{-1}\,\text{fw}^{-1}), \qquad (7)$$

$$\text{Chl}\,a + b = \left(7.15 \times A_{663}\right) + \left(18.71 \times A_{647}\right)(\text{mg g}^{-1}\,\text{fw}^{-1}). \qquad (8)$$

Measurement of free proline and soluble sugar contents

The proline content of the leaf extracts was measured according to Bates et al. (1973). Fresh leaf tissue (500 mg) was homogenized in 10 ml of 3% sulfosalicylic acid and the extract was filtered. Then, the mixture was centrifuged at $10,000 \times g$ for 10 min. The extract (2 ml) was added into ninhydrin reagent and glacial acetic (both in 2 ml). The reaction solution was heated in a water bath at 100 °C for 1 h. Then, the reaction mixture was extracted with toluene (4 ml). Finally, the absorbance was noted spectrophotometrically at 520 nm against toluene blank and expressed as $(\text{mg g}^{-1}\,\text{fw}^{-1})$.

The total soluble sugars of leaves were determined following the procedure described by Irigoyen et al. (1992). Dried leaf (500 mg) was mixed in ethanol (95%) and then the homogenized extract (0.1 ml) was blended with freshly prepared anthrone (3 ml). The reaction mixture was heated for 10 min in a laboratory water bath. Finally, the absorption of the obtained samples was recorded spectrophotometrically at 625 nm. The glucose standard curve was used for determination of total leaf soluble sugars content.

Estimation of flower yield

At flowering stage (60–70%), plants were harvested from the middle portion in each plot (2.5 m^2) for estimation of shoot top flower yield. The harvested herbs were air dried for ten continuous days and then flower dry weight was measured and expressed as kg ha^{-1}.

Table 2 The cumulative water used for irrigation treatments [I1, I2 and I3 defined as 30, 60 and 90% depletion of available soil water (ASW), respectively, during the years 2014 and 2015]

| Month | Irrigation water applied (mm) | | | | | |
| | 2014 | | | 2015 | | |
	I1	I2	I3	I1	I2	I3
April	203	195	188	210	198	190
May	396	382	312	391	379	353
June	553	524	483	567	542	496
July	705	615	575	714	632	565
August	874	764	664	886	720	611

Quantification of essential oil content and yield

The air-dried shoot samples (100 g) of *D. moldavica* L. were powdered for isolation of essential oil. The essential oil of the drug fraction was extracted by the hydrodistillation technique for 3 h using a Clevenger-type apparatus based on the procedure suggested in British Pharmacopoeia (British Pharmacopoeia 1993). The resulting essential oils were dried over Na_2SO_4 and kept in tightly closed dark vials at 4 °C until further analysis.

Measurement of irrigation water use efficiency (IWUE)

The IWUE is defined as the ratio of the plant yield to the value of water used (Howell 1994). IWUE values for flower yield ($IWUE_{FY}$) and essential oil yield ($IWUE_{EOY}$) were estimated by dividing flower yield (kg ha^{-1}) and oil yield (kg ha^{-1}) by total water (m^3) used in m^{-2} for each treatment of the irrigation regime, respectively (Askari and Ehsanzadeh 2015).

Statistical analysis

The normality of the data set was tested using skewness and kurtosis before analysis of variance (ANOVA) and mean (ANOM). Proper data transformation was done for certain traits that showed non-normal distributions. Data were subjected to ANOVA and ANOM using the MSTAT-C statistical software. The Pearson's correlation coefficient was used for determination of correlations among the various measured traits by SPSS software version 16 (SPSS Inc., Chicago, IL, USA). Means were compared by Duncan multiple range test (DMRT) at the 5% probability level ($p \leq 0.05$).

Results

All examined traits of *D. moldavica* L. in the present study such as leaf relative water content, chlorophyll a and b and total chlorophyll contents, leaf proline and soluble sugars, flower yield, essential oil content, essential oil yield, irrigation water use efficiencies for flower yield ($IWUE_{FY}$) and essential oil yield ($IWUE_{EOY}$) were significantly ($p < 0.05$) influenced by the experimental treatments (i.e. zeolite, biofertilizers and soil moisture) and their main and interaction effects (two-way or double, and three-way or triple interactions) in both years (Table 3). The main mean values for the aforesaid characteristics are presented first (Table 4) and the interaction means are explained afterward (Tables 5, 6, 7, 8).

Relative water content (RWC)

Averaged over years, mean RWC decreased significantly with increase in water-deficit stress (Table 4). The effect of zeolite application was significant for RWC, and the highest RWC value was observed at 8 t ha^{-1} zeolite. Also, RWC was significantly influenced by biofertilizer treatments; however, the maximum value was obtained from nitroxin × phosphate barvar-2 (73.778%) followed by nitroxin (73.528%) and phosphate barvar-2 (73.250%), and the minimum was recorded for the control (72.139%). The mean of RWC for double interaction effects is shown in Table 7. In addition, combined treatment of nitroxin × phosphate barvar-2 caused the maximum value of RWC under 30% depletion of ASW (Table 8). Furthermore, application of fertilizers increased RWC in the presence of zeolite in comparison with control. There was no significant difference among fertilizer treatments (nitroxin, phosphate barvar-2 and nitroxin × phosphate barvar-2) in both years. In triple interaction effects, nitroxin × phosphate barvar-2 with zeolite application under 30% depletion of ASW caused maximum percentage (91.5%) of RWC (Table 8).

Chlorophyll contents

Mean comparison showed that the maximum content of chlorophyll *a* and chlorophyll *b* was obtained in 30% depletion of ASW (Table 4). Application of zeolite had a significant effect only on chlorophyll *a* content. The maximum value of chlorophyll *a* and chlorophyll *b* was observed in plants treated with zeolite compared to the control.

Also, combined application of nitroxin and phosphate barvar-2 fertilizers showed maximum value of chlorophyll *a* and chlorophyll *b* contents. Furthermore, the two-way interactions of water deficit × either zeolite, and/or biofertilizers showed that the 30% depletion of ASW with zeolite and biofertilizer application caused the maximum amount of chlorophyll *a* and *b* (Table 6). In 2015, the highest value of chlorophyll *a* and *b* were related to plants subjected to the first level of irrigation regime (I1) and nitroxin × phosphate barvar-2, respectively (Table 5). The treatment combinations (triple interactions) of zeolite, nitroxin × phosphate barvar-2 and irrigation after depleting 30% of the ASW resulted in the highest chlorophyll *a* and *b* (1.682 and 1.705 mg g^{-1} fw^{-1}) contents, respectively (Table 8).

Total chlorophyll content was significantly impacted ($p < 0.01$) by all employed treatments, the two and three interactions between them in both years. Over both years, total chlorophyll content was increased with application of zeolite and biofertilizers under all irrigation treatments; however, the highest value of total chlorophyll (3.195 mg g^{-1} fw^{-1}) was obtained from plants exposed to nitroxin × phosphate barvar-2, 8 t ha^{-1} zeolite and 30% depletion of ASW, while

Table 3 Analysis of variance (ANOVA) for the main and interaction effects of zeolite and biofertilizers on the physiological characteristics in dragonhead (*D. moldavica* L.) under different irrigation regimes in the growing seasons of 2014 and 2015

Source of variation	df	Mean of squares										
		RWC	Chl a	Chl b	T Chl	Pro	SS	FY	EOC	EOY	$IWUE_{FY}$	$IWUE_{EOY}$
Year	1	126.563**	0.134**	0.001ns	0.191*	0.001ns	0.538**	61167.361ns	0.002*	0.006ns	0.168ns	0.163*
Rep (year)	4	0.806	0.034*	0.048ns	0.014ns	0.001ns	0.056ns	9353.576ns	0.001ns	0.250ns	6.739ns	0.023ns
I	2	11895.028***	2.762**	0.248*	4.403**	0.617**	18.911**	51294166.896**	0.051**	169.685**	2292.372**	48.236**
Year×I	2	46.583**	0.011ns	0.016ns	0.045ns	0.005ns	4.039**	67138.215ns	0.049**	31.812**	25.337*	0.052ns
Main error	8	1.878	0.008	0.031	0.019	0.002	0.016	16170.983	0.001	0.102	5.438	0.021
Z	1	403.340**	0.575**	0.004ns	0.502**	0.002ns	0.748**	810000.000**	0.001*	0.957*	258.459**	0.162*
Year×Z	1	85.563**	0.050**	0.011ns	0.124**	0.002ns	0.877**	191844.000**	0.001ns	0.137ns	4.594ns	0.020ns
I×Z	2	132.861**	0.062**	0.037*	0.172**	0.002ns	0.277**	510846.271**	0.001**	1.198**	192.911**	0.133**
Year×I×Z	2	67.000**	0.022*	0.018ns	0.029**	0.001ns	0.405**	49148.313**	0.001ns	0.350ns	2.347ns	0.150**
B	3	18.803**	1.195**	1.602**	6.735**	0.003*	0.001*	175572.720**	0.002**	13.365**	5415.179**	13.239**
Year×B	3	5.988ns	0.008ns	0.011ns	0.012*	0.002ns	0.142**	16273.484**	0.001ns	0.536*	4.938ns	0.094*
I×B	6	34.630**	0.167**	0.169**	0.306**	0.009**	0.008**	32442.537**	0.001*	2.116**	105.817**	1.297**
Year×I×B	6	3.759ns	0.007ns	0.007ns	0.004ns	0.001ns	0.009**	3534.352ns	0.001ns	0.478*	3.061ns	0.024ns
Z×B	3	1.581ns	0.015ns	0.053**	0.021**	0.001ns	0.001ns	15230.613**	0.001ns	0.075ns	6.017**	0.380**
Year×Z×B	3	3.581ns	0.001ns	0.008ns	0.009ns	0.001ns	0.001ns	6276.359ns	0.001ns	0.282ns	5.618ns	0.025ns
I×Z×B	6	7.630**	0.070**	0.037*	0.011*	0.001ns	0.001ns	15230.613**	0.001ns	0.366*	14.754**	0.178**
Year×I×Z×B	6	0.824ns	0.008ns	0.011ns	0.003ns	0.001ns	0.001ns	6276.359ns	0.001ns	0.224ns	3.127ns	0.038ns
Secondary error	84	2.394	0.006	0.012	0.005	0.001	0.002	2897.982	0.001	0.163	3.043	0.027
C.V. (%)		2.11	6.22	7.89	2.51	19.80	2.02	1.61	7.95	5.92	3.34	3.49

I different irrigation levels (drought stress), *Z* zeolite, *B* biofertilizer, *RWC* relative water content, *Chl a* chlorophyll *a*, *Chl b* chlorophyll *b*, *T Chl* total chlorophyll, *Pro* proline, *SS* soluble sugars, *FY* flower yield, *EOC* essential oil content, *EOY* essential oil yield, $IWUE_{SY}$ irrigation water use efficiency of flower yield, $IWUE_{EOY}$ irrigation water use efficiency of essential oil yield, *ns* non-significant, *df* degrees of freedom

*Significant at 0.01%; **significant at 0.05%

Table 4 Mean comparison for the effects of year, different irrigation regimes (I1, I2 and I3 defined as 30, 60 and 90% depletion of available soil water, respectively), zeolite and biofertilizers on the physiological characteristics of dragonhead

Treatment	RWC (%)	Chl a (mg g^{-1} fw^{-1})	Chl b (mg g^{-1} fw^{-1})	T Chl (mg g^{-1} fw^{-1})	Pro (mg g^{-1} fw^{-1})	SS (mg g^{-1} dw^{-1})	FY (kg ha^{-1})	EOC (%)	EOY (kg ha^{-1})	IWUE$_{FY}$ (g m^{-3})	IWUE$_{EOY}$ (ml m^{-3})
Year											
2014	74.111a	1.296a	1.418a	2.720a	0.135a	2.110b	3.366a	0.211a	6.805a	113.594a	2.106a
2015	72.236b	1.235b	1.413a	2.648b	0.138a	2.233a	3.323b	0.204b	6.818a	109.611b	2.094a
Irrigation regime											
I1	89.271a	1.480a	1.472a	2.924a	0.045c	1.449b	4.141a	0.170b	7.093b	124.726a	2.807b
I2	72.438b	1.311b	1.439a	2.784a	0.102b	2.486a	3.716b	0.222a	8.535a	118.342b	3.017a
I3	57.813c	1.007c	1.334b	2.344b	0.264a	2.580a	2.177c	0.230a	4.806c	79.674c	1.141c
Zeolite (t ha^{-1})											
0	71.500b	1.203b	1.410a	2.625b	0.141a	2.244a	3.270b	0.210a	6.730b	98.435b	2.023b
8	74.847a	1.329a	1.421a	2.743a	0.133a	2.099b	3.420a	0.204b	6.893a	121.814a	2.114a
Biofertilizer											
Control	72.139a	0.940c	1.102b	2.054c	0.124a	2.154a	3.096b	0.199a	6.063b	93.349c	2.243b
Nitroxin	73.528a	1.365b	1.483a	2.751b	0.139a	2.168a	3.383ab	0.207a	6.826ab	102.863b	2.522ab
Barvar-2	73.250a	1.425a	1.520a	2.945a	0.140a	2.178a	3.339ab	0.208a	6.801ab	107.521a	2.457ab
Nitroxin×barvar-2	73.778a	1.433a	1.556a	2.986a	0.144a	2.186a	3.561a	0.215a	7.555a	106.817a	2.604a

Means in each column followed by the same letter are not significantly different ($p < 0.05$)

RWC relative water content, *Chl a* chlorophyll *a*, *Chl b* chlorophyll *b*, *T Chl* total chlorophyll, *Pro* proline, *SS* soluble sugars, *FY* flower yield, *EOC* essential oil content, *EOY* essential oil yield, *IWUE$_{SY}$* irrigation water use efficiency of flower yield, *IWUE$_{EOY}$* irrigation water use efficiency of essential oil yield

Table 5 Interaction effects of year and irrigation regimes (I1, I2 and I3 defined as 30, 60 and 90% depletion of available soil water, respectively), zeolite and biofertilizers on the physiological characteristics of dragonhead

Treatment	RWC (%)		Chl a (mg g^{-1} fw^{-1})		Chl b (mg g^{-1} fw^{-1})		T Chl (mg g^{-1} fw^{-1})		Pro (mg g^{-1} fw^{-1})		SS (mg g^{-1} dw^{-1})		FY (kg ha^{-1})	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Irrigation regime														
I1	89.29a	89.25a	1.493a	1.466a	1.421b	1.456ab	2.925a	2.922a	0.053d	0.037d	1.432f	1.465e	4.149a	4.134a
I2	73.25b	71.63c	1.348b	1.273c	1.491a	1.453ab	2.843b	2.725c	0.101c	0.103c	2.115d	2.857a	3.780b	3.652c
I3	59.79d	55.83e	1.047d	0.966e	1.341c	1.328c	2.392d	2.295e	0.252b	0.275a	2.784b	2.375c	2.170d	2.183d
Zeolite (t ha^{-1})														
0	71.67c	71.33c	1.214c	1.191c	1.415a	1.427a	2.632c	2.618c	0.143a	0.139a	2.261a	2.227b	3.255d	3.285c
8	73.56a	73.14b	1.378a	1.280b	1.421a	1.398a	2.809a	2.677b	0.128a	0.138a	1.960c	2.239b	3.478a	3.362b
Biofertilizer														
Control	72.94cd	71.33e	0.963d	0.917d	1.093c	1.111c	2.082e	2.026f	0.134ab	0.113b	2.199c	2.172c	3.128e	3.063f
Nitroxin	74.56ab	72.50d	1.282c	1.248c	1.509ab	1.456b	2.796c	2.706d	0.134ab	0.114a	2.109d	2.199c	3.420c	3.346d
Barvar-2	75.22a	72.33de	1.476a	1.374b	1.524ab	1.515ab	3.002a	2.888b	0.134ab	0.114a	2.092d	2.245b	3.326d	3.352d
Nitroxin×barvar-2	73.72bc	72.78cd	1.464a	1.402b	1.544a	1.568a	3.002a	2.971a	0.138a	0.151a	2.042e	2.315a	3.592a	3.531b

Treatment	EOC (%)		EOY (kg ha^{-1})		IWUE$_{FY}$ (g m^{-3})		IWUE$_{EOY}$ (ml m^{-3})	
	2014	2015	2014	2015	2014	2015	2014	2015
Irrigation regime								
I1	0.173c	0.168d	7.218c	6.969d	132.467a	128.341b	2.814c	2.417d
I2	0.202b	0.258a	7.656b	9.413a	125.721bc	119.274c	3.617a	3.125b
I3	0.258a	0.186c	5.540e	4.072f	84.276e	95.327d	1.951e	1.627f
Zeolite (t ha^{-1})								
0	0.214a	0.207b	6.692b	6.767ab	104.268d	112.562c	2.108b	2.227b
8	0.208b	0.201c	6.917a	6.869ab	121.532b	124.271a	2.253a	2.364a
Biofertilizer								
Control	0.203b	0.194c	6.173d	5.952d	92.127e	78.127f	2.204d	2.127e
Nitroxin	0.201b	0.204b	6.821bc	6.862bc	109.272b	99.271d	2.571c	2.511c
Barvar-2	0.208b	0.208b	6.626c	6.976b	104.536c	107.462b	2.425cd	2.721b
Nitroxin×barvar-2	0.222a	0.208b	7.598a	7.512a	119.242a	121.721a	2.853ab	3.014a

Means in each column followed by the same letter are not significantly different ($p < 0.05$)

Different irrigation levels (I1, I2 and I3 defined as 30, 60 and 90% depletion of available soil water, respectively)

RWC relative water content, *Chl a* chlorophyll *a*, *Chl b* chlorophyll *b*, *T Chl* total chlorophyll, *Pro* proline, *SS* soluble sugars, *FOY*, *FY* flower yield, *EOC* essential oil content, *EOY* essential oil yield, *EOC* essential oil content, *EOY* essential oil yield, *IWUE$_{FY}$* irrigation water use efficiency of flower yield, *IWUE$_{EOY}$* irrigation water use efficiency of essential oil yield

Table 6 Interaction effects of irrigation regimes (I1, I2 and I3 defined as 30, 60 and 90% depletion of available soil water, respectively), zeolite and biofertilizers on the physiological characteristic of dragonhead

Treatment	RWC (%)			Chl a (mg g^{-1} fw^{-1})			Chl b (mg g^{-1} fw^{-1})			T Chl (mg g^{-1} fw^{-1})			Pro (mg g^{-1} fw^{-1})		
	I1	I2	I3	I1	I2	I3	I1	I2	I3	I1	I2	I3	I1	I2	I3
Zeolite (t ha^{-1})															
0	89.04a	70.04c	54.96e	1.448b	1.255d	0.904f	1.451ab	1.417bc	1.361cd	2.909a	2.750c	2.216e	0.044d	0.104c	0.274a
8	89.50a	74.83b	60.67d	1.511a	1.367c	1.109e	1.493a	1.461ab	1.308d	2.939a	2.819b	2.471d	0.045d	0.099c	0.253b
Biofertilizer															
Control	89.67e	71.67e	55.08h	1.180ef	1.144f	0.495h	1.035f	1.297e	0.974f	2.498g	2.185h	1.477i	0.0675d	0.0958c	0.2083b
Nitroxin	89.00b	71.75e	57.75g	1.497b	1.238de	1.061g	1.514cd	1.437d	1.497cd	2.931c	2.756d	2.566e	0.0375e	0.1033c	0.2755a
Barvar-2	87.33c	72.58de	59.75f	1.607a	1.428c	1.212de	1.657ab	1.453d	1.449d	3.090b	3.077b	2.668e	0.0383e	0.1042c	0.2750a
Nitroxin×barvar-2	91.08a	73.75d	58.67fg	1.636a	1.433c	1.258d	1.683a	1.568bc	1.417d	3.176a	3.119ab	2.663e	0.0367e	0.1033c	0.2933a

Treatment	SS (mg g^{-1} dw^{-1})			FY (kg ha^{-1})			EOC (%)			EOY (kg ha^{-1})			IWUE$_{FY}$ (g m^{-3})			IWUE$_{EOY}$ (ml m^{-3})		
	I1	I2	I3	I1	I2	I3	I1	I2	I3	I1	I2	I3	I1	I2	I3	I1	I2	I3
Zeolite (t ha^{-1})																		
0	1.450d	2.638a	2.643a	4.129a	3.522c	2.158e	0.168d	0.234b	0.228ab	6.987c	8.309b	4.893d	118.324b	96.461e	84.521f	2.115c	2.014d	1.783e
8	1.448d	2.334c	2.516b	4.154a	3.910b	2.195d	0.172d	0.236a	0.215c	7.199c	8.760b	4.719d	127.241a	112.734c	104.621d	2.532a	2.314b	2.142c
Biofertilizer																		
Control	1.484e	2.512c	2.561b	3.807d	3.391g	2.089j	0.152f	0.230ab	0.215c	5.870g	7.814d	4.504i	95.361g	82.781	69.401i	2.241d	2.071e	1.968f
Nitroxin	1.449ef	2.457d	2.556b	4.205b	3.758e	2.185i	0.167e	0.231ab	0.223bc	6.993f	8.636b	4.850h	119.014b	105.431d	97.315f	2.467c	2.856b	2.214d
Barvar-2	1.429f	2.491cd	2.585ab	4.194b	3.643f	2.179i	0.174e	0.225ab	0.225ab	7.370e	8.189c	4.843h	113.853c	102.641e	86.651h	2.567c	2.775b	2.081e
Nitroxin×barvar-2	1.735f	2.486cd	2.617a	4.359a	4.072c	2.253h	0.188d	0.233a	0.225ab	8.139d	9.499a	5.027h	122.256a	114.607c	99.142f	2.943b	3.145a	2.356d

I different irrigation levels, *RWC* relative water content, *Chl a* chlorophyll *a*, *Chl b* chlorophyll *b*, *T Chl* total chlorophyll, *Pro* proline

I different irrigation levels (I1, I2 and I3 defined as 30, 60 and 90% depletion of available soil water, respectively), *SS* soluble sugars, *FY* flower yield, *EOC* essential oil content, *EOY* essential oil yield, *IWUE$_{FY}$* irrigation water use efficiency of flower yield, *IWUE$_{EOY}$* irrigation water use efficiency of essential oil yield

Means in each column followed by the same letter are not significantly different ($p < 0.05$)

Table 7 Interaction effects of zeolite and biofertilizers on the physiological characteristics of dragonhead

Treatment	RWC (%)		Chl a (mg g⁻¹ fw⁻¹)		Chl b (mg g⁻¹ fw⁻¹)		TChl (mg g⁻¹ fw⁻¹)		Pro (mg g⁻¹ fw⁻¹)		SS (mg g⁻¹ dw⁻¹)		FY (kg ha⁻¹)	
Biofertilizer	Zeolite (t ha⁻¹)		Zeolite (t ha⁻¹)		Zeolite (t ha⁻¹)		Zeolite (t ha⁻¹)		Zeolite (t ha⁻¹)		Zeolite (t ha⁻¹)		Zeolite (t ha⁻¹)	
	0	8	0	8	0	8	0	8	0	8	0	8	0	8
Control	70.67d	73.61b	0.906e	0.973d	1.050e	1.154d	1.959f	2.148e	0.128a	0.136ab	2.262a	2.109b	3.023g	3.168f
Nitroxin	71.61cd	75.44a	1.199c	1.332b	1.506abc	1.459c	2.708d	2.794c	0.144a	0.134ab	2.232a	2.076c	3.328d	3.443c
Barvar-2	72.00c	75.56a	1.346b	1.504a	1.547ab	1.492bc	2.894b	2.996a	0.143a	0.128ab	2.235a	2.102bc	3.221e	3.457c
Nitroxin×barvar-2	71.72cd	74.78a	1.359b	1.507a	1.533abc	1.579a	2.938b	3.034a	0.142ab	0.120b	2.246a	2.111b	3.512b	3.611a

Treatment	EOC (%)		EOY (kg ha−1)		IWUE$_{FY}$ (g m−3)		IWUE$_{EOY}$ (ml m−3)	
Biofertilizer	Zeolite (t ha⁻¹)		Zeolite (t ha⁻¹)		Zeolite (t ha⁻¹)		Zeolite (t ha⁻¹)	
	0	8	0	8	0	8	0	8
Control	0.201cd	0.197d	5.999c	6.127c	79.542f	84.371e	1.628e	1.917d
Nitroxin	0.207bc	0.207bc	6.710b	6.943b	90.214d	102.241b	2.572c	2.892b
Barvar-2	0.211b	0.205bc	6.680b	6.922b	86.652e	105.252b	2.376c	3.124a
Nitroxin×barvar-2	0.209b	0.221a	7.530a	7.580a	98.621c	114.856a	2.798b	3.1652a

Means in each column followed by the same letter are not significantly different ($p < 0.05$)

RWC relative water content, *Chl a* chlorophyll *a*, *Chl b* chlorophyll *b*, *T Chl* total chlorophyll, *Pro* proline, *SS* soluble sugars, *FY* flower yield, *EOC* essential oil content, *EOY* essential oil yield, *IWUE$_{FY}$* irrigation water use efficiency of flower yield, *IWUE$_{EOY}$* irrigation water use efficiency of essential oil yield

Table 8 Triple interaction effects of irrigation regimes (I1, I2 and I3 defined as 30, 60 and 90% depletion of available soil water, respectively), zeolite and biofertilizers on the physiological characteristics in dragonhead

Zeolite (t ha⁻¹)	Biofertilizer	RWC (%) I1	I2	I3	Chl a (mg g⁻¹ fw⁻¹) I1	I2	I3	Chl b (mg g⁻¹ fw⁻¹) I1	I2	I3	T Chl (mg g⁻¹ fw⁻¹) I1	I2	I3
0	Control	87.00e	69.33j	52.33i	1.157fgh	1.060hij	0.502k	1.300f	1.022g	0.828h	2.460hi	2.087k	1.330m
0	Nitroxin	89.50bcd	68.33h	55.00k	1.492bc	1.128ghi	0.977j	1.462cde	1.600abc	1.457cde	2.952d	2.732ef	2.440i
0	Barvar-2	89.00bcd	70.00h	57.50j	1.590ab	1.358e	1.090hi	1.480cde	1.690a	1.472cde	3.067bc	3.042c	2.575g
0	Nitroxin×barvar-2	90.67ab	72.50g	55.00k	1.555bc	1.473cd	1.048ij	1.602abc	1.662ab	1.475cde	3.157ab	3.138ab	2.520ghi
8	Control	87.67de	74.00fg	57.83j	1.203fg	1.228f	0.488k	1.293f	1.048g	1.120g	2.537gh	2.283j	1.625l
8	Nitroxin	88.50cde	75.17f	60.50i	1.502bc	1.348e	1.145fghi	1.428def	1.412def	1.538bcd	2.910d	2.780e	2.692f
8	Barvar-2	90.33abc	75.17f	62.00i	1.658a	1.497bc	1.333e	1.623ab	1.427def	1.427def	3.113abc	3.113abc	2.760ef
8	Nitroxin×barvar-2	91.50a	75.00f	62.33i	1.682a	1.393de	1.268cd	1.705a	1.535bcd	1.358ef	3.195a	3.100bc	2.807f

Zeolite (t ha⁻¹)	Biofertilizer	Pro (mg g fw⁻¹) I1	I2	I3	SS (mg g dw⁻¹) I1	I2	I3	FY (kg ha⁻¹) I1	I2	I3
0	Control	0.067fg	0.098def	0.235abc	1.495g	2.658ab	2.632ab	3.788d	3.209g	
0	Nitroxin	0.038g	0.107def	0.230bcde	1.452gh	2.628ab	2.615b	4.216b	3.585e	
0	Barvar-2	0.036g	0.113d	0.233abcd	1.425h	2.633ab	2.647ab	4.162b	3.351f	
0	Nitroxin×barvar-2	0.036g	0.097def	0.237ab	1.427h	2.633ab	2.678a	4.349a	3.944c	
8	Control	0.068efg	0.093def	0.225bcdef	1.475gh	2.365e	2.490d	3.826d	3.573e	
8	Nitroxin	0.036g	0.100def	0.225bcde	1.447gh	2.285e	2.497d	4.195b	3.931c	
8	Barvar-2	0.040g	0.098def	0.225bcdef	1.433h	2.348e	2.523cd	4.227b	3.936b	
8	Nitroxin×barvar-2	0.036g	0.110de	0.232bcd	1.438gh	2.338e	2.555c	4.369a	4.200b	

Zeolite (t ha⁻¹)	Biofertilizer	EOC (%) I1	I2	I3	IWUE$_{FY}$ (g m⁻³) I1	I2	I3	EOY (kg ha⁻¹) I1	I2	I3	IWUE$_{EOY}$ (ml m⁻³) I1	I2	I3
0	Control	0.153k	0.235abc	0.215fg	87.213hi	76.381	69.154m	5.133k	7.667ef	4.462i	1.632ij	1.534j	1.264k
0	Nitroxin	0.167j	0.230bcde	0.225bcdef	103.531d	92.543fg	85.746i	6.262j	8.323cd	4.810i	1.963h	2.151fg	1.785i
0	Barvar-2	0.172ij	0.233abcd	0.228bcde	109.283c	98.631e	81.524k	6.657ij	7.918de	4.887i	2.452cd	2.284ef	1.501j
0	Nitroxin×barvar-2	0.182i	0.237ab	0.245a	114.183b	107.562cd	94.731ef	7.153i	9.327ab	5.415h	2.567bc	2.612b	1.952h
8	Control	0.150k	0.225bcdef	0.215fg	95.423ef	89.735gh	76.582l	5.231k	7.962cde	4.547i	2.215ef	2.013gh	1.918h
8	Nitroxin	0.167j	0.232bcd	0.220def	116.261b	103.571	95.869ef	6.764j	8.948b	4.890i	2.522bc	2.618b	2.015gh

Table 8 (continued)

Treatment		EOC (%)			EOY (kg ha⁻¹)			IWUE$_{FY}$ (g m⁻³)			IWUE$_{EOY}$ (ml m⁻³)		
Zeolite (t ha⁻¹)	Biofertilizer	I			I			I			I		
		I1	I2	I3	I1	I2	I3	I1	I2	I3	I1	I2	I3
8	Barvar-2	0.177ij	0.217efg	0.222cdef	7.073ij	8.460c	4.800i	112.921bc	104.781	98.672e	2.327de	2.727b	2.256ef
8	Nitroxin×bar-var-2	0.193h	0.230bcde	0.205g	7.891h	9.672a	4.638i	126.735a	115.632b	103.741d	2.653b	3.117a	2.238ef

Means in each column followed by the same letter are not significantly different ($p < 0.05$)

I different irrigation levels, *RWC* relative water content, *Chl a* chlorophyll *a*, *Chl b* chlorophyll *b*, *T Chl* total chlorophyll

I different irrigation levels (I1, I2 and I3 defined as 30, 60 and 90% depletion of available soil water, respectively), *Pro* proline, *SS* soluble sugars, *FY* flower yield, *EOC* essential oil content, *EOY* essential oil yield, *IWUE$_{FY}$* irrigation water use efficiency of flower yield, *IWUE$_{EOY}$* irrigation water use efficiency of essential oil yield

the lowest value (1.33 mg g⁻¹ fw⁻¹) was observed without application of zeolite and biofertlizers under severe water-deficit stress conditions (Table 8).

Proline

The mean leaf proline content was increased by 47.5% and 78.9% in 2014, and 64.07% and 86.5% in 2015, respectively, when dragonhead was grown under I2 and I3 irrigation regimes compared to I1 (Table 5). The effects of zeolite and biofertilizers were not statistically significant on free amino acid proline content; however, the highest proline content was obtained in plots without applying zeolite and also in combined application of nitroxin and phosphate barvar-2 (Table 4). Although, Table 8 presents the significant differences in triple interaction effects on proline content obtained by applying combined fertilizers without zeolite under stress caused by 90% depletion of ASW.

Soluble sugars

According to Table 3, the interaction of Z×B and I×Z×B on the content of soluble sugars was statically insignificant. Mean comparison of main effects of zeolite application and different biofertilization showed that the highest total soluble sugars were observed in control plants untreated with zeolite and in combined treatment of nitroxin×phosphate barvar-2, respectively (Table 4). In addition, in 2015, the highest value of total soluble sugars was observed under 60% depletion of ASW (Table 5). According to Table 6, soluble sugars increased with increasing severity water deficit stress, and zeolite application recorded minimum soluble sugar content compared to the respective control. The highest (2.678 mg g⁻¹ fw⁻¹) soluble sugar accumulation was observed in combined treatment of biofertilizers and 90% depletion of ASW without zeolite application (Table 8).

Flower yield

The flower yield was decreased by 10.26 and 47.4% with increasing drought intensity from 60 to 90% depletion of ASW compared to the first level of water availability (I1), respectively (Table 4). Mean comparison of zeolite treatment showed that the highest yield of flower belonged to the zeolite application.

The treatment combinations of nitroxin and phosphate barvar-2 resulted in the highest flower yield in both years, 2014 and 2015 by 12.9 and 13.2% compared to the non-fertilized control, respectively (Table 5). However, analysis of interaction effects showed that flower yield increased in all levels of soil moisture with zeolite application compared to the respective control (Table 6). In addition, combination of nitroxin and phosphate barvar-2 produced the

highest yield of flower in all levels of water deficit over the respective control (Table 6). Also, combined application of biofertilizers (nitroxin and phosphate barvar-2) along with zeolite caused the maximum flower yield (by 16.2%) compared to non-fertilized control without zeolite (Table 7). In the case of three interactions, the maximum amount of flower yield (4.369 kg ha^{-1}) was achieved by application of zeolite and a combination of nitroxin and phosphate barvar-2 in 30% depletion of ASW (Table 8).

Essential oil content

As Table 3 shows, essential oil percentage/content was significantly ($p < 0.01$) impacted by refrence treatments in both years. The moderate (I2) and severe (I3) water-deficit stress led to increase in essential oil content by 23.4 and 26% compared to mild water stress (I1), respectively; however, there was no significant difference between I2 and I3 treatments (Table 4).

Application of zeolite did not positively affect the essential oil content of dragonhead, as the highest essential oil content was obtained without zeolite application (Table 4). Although ANOVA identified significant difference among biofertilizer treatments on essential oil content (Table 3), there were no significant differences among means and they were placed in a similar statistical group (Table 4). Nevertheless, the maximum percentage of essential oil was achieved from nitroxin×phosphate barvar-2 treatment. Mean comparison of interaction effects indicated that the highest essential oil percentage (0.245%) was observed by 90% depletion of ASW without zeolite and combined application of nitroxin and phosphate barvar-2 (Table 8).

Essential oil yield

ANOVA results for essential oil yield response to drought stress, zeolite and biofertilizers in dragonhead plants are given in Table 3. As shown, the oil yield also affected the biofertilizer application (Table 3). Depletion of 60% soil moisture increased essential oil yield by 16.9 and 43.7% compared to plots subjected to 30 and 90% depletion of ASW, respectively (Table 4). Similar to other measured parameters, a combination of nitroxin and phosphate barvar-2 caused the highest yield of essential oil; however, no significant effect was observed among biofertilizer treatments (Table 4). According to Tables 5, 6, 7 and 8, the maximum essential oil yield in association with two-way and/or three-way interactions was obtained in plants exposed to zeolite and 60% depletion of ASW, and those treated with nitroxin×phosphate barvar-2 and 60% depletion of ASW.

Irrigation water use efficiency for flower yield (IWUE$_{FY}$) and essential oil (IWUE$_{EOY}$)

Over both years, water-deficit stress decreased IWUE$_{FY}$ with increasing stress severity. The lowest and the highest values for IWUE$_{FY}$ were observed in 2014, when plots were irrigated at 90 and 30% depletion of ASW, respectively (Table 5). The treatment combinations of nitroxin×phosphate barvar-2 with zeolite under 30% depletion of ASW (mild water stress) led to increase in IWUE$_{FY}$ by 31.1% compared with the control (Table 8). In general, treatment with biofertilizers had a greater effect (13.8%) in increasing IWUE$_{EOY}$ than application of zeolite (4.3%) in both years (Table 4).

Mean IWUE$_{EOY}$ was increased by 22.2% with increase in water-deficit stress from 30 to 60% depletion of ASW, and then dropped down (up to 46%) with an increase in severity of stress from 60 to 90% depletion of ASW in 2014, and the same change trend was found for IWUE$_{EOY}$ under the employed irrigation regimes in 2015 (Table 5).

The two-way interactions between water-deficit stress and zeolite (Table 6), and between water-deficit stress and biofertilization (Table 6) as well as between zeolite and biofertilozer application (Table 7) showed that IWUE$_{EOY}$ increased through application of zeloite or biofertilizers in all irrigation treatments compared to the respective control, while the minimum value of IWUE$_{EOY}$ in each level of water-deficit stress was observed without application of bacterial biofertilizers and zeloite (Table 6). According to Table 8, second-order interaction (i.e. three-way interaction) among treatment groups showed that the highest (3.117 ml m^{-3}) IWUE$_{EOY}$ was observed in interaction among nitroxin×phosphate barvar-2 and zeolite treatments with moderate water stress, while the lowest (1.264 ml m^{-3}) IWUE$_{EOY}$ was produced without zeolite and fertilizer application in plots exposed to severe water stress conditions (Table 8).

Figure 2 shows the relative comparison of the effects of reference treatments, zeolite and biofertilizer application under deficit irrigation, on some morpho-physiological characteristics of dragonhead plants. The maximum essential oil yield and IWUE$_{EOY}$ were observed in the plants inoculated with biofertilizer consortium (combined inoculation with nitroxin and phosphate barvar-2) under 60 depletion of ASW compared to the control (without zeolite and biofertilizers, and irrigation after 30% depletion of ASW).

Correlation coefficients between measured traits

There was a statistically significant positive correlation between leaf relative water content and flower yield ($r_{0.01} = 0.78$) and essential oil yield ($r_{0.05} = 0.61$) under experimental treatments (Table 9). Moreover, the flower

Fig. 2 The relative comparison of the effects of zeolite [0 (− Zeolite) and 8 t ha^{-1} (+ Zeolite)] and biofertilizers [nitroxine, phosphate barvar-2, and their mixture (nitroxine×phosphate barvar-2)] under different irrigation regimes (I1, I2 and I3 refer to 30, 60 and 90% depletion of available soil water, respectively) on some morpho-physiological characteristics of *Dracocephalum moldavica* L. (the values were averaged of 2 years). *RWC* relative water content, *T Chl* total chlorophyll, *FY* flower yield, *EOY* essential oil yield, *IWUE$_{SY}$* irrigation water use efficiency of flower yield
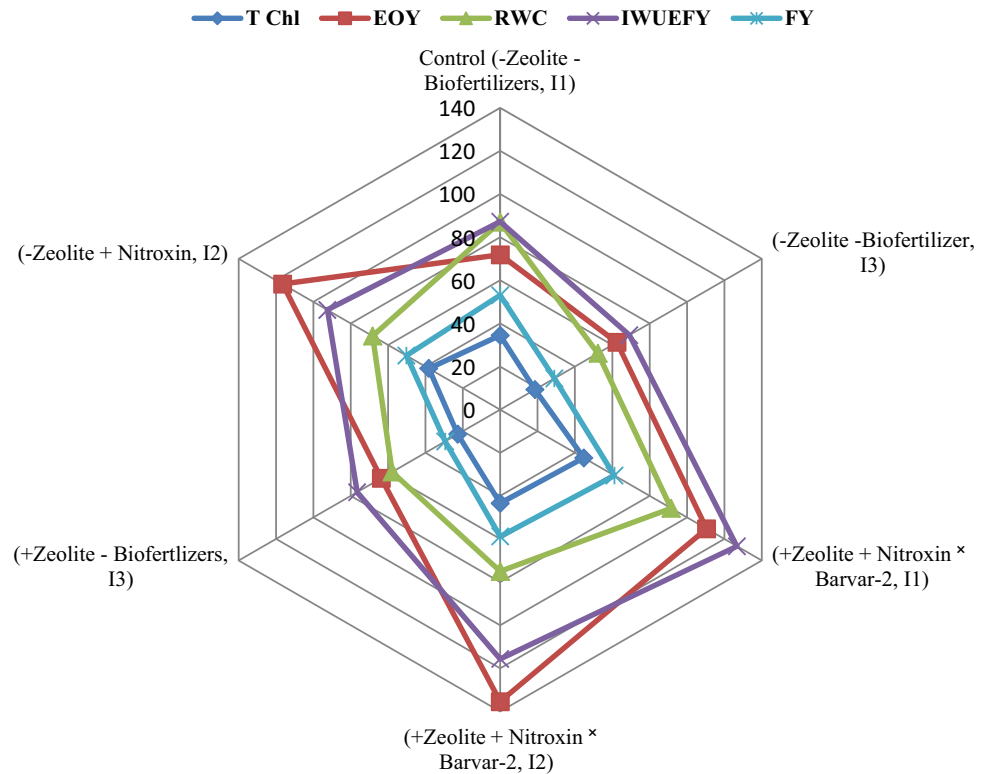
Table 9 Pearson's correlation coefficients among measured traits: relative water content (RWC), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (T Chl), proline (Pro), soluble sugars (SS), flower yield (FY), EOC (essential oil content), essential oil yield (EOY), irrigation water use efficiency of flower yield (IWUE$_{FY}$), and irrigation water use efficiency of essential oil yield (IWUE$_{EOY}$) in field-grown dragonhead treated with zeolite and biofertilizers under different irrigation regimes

Traits	RWC	Chl *a*	Chl *b*	T Chl	Pro	SS	FY	EOC	EOY	IWUE$_{FY}$	IWUE$_{EOY}$
RWC	1										
Chl a	0.48[ns]	1									
Chl b	0.38[ns]	0.94**	1								
T Chl	0.43[ns]	0.98**	0.83**	1							
Pro	0.66*	0.72**	0.57*	0.68*	1						
SS	0.59*	0.37[ns]	0.42[ns]	0.42[ns]	0.86**	1					
FY	0.78**	0.51[ns]	0.38[ns]	0.39[ns]	− 0.54*	− 0.32[ns]	1				
EOC	0.32 [ns]	0.35[ns]	0.26[ns]	0.35[ns]	0.55*	0.61*	0.67**	1			
EOY	0.61*	0.43[ns]	0.32[ns]	0.47[ns]	0.57*	0.54[ns]	0.85**	0.85**	1		
IWUE$_{FY}$	0.54*	0.36[ns]	0.53*	0.51*	− 0.69**	− 0.48[ns]	0.78**	0.96**	0.81**	1	
IWUE$_{EOY}$	0.69*	0.54*	0.49[ns]	0.63*	0.71*	0.54*	0.66*	0.98**	0.94**	0.95**	1

ns, *,**Non-significant and significant at 5 and 1% levels of probability, respectively

yield and the irrigation water use efficiency of flower yield were negatively correlated with the proline content ($r_{0.05} = 0.54$ and $r_{0.05} = 0.69$, respectively). A positive and highly significant correlation ($r_{0.01} = 0.85$ and 0.98) was obtained between essential oil content and essential oil yield and IWUE$_{EOY}$ in dragonhead plants under he employed treatments, respectively (Table 9).

Discussion

Generally, most of the examined traits were higher in 2014 than in 2015, which can be ascribed, to some extent, to the differences between weather in terms of temperature, precipitation, relative humidity and evapotranspiration. According to previous reports (Mokhtassi-Bidgoli et al.

2013; Baghbani-Arani et al. 2017), higher agronomic performance, physiological functions and crop yield were obtained from higher precipitation and lower temperature during the critical growth stage.

It appears from our results that RWC, chlorophyll (Chl *a*, Chl *b* and total Chl) contents, flower yield and IWUE$_{FY}$ decreased with increase in water stress intensity. Some of the basic/and or adaptive mechanisms in terrestrial plants to combat osmotic stress effects are known to be related to the stimulation of physiological responses such as changes in water and osmolyte status, soluble sugars, proline, photosynthetic pigments and secondary metabolite levels in cell and subcellular structures.

Leaf water content is positively associated with the tolerance of plants to stressors such as water deficit stress. The prolonged exposure to water-deficit stress reduces leaf water potential, which adversely affects plant growth processes such as leaf elongation, stem extension and root proliferation (due to reduction in the rate of expansion and cell division), limits photosynthesis (due to decreases in stomatal conductance), hampers plant water relations, and decreases water use efficiency and yield (Farooq et al. 2009; Askari and Ehsanzadeh 2015). In the present study, lower RWC in control plants and higher RWC in those treated with zeolite and biofertilizers were associated with lower and higher chlorophyll contents and flower yield, respectively.

However, application of zeolite duo to a particular structure plays a crucial and efficient role in modifying both the physico-chemical and biological features of the soil and can reduce leaching of nitrogen and increase the value of water availability and maintain soil fertility in the root zone through improvement of cation exchange capacity (Ippolito et al. 2011). In the current study, the maximum value of the examined physiological and phytochemical characteristics were obtained by application of 8 t ha^{-1} zeolite under 30% depletion of ASW (e.g. RWC, Chl *a* and *b*, and total chlorophyll values), under 60% depletion of ASW (e.g. essential oil content and yield) and under 90% depletion of ASW (e.g. proline and soluble sugars contents). Zeolites with three-dimensional, microporous and framework structures containing silicon, aluminium, oxygen, etc. absorb high humidity that could be efficient in reducing water stress effects on plants. Our results of interaction effects of zeolite × water-deficit stress were similar to the previous findings reported by Goksey et al. (2004), Mohammad et al. (2005), and Afsharmanesh (2009).

Chlorophyll content is an important physiological indicator that is strongly related to the photosynthetic efficiency and plant productivity (Askari and Ehsanzadeh 2015). The degree of drought-induced reduction in chlorophyll content is assumed to depend on the severity/intensity and duration of the water shortage stress. In the current work, Chl *a* and *b*, and total Chl of dragonhead were diminished when

plots were depleted by 60–90% ASW compared to 30%. The decrease in chlorophyll content upon exposure to drought stress may be attributed to extra generation of free radicals (ROS), alterations in the ratio of lipid to protein of light-harvesting complexes, enhanced expression of chlorophyllase activity and chlorophyll degradation, as well as inhibition of photosynthetic pigments biosynthesis (Haider et al. 2017; Meher et al. 2018).

According to the results, in contrast to decreases in RWC, proline and soluble sugar contents of the leaves were improved in dragonhead plants when grown under deficit water stress. A high correlation between RWC and proline and soluble sugars has been previously identified in other species such as *Glycine max* and *Aeluropus lagopoides* (Porcel and Ruiz-Lozano 2004; Mohsenzadeh et al. 2006). Organic solutes and their alterations in plant organs may be of potential application in elucidating plants productivity under different environmental perturbations. Osmotic adjustment induced by different types of compatible solutes and amino acids such as proline helps plant to maintain turgor and water potential, therefore, considered as a vital adaptive and protective approach by which plants combat adverse environmental conditions (Schutz and Fangmeier 2001). In addition, results showed that plots under 90% depletion of ASW without zeolite application imposed severe stress in dragonhead; nevertheless, enhancing soluble sugars and proline contents through osmotic adjustment process make vital contribution to maintain physiological functions of the plants for avoiding of water-deficit stress effects. It is noteworthy that the significant and positive correlation between RWC and proline is indicative of osmotic adjustment status in the water-stressed dragonhead; however, in our study a higher proportional increase in proline content is found under severe water-deficit stress compared to other conditions. Plants have improved a series of mechanisms for adaptation and survival under severe environmental perturbations. The capability of a plant to tolerate water-deficit stress is affected by several metabolic networks that intensify continuous water absorption, support cell organelles and structures, and regulate the ion homeostasis in cells (Mohammadi et al. 2018). The most common biochemical pathways are those that cause the biosynthesis of specific proteins and accumulation of cellular osmoprotectants or low molecular weight compounds (e.g. proline and SS), and activation of antioxidant defense enzymes, which subsequently maintain ion and water flux across the membrane and support highly efficient ROS-detoxifying machinery under various environmental stresses (Gholami Zali and Ehsanzadeh 2018). The same authors reported that drought stress causes significant changes in specific activities of antioxidant enzymes such catalase (CAT) in the absence and presence of proline. Interestingly, there was significant negative correlation between proline and malondialdehyde (MDA) and H$_2$O$_2$ contents

in *Thymus vulgaris* plants under drought stress conditions; however, in well-watered treatments, positive and significant correlation was obtained between proline and H_2O_2 and MDA contents (Mohammadi et al. 2018).

Biosynthesis of essential oils and or other bioactive compounds in medicinal plants is influenced by several factors such as genetics, physiological variations, edaphic and climatic conditions, geographic situations, environmental biotic and abiotic stresses and agronomical practices (Ramakrishna and Ravishankar 2011; Keshavarz Afshar et al. 2014). Also, the highest value of essential oil percentage (or content) was in the severe water-deficit stress conditions together with either zeolite and or biofertilizers (Nitroxin and phosphate barvar-2). These results are in accordance with Munne-Bosch and Alegre (2000); and Dadrasan et al. (2015), where they observed that the percentage of essential oil in *Melissa officinalis* L. and *Rosmarinus officinalis* L. (Lamiaceae), and in *Trigonella foenum-graecum* L. (Fabaceae), was increased under mild drought stress. In this study, the yield of essential oil was decreased with increase in water stress levels from 60% (I2) to 90% (I3) depletion of ASW, but the essential oil content was increased with increase in drought intensity from I1 to I2 (60% depletion of ASW).

It is remarkable that the essential oil content would not be constantly enhanced with raising stress intensity, because plants utilize more photosynthetic carbon assimilates to produce osmotically active substances including sugar compounds and proline, providing the vital situations for survival in such natural environments they are dealing with. These metabolites are expensive for plants and, therefore, plants pay back these expenses through diminishing growth, development and reproduction (Munns 1993). It has been acknowledged that the yield of essential oil was decreased under drought, but the percentage of oil was improved following stress treatment (Farahani et al. 2009). However, Sing Sangwan et al. (1994) showed that the production of essential oil was unchanged or enhanced under limited water conditions. Here, the maximum essential oil percentage value was gained at 90% depletion of ASW and the highest shoot top flower yield was produced at 30% depletion of ASW, but the highest oil yield oil was observed at 60% depletion of ASW; it appears that the stress of water deficit enhances the percentage of essential oil in medicinal plants, because under stress conditions, more secondary active compounds are synthesized and these substances can prevent the oxidation of other chemicals of the cells (Selmar et al. 2017). However, essential oil yield reduced under severe water deficit stress, due to the fact that interaction between the essential oil content and vegetative yield are two prominent subjects in calculation of oil yield. Therefore, watering at 60% depletion of ASW is suitable for creating a good

trade-off between the percentage of essential oil and aerial flower yield, leading to the maximum yield for essential oil.

The dual effects of water limitation on physiological and biochemical processes, yield attributes and essential oil production were previously reported in diverse species, for example in dragonhead (Rahbarian et al. 2010, 2014; Gholizadeh et al. 2010a, b), Balm (Abbaszadeh et al. 2008), Mint (Misra and Srivastava 2000), Basil (Hassani et al. 2004), Feverfew (Saharkhiz et al. 2007), and Peppermint (Alkire and Simon 1993).

According to Dadrasan et al. (2015) moderate and severe water deficit induction causes decrease (40 and 65%) in forage yield in fenugreek (*Trigonella foenum-graecum* L.), respectively. During water scarcity, plants tend to diminish water deprivation from leaves by closing the stomatal pores, which subsequently restricts the availability of carbon dioxide for photosynthetic process and biomass accumulation (Sun et al. 2013). Also, the mass overflow of nutrients occurs to the belowground part of plant and, therefore, acquisition of nutrient becomes restricted by severe stress which further reduces mass production (Diaz-Lopez et al. 2012). In the present work, nitroxin and phosphate barvar-2 can provide suitable nutritional conditions for dragonhead plants. Also, nitroxin and phosphate barvar-2 accompanied by zeolite increased the plant morpho-physiological traits, especially essential oil content and yield. Rahbarian et al. (2014) reported that the maximum RWC and oil yield in dragonhead were obtained under mild water-deficit stress via application of 30 t manure/ha. The minimum values of these traits were obtained under severe stress with manure application. Sharma et al. (2003) reported that combination of nitroxin and phosphate barvar-2 increased chlorophyll content and consequently photosynthesis potential and finally caused an increase in dragonhead yield. Mahfouz and Sharaf-Eldin (2007) pointed out that applying biological fertilizers such as *Azotobacter, Azospirillum* and *Bacillus* increased essential oil content in fennel plants. Reduction in RWC caused an increase in essential oil content. Similar findings related to increasing essential oil percentage under water stress were reported by Ranjbar et al. (2004) and Rahbarian et al. (2014). Furthermore, regarding significant interaction between biofertilizers and zeolite, it can be suggested that zeolite amendment with soil has a vital function in mitigating adverse impacts of deficit water stress and reducing soil fertilizer/nutrient leaching (Ippolito et al. 2011).

The $IWUE_{FY}$ was reduced with raising water stress intensity, but $IWUE_{EOY}$ was improved at mild deficit water stress compared to the other stress levels. On the hand, more favorable water availability conditions (30 and 60% depletion of ASW) were caused the maximum water use efficiency for $IWUE_{FY}$ and $IWUE_{EOY}$, respectively. Our results are in agreement with the previous reports (Lima et al. 2013; Silva et al. 2010). According to Lima et al. (2013), castor

(*Ricinus communis* L.) produced low yield and low water use efficiency in dryer years, showing that under low soil water availability the WUE of plants was very low. Furthermore, Silva et al. (2010), reported that the maximum WUE value for production of specific gel in Aloe vera (*Aloe barbadensis* Miller) was obtained in plants subjected to intermediate irrigation regime relative to extreme treatments. In contrary to the latter reports, however, Hazrati et al. (2017) demonstrated that the WUE of *Aloe vera* improved with water limitations. It may be due to the fact that *A. vera* L. plants (with CAM metabolism) are able to keep their stomata open under water scarcity conditions and continue carbon assimilation (Cousins and Witkowski 2012). The ability of plants to spend water effectively for the accumulation of dry weight extremely differs among different species. In our study, a reduction in WUE for flower yield shows that there was no desirable practical use of photo-assimilates by dragonhead reproductive parts under the prolonged water shortage stress conditions. Here, reduction in shoot top flower yield by water-deficit stress is a significant reason of reduced irrigation WUE of flower yield. In addition, deficiency of water during the reproductive growth stage affects early and late flower yield and dry matter.

The results of interaction means of different irrigation regimes and biofertilizers containing nitrogen stabilizing (nitroxin) and phosphat solubilizing (barvar-2) bacteria indicated that the maximum value of the examined traits recorded by combined application of nitroxin and phosphate barvar-2 under low (e.g. RWC, Chl *a* and *b*, and total Chl contents and flower yield) and medium (e.g. percentage and yield of essential oil) water-deficit stress conditions in both years. In the present study, the applied biofertilizers such as nitroxin (comprising *Azetobacter* sp., *Azospirillum* sp., and bacteria of *Pseudomonas* genus with phosphate-solubilizing ability) and phosphate barvar 2 (containing *Pantoea agglomerans* and *Pseudomonas putida*) significantly affected physiological functions and IWUE of flower and essential oil yield in dragonhead, which are in some parts comparable to those of Gharib et al. (2008), who obtained that inoculation of Majorana (*Majorana hortensis*) plants with biofertilizers significantly impacted the growth parameters as well as essential oil production.

Overall, the superiority of biofertilizers consortium inoculation over control or individual inoculation can be related to the positive and cumulative synergistic impacts of inoculation. These beneficial microorganisms enhance host plant growth and development via different direct and/or indirect mechanisms including bioavailability of P and N for plant absorption, production of antibiotics, secretion of vitamins, Fe sequestration by siderophores and chelates, synthesis of the enzymes and growth stimulators (auxins, cytokinins, and gibberellins), fixing atmospheric nitrogen and reduction in ethylene (an stress-accumulated hormone in plants) levels

inside plant cells using ACC deaminase (Glick 1995; Ghorbanpour et al. 2015).

Moreover, plant growth-promoting rhizobacteria (PGPRs) have been successfully applied as biotic elicitors for enhancement of secondary metabolite contents in aromatic medicinal herbs (Ghorbanpour et al. 2013). Due to the significant increasing costs of water consumption and disposal, enhanced water treatment technologies/strategies make economic and social sense. In the present study, regarding significant interaction between biofertilizers and zeolite, the treatment combinations of zeolite and nitroxin × phosphate barvar-2 could improve physiological functions and essential oil yield of dragonhead plants following irrigation after 60% depletion of ASW (the best treatment). Therefore, there is no doubt there are financial benefits of getting high essential oil yield with lower water consumption. The high amount of the essential oil yield following employed treatments, particularly following biofertlizers application and upon combination of zelolite and biofertlizers, were due to enhanced dry matter and flower yield and enhanced essential oil content. These findings are in agreement with Tahami et al. (2011), who reported that the essential oil of *Ocimum basilicum* L. plants increased following inoculation with biofertilizers under an organic farming system. Similar results were reported by Jahan and Jahan (2010) on German chamomile exposed to organic fertilizers.

Conclusions

The results exhibit that the employed biofertilizers [nitroxin (containing free-living nitrogen fixing bacteria including *Azetobacter* sp., *Azospirillum* sp., and phosphate-solubilizing bacteria from *Pseudomonas* genus) and phosphate barvar-2 containing *Pantoea agglomerans* (p5) and *Pseudomonas putida* (p13)] significantly affect quantitative and qualitative production of *D. moldavica* L. under different watering conditions. Application of nitroxin × phosphate barvar-2 with zeolite under 30% depletion of ASW (mild water deficit stress) caused maximum percentage of RWC, total chlorophyll content, shoot top flower yield and $IWUE_{FY}$. However, the highest essential oil percentage, proline and soluble sugars contents were observed when the available soil water depletion was reached at 90% (severe water deficit stress) without zeolite and combined application of nitroxin and phosphate barvar-2.

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