



Co-inoculation of Phosphate-Solubilizing Bacteria and Mycorrhizal Fungi: Effect on Seed Yield, Physiological Variables, and Fixed Oil and Essential Oil Productivity of Ajowan (*Carum copticum* L.) Under Water Deficit

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Abstract

Rhizosphere symbiotic microorganisms play a significant role in mitigating the effects of water deficit on plant production. This work explores the effects of co-inoculation of phosphate-solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AMF) on seed yield, essential oils (EOs), and oil composition of Ajowan under water deficit conditions. We assessed the response of Ajowan plants to individual or combined inoculation with AMF (*Rhizophagus intraradices*) and PSB (*Pseudomonas putida* + *Pantoea agglomerans*) under several irrigation regimes (irrigation at 100% FC, 75% FC, and 50% FC, corresponding to normal irrigation, moderate water stress, and severe water stress, respectively). Normal irrigation with AMF + PSB co-inoculation produced the highest seed yield (870 kg ha⁻¹). Moderate water stress with AMF + PSB co-inoculation yielded the most essential oil yield (22.3 kg ha⁻¹). For inoculated and uninoculated plants, severe water stress decreased chlorophyll a by 44.80% relative to normal irrigation. Proline, soluble sugars, and carotenoid contents, and peroxidase, superoxide dismutase, and catalase activities increased with increasing water deficit, more so with AMF + PSB co-inoculation than individual inoculations. Water deficit also increased the essential oil content in Ajowan seeds. Co-inoculation with AMF and PSB increased the percentage of major EO components—thymol (50.94%) and γ -terpinene (33.18%)—and the main fatty acids—petroselinic (64.28–70.11%), oleic (16.05–21.9%), and palmitic acids (6.50–9.10%)—under moderate water stress, relative to uninoculated plants. Co-inoculation with AMF and PSB significantly improved EO quantity and quality in Ajowan seeds under moderate water stress.

Keywords Antioxidant activities · Biofertilizers · Secondary metabolites · Thymol · Water limitation

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1 Introduction

There is continued consumer interest in herbal-based and organic products, especially from medicinal and aromatic plants (MAPs), used extensively in various industries, including pharmaceuticals, cosmetics, and food. Ajowan (*Carum copticum* L.), a well-known herb belonging to the Apiaceae family, is cultivated in many areas globally, including Iran, India, Pakistan, and Egypt (Niazian et al. 2019; Razavizadeh and Komatsu 2018). Ajowan seeds produce up to 5% essential oils (EO) and secondary metabolites, which are valuable as natural products in the food and flavoring industries and well-known for their antimicrobial and antiparasitic characteristics (Morsy 2020; Heidari et al. 2016). In addition, Ajowan has been traditionally used in Persian medicine to treat dyspepsia, colic and digestion-related conditions, and diarrhea (Rahimmalek et al. 2017; Zarshenas et al. 2013). Studies have reported that the main EO constituents of Ajowan are thymol, γ -terpinene, and p -cymene, which comprise 94–96% of the essential oil, but there is no complete information on the composition of Ajowan oil, especially under drought stress conditions (Soltani Howyzeh et al. 2018; Ghadimian and Esmaili 2016). Ghassemi-Golezani et al. (2018) reported that, under water stress, thymol and γ -terpinene were the major constituents of Ajowan seed essential oil. In addition, terpineol, trans-carveol, and tetrathiapentacene were induced by water stress. Many Ajowan essence compounds decreased, but some increased with water stress, including β -myrcene, cis-sabinene hydrate, terpinen-4-ol, 2-cyclohexen-1-ol, 2-propenoic acid, and carvacrol. Water stress also stimulated the synthesis of terpineol, trans-carveol, and tetrathiapentacene in Ajowan seeds.

Drought stress is one of the most important environmental stresses for agricultural productivity worldwide. In arid and semi-arid regions of Iran, water stress affects the growth of herbs and the quality and quantity of their EOs (Rezaei-Chiyaneh et al. 2018; Al-Huqail et al. 2020). Plants adapt to drought stress by adjusting their osmolytes, including the synthesis and accumulation of proline, glycine betaine, and soluble carbohydrates (Pashang et al. 2021; Chen and Jiang 2010). Plants are also equipped with efficient antioxidative defense systems, comprising non-enzymatic (ascorbic acid, carotenoids, and flavonoids) and enzymatic (catalase (CAT), superoxide dismutase (SOD), and peroxidase (POX)) components that scavenge reactive oxygen species (ROS) (Gill and Tuteja, 2010). The reduction in NADP⁺ as an electron acceptor and accumulation of NADPH and H⁺ under adverse drought stress require a high level of NADPH and H⁺ consumption by plants, which has a positive effect on the biosynthesis of secondary metabolites, such as phenols, terpenoids, alkaloids,

and thus EO content and components in medicinal plants (Kleinwachter et al. 2015). Therefore, enhancing the EO content of MAPs under drought stress is a viable option as this stress increases metabolite production in plants and prevents them from oxidizing in cells. However, EO yield, which is directly related to dry matter yield and EO content, decreases under water stress due to a reduction in biomass yield resulting from reduced nutrient uptake, especially P, from the soil. Decreased P uptake under water stress conditions could be attributed to the low solubility of organic matter and its slow release, reduced microbial activity, and reduced P mineralization and release in soils (Bista et al. 2018). Therefore, strategies for plants to better absorb nutrients under drought stress will increase the quantitative and qualitative yields of essential compounds in medicinal plants.

The use of rhizosphere microorganisms (arbuscular mycorrhizal fungi (AMF) and phosphate-solubilizing bacteria (PSB)) as sustainable components can mitigate abiotic stress and improve plant performance (Dimkpa et al. 2009; Rezaei-Chiyaneh et al. 2021a). AMF help direct water uptake and enhance drought tolerance in plants by regulating physiological and biochemical mechanisms, such as nutrient uptake and osmotic and antioxidant adjustments (Rahimzadeh and Pirzad 2017; Begum et al. 2019). Mycorrhizal fungi interact with numerous other beneficial soil microorganisms, including PSB (*Pseudomonas* and *Bacillus* strains), promoting plant growth through phosphate solubilization (Fitter and Garbaye 1994). In addition, the symbiotic association of plants and microbes (AMF and PSB) can affect the biosynthesis of various secondary metabolites and fatty acids in medicinal plants (Carlsen et al. 2008; Rahimzadeh and Pirzad 2019).

It is well established that water stress impacts the yield and quality of row crops, but the literature is scant on the effect of water stress on the yield and quality of medicinal plants, particularly in arid and semi-arid areas. In addition, it is well-known that inoculating plants with mycorrhizal fungi can increase plant resistance to water deficit, but less is known about co-inoculating with PSB and AMF, especially in medicinal plants such as Ajowan. To the best of our knowledge, this is the first study to evaluate whether co-inoculation of PSB and AMF positively impact Ajowan yield, essential oils, and oil composition under water deficit conditions.

We hypothesized that (i) moderate water stress reduces yield and yield components and negatively affects other agronomic variables, (ii) co-inoculation of PSB and AMF improves the quantity and quality of fatty acid constituents and EO in Ajowan under water stress, and (iii) water stress increases the physiological activities compared with non-stress conditions, and co-inoculation of PSB and AMF

increases plant resistance to water stress by increasing antioxidant enzyme activity.

2 Materials and Methods

2.1 Research Site and Experimental Design

A 2-year field trial was undertaken in the 2017 and 2018 growing seasons in Naqadeh, Iran (38.52° N, 45.24° E, 1320 m asl). This region has a cold semi-arid climate with annual precipitation of 275.5–352.5 mm and average annual air temperatures of 15.04–14.67 °C. The meteorological conditions of the experimental site during the study are in Table 1.

Soil sampling and analysis (0–30 cm depth) revealed that the soil is a silty clay (silt 40%, clay 43%, and sand 17%), with field capacity of 26%, electrical conductivity of 0.37 dS m⁻¹, pH 7.5, 1.09% organic carbon, 0.1% total N, 11.02 mg kg⁻¹ available P, and 566 mg kg⁻¹ available K (averaged over 2 years).

2.2 Plant Material

The experimental setup was factorial, based on a randomized complete block design, with three replications per treatment. The factors were field water capacity (FC) regime (irrigation at 100%, 75%, and 50% FC as full irrigation, moderate water stress, and severe water stress, respectively), and biofertilizer type (AMF (\pm *Rhizophagus intraradices*) and PSB (\pm *Pantoea agglomerans* strain P5 (with Bio Project code: PRJNA386632) and *Pseudomonas putida* strain P13 (16S rDNA sequence at GenBank with accession No. EU545414 a))) applied alone or in combination.

Irrigation water was measured using a volumetric flow meter installed at the entrance of the field. Volumetric soil water contents were measured using the gravitational

method at depths of 0.1, 0.2, 0.3, 0.4, 0.6, and 1 m in different irrigation treatments before each irrigation. The required normal irrigation depth (d) was estimated as follows for the normal irrigation treatments:

$$d = \sum_{i=1}^n (\theta_{fi} - \theta_i) \Delta z$$

where θ_{fi} and θ_i are field capacity and soil moisture content of soil layer i , respectively (m⁻³), n is the number of soil layers, and Δz is the thickness of each soil layer (m). For the stress treatments, water stress was started 4 weeks after germination.

The mycorrhizal inoculum (Strain DAOM 181,602/DAOM 197,198/MUCL 43,194: GCA_000439145.3 from ENA/EMBL: AUPC02000001-AUPC02001111) was a mixture of sterile sand, mycorrhizal hyphae, spores, and colonized root fragments prepared using the method described by Hajiboland et al. (2010). Each gram of inoculum media contained 120 living spores of *Rhizophagus intraradices*. The inoculum (40 g plant⁻¹) was banded directly below the seeds at sowing.

The bacterial inoculum (PSB) comprised a bacterial population of 5×10^8 colony-forming units (CFU) g⁻¹. Bacterial powder (Green Biotech Company, Iran) was mixed with water and uniformly sprayed to cover the seeds, before drying in the shade. Chemical nitrogen fertilizer (110 kg ha⁻¹ urea) was applied to the soil, once before sowing and again before stem elongation.

The experimental field was prepared by plowing, disk-ing, and leveling. All plots comprised eight crop rows (3 m long) spaced 20 cm apart, with plants spaced 40 cm apart within rows. The 36 plots and three blocks were spaced 2 and 3 m from one another, respectively, to prevent water runoff to adjacent plots. Seeds were sown 20 cm apart within each row on 17 March 2018 and 2019. For the stress treatments, water stress was started 3 weeks after germination. All plots were regularly irrigated after sowing until seedling establishment.

Table 1 Weather data from March to August 2017 and 2018 at the study site in Naqadeh, Iran

Year	March	April	May	June	July	August
Monthly average temperature (°C)						
2017	8	14.5	20.8	25.6	28.8	28.6
2018	12.8	16.3	25.5	29.9	32.8	26.1
2-yr average	10.4	15.4	23.15	27.75	30.8	27.35
10-yr average	8.9	14.4	19.5	24.6	27.4	27.1
Monthly average precipitation (mm)						
2017	38.1	38.6	12.8	1.7	2.2	0.6
2018	78.6	55.5	20.5	0.0	0.2	0.0
2-yr average	58.35	47.05	33.3	0.85	1.2	0.3
10-yr average	55.6	43.2	28.4	3.4	2.9	2.5

2.3 Harvest

At maturity (18 September 2017 and 2018), ten plants were randomly handpicked from each plot to determine yield-component traits (plant height, umbels per plant, seeds per umbel, and 1000-seed weight). Each sample was harvested from 2 m² within individual plots and threshed to separate seeds from the straw. Ajowan seeds were kept at room temperature (25 °C) for 12 days before determining seed yield and extracting essential oil.

2.4 Essential Oil Content and Yield

Air-dried ground seed samples (30 g) were hydro-distilled in a Clevenger-type apparatus with 500 mL deionized water for 3 h (Rezaei-Chiyaneh et al. 2020a). Extracted EOs transferred to an amber glass bottle, capped, and stored at 4 °C until gas chromatography-mass spectrometry (GC–MS) analysis.

The EO content and yield were determined using the following formulas (Amani Machiani et al. 2019):

$$\text{EO content (\%)} = \frac{\text{Extracted EO (\%)}}{30 \text{ g of Ajowan ground seed}} \times 100$$

$$\text{EO yield (kg ha}^{-1}\text{)} = \text{EO content (\%)} \times \text{seed yield (kg ha}^{-1}\text{)}$$

2.5 Essential Oil Analysis

Gas chromatography-mass spectrometry (GC–MS) analysis was undertaken using an Agilent 7890/5975C (Santa Clara, CA, USA) GC/MSD. An HP-5 MS capillary column (5% phenyl methyl polysiloxane, 30 m length, 0.25 mm i.d., 0.25 µm film thickness) was used to separate the EO components. The following oven temperature was applied: 1 min at 60 °C, before increasing by 5 °C min⁻¹ to 180 °C, and held for 2 min at 180 °C, finally in the last ramp, temperature increased at 10 °C min⁻¹ to 260 °C and held for 15 min. Helium was used as the carrier gas at a flow rate of 1 mL min⁻¹. The sample was injected (1 µL) in split mode (1:50). The EI mode was 70 Ev. Mass range was set from 40 to 550 m/z. The components were recognized by comparing the calculated Kovats retention indices from a mixture of n-alkane series (C8–C30, Supelco, Bellefonte, CA) and mass spectra (Adams, 2007; NIST, 2008). GC–FID analysis was done using an Agilent 7890 A instrument. The separation was performed in an HP-5 capillary column. The analytical conditions were the same as above. Quantification methods were the same as those reported

by Rezaei-Chiyaneh et al. (2020a) and Amani Machiani et al. (2019).

2.6 Fixed Oil Isolation

For measuring oil content, seeds of Ajowan were ground to extract the fixed oils; 10 g of ground seed was extracted in 250 mL of *n*-hexane in a Soxhlet apparatus. After 8 h of extraction, the solvent was removed from the oil by rotary evaporation. In addition, oil extraction from Ajowan seeds using the cold press method was also undertaken to determine oil composition. Oil yield was determined as follows (Rezaei-Chiyaneh et al. 2021b):

$$\text{Oil yield} = \text{seed yield (kg ha}^{-1}\text{)} \times \text{oil content (\%)}$$

2.7 Oil Analysis

The oil of Ajowan was analyzed using GC–MS (Wilmington, DE, USA) following the method reported by Rezaei-Chiyaneh et al. (2020b).

2.8 Root Colonization of AMF

Root colonization percentage was determined using ten plants per experimental plot—1-cm root pieces were placed into a formalin-acetone alcohol solution (13 mL formalin, 5 mL glacial acetic acid, and 200 mL of 50% ethanol) for 24 h. Samples were rinsed with distilled water and cleared in 10% KOH for 1 h at 90 °C. The roots were then placed in 1% hydrochloric acid for 3 min. The samples were stained with 0.05% trypan blue, boiled for 45 min, and placed in lactoglycerol for 24 h. The root colonization rate, expressed as a percentage, was measured as the ratio between the number of root segments containing vesicles, arbuscules, or hyphae and the total number of root segments sampled (Phillips and Hayman, 1970; Rezaei-Chiyaneh et al., 2021a).

2.9 Physiological Variables

Fresh leaf samples, randomly collected at the flower initiation stage in July 2018 and 2019, were stored at –80 °C for biochemical analysis.

2.9.1 Chlorophyll and Carotenoid Contents

Chlorophyll a and b and carotenoid contents were determined by homogenizing 0.2 g of the frozen leaf in 80% acetone, following Lichtenthaler's (1987) method. Each sample's absorbance was measured at 646.8, 663.2, and 470 nm, respectively, using a UV–visible spectrophotometer (Halo DB-20, UK) and reported as mg g⁻¹ fresh weight.

2.9.2 Proline Content

Proline content in leaf tissues was estimated according to the method of Bates et al. (1973). Briefly, 0.5 g of leaf sample was homogenized in 5 mL of 3% sulfosalicylic acid, and the extract centrifuged to obtain a clear supernatant. Then, a ninhydrin acid:glacial acetic acid (1:1) solution was mixed with 2 mL leaf extract. The prepared samples were heated at 100 °C for 1 h. The reaction was terminated on ice, before adding 4 mL toluene. After phase separation, the absorbance was determined at 520 nm. The amount of proline was calculated as $\mu\text{g g}^{-1}$ fresh weight of tissue.

2.9.3 Total Soluble Sugar Content

The phenol sulfuric acid method was followed to estimate total soluble sugar (TSS) in leaf samples (Irigoyen et al. 1992). Briefly, 0.5 g of fresh leaf was homogenized with ethanol. The clear homogenate was mixed with 5% phenol and 98% sulfuric acid. After cooling, the absorbance of the leaf extracts was recorded at 485 nm. The soluble sugar content was reported as mg g^{-1} fresh weight of tissue.

2.10 Enzyme Extraction and Assay

For enzyme extraction, 0.1 g of frozen leaf was homogenized in 2 mL sodium phosphate buffer (pH 7.0). The homogenate was centrifuged (MPW-260R, Poland) at 15,000 rpm for 20 min at 4 °C. The supernatant was used to measure antioxidant enzyme activities. All the enzymes were calculated as unit g^{-1} fresh weight min^{-1} .

2.10.1 Catalase Activity

CAT activity was measured using the disappearance rate of hydrogen peroxide (H_2O_2) (Aebi 1974). The reaction mixture comprised 50 mL enzyme extract, 0.1 mL H_2O_2 , and 50 mM phosphate buffer (pH 7.0). CAT activity was estimated as the absorbance at 240 nm.

2.10.2 Superoxide Dismutase Activity

SOD activity was assayed using the nitro blue tetrazolium (NBT) method described by Dhindsa et al. (1981). SOD activity (unit g^{-1} FW) was recorded by measuring the amount of enzyme required to inhibit NBT at 560 nm.

2.10.3 Peroxidase Activity

POX activity was measured using guaiacol and H_2O_2 as the substrates of the reaction mixture. The increase in absorbance due to tetraguaiacol formation was reported at 470 nm (Kar and Feierabend 1984).

2.11 Statistical Analysis

A combined analysis of variance across 2 years of data was conducted using a mixed linear model with PROC MIXED procedure in SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). The irrigation regimes and antioxidant treatments were considered fixed effects, while block, year, and their interactions were random effects. The means were compared using Duncan's multiple range test, and differences between individual means were considered significant at $P < 0.05$.

3 Results

Water deficit and AMF and PSB application, alone or in combination, affected plant growth and physiology in various capacities, with the results summarized below.

3.1 Agronomic Variables

Moderate and severe water stress significantly decreased plant height, umbels per plant, seeds per umbels, and 1000-seed weight in Ajowan, more so in severely stressed plants (50% FC). The addition of biofertilizers had a positive effect on these agronomic variables. The effects of irrigation and biofertilizer were significant ($P < 0.01$) for plant height, umbels per plant, seeds per umbel, seed yield, essential oil percentage and yield, chlorophyll, carotenoid, TSS, and proline contents, and enzymatic activities (Tables 2 and 3). Inoculation with AMF and PSB, alone or combined, significantly increased these variables, relative to uninoculated plants (Fig. 1). Water stress significantly reduced seed yield, relative to normal irrigation, by up to 49% under severe water stress (Fig. 2A). However, plants inoculated with AMF and PSB, alone or combined, under water stress had higher seed yields than uninoculated plants. Co-inoculation under severe water stress increased seed yield by 35% relative to uninoculated plants. Under normal irrigation, the co-inoculation treatments had the most root colonization (73.33%), while severe water stress without fertilization (control) had the lowest (5.5%). Non-stress conditions produced 17.37% and 43.20% higher root colonization than moderate and severe water stress, respectively. Inoculation with AMF, PSB, and AMF + PSB increased root colonization by 87.61%, 33.12%, and 89.50%, respectively, relative to the control (Fig. 2B).

Table 2 Effect of irrigation regime and biofertilizer application on agronomic characteristics of Ajowan (*Carum copticum* L.)

S.O.V	Plant height	Umbels per plant	Seeds per umbels	1000-seed weight	Yield	Root colonization	Oil content	Oil yield	Essential oil content
Year (Y)	NS	NS	NS	NS	NS	NS	NS	NS	NS
Irrigation (I)	**	**	**	**	**	**	**	**	**
Y × I	NS	NS	NS	NS	NS	NS	NS	NS	NS
Biofertilizer (B)	**	**	**	**	**	**	**	**	**
Y × B	NS	**	NS	NS	*	*	**	**	NS
I × B	**	**	**	NS	**	**	**	**	**
Y × I × B	NS	**	NS	NS	NS	NS	**	NS	NS

S.O.V, source of variation. NS, * and **: non-significant, significant at 5% and 1% probability level, respectively

Table 3 Effect of irrigation regime and biofertilizer application on physiological characteristics of Ajowan (*Carum copticum* L.)

S.O.V	Essential oil yield	Carotenoids	Chlorophyll a	Chlorophyll b	Soluble sugars	Proline	Catalase	Peroxidase	Super-oxide dismutase
Year (Y)	NS	NS	*	NS	NS	NS	NS	NS	NS
Irrigation (I)	**	**	**	**	**	**	**	**	**
Y × I	NS	NS	*	NS	NS	NS	*	NS	NS
Biofertilizer (B)	**	**	**	**	**	**	**	**	**
Y × B	NS	**	NS	NS	NS	NS	**	NS	NS
I × B	**	**	**	**	**	**	**	**	**
Y × I × B	NS	NS	NS	NS	NS	NS	**	NS	NS

NS, * and **: non-significant, significant at 5% and 1% probability level, respectively

3.2 Essential Oil Content and EO Yield

Averaged across all inoculation treatments, moderate water stress (75% FC) produced the highest EO content (2.5%), increasing to 3.2% in co-inoculated plants (Fig. 3A). Biofertilizer treatments significantly increased the essential oil yield of Ajowan seeds ($P < 0.01$). For all irrigation regimes, inoculation with AMF or PSB alone had a similar effect on increasing EO content, while co-inoculation had a greater effect than the individual treatments (Fig. 3B).

3.3 Essential Oil Composition

The GC–MS-based analysis detected 15 essential oil components in Ajowan seed (Table 4), with the major constituents being *p*-cymene (12.05–21.95%), γ -terpinene (26.13–33.18%), and thymol (44.17–50.94%). Irrigation, bacteria, and mycorrhizae affected EO composition in Ajowan. Moderate water stress produced the highest percentages of thymol and γ -terpinene, which increased with inoculation; co-inoculation produced the highest percentage of thymol (50.94%) and γ -terpinene (33.18%). Moreover,

inoculation had a greater effect under severe water stress than under moderate water stress, with co-inoculation producing the most *p*-cymene (21.95%). Co-inoculated plants under severe water stress produced the highest percentage of identified components (99.63%), followed by AMF inoculation alone (99.05%) (Table 4).

3.4 Fixed Oil Content and Oil Yield

Means comparison showed that non-stressed plants co-inoculated with AMF + PSB had the highest oil content (16.37%), while severe water stress without fertilization (control) produced the lowest oil content (12.45%). Non-stress conditions produced 10.8% and 23.8% higher oil content than moderate and severe water stress, respectively. Inoculation with AMF, PSB, and AMF + PSB increased the oil content by 3.2%, 4.4%, and 9.5%, respectively, relative to the control (Fig. 3C).

Similar results were obtained for oil yield. Non-stressed plants inoculated with AMF + PSB had the highest oil yield (142.3 kg ha⁻¹), while severe water stress without fertilization (control) produced the lowest oil content (50 kg ha⁻¹). Non-stress conditions produced 60.4% and

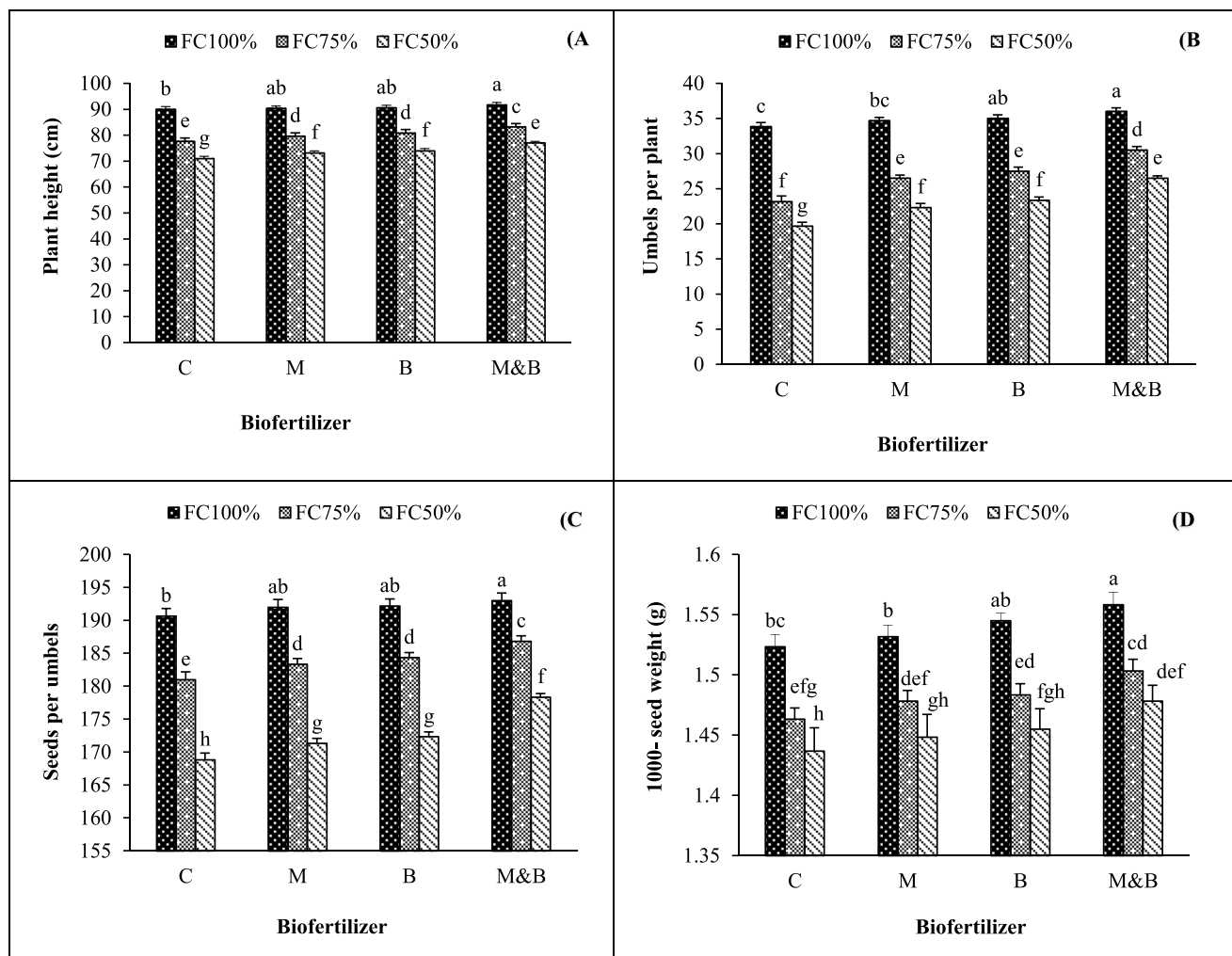


Fig. 1 Interaction effect of irrigation regime and biofertilizer application on **A** plant height, **B** umbels per plant, **C** seeds per umbels, and **D** 1000-seed weight in Ajowan. C, control; M, mycorrhizal; B, bacte-

rial. Different lower-case letters above bars indicate significant differences at $P < 0.05$

96% higher oil yield than moderate and severe water stress, respectively. Inoculation with AMF, PSB, and AMF+PSB increased the oil yield by 15.8%, 39.7%, and 26.6%, respectively, relative to the control (Fig. 3D).

3.5 Fatty Acid Composition

The fatty acid composition of the oil extracted from Ajowan seeds differed under the different irrigation regimes and inoculation treatments (Table 5). We identified five important fatty acids—petroselinic, oleic, and linoleic acids (unsaturated) and palmitic and arachidic acids (saturated)—in the seed oil. Co-inoculated plants under normal irrigation produced the highest percentage of identified components (99.94%), followed by PSB inoculation alone under normal irrigation (99.27%) and co-inoculated plants under moderate water stress (99.04%).

Co-inoculated plants under all three irrigation regimes had the highest petroselinic acid content, while those under moderate water stress had the highest oleic acid content (21.9%). Plants under moderate water stress had the highest percentage of total unsaturated oil. Co-inoculation—resulting in 91.21% total unsaturated fatty acids and 10.85% total saturated fatty acids—could be considered for improving the nutritional value of oils.

Heat map clustering classified essential oil (Fig. 8) and fatty acid composition (Fig. 9) into two main groups: (1) β -pinene, α -terpinene, β -phellandrene, α -terpineol, carvacrol, δ -carene, camphor, sabinene, γ -terpinene, β -myrcene, and α -thujene, with the lowest values under moderate water stress; (2) γ -terpinene and thymol, which increased under moderate water stress and decreased under severe water stress. The no fertilizer condition (control) for all three irrigation regimes produced the lowest values for the oil

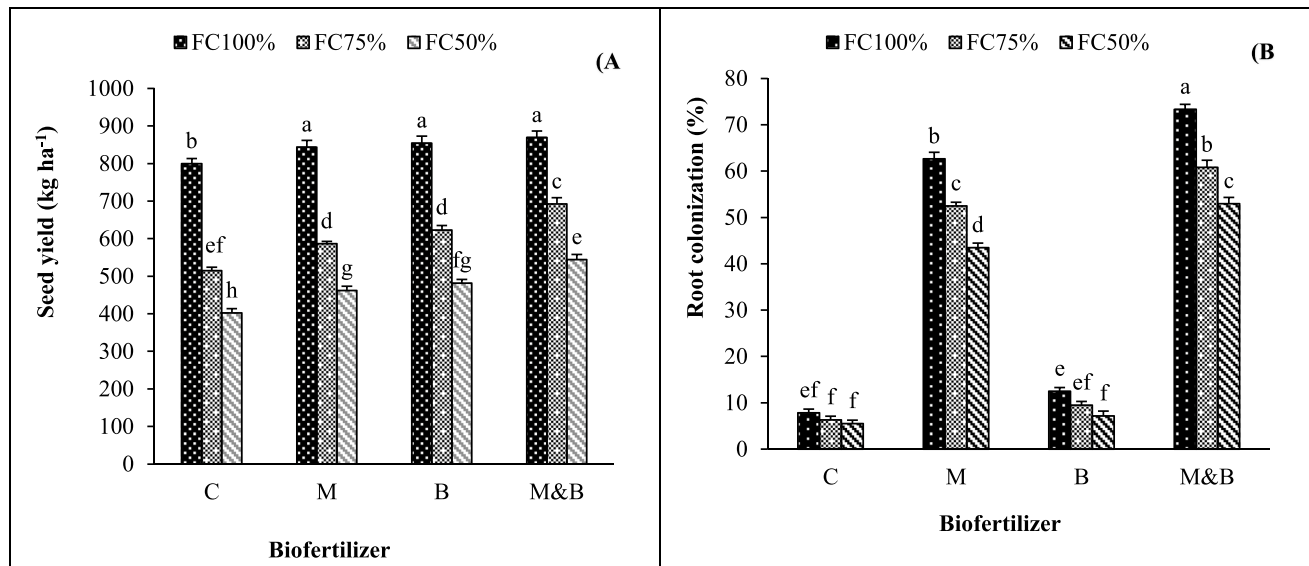


Fig. 2 Interaction effects of irrigation regime and biofertilizer application on **A** seed yield, and **B** root colonization of Ajowan. C, control; M, mycorrhizal; B, bacterial. Different lower-case letters above bars indicate significant differences at $P < 0.05$

components, including palmitic, linoleic, petroselinic, and oleic acids (Fig. 9).

Irrigation regime and biofertilizer treatment were classified into three main groups based on essential oil and fatty acid composition. Irrigation regime was grouped as a major factor affecting essential oil and fatty acid composition of Ajowan (Figs. 8 and 9).

3.6 Photosynthetic Pigments

Water stress reduced chlorophyll content. AMF or PSB alone had no significant effect on chlorophyll a content but significantly increased chlorophyll b content. Co-inoculation significantly increased leaf chlorophyll content under all three irrigation regimes (Fig. 4A and B). For all irrigation regimes, the biofertilizer treatments increased carotenoid content. The co-inoculated plants under severe water stress had the highest carotenoid content ($1.67 \text{ mg g}^{-1} \text{ FW}$), while uninoculated plants under normal irrigation had the lowest carotenoid content ($0.54 \text{ mg g}^{-1} \text{ FW}$), followed by inoculation with AMF, PSB, and AMF + PSB (Fig. 4C).

3.7 Osmotic Content

Water stress significantly increased osmolyte content. Proline and TSS contents increased with increasing severity of water stress for inoculated and uninoculated plants. Co-inoculation under severe water stress produced the highest proline ($4.02 \text{ mg g}^{-1} \text{ FW}$) and TSS ($4.13 \text{ mg g}^{-1} \text{ FW}$) contents in Ajowan leaves (Figs. 4D and 5A).

3.8 Antioxidant Activities

Increasing water stress increased antioxidant enzyme activities in inoculated and uninoculated Ajowan. Under normal irrigation, enzyme activities did not significantly differ between inoculated and uninoculated plants. Inoculation, alone or combined, increased all enzyme activities, with co-inoculation producing the highest CAT, POX, and SOD activities (2.8 , 2.0 , and $3.2 \text{ U min}^{-1} \text{ g}^{-1} \text{ FW}$, respectively) (Fig. 5B–D).

Heat map clustering of antioxidant activities and agro-morpho-physiological measurements was undertaken using Euclidean distance and Ward's minimum variance to visualize and clarify the findings (Fig. 6). The combinations of irrigation regime and biofertilizer treatment clustered into three main groups at an Euclidean distance of 8.0. Interestingly, all biofertilizer treatments with the same irrigation regime clustered in one main group, suggesting that the irrigation regimes had a greater impact on the evaluated variables than the biofertilizer treatments. The evaluated variables clustered into two major groups: (1) yield, oil yield, oil content, plant height, umbels per plant, chlorophyll a and b, 1000-seed weight, seeds per umbel, and essential oil yield; (2) proline, carotenoid and TSS contents, POX, CAT, and SOD activities, and essential oil content. With the highest values under normal irrigation, the first group of variables decreased markedly with increasing water stress. In contrast, the second group of variables significantly increased with increasing water stress.

The correlation analysis revealed significant positive correlations between seed yield and oil yield (1.00^{***}), oil

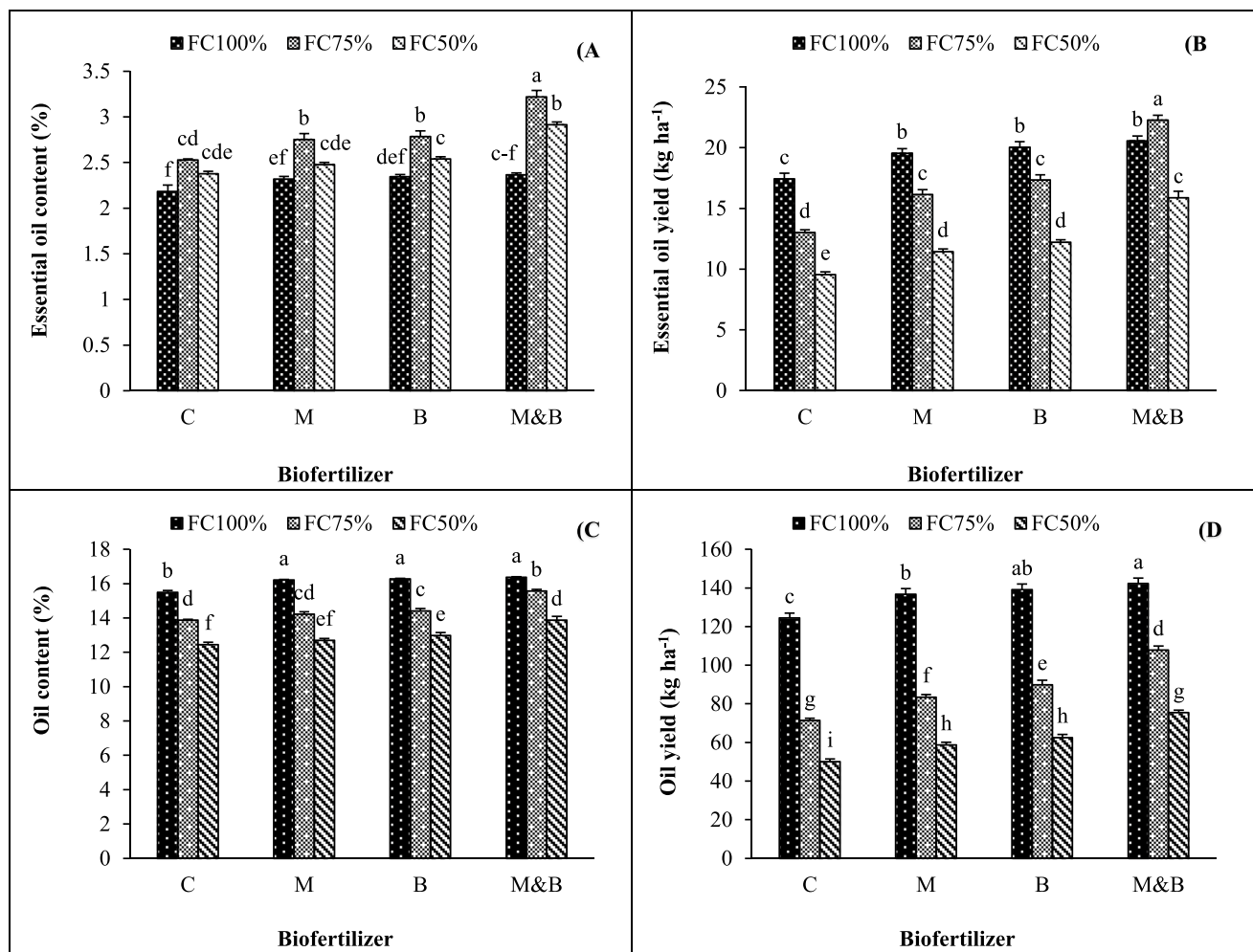


Fig. 3 Interaction effects of irrigation regime and biofertilizer application on **A** essential oil content, **B** essential oil yield, **C** oil content, and **D** oil yield in Ajowan. C, control; M, mycorrhizal; B, bacterial. Different lower-case letters above bars indicate significant differences at $P < 0.05$

content (0.98^{***}), plant height ($r = 0.99^{***}$), umbels per plant ($r = 0.99^{***}$), 1000-seed weight ($r = 0.98^{***}$), chlorophyll a ($r = 0.98^{***}$), chlorophyll b ($r = 0.98^{***}$), seeds per umbel ($r = 0.96^{***}$), essential oil yield ($r = 0.88^{***}$), TSS ($r = 0.74^{**}$), proline ($r = 0.72^{**}$), SOD ($r = 0.71^{**}$), CAT ($r = 0.65^{*}$), POX ($r = 0.65^{*}$), and carotenoids ($r = 0.60^{*}$) (Fig. 7).

4 Discussion

In this study, seed productivity of Ajowan decreased under water deficit conditions, which could be due to reduced photosynthetic carbon assimilation (the most important process determining plant productivity), resulting from suppressed leaf formation and expansion, induced leaf shedding, stomatal closure, and reduced efficiency of the carbon fixation process (Lee and Mudge 2013). Gholinezhad et al. (2020) reported that biomass and seed yield in sesame

(*Sesamum indicum* L.) significantly decreased with increasing water stress levels, but AMF inoculation enhanced seed productivity.

Microbial inoculation supports plant growth by improving plant tolerance to drought stress, suggesting that inoculation with AMF and PSB strains alters plant physiological and biochemical components under water stress, as reported elsewhere (Rahimzadeh and Pirzad 2019; Nacoon et al. 2020). Many factors influence plant growth, including soil nutrient availability, temperature, available water, and light. Nutrient accessibility plays an important role in the physiological and biochemical activities of plants. AMF increase nutrient availability for plants through mycelium expansion and root system development and could play a role in increasing plant performance under water stress. AMF hyphae also reduce the resistance of water transfer to roots by increasing nutrient uptake. Other factors that could be involved in increasing plant growth and yield under water stress include the dissolution of

Table 4 Effect of irrigation regime and biofertilizer on essential oil compounds of Ajowan (2-year average)

No	Component	RI ^a	Treatments ^b												Average of water stress
			100% FC + C	100% FC + M	100% FC + B	100% FC + C + M	75% FC + C	75% FC + M	75% FC + B	75% FC + C + M	50% FC + C	50% FC + M	50% FC + B	50% FC + C + M	
1	α -thujene	929	0.1 ± 0.03	0.32 ± 0.01	0.2 ± 0.004	0.97 ± 0.07	0.22 ± 0.01	0.31 ± 0.09	0.36 ± 0.05	0.30 ± 0.03	0.40 ± 0.09	0.19 ± 0.01	0.43 ± 0.02	0.48 ± 0.06	0.35
2	α -pinene	934	1.04 ± 0.02	1.12 ± 0.03	0.09 ± 0.01	0.46 ± 0.01	0.23 ± 0.05	0.191 ± 0.04	0.15 ± 0.02	0.01 ± 0.01	1.15 ± 0.03	–	0.19 ± 0.07	0.29 ± 0.01	0.44
3	Sabinene	969	0.1 ± 0.001	0.53 ± 0.02	0.21 ± 0.01	0.6 ± 0.05	0.20 ± 0.02	0.22 ± 0.01	0.30 ± 0.05	0.03 ± 0.01	1.29 ± 0.02	1.16 ± 0.01	0.36 ± 0.02	0.85 ± 0.08	0.48
4	β -pinene	973	1.74 ± 0.07	1.8 ± 0.01	1.32 ± 0.05	0.25 ± 0.03	1.48 ± 0.01	1.06 ± 0.07	0.65 ± 0.04	0.1 ± 0.02	1.82 ± 0.09	1.62 ± 0.09	1.02 ± 0.06	0.5 ± 0.03	1.11
5	β -myrcene	985	1.16 ± 0.06	0.6 ± 0.003	0.36 ± 0.01	0.86 ± 0.04	0.30 ± 0.002	0.34 ± 0.01	0.38 ± 0.01	0.03 ± 0.02	1.44 ± 0.08	1.29 ± 0.07	0.46 ± 0.02	0.49 ± 0.04	0.64
6	α -terpinene	1013	0.22 ± 0.002	0.76 ± 0.05	0.49 ± 0.01	0.14 ± 0.03	0.18 ± 0.02	0.22 ± 0.01	0.26 ± 0.01	0.02 ± 0.001	0.26 ± 0.02	0.19 ± 0.01	0.29 ± 0.03	0.44 ± 0.04	0.29
7	<i>p</i> -cymene	1021	12.05 ± 0.10	13.4 ± 0.19	12.29 ± 0.11	13.87 ± 0.17	13.23 ± 0.13	13.84 ± 0.11	14.25 ± 0.17	13.91 ± 0.12	16.06 ± 0.10	19.15 ± 0.17	20.70 ± 0.19	21.95 ± 0.15	15.40
8	β -phellandrene	1026	0.3 ± 0.001	1.14 ± 0.04	0.46 ± 0.03	0.95 ± 0.01	0.04 ± 0.04	0.04 ± 0.002	0.05 ± 0.003	0.01 ± 0.001	0.02 ± 0.006	0.3 ± 0.002	0.01 ± 0.004	0.02 ± 0.001	0.27
9	γ -terpinene	1055	26.13 ± 0.71	27.84 ± 0.82	28.11 ± 0.89	28.65 ± 0.77	29.97 ± 0.93	30.89 ± 1.02	32.01 ± 1.02	33.18 ± 0.90	24.90 ± 0.80	26.82 ± 0.74	26.46 ± 0.89	27.40 ± 0.96	28.53
10	DELTA-3-carene	1096	0.23 ± 0.04	–	0.05 ± 0.003	0.06 ± 0.001	–	0.01 ± 0.008	0.04 ± 0.001	0.02 ± 0.004	0.01 ± 0.01	0.03 ± 0.002	0.07 ± 0.004	0.11 ± 0.05	0.06
11	Terpinene-4-ol	1176	0.11 ± 0.06	–	0.31 ± 0.03	0.15 ± 0.08	–	0.01 ± 0.002	0.2 ± 0.01	0.08 ± 0.006	0.6 ± 0.01	1.2 ± 0.05	0.01 ± 0.006	0.43 ± 0.05	0.31
12	Camphor	1180	0.49 ± 0.06	0.18 ± 0.04	0.16 ± 0.02	0.11 ± 0.001	0.15 ± 0.03	0.01 ± 0.01	0.20 ± 0.04	0.10 ± 0.01	–	–	0.49 ± 0.02	0.18 ± 0.07	0.20
13	α -terpineol	1190	1.05 ± 0.09	–	0.06 ± 0.002	0.01 ± 0.004	0.5 ± 0.02	0.01 ± 0.05	0.09 ± 0.004	0.05 ± 0.001	0.03 ± 0.008	1.01 ± 0.06	0.04 ± 0.002	0.41 ± 0.05	0.29
14	Thymol	1288	46.74 ± 1.90	47.6 ± 1.80	49.05 ± 1.77	49.93 ± 1.90	48.70 ± 1.75	49.87 ± 1.83	49.71 ± 1.603	50.94 ± 1.65	44.30 ± 1.44	44.17 ± 1.30	45.20 ± 1.92	45.83 ± 1.87	47.67
15	Carvacrol	1303	1.33 ± 0.05	0.99 ± 0.01	0.82 ± 0.04	0.41 ± 0.002	0.31 ± 0.01	0.27 ± 0.03	0.29 ± 0.07	0.04 ± 0.06	0.28 ± 0.04	1.92 ± 0.09	0.24 ± 0.01	0.25 ± 0.08	0.59
Total identified (%)			92.79	96.28	93.98	97.42	95.51	97.291	98.94	98.82	92.56	99.05	95.97	99.63	96.63

^aRI, linear retention indices on DB-5 MS column, experimentally determined using homolog series of *n*-alkanes, data are mean ± SE (*n* = 3); the main components are shown in bold^bIrrigation at 100% FC, 75% FC, and 50% FC, corresponding to normal irrigation, moderate water stress, and severe water stress, respectively. C, control; M, mycorrhizal; B, bacterial

Table 5 Effect of irrigation regime and biofertilizer on oil compounds in Ajowan (2-year average)

No	Components	Treatments ^a											
		100% FC+C	100% FC+M	100% FC+B	100% FC+B+M	75% FC+C	75% FC+M	75% FC+B	75% FC+B+M	50% FC+C	50% FC+M	50% FC+B	50% FC+B+M
1	Palmitic acid	7.2 ± 0.17	8.82 ± 0.15	8.53 ± 0.10	7.61 ± 0.11	6.5 ± 0.13	8.52 ± 0.17	9.10 ± 0.10	7.38 ± 0.13	7.96 ± 0.19	8.05 ± 0.14	8.39 ± 0.11	8.90 ± 0.16
2	Petroselinic acid	64.28 ± 2.90	66.68 ± 2.15	67.85 ± 2.44	70.11 ± 2.89	64.59 ± 1.80	66.79 ± 2.54	67.05 ± 2.15	68.78 ± 1.89	62.29 ± 1.79	65.09 ± 2.09	65.04 ± 2.70	66.92 ± 2.09
3	Oleic acid	16.05 ± 0.87	17.82 ± 0.63	18.04 ± 0.76	18.89 ± 0.21	18.31 ± 0.56	19.05 ± 0.43	20.79 ± 0.50	21.9 ± 0.44	17.8 ± 0.23	18.19 ± 0.43	19.27 ± 0.57	19.74 ± 0.65
4	Linoleic acid	1.14 ± 0.02	1.65 ± 0.05	1.35 ± 0.01	1.75 ± 0.07	1.08 ± 0.03	1.02 ± 0.02	1.22 ± 0.03	0.53 ± 0.05	1.01 ± 0.07	1.86 ± 0.02	1.41 ± 0.08	1.25 ± 0.06
5	Arachidic acid	2.97 ± 0.08	0.75 ± 0.05	0.63 ± 0.07	1.58 ± 0.01	1.38 ± 0.04	1.73 ± 0.03	1.11 ± 0.04	0.45 ± 0.01	1.24 ± 0.052	1.15 ± 0.07	1.42 ± 0.03	1.95 ± 0.09
	Total identified (%)	91.64	95.72	96.4	99.94	91.86	97.11	99.27	99.04	90.3	94.34	95.53	98.76
	Total unsaturated fatty acids (TUF)	81.47	86.15	87.24	90.75	83.98	86.86	89.06	91.21	80.1	85.14	85.72	87.91
	Total saturated fatty acids (TSF)	10.17	9.57	9.16	9.19	7.88	10.25	10.21	7.83	10.2	9.2	9.81	10.85

^aIrrigation at 100% FC, 75% FC, and 50% FC, corresponding to normal irrigation, moderate water stress, and severe water stress, respectively. C, control; M, mycorrhizal; B, bacterial, data are mean ± SE (*n* = 3); the main components are shown in bold

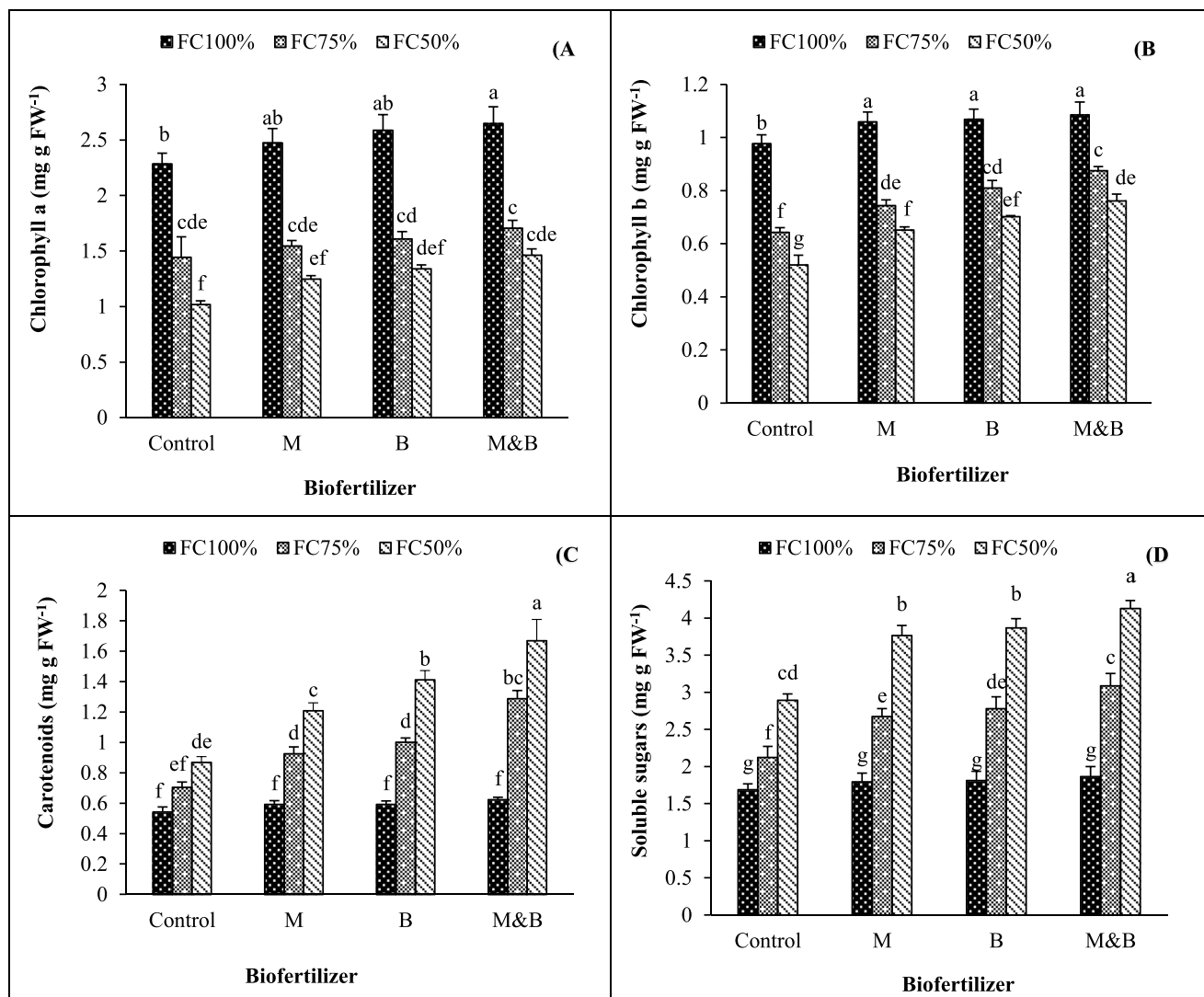


Fig. 4 Interaction effect of irrigation regime and biofertilizer application on **A** chlorophyll a, **B** chlorophyll b, **C** carotenoids, and **D** soluble sugars in Ajowan. C, control; M, mycorrhizal; B, bacterial. Different lower-case letters above bars indicate significant differences at $P < 0.05$

insoluble phosphate in soil, increased available P content from organic acid and extracellular phosphatase production, and increased PSB metabolism, producing a variety of plant hormones, acids, and vitamins (Ghorchiani et al. 2018; Miransari 2011). PSB can grow along AMF hyphae, using them to access additional soil volume containing insoluble P (Ordonez et al. 2016). Increasing P supply can significantly affect root colonization and improve plant production (Berta et al. 2014). In addition, PSB can develop a synergistic microbial interaction and increase plant growth variables through a stimulatory effect on native AMF establishment (Ordonez et al. 2016; Rahimzadeh and Pirzad 2017; Nanjundappa et al. 2019). The application of AMF and PSB improved plant growth by solubilizing nutrients for easy plant uptake, improving nutritional balance, and exuding plant growth regulators

and phytohormones (such as IAA, cytokinins, GA, and ethylene) (Begum et al. 2019). Rahimzadeh and Pirzad (2017) showed that AMF and PSB inoculation increased the yield and yield components of linseed (*Linum usitatissimum* L.) relative to untreated plants.

Co-inoculation with AMF and PSB improved oil and essential oil productivity under water deficit conditions. In thyme (*Thymus citriodorus*), water stress reduced leaf area and increased oil gland density, increasing oil accumulation (Tátrai et al. 2016), as also revealed in our results. Rahimzadeh and Pirzad (2019) reported that water stress might stimulate enzymatic activities for oil biosynthesis. Increased nutrient availability through the integrative application of AMF and PBS enhanced precursor essential oil compounds under water deficit conditions. Copetta et al. (2006) noted that the higher nutrient availability with AMF inoculation

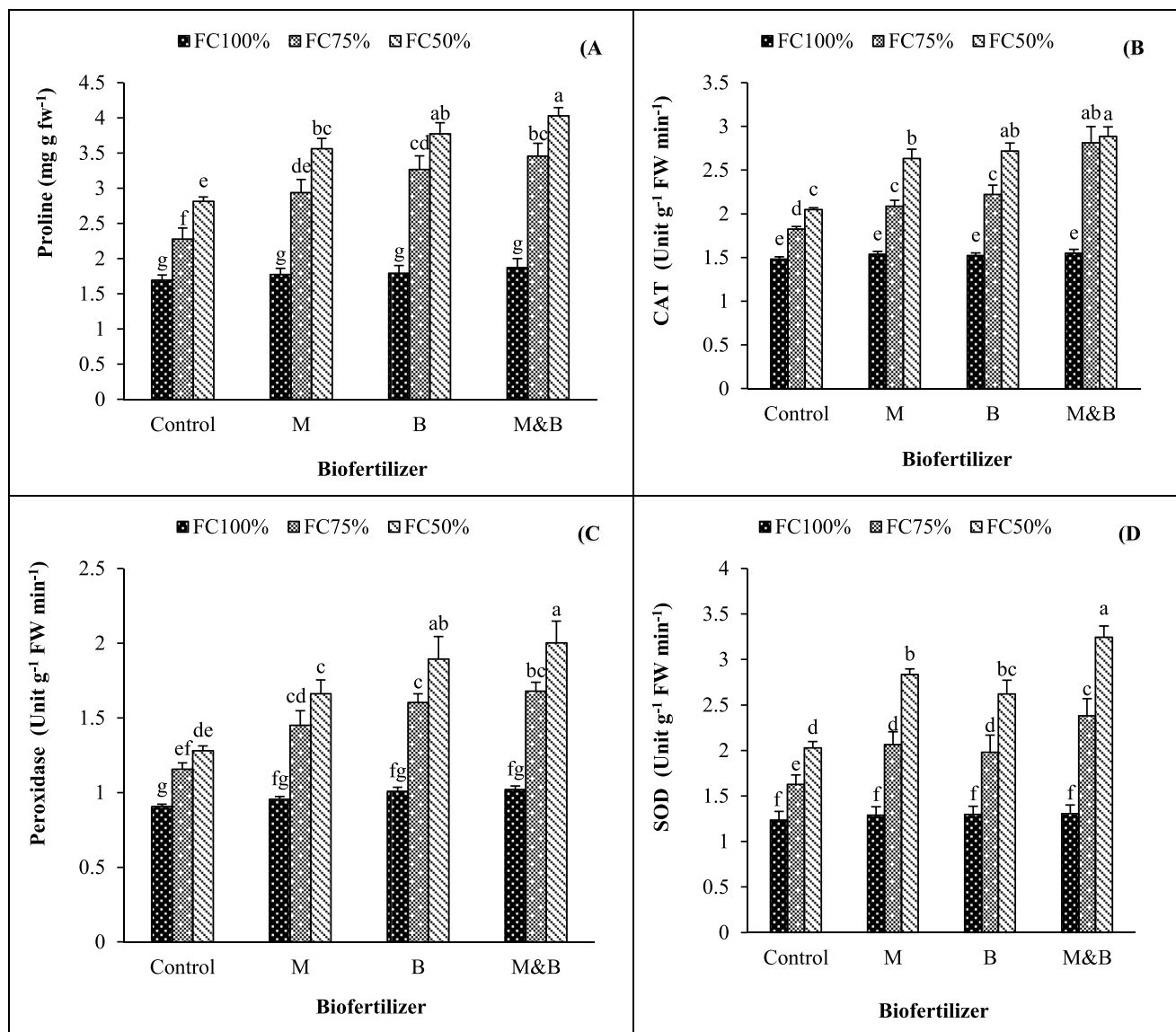


Fig. 5 Interaction effect of irrigation regime and biofertilizer application on **A** proline content, **B** catalase, **C** peroxidase, and **D** superoxide dismutase activities in Ajowan. C, control; M, mycorrhizal; B, bacte-

rial. Different lower-case letters above bars indicate significant differences at $P < 0.05$

enhanced glandular trichomes in basil (*Ocimum basilicum* L.) leaves, increasing essential oil productivity. Hence, the increased essential oil yield of Ajowan co-inoculated with AMF + PBS can be attributed to the positive role of AMF and PBS in increasing seed yield and essential content. Fatty acid composition improved in Ajowan co-inoculated with AMF + PSB under normal irrigation and moderate water stress (Figs. 8 and 9). This could be due to increased nutrient and water uptake, photosynthetic rate, and supply of carbon and other precursor compounds, including ATP and NADPH (Gholinezhad et al. 2020).

In this study, leaf proline and TSS contents accumulated in large quantities under water stress, which reduced

the detrimental effects of water stress on plant productivity (Masoudi Sadaghiani et al. 2011; Razavizadeh and Komatsu 2018). As a biochemical osmotic adjustment, the accumulation of proline and TSS is a main defense mechanism of plants in water stress conditions for mitigating oxidative damage (Rezaei-Chiyaneh et al. 2018). It seems that synthesis and accumulation of proline under stress conditions improve the ability of cells to retain water and maintain normal membrane function and reduce the negative impact of the stressful conditions by maintaining the osmotic balance and cell turgor, preventing electrolyte leakage, stabilizing membranes, and maintaining ROS levels in normal ranges

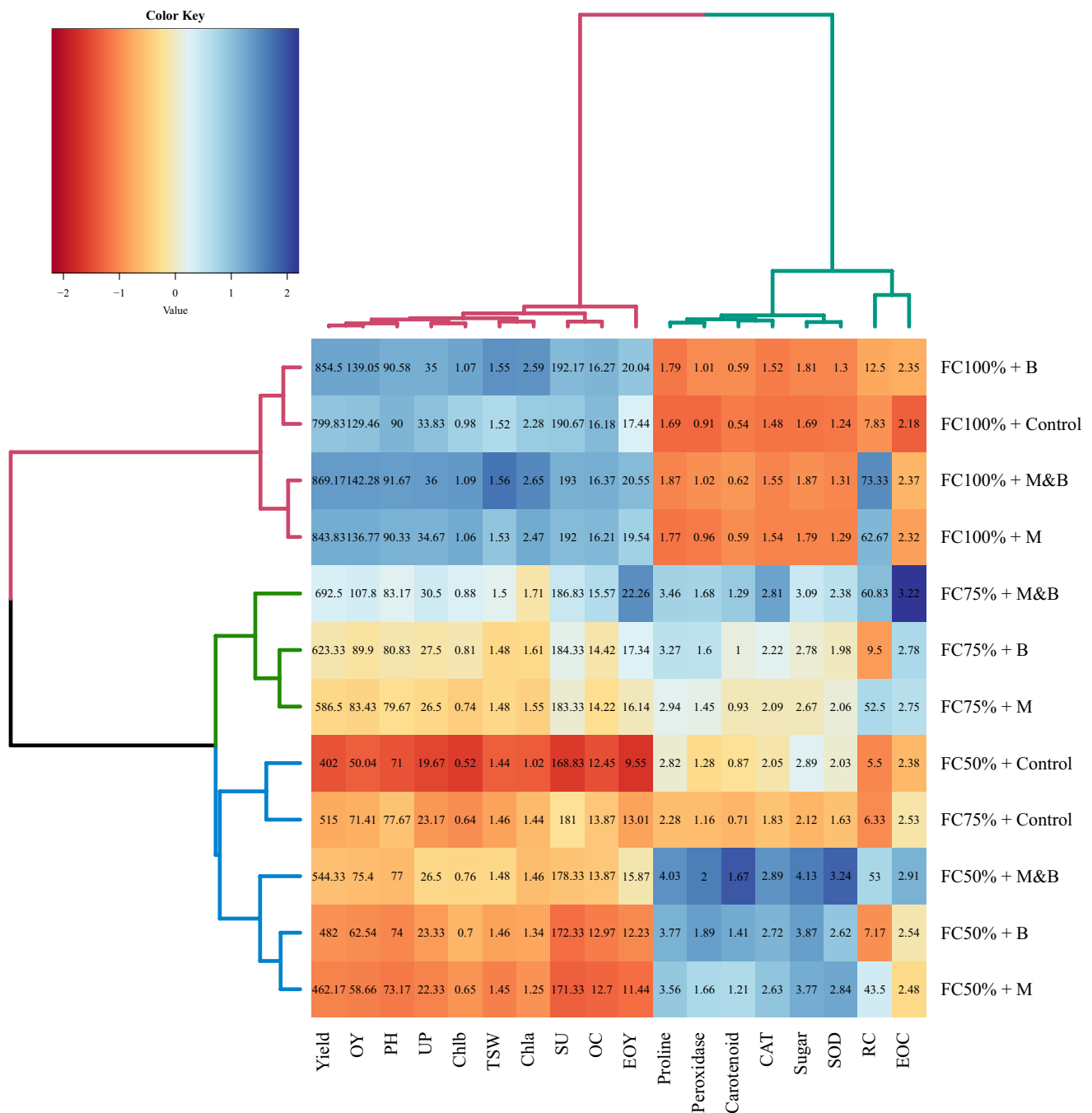


Fig. 6 Heat map clustering of irrigation regime and biofertilizer application (M, mycorrhizal; B, bacterial) based on morpho-physiological characteristics in Ajowan. The key color bar indicates stand-

ardized mean values (dark red indicates relatively low mean values; dark blue indicates relatively high mean values)

(detoxification of ROS) (Arabzadeh 2012; Rahmizadeh and Pirzad 2017).

Water stress significantly reduced chlorophyll a and b contents, mainly due to increased membrane lipid peroxidation and ultimately chlorophyll decomposition in plant leaves (Mohammadi et al. 2019). However, water stress increased carotenoid content due to increased

photo-oxidative stress (Farooq et al. 2019). Carotenoids have good biological activity as antioxidants for anti-aging and scavenging harmful free radicals. Therefore, increased carotenoid content under water stress conditions plays an important role as a non-enzymatic compound in reducing reactive oxygen radicals and increasing plant resistance to drought stress conditions. Co-inoculation produced the

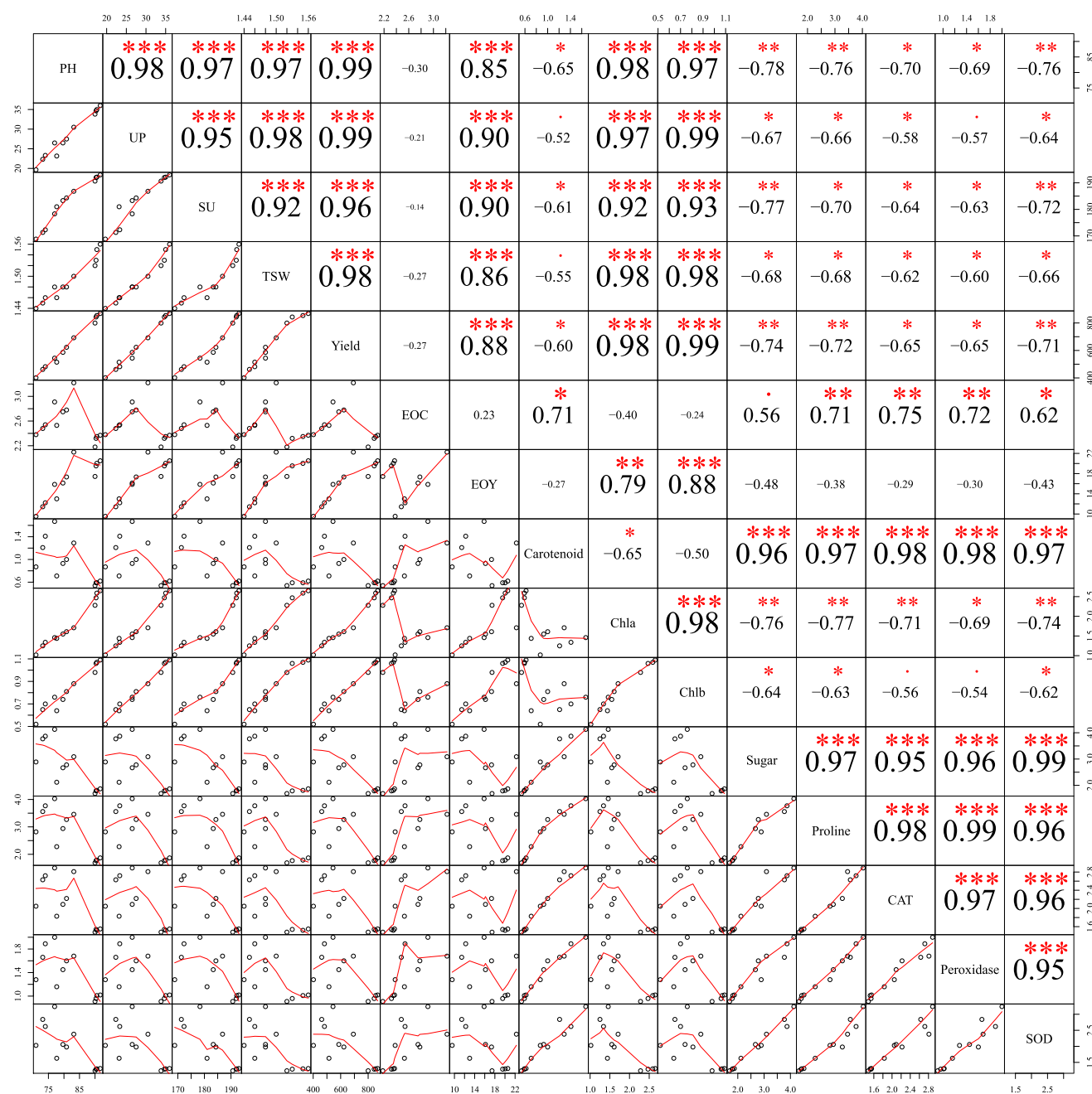


Fig. 7 Correlation coefficient of morpho-physiological characteristics in Ajowan

highest chlorophyll and carotenoid contents, which may be related to increased chloroplasts per bundle sheath in leaves (Rahimzadeh and Pirzad 2017), improving the rate of photosynthesis and thus increasing plant growth and development. Mahdavia et al. (2019) noted that AMF inoculation and bacterial biofertilizer improved chlorophyll content in basil (*Ocimum basilicum*) under water-limited conditions.

Generally, peroxidative damage under drought stress inhibits plant growth (Marulanda et al. 2009; Chang et al.

2018). Under increasing water stress, plants increase various antioxidant enzyme activities to aid in the removal of ROS, alleviating lipid peroxidation and stabilizing cell structure (Kusvuran et al. 2016). Microbial inoculants can help plants cope with severe water stress by reducing drought-induced oxidative damage (Miransari 2011; Nanjundappa et al. 2019). Similarly, the benefits of AMF and PSB symbiosis for plants include water absorption by hyphae and transfer to plants, increased water content, and decreased ROS generation (Bárcana et al. 2015; Gholinezhad et al. 2020). In

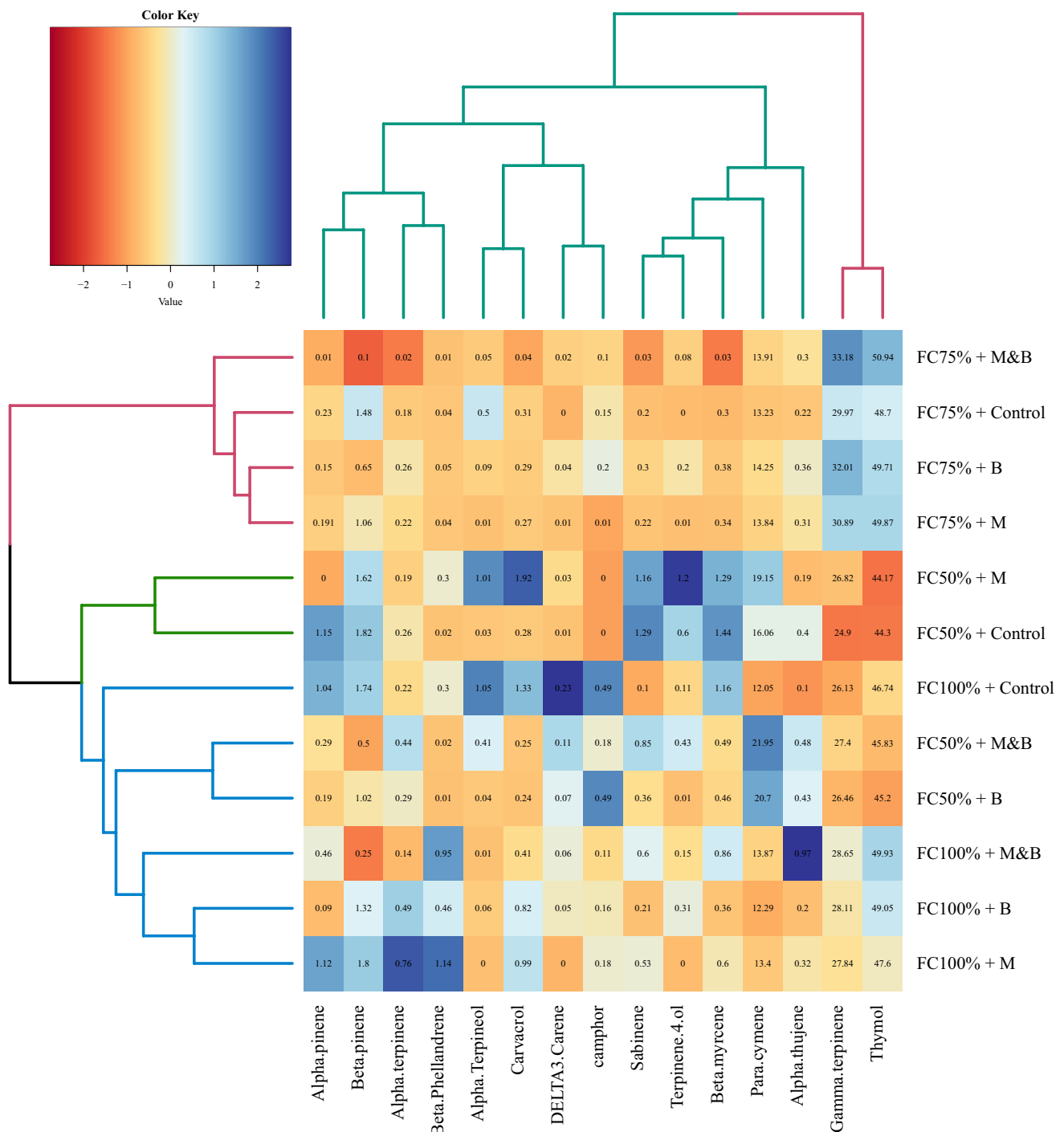


Fig. 8 Heat map clustering of irrigation regime and biofertilizer application (M, mycorrhizal; B, bacterial) sources based on essential oil constituents in Ajowan. The key color bar indicates standardized

mean values (dark red indicates relatively low mean values; dark blue indicates relatively high mean values)

this study, antioxidant enzyme activities (e.g., SOD, CAT, and POX) increased with increasing water stress, more so in inoculated than uninoculated plants, suggesting that inoculated plants activate these enzymes to counteract the damage induced by water stress (Marulanda et al. 2009;

Bárcana et al. 2015), such as oxidative damage. Al-Arjani et al. (2020) reported that AMF inoculation of *Ephedra foliata* decreased the negative impacts of water deficit stress by upregulating the antioxidant defense system. Mamnabi et al. (2020) indicated that vermicompost and PGPR, especially

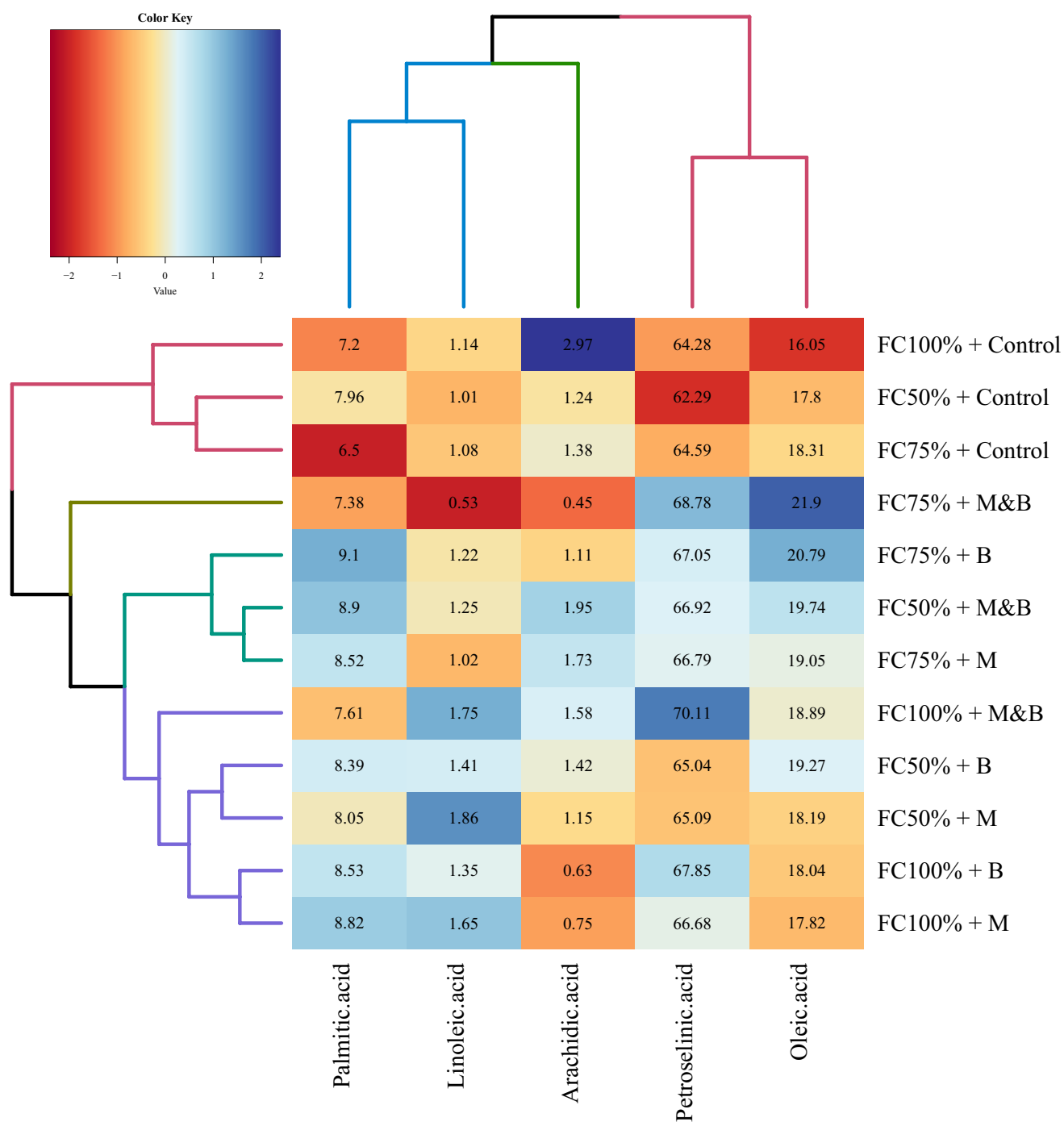


Fig. 9 Heat map clustering of irrigation regime and biofertilizer application (M, mycorrhizal; B, bacterial) sources based on oil components in Ajowan. The key color bar indicates standardized mean

values (dark red indicates relatively low mean values; dark blue indicates relatively high mean values)

as a combined fertilizer, under water deficit conditions increased antioxidant enzyme activities in rapeseed (*Brassica napus* L.), reducing lipid peroxidation and decreasing water stress.

Ajowan seed oil and essential oil components are important products for industrial use, with moderate water stress

producing the highest percentages in this study. Increased EO content under water stress was attributed to the function of secondary metabolites as self-defense components against environmental stress, suggesting that stressful conditions accelerate the biosynthesis of essential oils (Emami Bistgani et al. 2017). In addition, under drought stress, the ratio

of NADP⁺ (as electron acceptors)/NADPH⁺ H⁺ decreased, which adversely affected the photosynthetic process and plant performance (Foyer et al. 2012). Therefore, the EO productivity cells enhanced EO content and its constituents by increasing the consumption level of NADPH⁺ H⁺ and decreasing the adverse effects of drought stress (García-Caparrós et al. 2019). Co-inoculation with AMF + PSB and moderate water stress should increase the extracted essential oil yield in Ajowan. The GC–MS analyses revealed thymol, p-cymene, and γ -terpinene as the main essential oil constituents in Ajowan seeds, as reported by Razavizadeh and Komatsu (2018) and Zarshenas et al. (2014). Co-inoculated plants under moderate water stress produced the most thymol, followed by individual applications of PSB or AMF, compared to uninoculated plants (Table 4). Co-inoculated plants under moderate or severe water stress produced the greatest percentage of γ -terpinene and p-cymene, precursors of monoterpenoids in the essential oil (Table 4). The application of microorganisms to medicinal plants could increase some oil components while decreasing others (Rahimzadeh and Pirzad 2019; Nacoon et al. 2020). Tarraf et al. (2015) and Rahimzadeh et al. (2016) reported that essential oil components respond strongly to microorganism application, and specific inoculum can be used strategically to improve the chemical profile of essential oils.

The five dominant fatty acids of Ajowan are petroselinic, oleic, and linoleic (unsaturated) and palmitic and arachidic (saturated). The oil content significantly decreased under water stress, indicating that water stress has negative impact on fatty acid formation, decreasing oil content and quality (Ashrafi and Razmjoo 2010; Rydlova et al. 2011). The concentrations of petroselinic, oleic, and linoleic acids increased in the seed oil of inoculated plants, suggesting that appropriate inoculations can function as powerful tools for enhancing the health benefits of non-saturated fatty acids, making the seed oil more appropriate for human consumption (Rydlova et al. 2011). Oil quality is usually defined by the proportion and concentrations of linoleic and oleic acids (Connor and Sadras 1992). Our findings agree with Gholinezhad and Darvishzadeh (2021) who also found that biofertilizers significantly increased unsaturated fatty acids in sesame. Moderate water stress produced the highest oleic acid. Other studies have shown that water stress increased abscisic acid production—regulating seed desiccation tolerance and synthesizing seed storage lipids—thereby promoting fatty acid biosynthesis in the embryo (Jadhav et al. 2008; Rahimzadeh and Pirzad 2019).

5 Conclusions

This study provides clear evidence that Ajowan plants react to moderate and severe water stress by increasing total essential oil production, proline and TSS accumulation, and antioxidant enzyme activities, including CAT, SOD, and POX. Co-inoculation of AMF (*Rhizopagus intraradices*) and PSB (*Pseudomonas putida* plus *Bacillus lentus*) under water stress prevents oxidation by improving pharmacological components and secondary metabolite production in seeds, significantly affecting Ajowan yields. Co-inoculation of PSB and AMF increased plant yield and metabolite production under all irrigation regimes. Moderate water stress (75% FC) could be optimal for high-quality seed production in Ajowan, and further improved with the synergistic effects of co-inoculation of AMF and PSB. Future studies should examine the effect of synthetic and organic fertilizer sources, alone and combined with other fertilizers (such as other biofertilizers and nano-fertilizers) under water deficit on the yield, quality, and molecular properties of medicinal plants to holistically evaluate their economic and environmental benefits, particularly in arid and semi-arid areas.

Data Availability Data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Declarations

Conflict of Interest The authors declare no competing interests.

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