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ORIGINAL ARTICLE

Effect of Plant growth promoting rhizobacteria (PGPR) and mycorrhizal fungi inoculations on essential oil in *Melissa officinalis L.* under drought stress

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Abstract

Melissa officinalis L. (Lemon balm) is one of the most important medicinal plants in the *Lamiaceae* family, whose essential oil compounds are affected by the inoculation of beneficial microorganisms and different irrigation regimes. In the present study, the effect of inoculation of plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhiza fungi (AMF) and PGPR + AMF (co-microbial) on growth, photosynthetic pigments, relative water content (RWC), proline and essential oil of lemon balm subjected to 100 (control) and 50% (drought stress) of field capacity (FC) in greenhouse conditions was investigated. The results showed that 50% FC irrigation increased proline content (2.9-fold) and declined chlorophyll *a* (40.4%), *b* (40.2%), carotenoids (44.6%) and RWC (17.8%) compared to the well-watered plants. Plants watered with 50% FC showed a decrease in height, dry weight of roots and shoots by 28.9, 24.1 and 25.6%, respectively, over the control. However, inoculation of PGPR, AMF and co-microbial by improving photosynthetic pigments, proline content and RWC increased plant tolerance and thus restored the growth and biomass of lemon balm under drought stress. Co-microbial inoculation also altered the chemical composition of secondary metabolites and increased the essential oil yield of lemon balm leaves. PGPR + AMF inoculation in lemon balm plants is recommended to increase plant tolerance to limited water conditions and also to improve the yield of essential oils for medical purposes.

Keywords Drought stress · Essential oils · Lemon balm · Medicinal plant · Mycorrhiza fungi · PGPR

Introduction

Melissa officinalis L. (Lemon balm) is one of the valuable medicinal herbs of the family *Lamiaceae*, which is commonly cultivated in many countries, including Iran (Younesi and Moradi 2015). The essential oil of lemon balm is widely used in the production of products such as insect repellents, natural insecticides, flavoring agents and condiments (Abbaszadeh et al. 2009; Verma et al. 2015). Hesperidin, luteolin, flavonoids, tannins, citronellal, beta-carophyllene, apigenin, linalool, isoquercetin, caffeic acid, neral, isomers of citral (geranial and geraniol), flavo-glycoside acid and rosmarinic acid are the most important secondary metabolites identified from lemon balm leaves (Argyropoulos and Müller 2014). In order to provide the essential oils needed by the market and industry, sustainable and high-quality production of lemon balm, even in unfavorable conditions of abiotic stresses, should be specially considered. In aromatic and medicinal plants, the accumulation of secondary metabolites is significantly affected by adverse environmental conditions, so that it changes the composition of essential oils and, consequently, increases or decreases secondary metabolites (Bonacina et al. 2017; Gerami et al. 2018). Inhibitory effects on growth and alterations in essential oils of medicinal plants under various abiotic stresses such as salinity and drought have been previously reported (Khorasaninejad et al. 2010; Bonacina et al. 2017). Plants activate the defense networks in response to stressful conditions to maintain cell homeostasis and alleviate the harmful effects of stress (Ghorbani et al. 2020; Ghasemi-Omran et al. 2021). Drought stress has been reported to inhibit various cell metabolisms, reduce water potential, disrupt

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ionic homeostasis and the activity of various enzymes, thereby reducing plant growth and yield (Bistgani et al. 2017).

Today, plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhiza fungi (AMF) are used as biofertilizers in sustainable agricultural systems in order to improve the quality and yield of crops and horticultural products, especially medicinal plants (Beattie 2006). *Azospirillum brasilense* is a Gram-negative free-living nitrogen-fixing bacterium and one of the most studied PGPR bacteria (Housh et al. 2021). PGPR have been shown to improve plant tolerance under drought (Zahir et al. 2008), heavy metals (Sheng 2005) and salinity (Bidgoli et al. 2019) stresses as biocontrol agents. PGPR have been reported to restore plant growth under biotic and abiotic stresses by reducing ethylene levels and increasing auxin biosynthesis as well as nitrogen fixation in roots (Patten and Glick 2002; Glick et al. 2007). AMF are also beneficial soil microorganisms that effectively improve plant tolerance under stressful conditions by establishing a mutual symbiotic relationship with the roots (Selosse et al. 2006; Wu et al. 2021). Numerous studies have shown that AMF can improve plant growth and increase plant tolerance under various environmental stresses such as drought (Essahibi et al. 2018; Zhang et al. 2018), salinity (Hajiboland et al. 2015) and heavy metals (Abdelhameed and Rabab 2019). AMF has been reported to enhance host plant adaptation to drought stress by regulating leaf stomatal conductivity, improving root hydraulic conductivity, restoring ionic homeostasis, and adjusting biochemical mechanisms (antioxidant defense system and osmotic potential regulation) (Aroca et al. 2007; Bitterlich et al. 2018). Several reports have shown that inoculation of PGPR and AMF altered the biosynthesis and amount of essential oils of medicinal plants such as lavender (Tsuro et al. 2001), Hyssop (Sharifi 2017), rosemary (Bidgoli et al. 2019) and Satureja (Zakerian et al. 2020). Non-pathogenic microorganisms have been reported to induce the synthesis of secondary metabolites in the host plant through a mechanism called induced systemic resistance (ISR) (VanLoon and Glick 2004). It has also been indicated that the biological agents released by non-pathogenic microorganisms act as effective stimuli to induce key enzymes responsible for the synthesis of secondary metabolites (Chen et al. 2000; Çakmakçı et al. 2020).

Due to the problems of water shortage in many parts of Iran (Ghorbani et al. 2009) and the promising characteristics of PGPR and AMF, as well as the importance of the medicinal plant lemon balm in the pharmaceutical and food industry, the current study was conducted to investigate the effects of moderate drought stress alone and in combination with beneficial soil microorganisms of PGPR and AMF on the growth and composition of lemon balm essential oil.

Materials and methods

Microbial inoculum

In the present study, *Azospirillum brasilense* (Sp245 strain) was used as a PGPR. After culturing *A. brasilense* on Nitrogen-fixing bacteria (NFB) medium, PGPR inoculum was prepared in a bacterial concentration of 10^8 CFU/mL in deionized water. *Glomus mosseae* was used as AM fungi in this study, which was obtained from the Laboratory of Soil and Water Research Institute, Iran. The AMF inoculum included infected root fragments, mycelium and spores of *G. mosseae*. In order to inoculate, 100 g of AMF inoculum was added to the soil (1.5 kg) of each pot (Pan et al. 2020).

Plant materials and treatments

Lemon balm seeds were germinated in autoclaved in pots (17 cm height and 20 cm diameter) containing autoclaved clay, sand and humus (1:3:2) after surface sterilization with ethanol (70%) and chlorox (10%) solutions for 5 and 1 min, respectively, and washing with distilled water. In each pot, five seeds were planted which, after germination, were thinned to one seedling. Biofertilizer treatments include: (a) Non-inoculation treatment: The seeds were immersed in distilled water for 30 min and autoclaved AMF inoculum was added to the potting soil, (b) PGPR inoculation: soaking the seeds in PGPR inoculum (30 min) and adding autoclaved fungal inoculum to the soil, (c) AMF inoculation: soaking the seeds in distilled water and adding AMF inoculum to the soil, (d) Co-microbial inoculation: soaking the seeds in PGPR inoculum and adding AMF inoculum to the soil. The plants were grown at a 16/8 (light/dark) photoperiod with an intensity light of 350 μmol photon m^{-2} s^{-1}, 60 ± 5 relative humidity and 25/22 °C day/night. The pots were watered as needed and fed by half-strength Hoagland once a week. 30 days after sowing, lemon balm plants were treated with drought stress at two levels (100 and 50% of field capacity (FC)) according to the managed allowed depletion previously described by Allen et al. (2000). After weighing the pots daily and measuring the amount of water lost through soil leaching, evaporation and transpiration, the lost water was replaced. The plants were exposed to drought stress for 30 days and then sampling was performed.

Measurement of growth parameters

At the end of the experiment, the height and leaf numbers of lemon balm plants were measured. Then, to estimate the dry weight of roots and shoots, plant samples were divided into roots and shoots after sampling and weighed after being exposed to 70 °C for 72 h (Ghorbani et al. 2011).

Measurement of photosynthetic pigments, proline and relative water content (RWC)

The contents of chlorophyll (*a* and *b*) and carotenoids pigments were determined based on Wellburn (1994) and recording the optical density at 470, 652 and 665 nm as mg/g fresh weight.

Proline contents were measured by ninhydrin reagent (acetic acid + orthophosphoric acid (6 mM) + 125 g ninhydrin) according to Bates et al. (1973) method. After crushing the fresh leaves in sulfosalicylic acid and centrifuging at 10,000 × *g* for 15 min, ninhydrin reagent was added to the supernatants and incubated at 100 °C for 1 h. After cooling the samples on the ice bath and adding toluene to each, the spectrophotometric measurements were recorded at 520 nm absorbance.

The RWC leaves were obtained according to the method previously explained by Schonfeld et al. (1988) and according to the formula: (FM – DM) / (TM – DM) × 100. (FM: fresh mass weight, DM: dry mass weight, TM: turgid mass weight).

Extraction and assay of essential oil

After air-drying lemon balm leaves, the essential oil of the leaves was obtained by Clevenger device and hydrodistillation for 3 h. Gas chromatography-mass spectrometry (GC-MS) device (Varian 3400, USA) with a DB-5 fused silica column (30 m × 0.25 mm, film thickness 0.25 µm, J and W Scientific, Inc.) was used for GC-MS analysis. Dichloromethane with a ratio of 10:1 was used to dilute the essential oil. Helium was employed as the carrier gas, 1 mL/min flow rate. the injection volume and injector temperature were 2.0 µL and 220 °C, respectively. The temperature of the column was maintained at 60 °C for 5 min and then reached to 180 °C at the rate of 2 °C/min, to 260 °C at the rate of 10 °C/min, and finally heated at 40 °C/min to program 300 °C. Using the alkane series C7-C26, the retention indices (RIs) of the essential oil compounds were determined. The chemical compounds of the essential oils were identified by comparing the mass spectra of the essential oil compounds with the mass spectra of the NIST 11.0 library and comparing the IRs published in the literature.

Statistical analysis

All experiments were performed with four biological replications (mean morphological traits and RWC were calculated from 10 independent biological replications), each of which averaged three technical replications. Data analysis was carried out using SAS 9.1.3 software. The means were compared based on the least significant difference (LSD) test ($p < 0.05$).

Results

Plant growth dynamics

The results showed that drought stress (50% FC) reduced the height of lemon balm by 28.9% compared to the control. Under non-stress conditions, the application of biofertilizers (AMF, PGPR and co-microbial inoculation) significantly improved plant height compared to non-inoculated plants. An increase of 13.8, 17.3 and 17.6% were recorded in plants inoculated with PGPR, AMF and co-microbial over the non-inoculated plants. Three microbial treatments restored the height of lemon balm in drought-stressed plants, however, there were no significant differences among the three microbial-colonized plants ($p > 0.05$, Table 1). Inoculation of PGPR, AMF and PGPR + AMF significantly enhanced

Table 1 Effects of plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhiza fungi (AMF) on morphological traits in lemon balm plants under drought stress

Drought stress	Biofertilizer	Height (cm)	Root dry weight (RDW, g)	Shoot dry weight (SDW, g)	RDW/SDW ratio	Leaf numbers
100% filed capacity (FC)	non-inoculation	28.40 ± 1.15^{b}	1.16 ± 0.17^{b}	3.55 ± 0.14^{b}	0.327 ± 0.061^{c}	88.3 ± 7.8^{cd}
	PGPR	32.33 ± 1.15^{a}	1.56 ± 0.11^{a}	3.73 ± 0.14^{b}	0.418 ± 0.037^{ab}	102.7 ± 8.1^{ab}
	AMF	33.30 ± 0.99^{a}	1.70 ± 0.12^{a}	3.78 ± 0.13^{ab}	0.452 ± 0.039^{a}	100.0 ± 8.6^{abc}
	PGPR + AMF	33.40 ± 0.92^{a}	1.69 ± 0.15^{a}	4.01 ± 0.17^{a}	0.422 ± 0.055^{ab}	108.7 ± 7.5^{a}
50% filed capacity (FC)	non-inoculation	22.47 ± 1.25^{d}	0.88 ± 0.05^{c}	2.64 ± 0.13^{d}	0.335 ± 0.036^{c}	67.0 ± 5.6^{e}
	PGPR	26.63 ± 0.81^{bc}	1.13 ± 0.05^{b}	3.06 ± 0.12^{c}	0.368 ± 0.002^{bc}	81.0 ± 5.6^{d}
	AMF	26.43 ± 1.10^{c}	1.14 ± 0.05^{b}	2.97 ± 0.16^{c}	0.385 ± 0.006^{bc}	83.0 ± 6.1^{d}
	PGPR + AMF	26.97 ± 0.80^{bc}	1.22 ± 0.06^{b}	3.15 ± 0.13^{c}	0.388 ± 0.024^{abc}	92.0 ± 6.3^{bcd}

Values marked with the same alphabets are not significantly different (LSD, $p > 0.05$). All the values are means of 10 replicates ± SD the root dry weight (RDW) by 34.5, 46.6 and 45.7%, respectively in non-stressed plants. Drought stress declined the RDW by 24.1% over the control plants. However, three inoculation treatments significantly restored the RDW in drought-stressed plants, although there were no significant differences between them ($p > 0.05$, Table 1). In non-stressed plants, co-colonization of PGPR and AMF elevated the shoot dry weight (SDW) by 13%. Drought stress significantly reduced the SDW, while PGPR, AMF and PGPR + AMF colonization significantly improved the SDW of drought-stressed plants (12.5–19.3%) (Table 1). PGPR, AMF and PGPR + AMF treatments increased the RDW/SDW ratio by 27.8, 38.2 and 29.1%, respectively in comparison to no-inoculated plants under 100% FC. Drought stress had no significant effect on RDW/SDW ratio. Although the three inoculation treatments increased the RWD/SWD ratio in drought-stressed plants, however, no significant differences were observed between inoculated and non-inoculated plants under drought stress (Table 1). A 24.1% reduction was observed in the leaf numbers of lemon balm under drought stress compared to plants irrigated with 100% FC. However, inoculation of PGPR, AMF and PGPR + AMF significantly enhanced the numbers of leaves in 50% FC-stressed lemon balm plants and the highest increase was recorded in plants inoculated with PGPR + AMF (Table 1).

Photosynthetic pigments

As shown in Table 2, drought stress significantly reduced chlorophyll *a* and *b* content by 40.4 and 40.2 %, respectively in lemon balm leaves compared to non-inoculated plants watered with 100% FC. In drought stress-treated plants, PGPR, AMF and co-microbial colonization restored chlorophyll *a* content by 26.8, 22.8 and 42.3% and chlorophyll *b* by 32.7, 36.5 and 44.2%, respectively over non-inoculated plants under drought stress (Table 2). Drought stress also declined the carotenoids content over non-inoculated plants under 100% FC. However, three inoculation treatments caused a significant increase in the carotenoids content in drought stress-stressed plants over non-inoculated plants under drought stress, with the largest enhancement observed in co-microbial-inoculated plants (Table 2).

Relative water content (RWC) and proline content

Non-inoculated plants exposed to drought stress showed a 17.8% increase in RWC over non-inoculated plants watered with 100% FC. However, the colonization of PGPR, AMF and co-microbial significantly elevated RWC by 8.8, 10.3 and 11.7% in plants treated with drought stress over non-inoculated plants under 100% FC (Table 2). In Non-inoculated plants exposed to drought stress, all three inoculation treatments significantly enhanced the proline content in lemon balm leaves but there were no significant differences between them. Drought stress significantly increased the proline content over non-inoculated plants; however, the inoculation of PGPR, AMF and PGPR + AMF caused a further increase in proline content in drought-stressed plants that the highest raise in proline content was observed in co-microbial-inoculated plants (Table 2).

Yield and chemical composition of essential oil

The chemical composition and yield of essential oil have an important effect on the commercial value of medicinal plants. In the present study, the yield of essential oil in non-inoculated plants watered with 100% FC was 0.105% and the application of drought stress increased the yield of essential oil by 58.1% compared to well-irrigated non-inoculated plants. In plants watered with 100% FC, inoculation of PGPR, AMF and PGRP + AMF significantly enhanced the yield of lemon balm essential oil and the highest increase was related to the co-microbial inoculation treatment. In non-inoculated plants treated

Table 2 Effects of plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhiza fungi (AMF) on photosynthetic pigments, relative water content (RWC) and proline in lemon balm plants under drought stress

Drought stress	Biofertilizer	Chlorophyll *a* (mg/g.fw)	Chlorophyll *b*	Carotenoids	RWC (%)	Proline (µmol/g.fw)
100% filed capacity (FC)	Non-inoculation	2.05 ± 0.14^{a}	0.87 ± 0.07^{a}	0.637 ± 0.055^{a}	84.77 ± 0.61^{ab}	2.47 ± 0.16^{e}
	PGPR	2.03 ± 0.12^{a}	0.87 ± 0.09^{a}	0.613 ± 0.060^{ab}	83.93 ± 0.78^{b}	3.26 ± 0.25^{d}
	AMF	2.05 ± 0.16^{a}	0.88 ± 0.08^{a}	0.620 ± 0.056^{a}	85.80 ± 0.82^{a}	3.37 ± 0.23^{d}
	PGPR + AMF	2.00 ± 0.14^{a}	0.93 ± 0.08^{a}	0.650 ± 0.060^{a}	85.47 ± 1.17^{ab}	3.03 ± 0.16^{d}
50% filed capacity (FC)	Non-inoculation	1.23 ± 0.11^{d}	0.52 ± 0.04^{c}	0.353 ± 0.038^{d}	69.67 ± 1.19^{e}	7.26 ± 0.31^{c}
	PGPR	1.56 ± 0.07^{bc}	0.69 ± 0.05^{b}	0.477 ± 0.035^{c}	75.77 ± 1.15^{d}	9.41 ± 0.31^{b}
	AMF	1.51 ± 0.09^{c}	0.71 ± 0.04^{b}	0.463 ± 0.055^{c}	76.83 ± 1.08 c^{d}	9.24 ± 0.26^{b}
	PGPR + AMF	1.75 ± 0.09^{b}	0.75 ± 0.07^{b}	0.530 ± 0.046^{bc}	77.83 ± 1.17^{c}	$10.65 \pm 0.^{22a}$

Values marked with the same alphabets are not significantly different (LSD, $p > 0.05$). All the values are means of four replicates ± SD with drought stress, PGPR and AMF inoculation had no significant effects on essential oil yield, however, the co-microbial inoculation increased essential oil yield by 44.8% compared to non-inoculated plants treated with drought stress (Fig. 1).

Thirty-eight compounds were detected in the essential oil of lemon balm leaves, which belonged to four groups, i.e., oxygenated monoterpenes, oxygenated sesquiterpenes, monoterpene hydrocarbons and sesquiterpene hydrocarbons. The oxygenated monoterpenes group was the predominant class in lemon balm essential oil in all treatments. The results also showed that drought stress and all three microbial inoculation treatments increased the monoterpenes hydrocarbons and sesquiterpenes hydrocarbons class compounds compared to non-inoculated plants treated with 100% FC. However, the concentration of oxygenated monoterpenes compounds under drought stress and three microbial inoculation treatments decreased over non-inoculated plants treated with 100% FC (Table 3). The results showed that α-citral (40.25%), neral (28.11%) and citronellal (9.07%), as the most biologically active compounds in the essential oil of lemon balm leaves, were the most abundant compounds in non-inoculated plants treated with 100% FC. Drought stress diminished α-citral (36.18%), neral (25.44 %) and citronellal (7.01%) compounds over non-inoculated plants watered with 100% FC. In non-inoculated plants treated with 100% FC, microbial inoculation treatments enhanced citronellal and lowered α-citral and neral. In non-inoculated plants treated with drought stress, microbial inoculation treatments enhanced citronellal and reduced neral. However, PGPR and AMF colonizations enhanced citral content and co-microbial colonization declined citral content in non-inoculated plants treated with drought stress (Table 3).

Discussion

The results illustrated that plant height, total dry weight of roots and shoots and number of leaves in lemon balm showed a significant decrease under drought stress. Idrees et al. (2010) indicated that drought stress decreases plant biomass production and growth by reducing water potential, water absorption, closing stomatal apparatuses, reducing turgor pressure and cell enlargement. Negative effects of drought stress on plant growth, biomass production and leaf number have been previously reported by Saheri et al. (2020) and Khan et al. (2019). Therefore, the decrease in growth and total dry weight of lemon balm treated with drought stress can be due to the reduction in turgor pressure and water potential induced by water stress, which may reduce cell expansion and division and induce senescence in the leaves (Saheri et al. 2020). A similar reduction in growth occurred in medicinal plants, such as *Thymus daenensis* (Bistgani et al. 2017), *Portulaca oleracea* (Saheri et al. 2020), and *Rosmarinus officinalis* (Abbaszadeh et al. 2020). However, our results indicated that inoculation of PGPR, AMF, and PGPR + AMF improved the growth and biomass of lemon balm under drought stress, in accordance with other results Kang et al. 2014; Ganjeali et al. 2018; Mutumba et al. 2018; Zakerian et al. 2020). Boutasknit et al. (2020) showed that AM fungi restored the growth of drought-stressed Carob plants by improving efficiency of photosynthetic apparatuses, stomatal conductance, water content, and nutrient uptake. It has been reported that the positive effect of PGPR on plant growth may be due to increased activity of ACC (1-aminocyclopropane-1-carboxylic acid) deaminase enzymes and, consequently, decreased ethylene levels in the plant

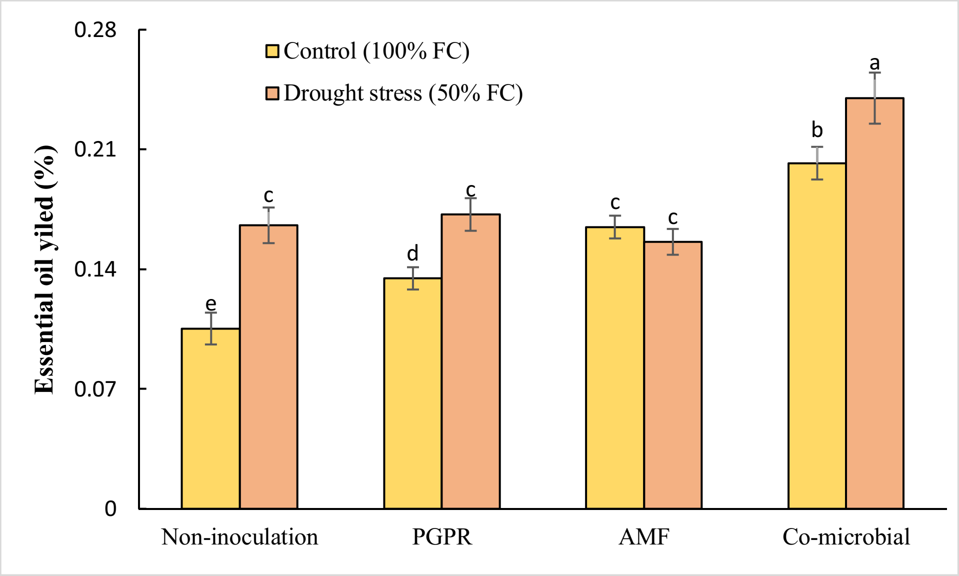

Fig. 1 Effects of plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhiza fungi (AMF) on yield of essential oils in lemon balm under drought stress. Values marked with same alphabets are not significantly different (LSD, $p < 0.05$). All the values are means of four replicates ± SD

Table 3 Effects of plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhiza fungi (AMF) on chemical composition of essential oils in lemon balm under drought stress

Compounds	RI[a]	Treatment							
		Control	PGPR	AMF	PGPR + AMF	Drought stress	Drought stress + PGPR	Drought stress + AMF	Drought stress + PGPR + AMF
Monoterpenes Hydrocarbons (%)									
α-pinene	936	0.31	0.29	0.33	0.36	0.35	0.34	0.38	0.38
Sabinene	976	0.04	0.07	0.04	0.07	0.12	0.08	0.09	0.13
β-pinene	979	0.19	0.15	0.14	0.18	0.15	0.18	0.21	0.17
Myrcene	991	4.01	5.11	5.16	5.32	5.39	5.48	5.52	5.53
α-phellandrene	1005	-	0.19	0.23	0.18	0.27	0.32	0.35	0.33
δ-3-carene	1011	0.08	-	-	0.05	0.05	-	-	-
p-cymene	1027	-	-	-	0.06	0.17	0.03	0.05	0.11
o-cymene	1027	0.22	0.11	0.07	0.05	0.27	0.13	0.07	0.21
Limonene	1031	-	-	-	-	0.07	-	0.05	0.11
β-phellandrene	1031	0.21	0.14	0.12	0.25	0.37	0.21	0.15	0.12
γ-terpinene	1033	0.48	0.32	0.33	0.27	0.41	0.25	0.27	0.37
Terpinolene	1100	-	1.02	0.77	1.11	0.61	1.14	0.85	1.04
Oxygenated Monoterpenes (%)									
Linalool	1104	1.12	0.59	0.62	0.44	0.58	0.42	0.36	0.41
Trans-thujone	1147	1.15	0.33	0.47	0.22	0.39	0.53	0.42	0.51
Cis-verbenol	1147	0.21	0.39	0.44	0.64	1.23	1.13	1.08	1.44
Camphor	1148	0.52	0.61	0.65	0.74	0.63	0.68	0.61	0.64
Isopulegol	1155	-	1.32	0.97	1.37	1.09	1.38	1.44	1.69
Citronellal	1167	9.07	11.26	12.03	13.19	7.01	8.14	9.24	9.85
Lavandulol	1169	-	0.28	0.36	0.44	0.41	0.39	0.45	0.69
Methylchavicol	1180	0.29	-	-	0.11	0.16	0.25	0.31	0.72
Estragol	1184	-	-	0.11	0.05	0.08	0.07	0.08	0.07
Citronellol	1198	2.34	1.87	1.64	2.05	2.11	1.54	1.61	2.41
Nerol	1198	-	0.31	0.27	0.07	0.61	0.21	0.15	0.32
Pulegone	1198	1.32	1.54	1.48	1.84	1.75	1.62	1.55	1.67
Neral	1230	28.11	23.12	22.11	22.56	25.44	20.25	20.09	19.19
Chavicol	1243	1.56	-	0.18	0.38	1.12	0.45	0.46	0.85
Geraniol	1247	-	1.82	1.64	2.17	2.15	1.87	1.95	2.46
α-citral	1276	40.25	37.06	37.51	36.29	36.18	36.58	37.19	36.08
Neryl acetate	1385	-	-	-	-	2.28	2.11	1.87	2.09
Geranyl acetate	1390	-	1.09	1.24	1.84	0.81	1.64	1.55	2.08
Sesquiterpenes Hydrocarbons (%)									
Trans-caryophyllene	1413	2.01	3.29	3.06	3.48	2.41	3.54	3.61	3.67
Aromadendrene	1452	-	-	-	0.22	-	0.14	0.09	0.32
α-curcumene	1484	-	0.28	0.37	-	0.24	0.45	0.51	0.34
α-amorphene	1494	0.16	0.19	0.24	0.38	0.28	0.21	0.28	0.42
δ-cadinene	1672	0.42	0.24	0.29	0.37	0.52	0.16	0.14	0.29
Oxygenated sesquiterpenes (%)									
Tumerol	1705	0.09	0.11	0.09	0.14	0.08	0.07	0.11	0.15
Caryophyllene-oxide	2108	2.14	1.56	1.87	2.32	2.24	2.34	1.97	2.35
Tumerone-dihydro	2109	0.21	0.53	0.42	0.52	0.29	0.48	0.35	0.55
Total identified (%)		96.51	95.19	95.25	99.73	98.32	94.81	95.46	99.76
Groups of compounds (%)									
Monoterpenes Hydrocarbons		5.54	7.4	7.19	7.9	8.23	8.16	7.99	8.5
Oxygenated Monoterpenes		85.94	81.59	81.72	84.4	84.03	79.26	80.41	83.17
Sesquiterpenes Hydrocarbons		2.59	4	3.96	4.45	3.45	4.5	4.63	5.04

Table 3 (continued)

Compounds	RI[a]	Treatment							
		Control	PGPR	AMF	PGPR + AMF	Drought stress	Drought stress + PGPR	Drought stress + AMF	Drought stress + PGPR + AMF
Oxygenated Sesquiterpenes		2.44	2.2	2.38	2.98	2.61	2.89	2.43	3.05

[a]RI = Retention indices calculated with n-alkanes (C7-C26)

(Glick et al. 2007; Zhang et al. 2019) indicated PGPR increase plant tolerance and growth under drought stress by preserving the thylakoid membrane structure of chloroplasts, improving the content of photosynthetic pigments, and increasing water use efficiency (WUE). Therefore, the improvement of growth in lemon balm by inoculation of PGPR and AMF indicates the ability of beneficial microorganisms to improve plant adaptation to conditions with limited water availability. The results also showed that growth improvement in plants inoculated by co-microbial (PGPR + AMF) was greater than inoculation with PGPR or AMF alone, indicating the synergistic effects of PGPR and AMF on the adaptation of lemon balm to drought stress, according to results previously reported by Ruíz-Sánchez et al. (2011).

Photosynthesis is an important plant process and sensitive to stressful conditions that directly affect plant growth and biomass (Ghorbani et al. 2019). The results illustrated that drought stress caused an evident decline in the contents of chlorophyll *a*, *b* and carotenoids, however, PGPR, AMF and co-microbial inoculation treatments were found to restore the contents of pigments in drought-stressed plants, which is consistent with data published by Zhang et al. (2019), Al-Arjani et al. (2020) and Sharifi (2017). Guler et al. (2016) demonstrated that PGPR restored the content of photosynthetic pigments by reducing oxidative stress and reactive oxygen species (ROS) levels, thereby improving plant adaptation to drought stress. Al-Arjani et al. (2020) showed that AMF increased chlorophyll content by enhancing magnesium uptake and, consequently, improved plant growth under drought stress. Increased chlorophyll synthesis by AM fungi has also been previously documented by Zhu et al. (2017) and Hashem et al. (2016). Improving the carotenoids content by inoculation of PGPR and AMF can protect the photosynthetic apparatus and, as a result, improve the efficiency of the photosynthetic process in drought-stressed plants. Therefore, our findings demonstrated that PGPR and AMF, by restoring photosynthetic pigments in drought-stressed plants, protected the photosynthetic apparatus and increased the efficiency of the photosynthesis process (Ghorbani et al. 2018a), which could improve plant growth under drought stress.

The relative water content (RWC) is used to evaluate the osmotic stress induced by stress conditions (Ghorbani et al. 2018b), which indicates the water balance in the plant. Plants with the accumulation of osmotic compounds such as proline, maintain plant water balance and thus improve adaptation to osmotic stress induced by abiotic stress such as drought stress (Ghorbani et al. 2021). The results of the present study showed that drought stress decreased RWC and increased proline accumulation in the leaves of lemon balm. However, PGPR, AMF and co-microbial inoculations improved RWC and proline content in drought-stressed plants. Similar results of improving RWC and proline content in potato by PGPR inoculation (Batool et al. 2020) and ephedra by AMF induction (Al-Arjani et al. 2020) have been documented. In addition to osmotic regulation, proline accumulation protects membranes and proteins against oxidative stress induced by abiotic stresses. Therefore, the findings showed that inoculation of PGPR and AMF by regulating proline metabolism, caused proline accumulation and, consequently, improved RWC, which can increase the adaptation of lemon balm plants under drought stress.

Shikimic acid and mevalonic acid are two distinct pathways for the biosynthesis of plant essential oils. Terpenoids are synthesized from the mevalonic pathway under the influence of photosynthesis, growth and assimilation, while phenolic compounds are synthesized from the shikimic pathway by the activity of the phenylalanine ammonia-lyase (PAL) enzyme (Gang et al. 2001). The results showed that the yield of lemon balm essential oil increased under drought stress, which suggests that adverse conditions, even with reduced photosynthesis and less available carbon, may have a positive effect on the production of secondary metabolites (Siemens et al. 2002; Brili et al. 2007). Similar increases occurred in the essential oils of *Cuminum cyminum* L. (Alinian et al. 2016) and *Pelargonium odoratissimum* (Khalid et al. 2010) under drought stress. However, Baghalian et al. (2011) and Bannayan et al. (2008) showed that drought stress had no effect on the essential oil of black cumin and German chamomile, respectively. Therefore, these results indicate that the essential oil of medicinal plants is affected by various factors such as growth conditions, genotype, species and drought stress level. The results also showed that co-microbial inoculation increased the yield of essential oil in lemon balm treated with 100% FC and drought stress, which indicates the positive effect of co-inoculation on the biosynthesis of lemon balm essential oil. Similar results have been reported by Zakerian et al. (2020), Bidgoli et al. (2019) and Sharifi

(2017) on the positive effect of PGPR and AMF inoculation on the essential oil content of medicinal plants. Due to the role of secondary metabolites as lipophilic compounds in reducing membrane lipid peroxidation and minimizing the accumulation of ROS and oxidative stress (Vickers et al. 2009), increasing the production of secondary metabolites could be a defense mechanism induced by microbial inoculation to improve plant tolerance under drought stress and alleviate oxidative stress induced by drought stress. It has been shown that the biosynthesis of secondary metabolites depends on photosynthesis, primary metabolism, and oxidation processes for the energy and carbon supply (Raklami et al. 2019; Azizi et al. 2021). Both PGPR and AMF are able to improve primary metabolites by increasing plant growth, mineral content and photosynthesis, suggesting that the improving effects of microbial inoculation on the synthesis of secondary metabolites may be due to the proper balance of carbon and nutrient supply (Vafadar et al. 2014). It has also been shown that volatile organic compounds (VOCs) released by PGPR induce the activity of enzymes involved in the synthesis of essential oils of *Mentha piperita* (Santoro et al. 2011). Therefore, the results revealed PGPR and AMF inoculations changed the concentration of essential oil compounds in non-inoculated plants treated with 100% FC and drought stress, which indicates the effects of microbial inoculation on the activity of enzymes involved in the synthesis of these compounds, which can be due to the production of elicitors produced by PGPR and AMF in the soil (Pan et al. 2020). However, due to the lack of available information on the role of PGPR and AMF on the expression or activity of enzymes responsible for the synthesis of essential oil in lemon balm, more accurate studies at the molecular levels are needed to accurately understand the effect of PGPR and AMF on essential oil accumulation.

Conclusions

Conclusively, drought stress (50% FC) significantly reduced the photosynthetic pigments and RWC and thus reduced the growth and biomass of lemon balm, however, the yield of essential oil under drought stress increased. Inoculation of PGPR, AMF and PGPR + AMF improved photosynthetic pigments and RWC and increased proline accumulation in drought-stressed plants, which was associated with improved plant growth and biomass, and the highest increase was observed in plants inoculated with co-microbial. Co-microbial inoculation also improved the essential oil yield in lemon balm treated with 100% FC and drought stress, which indicates the positive effect of microbial inoculation on the production of secondary metabolites in medicinal plants. Due to the synergistic effects of PGPR and AMF inoculation, co-microbial inoculation in lemon balm plants is recommended to enhance plant tolerance to drought stress and also to improve commercial value.

Declarations

Conflict of interest The authors declare no conflict of interests.

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