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Arbuscular Mycorrhizal Fungi (AMF) and Plant Growth-promoting Rhizobacteria (PGPR) as an Alternative to Mineral Fertilizers to Improve the Growth, Essential Oil Profile, and Phenolic Content of *Satureja Macrantha* L.

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Abstract
The use of biofertilizers as an alternative to mineral materials has been widely developed to reach optimum productivity in medicinal plants. The present study aimed to evaluate the effects Arbuscular mycorrhizal fungi (AMF) inoculation (*Rhizophagus intraradices*), plant growth-promoting rhizobacteria (PGPR: *Pseudomonas putida* and *Azospirillum lipoferum)*, and mineral fertilizer (NPK) on the plant growth, essential oil (EO) content, EO composition, and antioxidant activity of *Satureja macrantha* L on a loamy soil in semi-arid area of Iran. The results revealed that AMF, NPK, *A. lipoferum*, and *P. putida* increased dry weight yield (DWY) by 26%, 24%, 17%, and 22%, respectively, compared with control in August 2018. Mycorrhizal plants had higher chlorophyll (Chl) $a+b$ and total phenolic content (TPC) in comparison with no amendment application. Total flavonoid content (TFC) in plants supplied with AMF and NPK was higher than in other treated and untreated plants. In the second-year plants, the highest EO content and yield were observed in plants supplied with AMF followed by *P. putida, A. lipoferum*, and NPK, respectively. The GC/MS analysis showed that monoterpenes characterized the main EO profile of *S. macrantha* L., including *p*-cymene (22.14%–33.57%), γ-terpinene (22.26%–36.28%), thymol (0.11%–31.23%), and carvacrol (0.36%–36.56%). According to agglomerative hierarchical clustering (AHC), AMF and NPK were placed in the same cluster; therefore, AMF can be selected as a proper alternative to chemical fertilizer for improving plant yield and EO yield. By assisting biofertilizers in reaching their maximum performance of S. *macrantha* L. with significant pharmacological potential, the results may be useful for its development in semiarid regions.

Keywords Essential oil quality · Bacterial inoculation · Mycorrhiza symbiosis · Phenolic content

Introduction

Biofertilizer is characterized as a substance containing living *microorganisms*, which make interact with plant root and encourages growth by providing nutrients to the *host plant* (Yilmaz and Sönmez 2017). Different studies have shown the useful effects of biofertilizers on soil features such as improvement in *organic carbon* and aggregate stability of soils (Adedeji et al. 2020; Demir 2020). Arbuscular mycorrhizal fungi (AMF) are beneficial soil microorganisms, which have an effective role in plant growth because of their capacity for improving the uptake of minerals (Nanjundappa et al. 2019). The growth and performance of AMF can exclusively be done in the presence of plant roots, and this symbiosis is useful for plants especially for improving the uptake of the immobile and insoluble phosphate ions in the soil, where there are interacts with soil bi- and trivalent cations, mainly Ca^{2+}, Al^{3+}, and Fe^{3+} (Khaliq et al. 2022). This symbiosis is based on AMF's capacity to expand a network of external hyphae in developing the surface area and advancing nutrient uptake, which results in the enhancement of plant growth (Rouphael et al. 2015; Storer et al. 2018). The extraradical hyphae are eminent to enhance the uptake of ammonium, immobile micronutrients like Cu and Zn, and soil-derived mineral cations such as

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K^+, Ca^{2+}, and Mg^{2+} (Bhantana et al. 2021). AMF can act as biofertilizers by improvement of plant nutrition, behave as bioregulators via the phytohormone balance of the plant, thus increasing the plant development, and act as bioprotector by alleviating the adverse impacts of environmental stresses (Bhantana et al. 2021). *Plant growth-promoting rhizobacteria* (PGPR) are other important microorganisms for increasing the growth and yield of plants (Adedeji et al. 2020). The genus *Azospirillum* is the most used PGPB, and its association with plants results in increasing crop production. It can positively influence plant growth by promoting plant regulators and developing the root system (Goswami et al. 2016). *Pseudomonas* strains have been specified as phosphorus solubilizers with producing *organic acids* and *phosphatases* by facilitating the *solubilization* of phosphorus and other nutrients (Adedeji et al. 2020).

The Lamiaceae family has various genera, *Satureja* is a well-known genus comprising over 30 species, mainly distributed in the eastern part of the Mediterranean area (Bekut et al. 2018). *Satureja* species are annual or perennial semi-bushy aromatic plants that grow in arid and mountainous regions (Saki et al. 2019). This genus is characterized by highly aromatic and medicinal features. In folk medicine, the species of *Satureja* are widely used for treating various diseases. *S. macrantha* with up to 50 cm in height, widely grows in the northwest of Iran as well as in eastern parts of Iraq (Mirjalili et al. 2022). The leaves are coated with a large number of glands, which are corresponded to essential oil (EO) production. *S. macrantha* has certainly biological properties such as antimicrobial (Tepe and Cilkiz 2016; Karakaş and Bekler 2022) and *anti-epimastigote* (Jafari et al. 2016) activities.

Recently, climate changes and extravagant use of chemical fertilizers have dramatically considered *environmental* concerns in *agricultural lands*. However, the *global perspective* in producing medicinal plants is based on sustainable *agricultural operations* and proper *management strategies* such as bio-fertilizer application to alleviate environmental *threats* and enhance plant productivity. Based on the stated facts, bio-fertilizers have positive effects on the quality and quantity of medicinal plants. Previous studies have manifested (1) the useful effects of AMF symbiosis on the growth and EO quality and quantity of *Satureja macrostema* (Carreón-Abud et al. 2015) and *Leptospermum scoparium* (Wicaksono et al. 2018), and (2) the functional impacts of plant growth-promoting rhizobacteria (PGPR) on *Mentha piperita* (Santoro et al. 2016; del Rosario Cappellari et al. 2019) and *Ocimum basilicum* L. (Tahami et al. 2017; Yilmaz and Karik 2022). They have demonstrated that the application of proper portions for different bio-fertilizers, separately or in combination with inorganic fertilizers, can help the producers to reach the optimum products of medicinal plants.

Inorganic and biofertilizers can change the EO content and composition of medicinal plants. To our knowledge, there are no published reports on EO quality and antioxidant capacity of *S. macrantha* L. supplied by inorganic and biofertilizers. The hypothesis of the present study is that biofertilizers are superior to mineral fertilizers in terms of their ability to promote secondary metabolite production and plant growth in *S. macrantha* L. Therefore, the purposes of the present study were (1) to assess the effects of PGPR (*Pseudomonas putida* and *Azospirillum lipoferum*), AMF inoculation (*Rhizophagus intraradices*), and inorganic fertilizer (NPK) on plant growth, (2) to evaluate the effects of PGPR, AMF, and NPK on the EO content and composition, and (3) to assess the impacts of PGPR, AMF, and NPK on antioxidant activity of *S. macrantha* L.

Materials and Methods

Experimental Site

The study was carried out at the experimental farm in Alborz Research Station, Research Institute of Forests and Rangelands, Karaj (1312 m asl, 35°48′45″ N, 51°01′30″ E), Iran from February 20th 2017 to August 20th 2018. The soil in the experimental site included 34.5% clay, 47.5% silt, 18% sand with pH: 6.3, organic carbon:1.051%, nitrogen (N): 12%, available phosphorous (P): 13.5 mg Kg^{-1}, and potassium (K): 237 mg Kg^{-1}. The mean annual temperature was 13.7 and 13.9 °C for the first and second years, respectively. The annual rainfall was 258.5 and 261.4 mm in August 2017 and second years, respectively. The experiment started with seed cultivation from February 20th 2017, the first harvesting was done at August 20th 2017 and the second harvesting was conducted at August 20th 2018.

NPK and Soil Amendments

The recommended rate of nitrogen of 50 kg N ha^{-1} was applied as ammonium nitrate (33.5%); phosphorus was applied at a rate of 25 kg P ha^{-1} as calcium super phosphate (15.5%); and potassium was applied at a rate of 25 kg K ha^{-1} as potassium sulphate (48% K_2O).

The *R. intraradices* as a species of AMF was applied in the rhizosphere. The BLAST analysis unambiguously placed *Rhizophagus intraradices* as the closest relative of our *R. intraradices* CdG strain, with sequence accession number FR750209 (Estrada et al. 2013) having a 99% identity. The AM fungal strain has been incorporated into the collection of Zaidin Experimental Station, Granada, Spain, under accession EEZ 195. The mycorrhizal inocula (isolated from the endemic AMF of a maize farm originally), a combination of sterile sand, hyphae, and spores of AMF

(20 spores g^{-1} inoculum), and colonized fragments of the root, *were provided by* Research Institutes of Forest and Rangelands (RIFR), Iran.

Luria Broth medium was used to culture *P. putida* and stayed in an incubator (150 rpm) for 24 h at 27 ± 1 °C. *A. lipoferum* was cultured in Burk's N-free liquid medium and was kept for 24 h in a shaking incubator under 28 ± 1 °C and 150 rpm. In terms of bacterium application, 3×10^8 colony forming units (CFU)/mL of bacterial concentrations were utilized.

The experiment consisted of five treatments including NPK, AMF inoculation, *P. putida, A. lipoferum,* and control (untreated plants). The study was carried out in a randomized complete block design (RCBD) with three replications. *Foam* trays were filled with *vermiculite* and *peat moss* (1:1 by Volume) and were used for cultivating *S. macrantha* L. seeds. Two-month *seedlings* were transplanted in the open field at a spacing of 50 cm (row to row) × 50 cm (plant to plant) in April 2017. The area of each plot was 9 m^2 (3 × 3 m). During the experiment, the plants were irrigated at 7-day intervals with 50 mm irrigation water. The rhizosphere soil was combined with AMF, *P. putida,* and *A. lipoferum* when plants were transferred to the field. Mineral fertilizer was used as a recommended dosage of 50, 25, and 25 kg ha^{-1} for N, P, and K, respectively.

Plant Height and Weight

At the end of growing period, the plants were cut from the bottom and their height was measure by a ruler. After that, the samples were placed in paper bags in an oven set to 40 °C until they had a steady weight for recording dry weight (Khosropour et al. 2021).

Essential Oil Extraction

To measure the EO content, 100 g of dried aerial parts from each treatment were hydrodistilled in the Clevenger-type apparatus for 3 h and reported as v/w percentage (Sefidkon et al. 2006). The EO yield (kg ha^{-1}) was measured by multiplying the EO content with the plant yield of the experimental treatments. All the EO samples were stored at 4 °C for analysis by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

Gas Chromatography (GC)

UFC TYPE Rtx-5" (10 m length, 0.1 mm i.d., 0.4 µm film thickness for FID (Thermo SCIENTIFIC) was used to identify EOs. Oven temperature was maintained at 60 °C for 5 min and then programmed to 285 °C at a rate of 5 °C min^{-1}; flame ionization detector (FID) and injector temperature were 290 °C and 280 °C, respectively; helium was applied as carrier gas with a flow rate of 0.8 mL min^{-1} (Sefidkon et al. 2006).

Gas Chromatography—mass Spectrometry (GC-MS)

The EO composition was identified by GC-MS (Shimadzu, Japan). It was accomplished by Varian 3400 GC-MS system equipment with AOC-5000 auto-injector and DB-5 fused silica capillary column (30 m × 0.25 mm i.d.; film thickness 0.25 µm). The temperature was programmed from 60 °C to 250 °C with 3 °C min^{-1}; injector and interface temperatures were 260 °C and 270 °C, respectively; acquisition mass range of 40–340 amu; ionization voltage of 70 eV; the carrier gas was helium at a velocity of 45 cm sec^{-1}.

Measurement of Total Phenolic Content (TPC)

TPC was spectrophotometrically determined using Folin-Ciocalteu reagent. 100 µL of the MeOH solution of the precisely measured weight of investigated plant 1–10 (2.54, 2.58, 2.25, 4.03, 4.80, 2.13, 4.62, 1.47, 1.58, 15.05 mg mL^{-1}, respectively) were mixed with 0.75 mL of Folin-Ciocalteu reagent and allowed to stay at room temperature for 5 min. The mixture was enriched with 0.75 mL of $NaHCO_3$ solution and remained at a temperature room for 90 min. The absorbance was measured at 725 nm by UV-vis spectrophotometer (Varian Cary 50), and its standard curve was calibrated by gallic acid (GA) at 0–100 mg L^{-1}. The calibration curve revealed the linear regression at $r > 0.99$, and the outcomes were shown as mg GA g^{-1} dry weight (Khosropour et al. 2022).

Measurement of Total Flavonoid Content (TFC)

The aluminum chloride method was applied to measure the TFC (Khosropour et al. 2022). Briefly, 300 µL of $NaNO_2$ (1:20 w/v) were added to 0.5 mL of sample. The mixture was vortexed for 10 s and kept at room temperature for 5 min. Subsequently, 2 mL of NaOH (1 M), 300 µL of $AlCl_3$ (1:10 w/v), and 2 mL of distilled water. The absorbance was determined at 510 nm by UV-VIS spectrophotometer (Varian Cary 50). Quercetin concentrations at 0, 20, 40, 60, 80, 100, and 120 µg mL^{-1} were prepared and the linear fit was used for calibration of the standard curve.

Radical Scavenging Activity

To measure free radical scavenging activities, DPPH radical was used according to Brand-Brand-Williams et al. (1995). A mixture containing 0.1 mL of the extract solution, 1.0 mL of DPPH solution, and 4 mL of methanol was prepared. It was kept at ambient temperature for 30 min and measured at the absorbance at 517 nm by UV-VIS spectrophotometer

(Varian Cary 50). The scavenging effect was determined as follows:

$$DPPH\ scavenging\% = [1/(A517\,\text{nm, sample} - \text{A}\,517\,\text{nm, control})] \times 100 \quad (1)$$

Multivariate Analysis

Principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were performed by XLSTAT (Version 2009.6.03, Addinsoft, USA). In PCA, the data were mainly determined by two factors; the first factor was axis 1 (F1) and the second one was axis 2 (F2).

Statistical Analysis

The data ($n = 3$) were subjected to a one-way analysis of variance (ANOVA) using the SAS software package for Windows (SAS, version 9.3, SAS Institute, Cary, NC). The mean values were subjected to Duncan's multiple-range tests after the detection of statistical significance ($p < 0.05$).

Results

Plant Height and Dry Weight Yield (DWY)

Plant height was noticeably ($P \le 0.05$) improved by AMF, PGPR, and mineral fertilizers (Table 1). Plant height in August 2018 was greater than the first year by 18%. The highest plant height was observed in August 2018 with an application of AMF (42.66 cm) followed by NPK (41.66 cm), *A. lipoferum* (39.67), and *P. putida* (39.34 cm, Table 1).

DWY was influenced by the mineral and bio fertilizers ($P \le 0.05$, Table 1). In August 2017, the highest DWY was obtained in NPK treatment (1954 kg ha^{-1}), while its maximum amount was observed in AMF-inoculated plants

Table 1 The effect of chemical and bio fertilizers on plant height, dry weight yield, and photosynthesis pigments of *Satureja macrantha* L. during two harvesting time

Fertilizer	Year	Plant height (cm)	Dry weight yield (kg ha^{-1})	Chl $a+b$ (mg g^{-1} FW^{-1})	Carotenoid (mg g^{-1} FW^{-1})
Control	August 2017	30.65 ± 1.25e	1821 ± 13.4 h	1.01 ± 0.04c	0.43 ± 0.02 d
	August 2018	38.34 ± 1.34c	1971 ± 12.6e	1.33 ± 0.05b	0.48 ± 0.03bc
NPK	August 2017	35.65 ± 1.44 d	1954 ± 12.3ef	1.08 ± 0.06c	0.50 ± 0.04ab
	August 2018	41.66 ± 1.55ab	2570 ± 13.5b	1.43 ± 0.08ab	0.49 ± 0.02a–c
AMF	August 2017	35.33 ± 1.45c	1926 ± 14.9f	1.12 ± 0.06c	0.45 ± 0.05b–d
	August 2018	42.66 ± 2.11a	2633 ± 15.5a	1.60 ± 0.07a	0.54 ± 0.04a
AL	August 2017	34.34 ± 1.53 d	1871 ± 13.2 g	1.04 ± 0.04c	0.44 ± 0.02 cd
	August 2018	39.67 ± 1.75bc	2373 ± 16.8 d	1.40 ± 0.05b	0.51 ± 0.02ab
PP	August 2017	31.00 ± 1.27e	1825 ± 12.7 h	1.02 ± 0.05c	0.45 ± 0.04b–d
	August 2018	39.34 ± 1.85bc	2500 ± 14.3c	1.46 ± 0.05ab	0.52 ± 0.03a

Values are means ± standard deviation (SD) of three replications ($n = 3$). Different letters show statistically significant differences among treatments at $P \le 0.05$

Chl Chlorophyll, *FW* Fresh weight, *AMF* Arbuscular mycorrhizal fungi, *PP* Pseudomonas putida, *AL* Azospirillum lipoferum

Table 2 The effect of chemical and bio fertilizers on antioxidant capacity of *Satureja macrantha* L. during two harvesting time

Fertilizer	Year	TPC (mg GA g^{-1} FW)	TFC (mg QE g^{-1} FW)	DPPH radical scavenging activity (%)
Control	August 2017	45.90 ± 1.55 d	5.00 ± 0.55de	65.00 ± 2.15e
	August 2018	51.40 ± 1.35c	6.55 ± 0.75ab	73.33 ± 2.55c
NPK	August 2017	45.80 ± 1.65 d	5.60 ± 0.65 cd	70.33 ± 2.52 d
	August 2018	45.90 ± 1.25 d	7.23 ± 0.85a	77.66 ± 3.08b
AMF	August 2017	54.96 ± 1.24b	4.90 ± 0.65de	68.33 ± 2.15 d
	August 2018	59.23 ± 1.85a	7.36 ± 0.87a	81.00 ± 3.02a
AL	August 2017	45.03 ± 1.24 d	4.56 ± 0.54e	63.66 ± 2.35e
	August 2018	54.00 ± 1.62bc	6.06 ± 0.71bc	75.66 ± 2.85bc
PP	August 2017	45.46 ± 1.54 d	4.50 ± 0.45e	63.66 ± 2.45e
	August 2018	54.96 ± 1.76b	6.55 ± 0.78ab	77.66 ± 3.12b

Values are means ± standard deviation (SD) of three replications ($n = 3$). Different letters show statistically significant differences among treatments at $P \le 0.05$

TPC Total phenolic content, *TFC* Total flavonoid content, *AMF* Arbuscular mycorrhizal fungi, *PP* Pseudomonas putida, *AL* Azospirillum lipoferum

in August 2018 (2633 kg ha^{-1}). *P. putida* and *A. lipoferum* produced more DWY following AMF compared to control.

Photosynthesis Pigments

The harvesting time produced the remarkable fluctuations in Chl content. In comparison to plants from the first year, the second-year plants had greater Chl $a + b$ concentration. On the other hand, AMF was the only factor to achieve substantial increases in Chl $a + b$, with a 20% rise over the control, in August 2018. The maximum Chl $a + b$ was obtained at AMF inoculation in August 2018 year (1.60 mg g^{-1}) with 58% increase relative to its minimum amount in the control treatment (1.01 mg g^{-1}, Table 1). AMF and *P. putida* applications resulted in the greatest improvements for carotenoid, with 9 and 12% increases in comparison to the control in August 2018. Only NPK showed a substantial rise in carotenoid in August 2017, increasing it by 16% compared to the control (Table 1).

Antioxidant Capacity

Total phenolic content (TPC) was significantly ($P \leq 0.05$) changed by different treatments of PGPR, AMF, and NPK (Table 2). In August 2017, there was no significant difference among amendment treatments (Table 2). However, in August 2018, NPK, AMF, *A. lipoferum*, and *P. putida* led to increased TPC by 11, 15, 6, and 7%, respectively, relative to the non-fertilizer application (Table 2). Time of harvesting considerably boosted the total flavonoid content (TFC), enabling plants collected in August 2018 have greater TFC levels than those harvested in August 2107. The plants with the highest TFC were collected in August 2018 after receiving an AMF inoculation, with a 47% rise in TFC compared to August 2018 harvests of untreated plants. Without fertilizer application, TFC in August 2018 was 31% greater than it was in August 2017. The noticeable changes in DPPH radical scavenging activity were obtained in planted treated with the fertilizers and harvesting time (Table 2). In August 2018, it was discovered that plants inoculated by AMF had a maximal DPPH radical scavenging activity of 81% (Table 2).

Essential Oil (EO) Percentage and Yield

Although most fertilizers including NPK, *P. putida,* and *A. lipoferum* were unable to change the EO percentage relative to the control in August 2017, AMF inoculation increased it by 35%. However, EO percentages increased by 33, 16, and 18%, respectively, in the plants given AMF, *A. lipoferum*, and *P. putida* as compared to non-fertilizer application in August 2018 (Fig. 1a). EO yield in August 2018 remarkably was higher than August 2017 and its

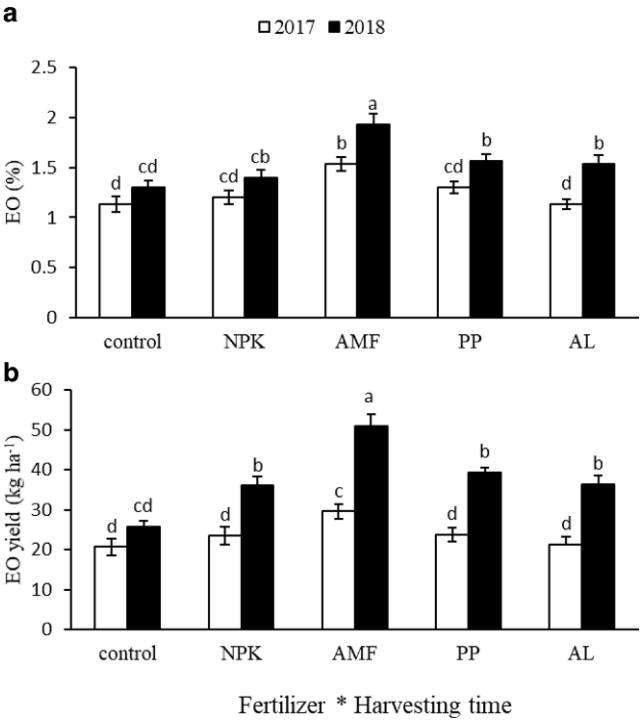

Fig. 1 The effect of chemical and biofertilizers on essential oil (EO) percentage (**a**) and EO yield (**b**) of *Satureja macrantha* L. during August 2017 and August 2018. Values are means ± standard deviation (SD) of three replications ($n = 3$). Different letters show statistically significant differences among treatments at $P \leq 0.05$. *AMF* Arbuscular mycorrhizal fungi, *PP* Pseudomonas putida, *AL* Azospirillum lipoferum

main changes under the fertilizers was obtained in August 2018. Only NPK had a significant role on EO yield in August 2017. Plants that received NPK, AMF, *P. putida*, and *A. lipoferum* in August 2018 exhibited greater EO yields than untreated plants by 40, 98, 53, and 42%, respectively (Fig. 1b).

EO Composition

The GC/MS analysis indicated that monoterpenes covered the most oil compounds of *S. macrantha* L. (Table 3). Accordingly, *p*-cymene (22.14%–33.57%), γ-terpinene (22.26%–36.28%), thymol (0.11%–31.23%), and carvacrol (0.36%–36.56%) were the main constitutes of *S. macrantha* EO (Table 3). *p*-cymene was altered during two experimental years and soil-amendment treatments. For the majority of treatments, the time of harvesting was the key factor in the rise, especially for *P. putida*, which represented a 23% increase in August 2018 compared to August 2017. It ranged from 22.14% in plants supplied with *P. putida* at August 2017 to 33.57% of AMF-inoculated plants in August 2017 (Table 3). Compared to the control, AMF recorded 29% increase in August 2017 to provide the largest rises in *p*-cymene. γ-Terpinene ranged from no

Table 3 Essential oil composition under different treatments during august 2017–2018

Compound (%)	RI	Terpene	C 2017	C 2018	NPK 2017	NPK 2018	AMF 2017	AMF 2018	PP 2017	PP 2018	Al 2017	Az 2018
α-thujene	937.55	M	0.72	1.38	1.24	2.18	1.72	1.51	0.79	1.47	1.09	1.47
α-pinene	949.28	M	1.47	2.44	1.86	1.41	1.67	2.08	0.93	2.28	1.25	2.27
Camphene	966.22	M	*	0.25	0.21	0.28	0.22	0.19	*	0.23	0.17	*
β-pinene	980.65	M	1.27	2.26	1.27	2.57	0.85	2.12	0.66	2.26	0.74	2.33
3-octanone	1007.94	M	*	1.50	0.18	0.59	0.20	1.21	0.14	1.32	0.16	1.36
Myrcene	1033.65	M	1.84	0.26	2.73	0.34	3.01	0.15	2.21	0.29	2.42	0.27
α-phellandrene	1043.61	M	*	2.36	*	3.49	*	2.46	*	2.38	*	2.53
α-terpinene	1048.53	M	1.43	1.64	2.14	0.19	1.47	0.72	1.04	0.51	0.46	0.88
p-cymene	1055.83	M	25.86	26.11	25.52	27.02	33.57	27.43	22.14	27.28	24.11	25.26
Limonene	1058.24	M	*	0.26	*	0.37	*	0.23	*	0.22	*	0.32
(Z)-β-ocimene	1060.64	M	*	2.11	2.77	0.35	1.71	0.93	1.16	0.65	0.53	1.02
(E)-β-ocimene	1063.04	M	0.14	0.22	0.19	0.26	0.19	0.17	0.13	0.14	0.14	0.15
γ-terpinene	1086.42	M	22.26	26.24	34.33	32.94	36.28	27.34	26.82	25.14	25.81	26.90
Cis-sabinene hydrate	1100.00	M	0.37	0.15	0.18	0.21	0.14	0.22	0.21	0.26	0.25	0.21
Borneol	1222.64	M	0.18	0.45	*	0.26	*	0.40	0.13	0.54	0.15	0.36
Thymol	1325.10	M	0.21	26.29	0.11	25.89	*	27.88	0.18	31.23	0.15	29.54
Carvacrol	1332.49	M	34.10	0.82	18.44	0.36	12.93	0.95	36.56	0.39	35.66	0.88
Thymol acetate	1363.79	M	0.69	0.18	1.02	*	0.42	0.27	0.59	0.24	0.64	0.19
(E)-caryophyllene	1474.38	S	1.49	1.01	1.79	0.57	1.20	0.84	1.41	0.80	1.41	1.18
Germacrene D	1530.53	S	0.20	1.08	0.14	0.21	*	0.61	0.14	0.53	0.12	0.75
β-bisabolene	1544.15	S	0.19	0.16	0.19	0.15	*	0.24	0.21	0.30	0.21	0.23
Spathulenol	1647.50	S	0.20	0.66	0.15	*	*	0.55	0.13	0.54	0.11	0.50
Caryophyllene oxide	1657.49	S	*	0.39	*	0.16	*	0.31	*	0.27	*	0.42
Sabinene	*	S	1.93	*	2.47	*	2.52	*	1.70	*	1.90	*
β-phellandrene	*	S	1.76	*	*	*	0.28	*	0.20	*	0.26	*
Terpinene-4-ol	*	S	0.22	*	0.16	*	0.13	*	0.17	*	0.18	*
Aromadendrene	*	S	0.17	*	0.16	*	*	*	0.17	*	0.18	*
Bicyclogermacrene	*	S	1.37	*	1.01	*	0.64	*	0.83	*	0.60	*
-cadinene	*	S	0.44	*	0.41	*	0.18	*	0.34	*	0.31	*
Total (%)	–	–	98.50	98.22	98.67	99.78	99.31	98.80	99.00	99.28	99.02	99.01

EO amount (%) for each compound shows the amount of total EO

M Monoterpene, *S* Sesquiterpene, *AMF* Arbuscular mycorrhizal fungi, *C* control (non-fertilizer), *PP* Pseudomonas putida, *AL* Azospirillum lipoferum, *RI* Retention Index

amendment application in August 2017 (22.26%) to AMF application in August 2017 (36.28%). Thymol and carvacrol have different behavior in during harvesting time. Thymol was found as the another major EO component, ranging from NPK application (25.89%) to *P. putida* application (31.23%) in August 2018. In contrast, carvacrol was described by the first year as the main component, differing from AMF application (12.98%) to the use of *P. putida* (36.56%).

Multivariate Analyses

The PCA of physiological and biochemical attributes revealed that Chl a + b, carotenoid, EO content, TFC, and DPPH scavenging activity were characterized by F1, while F2 explained plant height, DWY, EO yield, and TPC. In addition, AMF and NPK were specified by F1, while F2 mainly determined control and *A. lipoferum* (Fig. 2a). According to PCA of EO profile, p-cymene, thymol, and carvacrol were determined by F1, and γ-terpinene was expained by F2.

Acceding to AHC of physiological and biochemical attributes, three different clusters were identified; cluster 1 included AMF, NPK, and *P. putida*; cluster 2 explained *A. lipoferum;* control was placed in cluster 3 (Fig. 3a). The AHC of EO profile revealed three classes as cluster 1 for control, AMF, and *P. putida,* cluster 2 for NPK, and cluster 3 for *A. lipoferum* (Fig. 3b).

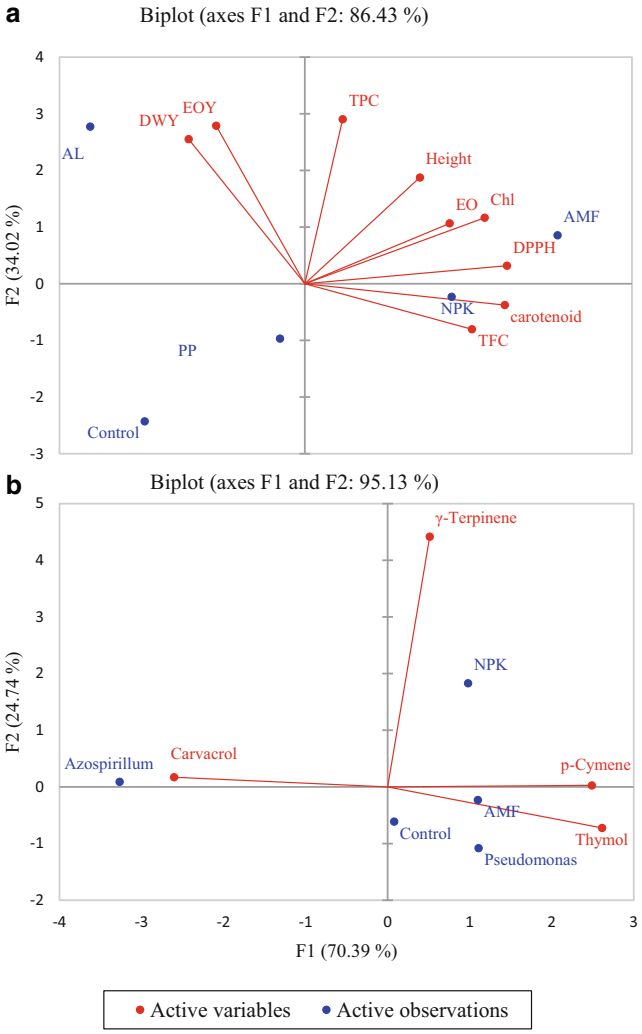

Fig. 2 Principal component analysis for physiological traits (**a**) and EO profile (**b**) of *Satureja macrantha* L. upon chemical and bio fertilizers. *EO* essential oil, *EOY* essential oil yield, *DWY* dry weight yield, *TPC* total phenolic content, *TFC* total flavonoid content, *DPPH* DPPH scavenging activity, *Chl* chlorophyll content, *AMF* Arbuscular mycorrhizal fungi, *PP* Pseudomonas putida, *AL* Azospirillum lipoferum

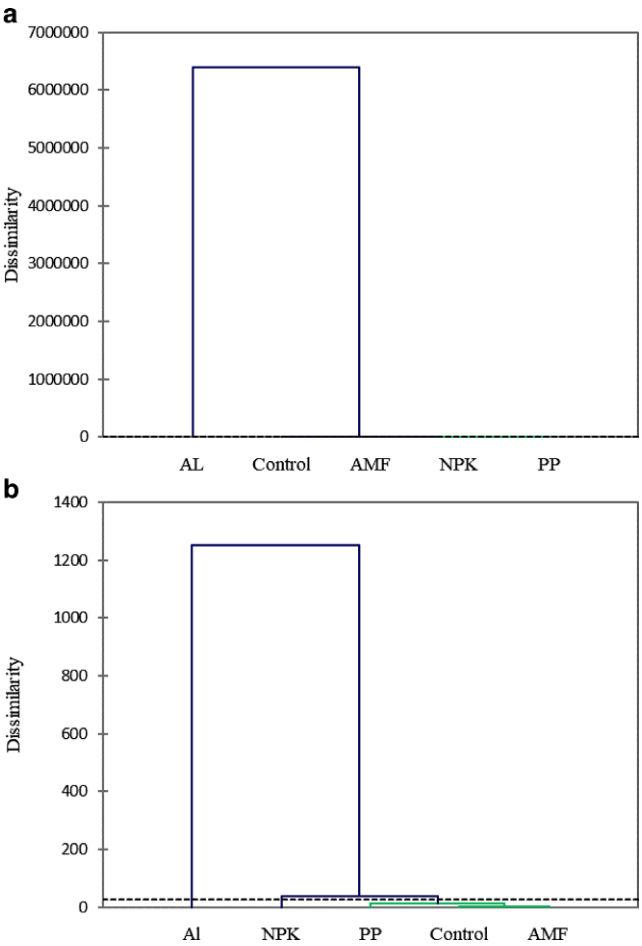

Fig. 3 Agglomerative hierarchical clustering for physiological traits (**a**) and EO profile (**b**) of *Satureja macrantha* L upon chemical and bio fertilizers. *AMF* Arbuscular mycorrhizal fungi, *PP* Pseudomonas putida, *AL* Azospirillum lipoferum

Discussion

While all biofertilizers are effective at boosting plant growth and yield, AMF had the greatest impact with 33% relative to the non-treated plants in August 2018 and 44% compared to the control in August 2017. Mycorrhizal roots due to their extramatrical hyphae can uptake and translocate more nutrients in comparison with non-Mycorrhizal roots (Darakeh et al. 2022). AMF can also improve the availability and supply of slowly diffusing ions like phosphate to plants (Makarov 2019). Control plants had very little soil microbial inoculation, which decreased their capacity to absorb nutrients and water, which further inhibited their ability to grow and develop. Previous research reveals that approximately 80% of the P absorbed by a mycorrhizal plant is provided by the corresponding fungus (Mitra et al. 2021). Additionally, AMF significantly improves the uptake of other macro- and micro-nutrients such as N, K, and Zn, especially in their less soluble forms (Wicaksono et al. 2018; Mitra et al. 2021). *P. putida* and *A. lipoferum* increased the plant growth and yield of *S. macrantha* L. The microorganisms presenting in *P. putida* and *A. lipoferum* induce a significant improvement in plant growth through the production of growth regulators and facilitation of the nutrient uptake by plants from the soil (Pérez-Rodriguez et al. 2020). Inoculation with PGPR improved plant growth by generating growth-promoting substances (Basu et al. 2021). It is well known that auxins and cytokinins significantly enhanced plant cell division and root enlargement (Ahmad et al. 2021). IAA has an important role in root initiation, cell division, and cell development, and boosts root surface area, resulting in high access to soil nutrients through improvement in the creation of lateral and adventitious roots (Borah et al. 2022). Cytokinins encourage cell division, cell development, and tissue enlargement in certain plant parts

in plants inoculated wit PGPRs (Wybouw and De Rybel 2019; Barzegari Barogh et al. 2023). Similar to our work, the positive effects of PGPR on plant growth and yield were reported in different medicinal plants such as *Melissa officinalis* L. (2022), *Bacopa monnieri* L. (Pankaj et al. 2020), and *Nigella sativa* L. (Darakeh et al. 2022).

Photosynthetic pigments increased by the application of soil amendments and time of harvesting, particularly with AMF inoculation in August 2018, which represents 58% and 25% increases in Chl $a+b$ and carotenoid, respectively, relative to untreated plants in August 2017. AMF due to its high potential in holding water and nutrients can provide a desirable condition for plants in regenerating their photosynthesis products (Yooyongwech et al. 2016). Our study revealed that *S. macrantha* L. can make a symbiosis with the AMF. The increased chlorophyll content was reported in potatoes (Yooyongwech et al. 2016) and basil (Hazzoumi et al. 2015) inoculated by AMF. Yadav et al. (2015) found that AMF with *Trichoderma viride* and *Pseudomonas fluorescens* make a higher amount of chlorophyll content compared to no treated plants.

Biofertilizers boosted the phenol and flavonoid content of savory plants. Mechri et al. (2015) reported that AMF inoculation accumulates TPC in olive tree roots. In addition, Darakeh et al. (2022) reported an increased TPC of block cumin after the co-application of AMF and vermicompost. Phenylalanine ammonia-lyase is the key enzyme in the biosynthesis of phenolic compounds, which is affected by AMF symbiosis (Begum et al. 2021). The mechanisms determining the synthesis of phenolic compounds in plants inoculated with AMF have not been clarified yet. AMF can change phenolic compounds of host roots that would influence the growth of hyphae. Zhang et al. (2013) indicated that AM fungi can increase phenolic synthesis in roots, probably through signaling pathways of salicylic acid (SA), hydrogen peroxide (H_2O_2), and nitric oxide (NO). However, the role of phenolic compounds in the vesicular-arbuscular mycorrhizal association is less well understood. Besides, flavonoids make a positive effect on hyphal growth during a symbiosis between AMF and plant (Mechri et al. 2015). The mycorrhizal symbioses caused cell type-specific differential expression in genes of phenylpropanoid/flavonoid/isoflavonoid biosynthesis, which is related to arbuscular development. Lima et al. (2017) revealed that the phenol content under mycorrhizal symbiosis increased, and glycosylated derivatives of a flavonoid were able to promote mycorrhiza formation, which are in line with results of the current study. Changes in the metabolic pathways of phenols and flavonoids may be the cause of the improvement of theses chemicals by AMF and PGPRs. For instance, the generation of Shikimic acid, a major pathway for phenolic compounds, was significantly impacted by the inoculation of AMF and PGPRs (Eshaghi Gorgi et al. 2022).

EO constitute had different responses to NPK, AMF, and bacterial inoculation. The EO yield was primarily improved by all biofertilizers, particularly AMF in plants collected at August 2018. The improvement in EO yield and the change in EO composition because of AMF symbiosis was reported in *Satureja macrostema* (Carreón-Abud et al. 2015), *Leptospermum scoparium* (Wicaksono et al. 2018), *Lavandula angustifolia* (Golubkina et al. 2020), *Ocimum basilicum* L. (Yilmaz and Karik 2022). Similarly, PGPR improves EO yield and alters the EO composition *of Mentha piperita* (Santoro et al. 2016), *Ocimum basilicum* L. (Tahami et al. 2017), Mentha x Piperita (del Rosario Cappellari et al. 2019). AM fungi increase EO production because mycorrhization allows the root system to exploit a greater volume of soil by (1) improving the physical structure of soil pores to be more available for root hairs; (2) expanding the root area; (3) increasing the production of extracellular acid phosphatases through acquiring organic phosphates (Darakeh et al. 2022). PGPRs in addition to improving essential oil yield, activate octadecanoid, shikimate, jasmonate, and terpenoid pathways. One of the benefits of replacing PGPRs is developing a stable formulation of antagonistic PGPR (Ghorbanpour et al. 2015). The signaling pathway of jasmonic acid (JA) is an integral signal in the biosynthesis of many plant secondary products like terpenoids, flavonoids, alkaloids, and phenylpropanoids. PGPRs promote JA biosynthesis in plants by accumulating the secondary metabolites in plants (del Rosario Cappellari et al. 2019). It should be mentioned that terpenoids, alkaloids, and phenolics are the three main groups of secondary plant metabolites used for pharmacological and therapeutical purposes (Ghorbanpour et al. 2015). The primary metabolism such as photosynthesis is corresponded to the biosynthesis of terpenoids (Shokati and Poudineh 2017).

According to the results of PCA, carotenoid and TFC mainly specified by NPK, while DPPH scavenging activity, EO content, and Chl content were justified by AMF. *A. lipoferum* had a notable role in DWY, EO yield, and TPC, which are beneficial for understanding the main changes of corresponding traits upon the fertilizers. P-cymene and thymol negatively correlated with carvacrol, which is important for producers of these EO compounds. Similar top our results, the changes in physiological and biochemical attributes by PCA and AHC have been reported by Al-Rowaily et al. (2020).

Conclusions

In the present study, we dissected the effects of different amendments on plant growth, antioxidant potential, and EO quantity and quality in a semiarid region. Although

the first harvesting (August 2017) was unable to represent the remarkable changes in growth and EO yield, the improvement in the second harvesting time (August 2018) was mainly better than the control. Among the fertilizers, inoculation with AMF is the most appropriate strategy for improving plant growth and finally EO yield of *S. macrantha* L. After AMF, it is possible to use *P. putida* as an alternative to inorganic fertilizer for obtaining the optimum traits of *S. macrantha* L. As a result, harvesting *S. macrantha* plants 18 months after cultivation with AMF inoculation is more advantageous economically than harvesting them after 6 months in a semiarid area with less than 300 mm precipitation per year. The food and pharmaceutical industries may benefit from the changes in EO composition and antioxidant capacity.

Conflict of interest M.B. Abkenar, H. Mozafari, K. Karimzadeh, F. Rajabzadeh and R. Azimi declare that they have no competing interests.

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