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Enhancement of Growth and Essential Oil Traits of Spearmint and Peppermint through Mycorrhizal and Trichoderma Inoculations

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ABSTRACT

This study evaluated the effects of arbuscular mycorrhizal fungi (AMF) and *Trichoderma harzianum* on growth, essential oil (EO) yield, and physiological traits in two spearmints (*Mentha spicata*) and one peppermint (*Mentha* × *piperita*) landrace. AMF treatments included *Glomus etunicatum* and *Glomus fasciculatum*, while *T. harzianum* was also tested. Combined inoculations significantly enhanced plant height, EO yield, and phytochemical composition. Spearmint plants treated with *G. fasciculatum* + *T. harzianum* reached the tallest height, and EO yields peaked at 2.8% in spearmint (Qaemshahr landrace) and 2.6% in spearmint (Vardavard landrace) under similar treatment. Limonene content in spearmint reached 20% with *G. fasciculatum*, while carvone peaked at 54% with *G. etunicatum* + *T. harzianum*. In peppermint, menthol content was highest (40.25%) with *T. harzianum* alone, and L-menthone peaked (34.9%) under *G. fasciculatum* + *T. harzianum*. Photosynthesis rates, stomatal conductance, and transpiration rates were highest in peppermint treated with *G. fasciculatum* + *T. harzianum* during the second harvest. Additionally, the greenness index peaked in peppermint treated with *T. harzianum* alone. These findings demonstrate the synergistic potential of AMF and *T. harzianum* in improving growth, physiological traits, and EO production in mint species, offering a promising strategy to enhance commercial mint cultivation.

Introduction

Lamiaceae is one of the largest families of angiosperms, encompassing 12 sub-families, 295 genera, and approximately 7,775 species (Barbosa Silva Cavalcanti et al., 2019). Among its genera, *Mentha* is particularly significant, comprising 25-30 species including spearmint (*Mentha spicata* L.) and peppermint (*Mentha piperita* L.) (Mehrnia et al., 2017).

Spearmint and peppermint, two prominent species in the Lamiaceae family, are extensively used in cosmetics, herbal medicines, and as flavorings in food and beverages (Moradi-Sadr et al., 2023). Spearmint, traditionally used as a carminative, anti-spasmodic, and for treating gastrointestinal and respiratory conditions, also addresses issues such as bad breath, diuretic needs, and sedative effects. Additionally, it is used to treat cough, asthma, cold, fever, jaundice, and digestive problems (Mahendran et al., 2021). Peppermint is renowned for its antioxidant, antimicrobial, and anti-cancer properties (Saqib et al., 2022).

The economic value of spearmint and peppermint EOs has driven ongoing research into these medicinal plants. In 2022, the top exporters of peppermint EOs were India ($61 million) and the United States ($60.9 million), while the top importers were the United States ($34.5 million) and Germany ($19.2 million) (Essential Oils of Peppermint | OEC - The Observatory of Economic Complexity, n.d.). In 2019, spearmint EO accounted

for 20% of the total EO market value ($1.3 billion), whereas the market shares for orange and peppermint EOs were 8 and 3%, respectively (Essential Oils (HS: 3301) Product Trade, Exporters and Importers | OEC - The Observatory of Economic Complexity, n.d.). Given the substantial economic value of these two medicinal herbs, understanding the factors affecting their growth and yield is of paramount importance.

A key point in the cultivation of spearmint and peppermint is the potential synergistic relationship between mycorrhizal fungi (MF) and Trichoderma fungi (TF) inoculation. The combined application of MF and TF has been shown to produce superior growth responses and phytochemical improvements compared to single inoculants. This dual inoculation approach can lead to enhanced morpho-physiological characteristics, such as increased leaf area, biomass, and essential oil (EO) content, which are crucial for the commercial viability of spearmint and peppermint cultivation (Elgharably and Nafady, 2021; Poveda and Eugui, 2022).

The inoculation of plants with beneficial microbes not only enhances growth but also fortifies stress tolerance by promoting biomass accumulation and modulating stress-responsive physiological traits. For instance, microbial symbiosis has been shown to significantly increase secondary metabolite production, such as enhanced artemisinin levels and guaiacol peroxidase activity in *Artemisia annua* (Zhao et al., 2022; Jemo et al., 2023). Additionally, cultivation techniques like aeroponics, hydroponics, and *in vitro* cultures have been successfully employed to boost secondary metabolite yields in MF-inoculated medicinal plants, thereby contributing to both improved crop productivity and pharmaceutical potential (Zydlik et al., 2021; Zhao et al., 2022).

Recent research has underscored the role of microbial communities in enhancing plant resilience under environmental stress. For example, a study on Persian walnut demonstrated that specific plant growth-promoting rhizobacteria (PGPR), particularly strains of *Bacillus velezensis* and *Bacillus amyloliquefaciens*, significantly improve drought tolerance through mechanisms such as indole-3-acetic acid (IAA) production and phosphorus solubilization (Lotfi et al., 2022). Similarly, dual microbial inoculation strategies involving arbuscular mycorrhizal fungi and PGPR have been found to enhance nutrient uptake, antioxidant enzyme activity, and biomass in plant, highlighting the synergistic potential of microbe-microbe interactions in medicinal plant cultivation (Lotfi et al., 2025). The concept of the holobiont plant, which views the host and its microbiome as an integrated biological unit, further reinforces the importance of a diverse and functionally rich microbial community in ensuring plant health and adaptability. These insights support the strategic use of microbial consortia to drive sustainable agriculture, especially in the face of increasing environmental stressors (Zenteno-Alegría et al., 2024).

In the current research, the effects of MF and TF inoculations on the morphological characteristics, greenness index, and photosynthesis rate of spearmint and peppermint were studied. Furthermore, the quantity and chemical profile of the obtained EOs were evaluated.

Materials and Methods

Experimental design

This experiment employed a Central Composite Design (CCD) as part of a factorial arrangement to optimize the interaction effects between plant species and microbial treatments. CCD was chosen due to its suitability for studying quadratic response surfaces and interaction effects, especially in biological systems with multiple factors. The experiment consisted of three main factors:

- Mint species: Two spearmint landraces (Vardavard and Qaemshahr) and one peppermint landrace.
- Mycorrhizal fungi (MF): At three levels – no inoculation (control), *Glomus etunicatum*, and *Glomus fasciculatum.*
- Trichoderma fungi (TF): At two levels – no inoculation (control) and inoculation with *Trichoderma harzianum*.

Each treatment was replicated three times, and two harvests were conducted at two-month intervals, resulting in a total of six biological observations per treatment to account for temporal variability.

Plant material preparation

Two high-quality spearmint landraces, confirmed by a previous study (Mokhtarikhah et al., 2022), were selected for this research, along with a local peppermint landrace. The culture medium used in the experiment consisted of 50% garden soil, 30% compost, and 20% perlite. To eliminate potential pathogens in the garden soil, formalin was applied as a disinfectant two weeks before planting, and the soil was thoroughly aerated to allow volatilization and prevent phytotoxic effects. The plants were cultivated in the research greenhouse of the Faculty of Agriculture at Tarbiat Modares University in Tehran, Iran. Samples were harvested twice at two-month intervals. After harvesting, the plants were shade-dried in a drying room maintained at 25 °C for one week.

Mycorrhizal and Trichoderma inoculations

Commercial formulations containing spores of MF and TF were provided by Turan Biotech Co. (Iran). The rhizomes used for planting were uniform in weight (~4 g) and length (~5 cm). Mycorrhizal fungi

were applied at a concentration of ~200 spores g^{-1} of soil at the time of planting. *Trichoderma harzianum* inoculum was applied at 1×10^7 CFU mL^{-1}, 20 mL per pot, immediately after planting and repeated 15 d later to ensure colonization. The treatments are shown in Table 1.

Table 1. Abbreviations of treatments.

Abbreviation	Treatments
VM0T0	non-Inoculated (control) spearmint (vardavard landrace)
VM0T1	spearmint (vardavard landrace) inoculated with *Trichoderma harzianum*
VET0	spearmint (vardavard landrace) inoculated with *Glomus etunicatum*
VET1	spearmint (vardavard landrace) inoculated with *Glomus etunicatum* and *Trichoderma harzianum*
VFT0	spearmint (vardavard landrace) inoculated with *Glomus fasciculatum*
VFT1	spearmint (vardavard landrace) inoculated with *Glomus fasciculatum* and *Trichoderma harzianum*
GMT0	non-inoculated (control) spearmint (Qaemshahr landrace)
GMT1	spearmint (Qaemshahr landrace) inoculated with *Trichoderma harzianum*
GET0	spearmint (Qaemshahr landrace) inoculated with *Glomus etunicatum*
GET1	spearmint (Qaemshahr landrace) inoculated with *Glomus etunicatum* and *Trichoderma harzianum*
GFT0	spearmint (Qaemshahr landrace) inoculated with *Glomus fasciculatum*
GFT1	spearmint (Qaemshahr landrace) inoculated with *Glomus fasciculatum* and *Trichoderma harzianum*
PET0	peppermint inoculated with *Glomus etunicatum*
PET1	peppermint inoculated with *Glomus etunicatum* and *Trichoderma harzianum*
PFT0	peppermint inoculated with *Glomus fasciculatum*
PFT1	peppermint inoculated with *Glomus fasciculatum* and *Trichoderma harzianum*
PM0T0	non-inoculated peppermint (control)
PM0T1	peppermint inoculated with *Trichoderma harzianum*

Morphological and physiological characteristics

At each harvest, the following parameters were measured per plant per treatment. Growth traits: plant height, number of leaves, side branches, nodes, internodes, fresh weight, dry weight, and leaf area (via Leaf Area Meter). Physiological traits: greenness index (SPAD-502 Plus; average of 7 randomly selected leaves) and photosynthesis rate (LI-6400XT, LICOR, USA). Photosynthesis rate measurements were taken between 10:00 and 13:00, under a light intensity of 20,000 lx, with each sample allowed to stabilize for 1–3 min before data capture.

EO isolation

From each treatment × harvest, 40 g of dried leaf material was subjected to hydro-distillation for 3 h using a Clevenger-type apparatus. The EO yield was expressed as % (v/w). EOs were dried over anhydrous sodium sulfate and stored at 4 °C in amber vials until analysis.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC–MS)

EO composition was determined using GC (Agilent 7890B) with FID and GC–MS (Thermoquest–Finnigan) equipped with HP-5 or HP-5ms capillary columns (30 m × 0.32 mm, 0.25 µm film thickness). Injector and FID temperatures were 250 and 280 °C, respectively. The oven temperature program started at 60 °C (2 min hold) and increased to 280 at 5 °C min^{-1}. The split ratio was 1:100, helium was used as the carrier gas at 1.1 mL min^{-1}, and mass spectra were recorded from 45 to 456 amu. Compounds were identified by comparing mass spectra with Wiley 7.0 and Adams libraries, as well as by calculating retention indices based on C8–C24 n-alkanes and literature data (Adams, 2007).

Statistical analysis

SAS software version 9.2 was used to analyze the obtained data and determine correlation coefficients between traits. Excel 2016 was employed to create graphs. Additionally, the comparison of the obtained means was performed using the Least Significant Difference (LSD) test.

Results

Morpho-physiological characteristics

Analysis of variance indicated that plant height, number of branches, greenness index, fresh weight of leaves, number of leaves, simple effect of treatments, simple harvest frequency, and the interaction effect of treatments in harvest frequency were significant at the 1% probability level (Table 2).

As shown in Table 3, the maximum plant height (64.17 cm) and leaf width (2.45 cm) were observed in the VTF0 treatment. The highest greenness indexes (39.15 and 38.44) were recorded in the PET0 and PM0T0 treatments, respectively. Additionally, the VFT0 and GMT0 treatments resulted in the maximum number of nodes (395 and 376, respectively). Traits such as node number, shoot and leaf fresh weights, and number of leaves were best in the GMT0 and GMT1 treatments during the second harvest frequency (Table 3). While the interaction effect of treatments on leaf length was not significant, leaf length increased in the second harvest frequency and in the VTF0 treatment.

Photosynthetic parameters

The highest rate of transpiration was observed in the VFT1 treatment during the second harvest frequency, reaching 1.35 mmol m^{-2} s^{-1} (Fig. 1a). Conversely, the lowest transpiration rate was recorded in the VM0T0 treatment during the first harvest, at 0.15 mmol $m^{-2}s^{-1}$ (Fig. 1a). The maximum stomatal conductance was measured in the VFT1 treatment during the second harvest frequency, with a value of 0.28 mol $m^{-2}s^{-1}$, while the lowest stomatal conductance was observed in the VM0T0, PM0T1, and GFT0 treatments, all recording a value of 0.04 mol m^{-2} s^{-1} (Fig. 1b). The highest intercellular CO_2 concentration was found in the VFT0 treatment, reaching 345.98 µmol mol^{-1}, and the lowest intercellular CO_2 concentration was recorded in the GET1 treatment, at 166.75 µmol mol^{-1} (Fig. 1c). The highest rate of photosynthesis was observed in the PFT1 treatment during the second harvest frequency (8.11 µmol m^{-2} s^{-1}), while the lowest rate of photosynthesis was recorded in the GFT0 treatment during the second harvest, at 1.53 µmol m^{-2} s^{-1} (Fig. 1d).

Phytochemical characterization and comparison of extracted oils

EO yield

As shown in Figure 2, the highest EO yield was observed in the GFT1 treatment for both the first and second harvest frequencies, with yields of 1.97 and 1.96%, respectively, which were not significantly different from each other. The lowest EO yield was recorded in the PM0T0 treatment at 1.24%.

EO composition

The analysis of EOs identified 30 compounds in spearmint and 31 compounds in peppermint samples inoculated with MF and TF (Tables 4 and 5). For spearmint EOs, limonene and carvone were identified as the principal constituents. In peppermint EOs, L-menthone and menthol were the dominant compounds (Table 5).

In spearmint EOs, the highest carvone concentrations were found in the VET1, GFT1, and GET0 treatments, with values of 54, 54, and 51.1%, respectively. Conversely, the lowest carvone content was recorded in GFT1 and VM0T0 treatments, both at 47%. The highest limonene content was observed in GFT0 and GFT1 treatments (20%), while the lowest was in VFT0 (Table 4).

In peppermint EOs, the highest menthol concentration was noted in the PET1 treatment, reaching 40.25%. The PET0 treatment had the highest amounts of menthofuran and piperitone, at 1.9% and 0.76%, respectively. The PFT1 treatment exhibited the highest level of L-menthone at 34.9%, while the PFT1 and PFT0 treatments showed the highest concentrations of 1,8-cineole, at 9.50 and 7.99%, respectively (Table 5).

Table 2. ANOVA (mean squares) of fungi inoculations effects on spearmint and peppermint morpho/physiological characteristics.

Source	Degrees of freedom	Plant height	Number of branches	Greenness index	Number of nodes	Number of internodes	Shoot Fresh weight	Leaf fresh weight	Number of leaves	Leaf length	Leaf width	Internode length
Treatments (T)	17	363.96**	338.59**	130.12**	37438.31**	8858.27**	21.66**	40.70**	36047.73**	1.42**	0.45**	0.53*
Harvest frequency (H)	1	7441.28**	8286.93**	3354.85**	2667.04^{ns}	2004.46^{ns}	28.02*	845.30*	84569.80**	15.91**	6.40**	0.24^{ns}
T*H	17	173.97**	90.4**	106.27**	29251.29**	3928.38**	11.32**	19.25**	32757.91**	0.34^{ns}	0.22**	0.46^{ns}
Repetition	2	14.87^{ns}	44.04^{ns}	0.97^{ns}	10580.18^{ns}	2818.17^{ns}	8.55^{ns}	22.73^{ns}	17224.81^{ns}	0.19^{ns}	0.03^{ns}	0.33^{ns}
Error	178	44.78	27.96	40.63	5120.31	1342.52	4.54	7.77	10114.41	0.22	0.06	0.28

^{ns} non-significant, * and ** are significant at 5 and 1 percent probability levels, respectively.

Table 3. Effect of fungi inoculations on morphological characteristics of spearmint and peppermint.

Treatments	Harvest frequency	Plant height (cm)	Number of branches	Greenness index	Number of nodes	Number of internodes	Shoot Fresh weight (g)	Leaf fresh weight (g)	Number of leaves	Leaf width (cm)
VM0T0	1	24.00±2.83^{j-l}	13.83±2.21^{f-j}	15.16±3.58^{h-j}	90.00±11.88^{lm}	45.00±5.94^{n}	2.05±0.46^{j}	3.08±0.81^{lm}	167.83±37.16^{ij}	1.52±0.03^{h-l}
VM0T1	1	25.33±1.63^{h-l}	26.17±3.59^{bc}	16.33±3.22^{f-j}	165.67±14.41^{g-l}	82.83±7.20^{h-n}	3.42±0.56^{e-j}	4.50±0.52^{i-m}	287.50±35.78^{c-h}	1.68±0.07^{f-j}
VET0	1	22.67±1.87^{kl}	21.17±3.36^{c-e}	20.15±2.63^{d-i}	145.±67±21.66^{h-m}	72.83±10.83^{j-n}	2.62±0.62^{hij}	3.52±0.55^{lm}	213.17±39.55^{e-j}	1.56±0.11^{g-k}
VET1	1	25.17±1.64^{h-l}	20.00±1.71^{de}	19.27±1.98^{d-i}	137.00±20.17^{i-m}	68.50±10.09^{l-n}	3.10±0.51^{f-j}	3.92±0.67^{k-m}	237.83±54.25^{d-j}	1.49±0.10^{h-m}
VFT0	1	30.50±2.05^{e-j}	17.50±3.18^{e-h}	19.69±2.18^{d-i}	120.00±9.05^{k-m}	60.00±4.52^{mn}	2.37±0.48^{ij}	3.92±0.26^{k-m}	226.00±23.50^{e-j}	1.69±0.05^{e-j}
VFT1	1	18.83±2.76^{l}	17.83±3.57^{e-g}	22.14±3.09^{d-h}	140.00±24.79^{i-m}	70.00±12.39^{k-n}	3.15±1.34^{f-j}	3.53±0.73^{lm}	176.67±35.91^{h-j}	1.51±0.03^{h-m}
GMT0	1	30.83±1.42^{e-j}	41.50±2.86^{a}	21.64±2.11^{d-h}	337.33±53.63^{ab}	168.67±26.82^{ab}	5.88±0.87^{bcd}	6.88±0.90^{d-k}	316.67±16.87^{a-e}	1.46±0.08^{i-m}
GMT1	1	29.17±2.81^{f-k}	27.667±5.30^{b}	20.54±2.69^{d-i}	275.33±8.24^{b-d}	137.67±4.12^{bcd}	4.37±0.96^{e-j}	5.35±0.51^{h-m}	239.33±25.30^{d-j}	1.55±0.19^{g-k}
GET0	1	28.83±1.94^{f-k}	28.00±2.96^{b}	15.93±2.52^{g-j}	274.00±36.30^{b-d}	137.00±18.15^{b-e}	4.65±1.07^{c-i}	5.40±1.32^{h-m}	255.50±73.59^{d-i}	1.16±0.05^{n}
GET1	1	25.83±1.17^{h-l}	25.17±1.40^{b-d}	20.78±2.66^{d-i}	251.00±26.83^{c-f}	125.50±13.41^{c-g}	3.00±0.25^{g-j}	5.13±0.41^{i-m}	268.33±38.24^{c-i}	1.31±0.08^{k-n}
GFT0	1	22.67±1.84^{kl}	24.17±2.87^{b-d}	15.13±2.82^{h-j}	225.00±43.21^{d-h}	112.50±21.60^{d-j}	5.17±1.25^{b-g}	3.95±1.00^{j-m}	196.50±34.15^{f-j}	1.25±0.03^{l-n}
GFT1	1	24.33±1.23^{i-l}	30.00±2.28^{b}	16.44±3.11^{f-j}	282.67±34.80^{b-d}	141.33±17.40^{a-d}	3.73±0.49^{d-j}	4.93±0.51^{i-m}	257.67±52.57^{d-i}	1.24±0.05^{mn}
PET0	1	36.33±4.18^{d-f}	11.83±0.91^{g-m}	22.24±3.73^{d-h}	242.67±11.97^{d-g}	121.33±5.99^{c-h}	5.62±1.20^{b-e}	6.02±1.17^{f-l}	207.50±24.44^{e-j}	1.94±0.22^{b-f}

PET1	1	31.83±2.21^{d-i}	20.50±2.81^{c-e}	20.85±1.79^{d-i}	264.67±42.96^{b-e}	132.33±21.48^{b-f}	7.22±1.26^{b}	6.18±0.78^{e-l}	377.67±44.05^{a-c}	1.67±0.13^{f-j}
PFT0	1	21.83±2.95^{kl}	15.17±0.87^{e-i}	10.67±1.87^{j}	186.33±20.81^{e-k}	93.17±10.40^{f-m}	4.75±0.88^{c-i}	2.55±0.38^{m}	191.67±40.37^{g-j}	1.23±0.09^{mn}
PFT1	1	30.50±2.78^{e-j}	12.67±2.43^{f-l}	18.74±1.83^{e-i}	225.67±30.26^{d-h}	112.83±15.13^{d-j}	5.33±0.87^{b-g}	3.90±0.42^{k-m}	199.17±39.07^{f-j}	1.463±0.14^{h-m}
PM0T0	1	35.50±3.78^{de-}	12.67±2.29^{f-l}	14.02±1.60^{ij}	208.33±17.38^{d-j}	104.17±8.69^{d-l}	6.47±1.41^{bc}	4.93±0.53^{i-m}	210.50±29.13^{e-j}	1.30±0.04^{k-n}
PM0T1	1	27.50±1.34^{g-k}	13.00±1.51^{f-k}	21.72±2.58^{d-h}	170.67±49.09^{f-l}	85.33±24.55^{g-n}	3.17±0.65^{f-j}	4.67±1.32^{i-m}	169.00±35.57^{ij}	1.31±0.06^{k-n}
VM0T0	2	38.58±1.02^{cd}	5.42±1.04^{n-p}	21.77±0.87^{d-h}	185.50±34.47^{e-k}	93.00±16.70^{f-m}	2.60±0.47^{h-j}	5.48±1.02^{g-m}	239.50±39.06^{d-j}	2.13±0.03^{b}
VM0T1	2	31.83±1.99^{d-i}	8.55±1.64^{j-p}	24.90±2.44^{b-e}	131.17±28.09^{j-m}	63.50±13.85^{l-n}	4.52±1.07^{c-i}	8.63±1.63^{b-g}	170.50±32.47^{ij}	1.97±0.12^{b-e}
VET0	2	37.00±2.19^{de}	7.40±1.15^{k-p}	22.28±1.06^{d-h}	179.00±12.81^{f-k}	87.67±7.05^{g-m}	3.67±0.54^{d-j}	7.37±1.16^{c-i}	232.00±24.39^{e-j}	1.99±0.04^{b-d}
VET1	2	34.08±2.89^{d-g}	6.97±1.41^{k-p}	23.79±0.40^{b-e}	187.83±24.41^{e-k}	94.83±12.29^{f-m}	3.27±0.46^{e-j}	7.13±1.42^{c-j}	256.00±32.32^{d-i}	2.03±0.10^{bc}
VFT0	2	64.17±1.64^{a}	11.77±0.54^{h-m}	23.14±1.88^{c-g}	395.00±13.84^{a}	141.50±20.53^{a-d}	4.28±0.17^{c-j}	7.35±1.66^{c-i}	276.67±47.15^{c-i}	2.45±0.14^{a}
VFT1	2	37.00±3.16^{de}	10.15±1.60^{i-p}	24.41±1.16^{b-e}	218.67±24.57^{d-hi}	114.50±12.73^{d-j}	5.07±1.14^{b-g}	9.98±1.60^{b-d}	300.17±34.08^{b-g}	1.74±0.14^{d-h}
GMT0	2	49.33±2.17^{b}	17.92±1.94^{ef}	22.90±1.05^{c-g}	224.50±45.57^{d-h}	142.83±21.67^{a-d}	10.67±1.72^{a}	16.75±1.88^{a}	430.00±55.30^{a}	2.14±0.13^{b}
GMT1	2	38.72±3.99^{cd}	11.38±1.65^{i-o}	25.72±2.78^{b-e}	376.33±26.54^{a}	180.00±10.46^{a}	6.65±1.12^{bc}	11.38±1.65^{b}	429.67±28.55^{a}	1.83±0.06^{c-g}
GET0	2	36.00±1.79^{d-f}	9.90±1.05^{i-p}	23.01±2.39^{c-g}	326.00±15.57^{a-c}	159.33±7.91^{a-c}	5.38±0.44^{b-g}	9.68±0.98^{b-d}	415.00±21.55^{ab}	1.73±0.08^{d-i}
GET1	2	32.67±1.15^{d-h}	11.43±1.03^{i-n}	21.78±1.12^{d-h}	216.33±22.24^{d-i}	111.83±9.41^{d-k}	5.97±0.65^{bcd}	11.40±1.01^{b}	349.17±11.05^{a-d}	1.72±0.09^{d-j}
GFT0	2	45.00±6.39^{bc}	8.57±1.93^{j-p}	26.11±3.60^{b-d}	227.50±27.89^{d-h}	115.83±13.61^{d-i}	5.90±1.10^{bcd}	10.23±1.43^{bc}	310.50±55.90^{b-f}	1.69±0.11^{e-j}
GFT1	2	30.75±2.08^{e-j}	9.23±0.94^{i-p}	22.19±0.78^{d-h}	247.33±36.44^{c-g}	124.17±18.06^{c-h}	5.53±0.70^{b-f}	9.33±1.06^{b-e}	293.83±45.76^{c-g}	1.62±0.07^{g-j}
PET0	2	39.17±3.08^{cd}	5.38±1.13^{op}	39.15±3.25^{a}	80.00±11.49^{m}	60.00±8.69^{mn}	4.33±0.58^{c-j}	4.53±1.16^{i-m}	125.17±27.02^{j}	1.55±0.07^{g-k}
PET1	2	35.17±2.10^{d-f}	7.82±1.42^{j-p}	29.97±3.74^{bc}	151.17±15.51^{h-m}	77.83±7.85^{i-n}	4.87±0.47^{b-h}	6.97±0.64^{d-k}	133.67±29.52^{j}	1.64±0.07^{g-j}
PFT0	2	30.75±4.72^{e-j}	5.10±1.28^{p}	23.42±2.28^{b-f}	157.17±46.75^{h-m}	93.33±21.60^{f-m}	3.15±0.65^{f-j}	4.87±1.51^{i-m}	282.83±76.03^{c-i}	1.457±0.10^{i-m}
PFT1	2	39.25±1.91^{cd}	6.00±1.23^{m-p}	29.77±5.16^{bc}	225.00±44.85^{d-h}	120.00±23.80^{c-h}	3.25±0.74^{e-j}	5.23±1.46^{h-m}	272.50±60.34^{c-i}	1.74±0.12^{d-h}
PM0T0	2	47.50±2.06^{b}	5.97±0.58^{m-p}	38.44±0.86^{a}	159.00±17.09^{h-m}	95.17±15.39^{e-m}	4.58±0.62^{c-i}	8.87±2.35^{b-f}	194.83±40.76^{g-j}	1.65±0.06^{g-j}
PM0T1	2	36.00±4.25^{d-f}	6.90±0.82^{l-p}	30.57±4.18^{b}	181.00±32.21^{f-k}	105.33±15.56^{d-l}	5.33±1.00^{b-g}	8.38±1.19^{b-h}	198.83±51.41^{f-j}	1.45±0.14^{j-m}

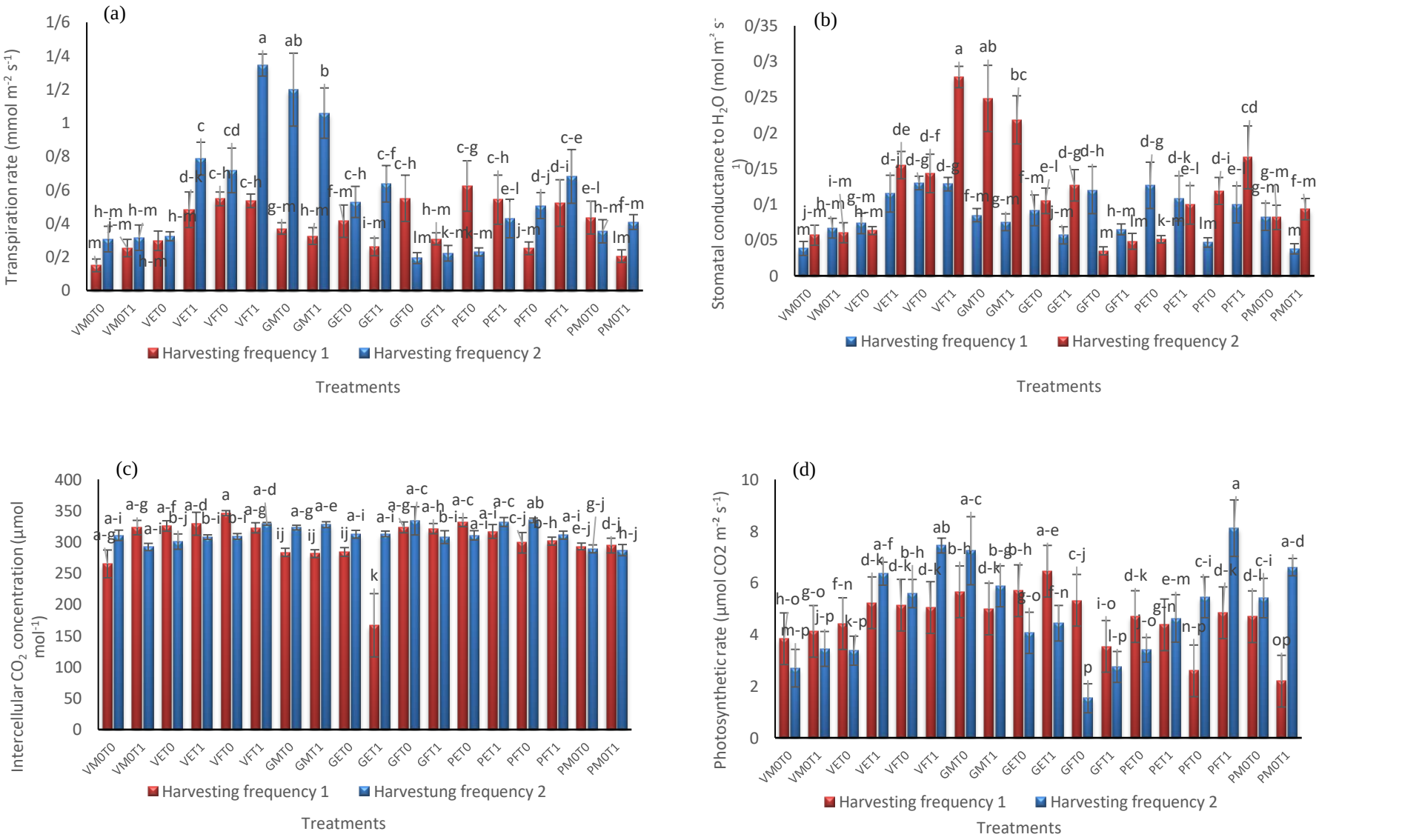

Fig. 1. Effect of treatments on photosynthetic parameters: (**a**) Transpiration rate; (**b**) Stomatal conductance to H_2O; (**c**) Intercellular CO_2 concentration; and (**d**) Photosynthetic rate.

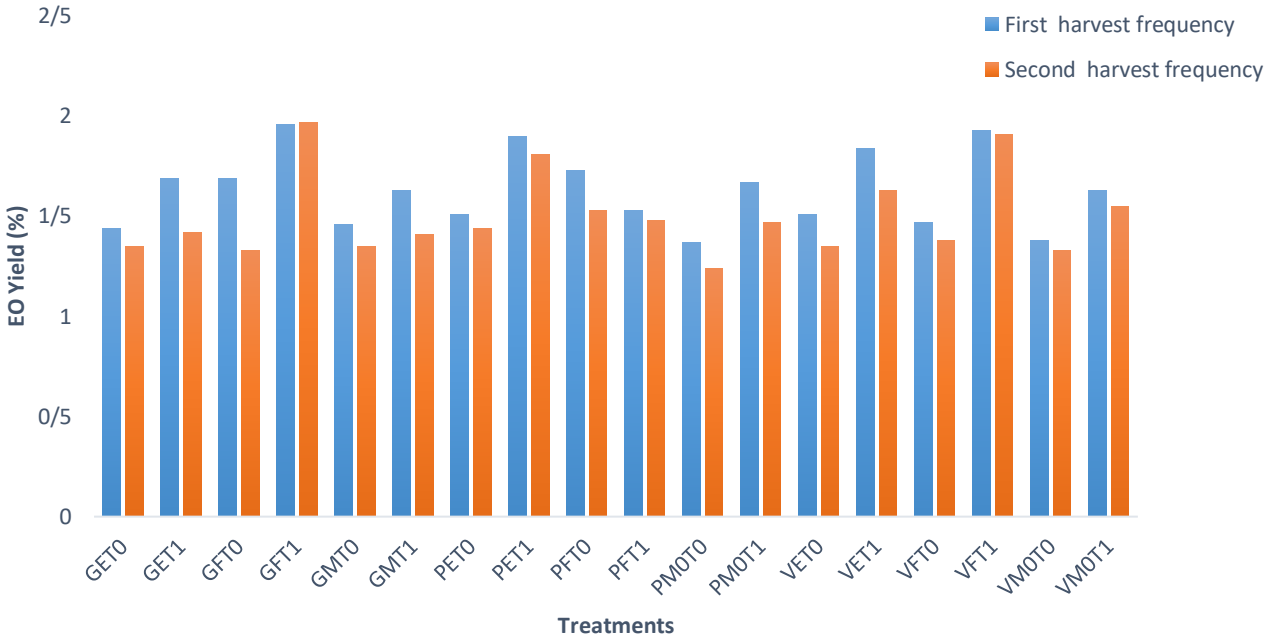

Fig. 2. Effect of various treatments on EO yield of spearmint and peppermint.

Table 4. Spearmint EOs constituents.

	Compounds	RI	VM0T0	VM0T1	VET0	VET1	VFT0	VFT1	GMT0	GMT1	GET0	GET1	GFT0	GFT1
1	α-Pinene	936	0.6	0.46	0.6	0.6	0.59	0.59	0.56	0.6	0.55	0.56	0.6	0.7
2	Camphene	954	0.15	0.18	0.11	0.15	0.17	0.18	0.15	0.14	0.15	0.18	0.15	0.16
3	Sabinene	976	0.65	0.7	0.69	0.71	0.7	0.7	0.7	0.45	0.67	0.65	0.69	0.68
4	β-Pinene	984	1.15	0.75	0.96	1.01	0.95	0.98	0.75	1.15	1	0.8	0.8	0.9
5	Myrcene	990	2.1	0.69	0.69	0.71	0.7	0.71	0.69	0.7	0.75	0.68	0.69	0.7
6	3-Octanol	999	0.25	0.4	0.27	0.4	0.42	0.3	0.4	0.39	0.34	0.43	0.4	0.13
7	p-Cymene	1029	0.1	0.11	0.11	0.1	0.13	0.1	0.11	0.11	0.1	0.1	0.12	0.09
8	Limonene	1035	18	19	18	18	17	19	18	19.21	18.46	18	20	20
9	1,8-Cineole	1039	3.9	4.9	4.21	4	1.4	5.01	6	3.85	5.07	5.7	3.6	4.2
10	*trans*-β-Ocimene	1047	0.2	0	0	0	0	0	0	0	0	0	0	0
11	Menthone	1164	4.4	5.3	1.3	3	5.3	5.3	1.3	3	5.3	5.3	5.3	3
12	Isomenthone	1174	0.42	0	0	0	0	0	0	0	0	0	0	0
13	Borneol	1182	0.7	0.6	0.6	0.7	0.6	0.6	0.7	0.5	0.6	0.6	0.7	0.5
14	Neoisomenthol	1208	2.96	3.2	3.2	3.1	3.1	3.2	3	3	3.2	3.2	3.1	3.12
15	*trans*-Dihydrocarveol	1215	0.3	0	0	0	0	0	0	0	0	0	0	0
16	*cis*-Carveol	1230	1.6	0.9	0.9	0.9	0.8	0.9	0.9	0.8	0.8	0.8	0.9	0.8
17	Phellandrene	1249	8.1	5.3	6.3	4.9	7.8	9.2	8.5	8.78	1.9	4.6	7.46	5.67
18	Carvone	1260	47	48	48	54	48	49	49	72.49		47	48	54
19	Piperitone	1265	0.03	0.4	0.5	0.5	0.54	0.4	0.4	0.54	0.4	0.5	0.5	0.4
20	Isodihydrocarveyl acetate	1328	0.2	0	0	0	0	0	0	0	0	0	0	0
21	Piperitone	1348	0.64	0.3	0.3	0.27	0.3	0.3	0.3	0.3	0.27	0.3	0.3	0.3
22	β-Bourbonene	1388	0.73	0.22	0.2	0.2	0.1	0.2	0.22	0.1	0.25	0.27	0.2	0.2
23	β-Elemene	2012	0.37	0.28	0.25	0.2	0.2	0.2	0.25	0.25	0.2	0.2	0.25	0.2
24	*cis*-Jasmone	1400	0.2	0.05	0.05	0.01	0.05	0.05	0.01	0.05	0.05	0.01	0.04	0.05
25	Transcaryophyllene	1425	2.5	1.68	1.94	1.35	1.64	1.76	1.25	1.34	0.9	1.1	1	0.7

26	*trans*-β-Farnesene	1453	0.25	0.1	0.1	0.12	0.1	0.15	0.15	0.15	0.1	0.12	0.11	0.15
27	α-Humulene	1461	0.34	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.2	0.3	0.2	0.3
28	Germacrene D	1486	1.36	1.9	1.2	2	1.9	1.9	1.9	1.2	2	2	9.1	1.2
29	Bicyclogermacrene	1500	0.4	0.1	0.11	0.1	0.15	0.15	0.1	0.1	0.1	0.12	0.1	0.1
30	*trans*-Calamenene	1526	0.1	0	0	0	0	0	0	0	0	0	0	0
	Total		98.36	94.01	93.51	97.04	93.81	99.36	95.05	96.53	99.96	94.09	95.59	98.96

Table 5. Peppermint EOs constituents.

	Compounds	**RI**	**PET0**	**PET1**	**PFT0**	**PFT1**	**PM0T0**	**PM0T1**
1	α-Pinene	936	0.55	0.7	0.54	0.67	0.5	0.58
2	Sabinene	976	0.38	0.2	0.62	0.6	0.57	0.6
3	β-Pinene	984	0.8	0.9	0.15	0.16	0.17	0.18
4	β-Myrcene	990	0.32	0.45	0.28	0.68	0.6	0.71
5	3-Octanol	999	0.29	0.3	0.76	0.44	1.12	1.02
6	Phellandrene	1002	0.06	0	0.7	0.07	0.69	0.71
7	α-Terpinene	1014	0.19	0.3	0.37	0.13	0.27	0.4
8	p-Cymene	1022	0.25	0.1	0.08	0.09	0.19	0.13
9	1,8-Cineole	1026	3.75	2.9	7.99	9.5	2.36	1.78
10	Limonene	1024	3.29	3.01	3.9	4.2	5.4	6.1
11	E-β-Ocimene	1044	0.2	0.01	0.03	0.04	0	0.02
12	Linalool	1095	0.39	0.12	0.5	0.3	3.6	3
13	L-Menthone	1148	28.9	32.1	30.08	34.9	31.68	24.06
14	Menthofuran	1159	1.9	0.4	0.7	0.5	0.7	0.6
15	Neomenthol	1161	1.03	0.6	1.2	2.12	3.15	2.95
16	Menthol	1167	36.9	40.25	33.99	26.84	28.87	38.78
17	Dihydrocarveol	1192	0.17	0.1	0.8	0.9	0.18	0.9
18	Carvone	1239	3.82	3.11	4.54	5.67	7.37	7.3
19	Hexenyl isovalerate	1243	0.57	0.22	0.25	0.59	0.56	0.25
20	Piperitone	1249	0.76	0.31	0.4	0.4	0.54	0.4
21	Hexenyl valerate	1279	0.06	0	0	0.09	0.08	0
22	Linalyl acetate	1282	1.26	1.11	0.25	0.3	0.27	0.3
23	Menthyl acetate	1294	4.54	5.01	0.1	5.2	0.22	0.2
24	Dihydrocarveol acetate	1306	0.14	0	0.2	0.3	0.3	0.35
25	β-Bourbonene	1388	0.33	0.5	0.04	0.05	0.01	0.04
26	trans-Caryophyllene	1425	1.61	1.25	1.78	0.7	1.27	2.1
27	*trans*-β-Farnesene	1453	0.24	0.12	0.1	0.25	0.12	0.11
28	Germacrene D	1387	1.3	1.5	0.2	1.5	1.9	0.3
29	Bicyclogermacrene	1500	0.27	0.3	0.1	2.1	0.7	1.8
30	γ-Cadinene	1518	0.06	0	0.12	0.1	0.1	0.1
31	*trans*-Calamenene	1526	0.2	0.3	0	0.4	0	0
	Total		94.53	96.17	90.77	99.79	92.99	95.77

Discussion

Numerous studies have examined the effects of introducing fungi to plants, demonstrating that plant-fungal partnerships can be highly beneficial. These partnerships can enhance morphological and physiological characteristics by boosting root growth and nutrient uptake, controlling pathogens or removing growth inhibitors from the soil, and inducing plant hormone production (Waheed et al., 2020). For example, the inoculation of Trichoderma fungi, rhizobium fungus, and mycorrhizal fungi (MF) in *Vigna radiata* improved leaf area and total soluble protein under drought stress (Kaur and Kumar, 2020). Similar improvements in growth and productivity, as well as reduced nematode population and penetration rates, were observed in tomato plants inoculated with *T. harzianum* and MF (Nafady et al., 2022). Due to the key roles of Trichoderma fungi, MF, and endophytic fungi in nematode control, they are also recognized as bio-control agents against plant-parasitic nematodes (Poveda et al., 2020). These enhancements are associated with increased phytohormone synthesis (e.g., auxins, cytokinins), improved phosphorus and nitrogen uptake, and enhanced root architecture, which collectively stimulate shoot growth and metabolic activity (Xia et al., 2023; Peng et al., 2024).It has been established that MF can boost photosynthesis in plants by reducing salt toxicity and improving nutrient absorption by roots. This leads to increased production of essential elements for chlorophyll synthesis, better light use efficiency, and more efficient photosynthetic processes, which help prevent damage to photosystem II (Peng et al., 2024). Additionally, MF modulates the source-sink relationship by enhancing nutrient exchange with the host, ultimately stimulating photosynthesis (Xia et al., 2023). MF has been shown to increase chlorophyll a, chlorophyll b, and total chlorophyll content in alfalfa and cotton leaves compared to non-inoculated samples, leading to increased photosynthetic rate, stomatal conductance, transpiration rate, and light energy utilization efficiency (Xia et al., 2023; Peng et al., 2024). Our findings are consistent with these observations, suggesting that fungal inoculation contributes to enhanced gas exchange and light capture in mint species.

Recent studies have confirmed that species like TF can protect tomato plants under stress from pathogens that limit the plant's ability to complete photosynthesis. In 2019, researchers showed that tomato plants exposed to a genetically engineered strain of *Trichoderma atroviride* were better able to handle the fungal pathogen *Verticillium dahliae* compared to the control group. Photosynthetic results revealed that samples treated with the genetically modified TF achieved heightened levels of chlorophyll content, increased rates of photosynthesis, and an improved state of photosystem II compared to untreated plants (Yao et al., 2023). Following this, a 2020 study investigated the impact of different TF species on tomato plants facing the bacterial pathogen *Pseudomonas syringae*. Inoculations with three different TF species—*T. harzianum*, *T. asperellum*, and *T. virens*—showed that treatment with *Trichoderma* distinctly improved photosynthesis, similar to the previous research. Treated plants exhibited chlorophyll content, enhanced photosynthesis rates, and increased stomatal conductance (Ferreira et al., 2024). These physiological improvements may be attributed to the modulation of antioxidant enzyme activity and reactive oxygen species detoxification, which contribute to better photosynthetic efficiency and stress resilience.

The symbiotic relationship between MF and plants can trigger defense responses, leading to the upregulation of defense mechanisms and an increase in the biosynthesis of secondary metabolites as a protective response. MF can also alter the expression of genes involved in the biosynthesis of secondary metabolites and EOs (Yuan et al., 2023). This transcriptional regulation is increasingly supported by omics-based studies that highlight the activation of terpene synthase genes and precursor pathways following fungal colonization.

Recent studies have shown that arbuscular mycorrhizal fungi (AMF) inoculation can significantly increase the EO yield of various aromatic plants. For instance, a 2020 study demonstrated that inoculation with MF and phosphorus increased the EO yield of *Plectranthus amboinicus* Lour. (Merlin et al., 2020). Additionally, combining MF with other beneficial microbes, such as nitrogen-fixing bacteria, can further enhance EO yield. A 2023 study found that the EO yield of *Dracocephalum moldavica* L. increased by 21.9% when inoculated with MF and nitrogen-fixing bacteria compared to chemical fertilizer alone (Amiriyan Chelan et al., 2023). Such synergistic microbial interactions may optimize both primary metabolism (for growth) and secondary metabolism (for EO biosynthesis), offering a sustainable alternative to chemical inputs.

While the beneficial effects of fungi inoculation on plants are well-documented, the extent of these effects can vary depending on the species. Different mint varieties may exhibit distinct growth responses and EO compositions following inoculation (Javanmard et al., 2022; Melato et al., 2024). This genotype-dependent variability highlights the importance of matching specific fungal strains to compatible host genotypes to achieve optimal outcomes in terms of EO quantity and quality.

MF inoculation has also been shown to affect EO content and composition in other plants, such as sage

(*Salvia officinalis*) (Ostadi et al., 2022), lemongrass (*Cymbopogon citratus*) (de Souza et al., 2022), and basil (*Ocimum basilicum* L.) (Yilmaz and Karik, 2022). A 2020 study reported that the combination of MF, growth-promoting bacteria, and mulch from plant residues increased anise EO production by 55% compared to the control (Ebrahimi et al., 2023). Our results align with these findings, suggesting that microbial inoculation can serve as a practical tool to tailor the phytochemical profile of mint for specific therapeutic or commercial goals.

The findings highlight that the content of peppermint's key compounds, such as menthol and menthone, which are crucial for its aromatic and therapeutic properties, can be significantly influenced by MF inoculation (Abdi-Moghadam et al., 2023). Additionally, previous studies corroborate that fungal inoculation can not only enhance EO yield but also modify the phytochemical profile, potentially improving bioactivity for applications in aromatherapy and natural pest control (Melato et al., 2024). This dual impact—on yield and composition—underscores the relevance of bio-inoculants in functional crop enhancement, especially within organic and low-input agricultural systems.

Conclusion

This study has demonstrated the significant role of Mycorrhizal Fungi (MF) and Trichoderma Fungi (TF) in enhancing the growth, physiological traits, and phytochemical properties of spearmint and peppermint. The application of *Glomus etunicatum*, *G. fasciculatum*, and *Trichoderma harzianum* not only improved EO yields but also influenced key constituents like limonene, carvone, menthol, and L-menthone, crucial for the commercial value of these plants. The findings suggest that MF and TF can effectively enhance photosynthesis, nutrient absorption, and overall plant health, making them valuable tools for sustainable agriculture.

Significant variations were observed across different treatments, with GFT1 and VFT1 yielding the highest EO concentrations and PET1 and PFT1 showing the highest levels of menthol and L-menthone. Moreover, the study highlighted the importance of specific fungal strains in achieving optimal results, emphasizing the need for tailored approaches depending on the target phytochemical outcomes and environmental conditions.

However, it is important to note certain limitations. First, the study was conducted under greenhouse conditions, which may not fully replicate field-level environmental variability. Second, the inoculated microbial consortia were not characterized at the community level, limiting insights into competitive or synergistic microbial dynamics. These factors should be considered when extrapolating the findings to open-field cultivation scenarios.

Future research should expand on these findings by exploring a wider range of MF and TF strains, as well as their effects on other bioactive compounds such as phenolics. In particular, integrating metabolomic and transcriptomic profiling could provide deeper insights into the regulatory pathways driving EO biosynthesis under fungal symbiosis. Additionally, studies investigating host–fungal genotype compatibility and long-term effects under field conditions would be valuable for optimizing strain selection and agronomic practices. Economic feasibility studies and microbial ecology analyses would also enhance the practical relevance of MF and TF applications.

This research provides a foundation for further exploration into the potential of MF and TF to revolutionize the cultivation of mint and other EO-producing plants.

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Author Contributions

Conceptualization and Supervision, ME; Methodology: ME and MA; Investigation, Writing - original draft: SH; Writing - review and editing, SH, ME, and MA; Data collection: S H; Data analysis: SH, ME, and MA; Funding acquisition and Resources: ME. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no competing interests.

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