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Research article

Bioprospecting of lemon balm (*Melissa officinalis* L.) inoculated with mycorrhiza under different rates of phosphorus for sustainable essential oil production

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Abstract: This study aimed to evaluate the yield and chemical composition of essential oil (EO) and the growth parameters of *Melissa officinalis* L. (lemon balm) inoculated with arbuscular mycorrhizal fungi (AMF) under different rates of phosphorus (P). Treatments comprised a high and low P rate combined or not with inoculation of *Rhizophagus clarus* (C. Walker & A. Schüßler) or *Claroideoglomus etunicatum* (C. Walker & A. Schüßler), arranged according to a 2 × 3 factorial design with 8 replications. At 4 months after transplanting, shoot fresh and dry weights increased in both AMF-inoculated treatments under a low P rate. There was an increase in shoot P content with *C. etunicatum* inoculation under a high P rate. EO yields ranged from 0.04% to 0.12% and increased with *R. clarus* inoculation. Eighteen compounds were identified in EOs. The major components were geranial (43.96%–54.93%), neral (29.95%–34.66%), geraniol (3.11%–12.85%), and (*E*)-caryophyllene (2.62%–6.66%). It was concluded that AMF inoculation increased plant growth, improved EO yield, and modified EO composition. It is recommended to inoculate lemon balm with *R. clarus* under low P rates.

Keywords: citral; medical plants; symbiosis; sustainability; terpenes

Abbreviations: AMF: Arbuscular mycorrhizal fungi; BSR: basal soil respiration; EO: essential oil; GC/MS: gas chromatography/mass spectrometry; MBC: microbial biomass carbon; N: nitrogen; P: phosphorus; PCA: principal component analysis; qCO_2: metabolic quotient; RDW: root dry weight; SFW: shoot fresh weight; SDW: shoot dry weight; TDW: total dry weight

1. Introduction

Medicinal, aromatic, and spice plants have fundamental value for humanity, having been widely used in traditional medicine for the treatment of diseases [1,2]. These plants are rich in essential oils (EOs) with great economic and industrial potential [3].

Melissa officinalis L. is an aromatic medicinal plant belonging to the family Lamiaceae. Native to Europe, the plant is commonly known as lemon balm, in allusion to its lemon-like odor stemming from the presence of volatile oils [4]. The aerial parts of lemon balm are consumed as herbal tea, prepared by infusion. This method prevents the decomposition of active compounds, particularly EO components [3].

Lemon balm has been used for the treatment of a multitude of diseases, with some reports dating back to more than 2,000 years [5]. In traditional medicine, the species is used to treat mental disorders such as anxiety and depression, as well as for memory improvement and prevention of heart disease. Such effects are attributed to the presence of flavonoids, alkaloids, polyphenols, and other products of secondary metabolism [3,6]. Lemon balm EO has high commercial value, as it finds application in the pharmaceutical, cosmetic, and food industries [7].

Studies indicated that lemon balm EO may be a promising antimicrobial agent [3,8]. In a clinical study [9], the EO was effective in preventing insulin resistance and obesity. Several pharmacological properties have been reported for lemon balm EO, including anxiolytic, antidepressant, neuroprotective, cardiovascular protective, cytotoxic, anti-inflammatory, and antioxidant, among others [3,5].

Given its several applications and widespread use, lemon balm should ideally be produced in sustainable systems. An interesting strategy is to apply arbuscular mycorrhizal fungi (AMF), organisms that form mutualistic symbiotic associations with the roots of most plant species. Such relationships favor plant growth and development by attenuating biotic and abiotic stresses, increasing the absorption of water and nutrients such as phosphorus (P) by plant roots, and enhancing secondary metabolism, ultimately improving EO production [10–16].

Assis et al. [17] investigated the inoculation of AMF together with organic fertilization in the cultivation of *M. officinalis* and identified that the chemical characteristics of the soil, such as P content, interfere with the colonization of roots by AMF. In addition, the combination of these practices increased the production of biomass and the active ingredient of the plants. Merlin et al. [18] also observed that inoculation with *R. clarus* and high phosphorus content in the cultivation of *Plectranthus amboinicus* (coarse mint) increased the dry matter content, however the extracted essential oil content was higher with *R. clarus* and low phosphorus.

The EO content of lemon balm ranges from 0.05% to 0.52%, which is low compared to that of other members of the family Lamiaceae [19]. In light of the growing industrial and consumer demands

for EOs, AMF inoculation has been proposed as a means of enhancing biomass production, increasing EO yield, and improving the chemical composition of medicinal and aromatic plants [13,17]. In addition to being an environmentally friendly practice, AMF treatment stimulates plant growth without compromising the quality of the plant or its EO [12,17,18–20].

Considering that AMF inoculation may improve plant growth, EO production, and EO quality, this study aimed to investigate the EO yield and chemical composition, as well as vegetative growth parameters, of lemon balm inoculated with *Rhizophagus clarus* or *Claroideoglomus etunicatum* under different P rates.

2. Materials and methods

2.1. Experimental design

The experiment was carried out at the Medicinal Garden (23°46′09.1″S, 53°16′38.4″W) of Paranaense University (UNIPAR), located in Umuarama, Paraná, Brazil. A mixture containing 50% substrate and 50% sand + vermiculite (1:1) was fumigated with 10 mL chloroform ($CHCl_3$) kg^{-1}, according to a method adapted Endlweber and Scheu [21]. The experimental design was completely randomized with 8 replications per treatment arranged in a 3 × 2 factorial (inoculation or not of *R. clarus* or *C. etunicatum* at high or low soil P levels), totaling 48 experimental units. Cuttings of lemon balm measuring about 5 cm in length were planted in 3.5 dm^{-3} pots and grown for 4 months.

2.2. Treatments

Inoculated treatments received approximately 200 spores of *R. clarus* or *C. etunicatum*, which were obtained from the Bank of Glomales of UNIPAR. For the pots assigned to the high P rate treatment, 0.88 g of monopotassium phosphate (KH_2PO_4) was added per kilogram of substrate, equivalent to 200 mg of P kg^{-1} of substrate, however the solution also contains about 253 mg of potassium (K) kg^{-1} of substrate Therefore, in the pots assigned to a low P rate were treated with 0.38 g of potassium chloride (KCl), corresponding to 199 mg of K kg^{-1} substrate. Thus, all treatments had approximately the same soil K content (at low and high P rate), differing only in P levels, according to the method described by Urcoviche et al. [22]. Plants were irrigated every two days (or as needed) with a modified P-free Hoagland and Arnon [23] nutrient solution.

2.3. Chlorophyll index and dry weight determination

Chlorophyll index were measured at the center of three leaves per plant using a chlorophyll meter (CFL 1030, ClorofiLOG®). When they complete their vegetative cycle, after 4 months of planting samples were dried in a forced-air oven at 65 °C for 24 h. Then, shoot dry weight, root dry weight, and total dry weight were quantified by weighing the samples on a semi-analytical scale.

2.4. Determination of shoot P and nitrogen (N) contents

Dried plant shoots were ground and subjected to determination of P and N [24,25]. The P was estimated by colorimetry with ammonium molybdate + ascorbic acid in a spectrophotometer, using a

red filter and a wavelength of 660 nm [24]. N content was determined by sulfuric acid digestion at 450 ℃, followed by the Kjeldahl method of distillation and titration with 0.05 mol·L^{-1} HCl [23,24].

2.5. Spore density and AMF colonization

AMF spores were extracted from 10 g subsamples of substrate by wet sieving (0.710–0.053 mm mesh) [26], and transferred to Petri dishes for counting under a stereoscopic microscope (40 × magnification) [15]. For determination of colonization percentage, fine roots were collected, bleached, stained, and acidified with trypan blue, according to the method of Phillips and Hayman [27]. The number of colonized root segments was counted using slides overlaid with coverslips [28]. In total, about 100 segments were numbered and counted using a stereoscopic microscope (40–100 × magnification) [15].

2.6. Determination of microbial biomass carbon, basal respiration, and metabolic quotient

Soil microbial biomass carbon (MBC) was determined by the fumigation-extraction method proposed by Vance et al. [29] and modified by Tate et al. [30]. Extracted MBC was estimated according to Lermen et al. [25] and Ferrari et al. [15]. Basal soil respiration (BSR) was determined according to Jenkinson and Powlson [31], using 30 g of soil sample. According to Anderson and Domsch [32], metabolic quotient (qCO_2) was estimated as the ratio of BSR to MBC.

2.7. EO extraction and chemical characterization

EO extraction, yield determination (%, g 100 g^{-1} fresh shoots), and chemical identification by gas chromatography/mass spectrometry (GC/MS) were performed according to Cruz et al. [33].

2.8. Statistical analysis

Data were subjected to analysis of variance (ANOVA). Means were compared by Duncan's test ($p \leq 0.05$) using SPSS for Windows version 22.0 (SPSS Inc. Chicago, IL, USA). Hierarchical clustering and principal component analysis (PCA) were performed using Statistica software version 13.0 Statsoft [34] to discriminate EO composition according to treatment.

3. Results

Shoot fresh and dry weights increased significantly with *R. clarus* and *C. etunicatum* inoculation and low P rate (Table 1). *R. clarus* inoculation and low P rate led to an increase in root dry weight and total dry weight. In the control treatment with low P, were observed a significant decrease in shoot fresh weight, shoot dry weight, root dry weight, and total dry weight (Table 1).

Shoot N and chlorophyll index did not differ significantly among the treatments. *R. clarus* + low P and *C. etunicatum* + high P increased significant P content in shoot (Table 2).

Spore density and root colonization were significantly higher in treatments with AMF inoculation, particularly under low P rates (Table 3). MBC was higher with AMF inoculation, regardless of P rates (Table 3). *C. etunicatum* inoculation combined with high P rate led to a significant

increase in BSR (Table 3). qCO_2 decreased significantly with AMF inoculation compared with the controls (Table 3).

Table 1. Shoot fresh weight (SFW, g·plant^{-1}), shoot dry weight (SDW, g·plant^{-1}), root dry weight (RDW, g·plant^{-1}), total dry weight (TDW, g·plant^{-1}) of lemon balm inoculated with arbuscular mycorrhizal fungi (*Rhizophagus clarus* or *Claroideoglomus etunicatum*) under low and high phosphorus rates.

Treatment	SFW	SDW	SDW	TDW
T1	12.28 ± 0.92c	2.85 ± 0.21c	0.68 ± 0.05c	3.53 ± 0.23c
T2	24.57 ± 1.62a	4.66 ± 0.40a	3.88 ± 0.67a	8.55 ± 0.71a
T3	24.57 ± 1.62a	4.25 ± 0.31ab	2.15 ± 0.57b	6.41 ± 0.70b
T4	18.59 ± 1.12b	3.41 ± 0.24bc	1.55 ± 0.15bc	4.97 ± 0.36bc
T5	19.56 ± 0.63b	3.63 ± 0.21bc	1.43 ± 0.27bc	5.06 ± 0.40bc
T6	15.99 ± 1.57bc	3.55 ± 0.34bc	2.39 ± 0.30b	5.94 ± 0.57b
p-value	< 0.001	0.002	< 0.001	< 0.001

Notes: Mean values (n = 8 ± standard error). Means within columns followed by the same letter are not significantly different by Duncan's test ($p \leq 0.05$). T1: uninoculated control under low P rate; T2: *R. clarus* under low P rate; T3: *C. etunicatum* under low P rate; T4: uninoculated control under high P rate; T5: *R. clarus* under high P rate; T6: *C. etunicatum* under high P rate.

Table 2. Chlorophyll index shoot nitrogen content (mg·g^{-1}), and shoot phosphorus content (mg·g^{-1}) of lemon balm inoculated with arbuscular mycorrhizal fungi (*Rhizophagus clarus* or *Claroideoglomus etunicatum*) under low and high phosphorus rates.

Treatment	Chlorophyll index	Shoot N content	Shoot P content
T1	26.28 ± 0.77a	33.87 ± 1.86a	1.56 ± 0.03b
T2	26.16 ± 0.69a	39.75 ± 3.27a	2.21 ± 0.03a
T3	26.27 ± 0.87a	40.06 ± 2.59a	1.42 ± 0.10b
T4	26.95 ± 0.68a	40.82 ± 2.35a	2.27 ± 0.08a
T5	26.64 ± 0.75a	40.36 ± 2.66a	1.57 ± 0.03b
T6	26.82 ± 0.74a	35.07 ± 1.41a	2.33 ± 0.07a
p-value	0.966	0.196	< 0.001

Notes: Mean values (n = 8 ± standard error). Means within columns followed by the same letter are not significantly different by Duncan's test ($p \leq 0.05$). T1: uninoculated control under low P rate; T2: *R. clarus* under low P rate; T3: *C. etunicatum* under low P rate; T4: uninoculated control under high P rate; T5: *R. clarus* under high P rate; T6: *C. etunicatum* under high P rate.

Table 3. Spore density (spores g^{-1} dry soil) and root colonization (%) by arbuscular mycorrhizal fungi, microbial biomass carbon (MBC, μg $CO_2 \cdot g^{-1}$), basal soil respiration (BSR, μg CO_2-$C \cdot g^{-1} \cdot h^{-1}$), and soil metabolic quotient (qCO_2, μg $CO_2 \cdot \mu g^{-1} \cdot C_{mic}$ h^{-1}) for lemon balm inoculated with *Rhizophagus clarus* or *Claroideoglomus etunicatum* under low and high phosphorus rates.

Treatment	Spore density	Root colonization	MBC	BSR	qCO_2
T1	0.05 ± 0.01c	1.62 ± 0.32c	88.57 ± 6.14b	0.94 ± 0.10ab	11.09 ± 1.56a
T2	1.94 ± 0.39b	63.86 ± 6.92ab	129.12 ± 8.81a	0.65 ± 0.11b	5.45 ± 1.22b
T3	3.48 ± 0.39a	72.64 ± 3.07a	129.22 ± 5.86a	0.81 ± 0.06ab	6.33 ± 0.55b
T4	0.05 ± 0.02c	1.87 ± 0.29c	80.88 ± 6.49b	0.76 ± 0.08ab	10.24 ± 1.81a
T5	2.06 ± 0.35b	55.66 ± 6.00b	148.79 ± 10.55a	0.97 ± 0.15ab	6.53 ± 0.91b
T6	2.40 ± 0.34b	67.05 ± 5.39ab	133.24 ± 4.67a	1.04 ± 0.07a	7.85 ± 0.51ab
p-value	< 0.001	< 0.001	< 0.001	0.046	0.009

Notes: Mean values (n = 8 ± standard error). Means within columns followed by the same letter are not significantly different by Duncan's test ($p \leq 0.05$). T1: uninoculated control under low P rate; T2: *R. clarus* under low P rate; T3: *C. etunicatum* under low P rate; T4: uninoculated control under high P rate; T5: *R. clarus* under high P rate; T6: *C. etunicatum* under high P rate.

EO yield ranged from 0.04% to 0.12% and was highest with *R. clarus* inoculation, regardless of P rates (Figure 1). In treatments inoculated with *C. etunicatum*, P rate did not significantly influence EO yield; nevertheless, the yields of *C. etunicatum*-inoculated plants were higher than those of the control.

GC/MS analysis revealed 18 chemical compounds in EOs (Table 4). The major compounds were geranial (43.96% to 54.93%), followed by neral (29.25% to 34.66%), geraniol (3.11% to 12.85%), and (*E*)-caryophyllene (2.62% to 6.66%).

Geranial and neral contents increased with AMF inoculation, regardless of P rates. However, the content of geraniol did not increase with inoculation, being higher in the control treatments, while (*E*)-caryophyllene increased with the inoculation of *C. etunicatum* under low P rate (Table 5). Data on EO composition (Table 5) were subjected to hierarchical cluster analysis to assess the variability in major compounds between treatments.

EO composition was grouped into two main groups (Figure 2) and plotted on a dendrogram obtained by the unweighted pair group method with arithmetic mean. The first cluster included plants inoculated with AMF and the second cluster comprised the controls.

PCA of EO data revealed two components (PCA1 and PCA2), which explained 82.50% and 16.47%, respectively, of the variance in chemical composition (Figure 3 and Table 5). As observed in the PCA loading biplot (Figure 3), control treatments (treatments 1 and 4) were clearly separated from treatments inoculated with AMF, corroborating the results of hierarchical clustering (Figure 2). Factor loadings demonstrated that geraniol was highly correlated with treatments 1 and 4 in PCA1 (plotted near −1). Citral, geranial, and neral were highly correlated with PC1 (plotted near +1). (*E*)-caryophyllene and treatment 3 were correlated with PCA2. Overall, the results of PCA underscore the effect of AMF inoculation (treatments 2, 3, 5 and 6) on the production of monoterpenes, such as geranial, which was lower in the controls (treatments 1 and 4) (Figure 3 and Table 5).

Table 4. Chemical composition (%) of essential oils from lemon balm inoculated with *Rhizophagus clarus* or *Claroideoglomus etunicatum* under low and high phosphorus rates.

Peak	Compound	RT literature	RT	T1	T2	T3	T4	T5	T6
1	*trans*-2-Hexenal	3.99	5.619	0.75	0.93	0.83	0.75	0.85	0.83
2	Methyl heptanoate	8.57	9.698	0.41	0.60	0.58	0.41	0.58	0.30
3	Citronellal	13.58	12.115	1.74	1.78	t	1.55	t	t
4	α-Cyclogeraniol	28.02	12.174	0.29	t	t	t	t	t
5	Linalool	11.32	13.743	0.96	0.83	0.97	0.96	0.55	0.90
6	Isocitral	14.82	16.597	0.42	0.55	1.51	0.42	1.76	t
7	Neral	17.32	18.763	29.25	34.62	32.48	29.25	34.66	34.66
8	Geraniol	17.95	19.286	12.85	3.11	3.28	12.85	3.51	3.31
9	Geranial	18.62	19.831	43.96	52.71	47.42	43.96	51.13	54.93
10	Linalool oxide acetate	19.64	20.091	t	0.79	t	t	t	t
11	Terpinyl acetate	20.22	20.222	1.19	0.64	1.53	1.19	0.58	1.24
12	Methyl geranate	21.19	21.458	0.49	0.77	0.80	0.49	0.87	0.58
13	2,6-Dimethyl-2,8-octanediol	22.83	22.049	t	t	0.38	t	t	t
14	8-Hydroxymenthol	21.44	23.245	t	t	0.54	t	0.57	t
15	(*E*)-caryophyllene	25.36	24.623	4.97	2.62	6.66	4.94	4.87	3.14
16	*ar*-Turmerone	35.51	31.982	1.53	t	t	2.23	T	t
17	Ledol	32,99	32.982	0.97	t	t	0.97	T	t
18	Farnesoic acid	40.98	40.991	t	t	1.00	t	T	t
	Citral (neral + geranial)			73.21	87.33	79.90	73.21	85.79	89.59
	Total identified			99.78	99.95	97.98	99.97	99.93	99.89

Notes: Compounds were identified by comparison of their retention indices and mass spectra with literature data [35]. RT: Retention time; T1: uninoculated control under low P rate; T2: *R. clarus* under low P rate; T3: *C. etunicatum* under low P rate; T4: uninoculated control under high P rate; T5: *R. clarus* under high P rate; T6: *C. etunicatum* under high P rate; t: traces.

Table 5. Major components (%) of essential oils from lemon balm inoculated with *Rhizophagus clarus* or *Claroideoglomus etunicatum* under low and high phosphorus rates.

Treatment	Neral	Geraniol	Geranial	(*E*)-caryophyllene	Citral (neral + geranial)	Yield
T1	29.25	12.85	43.96	4.97	73.21	0.065
T2	34.62	3.11	52.71	2.62	87.33	0.124
T3	32.48	3.28	47.42	6.66	79.9	0.072
T4	29.25	12.85	43.96	4.94	73.21	0.050
T5	34.66	3.51	51.13	4.87	85.79	0.121
T6	34.66	3.31	54.93	3.14	89.59	0.073
Literature, data from 32 articles						
Mean	26.07	2.21	37.65	5.14	62.35	0.32
SE	0.68	0.38	1.06	0.45	1.42	0.03
Range	4–38.9	0.12–19.16	4.6–63.7	0.3–45.07	8.6–92.46	0.008–1.2
n	144	75	153	146	142	114

Notes: T1: uninoculated control under low P rate; T2: *R. clarus* under low P rate; T3: *C. etunicatum* under low P rate; T4: uninoculated control under high P rate; T5: *R. clarus* under high P rate; T6: *C. etunicatum* under high P rate; SE: standard error; *n*: number of observations.

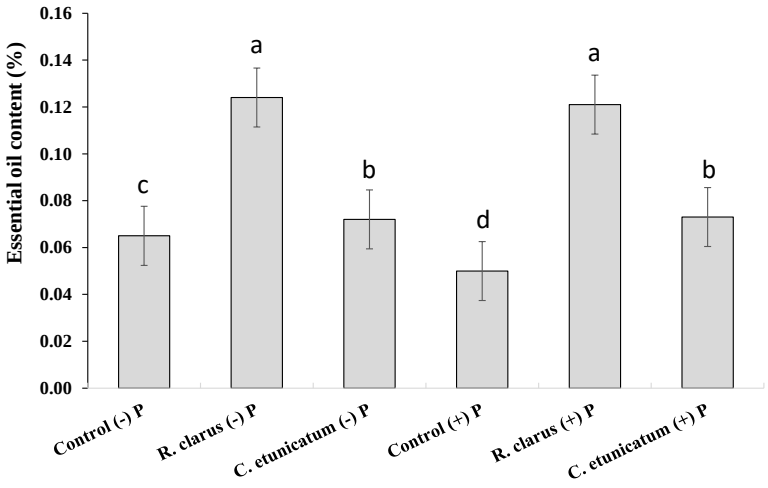

Figure 1. Essential oil content (%) of lemon balm inoculated with arbuscular mycorrhizal fungi (*Rhizophagus clarus* or *Claroideoglomus etunicatum*) under low (–) and high (+) phosphorus (P) rates.

Notes: Different letters indicate significant differences by Duncan's test ($p \leq 0.05$); Bars = standard error.

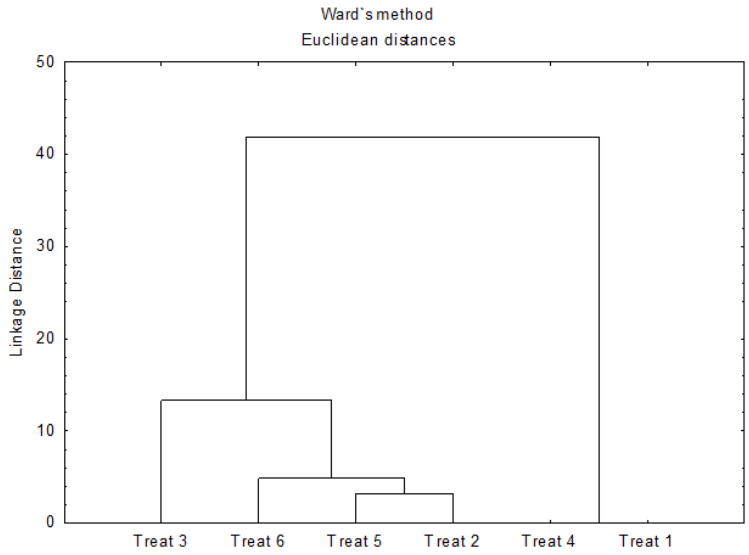

Figure 2. Hierarchical clustering dendrogram of essential oils of lemon balm inoculated with arbuscular mycorrhizal fungi (*Rhizophagus clarus* or *Claroideoglomus etunicatum*) under low and high phosphorus (P) rates.

Notes: Treat 1: uninoculated control under low P rate; Treat 2: *R. clarus* under low P rate; Treat 3: *C. etunicatum* under low P rate; Treat 4: uninoculated control under high P rate; Treat 5: *R. clarus* under high P rate; Treat 6: *C. etunicatum* under high P rate.

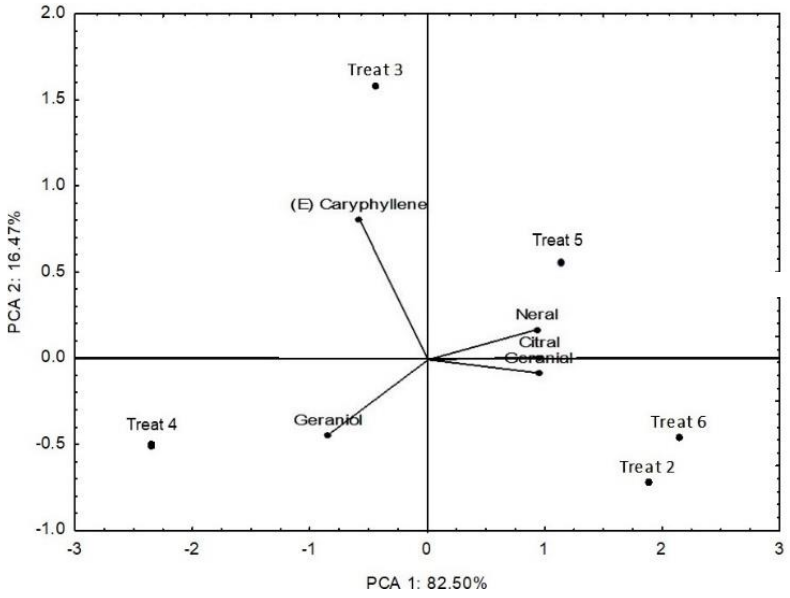

Figure 3. Principal component analysis (PCA) biplot for essential oils of lemon balm inoculated with arbuscular mycorrhizal fungi (*Rhizophagus clarus* or *Claroideoglomus etunicatum*) under low and high phosphorus (P) rates.

Notes: Treat 1: uninoculated control under low P rate; Treat 2: *R. clarus* under low P rate; Treat 3: *C. etunicatum* under low P rate; Treat 4: uninoculated control under high P rate; Treat 5: *R. clarus* under high P rate; Treat 6: *C. etunicatum* under high P rate.

4. Discussion

An increase in shoot fresh and dry weights was observed with *R. clarus* inoculation. Root dry weight and total dry weight increased with *R. clarus* inoculation and addition of P at a low rate. Salgado et al. [36] attributed an increase in root dry weight to *R. clarus* inoculation and enhanced nutritional status, leading to an overall improvement in plant development. Silva et al. [37] found similar results for total dry weight of tomato (*Solanum lycopersicum* L.) inoculated with *R. clarus*. AMF inoculation significantly increased the shoot dry weight of lemon balm plants in a study conducted by Shamizi et al. [38]. Engel et al. [1], however, observed no significant increase in shoot fresh or dry weight in lemon balm inoculated with AMF.

Addition of P did not stimulate biomass production. Similar results were reported by Carneiro et al. [39] for embauba (*Cecropia pachystachya* Trécul). The authors observed that AMF inoculation combined with absence of P_2O_5 enhanced shoot dry weight, given that, the higher the P rate, the lower the root colonization, leading to a decrease in plant growth [39]. By contrast, Urcoviche et al. [22] found that inoculation of *Mentha crispa* with AMF combined with high P rate increased shoot fresh weight.

An increase in shoot P content was observed with low P rate and *R. clarus* inoculation, as well as with *C. etunicatum* inoculation under high P rate. Lermen et al. [40], in assessing the effects of different P rates on *Cymbopogon citratus* (DC.) Stapf. (lemongrass), found similar root and shoot P contents in AMF-inoculated and uninoculated plants.

AMF inoculation combined with low P rate significantly increased spore density and root

colonization, which ranged from 55.66% to 72.64%. Engel et al. [1] observed a mean root colonization of 62% in lemon balm. Similar results were reported for *Plectranthus amboinicus* Lour. inoculated with *R. clarus* and *C*. *etunicatum* and treated with low P rates [20] and *Lippia alba* (Mill.) N.E.Br. [25].

P is essential for plant-soil-fungi interactions, which promote plant development and influence secondary metabolism [20,22,25]. However, addition of high P rates to the soil decreased spore density and root colonization by *R*. *clarus* and *C. etunicatum* (Table 3). Therefore, low P rates are sufficient to ensure good nutritional performance in plants inoculated with AMF.

MBC was higher in AMF-inoculated plants, demonstrating that inoculation favored plant development. High MBC rates are indicative of high absorption and storage of C, nutrients, and minerals in the soil [11,15,25]. Inoculation with *C*. *etunicatum* and high P rate increased BSR, resulting in greater loss of C to the atmosphere. As argued by Balota et al. [41], treatment of plants with high rates of inorganic nutrients, such as P, may lead to a reduction in soil C, possibly causing nutrient deficiencies.

qCO_2 decreased with AMF inoculation compared with the controls. This finding shows that AMF enhanced soil quality, given that the lower the qCO_2, the better the soil quality [15,42].

The EO yield (0.12%) of plants inoculated with *R*. *clarus* and treated with low P rates was below the average reported in the literature for lemon balm (0.32%). Even lower EO yields were observed in the controls, although values were within the range reported by Sodré et al. [43] and Szabó et al. [17], ranged from 0.07% to 0.3%. Cruz et al. [44], in studying *Salvia officinalis* L., and Merlin et al. [20], in studying *P. amboinicus*, observed that EO yield increased with *R*. *clarus* inoculation under both high and low P rates.

The main constituents of lemon balm EO are citral (geranial + neral), geranial, neral, geraniol, and (*E*)-caryophyllene (Table 5). Citral content generally ranges from 8.6% to 92.46%, with a mean of 62.35%; here, we obtained values of 73.21% to 89.59%, being slightly above the average (Table 5). Similar results were observed for geranial (43.96%–54.93%) and neral (29.25%–34.66%), whose contents were within ranges reported in the literature (Table 5) and in the studies of Sodré et al. [43], Szabó et al. [17], Abdellatif et al. [45], Silva et al. [46], Chrysargyris et al. [47], and Petrisor et al. [3]. Assis et al. [18] inoculated lemon balm with AMF and organic manure rates, but did not observe significant changes in geranial or neral contents with AMF inoculation. Karagiannidis et al. [19], in assessing the effects of AMF on oregano and mint development, found that inoculation promotes the growth of plants in soils with low fertility, minimizing the need for agricultural inputs and increasing EO production and quality.

The major EO component identified here was citral, as also observed in previous studies under different plant cultivation and processing methods [18,46] (Behbahani and Shahidi, 2019; Assis et al., 2020). Citral was the major compound of lemongrass EO [16,33]. This compound is responsible for the characteristic citric odor of lemon balm, very similar to that of lemongrass [48]. The cosmetic and perfume industries use citral in formulations of colognes, deodorants, body moisturizers, and soap. Citral is an important ingredient in the pharmaceutical industry, being used as a starting material for the production of ionone, vitamin A, and beta-carotene [5,45]. Its main pharmacological activities include antibacterial, antifungal and soothing effects [3,18,49].

5. Conclusions

AMF inoculation of lemon balm under low P rates increased shoot fresh and dry weights, soil

quality, and plant development. Furthermore, AMF inoculation enhanced root colonization and spore density.

EO yield ranged from 0.04% to 0.12% and increased with *R. clarus* inoculation. Eighteen compounds were identified in EOs, with the major compounds being geranial (43.96% to 54.93%), neral (29.95% to 34.66%), geraniol (3.11% to 12.85%), and (E)-caryophyllene (2.62% to 6.66%). On the basis of the results, it is recommended to inoculate lemon balm with *R. clarus* under low P conditions, being a sustainable, safe, and efficient strategy for farmers to reduce costs and increase yields.

Due to the increase in the global population, the demand for food, agro-industrial and medicinal products is increasing. Several studies have contributed to the investigation of AMFs in the cultivation of different medicinal species. This unprecedented study identified a significant increase in the EO yield and content of active compounds of *M. officinalis* when inoculated with *R. clarus*, opening opportunities for future research that may contribute to the implementation of more sustainable agronomic techniques in the production of industrially important species.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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