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Arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria promoted changes in plant metabolism and the volatile profile of *Piper callosum* Ruiz & Pav

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ABSTRACT

Piper callosum Ruiz & Pav. is known in the Amazon as a paregoric elixir and exists as a shrub native to the Neotropics. In traditional medicine, the tea from its leaves is used to treat pain in the digestive tract, rheumatism, and muscular aches. This study aimed to evaluate the influence of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) inoculation on the secondary metabolism of *P. callosum*. The plants were inoculated after 30 days of cultivation and kept in a greenhouse. Plant development parameters were monitored at 30-, 60-, and 90-days after inoculation (DAI) and indicated that AMF and PGPR favored plant growth throughout all stages of development. The leaf volatiles extracted by simultaneous distillation and extraction were analyzed by gas chromatography coupled with mass spectrometry and showed that phenylpropanoids and monoterpene hydrocarbons were predominant in all essential oils, revealing distinct abundance patterns across the different sampling points. The major volatile compounds identified were safrole, methyl eugenol, and β-pinene, and a reduction trend in their content was observed for safrole and methyl eugenol, especially at 90 DAI in both treatments. Principal component analysis revealed that germacrene D-4-ol and limonene were among the most influential in both treatments. Finally, the total phenolic content and the enzymatic activity of phenylalanine ammonia-lyase revealed different effect patterns for inoculation and time of evaluation on each treatment. All the data presented indicate that symbiotic inoculation can optimize the production of essential oil, which can improve the quality and productivity of *P. callosum* plants.

1. Introduction

Piper callosum Ruiz & Pav. is a native tree from Brazil, Bolivia, Peru, and Colombia and is naturally distributed across several states in Brazil, including the Pará State (Guimarães et al., 2015), where it is known by various names, including "elixir paregórico," "óleo-elétrico," "panquilé," "matricá," and "John-brandin'" (Andrade et al., 2009). It is used in popular medicine to treat rheumatic pain, as an astringent, local hemostatic, anti-blennorrhagic, anti-leukorrhea, anti-inflammatory, menstrual cramps, and to relieve toothache in indigenous populations (Sanz-Biset and Cañigueral, 2013; Andrade et al., 2009; Bernard et al., 2001; Martins et al., 2009; Pring, 1982). Previous studies on its chemical composition have identified amides, monoterpene hydrocarbons, oxygenated monoterpenoids, sesquiterpene hydrocarbons, and phenylpropanoids in the plant, which can be attributed to its biological applications (Andrade et al., 2009; Da Silva et al., 2017; Parmar et al., 1997).

The *P. callosum* essential oil (EO) is rich in safrole, and its chemical composition varies geographically. For example, the primary compounds in essential oils from the Brazilian Amazon are safrole (66.0%), methyl eugenol (10.2%), and elemicin (3.7%) (Da Silva et al., 2016). In contrast, those from the Peruvian Amazon are characterized by asaricin (35.9%), safrole (20.2%), methyl eugenol (9.7%), and (*E*)-asarone as the main constituents (Van Genderen et al., 1999). This variation

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contributes to the oil's diverse biological properties, including molluscicide, insecticide, antimicrobial, antifungal, and cytotoxic activities. These properties indicate the application potential of *P. callosum* essential oil in various fields (Souto et al., 2012; Rapado et al., 2011; Dorman and Deans, 2000; Da Silva et al., 2017; Alves et al., 2021).

The production of bioactive compounds in plants is specific to each species and is influenced by various environmental and physiological factors, particularly in the leaves and stems (Almeida et al., 2018; Souto et al., 2012). Environmental factors such as temperature, seasonality (De Castro et al., 2019), salinity (Jelali et al., 2011; Jha and Subramanian, 2016), and drought (Arpanahi and Feizian, 2019; Bistgani et al., 2017), as well as the plant's ecological interactions with symbionts and pathogens, can also affect this production (Da Luz et al., 2017; De Almeida et al., 2020; De Souza et al., 2022).

Plant-microbe interactions are not just significant, but they are a sustainable alternative, with the potential to stimulate the production of secondary compounds and thereby improve environmental adaptation (Kaur and Suseela, 2020). In this context, to further enhance the production of bioactive compounds, the inoculation of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) has emerged as a promising tool for modulating the yield and composition of essential oils in aromatic and medicinal plants (Alves et al., 2022; Khalediyan et al., 2021; Yilmaz and Karik, 2022)

AMF establish symbiotic interactions with approximately 80% of land plants and maintain an ecological balance in the soil (Bahadur et al., 2019; Weisany et al., 2016). Inoculation with AMF provides a potentially valuable strategy for increasing crop productivity and improving ecosystem sustainability (Schaefer et al., 2021; Weisany, 2024). This strategy favors the production of secondary metabolites and the growth, absorption, and translocation of nutrients to the host plant roots in exchange for carbon (Smith and Read, 2008).

AMF belonging to the Glomeromycota phylum include genera such as *Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis*, and *Scutellospora* (Oehl et al., 2011). Both *Rhizophagus clarus* and *Claroideoglomus etunicatum* were formerly classified into the *Glomus* genus (Schüssler and Walker, 2010; Stockinger et al., 2009) and can establish mutualistic relationships with a wide range of host plant species, facilitating enhanced nutrient uptake, particularly phosphorus, thereby promoting plant growth and resilience against abiotic stresses (Darakeh et al., 2022). Through this relationship, both AMF species can significantly alter the biosynthesis of secondary metabolites, enhancing the production of various bioactive compounds and contributing to the plant's defense mechanisms and overall health (Oliveira et al., 2019; Schweiger et al., 2014).

PGPR can enhance crop productivity, particularly in aromatic and medicinal species (Ahemad and Kibret, 2014). These microorganisms thrive in the nutrient-rich rhizosphere near the roots of plants and provide various benefits to their plant hosts (Cordovez et al., 2019). They fix atmospheric nitrogen (Monteiro et al., 2021), solubilize phosphorus and potassium (Sindhu et al., 2016; Walia et al., 2017), iron through siderophore production (Kloepper et al., 1980), and produce phytostimulators that directly promote plant growth (Bais et al., 2006). Additionally, the association between PGPR and plant roots can influence the plant's secondary metabolites (Yilmaz and Karik, 2022), impacting essential oil yield and composition (Banchio et al., 2009; Cappellari et al., 2015), as well as other non-volatile metabolites (Cappellari et al., 2017).

Enterobacter is a genus of Gram-negative bacteria with numerous strains known to enhance plant growth, further enhancing their role in agricultural applications (Jha et al., 2011). *Enterobacter asburiae* is a facultative anaerobic, oxidase-negative, non-motile, and non-pigmented species. It is commonly found in soil, water, and sewage and is generally considered an opportunistic pathogen (Kato, 2024; Paauw et al., 2008). However, several studies have reported its ability to promote plant growth and increase yields of various vegetable crops (Abraham and Silambarasan, 2015).

This study aims to demonstrate how arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) can significantly improve the growth and modify the secondary metabolism of *P. callosum* plants. We hypothesize that these interactions between the microorganisms and the plants can significantly enhance the medicinal value of *P. callosum*. Through a comprehensive analysis of the individual interactions, we seek to actively promote the use of biofertilizers for traditional medicinal crops in the Amazon region.

2. Materials and methods

2.1. Plant material and growth conditions

Piper callosum Ruiz & Pav. plants were collected in Belém, PA, Brazil, at different time points between 2015 and 2018. A voucher specimen was deposited and registered in the Herbarium of the Emílio Goeldi Museum (MPEG) under the accession number MG218523. Collected plant individuals from the sampling campaign were identified by morphological comparison with the authentic specimens previously registered.

Cuttings containing 1 to 2 nodes were grown in 15-cell polyethylene plastic trays, with each cell containing approximately 30 g of vermiculite type B (Urimamã Mineração Ltda, Santa Maria da Boa Vista, PE, Brazil) as substrate. The trays were placed in a greenhouse covered with anti-UV transparent polyethylene film and under a 26% shading screen. Irrigation was performed daily, and the substrate was maintained at field capacity. Seedlings were also fertilized every fifteen days with a commercial nutrient solution (5% N, 5% P_2O5, 5% K_2O, 1% S, 1.2% Mg, 0.3% Zn, 0.05% B, 0.05% Cu, 0.1% Fe, 0.06% Mn, and 0.01% Mo) to promote root development. After 21 days, the seedlings developed adequate root systems and were then transferred to polypropylene bags (approximately 9.0 cm in diameter) filled with a commercial substrate (limestone, castor oil, bone meal, and expanded vermiculite type B) previously autoclaved at 121 °C for 30 minutes.

2.2. Experimental design

Two separate experiments were conducted to evaluate the effects of arbuscular mycorrhizal fungi (AMF) inoculation and plant growth-promoting rhizobacteria (PGPR) inoculation on *P. callosum* seedlings. Each experiment employed a completely randomized design in a 2×3 factorial scheme. Factors consisted of both plant inoculation (inoculated or non-inoculated) and the plant sampling time (30, 60, and 90 days after inoculation). Each experiment had three distinct replicates, with a total of twenty-four *P. callosum* seedlings each. The seedlings were randomly assigned to each treatment and replicates to ensure unbiased distribution. Destructive samples were collected from *P. callosum* leaves and roots at each designated time point to assess development parameters and secondary metabolism.

2.3. Development of AMF spores and preparation of fungal inoculant

Spores from *Rhizophagus clarus* and *Claroideoglomus etunicatum* were isolated from rhizosphere soil samples in the Southeast Pará State, Amazon region (Brazil). They were cultivated in a greenhouse using sterile sand and *Brachiaria brizantha* (A. Rich.) Stapf. as a trap culture. Species identification was conducted through morphological comparison based on the International Collection of Cultures of Arbuscular Mycorrhizal (Vesicular) Fungi (INVAM). The final inoculum was prepared by mixing equal amounts of spores from *R. clarus* and *C. etunicatum* to achieve a final concentration of 90 spores/g. The seedlings were removed from the planting bags, and pits approximately 2.0 cm deep were opened. Then, each plant belonging to the inoculated group received 6.0 g of inoculum containing AMF directly to the roots, while control plants received only sterile sand.

2.4. Bacterial strain and inoculum preparation

The rhizobacterium *Enterobacter asburiae* strain EM56 (Alves et al., 2022) was provided by the Laboratory of Environmental Microbiology, located at Universidade Federal do Sul e Sudeste do Pará (UNIFESSPA), Marabá campus, Marabá, PA, Brazil. This strain was initially isolated from the rhizosphere of Paricá (*Schizolobium amazonicum* Huber ex Ducke). For the inoculum preparation, the strain was cultivated in 300 mL of LB medium for 4 h at 28 °C till an optical density (OD_{600}) of 0.40, containing approximately 10^8 colony-forming units per mL (CFU/mL). Then, the inoculum was centrifuged at 7600 *g* for 30 min, and the pellet was resuspended in 300 mL of 0.85% sterile saline solution (NaCl) with 0.5% cellulose to enhance bacterial adhesion and then stored at 10 °C for 24 h till inoculation. The growth curve of the strain was previously performed for inoculum concentration determination. The suspension was stored at 10 °C for 24 h. The bacterial inoculum (5 mL per plant) was applied 3 to 5 cm below the soil surface near the roots (Mafia et al., 2009), while control plants received only sterile saline solution.

2.5. Plant development evaluation

Each plant was evaluated for shoot height (cm), root length (cm), and the total fresh mass of leaves and roots (g). Shoot height was determined as the total length from the soil surface to the apex of the tallest leaf. To also measure the root length, the entire root system was excavated, and it was determined as the total length between the end of the shoot to the root tip. The production of total fresh biomass was based on the total weight per plant. Plant development parameters were measured at each sampling time point.

2.6. Extraction and identification of the volatile organic compounds in the essential oils

The EOs from each plant were extracted from fresh leaves (2.0 g) of *P. callosum* and obtained by simultaneous distillation–extraction process using a Likens–Nickerson apparatus for 2 h with *n*-pentane (3.0 mL) as solvent. Qualitative analysis, with an aliquot (1.0 μL) of the organic phase, was carried out by Gas Chromatography-Mass Spectrometry after extraction (GC–MS; Shimadzu QP2010 ultra, Shimadzu Corporation, Tokyo, Japan) under the following conditions: Rtx-5MS silica capillary column (30 m × 0.25 mm ID X 0.25 μm film thickness; Restek Corporation, Bellefonte, PA, USA); programmed temperature, 60–240°C (3°C/min); injector temperature, 200°C; carrier gas helium at a flow rate of 1.2 mL/min; injection type, splitless; septum purge flow was maintained at 10 mL/min; EIMS, electron energy, 70 eV; and temperature of the ion source and connection parts, 200°C. Retention indices were determined using a homologous series of *n*-alkanes (C8–C32; Sigma–Aldrich, St. Louis, MO, USA) (Van Den Dool and Kratz, 1963). The compounds were identified by comparing mass spectra and retention indices with library data ADAMS (Adams, 2007; Kramida et al., 2011). The component percentages are based on peak integrations without standardization.

2.7. Phenylalanine ammonia-lyase (PAL) activity

Fresh leaves were pulverized in liquid nitrogen, and the samples (1.0 g) were homogenized in 2.0 mL of 0.3 mM sodium borate buffer (pH 8.8) containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol (DTT), and 5% polyvinylpolypyrrolidone (PVP). The samples were centrifuged at 12,000 *g* and 4 °C for 15 min, and an aliquot (0.5 mL) of the supernatant was mixed with 1.0 mL of reaction buffer containing 0.3 mM sodium borate (pH 8.8) and 0.03 mM L-phenylalanine. The reaction was incubated for 15 min at room temperature, and then the absorbance was read at 290 nm in the UV/visible spectrophotometer (Amersham Biosciences, Little Chalfont, UK). PAL activity was analyzed based on the formation of (*E*)-cinnamic acid from the substrate, L-phenylalanine, with a specific molar coefficient of 9630 mol·L^{-1}·cm^{-1} (Vaganan et al., 2014). All reagents used in this experiment were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.8. Total phenolic content (TPC) determination

Fresh leaves (2.0 g) were extracted by percolation (96 h) with 50 mL of ethyl acetate. After solvent evaporation, the concentration of the total phenolics was determined using the Folin-Ciocalteu method (Sousa et al., 2007). An aliquot of 500 μL of extracts dissolved in methanol (20 mg/mL) was used to react with 250 μL of Folin-Ciocalteu reagent (1.0 N) and 1250 μL of sodium carbonate (75 g/L). After 30 min incubation in the dark, the absorbance of reaction was read at 760 nm using a UV/visible spectrophotometer (Amersham Biosciences, Little Chalfont, UK). The experimental calibration curve was set at concentrations of 0.5 to 10.0 mg/L, using gallic acid (Sigma Aldrich, St. Louis, MO, USA), and the results were expressed as gallic acid equivalents (GAE) in milligrams per gram of extract (mg GAE/g).

2.9. Statistical analysis

Statistical analyses and graphical visualization were performed on R using the R platform v. 4.3.1 (RStudio Team, 2023), together with 'ExpDes.pt' 1.2.2 (Ferreira et al., 2014), 'ARTool' 0.10.5 (Kay and Wobbrock, 2016), 'ggplot2' 3.1.0 (Wickham, 2016), 'factorextra' 1.0.7 (Kassambara and Mundt, 2017), and 'vegan' (Oksanen et al., 2020) packages. For the growth and biochemical parameters, the Shapiro-Wilk normality test and Bartlett's homogeneity test were performed to define the most appropriate statistical test to detect significant differences among treatments. When necessary, ANOVA of aligned rank-transformed data was used to investigate the effect of the inoculation of AMF and PGPR together with the sampling time (Wobbrock et al., 2011), later followed by pairwise comparisons using Tukey's adjustment. To address the compositional nature of the GC–MS chemical profile (Greenacre, 2021), the data were first transformed using the robust centered log-ratio (RCLR) followed by robust principal-component analysis (RPCA) (Martino et al., 2019). A permutational multivariate analysis of variance (perMANOVA) was also used to assess the effect of treatments in the EO profile.

3. Results

3.1. Plant growth and development

We assessed the impact of mycorrhizal (AMF) and rhizobacterial (PGPR) inoculation on plant growth parameters (Table 1). Mycorrhizal inoculation significantly increased shoot length ($F = 35.73$, $p < 0.001$), total fresh mass ($F = 269.366$, $p < 0.001$), and root length

Table 1
F statistics for the effect of the inoculation of either mycorrhiza or rhizobacteria (Inoculation), time of evaluation (DAI), and their interaction (Inoculation × DAI) on growth parameters of *Piper callosum* Ruiz et Pav. plants. DAI = Days after inoculation.

	Parameter	Inoculation	DAI	Inoculation x DAI
Mycorrhiza	Shoot length	35.73***	384.98***	3.49
	Total Fresh mass	269.366***	214.402***	92.852***
	Root length	116.256***	185.442***	22.958***
Rhizobacteria	Shoot length	33.681***	68.190***	16.888***
	Total Fresh mass	0.013	40.742***	5.222*
	Root length	4.8115	26.3561***	0.2018

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

(F = 116.256, $p < 0.001$). In contrast, rhizobacterial inoculation resulted in a significant increase only in shoot length (F = 33.681, $p < 0.001$).

The time factor ('DAI') was highly significant across all growth parameters for both mycorrhiza and rhizobacteria treatments ($p < 0.001$), indicating a solid temporal growth trend. Interaction effects between inoculation type and time were variable, indicating that the effect of both AMF and PGPR varied over the sampling periods; in the AMF treatment, a highly significant interaction was noted for total fresh mass (F = 92.852, $p < 0.001$) and root length (F = 22.958, $p < 0.001$) but not for shoot length (Table 1). In the rhizobacteria treatment, the interaction was highly significant for shoot length (F = 16.888, $p < 0.001$) and significant for total fresh biomass (F = 5.222, $p < 0.05$) but was not significant for root length. The inoculation of both microorganisms proved capable of affecting plant growth to some degree (Fig. 1), with AMF inoculation standing out with superior values for the different growth parameters over time.

3.2. Chemical characterization of the volatile compounds from leaves after colonization by AMF and PGPR

The evaluation of the organic volatile profile revealed effects in the chemical composition after the inoculation with either PGPR or AMF across the different sampling points (Fig. 2). In the group inoculated with PGPR, phenylpropanoids were slightly more abundant in inoculated plants at 30 DAI (91.79% control vs. 92.29% inoculated), a trend that was reversed at 60 DAI (94.34% control vs. 92.68% inoculated). By 90 DAI, the remaining inoculated plants showed a decrease in phenylpropanoids to 87.28%, while controls remained higher at 90.96%. Similarly, the AMF treatment showed lower phenylpropanoid levels than control plants at all time points, culminating in a decrease in inoculated plants to 38.22% at 90 DAI compared to 52.37% in controls.

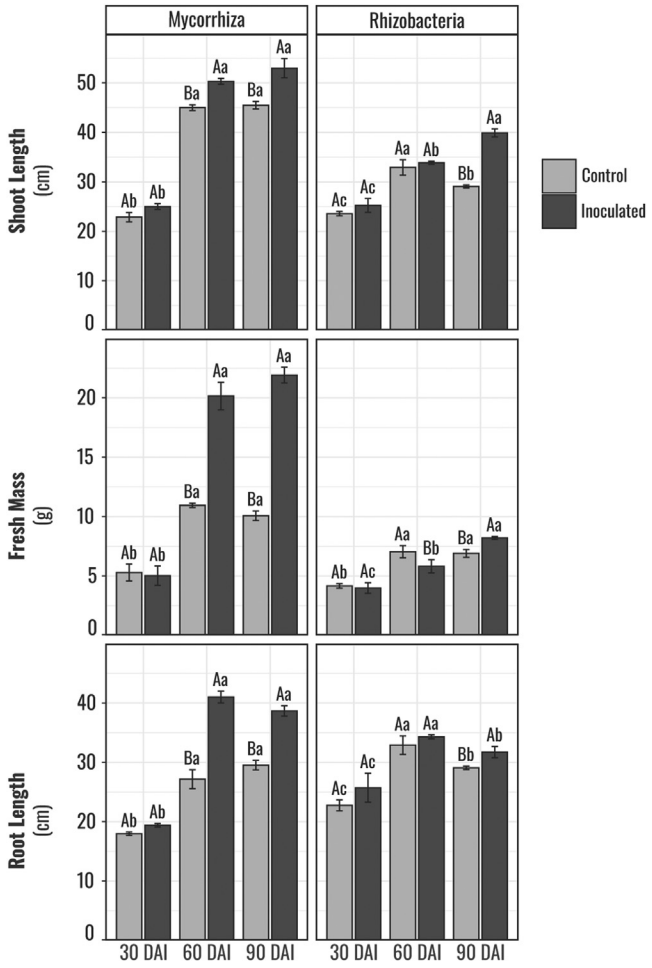

Fig. 1. Variation in plant growth and development parameters in *Piper callosum* inoculated (n = 3) with arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR). Values with different uppercase letters are significantly different ($p < 0.05$) based on Tukey's HSD test with control vs. inoculated within each sampling time. Values with different lowercase letters are significantly different ($p < 0.05$) based on Tukey's HSD test with each sampling time within each inoculation treatment. DAI = days after inoculation.

Monoterpene hydrocarbon levels in AMF-treated plants increased from 30 to 60 DAI in control (15.51% to 24.47%) and inoculated (16.47% to 27.43%) groups, followed by a peak at 90 DAI (31.79% control vs. 30.17% inoculated). In contrast, sesquiterpene hydrocarbons in the AMF treatment showed a decrease from 30 to 60 DAI (7.65% to 6.13%) and a spike from 60 to 90 DAI (6.13% to 9.98%), whereas control plants experienced a peak at 60 days (11.57%) before declining at 90 days (6.54%).

The dynamics of oxygenated monoterpenes and sesquiterpenes, along with unidentified compounds, also varied with inoculation and time in both AMF- and PGPR-inoculated plants, reflecting the complexity of plant-microbial interactions on the organic volatile chemical composition. We observed an increasing trend in oxygenated monoterpenes and sesquiterpenes content, mainly at 90 DAI in the AMF treatment, and an increase in monoterpene hydrocarbon content at 90 DAI. These observations suggest that microbial colonization may influence the volatile composition of plant leaves. The AMF treatment appeared to affect the dynamics of different classes of volatile compounds, particularly phenylpropanoids, in inoculated plants.

3.3. Volatile compound variation after inoculation with AMF and PGPR

In total, 62 individual volatile compounds were identified from the GC–MS data from the *P. callosum* leaf essential oils after the inoculation with the AMF *Rhizophagus clarus* and *Claroideoglomus etunicatum* (Appendix A. Supplementary Data – Table S1). The other 22 individual compounds were identified from the essential oils of plants after inoculation with the PGPR *Enterobacter asburiae* (Appendix A. Supplementary Data – Table S2). The major compounds found in all treatments were the phenylpropanoids safrole and methyl eugenol, together with the monoterpene hydrocarbon β-pinene (Fig. 3C).

In the control group of the AMF treatment, safrole was the predominant compound at 30 DAI, constituting approximately 50.97% of the volatile profile, followed by methyl eugenol at 17.54%. This profile exhibited a reduction in safrole to 37.38% in 60 days and then to 37.26% at 90 days. A similar downward trend was observed in methyl eugenol, which decreased to 13.07% at 60 days and 11.32% at 90 days. Inoculated samples reduced safrole content from 50.52% at 30 days to 35.20% at 90 days and methyl eugenol from 15.95% to 12.77% across the same timeframe.

The PGPR treatment displayed higher stability in safrole content, with the control samples starting at 82.83% at 30 days and only slightly decreasing to 81.99% by 90 days. Methyl eugenol levels were lower and showed a minor decline from 9.74% at 30 days to 9.30% at 90 days. Inoculated samples under the PGPR treatment maintained high safrole levels, slightly reducing from 82.02% at 30 days to 79.64% at 90 days, whereas methyl eugenol decreased from 8.18% to 7.49%.

The relative abundance of other volatile compounds, such as β-pinene, γ-terpinene, elemicin, and others, showed less significant changes across all treatments and time points. The bar chart visualization illustrates the dominance of safrole and methyl eugenol in the volatile profiles, their dynamic changes over time, and the influence of microbial treatments on these specific compounds (3).

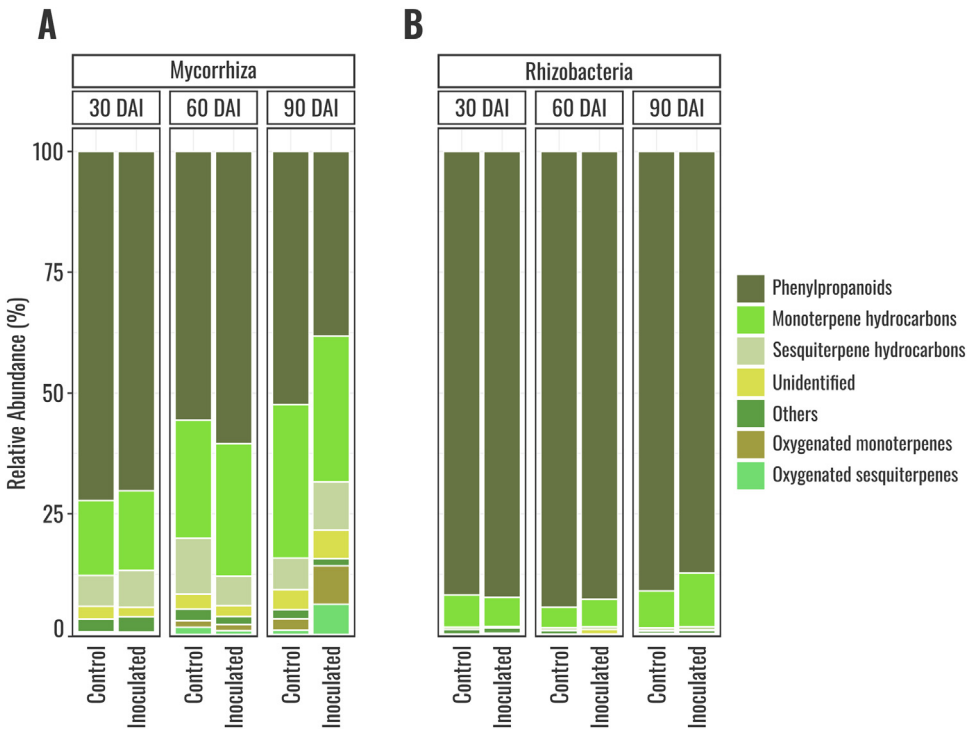

Fig. 2. Relative abundance of chemical classes found in the leaf extracts of *Piper callosum* plants inoculated (n = 3). (A) Inoculation with arbuscular mycorrhizal fungi (AMF); (B) Inoculation with plant growth-promoting rhizobacteria (PGPR). **DAI =** days after inoculation.

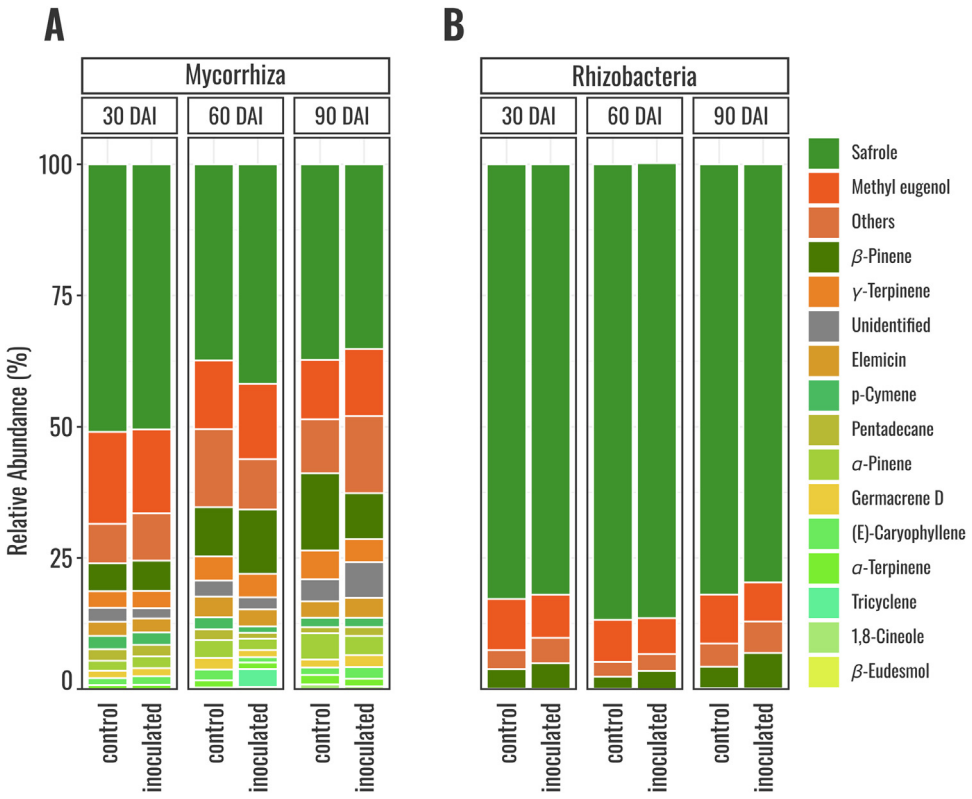

Fig. 3. Relative abundance of chemical compounds found in the leaf extracts of *Piper callosum* plants inoculated (n = 3) with arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR). DAI = days after inoculation.

3.4. Multivariate analysis of the volatile composition of plants inoculated with PGPR and AMF

To address the complex nature of GC–MS data, we used a multivariate approach to assess the effect of AMF and PGPR inoculation on the volatile compounds throughout the time. After central log-ratio transformation, Principal Component Analysis (PCA) of the compositional data was performed, and it comprised 50.03 and 61.22% of the total data variability for AMF and PGPR, respectively (Fig. 4).

The PCA biplots for both mycorrhiza and rhizobacteria treatments demonstrated a spatial separation of sample points, indicating distinct volatile profiles associated with each treatment and time point. In the AMF treatment, the first principal component (PC_1) accounted for 32.39% of the variance, while the second principal component (PC_2) accounted for 17.64%. Similarly, in the rhizobacteria treatment, PC_1 and PC_2 explained 34.54% and 22.68% of the variance, respectively.

The biplots revealed that for AMF treatment, compounds such as germacrene D-4-ol, 1,8-cineole, and limonene strongly contributed to the overall variance within and cluster separation within the data set. Control and inoculated samples did not show a clear separation at 30 DAI, implying an overlapping volatile profile between these groups at the beginning. However, at both 60 and 90 DAI this trend reversed, and the control and inoculated samples chemical profiles began to diverge. In the case of PGPR, octene, limonene, and pentanol were the major contributors to the variance and cluster separation. Notably, germacrene D-4-ol and limonene were among the most influential variables contributing to the variance in both treatments, indicating that changes in these compounds are associated with microbial colonization.

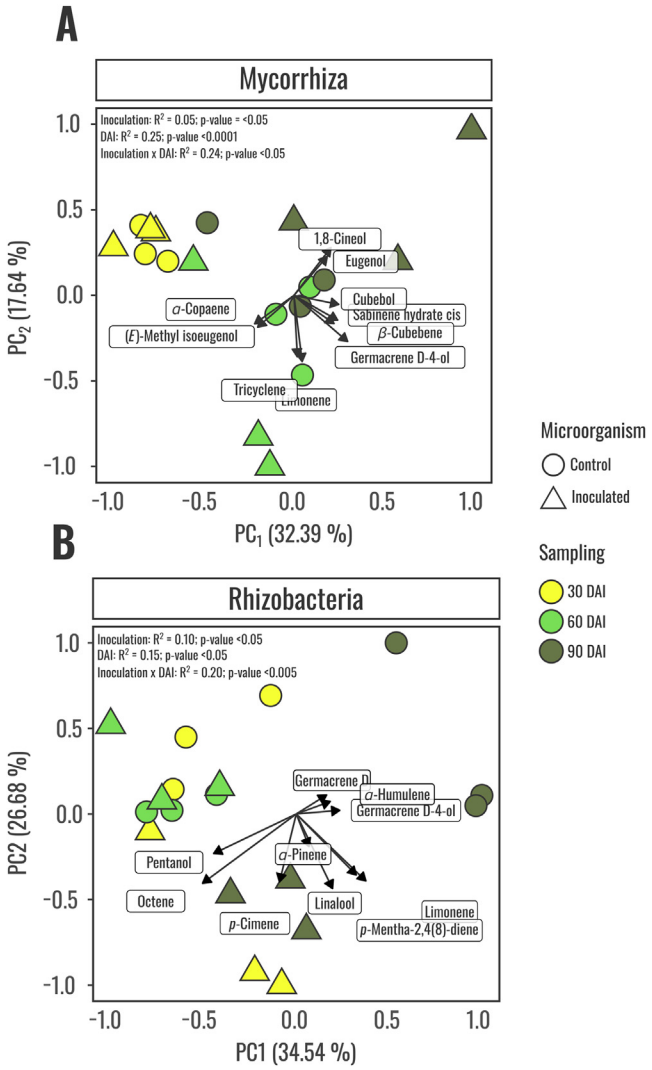

Fig. 4. Bi-dimensional plot of the two principal components (PC_1 and PC_2) obtained from the robust PCA analysis and the contribution of each volatile compound to the overall variance in control and inoculated *Piper callosum* plants inoculated with (A) AMF (B) PGPR. DAI = Days after inoculation.

3.5. Phenylalanine ammonia-lyase (PAL) activity and total phenolic content (TPC) determination

The inoculation with AMF or PGPR also influenced the enzymatic activity of PAL and TPC production (Table 2 and Fig. 5). Mycorrhizal inoculation only affected PAL activity ($F = 6.251$, $p < 0.05$) but did not affect phenolic content ($F = 1.674$, $p < 0.1$). In rhizobacteria, the inoculation factor did not significantly increase PAL or TPC ($p < 0.1$). The time of evaluation factor ('DAI') showed a significant alteration in PAL activity in mycorrhiza colonization ($F = 14.092$, $p < 0.01$) but did not significantly alter TPC ($F = 0.921$, $p < 0.1$).

Interaction effects between inoculation and time exhibited significant variations in PAL ($F = 12.067$, $p < 0.01$) and TPC ($F = 65.107$, $p < 0.001$) for mycorrhizal inoculation, indicating a strong trend for interaction effects. On the other hand, rhizobacterial inoculation did not present significant alterations for the interaction effects (PAL: $F = 37.343$, $p < 0.1$; TPC: $F = 69.423$, $p < 0.1$). The results indicate that AMF and PGPR inoculation can affect certain aspects of the secondary metabolism of *P. callosum* plants but in different effect patterns.

4. Discussion

Both AMF and PGPR are rhizospheric microorganisms that play crucial roles in supporting plant growth and development and have been used as tools to improve both fresh yield and key secondary metabolite production (Fasusi et al., 2021; Mishra et al., 2017). Thus, increasing both plant fresh yield and secondary metabolic activity is imperative to enhance the quantity and quality of the industrially valuable secondary metabolites produced by *P. callosum*. The results from the present study demonstrate that both AMF and PGPR are able to promote plant growth and induce changes in secondary metabolism.

4.1. Impact of AMF and PGPR Inoculation on Plant Growth and Development

The increase in fresh mass, shoot length, and root length in response mainly to AMF inoculation aligns with several studies that have demonstrated the plant growth-promoting effects of *R. clarus* and *C. etunicatum* in plants of the *Piper* genus, such as *P. aduncum* and *P. divaricatum* (Oliveira et al., 2019, 2022). The increase in plant growth and yield in response to mycorrhizal colonization can be attributed to enhanced nutrient and water uptake, as well as increased abiotic and biotic stress tolerance (Chen et al., 2018; Saia et al., 2014; Tarraf et al., 2017). The plant-mycorrhizal association can significantly increase the effective area explored by roots, as AMF hyphae can access pores that are too small for roots to penetrate, thereby establishing contact with unexplored mineral particles and organic residues in the soil (Johnson and Jansa, 2017). AMF hyphae are also able to synthesize and secrete organic acids, chelating agents, and other chemical compounds that may depolymerize organic compounds and solubilize mineral nutrients (Varma et al., 2018).

In contrast, inoculation with the previously described *E. asburiae* strain EM56 only resulted in a significant increase in shoot length (Fig. 1). Beneficial bacteria usually colonize the root surface and tissues of the host plant, where they can perform functions that improve the host's fitness (Andreote et al., 2014; Redman et al.,

Table 2
F statistics for the effect of the inoculation of either mycorrhiza or rhizobacteria (Inoculation), time of evaluation (DAI), and their interaction (Inoculation × DAI) on PAL and TPC of *P. callosum* plants. DAI = Days after inoculation.

	Parameter	Inoculation	DAI	Inoculation x DAI
Mycorrhiza	Total Phenolic Content (TPC)	1.674	0.921	65.107***
	Phenylalanine Ammonia-Lyase (PAL)	6.251*	14.092**	12.067**
Rhizobacteria	Total Phenolic Content (TPC)	1.1065	6.9423*	1.4666
	Phenylalanine Ammonia-Lyase (PAL)	0.8504	8.3960*	3.7343

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

2011). However, the rhizocompetence (i.e., the ability of rhizobacteria to colonize plant roots and tissues in natural soil environments alongside the indigenous soil microbiota) and the expression of plant growth-promoting traits of rhizobacteria isolated from the rhizosphere of distantly related plants can be ephemeral. This is due to host selectivity and an inability to effectively establish in the new host rhizosphere (Compant et al., 2005; Helal et al., 2022).

4.2. Alterations in Secondary Metabolism and Plant Defense Mechanisms Post-Inoculation

The alteration of secondary metabolism post-inoculation reveals a sophisticated level of biochemical modulation by the plant-microbial interaction. The major compounds found in all extracts were the phenylpropanoids safrole and methyl eugenol, and the monoterpene hydrocarbon β-pinene (Fig. 3). Phenylpropanoids and monoterpene hydrocarbons compose the majority of the volatile organic compound profile of a wide range of *Piper* species (Takeara et al., 2017). In the betel vine (*Piper betle* L.), a plant commonly found in Asian countries, methyl eugenol is one of the main compounds in the leaves (Guha and Nandi, 2019). This phenylpropanoid, as well as safrole, can also be found in the leaves of *P. divaricatum* (Barbosa et al., 2012; Corpes et al., 2019; Da Silva et al., 2010). The monoterpene hydrocarbon β-pinene can usually be found in the fruit extract and the EO of several black pepper (*Piper nigrum* L.) cultivars found in the Brazilian Amazon Forest (Barata et al., 2021; Dosoky et al., 2019).

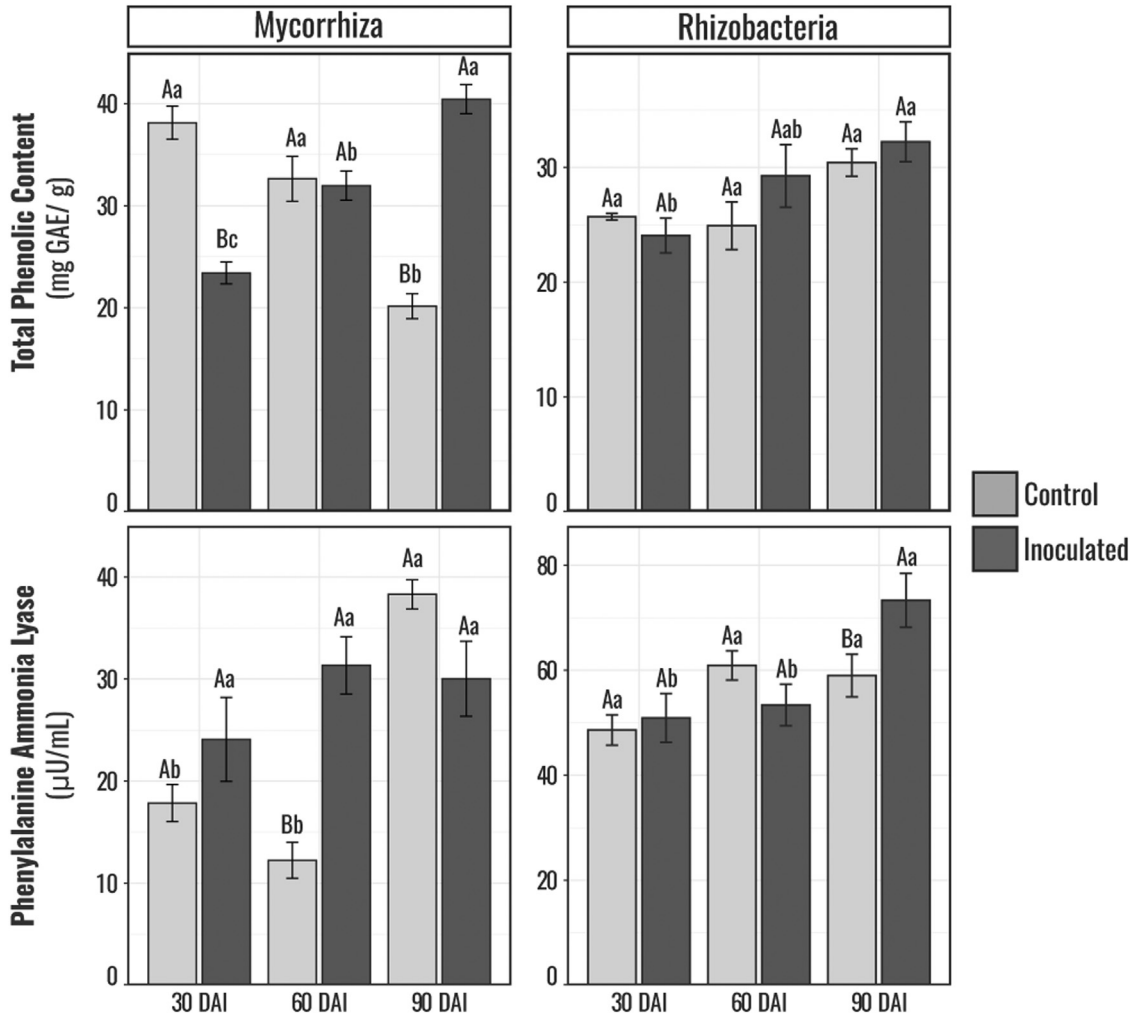

Fig. 5. Variation in TPC production and PAL enzyme activity in *Piper callosum* inoculated (n = 3) with arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR). Values with different uppercase letters are significantly different ($p < 0.05$) based on Tukey's HSD test with control vs. inoculated within each sampling time. Values with different lowercase letters are significantly different ($p < 0.05$) based on Tukey's HSD test with each sampling time within each inoculation treatment. **DAI** = days after inoculation.

Upon root colonization, these microorganisms trigger plant defense mechanisms and signaling pathways that can result in many changes in primary (Mhlongo et al., 2020; Saia et al., 2015) and secondary metabolism (Asghari et al., 2020; Noceto et al., 2021), resulting in significant fluctuations in the major components, especially safrole and methyl eugenol (Figure).

AMF symbiosis can provide protection from a wide range of pathogens, including viruses, necrotrophic and biotrophic fungi, nematodes, and insects (Cameron et al., 2013; Campos–Soriano et al., 2012; Fiorilli et al., 2019; Miozzi et al., 2019). After root colonization, the plant-AMF symbiosis induces a physiological state in plants known as "priming" (Goddard et al., 2021), leading to an alert state capable of producing a rapid and intense induction of various cellular defense responses upon exposure to pathogens or abiotic stress, triggering the jasmonate (JA)-dependent signaling pathway in the host plants (Mauch-Mani et al., 2017). AMF colonization triggers the terpene and phenylpropanoid pathways, increasing the production of bioactive molecules and changing the relative abundance of the compounds present in the leaf extract (Adolfsson et al., 2017; Bruisson et al., 2016).

The plant responses triggered by AMF inoculation include characteristics of both systemic acquired resistance (SAR), which occurs after pathogen infection in plants, and induced systemic resistance (ISR) that follows root colonization by non-pathogenic bacteria like the PGPR (Cameron et al., 2013). However, after inoculation with PGPR, the leaves of *P. callosum* plants exhibited a slight decrease in phenylpropanoid relative abundance and a moderate increase in monoterpene hydrocarbons (Fig. 2). The plant response to the PGPR inoculations was magnitudes weaker than to the AMF, maintaining the previously reported high proportion of the phenylpropanoids safrole and methyl eugenol in the essential oil of *P. callosum* leaves (Andrade et al., 2009; Da Silva et al., 2017; Parmar et al., 1997). The strength of the response to the inoculation of *E. asburiae* may relate to its origin. The strain EM56 of *E. asburiae* was first isolated from the rhizosphere of *S. amazonicum* trees (Alves et al., 2022). The phylogenetic distance between hosts may render the isolate unable to effectively colonize and express plant growth-promoting traits (Helal et al., 2022), leading to a relatively weak response when compared with AMF colonization.

Particular volatile compounds, such as germacrene D-4-ol, 1,8-cineole, and limonene in AMF-treated plants, and octene, limonene, and pentanol in PGPR-treated plants, are significant contributors to the observed variability (Fig. 4) (Dudareva et al., 2004; Pichersky and Raguso, 2018). These compounds are exceptionally responsive to microbial presence, which could be indicative of their roles in signaling or defense (Junker and Tholl, 2013). The prominence of germacrene D-4-ol and limonene is noteworthy and reinforces their potential importance in plant-microbe interactions (Huang et al., 2012; Junker and Tholl, 2013).

Determination of phenylalanine ammonia-lyase (PAL) activity and total phenolic content (TPC) revealed that the secondary metabolism of inoculated plants is affected by the colonization of AMF and PGPR, especially in the early stages of inoculation (Fig. 5). However, it was noted that the effects of inoculation and time alter the plant metabolism differently. The time effect had a greater impact on rhizobacterial inoculation, whereas the interaction between inoculation and time was stronger in mycorrhizal inoculation.

The PAL enzyme is related to the induction of plant defense responses because it converts the amino acid phenylalanine into (*E*)-cinnamic acid, a critical regulatory point in the phenylpropanoid metabolic pathway, which can cause accumulation of lignins and phytoalexins that are responsible for disease resistance (Kim and Hwang, 2014; Ngadze et al., 2012). Meanwhile, phenolic compounds are produced through the shikimate and pentose phosphate pathways following the metabolization of phenylpropanoids and are known for their various biological activities, including roles in plant defense (Lin et al., 2016; Zaynab et al., 2018).

Taken together, the data suggest that microbial colonization can modulate plant volatile profiles and secondary metabolism, with certain compounds being more responsive to microbial interactions than others. This modulation could have significant implications for plant health and stress responses, as well as for the ecological interactions between plants and their environment (Baldwin et al., 2006; Heil and Silva Bueno, 2007). Understanding these interactions can provide valuable insights for agricultural biotechnology, particularly in the development of microbial inoculants aimed at enhancing crop resilience and yield (Backer et al., 2018; Berg, 2009).

5. Conclusion

PGPR and AMF inoculation in *P. callosum* specimens promoted significant changes in different plant developmental parameters and secondary metabolism, with AMF presenting a more significant role in plant growth promotion and secondary metabolism modulation. All the data presented indicate that the inoculation of AMF can optimize the volatile organic compounds present in the essential oil of *P. callosum* plants, improving its biotechnological qualities. However, we also highlight the importance of the bacterial strain selection for more substantial changes in *P. callosum* essential oil composition. Thus, inoculation can be an efficient biotechnological tool for stimulating plant growth and optimize certain compounds in the essential oil. In addition, further experiments on *P. callosum* plants are needed for the fine tuning and agricultural scale up.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Maycow Marcos L. de Azevedo: Writing – original draft, Methodology, Formal analysis, Data curation. **Joyce Solange F. de Oliveira:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Luccas M. Barata:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis. **Gabriel Gustavo T.N. Monteiro:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis. **Eloisa Elena A. Andrade:** Funding acquisition, Formal analysis. **Pablo Luis B. Figueiredo:** Writing – original draft, Formal analysis, Data curation. **William N. Setzer:** Writing – review & editing, Visualization, Investigation. **Joyce Kelly R. da Silva:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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Supplementary materials

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References

Abraham, J., Silambarasan, S., 2015. Plant growth promoting bacteria enterobacter asburiae JAS5 and enterobacter cloacae JAS7 in mineralization of endosulfan. Appl. Biochem. Biotechnol. 175, 3336–3348. https://doi.org/10.1007/s12010-015-1504-7.

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry, 4th ed. Allured Publishing Corporation, Carol Stream, IL.

Adolfsson, L., Nziengui, H., Abreu, I.N., Šimura, J., Beebo, A., Herdean, A., Aboalizadeh, J., Široká, J., Moritz, T., Novák, O., Ljung, K., Schoefs, B., Spetea, C., 2017. Enhanced secondary- and hormone metabolism in leaves of arbuscular mycorrhizal medicago truncatula. Plant Physiol. 175, 392–411. https://doi.org/10.1104/pp.16.01509.

Ahemad, M., Kibret, M., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J. King. Saud. Univ. Sci. 26, 1–20. https://doi.org/10.1016/j.jksus.2013.05.001.

Almeida, C.A., Azevedo, M.M.B., Chaves, F.C.M., Roseo de Oliveira, M., Rodrigues, I.A., Bizzo, H.R., Gama, P.E., Alviano, D.S., Alviano, C.S., 2018. Piper essential oils inhibit rhizopus oryzae growth, biofilm formation, and rhizopuspepsin activity. Can. J. Infect. Dis. Med. Microbiol. 2018, 1–7. https://doi.org/10.1155/2018/5295619.

Alves, C.M.G., Nogueira, J.N., Luz, J.G.R., Chaves, F.C.M., Tavares-Dias, M., 2021. Essential oil of Piper callosum, Piper hispidum and Piper marginatum (Piperaceae) possesses in vitro efficacy against monogeneans of Colossoma macropomum (tambaqui). Aquac. Res. 52, 6107–6116. https://doi.org/10.1111/are.15473.

Alves, N.S.F., Kaory Inoue, S.G., Carneiro, A.R., Albino, U.B., Setzer, W.N., Maia, J.G., Andrade, E.H., da Silva, J.K.R., 2022. Variation in Peperomia pellucida growth and secondary metabolism after rhizobacteria inoculation. PLoS. One 17, e0262794. https://doi.org/10.1371/journal.pone.0262794.

Andrade, E.E.A., Guimarães, E.F., Maia, J.G.S., 2009. Variabilidade Química Em óleos essenciais De Espécies De Piper da Amazônia. FEQ/UFPA, Belém, Pará.

Andreote, F.D., Gumiere, T., Durrer, A., 2014. Exploring interactions of plant microbiomes. Sci. Agric.. https://doi.org/10.1590/0103-9016-2014-0195.

Arpanahi, A.A., Feizian, M., 2019. Arbuscular mycorrhizae alleviate mild to moderate water stress and improve essential oil yield in thyme. Rhizosphere 9, 93–96. https://doi.org/10.1016/j.rhisph.2018.10.003.

Asghari, B., Khademian, R., Sedaghati, B., 2020. Plant growth promoting rhizobacteria (PGPR) confer drought resistance and stimulate biosynthesis of secondary metabolites in pennyroyal (Mentha pulegium L.) under water shortage condition. Sci. Hortic. 263, 109132. https://doi.org/10.1016/j.scienta.2019.109132.

Backer, R., Rokem, J.S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., Smith, D.L., 2018. Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front. Plant Sci. 9, 1473. https://doi.org/10.3389/fpls.2018.01473.

Bahadur, A., Batool, A., Nasir, F., Jiang, S., Mingsen, Q., Zhang, Q., Pan, J., Liu, Y., Feng, H., 2019. Mechanistic insights into arbuscular mycorrhizal fungi-mediated drought stress tolerance in plants. Int. J. Mol. Sci. 20, 4199. https://doi.org/10.3390/ijms20174199.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev. Plant Biol. 57, 233–266. https://doi.org/10.1146/annurev.arplant.57.032905.105159.

Baldwin, I.T., Halitschke, R., Paschold, A., von Dahl, C.C., Preston, C.A., 2006. Volatile signaling in plant-plant interactions: "talking trees" in the genomics era. Science (1979) 311, 812–815. https://doi.org/10.1126/science.1118446.

Banchio, E., Xie, X., Zhang, H., Paré, P.W., 2009. Soil bacteria elevate essential oil accumulation and emissions in sweet basil. J. Agric. Food Chem. 57, 653–657. https://doi.org/10.1021/jf8020305.

Barata, L.M., Andrade, E.H., Ramos, A.R., De Lemos, O.F., Setzer, W.N., Byler, K.G., Maia, J.G.S., Da Silva, J.K.R., 2021. Secondary metabolic profile as a tool for distinction and characterization of cultivars of black pepper (Piper nigrum L.) cultivated in Pará State, Brazil. Int. J. Mol. Sci. 22, 890. https://doi.org/10.3390/ijms22020890.

Barbosa, Q.P.S., Da Câmara, C.A.G., Ramos, C.S., Nascimento, D.C.O., Lima-Filho, J.V., Guimarães, E.F., 2012. Chemical composition, circadian rhythm and antibacterial activity of essential oils of Piper divaricatum: a new source of safrole. Quim. Nova 35, 1806–1808. https://doi.org/10.1590/S0100-40422012000900019.

Berg, G., 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl. Microbiol. Biotechnol. 84, 11–18. https://doi.org/10.1007/s00253-009-2092-7.

Bernard, P., Scior, T., Didier, B., Hibert, M., Berthon, J.Y., 2001. Ethnopharmacology and bioinformatic combination for leads discovery: application to phospholipase A2 inhibitors. Phytochemistry 58, 865–874. https://doi.org/10.1016/S0031-9422(01)00312-0.

Bistgani, Z.E., Siadat, S.A., Bakhshandeh, A., Pirbalouti, A.G., Hashemi, M., 2017. Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of Thymus daenensis Celak. Crop J. 5, 407–415. https://doi.org/10.1016/J.CJ.2017.04.003.

Bruisson, S., Maillot, P., Schellenbaum, P., Walter, B., Gindro, K., Deglène-Benbrahim, L., 2016. Arbuscular mycorrhizal symbiosis stimulates key genes of the phenylpropanoid biosynthesis and stilbenoid production in grapevine leaves in response to downy mildew and grey mould infection. Phytochemistry 131, 92–99. https://doi.org/10.1016/j.phytochem.2016.09.002.

Cameron, D.D., Neal, A.L., van Wees, S.C.M., Ton, J., 2013. Mycorrhiza-induced resistance: more than the sum of its parts? Trends. Plant Sci. 18, 539–545. https://doi.org/10.1016/j.tplants.2013.06.004.

Campos-Soriano, L., García-Martínez, J., Segundo, B.S., 2012. The arbuscular mycorrhizal symbiosis promotes the systemic induction of regulatory defence-related genes in rice leaves and confers resistance to pathogen infection. Mol. Plant Pathol. 13, 579–592. https://doi.org/10.1111/j.1364-3703.2011.00773.x.

Cappellari, L.del R., Chiappero, J., Santoro, M.V., Giordano, W., Banchio, E., 2017. Inducing phenolic production and volatile organic compounds emission by inoculating Mentha piperita with plant growth-promoting rhizobacteria. Sci. Hortic. 220, 193–198. https://doi.org/10.1016/j.scienta.2017.04.002.

Cappellari, L.del R., Santoro, M.V., Reinoso, H., Travaglia, C., Giordano, W., Banchio, E., 2015. Anatomical, Morphological, and Phytochemical Effects of Inoculation with Plant Growth- Promoting Rhizobacteria on Peppermint (Mentha piperita). J. Chem. Ecol. 41, 149–158. https://doi.org/10.1007/s10886-015-0549-y.

Chen, M., Arato, M., Borghi, L., Nouri, E., Reinhardt, D., 2018. Beneficial services of arbuscular mycorrhizal fungi – from ecology to application. Front. Plant Sci.. https://doi.org/10.3389/fpls.2018.01270.

Compant, S., Duffy, B., Nowak, J., Clément, C., Barka, E.A., 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol.. https://doi.org/10.1128/AEM.71.9.4951-4959.2005.

Cordovez, V., Dini-Andreote, F., Carrión, V.J., Raaijmakers, J.M., 2019. Ecology and evolution of plant microbiomes. Annu. Rev. Microbiol. 73, 69–88. https://doi.org/10.1146/annurev-micro-090817-062524.

Corpes, R., Monteiro, L., Andrade, E.H., Maia, J.G., Menezes, I., Setzer, W.N., Ramos, A.R., Da Silva, J.K.R., 2019. Comparison of volatile profile and antioxidant activity of Piper divaricatum G. Meyer (Piperaceae) using cuttings and cell tissue. J. Braz. Chem. Soc. 30, 2291–2298. https://doi.org/10.21577/0103-5053.20190128.

Da Luz, S.F.M., Yamaguchi, L.F., Kato, M.J., De Lemos, O.F., Xavier, L.P., Maia, J.G.S., Ramos, A.de R., Setzer, W.N., Da Silva, J.K.do R., 2017. Secondary metabolic profiles of two cultivars of Piper nigrum (black pepper) resulting from infection by Fusarium solani f. sp. piperis. Int. J. Mol. Sci. 18, 2434. https://doi.org/10.3390/ijms18122434.

Darakeh, S.A.S.S., Weisany, W., Tahir, N.A.R., Schenk, P.M., 2022. Physiological and biochemical responses of black cumin to vermicompost and plant biostimulants: Arbuscular mycorrhizal and plant growth-promoting rhizobacteria. Ind. Crops. Prod. 188. https://doi.org/10.1016/j.indcrop.2022.115557.

Da Silva, J., Da Trindade, R., Alves, N., Figueiredo, P., Maia, J., Setzer, W., 2017. Essential oils from neotropical piper species and their biological activities. Int. J. Mol. Sci. 18, 2571. https://doi.org/10.3390/ijms18122571.

Da Silva, J.K.R., Andrade, E.H.A., Guimarães, E.F., Maia, J.G.S., 2010. Essential oil composition, antioxidant capacity and antifungal activity of piper divaricatum. Nat. Prod. Commun. 5, 477–480. https://doi.org/10.1177/1934578×1000500327.

Da Silva, J.K.R., Silva, N.N.S., Santana, J.F.S., Andrade, E.H.A., Maia, J.G.S., Setzer, W.N., 2016. Phenylpropanoid-rich essential oils of Piper species from the Amazon and their antifungal and anti-cholinesterase activities. Nat. Prod. Commun. 11, 1907–1911. https://doi.org/10.1177/1934578×1601101233.

De Almeida, J.D., Alberton, O., Otênio, J.K., Carrenho, R., 2020. Growth of chamomile (Matricaria chamomilla L.) and production of essential oil stimulated by arbuscular mycorrhizal symbiosis. Rhizosphere 15, 100208. https://doi.org/10.1016/j.rhisph.2020.100208.

De Castro, J.A.M., Monteiro, O.S., Coutinho, D.F., Rodrigues, A.A.C., Da Silva, J.K.R., Maia, J.G.S., 2019. Seasonal and circadian study of a thymol/γ-terpinene/p-cymene type oil of ocimum gratissimum l. and its antioxidant and antifungal effects. J. Braz. Chem. Soc. 30, 930–938. https://doi.org/10.21577/0103-5053.20180237.

De Souza, B.C., Da Cruz, R.M.S., Lourenço, E.L.B., Pinc, M.M., Dalmagro, M., Da Silva, C., Nunes, M.G.I.F., De Souza, S.G.H., Alberton, O., 2022. Inoculation of lemongrass with arbuscular mycorrhizal fungi and rhizobacteria alters plant growth and essential oil production. Rhizosphere 22, 100514. https://doi.org/10.1016/J.RHISPH.2022.100514.

Dorman, H.J.D., Deans, S.G., 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88, 308–316. https://doi.org/10.1046/J.1365-2672.2000.00969.X.

Dosoky, N.S., Satyal, P., Barata, L.M., Da Silva, J.K.R., Setzer, W.N., 2019. Volatiles of black pepper fruits (Piper nigrum L.). Molecules. 24, 4244. https://doi.org/10.3390/molecules24234244.

Dudareva, N., Pichersky, E., Gershenzon, J., 2004. Biochemistry of plant volatiles. Plant Physiol. 135, 1893–1902. https://doi.org/10.1104/pp.104.049981.

Fasusi, O.A., Cruz, C., Babalola, O.O., 2021. Agricultural sustainability: microbial biofertilizers in rhizosphere management. Agriculture 11, 163. https://doi.org/10.3390/agriculture11020163.

Ferreira, E.B., Cavalcanti, P.P., Nogueira, D.A., Ferreira, E.B., Cavalcanti, P.P., Nogueira, D.A., 2014. ExpDes: an R package for ANOVA and experimental designs. Appl. Math. (Irvine) 5, 2952–2958. https://doi.org/10.4236/AM.2014.519280.

Fiorilli, V., Wang, J.Y., Bonfante, P., Lanfranco, L., Al-Babili, S., 2019. Apocarotenoids: old and new mediators of the arbuscular mycorrhizal symbiosis. Front. Plant Sci. 10, 1186. https://doi.org/10.3389/fpls.2019.01186.

Goddard, M.-L., Belval, L., Martin, I.R., Roth, L., Laloue, H., Deglène-Benbrahim, L., Valat, L., Bertsch, C., Chong, J., 2021. Arbuscular mycorrhizal symbiosis triggers major changes in primary metabolism together with modification of defense responses and signaling in both roots and leaves of vitis vinifera. Front. Plant Sci. 12, 721614. https://doi.org/10.3389/fpls.2021.721614.

Greenacre, M., 2021. Compositional data analysis. Annu Rev. Stat. Appl. 8, 271–299. https://doi.org/10.1146/ANNUREV-STATISTICS-042720-124436.

Guha, P., Nandi, S., 2019. Essential oil of betel leaf (Piper betle L.): a novel addition to the world food sector. In: Malik, S. (Ed.), Essential Oil Research. Springer, Cham, pp. 149–196. https://doi.org/10.1007/978-3-030-16546-8_5.

Guimarães, E.F., Medeiros, E.V.S.S., Queiroz, G.A., 2015. Piper in Flora e Funga Do Brasil [WWW Document]. Jardim Botânico do Rio de Janeiro.. URL https://floradobrasil.jbrj.gov.br/FB12735 (accessed 8.16.23).
Heil, M., Silva Bueno, J.C., 2007. Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. Proc. Nat. Acad. Sci. 104, 5467–5472. https://doi.org/10.1073/pnas.0610266104.
Helal, D.S., El-khawas, H., Elsayed, T.R., 2022. Molecular characterization of endophytic and ectophytic plant growth promoting bacteria isolated from tomato plants (Solanum lycopersicum L.) grown in different soil types. J. Genet. Eng. Biotechnol. 20. https://doi.org/10.1186/s43141-022-00361-0.
Huang, M., Sanchez-Moreiras, A.M., Abel, C., Sohrabi, R., Lee, S., Gershenzon, J., Tholl, D., 2012. The major volatile organic compound emitted from Arabidopsis thaliana flowers, the sesquiterpene (E)-β-caryophyllene, is a defense against a bacterial pathogen. New Phytol. 193, 997–1008. https://doi.org/10.1111/j.1469-8137.2011.04001.x.
Jelali, N., Dhifi, W., Chahed, T., Marzouk, B., 2011. Salinity effects on growth, essential oil yield and composition and phenolic compounds content of marjoram (Origanum majorana L.) leaves. J. Food Biochem. 35, 1443–1450. https://doi.org/10.1111/J.1745-4514.2010.00465.X.
Jha, C.K., Aeron, A., Patel, B.V., Maheshwari, D.K., Saraf, M., 2011. Enterobacter: Role in Plant Growth Promotion. Bacteria in Agrobiology: Plant Growth Responses. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 159–182. https://doi.org/10.1007/978-3-642-20332-9_8.
Jha, Y., Subramanian, R.B., 2016. Rhizobacteria enhance oil content and physiological status of Hyptis suaveolens under salinity stress. Rhizosphere 1, 33–35. https://doi.org/10.1016/J.RHISPH.2016.07.006.
Johnson, N.C., Jansa, J., 2017. Mycorrhizas: At the Interface of Biological, Soil, and Earth Sciences. Mycorrhizal Mediation of Soil: Fertility, Structure, and Carbon Storage. Elsevier Inc., pp. 1–6. https://doi.org/10.1016/B978-0-12-804312-7.00001-2.
Junker, R.R., Tholl, D., 2013. Volatile organic compound mediated interactions at the plant-microbe interface. J. Chem. Ecol. 39, 810–825. https://doi.org/10.1007/s10886-013-0325-9.
Kassambara, A., Mundt, F., 2017. Factoextra: Extract and Visualize the Results of Multivariate Data Analyses [R package factoextra version 1.0.7] [W WW Document]. URL http://ftp.udc.es/CRAN/web/packages/factoextra/index.html (accessed 8.7.23).
Kato, A., 2024. Isolation and draft genome sequence of Enterobacter asburiae strain i6 amenable to genetic manipulation. J. Genomics. 12, 26–34. https://doi.org/10.7150/jgen.91337.
Kaur, S., Suseela, V., 2020. Unraveling arbuscular mycorrhiza-induced changes in plant primary and secondary metabolome. Metabolites. 10, 1–30. https://doi.org/10.3390/metabo10080335.
Kay, M., Wobbrock, J.O., 2016. Package 'ARTool.' CRAN Repository, Aligned Rank Transform. Association for Computing Machinery. https://doi.org/10.1145/1978942.1978963.
Khalediyan, N., Weisany, W., Schenk, P.M., 2021. Arbuscular mycorrhizae and rhizobacteria improve growth, nutritional status and essential oil production in Ocimum basilicum and Satureja hortensis. Ind. Crops. Prod. 160. https://doi.org/10.1016/j.indcrop.2020.113163.
Kim, D.S., Hwang, B.K., 2014. An important role of the pepper phenylalanine ammonia-lyase gene (PAL1) in salicylic acid-dependent signalling of the defence response to microbial pathogens. J. Exp. Bot. 65, 2295–2306. https://doi.org/10.1093/jxb/eru109.
Kloepper, J.W., Leong, J., Teintze, M., Schroth, M.N., 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286, 885–886. https://doi.org/10.1038/286885a0.
Kramida, A., Ralchenko, Yu., Reader, J., 2011. Atomic Spectra Database | NIST [WWW Document]. National Institute of Standards and Technology. https://doi.org/10.18434/T4W30F.
Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H., Chen, S., 2016. An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules. 21. https://doi.org/10.3390/molecules21101374.
Mafia, R.G., Alfenas, A.C., Ferreira, E.M., Binoti, D.H.B., Mafia, G.M.V., Mounteer, A.H., 2009. Root colonization and interaction among growth promoting rhizobacteria isolates and eucalypts species. Revista Árvore 33, 1–9. https://doi.org/10.1590/S0100-67622009000100001.
Martino, C., Morton, J.T., Marotz, C.A., Thompson, L.R., Tripathi, A., Knight, R., Zengler, K., 2019. A novel sparse compositional technique reveals microbial perturbations. mSystems. 4. https://doi.org/10.1128/MSYSTEMS.00016-19/SUPPL_FILE/MSYSTEMS.00016-19-ST002.XLSX.
Martins, A.S., Alves, C.N., Lameira, O.A., Santos, A.S., Müller, R.C.S., 2009. Minerals evaluation in Amazonian medicinal plants. Revista Brasileira de Farmacognosia 19, 621–625. https://doi.org/10.1590/S0102-695×2009000400020.
Mauch-Mani, B., Baccelli, I., Luna, E., Flors, V., 2017. Defense Priming: An Adaptive Part of Induced Resistance. Annu Rev. Plant Biol. 68, 485–512. https://doi.org/10.1146/annurev-arplant-042916-041132.
Mhlongo, M.I., Piater, L.A., Steenkamp, P.A., Labuschagne, N., Dubery, I.A., 2020. Metabolic profiling of PGPR-treated tomato plants reveal priming-related adaptations of secondary metabolites and aromatic amino acids. Metabolites. 10, 210. https://doi.org/10.3390/metabo10050210.
Miozzi, L., Vaira, A.M., Catoni, M., Fiorilli, V., Accotto, G.P., Lanfranco, L., 2019. Arbuscular mycorrhizal symbiosis: plant friend or foe in the fight against viruses? Front. Microbiol. 10, 1238. https://doi.org/10.3389/fmicb.2019.01238.
Mishra, J., Singh, R., Arora, N.K., 2017. Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. Front. Microbiol. 8, 1706. https://doi.org/10.3389/fmicb.2017.01706.
Monteiro, G., Nogueira, G., Neto, C., Nascimento, V., Freitas, J., 2021. Promotion of nitrogen assimilation by plant growth-promoting rhizobacteria. Nitrogen Agricult. - Physiol., Agricult. Ecol. Aspects. IntechOpen.. https://doi.org/10.5772/intechopen.96634.
Ngadze, E., Icishahayo, D., Coutinho, T.A., Van Der Waals, J.E., 2012. Role of polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, chlorogenic acid, and total soluble phenols in resistance of potatoes to soft rot. Plant Dis. 96, 186–192. https://doi.org/10.1094/PDIS-02-11-0149.
Noceto, P.-A., Bettenfeld, P., Boussageon, R., Hériché, M., Sportes, A., van Tuinen, D., Courty, P.-E., Wipf, D., 2021. Arbuscular mycorrhizal fungi, a key symbiosis in the development of quality traits in crop production, alone or combined with plant growth-promoting bacteria. Mycorrhiza 31, 655–669. https://doi.org/10.1007/s00572-021-01054-1.
Oehl, F., Sieverding, E., Palenzuela, J., Ineichen, K., da Silva, G.A., 2011. Advances in Glomeromycota taxonomy and classification. IMa Fungus. 2, 191–199. https://doi.org/10.5598/imafungus.2011.02.02.10.
Oksanen, A.J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., Hara, R.B.O., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., 2020. Vegan: community ecology package. R Package [WWW Document] URL http://cran.rproject.org/package=vegan (accessed 8.7.23).
Oliveira, J.S., Ramos, N.P., Júnior, J.L., Xavier, L.P., Andrade, E.H., Mello, A.H., Setzer, W.N., Da Silva, J.K.R., 2022. Secondary metabolism and plant growth of Piper divaricatum (Piperaceae) inoculated with arbuscular mycorrhizal fungi and phosphorus supplementation. Agronomy 12, 596.
Oliveira, J.S., Xavier, L.P., Lins, A., Andrade, E.H.A., Maia, J.G.S., de Mello, A.H., Setzer, W.N., Ramos, A.R., da Silva, J.K.R., 2019. Effects of inoculation by arbuscular mycorrhizal fungi on the composition of the essential oil, plant growth, and lipoxygenase activity of Piper aduncum L. AMB Express. 9, 1–12.
Paauw, A., Caspers, M.P.M., Schuren, F.H.J., Leverstein-van Hall, M.A., Delétoile, A., Montijn, R.C., Verhoef, J., Fluit, A.C., 2008. Genomic diversity within the enterobacter cloacae complex. PLoS. One 3. https://doi.org/10.1371/journal.pone.0003018.
Parmar, V.S., Jain, S.C., Bisht, K.S., Jain, R., Taneja, P., Jha, A., Tyagi, O.D., Prasad, A.K., Wengel, J., Olsen, C.E., Boll, P.M., 1997. Phytochemistry of the genus Piper. Phytochemistry 46, 597–673. https://doi.org/10.1016/S0031-9422(97)00328-2.
Pichersky, E., Raguso, R.A., 2018. Why do plants produce so many terpenoid compounds? New Phytologist 220, 692–702. https://doi.org/10.1111/nph.14178.
Pring, B.G., 1982. Isolation and identification of amides from Piper callosum. Synthesis of pipercallosine and pipercallosidine. J. Chem. Soc. Perkin. 1 1, 1493–1498. https://doi.org/10.1039/P19820001493.
Rapado, L.N., Nakano, E., Ohlweiler, F.P., Kato, M.J., Yamaguchi, L.F., Pereira, C.A., Kawano, T., 2011. Molluscicidal and ovicidal activities of plant extracts of the Piperaceae on Biomphalaria glabrata (Say, 1818). J. Helminthol. 85, 66–72. https://doi.org/10.1017/S0022149×10000258.
Redman, R.S., Kim, Y.O., Woodward, C.J.D.A., Greer, C., Espino, L., Doty, S.L., Rodriguez, R.J., 2011. Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: A strategy for mitigating impacts of climate change. PLoS. One 6. https://doi.org/10.1371/journal.pone.0014823.
RStudio Team, 2023. RStudio: integrated development for R.
Saia, S., Amato, G., Frenda, A.S., Giambalvo, D., Ruisi, P., 2014. Influence of arbuscular mycorrhizae on biomass production and nitrogen fixation of berseem clover plants subjected to water stress. PLoS. One 9, e90738. https://doi.org/10.1371/journal.pone.0090738.
Saia, S., Ruisi, P., Fileccia, V., Di Miceli, G., Amato, G., Martinelli, F., 2015. Metabolomics suggests that soil inoculation with arbuscular mycorrhizal fungi decreased free amino acid content in roots of durum wheat grown under N-limited, P-rich field conditions. PLoS. One 10, e0129591. https://doi.org/10.1371/journal.pone.0129591.
Sanz-Biset, J., Cañigueral, S., 2013. Plants as medicinal stressors, the case of depurative practices in Chazuta valley (Peruvian Amazonia). J. Ethnopharmacol. 145, 67–76. https://doi.org/10.1016/J.JEP.2012.09.053.
Schaefer, D.A., Gui, H., Mortimer, P.E., Xu, J., 2021. Arbuscular mycorrhiza and sustainable agriculture. Circul. Agricult. Syst. 1, 1–7. https://doi.org/10.48130/cas-2021-0006.
Schüssler, A., Walker, C., 2010. The Glomeromycota: a species list with new families and new genera.
Schweiger, R., Baier, M.C., Müller, C., 2014. Arbuscular mycorrhiza-induced shifts in foliar metabolism and photosynthesis mirror the developmental stage of the symbiosis and are only partly driven by improved phosphate uptake. Mol. Plant-Microbe Interact. 27, 1403–1412. https://doi.org/10.1094/MPMI-05-14-0126-R.
Sindhu, S.S., Parmar, P., Phour, M., Sehrawat, A., 2016. Potassium-solubilizing microorganisms (KSMs) and its effect on plant growth improvement. Potassium Solubilizing Microorganisms for Sustainable Agriculture. Springer India, New Delhi, pp. 171–185. https://doi.org/10.1007/978-81-322-2776-2_13.
Smith, S.E., Read, D., 2008. Mycorrhizal symbiosis, Mycorrhizal Symbiosis. 3rd ed. Elsevier. https://doi.org/10.1016/B978-0-12-370526-6.X5001-6.
Sousa, C.M.D.M., Silva, H.R.E., Vieira, G.M., Ayres, M.C.C., Da Costa, C.L.S., Araújo, D.S., Cavalcante, L.C.D., Barros, E.D.S., Araújo, P.B.D.M., Brandão, M.S., Chaves, M.H., 2007. Fenóis totais e atividade antioxidante de cinco plantas medicinais. Quim. Nova 30, 351–355. https://doi.org/10.1590/S0100-40422007000200021.
Souto, R.N.P., Harada, A.Y., Andrade, E.H.A., Maia, J.G.S., 2012. Insecticidal activity of piper essential oils from the amazon against the fire ant solenopsis saevissima (smith) (hymenoptera: formicidae). Neotrop. Entomol. 41, 510–517. https://doi.org/10.1007/s13744-012-0080-6.

Stockinger, H., Walker, C., Schüßler, A., 2009. Glomus intraradices DAOM197198", a model fungus in arbuscular mycorrhiza research, is not Glomus intraradices. New Phytologist 183, 1176–1187. https://doi.org/10.1111/j.1469-8137.2009.02874.x.

Takeara, R., Gonçalves, R., Ayres, V.F.dos S., Guimarães, A.C., 2017. Biological properties of essential oils from the piper species of brazil: a review. Aromatic Med. Plants - Back Nature. InTech.. https://doi.org/10.5772/66508.

Tarraf, W., Ruta, C., Tagarelli, A., De Cillis, F., De Mastro, G., 2017. Influence of arbuscular mycorrhizae on plant growth, essential oil production and phosphorus uptake of Salvia officinalis L. Ind. Crops. Prod. 102, 144–153. https://doi.org/10.1016/j.indcrop.2017.03.010.

Vaganan, M.M., Ravi, I., Nandakumar, A., Sarumathi, S., Sundararaju, P., Mustaffa, M.M., 2014. Phenylpropanoid enzymes, phenolic polymers, and metabolites as chemical defenses to infection of Pratylenchus coffeae in roots of resistant and susceptible bananas (Musa spp.). Indian J. Exp. Biol. 52, 252–260.

Van Den Dool, H., Dec Kratz, P., 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. A 11, 463–471. https://doi.org/10.1016/s0021-9673(01)80947-x.

Van Genderen, M.H.P., Leclercq, P.A., Delgado, H.S., Kanjilal, P.B., Singh, R.S., 1999. Compositional analysis of the leaf oils of Piper callosum Ruiz & Pav. from Peru and Michelia montana Blume from India. J. Spectrosc. 14, 51–59. https://doi.org/10.1155/1999/473812.

Varma, A., Prasad, R., Tuteja, N., 2018. Mycorrhiza - Nutrient uptake, biocontrol, ecorestoration: Fourth edition, Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration, 4th Ed. Springer International Publishing. https://doi.org/10.1007/978-3-319-68867-1.

Walia, A., Guleria, S., Chauhan, A., Mehta, P., 2017. Endophytic bacteria: role in phosphate solubilization. pp. 61–93. https://doi.org/10.1007/978-3-319-66544-3_4

Weisany, W., 2024. Arbuscular mycorrhizal fungi inoculation in the modulation of plant yield and bioactive compounds. Biostimulants in Plant Protection and Performance. Elsevier, pp. 255–271. https://doi.org/10.1016/B978-0-443-15884-1.00002-6.

Weisany, W., Raei, Y., Salmasi, S.Z., Sohrabi, Y., Ghassemi-Golezani, K., 2016. Arbuscular mycorrhizal fungi induced changes in rhizosphere, essential oil and mineral nutrients uptake in dill/common bean intercropping system. Ann. Appl. Biol. 169, 384–397. https://doi.org/10.1111/aab.12309.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis, 2nd ed Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-24277-4. Use R!.

Wobbrock, J.O., Findlater, L., Gergle, D., Higgins, J.J., 2011. The aligned rank transform for nonparametric factorial analyses using only ANOVA procedures. Conference on Human Factors in Computing Systems - Proceedings, pp. 143–146. https://doi.org/10.1145/1978942.1978963.

Yilmaz, A., Karik, Ü., 2022. AMF and PGPR enhance yield and secondary metabolite profile of basil (Ocimum basilicum L.). Ind. Crops. Prod. 176, 114327. https://doi.org/10.1016/j.indcrop.2021.114327.

Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M.H., Bahadar, K., 2018. Role of secondary metabolites in plant defense against pathogens. Microb. Pathog. 124, 198–202. https://doi.org/10.1016/j.micpath.2018.08.034.