RESEARCH ARTICLE

Variation in *Peperomia pellucida* growth and secondary metabolism after rhizobacteria inoculation

Nayara Sabrina Freitas Alves¹, Suzana G. Kaory Inoue², Adriana Ribeiro Carneiro¹,², Ulisses Brigatto Albino³, William N. Setzer⁴,⁵, José Guilherme Maia⁶, Eloisa Helena Andrade⁶, Joyce Kelly R. da Silva¹,²,⁶

¹ Programa de Pós-Graduação em Biotecnologia, Universidade Federal do Pará, Belém, Brazil, ² Faculdade de Biotecnologia, Universidade Federal do Pará, Belém, Brazil, ³ Instituto de Ciências Exatas, Universidade Federal do Sul e Sudeste do Pará, Marabá, Brasil, ⁴ Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL, United States of America, ⁵ Aromatic Plant Research Center, Lehi, UT, United States of America, ⁶ Programa de Pós-Graduação em Química, Universidade Federal do Pará, Belém, Brazil

*joycekellys@ufpa.br

Abstract

*Peperomia pellucida* L. Kunth is a herb well-known for its secondary metabolites (SM) with biological potential. In this study, the variations in the SM of *P. pellucida* during association with rhizobacteria were evaluated. Plants were inoculated with *Enterobacter asburiae* and *Klebsiella variicola*, which were identified by sequencing of the 16S rRNA gene. The data were evaluated at 7, 21, and 30-day post inoculation (dpi). Plant-bacteria symbiosis improved plant growth and weight. Total phenolic content and phenylalanine ammonia lyase enzyme activity had a significant increase mainly at 30 dpi. *P. pellucida* was mainly composed of phenylpropanoids (37.30–52.28%) and sesquiterpene hydrocarbons (39.28–49.42%). The phenylpropanoid derivative 2,4,5-trimethoxy-styrene (ArC2), the sesquiterpene hydrocarbon ishwarane, and the phenylpropanoid dillapiole were the major compounds. Principal component analysis (PCA) of the classes and compounds > 2.0% indicated that plants colonized by *E. asburiae* had a reduction in the content of sesquiterpene hydrocarbons and an increase in phenylpropanoids and derivatives. Plants treated with this bacterium also had an increase in the content of 2,4,5-trimethoxystyrene at 30 dpi. Plants inoculated with *K. variicola* had significant increases only in the content of the classes monoterpenoid hydrocarbons and ‘other compounds’ (hydrocarbons, esters, ketones, etc.). These data suggest that the production of plant secondary metabolites can be modified depending on the type of rhizobacteria inoculated.

1. Introduction

Piperaceae is a family of angiosperms composed of approximately 3,700 species from which 2,000 species belong to the genus *Piper* and 1,600 to the group *Peperomia*. The species
Peperomia pellucida (L.) Kunth can be found in the Neotropics, Africa, Southeast Asia, and Oceania [1]. It is well-known for its biological activities such as cytotoxicity (leukemia HL-60, cervical HeLa, and breast MCF-7) [2], fracture healing [3], analgesic [4], antimicrobial [5] and anti-inflammatory [6]. In addition, the plant is used in popular medicine to treat inflammation, hypertension, cough, cardiac arrhythmia, skin bruises, and several other health problems [1].

Peperomia pellucida essential oil (EO) is mainly characterized by the presence of dillapiole (20.7–55.3%) [7,8]. Plants from the Brazilian Amazon, for example, are mostly composed of dillapiole (39.7–55.3%), β-caryophyllene (10.7–14.3%) and carotol (0–8.1%) [7,9]. Its extracts are dominated by phenylpropanoid pathway derivatives with a wide variety of lignans with different skeletons [1,10]. The biosynthesis of secondary metabolites can be influenced by physiological and environmental factors such as temperature, seasonality [11], type of soil [12], salinity [13], and symbiosis with microorganisms [14,15].

Plant growth-promoting rhizobacteria (PGPR) flourish in the rhizosphere of plants by growing inside or around their tissues. These microorganisms are well-known for their potential to fix atmospheric nitrogen [16], solubilize phosphorus and produce siderophores that sequester iron [17], including species of the genera Pseudomonas, Bacillus, Enterobacter and Klebsiella (Section 4). PGPR can act as biofertilizers, increasing the availability and absorption of important minerals [18], and in the biological control of phytopathogens [19] and production of phytohormones [20].

In addition, plant association with bacteria can affect plant secondary metabolism. Specifically, these microorganisms can alter plant essential oil yield and composition, as well as its non-volatile compounds [21,22]. Seedlings of Origanum majorana L. inoculated with Pseudomonas fluorescens had an increase in essential oil (EO) yield from 0.05% to 0.14% [23]. Leaf spraying and micro-injection of P. fluorescens and P. aeruginosa in chickpeas (Cicer arietinum) infected with Sclerotinia sclerotiorum induced phenylalanine ammonia-lyase (PAL) activity and phenolic compounds [24].

Therefore, this study aimed to evaluate changes in the secondary metabolism of P. pellucida during association with rhizobacteria. The bacterial strains EM56 and EM09 were isolated from the rhizosphere of Schizolobium amazonicum Huber ex Ducke (State of Pará, Amazon region, Brazil) and identified by sequencing of the 16S rRNA gene as Enterobacter asburiae strain EM56 and Klebsiella variicola strain EM09. These microorganisms were used in this study because of their potential for nitrogen fixation, phosphate solubilization and plant-growth promotion [25–28]. Additionally, plant inoculation with PGPR can improve both plant growth and change plant secondary metabolism depending on the bacterial species inoculated.

2. Materials and methods

2.1 Plant sample collection and experimental procedure

Peperomia pellucida plants (30–40 days old) were collected at the Universidade Federal do Pará (UFPA), campus Belém, Pará, Brazil, in the location site 1 (UTM coordinate: 22S 9837030 782873) and 2 (UTM coordinate: 22S 9836945 783313). A collection authorization was not required since it is an invasive plant which was not collected in a protected area. The experiment was conducted in a greenhouse located in the Institute of Biological Sciences (ICB) of UFPA under 70% of shading, in the same coordinate of location 1. Inoculated (PpI) and control (PpC) plants were named according to the day of collection: PpI-7 and PpC-7; PpI-21 and PpC-21; and PpI-30 and PpC-30. All analyses were performed at 7, 21 and 30 days post inoculation (dpi). The experiment was conducted according to the following Sections.
2.2 Bacteria isolation

The bacterial strains EM09 and EM56 were isolated from the rhizosphere of ‘paricá da Amazônia’ (Schizolobium amazonicum Huber ex Ducke) present in forest fragments of the territory Transamazônica-Xingu, State of Pará, Brazil. Paricá plants (30–40 cm long) were collected with roots and root soil, stored in plastic bags and maintained on ice. In the laboratory, 10 g of soil was diluted in 9 mL of sterile 0.85% NaCl solution (Sigma-Aldrich Brasil Ltda, São Paulo, Brazil), and 1 mL was used in a serial dilution of $10^{-4}$. Around 50 μL from the forth dilution was plated in two Petri dishes containing Luria-Bertani (LB) agar medium (Sigma-Aldrich Inc., St. Louis, Missouri, USA), using the spread plating method in a vertical laminar flow hood under sterile conditions [29,30].

Plant roots were washed with tap water to remove soil, cut into small pieces of approximately 2 cm, and immersed in 70% ethyl alcohol for 5 minutes to eliminate the external microbiota. The material was rinsed 6 times with sterile distilled water, and 10 g macerated with a sterile glass rod in test tubes containing 9 mL of sterile 0.85% NaCl solution. The material was submitted to a serial dilution of $10^{-4}$ and plated as previously described. Plates were incubated at 28˚C for 24 h [29,30]. All bacteria isolation experiments were performed in biological triplicates.

2.3 DNA extraction, 16S gene PCR and sequencing

The bacterial strains E. asburiae and K. variicola were grown in 10 mL of LB liquid medium and incubated at 28˚C for 24 h. The microbial suspensions were centrifuged at a gravitational force (Force G) of 6000 g at 4˚C for 10 min to obtain the pellet that was used for DNA extraction by the DNeasy Blood and Tissue Kit (Qiagen®). The 16S rRNA gene was amplified using the universal primers 8F (5’AGAGTTTGATCCTGGCTCAG3’) and 1492R (5’TACGGYTACCTTGTTACGACTT3’). PCR was carried out in 50-μL reaction mixtures containing 0.2 mM of DNTP solution, 1.5 mM of MgCl$_2$ solution, 0.2 pmol of each primer, 1 U of Taq DNA polymerase (Invitrogen, Carlsbad, California, USA) and 50 ng of DNA. Cycling conditions had an initial denaturation step (95˚C, 5 min) followed by 35 cycles of annealing (95˚C, 1 min), extension (60˚C, 1 min) and denaturation (72˚C, 1 min). The process was terminated by a final extension step (72˚C, 7 min) [31].

Sequencing was performed by the Sanger method at ACTGene Análises Moleculares, Rio Grande do Sul, Brazil, with a 50 cm capillary sequencer and AB 3500 platform. The sequences were visualized in the program BioEdit and identified through local alignment using the National Center for Biotechnology Information (NCBI) tool named BLAST. The evolutionary history of the strains was evaluated using the program MEGA6 [32] by the neighbor-joining method [33]. The phylogeny test applied was the Bootstrap and the evolutionary distances were calculated by the p-distance model [34].

2.4 Preparation of bacterial inoculum

The strains E. asburiae and K. variicola were individually cultivated in 300 mL of LB medium for 4 h at 28˚C. Approximately 1 mL of each microbial culture was regularly collected from the Erlenmeyer flask in a laminar flow hood and the density measured until the absorbance at 600 nm (OD$_{600}$) reached 0.4, which contained around $10^8$ Colony Forming Units (CFU) per mL [35]. The grown medium was centrifuged at 7600 g for 30 min, the supernatant discarded and the pellet homogenized with 300 mL of sterile 0.85% NaCl solution containing 0.5% of cellulose [36].

2.5 Plant cultivation and inoculation

Peperomia pellucida cuttings containing 3 or 4 nodes and ½ of a leaf were propagated in sterile vermiculite type B (Urimamã Mineração Ltda, Santa Maria da Boa Vista, Brazil). Nutrient
solution (Biofert Root) containing N, P₂O₅, K₂O, S, B, Cl, Cu, Fe, Mn, Mo and Zn was applied at every 15 days. After 30 days of cultivation, plants were inoculated with 5 mL of the bacterial inoculum 3 to 5 cm below the soil surface. Control plants (non-inoculated with bacteria) received only 5 mL of sterile 0.85% NaCl solution with 0.5% cellulose (Section 2.3) [37]. The experiment was performed in vermiculite since plants were supplemented with nutrient solution containing nutrients also important to the microorganisms studied. This strategy has also been used in other similar studies with herbs since their growth conditions are harder to be reproduced in a greenhouse [21,38–40].

2.6 Plant development evaluation
The following plant growth parameters were evaluated: number of leaves, number of nodes, height (cm), root length (cm), leaves and roots fresh biomass (g). Height was measured from the plant collar to the end of the terminal bud of the main branch. Leaf and node numbers were counted in the collection site. Plant leaves and roots were collected in aluminum foil, maintained on ice, weighted and conserved under refrigeration at -20°C.

2.7 Extraction and analysis of volatile compounds
Leaf volatile compounds were extracted by simultaneous distillation using the Likens-Nickerson extractor for 2 h with 3 mL of n-pentane. Aliquots of 1 μL of the resulting organic fraction were analyzed by GC-MS. The qualitative analysis was carried out on a Shimadzu QP2010 plus instrument under the following conditions: Rtx 5MS silica capillary column (30 m × 0.25 mm × 0.25 μm); programmed temperature of 60–240°C (3°C/min); carrier gas helium with velocity of 32 cm/s; type of injection splitless and ionization by electronic impact (70 eV); injector temperature of 250°C; ion source and transfer line temperature of 200°C. The identification of compounds was performed by comparison of mass spectrum and retention index (RI) with data present in the libraries NIST [41] and Adams [42]. The RIs were calculated using a homologous series of n-alkanes (C8–C20, Sigma–Aldrich) [43].

2.8 Determination of total phenolic content (TPC)
The extract fractions from fresh leaves (2 g) were obtained by percolation (96 h) with 50 mL of methanol. After solvent evaporation, the Folin-Ciocalteu method was used to determine total phenolic content (TPC) [44]. The extracts were solubilized again in methanol at a concentration of 20 mg/mL and then diluted 30 times in water because of our samples reactivity. This dilution should be tested for each type of plant sample in order to maintain an absorbance between 0.3 and 0.7. Aliquots of 500 μL of the diluted sample received 250 μL of Folin-Ciocalteu (1 N) and then 1,250 μL of Na₂CO₃ (75 g/L). After 30 min of incubation in the dark, the absorbance was read at 760 nm using a UV-Vis spectrophotometer (Ultrospec 5300 pro, Amersham Biosciences, Little Chalfont, Reino Unido). The experimental calibration curve was prepared using gallic acid. TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg/GAE g⁻¹) [44].

2.9 In vitro phenylalanine ammonia-lyase (PAL) activity
Leaves were frozen and then macerated in liquid nitrogen. An amount of 250 mg of macerated leaves was homogenized in 1 mL of sodium borate buffer solution (0.3 mM, pH 8.8), 1 mM EDTA, 1 mM DTT and 5% polyvinylpyrrolidone. The material was centrifuged at 13,000 g for 20 minutes at 4°C. An aliquot of 0.5 mL of the supernatant was mixed with 1 mL of 0.3 mM sodium borate buffer at pH 8.8 with 0.03 mM L-phenylalanine and incubated for 15
minutes at 25˚C. The activity was evaluated in a UV-Visible spectrophotometer at 290 nm by quantification of (E)-cinnamic acid produced from L-phenylalanine. The blank had only the sodium borate buffer with L-phenylalanine and water [45]. The molar extinction coefficient of (E)-cinnamic acid \((9630\ \text{mol}^{-1}\cdot\text{L}^{-1}\cdot\text{cm}^{-1})\) was applied to determine the enzyme activity [45,46].

### 2.10 Statistical analysis

The experiment was performed in completely randomized blocks with 20 plants for treatment with a total of 120 individuals (60 controls and 60 inoculated). All analyses were performed in triplicate, compared with the control group and expressed as means ± standard deviation. Analyses of variance of plant developmental parameters, PAL enzyme activity, TPC and major volatile compounds were conducted by Bonferroni test, Two-way ANOVA, using the software GraphPad Prism 7.0. Differences at \(p < 0.05\) were considered statistically significant.

Volatile compounds were also submitted to a multivariate analysis using as variables the components with percentages \(\geq 2.0\%\) and the total sum of the classes of compounds (monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, phenylpropanoids and derivatives, and other compounds). The data matrix was standardized by subtracting the mean from each value and dividing it by the standard deviation. Principal Component Analysis (PCA) was performed in the Software Minitab (free version 390, Minitab Inc., State College, PA, USA) [47–49].

### 3. Results

#### 3.1 Identification of the bacteria by 16S rRNA gene sequencing

The bacteria EM56 and EM09 were isolated from roots and soil of *paricá*. The 16S rRNA gene was amplified by PCR and the amplicon sequenced. EM56 and EM09 had a sequence size of 1359 pb and 1393 pb, respectively, which were deposited in the GenBank and assigned with the accession numbers MT279982 and MT279983. Both DNA sequences were submitted to a search for homology on the tool BLAST of the NCBI. EM56 showed 99.85% of similarity with *Enterobacter asburiae* and isolate EM09 indicated 99.93% of similarity with *Klebsiella variicola*. The phylogenetic analysis was performed to show the bacterial species and their relation with other microorganisms (Fig 1). Both microorganisms are well-known for their potential for nitrogen fixation, phosphate solubilization and siderophore production [27,50].

![Phylogenetic tree of the bacteria](https://doi.org/10.1371/journal.pone.0262794.g001)

**Fig 1.** Phylogenetic tree of the bacteria *Enterobacter asburiae* strain EM09 and *Klebsiella variicola* strain EM56 based on sequencing of the 16S rRNA gene. The analysis contained 9 nucleotide sequences with 1,354 positions in the final data set. The *Escherichia coli* sequence was used as an external group. The scale bar represents approximately 5 base substitutions per 1000 nucleotide positions.
3.2 Comparative analysis of plant development

Plants inoculated with *E. asburiae* (Table 1) displayed an increase of 24.82% and 34.48% in the number of leaves and leaf weight at 21 dpi, respectively. Nonetheless, at 30 dpi there was a reduction of 13.20% in the number of leaves and an increase of 61.10% in their weight since they became larger. There was a rise in the number of nodes at 21 and 30 dpi (46.70% and 32.40%). However, no significant change was found for plant height, which indicates that inoculated plants had shorter internodes. The parameter root length was not affected by bacteria colonization, but root weight increased by 66.70% at 21 dpi and 85.70% at 30 dpi.

Plants inoculated with *K. variicola* exhibited an increase of 32.80% and 19.80% in the number of leaves at 21 and 30 dpi, respectively (Table 2). However, leaf weight was only improved at 30 dpi (50.0%). Furthermore, the number of nodes increased by 52.80% at 21 dpi and by 75.60% at 30 dpi. Height was improved in all days analyzed (18.30%, 30.30% and 25.0%). There was also a 33.30% increase in root length and a 200.0% increase in root weight at 21 dpi. No significant changes were observed at 30 dpi.

3.3 Phenylalanine ammonia-lyase (PAL) activity

Plant inoculation induced a higher production of PAL enzyme in the leaves. Propagules colonized by *E. asburiae* had an increase in the unit of enzyme/mL of extract of 34.0% and 38.0% at 21 and 30 dpi (23.0–30.80 and 29.0–40.0 μU/mL, respectively) (Fig 2A). Similarly, herbs treated with *K. variicola* had a rise of 36.80% (18.35–25.10 μU/mL) and 55.32% (19.25–29.90 μU/mL) in the enzyme activity at 7 and 30 dpi, respectively (Fig 2B).

3.4 Total phenolic determination

The concentration of total phenolic compounds in leaf extracts was improved by both inoculations. Plants treated with *E. asburiae* displayed an increase of 11.40% and 30.50% in comparison to control groups at 21 and 30 dpi (26.40–29.40 and 26.90–35.10 mg EAG/g of extract, respectively) (Fig 3A). *Peperomia pellucida* colonized by *K. variicola* had an increase in TPC of 31.20% at 21 dpi (21.50–28.20 mg) and of 30.0% at 30 dpi (24.0–31.20 mg EAG/g of extract) (Fig 3B).

| Table 1. Developmental parameters of *Peperomia pellucida* after inoculation with *Enterobacter asburiae*. |
|---|---|---|---|---|---|---|---|---|
| dpi | Treatment | Leaves (n*) | CV value (%) | Nodes (n*) | CV value (%) | Height (cm) | CV value (%) | Root (cm) | CV value (%) | Fresh weight–leaves (g) | CV value (%) | Fresh weight–root (g) | CV value (%) |
| 07 | PpC | 24.3±1.8 | 7.3 | 17.0±1.2 | 7.2 | 20.1±1.2 | 5.8 | 9.8±0.8 | 8.3 | 0.6±0.1 | 8.6 | 0.2±0.1 | 35.5 |
| | PpIE | 26.3±4.5 | 17.1 | 19.7±1.2 | 6.3 | 18.3±1.0 | 5.6 | 11.5±0.8 | 6.5 | 1.4±0.3* | 23.3 | 0.3±0.1 | 19.5 |
| 21 | PpC | 71.7±4.2 | 5.8 | 46.0±2.2 | 4.7 | 36.8±0.8 | 2.3 | 13.3±1.3 | 9.8 | 2.9±0.1 | 2.7 | 0.9±0.1 | 6.1 |
| | PpIE | 89.5±0.4* | 0.5 | 67.5±1.2* | 1.8 | 34.5±3.4 | 9.8 | 14.8±1.0 | 6.9 | 3.9±0.1* | 2.7 | 1.5±0.4* | 29.4 |
| 30 | PpC | 75.7±3.3* | 4.4 | 39.5±2.9 | 7.2 | 36.8±2.5 | 6.7 | 14.8±1.4 | 9.7 | 1.8±0.3 | 17.4 | 0.7±0.1 | 20.1 |
| | PpIE | 65.7±2.4 | 3.6 | 52.3±2.4* | 4.5 | 36.7±0.6 | 1.7 | 15.2±2.0 | 13.3 | 2.9±0.2* | 7.6 | 1.3±0.2* | 13.3 |
| DF values | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |

*mean ± standard deviation (n = 3).
*Statistically different according to Bonferroni-test (p < 0.05).
dpi: Days post inoculation. PpC: *P. pellucida* control. PpIE: *P. pellucida* inoculated with *Enterobacter asburiae*. CV: Coefficient of variation. DF: Degree of freedom. Note: Each variable is followed by its CV value.

https://doi.org/10.1371/journal.pone.0262794.t001
3.5 Analysis of the volatile compounds

The analysis of the volatile compounds indicated 24 and 53 compounds for plants inoculated with *E. asburiae* and *K. variicola*, respectively (Tables 3 and 4). From 93.3 to 100.0% of the components were identified with a predominance of phenylpropanoids and derivatives (37.30–52.28%), followed by sesquiterpene hydrocarbons (39.28–49.42%). The components with contents above 2% were the phenylpropanoid dillapiole (16.72–24.39%), the phenylpropanoid derivative (ArC2) 2,4,5-trimethoxystyrene (18.03–31.35%), and the sesquiterpene hydrocarbons ishwarane (19.37–27.58%), β-caryophyllene (9.69–12.21%), β-elemene (0.83–3.54%) and (E,E)-α-farnesene (2.93–6.56%).

The percentage of the four major compounds dillapiole, 2,4,5-trimethoxystyrene, ishwarane and β-caryophyllene were submitted to an analysis of variance and a significant variation was observed only for plants inoculated with *E. asburiae*. This included the compound ishwarane.

### Table 2. Developmental parameters of *Peperomia pellucida* after inoculation with *Klebsiella variicola*.

| dpi | Treatment | Evaluation parameters † |
|-----|-----------|-------------------------|
|     |           | Leaves (n°) | CV value (%) | Nodes (n°) | CV value (%) | Height (cm) | CV value (%) | Root (cm) | CV value (%) | Fresh weight–leaves (g) | CV value (%) | Fresh weight–root (g) | CV value (%) |
| 07  | PpC       | 24.0±1.6   | 6.8          | 17.0±1.4  | 8.3         | 17.5±1.1  | 6.2         | 8.3±0.2  | 2.8         | 1.1±0.1             | 9.5         | 0.2±0.03              | 14.2        |
|     | PpIK      | 31.7±0.5   | 1.5          | 22.7±1.2  | 5.5         | 20.7±1.3  | 6.4         | 10.4±1.0 | 0.9         | 1.9±0.2             | 8.8         | 0.4±0.1              | 19.0        |
| 21  | PpC       | 53.7±2.9   | 5.3          | 43.0±7.9  | 18.3        | 19.8±0.2  | 1.0         | 10.2±1.9 | 19.0        | 2.0±0.1             | 4.4         | 0.2±0.2              | 10.4        |
|     | PpIK      | 71.3±5.4   | 7.6          | 65.7±8.5  | 12.9        | 25.8±0.5  | 1.8         | 13.6±1.6 | 11.8        | 2.8±0.5             | 19.0        | 0.6±0.1*              | 15.5        |
| 30  | PpC       | 76.3±10.6  | 13.9         | 39.7±7.3  | 18.4        | 26.8±1.3  | 5.0         | 11.2±0.5 | 4.3         | 2.8±0.1             | 2.3         | 0.6±0.1              | 18.9        |
|     | PpIK      | 91.4±2.7   | 3.0          | 69.7±4.0  | 5.8         | 33.5±0.4  | 1.2         | 13.3±0.5 | 3.5         | 4.2±0.8*            | 17.8        | 0.8±0.2              | 23.8        |
|     | DF values | 2.0         | 2.0          | 2.0        | 2.0         | 2.0        | 2.0         | 2.0       | 2.0         | 2.0                  | 2.0         | 2.0                   | 2.0         |

§mean ± standard deviation (n = 3).
†Statistically different according to Bonferroni-test (p < 0.05). dpi: Days post inoculation. PpC: *P. pellucida* control. PpIK: *P. pellucida* inoculated with *Klebsiella variicola*. CV: Coefficient of variation. DF: Degree of freedom. Note: Each variable is followed by its CV value.

https://doi.org/10.1371/journal.pone.0262794.t002

Fig 2. Variation in the PAL enzyme activity in plants inoculated (n = 3) with *Enterobacter asburiae* (A) and *Klebsiella variicola* (B). *Statistically different according to Bonferroni test (p < 0.05). dpi: Days post inoculation. PpC: *P. pellucida* control. PpIE: *P. pellucida* inoculated with *Enterobacter asburiae*. PpIK: *P. pellucida* inoculated with *Klebsiella variicola*.

https://doi.org/10.1371/journal.pone.0262794.g002
which decreased at 7 (27.51–24.85%) and 30 dpi (27.58–23.11%), and 2,4,5-trimethoxystyrene, which was increased by 20.0% (26.09–31.35%) at 30 dpi (Fig 4A and 4B).

### 3.6 Multivariate analysis of the volatile composition of plants inoculated with *Enterobacter asburiae* and *Klebsiella varicola*

PCA (Principal Component Analysis) analyses were performed using as variables the total percentages of the majority classes and the constituents identified in the volatile fraction (content ≥ 2.0%).

PC1 and PC2 of the compound classes accounted for 85.7% of the data variance (Fig 5). PC1 separated samples inoculated with *E. asburiae* (negative loadings) from samples treated with *K. varicola* (positive loadings). Plants colonized by *E. asburiae* and the control samples were divided into four groups named from Groups E-I to IV and displayed similar loadings at 7 and 21 dpi. However, inoculated samples at 30 dpi (E-IV) had the greatest distance from its respective control group (E-III) with loadings of -0.57 in PC1 and 1.76 in PC2 which are mainly related to the reduction in the amount of sesquiterpenes hydrocarbons (49.42–42.83%) and increase in the phenylpropanoids and derivatives concentrations (47.35–52.07%) (Table 3).

Plants treated with *K. varicola* at 7 dpi and all control groups of this experiment formed a single group with negative loadings in PC2 and positive in PC1 (Group K-I). The samples PPIK-21 and PPIK-30 were individually separated from all the control samples and PPIK-7. The group PPIK-21 exhibited positive loadings in both PC1 and PC2 (2.84 and 1.63) while PPIK-30 had positive loading in PC1 and negative in PC2 (3.56 and -0.30), which are related to the increase in the concentration of monoterpen hydrocarbons (0.10–0.62% and 0.13–1.26%) and ‘other compounds’ (hydrocarbons, esters, ketones, etc.) (4.74–10.80% and 5.77–9.46%) in comparison to the controls, respectively. The sample PPIK-30 was also close to the Group K-I because of their similar content in oxygenated sesquiterpenes (Table 4).

PC1 and PC2 analysis of the volatile compounds (content ≥ 2.0%) comprised 79.3% of the total variability and separated plants colonized with *E. asburiae* and *K. varicola* in PC1 into positive and negative loadings, respectively (Fig 6).

The experiment carried out with *E. asburiae* effectively separated inoculated samples from the control samples. The Group E-I (PpC-7, PpC-21 and PpC-30) had a predominance of the phenylpropanoid dillapiole (22.45%, 22.33%, and 20.31%) and the sesquiterpene hydrocarbons...
Table 3. Comparison of volatile compounds produced in leaves of Peperomia pellucida inoculated and non-inoculated with Enterobacter asburiae.

| Compounds                  | RI(C) | RI(L) | PpIE 7 dpi | PpC 7 dpi | PpIE 21 dpi | PpC 21 dpi | PpIE 30 dpi | PpC 30 dpi |
|----------------------------|-------|-------|------------|-----------|-------------|------------|-------------|------------|
| (E)-β-Ocimene              | 1042  | 1044a | 0.05±0.07  | 0.45±0.39 | 0.27±0.19   | 0.71±0.38  | 0.24±0.34   |
| 4-Methyldecane             | 1053  | 1051a | 0.24±0.10  | 0.08±0.11 | 0.13±0.10   | 0.15±0.05  | 0.30±0.03   | 0.15±0.04  |
| α-Undecane                 | 1103  | 1100a | 0.15±0.21  | 0.16±0.18 | 0.13±0.11   |            |             |            |
| Hexyl butanoate            | 1194  | 1191a | 0.08±0.12  |           |             |            |             |            |
| δ-Decanal                  | 1208  | 1201a | 0.65±0.20  | 0.60±0.10 | 1.02±0.45   | 0.53±0.11  | 1.26±0.21   | 0.81±0.38  |
| Octyl acetate              | 1214  | 1211a | 1.07±0.10  | 1.38±0.29 | 1.34±0.32   | 1.00±0.10  | 1.78±0.44   | 1.00±0.41  |
| 4,6-Dimethyldecane         | 1281  | 1285a | 0.14±0.05  | 0.15±0.02 | 0.05±0.04   | 0.05±0.05  | 0.14±0.02   | 0.08±0.03  |
| Hexyl hexanoate            | 1386  | 1382a | 0.06±0.08* |           |             |            |             |            |
| Butanoic acid              | 1389  | 1381a | 0.08±0.11* |           |             |            |             |            |
| β-Elemene                  | 1395  | 1389a | 2.02±0.20  | 0.98±0.69 | 2.62±0.53   | 0.83±1.14  | 2.29±0.11   | 2.08±0.04  |
| β-Caryophyllene            | 1424  | 1417a | 10.31±0.23 | 11.43±1.11| 10.14±0.77  | 11.44±1.11| 10.37±0.91  | 11.70±0.88 |
| α-Humulene                 | 1459  | 1452a | 0.31±0.05  | 0.28±0.04 | 0.51±0.20   | 0.53±0.20  | 0.41±0.02   | 0.42±0.03  |
| Ishwarane                  | 1469  | 1465a | 24.85±0.72 | 27.51±0.20| 26.06±1.39  | 27.44±1.21| 23.11±0.20  | 27.58±0.36 |
| Germacrene D               | 1487  | 1484a | 0.82±0.09  | 0.67±0.34 | 0.94±0.20   | 1.49±0.57  | 0.79±0.06   | 1.07±0.05  |
| Aristolochene              | 1490  | 1487a | 0.08±0.11* |           |             |            |             |            |
| Valencene                  | 1499  | 1495a | 0.40±0.56* |           |             |            |             |            |
| γ-Aromphene                | 1499  | 1495a | 0.59±0.12  | 0.26±0.19 | 0.47±0.38   | 0.46±0.37  | 0.70±0.03   | 0.70±0.01  |
| Pentadecane                | 1503  | 1500a | 0.05±0.04  | 0.06±0.05 | 0.07±0.06   | 0.10±0.00  | 0.06±0.04   |            |
| (E,E)-α-Farnesene          | 1512  | 1505a | 6.07±0.47  | 3.10±0.80 | 6.56±1.53   | 6.19±2.81  | 5.16±0.62   | 5.87±0.02  |
| Myristicin                 | 1525  | 1517a | 1.29±0.22  | 0.25±0.20 | 0.43±0.43   | 0.31±0.44  | 1.09±0.56   | 0.90±0.13  |

RI(C): Retention index calculated; RI(L): Retention index of library; a: Adams; n; NIST. *Compounds with low representativeness. **Identification tentative—See Section 4 for more details. dpi: Days post inoculation. PpC: P. pellucida control (non-inoculated). PpIE: P. pellucida inoculated with Enterobacter asburiae (n = 3).

2,4,5-Trimethoxystyrene** 1566 1621n 28.44±1.67 29.58±1.27 27.57±2.10 25.21±2.13 31.35±0.98 26.09±0.84
Carotol                    1604 1594a 0.36±0.03 0.35±0.15 0.52±0.34 0.73±0.16 0.68±0.06 0.87±0.03
Dillapiole                 1629 1620a 22.01±1.23 22.45±0.14 19.87±1.11 18.96±0.98 19.57±1.19 20.31±0.27
Apiole                     1685 1677a 0.12±0.12 0.10±0.07 0.06±0.05 0.05±0.07
Monoterpe hydrocarbons     | 0.05±0.00 | 0.45±0.00 | 0.27±0.00 | 0.71±0.00 | 0.24±0.00 |
Sesquiterpe hydrocarbons   | 44.97±8.25 | 44.23±4.90 | 47.78±8.03 | 48.38±9.19 | 42.83±7.68 | 49.42±9.17 |
Oxygenated sesquiterpenes  | 0.36±0.00 | 0.35±0.00 | 0.52±0.00 | 0.73±0.00 | 0.68±0.00 | 0.87±0.00 |
Phenylpropanoids and derivatives 51.74±11.58 52.28±12.49 47.99±12.03 44.58±11.16 52.07±13.13 47.35±11.55
Others                     2.10±0.37 2.41±0.47 2.60±0.55 2.18±0.30 3.71±0.66 2.10±0.40
Total (%)                  99.22 99.27 99.34 99.39 96.14 100.00 99.98

RI(C): Retention index calculated; RI(L): Retention index of library; a: Adams; n; NIST. *Compounds with low representativeness. **Identification tentative—See Section 4 for more details. dpi: Days post inoculation. PpC: P. pellucida control (non-inoculated). PpIE: P. pellucida inoculated with Enterobacter asburiae (n = 3).

https://doi.org/10.1371/journal.pone.0262794.t003

ishwarane (27.5%, 27.4%, and 27.6%) and β-caryophyllene (11.43%, 11.44%, and 11.70%). Group E-II, composed by inoculated samples (PpIE-7, PpIE-21 and PpIE-30), was mainly characterized by an initial increase in the content of the sesquiterpenes hydrocarbon (E,E)-α-farnesene and decrease at 30 dpi (5.89–5.16%). In addition, the samples had an increase in the concentration of the phenylpropanoid derivative (ArC2) 2,4,5-trimethoxystyrene mainly at 30 dpi (26.09–31.35%), which corroborate with the results presented in the Section 3.5. These compounds had positive loadings in PC1 and PC2.

Plants colonized with K. variicola and its respective controls were distributed into two groups: Group K-I was composed of PpC-7, PpI-7 and PpC-21 and characterized by the predominance of β-caryophyllene (12.21%, 12.14%, and 11.34%) while Group K-II, consisting of
Table 4. Comparison of volatile compounds produced in leaves of *Peperomia pellucida* inoculated and non-inoculated with *Klebsiella variicola*.

| Compounds                  | RI(C) | RI(L) | 7 dpi | 21 dpi | 30 dpi |
|----------------------------|-------|-------|-------|--------|--------|
| n-Octane                   | 814   | 800*  | 0.29±0.20 | 0.39±0.13 | 2.53±2.80 | 0.36±0.27 | 2.56±1.27 | 0.86±0.40 |
| (2E)-Hexenal               | 841   | 846*  | 0.14±0.10 | 0.18±0.21 | 0.07±0.06 |
| 2-Methyloctane             | 854   | 852*  | 0.29±0.30 | 0.23±0.19 | 0.10±0.06 |
| n-Nonane                   | 897   | 900*  | 0.07±0.10 | 1.12±1.58 | 1.45±0.65 | 0.51±0.42 |
| None                       | 928   | 924*  | 0.20±0.29* |
| Tetrahydrocitronellene     | 935   | 930*  | 0.06±0.09 | 0.39±0.15 | 0.08±0.06 |
| 4-Methylnonane             | 949   | 951*  | 0.05±0.07* |
| Mesitylene                 | 993   | 994*  | 0.10±0.14* | 0.12±0.06* |
| n-Decane                   | 998   | 1000* | 0.30±0.43 | 0.42±0.14 | 0.14±0.11 |
| Acetophenone               | 1021  | 1029* | 0.28±0.23* |
| (E)-β-Ocimene              | 1046  | 1044* | 0.12±0.09 | 0.24±0.34 | 0.56±0.33 | 0.10±0.02 | 0.88±0.45 | 0.05±0.04 |
| 4-Methyldecane             | 1055  | 1051* | 0.75±0.07 | 0.66±0.18 | 0.89±0.47 | 0.74±0.11 | 0.13±0.03 | 0.41±0.27 |
| 2-Methyldecane             | 1061  | 1051* | 0.16±0.01 | 0.15±0.03 | 0.06±0.08 | 0.18±0.03 | 0.09±0.07 |
| n-Octanol                  | 1067  | 1063* | 0.17±0.05 | 0.23±0.28 | 0.25±0.04 | 0.16±0.11 |
| n-Undecane                 | 1102  | 1100* | 0.67±0.01 | 0.71±0.12 | 0.75±0.24 | 0.70±0.12 | 0.40±0.06 | 0.50±0.31 |
| Naphthalene                | 1182  | 1178* | 0.34±0.06 | 0.32±0.11 | 0.37±0.28 | 0.40±0.06 | 0.20±0.16 |
| Hexyl butanoate            | 1191  | 1191* | 0.07±0.05* | 0.06±0.04* |
| n-Decanal                  | 1205  | 1201* | 0.84±0.08 | 1.25±0.11 | 1.14±0.56 | 0.44±0.13 | 1.24±0.21 | 0.66±0.18 |
| Capryl acetate             | 1210  | 1214* | 0.82±1.16 | 0.70±0.99 | 0.56±0.46 |
| Octyl acetate              | 1211  | 1211* | 1.17±0.16 | 1.39±0.16 | 0.68±0.48 | 0.74±0.22 | 0.92±0.65 | 0.37±0.30 |
| Isoamyl hexanoate          | 1252  | 1246* | 0.11±0.10* |

RI(C): Retention index calculated; RI(L): Retention index of library; a: Adams; n: NIST. *Compounds with low representativeness. † Identification tentative–See Section 4 for more details. dpi: Days post inoculation. PpC: *P. pellucida* control (non-inoculated). PpIK: *P. pellucida* inoculated with *Klebsiella variicola* (n = 3).

(Continued)
#### Table 4. (Continued)

| Compound                          | Retention index calculated; RI(C): | Retention index of library; RI(L): | Others |
|-----------------------------------|------------------------------------|-----------------------------------|---------|
| Myristicin                        | 1522                               | 1517*                             | 0.13±0.37|
| 2,4,5-Trimethoxystyrene**         | 1586                               | 1582*                             | 0.25±0.10|
| Caryophyllene oxide               | 1586                               | 1582*                             | 0.25±0.10|
| Hexanoic acid                     | 1583                               | 1580*                             | 0.37±0.12|
| Carotol                           | 1600                               | 1594*                             | 1.26±0.38|
| Dillapiol                         | 1626                               | 1620*                             | 1.26±0.38|
| 14-Hydroxy-9-epi-(E)-caryophyllene| 1672                               | 1668*                             | 1.26±0.38|
| Apiole                            | 1683                               | 1677*                             | 0.57±0.20|
| Total (%)                         |                                     | 97.26                             | 97.84   |

RI(C): Retention index calculated; RI(L): Retention index of library; a: Adams; n: NIST. *Compounds with low representativeness. **Identification tentative—See Section 4 for more details. dpi: Days post inoculation. PpC: *P. pellucida* control (non-inoculated). PpIK: *P. pellucida* inoculated with *Klebsiella variicola* (n = 3).

4. Discussion

The genera *Enterobacter* and *Klebsiella* are well-known for their potential for plant growth promotion and biocontrol of agricultural diseases [51–57]. *E. asburiae* was reported to improve maize [58], peppers, lettuces, cucumbers and tomatoes development [26] while *K. variicola* is well-known for promoting soybean [59], maize [60] and wheat growth [61]. Both PpI-21, PpC-30 and PpI-30, stood out mainly for the similar concentration of β-elemene (3.06%, 3.54%, and 3.49%). These groups’ formation indicates that there was no significant difference in the contents of the components of plants inoculated with *K. variicola*.

![Figure 4](https://doi.org/10.1371/journal.pone.0262794.g004)
E. asburiae and K. variicola indicated potential to increase sugarcane growth [25,28]. E. asburiae strain EM56 and K. variicola strain EM09 were both isolated from S. amazonicum rhizosphere and used to inoculate P. pellucida because PGPR can be applied in all groups of plants. However, these microorganisms may have different effects on plant development and secondary metabolism.
Bacteria inoculation caused significant variations in *P. pellucida* developmental parameters. Specimens colonized by *E. asburiae* had the number of leaves reduced at 30 dpi while leaf weight was increased at 21 and 30 dpi. *K. variicola* caused a rise in the number of leaves at 21 and 30 dpi and improved leaf weight at 30 dpi. Tomato seedlings (*Solanum lycopersicum* L.) treated with different *Streptomyces* spp. (Stm) strains had no changes in the number of leaves but displayed a rise in leaf weight at 30 dpi [62]. Likewise, the inoculation of *Bacillus subtilis* in *Ocimum basilicum* increased leaf fresh weight at 30 dpi [14].

Furthermore, *E. asburiae* inoculation increased the number of nodes at 21 and 30 dpi but had no significant effect on height. Plant symbiosis with *K. variicola* enhanced the number of nodes at 21 and 30 dpi and plant height in all days analyzed. Similarly, marjoram seedlings (*Origanum majorana* L.) treated with *Pseudomonas fluorescens* and *Bradyrhizobium* sp. exhibited an improvement of 33.80% and 23.20% in the number of nodes, respectively [23]. The association of *B. subtilis* with *O. basilicum* also improved plant height by 16 mm at 14 dpi [14]. Plants colonized by *E. asburiae* had root weight increased at 21 and 30 dpi, but root length was not affected. The second inoculation caused a growth in root length and weight at 21 dpi, but no significant changes were found at 30 dpi. *Vigna radiata* (L.) R.Wilczek colonized by *P. aeruginosa* and *B. subtilis* showed a growth of 84.6% and 61.9% on root length and 369.1% and 239.8% on its fresh weight at 35 dpi, respectively [63]. Hydroponic beans (*Vigna radiata*) associated with *Enterobacter sp. P36* exhibited a raise of 64.20% in root weight [64].

These rhizobacteria potentials to improve plant growth can be explained by the presence of several genes with plant-beneficial functions. The genome of *K. variicola* was reported to contain *nif* cluster, indole-3-pyruvate decarboxylase (*ipdC*), siderophore enterobactin synthesis genes (*entABCDEF*) and enterobactin exporter gene (*ents*), and pyrroloquinoline quinone synthesis genes (*pqqBCDEF*), which are responsible for its N$_2$ fixation, indole-3-acetic acid (IAA) production, siderophore production, and phosphate solubilization properties [27]. *E. asburiae* was also found to have genes involved in N$_2$ fixation, auxin synthesis (*iaa* and *ipdC*), phosphorus metabolism and siderophore biosynthesis. All these genes together promote plant-bacteria communication and symbiosis [50,65].

The enzyme PAL plays an important role in inducing plant defense responses [66,67] since it is the first enzyme in the phenylpropanoid metabolic pathway which converts the amino acid phenylalanine into (E)-cinnamic acid [67,68]. Since it is involved in the regulation of this metabolic pathway, PAL can cause accumulation of lignins and phytoalexins that induce disease resistance [69]. The inoculation of *P. pellucida* with *E. asburiae* and *K. variicola* increased enzymatic activity mainly at 30 dpi (Fig 2). Similarly, *Mentha piperita* inoculated and co-inoculated with different rhizobacteria species showed a growth of approximately 300% in the enzyme activity [39].

Phenolic compounds are secondary metabolites produced by the shikimate pathway and pentose phosphate pathway through the metabolization of phenylpropanoids [70–72]. They are well known for their antioxidant, antibacterial, antifungal, and UV protection activities. They can also act as defense agents in plants [72,73]. Inoculated propagules showed a growth in TPC at 21 and 30 dpi (Fig 3). Likewise, specimens of chickpea (*Cicer arietinum* L.) inoculated and co-inoculated with *P. fluorescens* and *P. aeruginosa* displayed an increase in phenolic content in various growth stages [74]. These compounds have the potential to induce seed germination and improve plant development [75–79]. They can also contribute to plant defense against phytopathogens and can act as signaling molecules for symbiont recognition [39,80–82]. The increase in PAL activity and TPC may be related to both of their roles in inducing plant defense responses since colonization by symbiotic microorganisms may be initially recognized as a pathogen infection, causing a biotic stress [66,83].
The occurrence of dillapiole, 2,4,5-trimethoxystyrene, and β-caryophyllene as the major compounds of *P. pellucida* has been previously reported. For instance, EOs of plants collected in the northern region of Brazil had dillapiole (39.70–55.30%) and β-caryophyllene (10.70–14.3%) as some of their main components [1, 7, 9]. The compound 2,4,5-trimethoxystyrene was reported for *P. pellucida* collected in the Philippines [84], Belém [85] and São Paulo, in Brazil [86]. In this study, 2,4,5-trimethoxystyrene retention index (RI) of library (1621, NIST) was much higher than the RI calculated (1566), which probably happened because the library value was defined using a DB-1 capillary column. However, the RI values 1565 [87], 1551 [88] and 1552 [89] have also been reported for this compound. The indices found in these studies are much closer to our calculated RI, which explains this tentative compound identification (Tables 3 and 4).

The analysis of variance of the major compounds showed significant changes only for plants colonized by *E. asburiae* which had a decrease in the concentration of ishwarane and an increase in 2,4,5-trimethoxystyrene (Fig 4). The growth in the phenylpropanoid derivative content was an unexpected finding since monoterpene and sesquiterpene contents are usually the ones affected by microorganism inoculation [21, 38, 90]. The increase in this compound content may not have been reported for plant-microbe symbiosis, but 2,4,5-trimethoxystyrene could be involved in plant-defense since it has insecticidal activity [91].

The compound classes and the components with percentages ≥ 2.0% were submitted to multivariate analysis (Figs 5 and 6). Plants inoculated with *E. asburiae* were mainly characterized by the decrease and increase of sesquiterpene hydrocarbons and phenylpropanoids and derivatives at 30 dpi, respectively (Fig 5 and Table 3). The compound 2,4,5-trimethoxystyrene had positive loadings in both components (PC1 and PC2) and contributed the most in the separation of inoculated samples from control samples mainly at 30 dpi (Fig 6), which confirms the results expressed by the analysis of variance (Fig 4). Likewise, individuals colonized by *K. variicola* had a predominance of monoterpene hydrocarbons and ‘other compounds’ such as hydrocarbons, esters, ketones, and so on (Fig 5 and Table 4). The contents of compounds ≥ 2.0% were not affected by this bacterial colonization in comparison to the control groups (Fig 6).

The compound dillapiole is a phenylpropanoid that has antioxidant, antimicrobial, insecticidal, antitumor and anti-inflammatory activity [92–95]. Although it has been reported as the main component of *P. pellucida* EOs occurring in northern Brazil [1, 7, 9], plant colonization by *E. asburiae* and *K. variicola* did not affect its concentration. This probably happened because plant colonization by symbiotic microorganisms usually affects species rich in terpenes [14, 21, 38, 90]. Similar effects have also been observed after herbivore attack [96–98]. These organisms affect the content of plant compounds by upregulating the expression of genes related to terpenoids, phenylpropanoids and other classes of compounds metabolic pathways [99].

Terpenes are characterized by having basic isoprene structures (C5) and are toxic substances that can stop herbivore attack [72]. Components of this group are usually related to plant defense mechanisms during colonization and infection [72, 100] since they have insecticidal, fungicidal and antibacterial activity [101–104]. This study showed improvements in the concentrations of some classes of terpenes after bacteria symbiotic association with *P. pellucida*. It also indicated that plant colonization by rhizobacteria may increase phenylpropanoids contents since there was a rise in the concentration of the phenylpropanoid derivative 2,4,5-trimethoxystyrene.
5. Conclusions

The inoculation of *P. pellucida* with *E. asburiae* strain EM56 and *K. variicola* strain EM09 proved to be an efficient alternative to promote plant growth in this species since these microorganisms improved plant development. Furthermore, these bacteria increased PAL enzyme activity and total phenolic content which are both related to plant defense mechanisms during biotic stress.

*E. asburiae* inoculation caused an increase mainly in the content of 2,4,5-trimethoxystyrene, while *K. variicola* inoculation did not show any significant variations in the concentrations of the major compounds. Both inoculations affected the classes of terpenes, but only *E. asburiae* treatment increased the content of the class of phenylpropanoids and derivatives. These data show that the production of secondary metabolites in *P. pellucida* can be optimized by rhizobacteria inoculation, but factors such as the microorganism, the plant species and the plant chemical profile should be considered. The next step of this research will be the inoculation of both bacteria since it could improve even more plant growth and the production of secondary metabolites.

Supporting information

S1 File.
(DOCX)

S2 File.
(XLSX)

S1 Graphical abstract.
(TIF)

Acknowledgments

The authors are thankful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES—Coordination for the Improvement of Higher Education Personnel) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq—National Council for Scientific and Technological Development) for providing a scholarship to N.S.F. Alves and S. G.K. Inoue, respectively.

Author Contributions

**Conceptualization:** Nayara Sabrina Freitas Alves, Joyce Kelly R. da Silva.

**Data curation:** Nayara Sabrina Freitas Alves, Eloisa Helena Andrade.

**Formal analysis:** Nayara Sabrina Freitas Alves, Suzana G. Kaory Inoue, Adriana Ribeiro Carneiro, Ulisses Brigatto Albino, William N. Setzer, Eloisa Helena Andrade, Joyce Kelly R. da Silva.

**Funding acquisition:** Adriana Ribeiro Carneiro, José Guilherme Maia, Joyce Kelly R. da Silva.

**Investigation:** Nayara Sabrina Freitas Alves, Suzana G. Kaory Inoue, William N. Setzer, Joyce Kelly R. da Silva.

**Methodology:** Nayara Sabrina Freitas Alves, Suzana G. Kaory Inoue, Adriana Ribeiro Carneiro, Ulisses Brigatto Albino.

**Project administration:** Joyce Kelly R. da Silva.
Resources: Adriana Ribeiro Carneiro.

Supervision: Joyce Kelly R. da Silva.

Writing – original draft: Nayara Sabrina Freitas Alves.

Writing – review & editing: Nayara Sabrina Freitas Alves, William N. Setzer, José Guilherme Maia, Joyce Kelly R. da Silva.

References

1. Alves NSF, Setzer WN, Da Silva JKR. The chemistry and biological activities of Peperomia pellucida (Piperaceae): A critical review. J Ethnopharmacol. 2019; 232: 90–102. https://doi.org/10.1016/j.jep.2018.12.021 PMID: 30562552

2. Xu S, Li N, Ning M, Zhou C, Yang Q, Ming-Wei W. Bioactive compounds from Peperomia pellucida. J Nat Prod. 2006; 69: 247–250. https://doi.org/10.1021/np050457s PMID: 16499324

3. Florence NT, Huguette STS, Hubert DJ, Raceline GK, Desire DDP, Pierre K, et al. Aqueous extract of Peperomia pellucida (L.) HBK accelerates fracture healing in Wistar rats. BMC Complement Altern Med. 2017; 17: 1–9. https://doi.org/10.1186/s12906-016-1505-2 PMID: 28049463

4. Aziba PI, Adedeji A, Ekor M, Adeyemi O. Analgesic activity of Peperomia pellucida aerial parts in mice. Fitoterapia. 2001; 72: 57–58. https://doi.org/10.1016/s0367-326x(00)00249-5 PMID: 11163942

5. Khan MR, Omoloso AD. Antibacterial activity of Hygrophila stricta and Peperomia pellucida. Fitoterapia. 2002; 73: 251–254. https://doi.org/10.1016/s0367-326x(02)00066-7 PMID: 12048020

6. Arrigoni-Blank MDF, Dmitrieva EG, Franzotti EM, Antoniolli AR, Andrade MR, et al. Anti-inflammatory and analgesic activity of Peperomia pellucida (L.) HBK (Piperaceae). J Ethnopharmacol. 2004; 91: 215–218. https://doi.org/10.1016/j.jep.2003.12.030 PMID: 15120441

7. De Lira PN, Da Silva JK, Andrade EH, Sousa PJC, Maia JGS. Essential oil composition of three Peperomia species from the Amazon, Brazil. Na. Prod Comm. 2009; 4: 427–430. https://doi.org/10.1077/1934578X090040323 PMID: 19413127

8. Verma RS, Padalia RC, Goswami P, Chauhan A. Essential oil composition of Peperomia pellucida (L.) Kunth from India. J Essent Oil Res. 2014; 27: 1–7. https://doi.org/10.1080/10412905.2014.982878

9. Da Silva MHL, Zoghbi MDGB, Andrade EHA, Maia JGS. The essential oils of Peperomia pellucida Kunth and P. circinnata (L.) Link var. circinnata. Flavour Fragr J. 1999; 14: 312–314. https://doi.org/10.1002/(SICI)1099-1026(19990910)14:5<312::AID-FFJ835>3.0.CO;2-B

10. Parmar VS, Jain SC, Bishit KS, Jain R, Taneja P, Jha A, et al. Phytochemistry of the genus Piper. Phytochemistry. 1997; 46: 597–673. https://doi.org/10.1016/S0031-9422(97)00328-2

11. De Castro JAM, Monteiro OS, Coutinho DF, Rodrigues AAC, Da Silva JKR, Maia JGS. 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. 2019; 30: 930–938. https://doi.org/10.21577/0103-5053.20180237

12. Hendawy SF, Hussein MS, Amer HM, El-Gohary AE, Soliman WS. Effect of soil type on growth, productivity, and essential oil constituents of rosemary, Rosmarinus officinalis. Asian J Agri & Biol. 2017; 5: 303–311.

13. Jelali N, Dhifi W, Chaheed T, Marzouk B. Salinity effects on growth, essential oil yield and composition and phenolic compounds content of marjoram (Origanum majorana L.) leaves. J Food Biochem. 2011; 35: 1443–1450. https://doi.org/10.1111/j.1745-4514.2010.00465.x

14. Banchio E, Xie X, Zhang H, Pare PW. Soil bacteria elevate essential oil accumulation and emissions in sweet basil. J Agr Food Chem. 2009; 57: 653–657. https://doi.org/10.1021/jf802035p PMID: 19128010

15. Da Trindade R, Almeida L, Xavier L, Lins AL, Andrade EH, Maia JG, et al. Arbuscular mycorrhizal fungi colonization promotes changes in the volatile compounds and enzymatic activity of lipooxygenase and phenylalanine ammonia lyase in Piper nigrum L. ‘Bragantina’. Plants. 2019; 8: 1–15. https://doi.org/10.3390/plants8110442 PMID: 31652848

16. Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil. 2009; 321: 305–339. https://doi.org/10.1007/s11104-009-9895-2

17. Glick BR, Patten CL, Holguin G, Penrose DM. Biochemical and genetic mechanisms used by plant growth-promoting bacteria. 1st ed. London, UK: Imperial College Press; 1999.
18. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 2003; 255: 571–586. https://doi.org/10.1023/A:1026037216893

19. Meng Q, Hanson LE, Douches D, Hao JJ. Managing scab diseases of potato and radish caused by Streptomyces spp. using Bacillus amyloliquefaciens BAC03 and other biomaterials. Biol Control. 2013; 67: 373–379. https://doi.org/10.1016/j.biocontrol.2013.09.009

20. Patel T, Saraf M. Biosynthesis of phytohormones from novel rhizobacteria isolates and their in vitro plant growth-promoting efficacy. J Plant Interact. 2017; 12: 480–487. https://doi.org/10.1080/17429145.2017.1392625

21. Banchio E, Bogino PC, Santoro M, Torres L, Zygadlo J, Giordano W. Systemic induction of monoterpene biosynthesis in Origanum × majoricum by soil bacteria. J Agr Food Chem. 2010; 58: 650–654. https://doi.org/10.1021/jf9030629 PMID: 20000572

22. Santoro MV, Bogino PC, Nocelli N, Cappellari LDR, Giordano WF, Banchio E. Analysis of plant growth-promoting effects of fluorescent Pseudomonas strains isolated from Mentha piperita rhizosphere and effects of their volatile organic compounds on essential oil composition. Front Microbiol. 2016; 7: 1–17. https://doi.org/10.3389/fmicb.2016.00001 PMID: 26834723

23. Banchio E, Bogino PC, Zygadlo J, Giordano W. Plant growth promoting rhizobacteria improve growth and essential oil yield in Origanum majorana L. Biochem Syst Ecol. 2008; 36: 766–771. https://doi.org/10.1016/j.bse.2008.08.006

24. Basha SA, Sarma BK, Singh DP, Annapurna K, Singh UP. Differential methods of inoculation of plant growth-promoting rhizobacteria induce synthesis of phenylalanine ammonia-lyase and phenolic compounds differentially in chickpea. Folia Microbiol. 2006; 51: 463–468. https://doi.org/10.1007/s00374-013-0878-3

25. Jetiyanon K. Multiple mechanisms of Enterobacter asburiae strain RS83 for plant growth enhancement. Songklanakarin J. Sci. Technol. 2015; 37: 29–36. https://doi.org/10.3390/microorganisms8060948 PMID: 32599701

26. Lin L, Wei C, Chen M, Wang H, Li Y, Li Y, et al. Complete genome sequence of endophytic nitrogen-fixing Klebsiella variicola strain DX120E promotes sugarcane growth. Biol. Fertil. 2014; 50: 657–666. https://doi.org/10.1007/s00374-013-0783-3

27. Silva RLO, Luz JS, Da Silveira EB, Cavalcante UMT. Fungos endofílicos em Annona spp.: isolamento, caracterização enzimática e promoção do crescimento em mudas de pinha (Annona squamosa L.). Acta Bot Bras. 2006; 20: 649–655. https://doi.org/10.1590/S0102-33062006000300015

28. Khan FM, Inamul H., Nimatullah TS, Muhammad F, Ohia C, Tauseef A. Isolation, characterization and identification of plant growth promoting rhizobacteria from cauliflower (Brassica oleracea). Arch. Basic Appl. Med. 2018; 6: 1–15.

29. Freitas DY, Araújo S, Folador ARC, Ramos RTJ, Azevedo JSN, Tacão M, et al. Extended spectrum beta-lactamase-producing Gram-negative bacteria recovered from an Amazonian lake near the city of Belem, Brazil. Front Microbiol. 2019; 10: 1–13. https://doi.org/10.3389/fmicb.2019.00001 PMID: 30728808

30. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013; 30: 2725–2729. https://doi.org/10.1093/molbev/mst197 PMID: 24132122

31. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4: 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454 PMID: 3447015

32. Nei M, Kumar S. Molecular Evolution and Phylogenetics. New York, USA: Oxford University Press; 2000.

33. Kim W, Conery AL, Rajamuthirah R, Fuchs BB, Ausubel FM, Mylonakis E. Identification of an antimicrobial agent effective against methicillin-resistant Staphylococcus aureus persisters using a fluorescence-based screening strategy. PLoS One 2015; 10: 1–15. https://doi.org/10.1371/journal.pone.0127640 PMID: 26039584

34. Mafia RG, Alfenas AC, Maffia LA, Ferreira EM, Binoti DHB, Mafia GMV. Plant growth promoting rhizobacteria as agents in the biocontrol of eucalyptus mini-cutting rot. Trop Plant Pathol. 2009; 34: 10–17. https://doi.org/10.1590/2178-56762009001000002
37. Kang AL, Khan AL, Hamayun M, Shinwari ZK, Kim YH, Joo GJ, et al. *Acinetobacter calcoaceticus* ameliorated plant growth and influenced gibberellins and functional biochemical. Pak J Bot. 2012; 44: 365–372.

38. Cappellari LDR, Santoro MV, Nievas F, Giordano W, Banchio E. Increase of secondary metabolite content in marigold by inoculation with plant growth-promoting rhizobacteria. Appl Soil Ecol. 2013; 70: 16–22. https://doi.org/10.1016/j.apsoil.2017.04.002

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

40. Cappellari LR, Santoro MV, Schmidt A, Gershenson J, Banchio E. Induction of essential oil production in *Mentha × piperita* by plant growth promoting bacteria was correlated with an increase in jasmonate and salicylate levels and a higher density of glandular trichomes. Plant Physiol. Biochem. 2019; 141: 142–153. https://doi.org/10.1016/j.plaphy.2019.05.030 PMID: 31163341

41. NIST (National Institute of Standards and Technology). Mass Spectral Library. The NIST Mass Spectrometry Data Center. 2011 [Cited 2021 July 30]. Available from: https://www.nist.gov/ (accessed on 11 April 2020).

42. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. 4th ed. Carol Stream, USA: Allured Publishing Corporation; 2007.

43. Van Den Dool H, Kratz PD. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. J Chromatogr A. 1963; 11: 463–471. https://doi.org/10.1016/S0021-9673(01)80947-X

44. Singleton VL, Rossi JA. Colorimetric of total phenolic s with phosphomolybdic-phosphotungs tic acid reagents. Am J Enol Vitic. 1965; 16: 144–158.

45. Vaganan MM, Ravi I, Nandakumar A, Sarumathi S, Sundararaju P, Mustaffa MM. Phenylpropanoid enzymes, phenolic polymers and metabolites as chemical defenses to infection of *Pratylenchus coco*ae in roots of resistant and susceptible bananas (*Musa spp.*). Indian J Exp Biol. 2014; 52: 252–260. PMID: 24696668

46. Dickerson D.P., Pascholati SF, Hagerman A.E., Butler L.G., Nicholson R.L. Phenylalanine ammonia-lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. Physiol. Plant Pathol. 1984, 25, 111–123. https://doi.org/10.1016/0048-4059(84)90050-x

47. Pearson K. On lines and planes of closest fit to systems of points in space. Philos Mag. 1901; 2: 559–572. https://doi.org/10.1080/14786440109462720

48. Abdi H, Williams LJ. Principal component analysis. WIREs Comp Stats. 2010; 2: 433–459. https://doi.org/10.1002/wics.101

49. Figueiredo PLB, Pinto LC, Da Costa JS, Da Silva ARC, Moura RHV, Montenegro RC, et al. Composition, antioxidant capacity and cytotoxic activity of *Eugenia uniflora* L. chemotytes-oils from the Amazon. J. Ethnopharmacol. 2019; 232: 30–38. https://doi.org/10.1016/j.jep.2018.12.011 PMID: 30543916

50. Yaish MW. Draft genome sequence of endophytic bacterium *Enterobacter asburiae* PDA134, isolated from date palm (*Phoenix dactylifera* L.) roots. Genome Announc. 2016; 4: 1–2. https://doi.org/10.1128/genomeA.0048-16 PMID: 27540071

51. Jha CK, Aeron A, Patel BV, Maheshwari DK, Saraf M. *Enterobacter*: role in plant growth promotion. In: Maheshwari DK, editor. Bacteria in agrobiology: Plant growth responses. Berlin, Germany: Springer; 2011. pp. 159–182. https://doi.org/10.1007/978-3-642-20332-9_8

52. Khalifa AY, Aisyehem AM, Almalki MA, Saleh FA. Characterization of the plant growth promoting bacterium, *Enterobacter cloacae* MSR1, isolated from roots of non-nodulating *Medicago sativa*. Saud. J Biol Sci. 2016; 23: 79–86. https://doi.org/10.1016/j.sjbs.2015.06.008 PMID: 26858542

53. Mazumdar D, Saha SP, Ghosh S. *Klebsiella pneumoniae* rs26 as a potent PGPR isolated from chick-pea (*Cicer arietinum*) rhizosphere. Pharma Innovation. 2018; 7: 56–62.

54. Liu D, Chen L, Zhu X, Wang Y, Xuan Y, Liu X, et al. *Klebsiella pneumoniae* SnbE/YK mediates resistance against *Heterodera glycines* and promotes soybean growth. Front Microbiol. 2018; 9: 1–13. https://doi.org/10.3389/fmicb.2018.00001 PMID: 29403456

55. Rajkumari J, Singha LP, Pandey P. Genomic insights of aromatic hydrocarbon degrading *Klebsiella pneumoniae* AWD5 with plant growth promoting attributes: a paradigm of soil isolate with elements of biodegradation. 3 Biotech. 2018; 8: 1–22. https://doi.org/10.1007/s13205-017-1019-8 PMID: 29201587

56. Ludueña LM, Anzuay MS, Angelini JG. McIntosh M, Becker A, Rupp O., et al. Genome sequence of the endophytic strain *Enterobacter* sp. J49, a potential biofertilizer for peanut and maize. Genomics. 2019; 111: 913–920. https://doi.org/10.1016/j.ygeno.2018.05.021 PMID: 29857118
Changes in Peperomia pellucida after rhizobacteria inoculation

57. Ye J, Kostrynska M, Dunfield K, Warriner K. Evaluation of a biocontrol preparation consisting of Enterobacter asburiae AX1 and a lytic bacteriophage cocktail to suppress the growth of Salmonella javiana associated with tomatoes. J Food Prot. 2009; 72: 2284–2292. https://doi.org/10.4315/0362-028X-72.11.2284 PMID: 19903390

58. Ogbo F, Okonkwo J. Some characteristics of a plant growth promoting Enterobacter sp. isolated from the roots of maize. Adv Microbiol. 2012; 2: 368–374. https://doi.org/10.4172/aim.2012.23046

59. Kim AY, Shahzad R, Kang SM, Seo CW, Park YG, Park HJ, et al. IAA-producing Klebsiella variicola AY13 reprograms soybean growth during flooding stress. J. Crop Sci. Biotechnol. 2017; 20: 235–242. https://doi.org/10.1007/s12892-017-0041-0

60. Yang L, Yang K. Biological function of Klebsiella variicola and its effect on the rhizosphere soil of maize seedlings. Peer J. 2020; 8: 1–24. https://doi.org/10.7717/peerj.9894 PMID: 32995084

61. Kusale SP, Attar YC, Sayyed RZ, Malek RA, Ilyas N, Suriani NL, et al. Production of plant beneficial and antioxidant metabolites by Klebsiella variicola under salinity stress. Molecules 2021; 26: 1–16. https://doi.org/10.3390/molecules26071894 PMID: 33810565

62. Dias MP, Bastos MS, Xavier VB, Cassel E, Astartita LV, Santarém ER. Plant growth and resistance promoted by Streptomyces spp. in tomato. Plant Physiol Bioch. 2017; 118: 479–493. https://doi.org/10.1016/j.plaphy.2017.07.017 PMID: 28756346

63. Kumari P, Meena M, Gupta P, Dubey MK, Nath G, Upadhyay RS. Plant growth promoting rhizobacteria and their biopriming for growth promotion in mung bean (Vigna radiata L.) R. Wilczek. Biocatal Agric Biotechnol. 2018; 16: 163–171. https://doi.org/10.1016/j.cbcar.2018.07.030

64. Sharaff M, Kamat S, Archana G. Analysis of copper tolerant rhizobacteria from the industrial belt of Gujarat, western India for plant growth promotion in metal polluted agriculture soils. Ecotoxicol Environ Saf. 2017; 138: 113–121. https://doi.org/10.1016/j.ecoenv.2016.12.023 PMID: 28038338

65. François CJ, Batinovic S, Petrovski S, Gendall AR. Draft genome sequence of Enterobacter asburiae NCR1, a plant growth-promoting rhizobacteria isolated from a cadmium-contaminated environment. Microbiol. Resour. Announc. 2021; 10: 1–2. https://doi.org/10.1128/MRA.00478-21 PMID: 34472973

66. Compan S, Duffy B, Nowak J, Clément C, Barka EA. Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. Appl Environ Microbiol. 2005; 71: 4951–4959. https://doi.org/10.1128/AEM.71.9.4951-4959.2005 PMID: 16151072

67. Kim DS, Hwang BK. 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. 2014; 65: 2295–2306. https://doi.org/10.1093/jxb/eru109 PMID: 24642849

68. Yamada S, Nabe K, Izuo N, Nakamichi K, Chibata I. Production of L-phenylalanine from trans-cinnamic acid with Rhodotorula glutinis containing L-phenylalanine ammonia-lyase activity. Appl Environ Microbiol. 1981; 42: 773–778. https://doi.org/10.1128/aem.42.5.773-778.1981 PMID: 16345879

69. Abhayashree MS, Murali M, Thriveni MC, Sindhu GM, Amruthesh KN. Crude oligosaccharides mediated resistance and histo-chemical changes in Colletotrichum capsici infected Vigna radiata. Plant Biosyst. 2016; 151: 221–233. https://doi.org/10.1080/11263504.2016.1150361

70. Randhir R, Lin YT, Shetty K. Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. Process Biochem. 2004; 39: 637–646. https://doi.org/10.1016/S0032-9592(03)00197-3

71. Michalak A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Pol J Environ Stud. 2006; 15: 523–530.

72. Zaynab M, Fatima M, Abbas S, Sharif Y, Umair M, Zafar MH, et al. Role of secondary metabolites in plant defense against pathogens. Microb Pathog. 2018; 124: 198–202. https://doi.org/10.1016/j.micpath.2018.08.034 PMID: 30145251

73. Lin JT, Liu SC, Hu CC, Shyu YS, Hsu CY, Yang DJ. Effects of roasting temperature and duration on fatty acid composition, phenolic composition, Maillard reaction degree and antioxidant attribute of almond (Prunus dulcis) kernel. Food Chem. 2016; 190: 520–528. https://doi.org/10.1016/j.foodchem.2015.06.004 PMID: 26213005

74. Singh UP, Sarma BK, Singh DP. Effect of plant growth-promoting rhizobacteria and culture filtrate of Sclerotium rolfsii on phenolic and salicylic acid contents in chickpea (Cicer arietinum). Curr. Microbiol. 2003; 46: 131–140. https://doi.org/10.1007/s00284-002-3834-2 PMID: 12520369

75. Tudose I. Dynamics of accumulation of polyphenolic compounds in Vitis vinifera and some aspects of their bioactive properties. Doctorate thesis, Technical University of Lasi. 2002.

76. Stingu A, Volf I, Popa IV. Spruce bark extract as modulator in rape plant copper bioaccumulation. Cellul Chem Technol. 2010; 45: 281–86.
77. Tanase C, Vintu S, Volf I, Popa IV. Potential applications of wastes from energy and forestry industry in plant tissue culture. Cellul Chem Technol. 2013; 47: 553–63.

78. Tanase C, Oroian S, Cosarca S, Popa IV. Wastes from forestry and energy industries as potential bioregulators in soybean (Glycine max L.) plants. Cellul Chem Technol. 2016; 50: 529–34.

79. Tanase C, Bujor OC, Popa VI. Phenolic natural compounds and their influence on physiological processes in plants. In: Watson R.R., editor. Polyphenols in Plants. 2nd ed. Cambridge, USA: Academic Press; 2019. pp. 45–58. https://doi.org/10.1016/B978-0-12-817368-0.00003–7

80. Naczk M, Shahidi F. Phenolics in cereals, fruit and vegetables: occurrence, extraction and analysis. J Pharm Biomed Anal. 2006; 41: 1523–42. https://doi.org/10.1016/j.jpb.2006.04.002 PMID: 16753277

81. Dai J, Mumper JR. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. J Agric Food Chem. 2008; 56: 5713–22. https://doi.org/10.1021/jf802140c PMID: 19069929

82. Asensi M, Ortega A, Mena S, Feddi F, Estrela JM. Natural polyphenols in cancer therapy. Crit Rev Clin Lab Sci. 2011; 48: 197–216. https://doi.org/10.3109/10408363.2011.631268 PMID: 22141580

83. Ngadze E, Icishahayo D, Coutinho TA, Van Der Waals JE. Role of polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, chlorogenic acid, and total soluble phenols in resistance of potatoes to soft rot. Plant Dis. 2012; 96: 186–192. https://doi.org/10.1094/PDIS-02-11-0149 PMID: 30731807

84. Manalo JB, Han BH, Han YH, Park MH, Anzaldo FE. Studies on ether-soluble neutral compounds of Peperomia pellucida. Arch Pharm Res. 1983; 6: 133–136. https://doi.org/10.1007/BF02857191

85. Bayma DCJ, Arruda MSP, Müller AH, Arruda AC, Arruda HJ. A dimeric ArC2 compound from Peperomia pellucida. Phytochemistry 2000; 55: 779–782, 2000. https://doi.org/10.1016/S0031-9422(00)00224-7 PMID: 11190395

86. Moraes MM. de. Biossíntese da pellucidina A em Peperomia pellucida (L.) HBK. Doctorate thesis, Universidade de São Paulo. 2016.

87. Ribeiro LP, Domingues VC, Gonçalves GL, Fernandes JB, Gloria EM, Vendramim JD. Essential oil from Duguetia lanceolata St.-Hil. (Annonaceae): Suppression of spoilers of stored-grain. Food Biosci. 2020; 36: 1–9. https://doi.org/10.1016/j.ibio.2020.100653

88. Saldanha AA, Vieira L, Ribeiro RMA, Thorné RG, HB Dos Santos, Silva DB, et al. Chemical composition and evaluation of the anti-inflammatory and antinociceptive activities of Duguetia furfuracea essential oil: Effect on edema, leukocyte recruitment, tumor necrosis factor alpha production, iNOS expression, and adenosinergic and opioidergic systems. J Ethnopharmacol. 2019; 231: 325–336. https://doi.org/10.1016/j.jep.2018.11.017 PMID: 30445104

89. De Oliveira JSF, Xavier LP, Lins A, Andrade EHA, Maia JGS, De Mello AH, et al. Effects of inoculation by arbuscular mycorrhizal fungi on the composition of the essential oil, plant growth, and lipoxygenase activity of Piper aduncum. AMB Express. 2019; 9: 1–12. https://doi.org/10.1186/s13568-018-0728-7 PMID: 30607514

90. Koon P, Bouda H. Activity of 2, 4, 5-trimethoxystyrene from Pachypodium staudtii against two stored product pests. Trop Sci. 2004; 44: 120–123. https://doi.org/10.1002/ts.151

91. Bernard CB, Krishnamurty HG, Chauret D, Durst T, Philogene BJR, Sanchez-Vindas P, et al. Insecticidal defenses of Piperaceae from the Neotropics. J Chem Ecol. 1995; 21: 801–814. https://doi.org/10.1007/BF02985719

92. Zebelo SA, Bertea CM, Bossi S, Occhipinti A, Gnawi G, Maffei ME. Chrysolina herbacea modulates terpenoid biosynthesis of Mentha aquatica. L. PLoS One. 2011; 6: 1–10. https://doi.org/10.1371/journal.pone.0017195 PMID: 21408066

93. Parise-Filho R, Pastrello M, Camerlingo CEP, Silva GJ, Agostinho LA, De Souza T, et al. The anti-inflammatory activity of diallilole and some semisynthetic analogues. Pharm Biol. 2011; 49: 1173–1179. https://doi.org/10.3109/13880209.2011.575793 PMID: 22014265

94. Banchio E, Zygadlo J, Valladares G. Quantitative variations in the essential oil of Minthostachys mollis (Kunth.) Griseb. in response to insects with different feeding habits. J Agric Food Chem. 2005; 53: 6903–6906. https://doi.org/10.1021/jf051157j PMID: 16104818

95. Bayma DCJ, Arruda MSP, Muller AH, Arruda AC, Canto WC. A dimeric ArC2 compound from Peperomia pellucida. Phytochemistry 2000; 55: 779–782, 2000. https://doi.org/10.1016/S0031-9422(00)00224-7 PMID: 11190395
98. Suárez-Vidal E, Sampedro L, Voltas J, Serrano L, Notivon E, Zas R. Drought stress modifies early effective resistance and induced chemical defences of Aleppo pine against a chewing insect herbivore. Environ Exp Bot. 2019; 162: 550–559. https://doi.org/10.1016/j.envexpbot.2019.04.002

99. Etalo DW, Jeon JS, Raaijmakers JM. Modulation of plant chemistry by beneficial root microbiota. Nat. Prod. Rep. 2018; 35: 398–409. https://doi.org/10.1039/c7np00057j PMID: 29722774

100. Harrewijn P, Van Oosten AM, Piron PGM. Natural Terpenoids as Messengers: A multidisciplinary Study of their Production, Biological Functions, and Practical Applications. 1st ed. Netherlands: Springer Science & Business Media; 2001.

101. Sangwan NS, Farooqi AHA, Shabih F, Sangwan RS. Regulation of essential oil production in plants. Plant Growth Regul. 2001; 24: 3–21. https://doi.org/10.1023/A:1013386921596

102. Nikkon F, Habib RM, Karim R, Motiur ZF, Ekramul MR, Haque M. Insecticidal activity of flower of Tagetes erecta L. against Tribolium castaneum (Herbst). J Agric Biol Sci. 2009; 5: 748–753.

103. Brazão MAB, Brazao FV, Guilherme J, Monteiro MC. Antibacterial activity of the Piper aduncum oil and dillapiole, its main constituent, against multidrug-resistant strains. B Latinoam Caribe Pl. 2014; 13: 517–526.

104. Vizcaíno-Páez S, Pineda R, García C, Gil J, Durango D. Metabolism and antifungal activity of saffrole, dillapiole, and derivatives against Botryodiplodia theobromae and Colletotrichum acutatum. B. Latinoam. Caribe Pl. 2016; 15: 1–17.