



The LifeWatch ERIC Biodiversity
& Ecosystem eScience Conference

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Biostimulants' Impact on the *Lippia dulcis* Antioxidant Capacity

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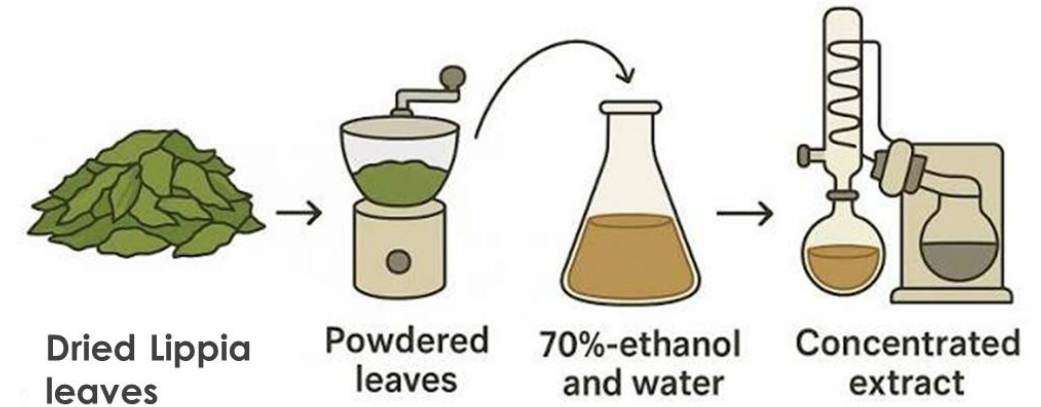
Lippia dulcis Trevir. (*Phyla dulcis*)

- *L. dulcis* is an aromatic herb native to parts of Mexico, Central America, and the Caribbean and belonging to the tropical plant family Verbenaceae.
- *L. dulcis* has green-purple leaves and can propagate low to the ground by stolons.
- It is frost-tender and grows best as a perennial in tropical/subtropical climates, or as an annual/indoor plant in colder regions.
- Prefers full sun to partial shade in moist, well-draining soil.



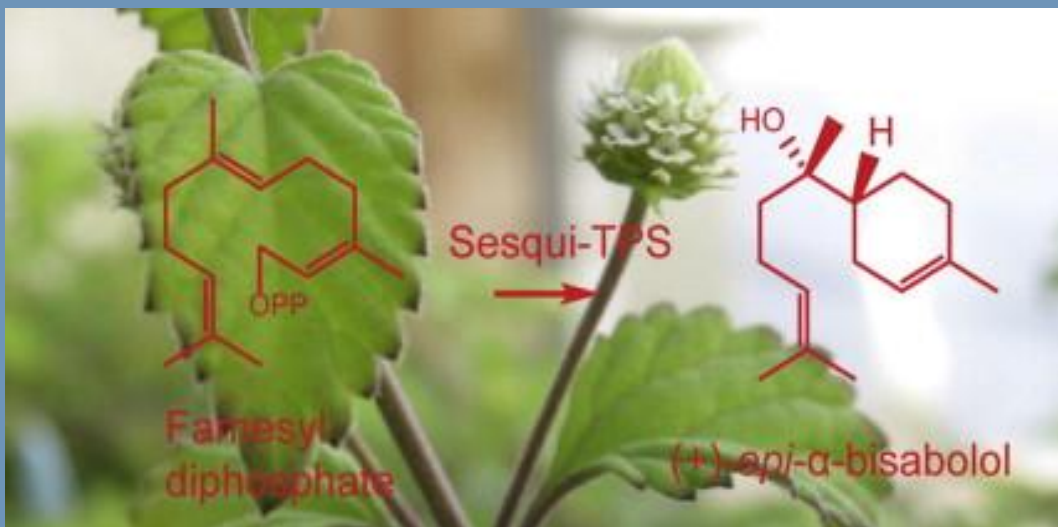
Main medicinal properties

- Anti-inflammatory effect: ethanol extracts significantly reduce swelling and tissue inflammation.
- Digestive system: Infusions of the leaves are used in South America to relieve stomach pain, colic, indigestion and diarrhea.
- Antimicrobial effect: Laboratory tests show that the plant helps fight some gram-positive bacteria that cause respiratory infections.
- Obstetrics: abortifacient
- Respiratory diseases: It is used as an expectorant for persistent cough, whooping cough, asthma, acute colds and chronic bronchitis.



⚠ *Lippia dulcis* is not suitable for regular use as a sugar substitute. The essential oil in the leaves contains large amounts of camphor. In large doses or with prolonged use, camphor is highly toxic to the nervous system, can cause seizures, and is especially dangerous for children and pregnant women. The herb should only be taken in minimal amounts and for short periods.

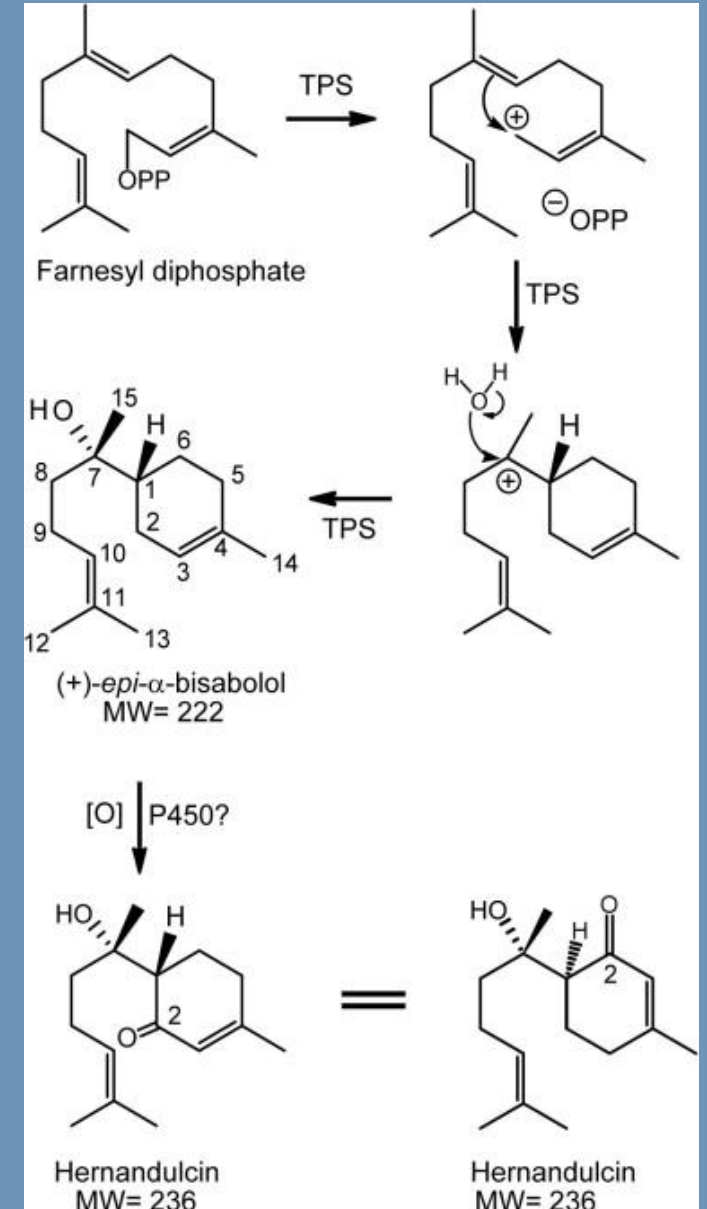
The metabolic profile of *L. dulcis*



- Many monoterpenoids, including camphor, camphene, and α -pinene, as well as many sesquiterpenoids, including caryophyllene, germacrene D, and α -bisabolol.
- The most notable metabolite is the sesquiterpene ketone, hernandulcine, the discovery as a potent natural sweetener of which dates back five hundred years, discovered by Spanish physician and naturalist Francisco Hernandez.
- Located in the leaves and flowers, it has been found to be 1000 times sweeter than sucrose, although it has a slight bitter aftertaste and does not cause mutagenic or toxic activity.

Hernandulcin biosynthesis

- The core biochemical steps:
- **Isoprenoid Precursor Synthesis:** The pathway initiates via the cytosolic mevalonate (MVA) pathway or plastidial MEP pathway, generating the universal 15-carbon precursor farnesyl pyrophosphate (FPP).
- **Cyclization to (+)-epi- α -bisabolol:** The enzyme **epi- α -bisabolol synthase (BBS)** converts FPP into (+)-epi- α -bisabolol, which forms the carbon backbone of the sweetener.
- **Oxidation via Cytochrome P450:** Specific CYP450 monooxygenases in the plant hydroxylate and oxidize the (+)-epi- α -bisabolol ring structure. This step yields the final sweet-tasting bisabolane-type sesquiterpene ketone.



The aim of the present work:

To investigate the potential biostimulatory role of two species of rhizosphere microorganisms (arbuscular mycorrhizal fungi *Claroideoglomus claroideum* and free-living nitrogen fixers *Azotobacter chroococcum*) on physiological changes and antioxidant potential of plants.

Measurement of
fresh and dry
weight of
aboveground parts

Determination of
the content of total
protein and plastid
pigments

Quantitative determination
of secondary metabolites:
phenols, flavonoids, water-
and lipid-soluble
antioxidants

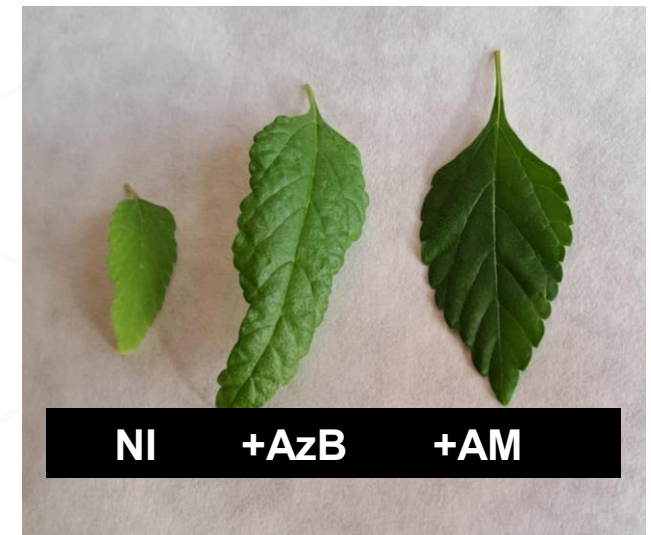
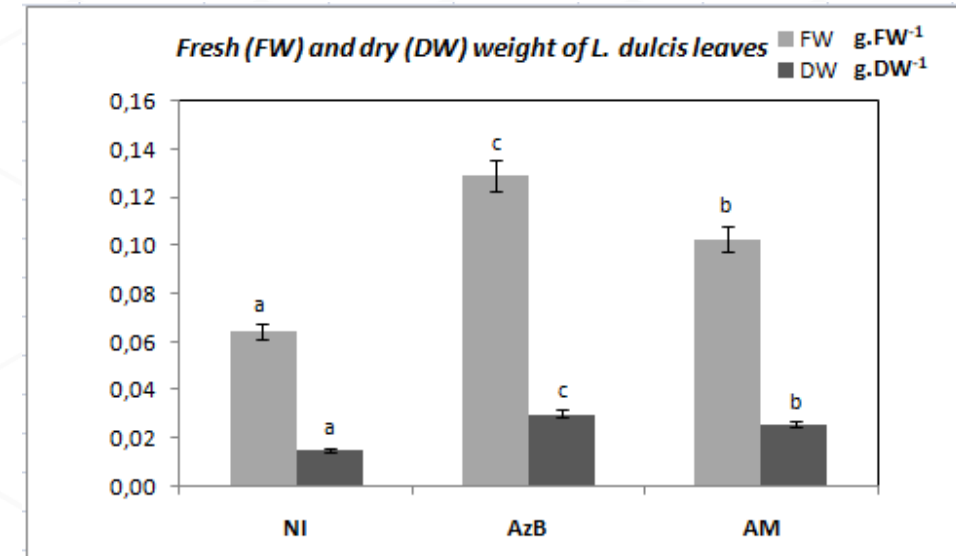
Assessment of
antioxidant status
by FRAP analysis

EXPERIMENTAL TASKS

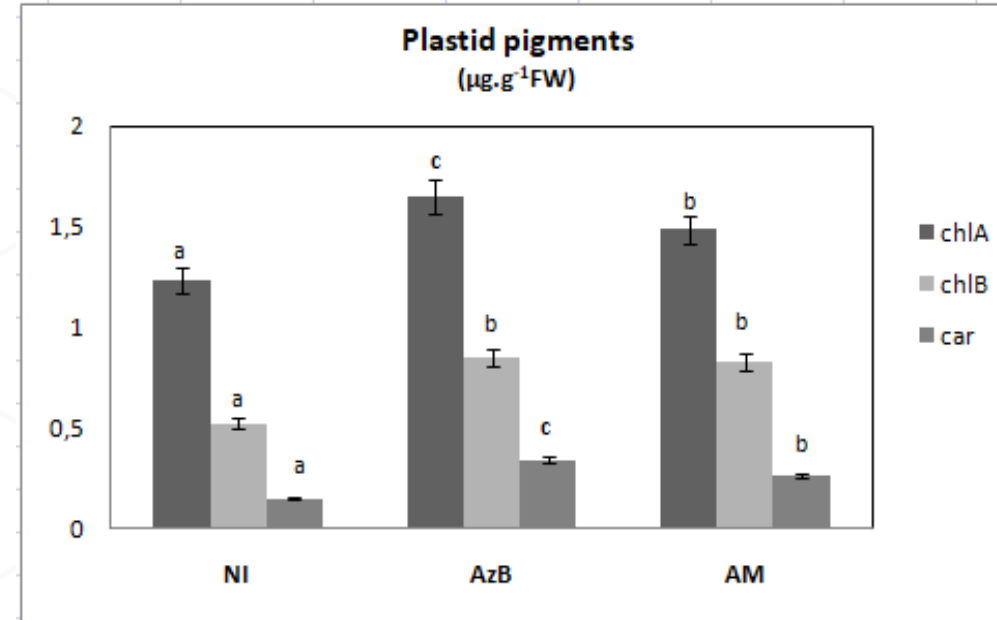
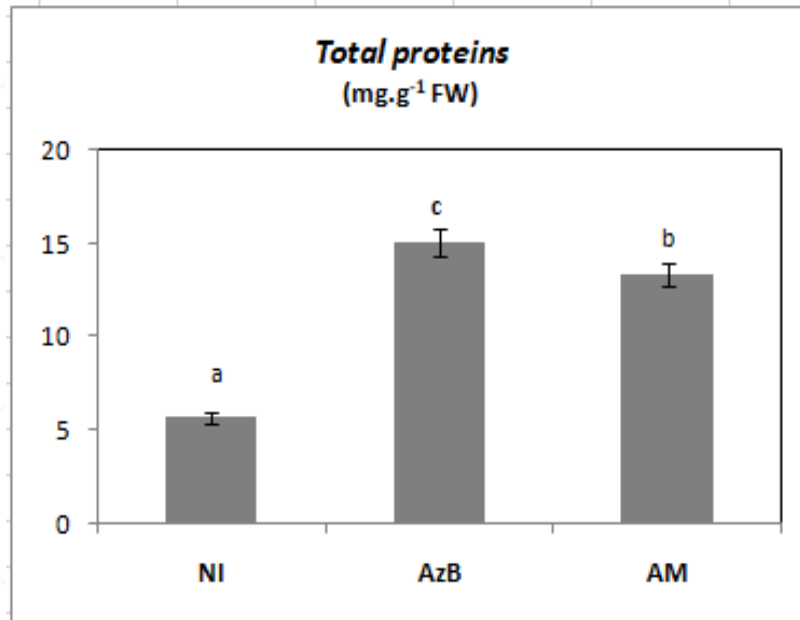
Growth and physiological development of *L. dulcis* plants

The following experimental variants were defined:

- NI/K - Non-inoculated *Lippia* plants
- AzB - Plants grown on soil substrate containing bacteria *Azotobacter chroococcum*
- AM – *Lippia* plants grown on soil substrate with added mycorrhizal strain *Claroideoglomus claroideum*

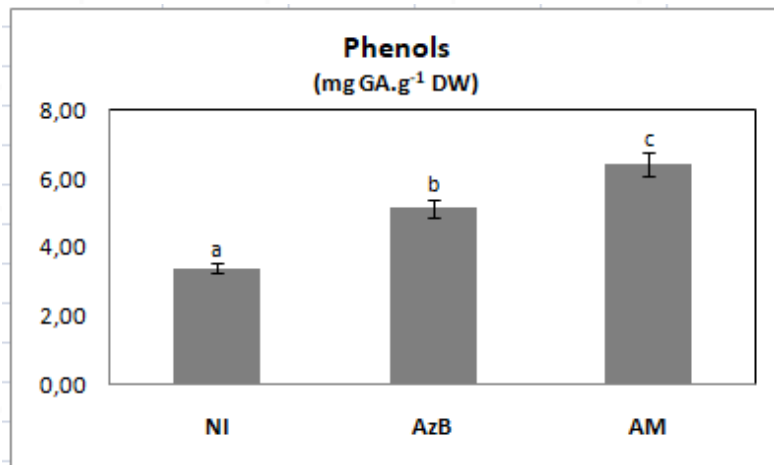
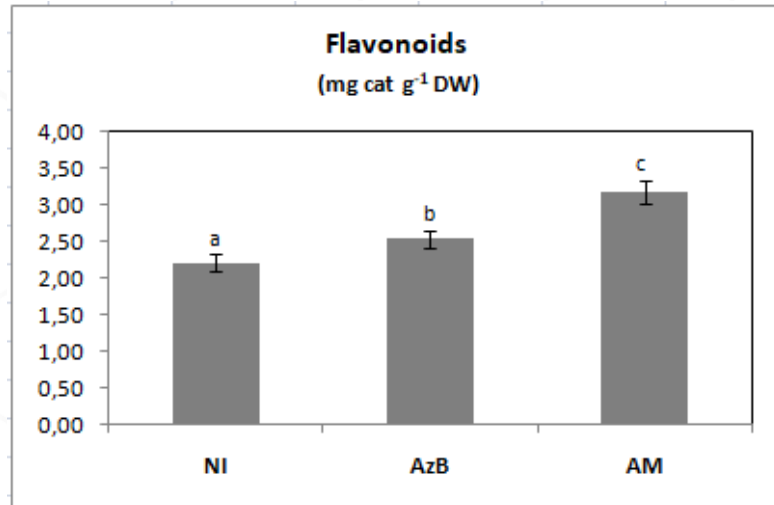


Determining the content of total proteins and plastid pigments: chlorophylls and carotenoids



Azotobacter bacteria promote growth, mainly due to their role in nitrogen fixation. Nitrogen is an element of primary importance for protein synthesis in plants, which is directly related to biomass accumulation. The improved nitrogen uptake achieved by inoculation with the bacterial species in the present work directly translates into improved growth and higher plant productivity, reflected in biomass accumulation. This element is also an important component of chlorophyll and is the most important pigment needed for photosynthesis.

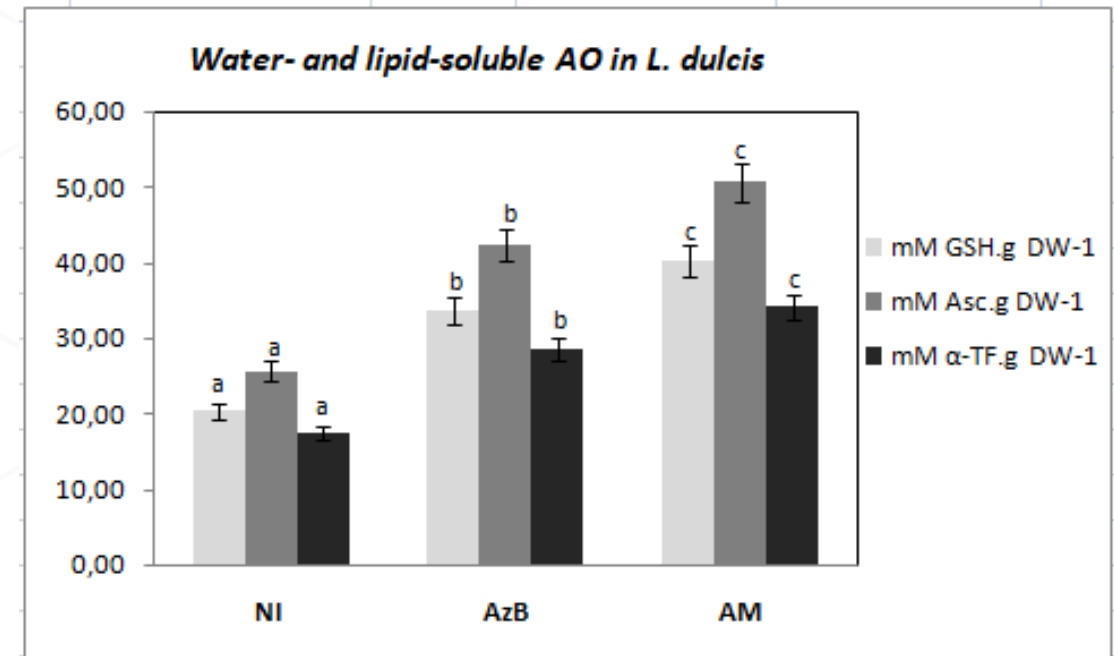
Evaluation of *L. dulcis* T. antioxidant status modified by the studied rhizosphere microorganisms



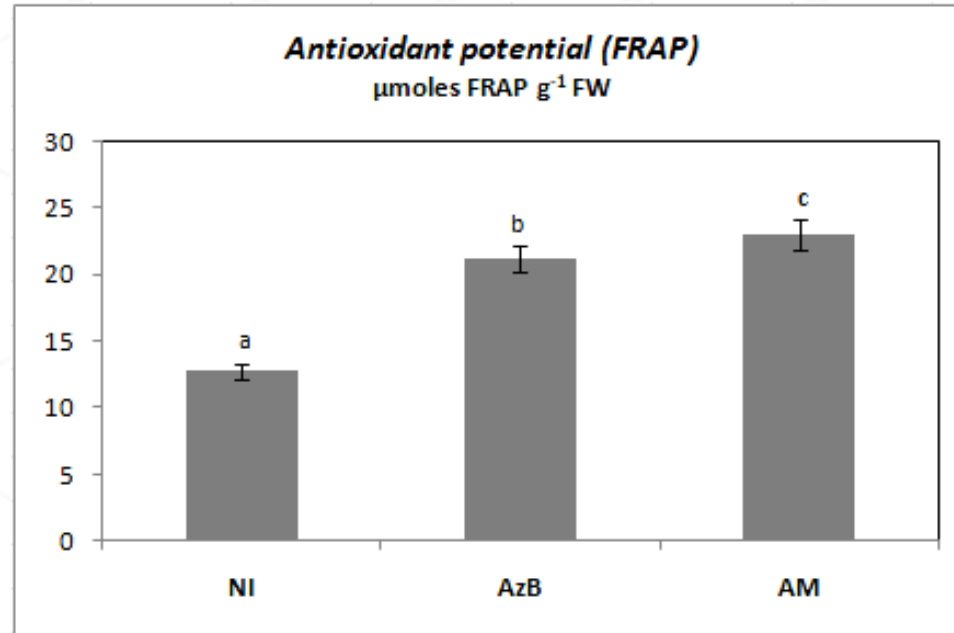
- The results show a statistically significant increase in the amount of both studied secondary metabolites in plants with added inoculants compared to the control sample, and in both cases AM has a significant effect on the production of phenols and flavonoids, respectively, compared to *Azotobacter chroococcum*.
- *Azotobacter* inoculation generally enhances the production of plant secondary metabolites, frequently boosting the concentrations of total phenols and flavonoids by enhanced nitrogen fixation, phytohormone production and induced systemic resilience.
- AMF influence plant phenol and flavonoid content through phosphorus (P) acquisition via directly altering plant secondary metabolism. The presence of mycorrhizae up-regulates the expression of key biosynthetic genes and enzymes, particularly phenylalanine ammonia-lyase (PAL) and chalcone synthase.

Concentration of non-enzymatic antioxidant water- and lipid-soluble antioxidants (WS- and LS-AO)

- Both soil rhizobacteria increase the total AO status of *Lippia* plants, with a more significant effect of mycorrhizal fungi compared to *Azotobacter chroococcum*, primarily by modulating the host plant's reactive oxygen species (ROS) signaling and improving root architecture and nutrient uptake:
- AMF significantly increases the uptake of essential micronutrients (such as Zinc), which are cofactors for enzymes that drive the synthesis of glutathione and ascorbate. Also, upregulate plant genes encoding enzymes such as glutathione reductase (GR) and dehydroascorbate reductase (DHAR).
- *Azotobacter* acts primarily by fixing atmospheric nitrogen and producing phytohormones like auxins (IAA), gibberellins, and cytokinins. These changes optimize the plant's metabolic capacity and activate systemic defenses, which consequently influence antioxidant pathways



The antioxidant capacity determined by Ferric Reducing Antioxidant Power (FRAP)



The mycorrhizal association has been shown to enhance the antioxidant capacity, as indicated by the FRAP test in *L. dulcis*. The antioxidant capacity is significantly determined by the overall presence of antioxidants in the samples. The findings from this analysis are closely linked to the measured total antioxidant activity and the levels of secondary metabolites like phenols and flavonoids, which are essential compounds known for their strong antioxidant effects. Understanding these relationships highlights the importance of antioxidants in improving plant health and combating oxidative stress.

CONCLUSION AND PERESPECTIVES

- Targeted inoculation with specific strains highlights the biostimulants' benefits in *Lippia dulcis* cultivation, resulting in increased biomass production and changes in the concentration and composition of primary and secondary metabolites, thereby increasing the overall antioxidant status of the plants.
- Applying biostimulants to *Lippia dulcis* offers a promising perspective for improving crop yield and increasing the concentration of essential oils.
- The plant's primary commercial value comes from the secondary metabolites hernandulcin (a natural sweetener) and biostimulants help optimize the metabolic pathways required to concentrate this sweetening agent while managing the levels of less desirable compounds like camphor.
- As a medicinal plant native to tropical and subtropical climates, *Lippia dulcis* can be susceptible to environmental stresses (drought, extreme temperatures). Biostimulant treatments act as "pre-stress conditioners," allowing the plant to better maintain photosynthetic efficiency and growth under suboptimal conditions in new habitats.
- Utilizing biostimulants supports sustainable agricultural development by improving nitrogen uptake efficiency, conserving soil health, and reducing the reliance on synthetic chemical fertilizers.



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THANK YOU!

Questions?

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