CHAPTER 3

MYCORRHIZA APPLICATION TO ENHANCE THE PRODUCTION OF PLANT BIOACTIVE COMPOUNDS: A REVIEW OF STUDIES DEVELOPED IN THE BRAZILIAN SOUTH AND SOUTHEAST

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ABSTRACT: The South is the second largest region in terms of Brazilian studies conducted on the phytochemistry of mycorrhizal species. Along with the Southeast, most of the research focuses on evaluating the production of plant bioactive compounds in medicinal species. However, the studies on strawberry plants are relevant because they address the effect of mycorrhization on fleshy fruit quality

parameters and are the only ones to evaluate this plant organ in the region. In addition, most of the studies were conducted with *Cymbopogon citratus* [DC.] Stapf. and the mycorrhizal inocula most often tested were from representatives of *Entrophosporaceae*. Mycorrhizal colonization has been evaluated in most of the studies, nevertheless, data on the production of glomalin-related soil proteins and glomerospores remains relatively uncommon. In this regard, mycorrhizal technology has the potential to provide plant material from medicinal species with an outstanding accumulation of metabolites related to bioactive properties.
KEYWORDS: *Entrophospora*; essential oils, medicinal plants, secondary metabolites.

1. INTRODUCTION

The Southeast region of Brazil is the most populous and the richest in the country (IBGE, 2022a,b). The states of Espírito Santo, São Paulo, Minas Gerais, and Rio de Janeiro are mostly dominated by regions with a subtropical climate, with average annual temperatures of 20 to 23 ºC (Alvares *et al.*, 2013a,b). In agribusiness, sugarcane, tomatoes and coffee are the most economically important crops in the region (IBGE, 2022c). However, approaches using mycorrhizal technology, considering phytochemical studies, are mainly focused on the potential for optimizing the production of bioactive compounds in species of medicinal relevance.

The South of Brazil comprises the states of Paraná, Santa Catarina, and Rio Grande do Sul. Despite having the smallest territorial extension compared to other Brazilian regions, is economically important in the country (IBGE, 2022b). The fruit-growing market generates billions of *reais* per year, especially for apple, grape, and strawberry crops (Lima *et al.*, 2021; IBGE, 2022c). This condition is likely due to the mild climate, with average annual temperatures of 14 to 23 ºC (Alvares *et al.*, 2013a) and classified as subtropical (Alvares *et al.*, 2013b). In this context, arbuscular mycorrhizal fungi can be an alternative for enhancing phytochemical and fruit quality parameters (Chiomento *et al.,* 2021), an approach that has been carried out in some studies in this region.

2. RESULTS AND DISCUSSION: THE USE OF MYCORRHIZAL FUNGI TO INCREASE PHYTOCHEMICAL PRODUCTION IN SOUTHERN AND SOUTHEAST BRAZIL

The South has the second-highest number of Brazilian studies on the phytochemistry of plants associated with AMF. In this region, the most investigated plants were *Cymbopogon citratus* [DC.] Stapf. (*Poaceae*) (Lermen *et al.*, 2015; Cruz *et al.*, 2020; Silva *et al.*, 2021a,b; Souza *et al.*, 2022) and *Fragaria* × *ananassa* (Duchesne ex Weston) Duchesne ex Rozier (*Rosaceae*) (Cordeiro *et al.*, 2019; Chiomento *et al.*, 2019;2021;2022; Nardi *et al.*, 2024).

Phytochemical studies of mycorrhizal plants conducted in this region have evaluated the effect of AMF on the production of metabolites in medicinal plants (Silva *et al.*, 2008; Lermen *et al.*, 2015; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Cruz *et al.*, 2019;2020;

Almeida *et al.*, 2020; Merlin *et al.*, 2020; Ferrari *et al.*, 2020; Silva *et al.*, 2021a,b; Silva *et al.*, 2021c; Pinc *et al.*, 2022; Souza *et al.*, 2022; Lermen *et al.*, 2023; Melato *et al.*, 2024) (Table 1), with the main focus on evaluating the biosynthesis of essential oils (Lermen *et al.*, 2015; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Cruz *et al.*, 2019;2020; Almeida *et al.*, 2020; Merlin *et al.*, 2020; Ferrari *et al.*, 2020; Silva *et al.*, 2021a,b; Silva *et al.*, 2021c; Pinc *et al.*, 2022; Souza *et al.*, 2022; Lermen *et al.*, 2023; Melato *et al.*, 2024) and oil-resin (Silva *et al.*, 2008).

In the south of the country, the main AMF isolates evaluated were *E. etunicata* and *R. clarum*, fungi that made up 61.90% of the experiments in this region (Lermen *et al.*, 2015; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Cruz *et al.*, 2019;2020; Merlin *et al.*, 2020; Ferrari *et al.*, 2020; Chiomento *et al.*, 2021;2022; Silva *et al.*, 2021a,b; Silva *et al.*, 2021c; Pinc *et al.*, 2022; Souza *et al.*, 2022; Lermen *et al.*, 2023; Melato *et al.*, 2024; Nardi *et al.*, 2024).

As in the other regions, most of the research was conducted in a greenhouse; however, only Cordeiro *et al.* (2019) evaluated the effect of mycorrhizal biostimulants under field conditions (Table 2). Leaves and other vegetative aerial parts were the most evaluated materials, accounting for around 85% of the research. Of these, only one study used the inflorescences to quantify metabolites (Almeida *et al.*, 2020).

The fruit was the second most studied plant organ (Cordeiro *et al.*, 2019; Chiomento 2019;2021; Nardi *et al.*, 2024), followed by the roots (Andrade *et al.*, 2013; Chiomento *et al.*, 2022; Nardi *et al.*, 2024) and the rhizome (Silva *et al.*, 2008; Ferrari *et al.*, 2020). Only four studies evaluated more than one plant organ at the same time (Andrade *et al.*, 2013; Ferrari *et al.*, 2020; Chiomento *et al.*, 2022; Nardi *et al.*, 2024), an approach that is relevant to understanding the physiology of mycorrhizal plants, which can be used in future studies.

Table 1. Overview of phytochemical studies on mycorrhizal plants conducted in the Brazilian South

Plant species	Plant part	Evaluated compound group	AMF species	Mycorrhizal parameters	References
Zingiber officinale Roscoe	Rhizome	Terpenes	*Dentiscutata heterogama* (T.H. Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl; *Gigaspora decipiens* I.R. Hall & L.K. Abbott; *Acaulospora koskei* Blaszk.; *Acaulospora colombiana* (Spain & N.C. Schenck) Kaonongbua, J.B. Morton & Bever	Mycorrhizal colonization; Glomerospores	Silva *et al.* (2008)
Cymbopogon citratus [DC.] Stapf.	Aerial part	Terpenes	*Rhizoglomus clarum* (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl	None	Lermen *et al.* (2015)
Mentha crispa L.	Aerial part	Terpenes	*Entrophospora etunicata* (W.N. Becker & Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno; *R. clarum*	Mycorrhizal colonization; Glomerospores	Urcoviche *et al.* (2015)
Ocimum basilicum L.	Aerial part	Terpenes	*E. etunicata*	Mycorrhizal colonization; Glomerospores	Morelli *et al.* (2017)
Fragaria x *ananassa* (Duchesne ex Weston) Duchesne ex Rozier	Fruits	Phenols	AMF community	None	Chiomento *et al.* (2019)
Fragaria x *ananassa*	Fruits	Phenols	AMF community	Mycorrhizal colonization	Cordeiro *et al.* (2019)
Salvia officinalis L.	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization; Glomerospores	Cruz *et al.* (2019)
Matricaria chamomilla L.	Inflorescence	Terpenes	AMF community	Mycorrhizal colonization	Almeida *et al.* (2020)
C. citratus	Aerial part	Phenols; Terpenes	*E. etunicata*; *R. clarum*	None	Cruz *et al.* (2020)
Curcuma longa L.	Rhizome and leaves	Phenols; Terpenes	*E. etunicata*; *R. clarum*	None	Ferrari *et al.* (2020)
Plectranthus amboinicus (Lour.) Spreng.	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization; Glomerospores	Merlin *et al.* (2020)
Fragaria x *ananassa*	Fruits	Phenols	*E. etunicata;* AMF community	Mycorrhizal colonization	Chiomento *et al.* (2021)
O. basilicum	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization; Glomerospores	Silva *et al.* (2021c)
C. citratus	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization; Glomerospores	Silva *et al.* (2021b)
C. citratus	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization	Silva *et al.* (2021a)

Fragaria x *ananassa*	Leaves and roots	Phenols	*Entrophospora claroidea* (N.C. Schenck & G.S. Sm.) Błaszk.; *E. etunicata*; *Funneliformis geosporum* (T.H. Nicolson & Gerd.) C. Walker & A. Schluessler; *Diversispora versiformis* (P. Karst.) Oehl, G.A. Silva & Sieverd; *Glomus* sp.	Mycorrhizal colonization	Chiomento *et al.* (2022)
Melissa officinalis L.	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization; Glomerospores	Pinc *et al.* (2022)
C. citratus	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization; Glomerospores	Souza *et al.* (2022)
Lippia alba (Mill.) N.E.Br. ex Britton & P.Wilson	Aerial part	Terpenes	R. clarum	None	Lermen *et al.* (2023)
Fragaria × ananassa	Roots, aerial part, fruits, and crown	Phenols	*Acaulospora mellea* Spain & N.C. Schenck; *Acaulospora longula* Spain & N.C. Schenck; *Cetraspora pellucida* (T.H. Nicolson & N.C. Schenck) Oehl, F.A. Souza & Sieverd.; *E. etunicata*; *Glomus* sp.; *Septoglomus viscosum* (T.H. Nicolson) C. Walker, D. Redecker, Stiller & A. Schüßler	Mycorrhizal colonization	Nardi *et al.* (2024)
Ruta graveolens L.	Aerial part	Terpenes	*R. clarum*; *E. etunicata*	Mycorrhizal colonization; Glomerospores	Melato *et al.* (2024)

AMF: arbuscular mycorrhizal fungi

In the Brazilian state, species from *Zingiberaceae* were also tested. *Zingiber officinale* Roscoe and *Curcuma longa* L., species listed in RENISUS (*Relação Nacional de Plantas de Interesse ao Sistema Único de Saúde*) (Brasil, 2009), produce around thrice more bioactive compounds when associated with selected AMF (Silva *et al.*, 2008; Ferrari *et al.*, 2020). Other plants of phytotherapeutic relevance evaluated by researchers in this region were *Lippia alba* (Mill.) N.E.Br. ex P. Wilson (*Verbenaceae*) (Lermen *et al.*, 2023), *Matricaria chamomilla* L. (*Asteraceae*) (Almeida *et al*., 2020) and the *Lamiaceae*, *Mentha crispa* L. (Urcoviche *et al.*, 2015), *Ocimum basilicum* L. (Morelli *et al*., 2017; Silva *et al.*, 2021c), *Salvia officinalis* L. (Cruz *et al.*, 2019), *Melissa officinalis* L. (Pinc *et al.*, 2022), and *Plectranthus amboinicus* (Lour.) Spreng. (Merlin *et al.*, 2020).

The studies conducted with strawberries reported greater accumulation of phenolic compounds (Cordeiro *et al.,* 2019; Chiomento *et al.,* 2019), something also reported in other organs (Chiomento *et al.,* 2022). These studies also differ from others due to the use of a greater number of mycorrhizal isolates (11 species) (Cordeiro *et al.*, 2019), as well as mixed inocula or those obtained from agricultural or forest sites (Chiomento *et al.,* 2019; 2021;2022). These, together with the study of Almeida *et al.* (2020), were the only ones that evaluated the effect of mycorrhizal communities in enhancing the production of bioactive compounds.

On the other hand, in the Southeast, different research groups have evaluated the potential of AMF to improve the concentration of compounds in phytomass, representing around 12% of national production. Species such as *Baccharis trimera* (Less.) DC., *Acmella oleracea* (L.) R.K. Jansen, *Mikania glomerata* Spreng., *Mikania laevigata* Sch. Bip. ex (*Asteraceae*) (Freitas *et al*., 2004a; Vieira *et al*., 2021; Almeida *et al*., 2018), *Mentha arvensis* L. (*Lamiaceae*) (Freitas *et al.*, 2004b), *Canavalia ensiformis* (L.) D.C. (*Fabaceae*) (Andrade *et al.*, 2010), *Catharanthus roseus* (L.) G. Don (*Apocynaceae*), *Nicotiana tabacum* L. (*Solanaceae*) (Andrade *et al*., 2013), *P. alata* (*Passifloraceae*) (Riter Netto *et al*., 2014), and *Toona ciliata* M. Roem. (*Meliaceae*) were evaluated (Table 2).

Table 2. Studies conducted in the southeast Brazil that evaluated the effect of arbuscular mycorrhizal fungi (AMF) inoculation on phytochemistry

Plant species	Plant part	Evaluated compound group	AMF species	Mycorrhizal parameters	Reference
Baccharis trimera (Less.) DC.	Aerial part	Phenols	*Rhizoglomus clarum* (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl; *Entrophospora etunicata* (W.N. Becker & Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno; *Gigaspora margarita* W.N. Becker & I.R. Hall; *Acaulospora scrobiculata* Trappe	Mycorrhizal colonization	Freitas *et al.* (2004a)
Mentha arvensis L.	Aerial part	Terpenes	*R. clarum*; *E. etunicata*; *G. margarita*; *A. scrobiculata*	Mycorrhizal colonization	Freitas *et al.* (2004b)
Canavalia ensiformis (L.) DC.	Leaves	Phytochelatins	*E. etunicata*	Mycorrhizal colonization	Andrade *et al.* (2010)
Catharanthus roseus (L.) G.Don; *Nicotiana tabacum* L.	Leaves and roots	Alkaloids	*E. etunicata*; *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl	Mycorrhizal colonization	Andrade *et al.* (2013)
Passiflora alata Curtis	Aerial part	Phenols	*R. clarum*; *G. margarita*; *R. intraradices*; *E. etunicata*	Mycorrhizal colonization	Riter Netto *et al.* (2014)
Toona ciliata M. Roem.	Aerial part	Phenols	*R. clarum*; *G. margarita*; *E. etunicata*	Mycorrhizal colonization	Lima *et al.* (2015)
Mikania glomerata Spreng.; *Mikania laevigata Sch.Bip. ex Baker*	Leaves	Phenols; Terpenes	*Rhizoglomus irregulare* (Błaszk., Wubet, Renker & Buscot) Sieverd., G.A. Silva & Oehl	Mycorrhizal colonization	Almeida *et al.* (2018)
Acmella oleracea (L.) R.K.Jansen	Aerial part	Terpenes	*E. etunicata*; *R. clarum*	Mycorrhizal colonization; Glomerospores	Vieira *et al.* (2021)

In Rio de Janeiro, Freitas *et al.* (2004a) documented the enhanced production of phenols in *B. trimera*, a pioneer study in Brazil. This and other research conducted in the Southeast have evaluated the combined effects of mycorrhization and fertilization on the production of bioactive compounds in species with medicinal (Freitas *et al.*, 2004a,b; Riter Netto *et al.*, 2014; Vieira *et al.*, 2021), timber (Lima *et al.*, 2015), and food (Andrade *et al.*, 2010) applications.

In addition to this research, others with *Asteraceae* have been carried out (Veira *et al.*, 2021; Almeida *et al.*, 2018) and made it possible to select *R. clarum* and *E. etunicata* as biostimulants for phenol production (Vieira *et al.,* 2021). However, it is advisable to evaluate more AMF isolates, since in *guaco* species (*M. glomerata* and *M. laevigata*), which produce phenolics and are from the same botanical family, the inoculation of *Rhizoglomus irregulare* (Błaszk., Wubet, Renker & Buscot) Sieverd., G.A. Silva & Oehl did not influence or reduce secondary anabolism, depending on the bioactive metabolite considered (Almeida *et al.,* 2018). Other inocula, with isolated AMF species and a mix of *R. clarum, E. etunicata, G. margarita*, and *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl, resulted in higher levels of metabolites when inorganic P was jointly applied to mycorrhizal plants (Freitas *et al.*, 2004a; Riter Netto *et al.*, 2014; Vieira *et al.*, 2021).

However, depending on the plant species and the group of secondary compounds, mycorrhization either dispensed fertilization supply (Freitas *et al.*, 2004a,b; Riter Netto *et al.*, 2014; Lima *et al.*, 2015; Vieira *et al.*, 2021) or was less efficient than chemical fertilizers (Andrade *et al.*, 2013) to optimize the biosynthesis of plant bioactive compounds. Although these results can be expected, the selection of adapted fungi to conditions of high fertility should be encouraged, considering that this condition is common in cultivated soils.

In the studies conducted in South and Southeast Brazil, mycorrhizal colonization was estimated in around 83% of the papers; of these, approximately 31% also quantified glomerospores; on the other hand, Glomalin-Related Soil Proteins (GRSP) production was not measured in soils from these experiments. As a result, there are still gaps regarding GRSP and glomerospore production in studies that aim to increase the content of bioactive compounds in plants associated with AMF in this region.

3. CONCLUSIONS AND PERSPECTIVES

The South and Southeast regions have crops of great economic importance for producing food plants. However, the main focus of studies involving the phytochemistry of mycorrhizal species has been to evaluate the production augmentation of bioactive compounds in medicinal species. An exception is the research on strawberry plants, which shows the efficiency of mycorrhizal technology in improving fruit quality parameters. Nevertheless, it is still necessary to establish efficient and well-characterized inocula for recommendation in different environmental conditions of this region.

Furthermore, research evaluating the effect of mycorrhization on the production of bioactive metabolites in maize and wheat crops, especially under field conditions, could favor cultivation protocols for some of the major agricultural products in the region.

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