CHAPTER 1

OVERVIEW OF BRAZILIAN STUDIES ON PHYTOCHEMISTRY OF MYCORRHIZAL SPECIES

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ABSTRACT: Mycorrhizal technology to enhance the production of plant bioactive compounds in Brazil has been studied for around 20 years and has given promising results for the Brazilian industry. Therefore, this review aimed to present the research on the phytochemistry of species inoculated with arbuscular mycorrhizal fungi (AMF) in Brazil, to assist research groups in selecting isolates that are effective in boosting the production of bioactive compounds. Based on database searches (Web of Science and National Center for Biotechnology Information), 66 experimental papers, four reviews, one editorial, and one opinion paper were selected. An overview of AMF species, botanical families, regions where studies have been carried out, experimental methods, main groups of biomolecules, and the most evaluated mycorrhizal parameters in the country were summarized. It was observed that Northeast Brazil accounts for more than 50% of all studies on the phytochemical aspect of mycorrhizal plants. The isolates of *Entrophospora etunicata* (W.N. Becker & Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno, *Acaulospora longula* Spain & N.C. Schenck, and *Gigaspora albida* N.C. Schenck & G.S. Sm. are the most tested in phytochemical studies of mycorrhizal species in the country, with results mainly reported under greenhouse conditions, as only six studies have been carried out under field conditions. The application of AMF can potentially increase the production of secondary compounds in plants, especially in *Fabaceae* representatives, which occur in Brazil, becoming an agronomic tool for the Brazilian pharmaceutical and cosmetic industries.

KEYWORDS: *Entrophospora*, mycorrhizal fungi, *Glomeromycota*, secondary metabolites.

1. INTRODUCTION

The supply of raw materials from plants is essential to meet the global demand for food and medicine (Maroyi, 2022). From this perspective, Brazil has a high potential as the country with the world's greatest biodiversity, due to its vast plant genetic heritage (Brasil, 2016), including medicinal resources for the industry.

Raw plant materials can be used to formulate medicines due to the presence of pharmacologically active compounds (Bernardes *et al.*, 2017). Examples of these are products marketed by pharmaceutical companies, such as coumarins obtained from *Mikania laevigata* Sch. Bip. ex Baker, vitexin found in Passiflora alata Curtis, senosides A and B produced by *Senna alexandrina* Mill. and valerenic acid, extracted from Valeriana officinalis L. (ANVISA, 2019). These compounds, among others found in products of plant origin, contributed to a revenue of more than R\$300 million in Brazil in 2019 (ANVISA, 2021).

In addition to herbal medicines, cosmetic products with moisturizing, depigmenting, anti-acne, repairing, and sun protection properties can also contain plant-derived ingredients. In such products, species like *Aloe vera* (L.) Burm. f. (Nivea®) (www.niveausa.com), *Melaleuca alternifolia* Cheel (Sallve®) (www.sallve.com.br), *Agathosma betulina* (Bergius) Pillans (www.sallve.com.br), *Rosa canina* L. (Sallve®) (www. sallve.com.br), *Bidens pilosa* L. (Sallve®) (www.sallve.com.br), *Centella asiatica* L. (La Roche-Posay®) (www.larocheposay.pt), and *Theobroma cacao* L. (Natura®) (www.natura.com.br) are used by national and international companies. However, it is important to improve the quality of the raw materials used to manufacture these and other products, as they can vary in metabolite content (Barbosa *et al.*, 2008).

Among the agro-biotechnologies available to improve plant production, beneficial microorganisms are promising, especially those that form mutualistic associations, such as arbuscular mycorrhizal fungi (AMF). This biotechnology has been tested to promote

the biosynthesis of secondary plant metabolites in Brazil for over 20 years (Freitas *et al.*, 2004a). It generates yields that exceed 500% to produce pharmaceutically and cosmetically relevant phytochemicals (Falcão *et al.*, 2022).

AMF are obligate biotrophs (Redecker *et al.*, 2013), belonging to the phylum *Glomeromycota* and classified into 17 families and 50 genera (Wijayawardene *et al.*, 2022). In Brazil, 38 AMF genera are present in national biomes (Maia *et al.*, 2020), with most species belonging to *Glomeraceae* and *Acaulosporaceae* (Maia *et al.*, 2020). These fungi form a symbiotic association from the emission of the germ tube (Tanaka *et al.*, 2022), an asymbiotic hypha that comes into contact with the root (Hepper, 1985) and differentiates into an appressorium (Mosse; Hepper, 1975). After penetration, the hyphae grow through the root cortex into the intercellular (Cox; Sanders, 1974; Mosse; Hepper, 1975) and intracellular spaces, where arbuscules are formed; in these, nutrient exchange takes place between the fungus and the host (Cox; Sanders, 1974; Marx *et al.*, 1982). An external mycelium is formed after establishing intracellular root colonization, which commonly restarts the life cycle, producing new glomerospores (Mosse; Hepper, 1975).

To apply these fungi, it is recommended to produce considerable amounts of AMF inoculum containing spores, hyphae, and fragments of colonized roots. They are obtained through substrate cultivation (Silva *et al.*, 2014a; Selvakumar *et al.*, 2016;2018a), which can be by monosporic culture (Selvakumar *et al.*, 2018b), transformed roots (Srinivasan *et al.*, 2014), in aeroponic (Mohammad *et al.*, 2000) or hydroponic systems (Nurbaity *et al.*, 2019).

The cost of producing soil-inoculum is relatively low and can range from 0.02 - 1.30 USD per pot (Santana *et al.*, 2014; Silva; Silva, 2020). However, this technology has not been commercialized in Brazil yet. Considering the diversity of AMF representatives in Brazilian soils (Maia *et al.*, 2020) with recognized efficiency (Pedone Bonfim *et al.*, 2015; Falcão; Silva, 2023), the use of these microorganisms should be encouraged without sticking only to isolates marketed abroad (Basiru *et al.*, 2021). When AMF propagules are applied (Muniz *et al.*, 2021), the fungi benefits to the host plant can be identified (Chen *et al.*, 2017; Mathur *et al.*, 2018); among these, the enhanced production of metabolites stands out, which can be explained by nutritional, physiological and molecular modulations in the photobiont, as summarized in Figure 1.

The benefits of applying AMF in the production of bioactive compounds can be numerous (Wu *et al.*, 2023; Falcão *et al.,* 2023a), considering studies conducted in Brazil. Notwithstanding, compiled data on such symbiotic efficiency are not available, even though comprehensive reviews have been published worldwide (Pedone Bonfim *et al.*, 2015; Sharma *et al.*, 2017; Kaur; Suseela, 2020; Zhao *et al.*, 2022; Thokchom *et al.*, 2023; Falcão; Silva, 2023; Falcão *et al.,* 2024a). Therefore, this review aimed to compile papers on the phytochemistry of mycorrhizal plants from studies conducted in Brazil.

Figure 1. Mechanisms that explain the improved biosynthesis of secondary compounds in response to mycorrhization (Lohse *et al*., 2005; Kapoor *et al.*, 2007; Zubek *et al.*, 2010; Mandal *et al.*, 2013; Zhang *et al.*, 2013; Torres *et al.*, 2015; Sharma *et al.*, 2017; Cui *et al.*, 2019; Ran *et al.*, 2021; Cela *et al.*, 2022; Falcão *et al.*, 2022;2023b;2024b; Muniz *et al.*, 2023). Icons: canva.com

2. MATERIAL AND METHODS

A descriptive review was conducted using combinations of descriptors related to studies on AMF and phytochemistry, with terms in English and research time interval of 22 years (2002 to June 2024), as shown in Figure 2. In total, 433 articles were found, considering the search on the National Center for Biotechnology Information (NCBI) and Web of Science platforms, disregarding those repeated in both databases. After initial screening of titles, abstracts, keywords, and methodology, the aims of the papers were also assessed so that only those that focused on increasing the production of biomolecules with mycorrhizal inoculation and were setup in Brazil were included in this review. Thus, 72 papers were selected (Figure 2).

Figure 2. Flowchart of the search based on descriptors related to studies of arbuscular mycorrhizal fungi and the evaluation of the phytochemistry of inoculated plants whose research was conducted in Brazil. NCBI= National Center for Biotechnology Information.

The data from the selected papers were quantified, and the results were expressed as percentages and plotted on graphs. However, of these 72 papers, four were review papers (Pedone Bonfim *et al.*, 2015; Santos *et al.*, 2021a; Falcão; Silva, 2023; Falcão *et al*., 2024a), one opinion paper (Falcão *et al.,* 2023a), and one was published as an editorial (Wu *et al.*, 2023) so they were not included in the counting presented. In addition, a map was built to plot the distribution of all studies by region and Brazilian states, using Canva (canva.com) (see chapter 2).

3. RESULTS AND DISCUSSION: OVERVIEW OF BRAZILIAN STUDIES ON THE PHYTOCHEMISTRY OF MYCORRHIZAL SPECIES

In Brazil, the main AMF isolates used to increase the production of plant bioactive compounds were *Entrophospora etunicata* (W.N. Becker & Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno (previously classified as *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler or *Glomus etunicatum* W.N. Becker & Gerd.), *Acaulospora longula Spain* & N.C. Schenck (also considered *Acaulospora morrowiae* Spain & N.C. Schenck),

Gigaspora albida N.C. Schenck & G.S. Sm. and *Rhizoglomus clarum* (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl (previously classified as *Rhizophagus clarus* (T.H. Nicolson & N.C. Schenck) C. Walker & A. Schüßler or *Glomus clarus* T.H. Nicolson & N.C. Schenck) (Figure 3). This pattern was partially observed in the review by Zhao *et al.* (2022), which systematized studies conducted worldwide.

Other species evaluated were *Acaulospora colombiana* (Spain & N.C. Schenck) Kaonongbua, J.B. Morton & Bever (previously classified as *Entrophospora colombiana* Spain & N.C. Schenck), *Acaulospora koskei* Błaszk, *Acaulospora scrobiculata* Trappe, *Dentiscutata heterogama* (T.H. Nicolson & Gerd.) Sieverd, F.A. Souza & Oehl [previously classified as *Scutellospora heterogama* (Nicol. & Gerd.) Sieverd., Souza & Oehl], *Diversispora versiformis* (P. Karst.) Oehl, G.A. Silva & Sieverd. [previously classified as *Glomus versiforme* (P.Karst.) S.M. Berch], *Entrophospora claroidea* (N.C. Schenck & G.S. Sm.) Błaszk., Niezgoda, B.T. Goto & Magurno, *Funneliformis geosporum* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, *Fuscutata heterogama* Oehl, F.A. Souza, L.C. Maia & Sieverd., *Gigaspora decipiens* I.R. Hall & L.K. Abbott, *Gigaspora margarita* W.N. Becker & I.R. Hall, *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl [previously classified as *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.)] C. Walker & A. Schüßler or *Glomus intraradices* N.C. Schenck & G.S. Sm.), *Rhizoglomus irregulare* (Błaszk., Wubet, Renker & Buscot) Sieverd., G.A. Silva & Oehl [also known as *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler], *Cetraspora pellucida* (T.H. Nicolson & N.C. Schenck) Oehl, F.A. Souza & Sieverd.*, [Acaulospora mellea](http://www.mycobank.org/MycoTaxo.aspx?Link=T&Rec=105903)* [Spain & N.C. Schenck](http://www.mycobank.org/MycoTaxo.aspx?Link=T&Rec=105903)*, Septoglomus viscosum* (T.H. Nicolson) C. Walker, D. Redecker, Stiller & A. Schüßler*,* and *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders (Figure 3).

Figure 3. Tested AMF species in studies carried out in Brazil using arbuscular mycorrhizal fungi (AMF) to increase the production of phytochemicals. Number of experimental studies= 66. *Acaulospora colombiana* (Spain & N.C. Schenck) Kaonongbua, J.B. Morton & Bever, *Acaulospora koskei* Błaszk., *Acaulospora longula* Spain & N.C. Schenck, *Acaulospora scrobiculata* Trappe, *Dentiscutata heterogama* (T.H. Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl, *Diversispora versiformis* (P. Karst.) Oehl, G.A. Silva & Sieverd., *Entrophospora claroidea* (N.C. Schenck & G.S. Sm.) Błaszk., Niezgoda, B.T. Goto & Magurno, *Entrophospora etunicata* (W.N. Becker & Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno, *Funneliformis geosporum* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, *Fuscutata heterogama* Oehl, F.A. Souza, L.C. Maia & Sieverd., *Gigaspora albida* N.C. Schenck & G.S. Sm., *Gigaspora decipiens* I.R. Hall & L.K. Abbott, *Gigaspora margarita* W.N. Becker & I.R. Hall, *Rhizoglomus clarum* (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl, *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl, *Rhizoglomus irregulare* (Błaszk., Wubet, Renker & Buscot) Sieverd., G.A. Silva & Oehl, *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders, *Cetraspora pellucida* (T.H. Nicolson & N.C. Schenck) Oehl, F.A. Souza & Sieverd.*, [Acaulospora mellea](http://www.mycobank.org/MycoTaxo.aspx?Link=T&Rec=105903)* [Spain & N.C.](http://www.mycobank.org/MycoTaxo.aspx?Link=T&Rec=105903) [Schenck](http://www.mycobank.org/MycoTaxo.aspx?Link=T&Rec=105903)*, Septoglomus viscosum* (T.H. Nicolson) C. Walker, D. Redecker, Stiller & A. Schüßler (Freitas *et al.,* 2004a,b; Andrade *et al.*, 2010;2013; Oliveira *et al.*, 2013;2015a,b,c;2019a,b;2020;2022; Pedone Bonfim *et al.*, 2013;2018; Riter Netto *et al.*, 2014; Silva *et al.*, 2014a,b,c,d;2018a,b,c;2019;2021a,b,c,d; Lermen *et al.*, 2015;2023; Lima *et al.*, 2015a,b;2017; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Santos *et al.*, 2017;2020;2021b; Silva; Silva, 2017;2020; Almeida *et al.*, 2018;2020; Silva; Maia, 2018; Chiomento *et al.*, 2019;2021;2022; Cordeiro *et al.*, 2019; Cruz *et al.*, 2019;2020; Ferrari *et al.*, 2020; Merlin *et al.*, 2020; Vieira *et al.*, 2021; Trindade *et al.*, 2021; Muniz *et al.*, 2021;2022a,b;2023; Marcolino *et al.*, 2021; Falcão; Silva, 2022; Falcão *et al.*, 2022;2023b;2024b; Palhares Neto *et al.*, 2022; Pinc *et al.*, 2022; Souza *et al.*, 2022; Luz *et al.*, 2023; Nardi *et al.*, 2024; Melato *et al.,* 2024).

When the distribution of studies by region was considered, *A. longula* and *G. albida,* which often promote plant anabolism, were the most applied fungi in research conducted in Northeast Brazil, region with the highest number of published papers (Figure 4) (Oliveira *et al.*, 2013; Pedone Bonfim *et al.*, 2013;2018; Lima *et al.*, 2015a,2017; Silva *et al.*, 2014a,b,c,d;2018;2019;2021a; Oliveira *et al.*, 2015a,b,c;2019a;2020; Santos *et al.*, 2017;2020;2021b; Silva; Silva, 2017;2020; Silva; Maia, 2018; Muniz *et al.*, 2021;2022a,b;2023; Marcolino *et al.*, 2021; Falcão; Silva, 2022; Falcão *et al.*, 2022;2023b;2024b; Luz *et al.*, 2023).

Figure 4. Studies conducted in Brazil that investigated the use of arbuscular mycorrhizal fungi to increase the production of phytochemicals (Freitas *et al.,* 2004a,b; Andrade *et al.*, 2010;2013; Oliveira *et al.*, 2013;2015a,b,c;2019a,b;2020;2022; Pedone Bonfim *et al.*, 2013;2018; Riter Netto *et al.*, 2014; Silva *et al.*, 2014a,b,c,d;2018a,b,c;2019;2021a,b,c,d; Lermen *et al.*, 2015;2023; Lima *et al.*, 2015a,b;2017; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Santos *et al.*, 2017;2020;2021b; Silva; Silva, 2017;2020; Almeida *et al.*, 2018;2020; Silva; Maia, 2018; Chiomento *et al.*, 2019;2021;2022; Cordeiro *et al.*, 2019; Cruz *et al.*, 2019;2020; Ferrari *et al.*, 2020; Merlin *et al.*, 2020; Vieira *et al.*, 2021; Trindade *et al.*, 2021; Muniz *et al.*, 2021;2022a,b;2023; Marcolino *et al.*, 2021; Falcão; Silva, 2022; Falcão *et al.*, 2022;2023b;2024b; Palhares Neto *et al.*, 2022; Pinc *et al.*, 2022; Souza *et al.*, 2022; Luz *et al.*, 2023; Nardi *et al.*, 2024; Melato *et al.,* 2024).

Among the most evaluated botanical families, representatives of *Fabaceae*, Passifloraceae, and *Lamiacaeae* were the most tested for the quantification of bioactive compounds in mycorrhizal species (Figure 5). Within the Passifloraceae family, only Passiflora species have been evaluated, mainly the leaves of *P. alata* (Oliveira *et al.*, 2015a,b; Muniz *et al.*, 2021;2022a), Passiflora edulis f. flavicarpa Deg. (Oliveira *et al.*, 2019a;2020), Passiflora cincinnata Mast. (Falcão; Silva, 2022), and Passiflora setacea DC. (Muniz *et al.*, 2022b) and some of these species are used in the anxiolytic herbal medicine industry (Fonseca *et al.*, 2020; Oliveira *et al.*, 2020).

Figure 5. Botanical families studied in Brazil using arbuscular mycorrhizal fungi (AMF) to increase the production of phytochemicals. Number of experimental studies= 66 (Freitas *et al.,* 2004a,b; Andrade *et al.*, 2010;2013; Oliveira *et al.*, 2013;2015a,b,c;2019a,b;2020;2022; Pedone Bonfim *et al.*, 2013;2018; Riter Netto *et al.*, 2014; Silva *et al.*, 2014a,b,c,d;2018a,b,c;2019;2021a,b,c,d; Lermen *et al.*, 2015;2023; Lima *et al.*, 2015a,b;2017; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Santos *et al.*, 2017;2020;2021b; Silva; Silva, 2017;2020; Almeida *et al.*, 2018;2020; Silva; Maia, 2018; Chiomento *et al.*, 2019;2021;2022; Cordeiro *et al.*, 2019; Cruz *et al.*, 2019;2020; Ferrari *et al.*, 2020; Merlin *et al.*, 2020; Vieira *et al.*, 2021; Trindade *et al.*, 2021; Muniz *et al.*, 2021;2022a,b;2023; Marcolino *et al.*, 2021; Falcão; Silva, 2022; Falcão *et al.*, 2022;2023b;2024b; Palhares Neto *et al.*, 2022; Pinc *et al.*, 2022; Souza *et al.*, 2022; Luz *et al.*, 2023; Nardi *et al.*, 2024; Melato *et al.,* 2024).

From the studies on terpene production in mycorrhizal *Lamiaceae*, three of them evaluated *Mentha* species (Freitas *et al.,* 2004b; Silva *et al.,* 2014b; Urcoviche *et al.,* 2015), two of them studied *Ocimum basilicum* L. (Morelli *et al.,* 2017; Silva *et al.,* 2021b), in addition to assays using Salvia officinalis L. (Cruz *et al.,* 2019), *Plectranthus amboinicus* (Lour.) Spreng (Merlin *et al.,* 2020), and Melissa officinalis L. (Pinc *et al.*, 2022). These studies are relevant, considering that essential oils have potential in the food industry due to their antimicrobial and antioxidant properties (Inanoglu *et al.,* 2023).

It was expected that the most evaluated legumes would be those of food and economic importance, nevertheless, the most studied were those of ethnobotanical relevance, such as *Libidibia ferrea* (Mart. ex Tul.) L. P. Queiroz, *Anadenanthera colubrina* (Vell.) Brenan, *Inga vera* Willd., and *Hymenaea martiana* Hayne (Pedone Bonfim *et al.*, 2013; Lima *et al.*, 2015; Silva *et al.*, 2014a,b;2018a;2021a; Santos *et al.*, 2017;2020;2021b; Falcão *et al.*, 2022;2023b;2024b; Muniz *et al.*, 2023). In addition, all experiments on the phytochemistry of mycorrhizal legumes were conducted in the Northeast of Brazil, which hosts over 1179 species from this plant family (Flora e Funga do Brasil, 2024).

The most studied plant parts were the leaves alone and the aerial part (Figure 6a), with the inflorescence being one of the least studied organs (1.4% of the studies). The more significant number of studies on leaves likely reflects the potential of this organ to produce and present an optimized anabolism due to mycorrhizal inoculation, which could make up herbal medicines. Although Brazilian studies on the phytochemistry of mycorrhizal species represent approximately 10% of the research in this area of mycorrhizology, there is a need to validate the benefits reported in greenhouses under field conditions (Figure 6b). Thus, only 9.1% of the studies have been conducted in experimental fields (Cordeiro *et al.*, 2019), especially for *L. ferrea* (Silva *et al.*, 2018a; Santos *et al.*, 2017;2020;2021b). This reflects the need to plan studies that consider field conditions to develop protocols that can be reproduced in cultivation sites established by companies that manufacture and market phytoformulations.

To assess the mycorrhizal efficiency in the production of secondary metabolites, compounds from phenolic origin were estimated in more than 55% of the studies, followed by the terpene group (39.8%) (Figure 7a). However, alkaloids, which are extremely important in chemotherapy treatments (Dhyani *et al.*, 2022), were only quantified in the studies by Andrade *et al.* (2013) and Luz *et al.* (2023), confirming the need for more research into this compound group, which are barely addressed from a mycorrhizal perspective.

Figure 6. a) Plant parts used to assess bioactive compounds in mycorrhizal species. b) Sites where the experiments were conducted to quantify the phytochemistry of mycorrhizal species in studies developed in Brazil. Number of experimental studies= 66 (Freitas *et al.,* 2004a,b; Andrade *et al.*, 2010;2013; Oliveira *et al.*, 2013;2015a,b,c;2019a,b;2020;2022; Pedone Bonfim *et al.*, 2013;2018; Riter Netto *et al.*, 2014; Silva *et al.*, 2014a,b,c,d;2018a,b,c;2019;2021a,b,c,d; Lermen *et al.*, 2015;2023; Lima *et al.*, 2015a,b;2017; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Santos *et al.*, 2017;2020;2021b; Silva; Silva, 2017;2020; Almeida *et al.*, 2018;2020; Silva; Maia, 2018; Chiomento *et al.*, 2019;2021;2022; Cordeiro *et al.*, 2019; Cruz *et al.*, 2019;2020; Ferrari *et al.*, 2020; Merlin *et al.*, 2020; Vieira *et al.*, 2021; Trindade *et al.*, 2021; Muniz *et al.*, 2021;2022a,b;2023; Marcolino *et al.*, 2021; Falcão; Silva, 2022; Falcão *et al.*, 2022;2023b;2024b; Palhares Neto *et al.*, 2022; Pinc *et al.*, 2022; Souza *et al.*, 2022; Luz *et al.*, 2023; Nardi *et al.*, 2024; Melato *et al.,* 2024).

Figure 7. a) Compounds evaluated in phytochemical studies and b) parameters used to evaluate mycorrhizal activity in plants inoculated with arbuscular mycorrhizal fungi (AMF) based on studies conducted in Brazil. GRSP= Glomalin-Related Soil Proteins; Glomerospores= Glomerospore production; Colonization= Colonization percentage. Number of experimental studies= 66 (Freitas *et al.,* 2004a,b; Andrade *et al.*, 2010;2013; Oliveira *et al.*, 2013;2015a,b,c;2019a,b;2020;2022; Pedone Bonfim *et al.*, 2013;2018; Riter Netto *et al.*, 2014; Silva *et al.*, 2014a,b,c,d;2018a,b,c;2019;2021a,b,c,d; Lermen *et al.*, 2015;2023; Lima *et al.*, 2015a,b;2017; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Santos *et al.*, 2017;2020;2021b; Silva; Silva, 2017;2020; Almeida *et al.*, 2018;2020; Silva; Maia, 2018; Chiomento *et al.*, 2019;2021;2022; Cordeiro *et al.*, 2019; Cruz *et al.*, 2019;2020; Ferrari *et al.*, 2020; Merlin *et al.*, 2020; Vieira *et al.*, 2021; Trindade *et al.*, 2021; Muniz *et al.*, 2021;2022a,b;2023; Marcolino *et al.*, 2021; Falcão; Silva, 2022; Falcão *et al.*, 2022;2023b;2024b; Palhares Neto *et al.*, 2022; Pinc *et al.*, 2022; Souza *et al.*, 2022; Luz *et al.*, 2023; Nardi *et al.*, 2024; Melato *et al.,* 2024).

In studies on mycorrhizal benefits in the production of plant bioactive compounds, the most common method used to assess the presence of the fungus in the root was to estimate mycorrhizal colonization using the methods of Giovannetti; Mosse (1980) and McGonigle *et al.* (1990). However, around 16% of the studies did not investigate mycorrhizal parameters, which is a concern because many of the explanations for how AMF can enhance the synthesis of bioactive compounds have been attributed to mycorrhizal activity in roots (Oliveira *et al.*, 2015a) and rhizosphere (Hristozkova *et al.*, 2017; Falcão *et al.*, 2023b).

Another important aspect of academic productivity is the establishment of partnerships between research groups from different countries (Rostan; Ceravolo, 2015), which seems to be a limitation for most Brazilian groups in the field of mycorrhizal plant phytochemistry that often do not have a national and/or international network. In any case, the number of papers with international partnerships is on the rise, as seen in the papers by Trindade *et al.* (2021), Oliveira *et al.* (2022), Falcão *et al.* (2023b; 2024b), Muniz *et al.* (2023), Wu *et al.* (2023), Luz *et al.* (2023), Nardi *et al*. (2024), and Falcão *et al.* (2024a,b), which had the collaboration of researchers from universities in the United States, Canada, China, India, and Spain.

Based on the continental dimensions of Brazil, data are presented on the publication of papers on the phytochemistry of mycorrhizal species in the five geographical regions of this country. Thus, these results will be presented in the next chapters, considering the overview of the evaluated studies.

4. CONCLUSION AND PERSPECTIVES

Mycorrhizal biotechnology is an essential tool that can help to obtain a high-quality plant material that can be used to produce food, cosmetics, and medicines. Currently, several studies on the phytochemistry of mycorrhizal species have been conducted in Brazil, mainly in the Northeast (Figure 8); however, the recommendation for use must still be evaluated with caution, given the factors that can regulate the AMF efficiency, including climate, soil characteristics, and symbiotic partners. Given this, the lack of studies in the Central-West region, from a phytochemical perspective, needs to be encouraged, as this location has other climatic characteristics.

Given that the focus of research has been on phenolics and terpenes, it is important to fill the gap to understand how the production of Nitrogen compounds occurs, which have been poorly evaluated. In addition, it is necessary to explore the varied species of AMF occurring and isolated in the country, whose relationships with some plants are not yet known and could provide advantageous information to increase the production of biomolecules of industrial interest.

In addition, it is essential to develop new field studies aimed not only at improving the synthesis of molecules but also at understanding the mechanisms involved, the relationships established by the soil microbiota, and the ideal conditions for plant production, thus enabling the development of specific protocols that can meet the need of farmers and large industries.

This review aimed to compile the various aspects covered in studies of the phytochemistry of mycorrhizal species in Brazil and thus serve as an incentive for the creation of new research groups distributed throughout the country, which will help to clarify the role of mycorrhizal symbiosis in improving the plant biomass used in various industry sections.

The potential of mycorrhizal technology in improving the production of plant bioactive compounds: What is the overview of studies conducted in Brazil?

- Brazilian regions:
- Experimental sites: $\frac{1}{2}$ greenhouse or $\frac{1}{2}$ field conditions;
- Botanical families: *A Poaceae, A Fabaceae,* **K** Passifloraceae, **A** Lamiaceae, and \mathscr{C} are \mathscr{C} others [Solanaceae, Piperaceae, Verbenaceae, Lytaceae, Zingiberaceae, Asteraceae, and others];
- Compound groups: \circlearrowleft phenolics, \mathcal{L} terpenes, and \mathbb{Q}_1 alkaloids;
- Plant parts: leaves, vaerial part, Tiruit, Shark, and Troots;
- Tested arbuscular mycorrhizal fungi.

Figure 8. Overview of mycorrhizal species phytochemistry studies in Brazil (Freitas *et al.,* 2004a,b; Andrade *et al.*, 2010;2013; Oliveira *et al.*, 2013;2015a,b,c;2019a,b;2020;2022; Pedone Bonfim *et al.*, 2013;2018; Riter Netto *et al.*, 2014; Silva *et al.*, 2014a,b,c,d;2018a,b,c;2019;2021a,b,c,d; Lermen *et al.*, 2015;2023; Lima *et al.*, 2015a,b;2017; Urcoviche *et al.*, 2015; Morelli *et al.*, 2017; Santos *et al.*, 2017;2020;2021b; Silva; Silva, 2017;2020; Almeida *et al.*, 2018;2020; Silva; Maia, 2018; Chiomento *et al.*, 2019;2021;2022; Cordeiro *et al.*, 2019; Cruz *et al.*, 2019;2020; Ferrari *et al.*, 2020; Merlin *et al.*, 2020; Vieira *et al.*, 2021; Trindade *et al.*, 2021; Muniz *et al.*, 2021;2022a,b;2023; Marcolino *et al.*, 2021; Falcão; Silva, 2022; Falcão *et al.*, 2022;2023b;2024b; Palhares Neto *et al.*, 2022; Pinc *et al.*, 2022; Souza *et al.*, 2022; Luz *et al.*, 2023; Nardi *et al.*, 2024; Melato *et al.,* 2024). Icons: canva.com

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