academicJournals

Vol. 9(24), pp. 712- 718, 25 June, 2015
DOI: 10.5897/JMPR2015.5798
Article Number: EB4FB9E53878
ISSN 1996-0875
Copyright © 2015
Author(s) retain the copyright of this article
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Foliar bioactive compounds in *Amburana cearensis* (Allemao) A.C. Smith seedlings: Increase of biosynthesis using mycorrhizal technology

Paula Tarcila Félix de Oliveira[1,3], Gilberto Dias Alves[2], Francineyde Alves da Silva[3] and Fábio Sérgio Barbosa da Silva[1,3]*

[1]Programa de Pós-Graduação em Biologia Celular e Molecular Aplicada, Instituto de Ciências Biológicas, Universidade de Pernambuco, Rua Arnóbio Marques, 310, Santo Amaro – 50100130 - Recife, PE-Brasil.
[2]Laboratório de Fisiologia Vegetal, Instituto de Ciências Biológicas, *Campus* Santo Amaro, Universidade de Pernambuco, Rua Arnóbio Marques, 310, Santo Amaro – 50100130 - Recife, PE-Brasil.
[3]Laboratório de Enzimologia e Fitoquímica Aplicada a Micologia, Universidade de Pernambuco, *Campus* Petrolina, 56328-900, Petrolina, PE-Brasil.

Received 19 March, 2015; Accepted 10 June, 2015

***Amburana cearensis* (Allemao) A.C. Smith is a widely used legume by the population due to its medicinal properties. This species establish symbiosis with the arbuscular mycorrhizal fungi (AMF) that can increase the production of secondary metabolites, a fact which has not been clarified for this plant. Therefore, the aim of this study was to examine the contribution of the AMF in the production increase of foliar bioactive compounds in *A. cearensis* seedlings. The experiment which under-goes protected roofing was carried out using four inoculation treatments: non-inoculated control treatment, inoculated with *Gigaspora albida*, inoculated with *Claroideoglomus etunicatum* and inoculated with *Acaulospora longula.* After 160 days, the following was examined: dry matter of the aerial part, chlorophylls *a*, *b* and total, soluble carbohydrates, total proteins, total phenols, total flavonoids and total tannins. *A. cearensis* seedlings inoculate with *C. etunicatum* accumulated more dry matter of the aerial part (78.38%), total chlorophylls (24.28%) and chlorophylls *b* (53.63%), total phenols (47.82%), total flavonoids (32.28%) and total tannins (61.58%) in relation to the control treatment. Mycorrhizal technology using the *C. etunicatum* fungus is an alternative to increase the levels of foliar bioactive compounds in *A. cearensis* seedlings.**

Key words: Caatinga, phenolic compounds, arbuscular mycorrhizal fungi (AMF).

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are inhabitants of the soil and belong to the Phylum Glomeromycota (Schubler et al., 2001). Such organisms are obligatory symbionts because they complete their life-cycle only in the presence of a host plant (Souza et al., 2008). After the fungus has established on the root, the AMF absorb

*Corresponding author. E-mail: fabio.barbosa@pesquisador.cnpq.br.
Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

water and nutrients from the soil and in exchange the phytobiont makes about 20% of carbon available for the development of the fungus (Smith and Read, 2008).

Various studies relate the benefits of mycorrhizal association with legumes and point out the increased vegetable growth and optimized production of primary (Manoharan et al., 2010) and secondary metabolites (Silva et al., 2014a; Nisha and Rajeshkumar, 2010; Kapoor et al., 2004).

The increase in the production of secondary compounds in plants associated with AMF may be due to the increased nutritional supply (Toussaint et al., 2007), hormonal changes, enzymatic activation (Zhang et al., 2013) and increased activity of plastidial and mitochondrial pathways (Lohse et al., 2005), however, the effects seem to be somatory and multifactorial (Toussaint et al., 2007).

The Caatinga is a biome that is rich in leguminous species with medicinal properties that are widely used by the local population as phytotherapeutic drugs (Agra et al., 2007, 2008). *Amburana cearensis* is found among the medicinal plants of the Caatinga, a legume that is used by the local population. Parts of this plant, such as the stem, the seeds and bark are used in the production of pastilles, syrups and teas for the treatment of various diseases due to their antioxidant (Leal et al., 2003), anti-inflammatory (Leal et al., 2008), antifungal (Santos et al., 2009), antibacterial (Figueiredo et al., 2013) and antineoplastic (Costa-Lotufo et al., 2003) properties. Such therapeutic benefits have been attributed to the presence of secondary compounds, especially phenolic compounds (Canuto and Silveira, 2006; Bravo et al., 1999). However, it is unknown in mycorrhizal that inoculation influences the increase in the production of secondary metabolites in *A. cearensis* seedlings. Therefore, the following hypothesis was tested: inoculation with AMF increases the production of bio-active compounds in *A. cearensis* with the benefits depending on the fungus that was tested. The aim of this study was to examine the efficiency of the AMF in increasing the production of foliar bioactive compounds in *A. cearensis* seedlings.

MATERIALS AND METHODS

Plant, AMF and experimental implementation

A. cearensis seeds were disinfected with 20% of NaClO (2% of active chlorine) for 2 min, washed in distilled water and put to germinate in plastic pots containing sterilized soil (autoclave at 121°C/30 min/2 consecutive days).

Three AMF isolates were tested: *Acaulospora longula* Spain & N.C. Schenck (UFPE 21), *Claroideoglomus etunicatum* (W. N. Becker & Gerdemann) C. Walker & A. Schussler (UFPE 06) and *Gigaspora albida* N.C. Schenck & G.S. Sm. (UFPE 01). The inoculums were supplied by the Department of Mycology from the Federal University of Pernambuco, Brazil, multiplied on millet (*Panicum miliaceum* L.) and stocked at 4°C, for 26 months, until the moment of inoculation.

Black polyethylene pots were filled with non-sterilized soil, which was collected from the Caatinga region and showed the following chemical characteristics: organic material, 3.21 g kg^{-1}; pH, 5.2; electric conductivity, 3.53 dSm^{-1}; P, 12.68 mg dm^{-3}; K, 0.26 $cmol_c$ dm^3; Ca, 2.7 $cmol_c$ dm^{-3}; Mg, 1.8 $cmol_c$ dm^{-3}; Na, 0.49 $cmol_c$ dm^{-3}; Al, 0.05 $cmol_c$ dm^{-3}. The following AMF were identified in this soil: *Appendicispora appendicula* Spain, Sieverd. & Shenck, *Acaulospora scrobiculata* Trappe, *Acaulospora* sp.1, *Glomus macrocarpum* Tul. & Tul., *Glomus* sp.1 and *Scutellospora* sp.1 (Lima, 2014).

Plantlets with two definite leaves were transferred to the pots and inoculated at the root region with soil-inoculum of the tested AMF (200 glomerospores + colonized roots + hyphae). *A. cearensis* seedlings remained under experimental roofing for 160 days at the University of Pernambuco – Campus Petrolina, Brazil, under ambient temperature conditions (minimum: 21.7°C and maximum: 29.7°C), relative air humidity (42%) and an average global radiation (461.8 ly/day).

Evaluation of the experiment and preparation of the extract

The experiment was evaluated 160 days after inoculation. Chlorophylls (total, *a* and *b*) were tested *in vivo*, using the CFL1030 – an electronic chlorophyll level meter ClorofiLOG (Silva et al., 2014a). After examining chlorophyll, the aerial part was separated from the roots and dried (45°C) for 3 consecutive days to determine the dry matter of the aerial part. The subterranean part was removed from the substrate and the fine roots were separated from the stylopodium, washed and preserved in ethanol (50%) until examination.

Aliquots (100 mg) of the leaves were punctured and put in amber flasks containing 20 ml of ethanol (95% v/v) and maceration lasted 12 days at 25°C. After this period, the extract was filtered with gauze and refiltered with qualitative paper filter and stocked in amber flasks (- 4°C) (Brito et al., 2008). The extract was used to quantify the biomolecules.

Analysis of soluble carbohydrates and total proteins

Total proteins were quantified by a modification of the Bradford (1976): 50 µl of the extract was added to 2.5 ml of Bradford reagent and readings were taken with a spectrophotometer (595 nm) with a standard BSA curve (Bovine Serum Albumin). Total soluble carbohydrates were determined by a modification of the Dubois et al. (1956) method. The following was added to a test tube: 20 µl of the plant extract, 95 µl of distilled water, 50 µl of 80% phenol (w/v) and 2 ml of sulfuric acid. Readings were taken with a spectrophotometer (490 nm) and glucose was used to prepare the standard curve.

Analysis of phenols, flavonoids and total tannins

Total phenols were determined by a modification of the Folin-Ciocalteu method (Monteiro et al., 2006). The following was added to 100 ml volumetric balloons: 1 ml of the plant extract, 5 ml of the Folin-Ciocalteu reagent (10%, w/v) and 10 ml of sodium carbonate solution (7.5%, w/v) and the volume was completed with distilled water. Readings were taken with a spectrophotometer (760 nm) and tannic acid was used to prepare the standard curve.

Total flavonoids were quantified by a modification of the Araújo et al. (2008) method. The following was added to 25 ml flasks: 1 ml of the plant extract, 0.6 ml of glacial acetic acid, 10 ml of pyridine-methanol solution (2:8, v/v) and 2.5 ml of aluminum chlorate (5% w/v, in absolute methanol) and the volume was completed with distilled water. Readings were taken with a spectrophotometer

Table 1. Analysis of variance for the studied variables.

Variable	Effect
Dry matter of the aerial part	**
Total chlorophyll	*
Chlorophyll *a*	ns
Chlorophyll *b*	**
Mycorrhizal colonization	**
Concentration of total proteins	ns
Content of total proteins	*
Concentration of soluble carbohydrates	ns
Content of soluble carbohydrates	ns
Concentration of total phenols	*
Content of total phenols	**
Concentration of total flavonoids	**
Content of total flavonoids	**
Concentration of total tannins	*
Content of total tannins	**

*p ≤ 0.05; **p ≤ 0.01; ns: Non-significant.

(420 nm) and rutin was used to prepare the standard curve.

Analysis of total tannins was carried out with a modification of the Monteiro et al. (2006) method: 3 ml of the plant extract was mixed with 0.5 g of casein and the mixture was kept under agitation for 3 h at 25°C (160 rpm). After this period, the material was filtered with qualitative paper filter and the resulting volume was transferred to 25 ml volumetric balloons and completed with distilled water. Analysis of the remaining phenols was carried out by the Folin-Ciocalteu method and the concentration of total tannins corresponded to the difference between the levels found in this analysis and those found during quantification of total phenols.

Mycorrhizal colonization

For examination, the roots were bleached with KOH (10%, w/v, for 22 h), hydrogen peroxide (H_2O_2 10% v/v, for 20 min), acidified (HCl 1% v/v, for 5 min) and stained with Trypan blue (0.05% in lactoglycerol w/v, for 22 h) (Phillips and Hayman, 1970). A physical examination is carried out using the intersection of quadrants method (Giovannetti and Mosse, 1980).

Reagents and equipment used

The following reagents were used: glacial acetic acid, sulfuric acid, ethyl alcohol, methyl alcohol, sodium carbonate, glycerin and hydrogen peroxide (F Maia, Cotia, Brazil); bovine serum albumin and rutin hydrate (Sigma-Aldrich, São Paulo, Brazil); lactic acid, tannic acid, casein, aluminum chloride, Coomassie blue G-250, Trypan blue, glucose, phosphoric acid, phenol, pyridine (Vetec, Duque de Caxias, Brazil) and Folin-Ciocalteu reagent (Merck, Rio de Janeiro, Brazil).

The following equipment was used: a vortex shaker (Vision Scientific, Korea), a magnetic stirrer with heating (Quimis, Diadema, Brazil), a vertical autoclave (Phoenix, Araraquara, Brazil), semi-analytic scales (Bel Engineering, Italy), a digital spectrophotometer (Biospectro, Curitiba, Brazil), a drying oven (Biopar, Porto Alegre, RS, Brazil), an electronic chlorophyll content meter – ClorofiLOG -CFL 1030 (Falker, Porto Alegre, Brazil) and an orbital (Marconi, Piracicaba, Brazil).

Experimental outline and statistical analysis

The experimental outline was entirely randomized with four inoculation treatments (AMF control, inoculated with *G. albida*, inoculated with *A. longula* or inoculated with *C. etunicatum*), with five repetitions, totaling 20 experimental units. The data were submitted for analysis of variance (ANOVA) and the means were compared by the Tukey test (5%) using the Assistat program (2013).

RESULTS AND DISCUSSION

The mycorrhizal treatments had no effect on the chlorophyll *a* content, on the concentration of total proteins and on the concentration and content of total carbohydrates (Table 1).

The dry matter of the aerial part (DMAP) increased when the seedlings were colonized by *G. albida* (52.70%) and *C. etunicatum* (78.37%), in relation to the non-inoculated control treatment (Table 2), which means that the mycorrhization with *G. albida* and *C. etunicatum* was beneficial for the growth of *A. cearensis*. Similar results were found by Araim et al. (2009), Baslam et al. (2011) and Toussaint et al. (2007), for *Echinacea purpurea*, in varieties of *Lactuta sativa* and in *Ocimum basilicum*, respectively.

Increased values of mycorrhizal colonization were found in the roots of inoculated plants in relation to the control (Table 2), which supports the results obtained for other Leguminosae, such as *Libidibia ferrea* (Silva et al., 2014a).

Table 2. Dry matter of the aerial part (DMAP), total chlorophyll *a* and *b* and mycorrhizal colonization (MC) in *Amburana cearensis* seedlings, inoculated or non-inoculated with arbuscular mycorrhizal fungi, 160 days after inoculation under experimental roofing.

Inoculation treatment	DMAP (g)	Total (FCI)	Chlorophyll *a* (FCI)	Chlorophyll *b* (FCI)	MC (%)
Control	0.74[b]	36.98[b]	29.26[a]	7.72[b]	7.40[b]
Acaulospora longula	0.69[b]	44.02[ab]	31.82[a]	10.40[ab]	32.60[a]
Gigaspora albida	1.13[a]	45.88[a]	34.16[a]	1.72[a]	36.78[a]
Claroideoglomus etunicatum	1.32[a]	45.96[a]	34.18[a]	11.86[a]	33.68[a]

Means followed by the same letter do not differ from the Tukey test (5 %). FCI: Falker chlorophyll index.

Table 3. Concentration and content of total proteins and foliar soluble carbohydrates in *Amburana cearensis* seedlings, inoculated or non-inoculated with arbuscular mycorrhizal fungi, 160 days after inoculation under experimental roofing.

Inoculation treatment	Total proteins		Soluble carbohydrates	
	Concentration (mg g plant^{-1})	Content (mg plant^{-1})	Concentration (mg g plant^{-1})	Content (mg plant^{-1})
Control	67.80[a]	47.35[ab]	150.79[a]	112.03[a]
Acaulospora longula	39.50[a]	29.10[b]	255.49[a]	171.64[a]
Gigaspora albida	73.90[a]	84.08[a]	168.38[a]	198.13[a]
Claroideoglomus etunicatum	65.70[a]	68.63[ab]	453.19[a]	407.91[a]

Means followed by the same letter do not differ from the Tukey test (5%).

Inoculation with *G. albida* and *C. etunicatum* increased by 24.06 and 24.28% the concentration of total chlorophyll in relation to the non-inoculated control, respectively. Similar results were obtained for chlorophyll *b* (Table 2). On the other hand, the benefits of inoculation for chlorophyll *a* (Table 2) were not documented. As was suggested by Singh et al. (2012), the increase in chlorophyll content may be related to the increased nutrient absorption, taking into consideration that various studies indicate maximization in the production of photosynthetic pigments in terms of mycorrhization, which leads to an improvement of the nutritional status of the host (Selvaraj et al., 2009; Singh et al., 2012).

Mycorrhization did not alter the concentration and the total foliar protein content and soluble carbohydrates in *A. cearenis* (Table 3); on the other hand, there are situations in which inoculation with AMF favors the accumulation of proteins and plant sugars, as was documented by Ratti et al. (2010) and Baslam et al. (2011). There are situations in which the increase in the content of primary metabolites directs the synthesis of secondary compounds (Oliveira et al., 2013), a fact that has not been documented in this study (Tables 2, 3 and 4).

Mycorrhization with *C. etunicatum* increased in relation to the non-inoculated control, the production of total foliar phenolic compounds in the *A. cearensis* seedlings, both in content (198.92%) and concentration (47.82%) (Table 4). Levels of phenolic compounds also varied because of mycorrhizal inoculation, as was documented by Araim et al. (2009), Ceccarelli et al. (2010) and Singh et al. (2012), which makes the use of mycorrhizal technology an alternative to increase the production of such compounds with pharmacological importance.

The use of *C. etunicatum* maximized the content of total foliar flavonoids in relation to the non-inoculated control (Table 4). Possibly, mycorrhization lead to an increased absorption of nutrients, increasing the synthesis of production precursors of such compounds, such as the enzyme Chalcone synthase (*Chs*), which regulates the biosynthesis of this group of phenols (Zhang et al., 2013). An increase in the production of this group of phenolic compounds was also found in other situations (Antunes et al., 2006; Larose et al., 2002), as well as in other Leguminosae species in the Caatinga (Pedone-Bonfim et al., 2013).

Inoculation increased the production of total tannins when *C. etunicatum* was used (Table 5). Nisha and Rajeshkumar (2010) also observed an increase in the biosynthesis of tannins in *Wedilla chinensis* seedlings when inoculated with *Glomus aggregatum*. It is probable that intermediaries of biosynthetic pathways of the tannins, such as gallic acid have optimized the production through mycorrhization, as has been recently documented for the Leguminosae *L. ferrea* (Silva et al., 2014b).

Various mechanisms, nutritional and non-nutritional, have been suggested to explain the effects of mycorrhization on the increase in the biosynthesis of secondary compounds (Mandal et al., 2013; Zhang et al., 2013). Taking into consideration that mycorrhization did not alter the production of primary metabolites (Table 3), it is probable that the mechanisms that are involved in the

Table 4. Concentration of total foliar content of phenols and flavonoids in *Amburana cearensis* seedlings, inoculated or non-inoculated with arbuscular mycorrhizal fungi, 160 days after inoculation under experimental roofing.

Inoculation treatment	Total phenols		Total flavonoids	
	Concentration (mg g plant^{-1})	Content (mg plant^{-1})	Concentration (µg g plant^{-1})	Content (µg plant^{-1})
Control	7.11^{b}	4.67^{b}	627.14^{b}	393.92^{b}
Acaulospora longula	8.99^{ab}	6.23^{b}	843.75^{a}	582.76^{b}
Gigaspora albida	7.53^{b}	8.50^{b}	522.08^{b}	594.59^{b}
Claroideoglomus etunicatum	10.51^{a}	13.96^{a}	829.59^{a}	1098.08^{a}

Means followed by the same letter do not differ from the Tukey test (5%).

Table 5. Concentration of total foliar content of tannins in *Amburana cearensis* seedlings, inoculated or non-inoculated with arbuscular mycorrhizal fungi, 160 days after inoculation under experimental roofing.

Inoculation treatment	Total tannins	
	Concentration (mg g plant^{-1})	Content (mg plant^{-1})
Control	6.09^{b}	4.06^{b}
Acaulospora longula	8.33^{ab}	5.90^{b}
Gigaspora albida	6.61^{b}	7.51^{b}
Claroideoglomus etunicatum	9.84^{a}	12.05^{a}

Means followed by the same letter do not differ from the Tukey test (5%).

foliar phenols increase in *A. cearensis* are non-nutritional as was suggested by Toussaint et al. (2007). Such mechanisms involve an increase in the enzymatic activity, increase in the gene expression, maximized activation of the metabolic pathways and optimized biosynthesis of signaling in mycorrhizal plants (Walter et al., 2000; Lohse et al., 2005; Zhang et al., 2013). Furthermore, it is probable that the inoculated AMF increased the absorption of P, a fact that is well documented for mycorrhizal plants (Smith and Read, 2008), which is an important requirement for the biosynthetic pathways of phenolic compounds (Heldt, 2005).

Benefits of the mycorrhizal technology for the production of bioactive compounds were found for other plants from the Caatinga, as was referred to by Pedone-Bonfim et al. (2013), Oliveira et al. (2013) and Silva et al. (2014a), for *Anadenanthera colubrina, Myracrodruon urundeuva* and *L. ferrea*, respectively. Such benefits were also observed for the first time in *A. cearensis,* which confirms the initial working hypothesis.

The mycorrhizal technology, employing selected AMF, favored the production of the phytomass of *A. cearensis* with an elevated concentration of bioactive compounds, which possess various therapeutic properties. Therefore, the fungus *C. etunicatum* is recommended as a biotechnological alternative to maximize the production of foliar bioactive compounds in *A. cearensis* seedlings. This way, a low cost biotechnological protocol was established to maximize the production of plant biomolecules that are important to the phytotherapeutic industry. Other experiments have to be carried out to elucidate the benefits under field conditions and to determine whether there is a specific increase of molecules that are of industrial interest, such as vanillic acid.

ACKNOWLEDGEMENTS

The authors acknowledged Cleiton Santos Lima for supplying the seeds and his help in conducting the experiment; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting scholarships to PTF Oliveira; and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for their financial support.

Conflict of interest

The authors do not have any conflicts of interest.

REFERENCES

Agra MDF, Silva KN, Basílio IJLD, Freitas PF, Barbosa-Filho JM (2008). Survey of medicinal plants used in the region Northeast of Brazil. Rev. Bras. Farmacogn. 18:472–508.

Agra MDF, Freitas PF, Barbosa-filho JM (2007). Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. Rev. Bras. Farmacogn. 17:114-140.

Antunes PM, Varennes A, Rajcan I, Goss MJ (2006). Accumulation of specific flavonoids in soybean (*Glycine max* (L.) Merr.) as a function of the early tripartite symbiosis with arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* (Kirchner) Jordan. Soil Biol. Biochem. 38:1234-1242.

Araim GA, Saleem JTA, Charest C (2009). Root colonization by an arbuscular mycorrhizal (AM) fungus increase growth and secondary metabolism of purple coneflower, *Echinacea purpurea* (L.) Moench. J. Agric. Food Chem. 57:2255-2258.

Araújo TAS, Alencar NL, Amorim ELC, Albuquerque UP (2008). A new approach to study medicinal plants with tannins and flavonoids contents from the local knowledge. J. Ethnopharmacol. 120:72-80.

Assistat Program (2013). Statistical Assistance. Universidade Federal de Campina Grande, Campina Grande, Paraíba, Brazil. Available at: http://www.assistat.com/indexi.html

Baslam M, Garmendia I, Goicoechea N (2011). Arbuscular mycorrhizal fungi (AMF) improved growth and nutritional quality of greenhouse-grown lettuce. J. Agric. Food Chem. 59:5504-5515.

Bradford MM (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72(1-2):248-254.

Bravo JA, Sauvain M, Gimenez AT, Muñoz VO, Callapa J, Men-Olivier LL, Massiot G, Lavaud C (1999). Bioactive phenolic glycosides from *Amburana cearensi*s. Phytochemistry 50:71-74.

Brito HO, Noronha EP, França LM, Brito LMO, Prado SA (2008). Análise da composição fitoquímica do extrato etanólico das folhas de *Annona squamosa* (ATA). Rev. Bras. Farm. 89:180-184.

Canuto KM, Silveira ER (2006). Constituintes químicos da casca do caule de Amburana cearensis A.C. Smith. Química Nova 29:1241-1243.

Ceccarelli N, Curadi M, Martelloni L, Sbrana C, Picciarelli P, Giovannetti M (2010). Mycorrizal colonization impacts on phenolic content and antioxidant properties of artichoke leaves and flower heads two years after field transplant. Plant Soil 335:311-323.

Costa-Lotufo LV, Jimenez PC, Wilke DV, Leal LKAM, Cunha GMA, Silveira ER, Canuto KM, Viana GSB, Moraes MEA, Moraes MO, Pessoa C (2003). Antiproliferative effects of several compounds isolated from *Amburana cearensis* A. C. Smith. J. Biosci. 58:675-680.

Dubois M, Guiles A, Hamilton JK, Rebers PA, Smith F (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-355.

Figueiredo FG, Ferreira EO, Lucena BFF, Torres CMG, Lucetti DL, Lucetti ECP, Silva JMFL, Santos FAV, Medeiros CR, Oliveira GMM, Colares AV, Costa JGM, Coutinho HDM, Menezes IRA, Silva JCF, Kerntopf MR, Figueiredo PRL, Matias EFF (2013). Modulation of the antibiotic activity by extracts from *Amburana cearensis* A. C. Smith and *Anadenanthera macrocarpa* (Benth.) Brenan. Biomed Res. Int. I:1-5.

Giovannetti M, Mosse B (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. N. Phytol. 84:489-500.

Heldt HW (2005). Plant Biochemistry. Thrid edition, Elsevier Academic Press.

Kapoor R, Giri B, Mukerji KG (2004). Improved growth and essential oil yield and quality in *Foeniculum vulgare* Mill on mycorrhizal inoculation supplemented with P-fertilizer. Bioresour. Technol. 93:307-311.

Larose G, Chênevert R, Moutoglis P, Gagné S, Piché Y, Vierheilig H (2002). Flavonoid levels in roots of *Medicago sativa* are modulated by the developmental stage of the symbiosis and the root colonizing arbuscular mycorrhizal fungus. J. Plant Physiol. 159:1329-1339.

Leal LKAM, Fonseca FN, Pereira FA, Canuto KM, Felipe CFB, Fontenele JB, Pitombeira MV, Silveira ER, Viana GSB (2008). Protective effects of amburoside A, a phenol glucoside from *Amburana cearensis*, against CCl4-induced hepatotoxicity in rats. Planta Med. 74:497-502.

Leal LKAM, Nechio N, Silveira ER, Canuto KM, Fontenele JB, Ribeiro RA, Viana GSB (2003). Anti-inflammatory and smooth muscle relaxant activities of the hydroalcoholic extract and chemical constituents from *Amburana cearensis* A C Smith. Phytother. Res. 17:335-340.

Lima CS (2014). Tecnologia micorrízica para maximização da produção de biomoléculas foliares em mudas de umburana-de-cambão e de ingazeira. Dissertação de Mestrado, Universidade de Pernambuco, Brasil.

Lohse S, Schliemann W, Ammer C, Kopka J, Strack D, Fester T (2005). Organization and metabolism of plastids and mitochondria in arbuscular mycorrhizal roots of *Medicago truncatula.* Plant Physiol. 139:329-340.

Mandal S, Evelin H, Giri B, Singh VP, Kapoor R (2013). Arbuscular mycorrhiza enhances the production of stevioside and rebaudioside-A in *Stevia rebaudiana* via nutritional and non-nutritional mechanisms. Appl. Soil Ecol. 72:187-194.

Manoharan PT, Shanmugaiah V, Balasubramanian N, Gomathinayagam S, Sharma MP, Muthuchelian K (2010). Influence of AM fungi on the growth and physiological status of *Erythrina variegata* Linn. grown under different water stress conditions. Eur. J. Soil Biol. 46:151-156.

Monteiro JM, Almeida CFCBR, Albuquerque UP, Lucena RFP, Florentino ATN, Oliveira RLC (2006). Use and traditional management of *Anadenanthera colubrina* (Vell.) Brenan in the semi-arid region of northeastern Brazil. J. Ethnobiol. Ethnomed. 7:1-7.

Nisha MC, Rajeshkumar S (2010). Influence of arbuscular mycorrhizal fungi on biochemical changes in *Wedilla Chinensis* (Osbeck) Merril. Anc. Sci. Life 29:26-29.

Oliveira MS, Albuquerque UP, Campos MAS, Silva FSB (2013). Arbuscular mycorrhizal fungi (AMF) affects biomolecules content in *Myracrodruon urundeuva* seedlings. Ind. Crop Prod. 50:244-247.

Pedone-Bonfim MVL, Lins MA, Coelho IR, Santana AS, Silva FSB, Maia LC (2013). Mycorrhizal technology and phosphorus in the production of primary and secondary metabolites in cebil (*Anadenanthera colubrina* (Vell.) Brenan) seedlings. J. Sci. Food Agric. 93:1479-1484.

Phillips JM, Hayman D (1970). Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158-161.

Ratti N, Verma HN, Gautam SP (2010). Effect of *Glomus* species on physiology and biochemistry of *Catharantus roseus.* Indian J. Microbiol. 50:355-360.

Santos PHA, Santos IS, Melo VMM, Vasconcelos IM, Carvalho AO, Gomes VM (2009). Partial characterization and antimicrobial activity of peptides from *Amburana cearensis* seeds against phytopathogenic fungi and yeasts. Acta Physiol. Plant. 32:597-603.

Schubler A, Schwarzott D, Walker C (2001). A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol. Res. 105:1413-1421.

Selvaraj T, Nisha MC, Rajeshkumar S (2009). Effect of indigenous arbuscular mycorrhizal fungi on some growth parameters and phytochemical constituents of *Pogostemon patchouli* Pellet. J. Sci. Technol. 3:222-234.

Singh NV, Singh SK, Singh AK, Meshram DT, Suroshe SS, Mishra DC (2012). Arbuscular mycorrhizal fungi (AMF) induced hardening of micropropagated pomegranate (*Punica granatum* L.) plantlests. Sci. Hortic. 136:122-127.

Silva FA, Silva FSB, Maia LC (2014a). Biotechnical application of arbuscular mycorrhizal fungi used in the production of foliar biomolecules in ironwood seedlings [*Libidibia ferrea* (Mart. ex Tul.) L.P.Queiroz var. *ferrea*]. J. Med. Plants Res. 8:814-819.

Silva FA, Ferreira, MRA, Soares LAL, Sampaio EVSB, Silva FSB, Maia LC (2014b). Arbuscular mycorrhizal fungi increase gallic acid production in leaves of field grown *Libidibia ferrea* (Mart. *ex*. Tul.) L. P. Queiroz. J. Med. Plant. Res. 8:1110-115.

Smith SE, Read DJ (2008). Mycorrhizal symbiosis. Third edition. Academic Press, London.

Souza FA, Silva ICL, Berbara RLL (2008). Fungos micorrízicos arbusculares: muito mais diversos do que se imaginava. In: Moreira FMS, Siqueira JO, Brussaard L. (eds.) Biodiversidade do solo em ecossistemas brasileiros. Lavras, Ed. UFLA. pp. 483-536.

Toussaint JP, Smith FA, Smith SE (2007). Arbucular mycorrhizal fungi can induce the production of phytochemicals in sweet basil irrespective of phosphorus nutrition. Mycorrhiza 17:291-297.

Walter MH, Fester T, Strack D (2000). Arbuscular mycorrhizal fungi induce the non-mevalonate methylerythritol phosphate pathway os isoprenoid biosynthesis correlated with accumulation of the 'yellow pigment' ond other apocarotenoids. Plant J. 21:571-578.

Zhang RQ, Zhu HH, Zhao HQ, Yao Q (2013). Arbuscular mycorrhizal fungal inoculation increases phenolic synthesis in clover roots via hydrogen peroxide, salicylic acid and nitric oxide signaling pathways. J. Plant Physiol. 170:74-79.