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# Endophytic mycobiota from leaves of *Indigofera suffruticosa* Miller (Fabaceae): The relationship between seasonal change in Atlantic Coastal Forest and tropical dry forest (Caatinga), Brazil

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**Endophytic fungi were isolated from leaves of the medicinal plant, *Indigofera suffruticosa* collected at the Atlantic Coastal Forest and tropical dry forest (Caatinga), Pernambuco, Brazil, during the dry and rainy seasons. A total of 107 fungal isolates, representing nine fungal taxa, were obtained and classified as Ascomycota, among them *Colletotrichum gloeosporioides* with relative frequency (fr) 27.1% and *Pseudocochliobolus pallescens* with fr 16.82% were the most frequent. *Curvularia australiensis* and *Chaetomella raphigera* were isolated only in Caatinga during rainy and dry seasons, respectively, and for the first time they were isolated from a Caatinga plant. *Lasiodiplodia theobromae* was found only in Atlantic Coastal Forest in dry season, and according to Simpson (*D'*) and Shannon-Wiener (*H'*) indices fungal diversity were considered statistically significant in this forest. Besides, a greater similarity was observed between fungi isolated in Atlantic Coastal Forest and Caatinga in the dry season, suggesting the predominance of seasonality rather than geographical factor. This study is the first report on endophytes fungi from *I. suffruticosa*, and the results represent an important basis for further studies in the fields of ecology and biotechnology, since these endophytic fungi may be important source for future study in searching for new natural compounds with biological activities.**

**Key words:** Ascomycota, Caatinga, endophytic mycobiota, fungal diversity, *Indigofera suffruticosa*, seasonal predominance.

## INTRODUCTION

Endophytic fungi are microorganisms that, during part or all of their life cycle, colonize inter and/or intracellularly healthy plant tissues, in an asymptomatic manner, without causing any apparent damage to their host (Tan and Zou, 2001), many of which are able to produce secondary metabolites that may offer protection against different phytopathogens and herbivores (Rivera-Orduña et al., 2011). The endophytic fungi represent a wide diversity of microbial adaptations that have developed in special and unusual environments, making them a great source of study and research for new chemicals for medicinal, industrial and agricultural uses (Aly et al., 2011; Kusari and Spiteller, 2011; Rajulu et al., 2011; Li et al., 2012; Kusari et al., 2013; Lou et al., 2013; Pharamat et al., 2013; Teiten et al., 2013). Furthermore, the production by the endophytic fungi of a variety of secondary compounds, such as alkaloids, terpenoids, steroids and aromatic compounds that are repellent or toxic to their enemies, gives greater competitive ability to colonized plants due to this symbiosis (Redman et al., 2002; Arnold et al., 2003; Rodriguez and Redman, 2008; Porras-Alfaro and Bayman, 2011).

Clarke et al. (2006) demonstrated that plants colonized by endophytes have greater resistance as compared to non-colonized plants, and that endophytic fungi may be used to suppress plant diseases in various environments. Although this resistance mechanism is not fully understood, some studies have shown that climate variation and cultivation conditions influence vegetable matabolism (Simões-Ambrosio et al., 2010; Zalamea et al., 2013) and that seasonal variation and geographical location affect endophytic colonization (Martín et al., 2004; Göre and Bucak, 2007; Guo et al., 2008). Therefore, studies on endophytic fungi are of great importance for providing essential information for assessing fungal diversity under the influence of geographical and seasonal factors.

Few studies have been conducted with the communities of endophytic fungi in leaves of plants of the same species growing in different locations with distinct ecological characteristics (Collado et al., 1999; Martín et al., 2004; Göre and Bucak, 2007; Vaz et al., 2014). However, the composition of the endophytic mycobiota from different locations is very important for the understanding of the relationship between endophytic fungal populations and the establishment of plants subjected to distinct selection pressures in different ecosystems, mainly considering that endophytes may also increase the resistance of plants against biotic and abiotic stresses and produce compounds of biotechnological interest (Azevedo, 2014).

*Indigofera suffruticosa* Mill. is a shrub 1-2 m high originally from the Antilles and Central America, which was introduced and cultivated in Brazil on a large scale for the extraction of natural indigo dye for the textile industry, but in the 1980s this natural dye was replaced by an artificially produced pigment (Alzugaray and Alzugaray, 1988). *I. suffruticosa* is found in different biomes of Brazil, among these tropical dry forests (Caatinga) and Atlantic Coastal Forest. This plant is used in the folk medicine as a febrifuge, purgative, sedative (Almeida, 1993; Hastings, 1990) and to treat epilepsy, infection and inflammation such as gastrointestinal pain (Roig, 1988; Matos, 1999; Agra et al., 2007). Previous studies have shown that the leaves of *I. suffruticosa* have embryotoxic effects (Leite et al., 2004), antimicrobial (Leite et al., 2006; Bezerra dos Santos et al., 2015) and anticonvulsant (Almeida et al., 2013) activities, and act as gastroprotective agent (Luiz-Ferreira et al., 2011).

Almost half of the world's tropical forests are represented by tropical dry forests. In Brazil, the tropical dry forests is named Caatinga due to the predominant type of vegetation (Albuquerque et al., 2012), and climate marked by high temperatures with sparse and irregularly distributed rains, with annual average precipitation ranging between 250 and 500 mm (Basso et al., 2005). The Caatinga soils are of different origins, and as a rule, are chemically fertile, well drained and oxygenated. Water bodies are rarely permanent, drying completely during the summer (Basso et al., 2005), but has faced intensive degradation from exhaustion due to deliberate introductions of exotic plants for giving support to farming activities (Leal et al., 2005). The rapid reduction of forests in tropical areas of Brazil is a major problem since this situation could result in the extinction of many endophytic fungi with the loss of potentially important products for use in agricultural, pharmaceutical, environmental and other fields of interest (Azevedo, 2014).

Another important biome of Brazil is the Atlantic Coastal Forest one of the most widely distributed tropical forests in Southern America, occupying almost all Brazilian Eastern coast besides inland areas and is marked by the occurrence of three important forest types (Oliveira-Filho and Fontes, 2000). That is characterized by a high biodiversity, and by proximity to the Atlantic Ocean, which provides a stable source of humidity, allowing high vegetation density. A floristic survey of the southern limit of Atlantic Forest area has been carried out, revealing several species of economic interest, many of which exhibit medicinal properties (Basso et al., 2005). Ribeiro et al. (2009) reported that due to the fragmentation process only 11.73% of the Atlantic Coastal Forest remains in Brazil, of which 12.1% is in Pernambuco State, where the sugar-cane plantations is among one of the main factors responsible for the fragmentation (Lôbo et al., 2011).

The isolation and identification of endophytic mycobiota is necessary, since the medicinal properties of a plant may be due to the ability of their endophytic microorganisms to produce biologically active secondary metabolites of medical and industrial interest, e.g. the taxol, which an anticancer agent produced by *Taxus brevifolia* Nutt., and by its endophyte fungus *Taxomyces andreanae* Strobel, A. Stierle, D. Stierle & W.M. Hess (Stierle et al., 1993; Bhardwaj and Agrawal, 2014).

In this context, the results of this research may

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contribute to future search on bioactive compounds derived from endophytic fungi species, since this is the first study on endophytic fungi colonization associated with *I. suffruticosa*. Thus, the objective of this study was to evaluate the relative geographical influence of two different locations and seasonality on the endophytic mycobiota associated with *I. suffruticosa*.

## MATERIALS AND METHODS

### Plant material and study area

The collection of the plant material was done at natural growing in two areas studied in the rainy season (June) of 2009 and dry season (January) of 2010, at two different ecosystems. One collection site is located at the municipality of Caatinga (08°19'33"S, 36°04'21"W) in semiarid region of Pernambuco State, with average annual precipitation of 526.2 mm and a dry season that typically lasts nine months per year. The other site is in the municipality of Atlantic Coastal Forest (07°50'00"S, 34°54'30"W) in the coast of the Pernambuco State, with average annual precipitation of 1709.2 mm and a dry season that typically lasts three months per year (Rodal et al., 2008; APAC, 2013). Leaves of three health specimens of *I. suffruticosa* were randomly collected, in three different points of each areas studied and were put in plastic bags, transported to the laboratory, processed on up to 48 h for isolation and characterization of endophytic fungi according to methodologies established previously (Araújo et al., 2002). The plant material was authenticated by the Biologist Marlene Barbosa from the Botanic Department, Universidade Federal de Pernambuco (UFPE). A voucher specimen number 45217 has been deposited at the UFP Geraldo Mariz Herbarium of UFPE.

### Isolation of endophytic fungi

The plant material was subjected to a surface sterilization process in accordance with the methodology described by Araújo et al. (2002), where healthy leaves of *I. suffruticosa* were washed with running water, followed by immersion in 70% ethanol for one minute, sodium hypochlorite (2-2.5% active chlorine) for four minutes and washed three times in sterilized, distilled water. After surface sterilization, the samples were cut into fragments of 0.5 $cm^2$ and transferred aseptically to Petri dishes containing potato dextrose agar (PDA) culture medium supplemented with chloramphenicol (50 mg $L^{-1}$) to suppress bacterial growth. The Petri dishes were inoculated each with six leaf fragments from different points of each area studied, in triplicate, were incubated at room temperature (28 ± 2°C) for 30 days, analyzed daily and any fungal colony present was isolated, purified and kept in PDA medium for subsequent identification. The control of efficiency of the sterilization method was confirmed by seeding 1 mL of the last washing in Petri dishes containing PDA medium.

### Identification of endophytic fungi

The morphological identification of endophytic fungi from *I. suffruticosa* was performed at the Micoteca URM, Department of Mycology, Federal University of Pernambuco, Recife, Brazil. The macro and micro morphological characteristics were observed based in technics and literature specific (Morton and Smith, 1963; Ellis, 1971, 1976; Sutton, 1980; Barnett and Hunter, 1987; Hanlin, 2000). After morphological identification, the representative fungi cultures were preserved in the Culture Collection – Micoteca URM (WDCM604), Department of Mycology, Federal University of Pernambuco, Brazil.

### Data analysis

The frequencies of isolation of endophytic fungi were calculated. The absolute frequency (f) was estimated as the total number of endophytes isolates, and the relative frequency (fr) was the number of endophytes of each species divided by the total number of endophytic fungi. The rate of colonization was estimated as the total number of fragments of leaves colonized by fungi, divided for total number of fragments used for isolation of endophytes, as reported by Larran et al. (2002).

The number of isolates obtained was used to calculate the components of diversity: richness (S), number of different species found at each site and in each periods of the year, and evenness (*J'*), the Simpson (*D'*) and Shannon-Wiener (*H'*) diversity indices, as described by Magurran (1988) and the similarity matrix was constructed from the Sørensen index, which was grouped using UPGMA as clustering algorithm.

$$\text{Shannon-Wiener index } (H') = -\sum_{i=1}^{s} pi \ln pi$$

$$\text{Simpson index } (D') = 1 / \sum_{i=1}^{s} pi^2$$

evenness (*J'*) = *H'* / ln N

In the Shannon index, p is the proportion (n/N) of fungi of one particular species found (n) divided by the total number of fungi found (N), ln is the natural log, Σ is the sum of the calculations, and s is the number of species.

In the Simpson index, p is the proportion (n/N) of individuals of one fungal species found (n) divided by the total number of fungi found (N), Σ is still the sum of the calculations, and s is the number of species. The evenness was the ratio of observed diversity to maximum diversity (ln N), N is the total number of fungi found.

### Statistical analysis

Data were analyzed using Bioestat v. 5.0 by one-way analysis of variance (ANOVA) and Tukey test to determine statistical significance. A *p*-value of <0.05 was considered to be statistically significant.

## RESULTS AND DISCUSSION

From 216 fragments analyzed, of which 54 were collected at each site and in different periods of the year, a total of 107 fungal isolates, representing 9 fungal taxa, were obtained from isolations. The isolates were identified as Ascomycota and belong to the groups Pleosporales, Sordariomycetidae, Xylariales, Diaporthales, Leotiomycetes and Bryosphaeriales. Among them, the *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. was the taxon most frequent (27.41%), followed by *Pseudocochliobolus pallescens* Tsuda and Ueyama (16.82%), *Khuskia oryzae* H.J. Hunds (14.95%)

**Table 1.** Absolute (f) and relative (fr) frequency of endophytic fungi isolated from *I. suffruticosa* at Atlantic Coastal Forest and Caatinga (tropical dry forest) in rainy and dry season (Brazil).

| **Endophytic fungi** | **Atlantic Coastal Forest** | | | | **Caatinga** | | | | **Total** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Rainy** | | **Dry** | | **Rainy** | | **Dry** | | | |
| | **f** | **fr** | **f** | **fr** | **f** | **fr** | **f** | **fr** | **f** | **fr** |
| *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. | | | 8 | 23.55 | 6 | 37.5 | 15 | 57.7 | 29 | 27.10 |
| *Colletotrichum dematium* (Pers.) Grove (URM-6063) | 4 | 12.9 | | | 2 | 12.5 | | | 6 | 5.60 |
| *Pseudocochliobolus pallescens* Tsuda & Ueyama (URM-6064) | 14 | 45.17 | | | 4 | 25.0 | | | 18 | 16.82 |
| *Phomopsis archeri* B. Sutton (URM-5630) | 2 | 6.45 | | | 2 | 12.5 | | | 4 | 3.74 |
| *Curvularia australiensis* (Tsuda & Ueyama) Manamgoda, L. Cai & K.D. Hyde | | | | | 2 | 12.5 | | | 2 | 1.87 |
| *Pestalotiopsis maculans* (Corda) Nag Raj (URM-6061) | 9 | 29.03 | 6 | 17.64 | | | | | 15 | 14.02 |
| *Khuskia oryzae* H.J. Huds. (URM-6060) | 2 | 6.45 | 6 | 17.64 | | | 8 | 30.77 | 16 | 14.95 |
| *Chaetomella raphigera* Swift | | | | | | | 3 | 11.53 | 3 | 2.80 |
| *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. | | | 3 | 8.82 | | | | | 3 | 2.80 |
| Sterile mycelia | | | 11 | 32.35 | | | | | 11 | 10.30 |
| Total of endophytic fungi | 31 | 100 | 34 | 100 | 16 | 100 | 26 | 100 | 107 | 100 |

and *Pestalotiopsis maculans* (Corda) Nag Raj (14.02%). The isolates that did not develop reproductive structures in medium culture were grouped as sterile mycelia (Table 1).

The species *P. pallescens*, *Phomopsis archeri* B. Sutton and *Colletotrichum dematium* (Pers.) Grove were detected in both sites and in particular only during the rainy season. Also, *Colletotrichum gloeosporioides* and *Khiskia oryzae* were detected in both sites, but not season association was observed. The species *P. maculans* presented locality specificity, since this species was isolated only in collections made in the Atlantic forest in both periods of the year (Table 1). Moreover, *Chaetomella raphigera* Swift, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. and *Curvularia australiensis* (Tsuda & Ueyama) Manamgoda, L. Cai & K.D. Hyde exhibit specificity regarding the locality and period of the year. Specifically, *C. australiensis* and *C. raphigera* were isolated only in Caatinga during the rainy season and in the dry season, respectively, whilst *L. theobromae* was found only in Atlantic forest during the dry season (Table 1).

Incidental species are frequently observed in studies with endophytic fungi and represent those which have been isolated in a small number, as described by Siqueira et al. (2011). Incidental species were observed in the present study and included *C. australiensis*, *C. raphigera* and *L. theobromae*. The genus *Curvularia* was also found as incidental species in *Lippia sidoides* Cham. (Siqueira et al., 2011), and in the study of Bezerra et al. (2013) from the analysis of the endophytic fungi isolated from cactus *Cereus jamacaru* DC. growing in Caatinga, Brazil. The species *L. theobromae* has been isolated as endophytes associated with *Piper hispidum* Sw. (Piperaceae) leaves (Orlandelli et al., 2012) and *C. raphigera* were also found as incidental species in *Catharanthus roseus* (L.) G. Don and *Cassia tora* L., herbaceous medicinal plants from the Malnad region, southern India (Krishnamurthy et al., 2008). Investigation of fungi isolated from plants that live in extreme environments is of paramount importance, since some studies have raised the hypothesis that endophytic fungi can alter the levels of the plant hormones that control stomatal behavior and osmotic adjustment (Malinowski and Belesky, 2000; Mandyam and Jumpponen, 2005). Given that plants are exposed to environmental stress due to factors such as low water availability, high salinity, high irradiance and nutrient deprivation, the association with these microorganisms can be the determining factor in the adaptation of plants to environmental conditions adverse.

In our study, the properties of the Atlantic Coastal Forest and Caatinga ecosystems present differences in several aspects. In terms of altitude, the municipality of Atlantic Coastal Forest is approximately 19 m a.s.l. (above sea level) and Caatinga approximately 552 m a.s.l. Comparing the type of soil, in Atlantic Coastal Forest, there is a predominance of floodplain type soil, while in Caatinga, clay types predominate. In terms of the vegetation around the site, the Caatinga is generally described as a woody vegetation with discontinuous canopy, variable in both height (3-9 m) and density, composed mostly of succulent (cacti essentially) and nonsucculent shrubs and trees, most of which are armed with either thorns or prickles and bear microphyllous foliages, though they are leafless during the long-lasting periods of drought; the ground layer is rich in bromeliads annual herbs, and geophytes (Ab'Saber, 1974; Prado,2003; Cardoso and Queiroz, 2011). According to data shown in Table 1, it can be seen that the environmental conditions found in Atlantic Coastal Forest may have favored more endophytic colonization than those found in Caatinga, since the number of fungi isolated in Atlantic Coastal Forest in the two seasons studied, was higher than the number of isolates from Caatinga in the same periods. Differences related to the number of species and specimens of endophytic fungi, in studies conducted in different locations and periods of the year, can be explained by the fact that the species involved can vary according to the host, geographic distribution, host plant age, ecological and seasonal conditions, including altitude and precipitation, and differences in the vegetation around the local where the plant was collected (Arnold et al., 2003; Helander et al., 2007).

Our results are in agreement with those observed in other studies, where it is reported that the population of endophytic fungi is directly related to the difference in intensity and period of exposure of these organisms to solar radiation, which generally occurs in areas that have differences in climatic factors (Collado et al., 1999; Martín et al., 2004; Martin Pinto et al., 2006; Hashizume et al., 2010). In addition, other environmental parameters such as soil, amount of available water, plant physiological condition at the time of collection and management, can also influence endophytic colonization (Hashizume et al., 2008).

The dominant endophytic fungi differed according to the collection sites and the period of the year (Table 1). In the municipality of Atlantic Coastal Forest, it was observed that the dominant endophytes differed according to the period of the year in which the collections were made. The fungus *C. gloeosporioides* was the dominant species in Caatinga in both periods of the year, unlike Atlantic Coastal Forest where *P. pallescens* was the dominant species in the rainy season and sterile mycelia was dominant in the dry season.

These results are in accordance with other studies of endophytes where it has been reported that many species of fungi can be isolated from a certain plant, but only a few are frequently found. This was the case, for example, in the study by Xing et al. (2010), which investigated the distribution and diversity of endophytic fungi in different tissues and ages of American ginseng. Among the 27 taxa isolated, *Glomerella* sp. was the dominant fungus in most tissues. In addition, Siqueira et al. (2011) analysing the endophytic mycobiota of leaves and stems of *L. sidoides* observed that *C. gloeosporioides* was the most frequent species in the leaves, while *Alternaria alternata* (Fr.) Keissl. was most frequent in stems. In a recent study, Vaz et al. (2014) observed that *Pseudocercospora basintrucata* Crous and *Xylaria* sp. were the most frequent taxa isolated from the *Luma apiculata* (DC.) Burret in Andean Patagonian forest, while *Colletotrichum* sp. was the most frequent fungal species isolated from *Eugenia neomyrtifolia* M. Sobral in Atlantic rainforest, Brazil.

Studies in the semiarid region of the Brazilian northeast showed the endophytic associations of *Opuntia ficus-indica* (L.) Mill. and *Cereus jamacaru* with isolates of *K. oryzae* and species of the genus *Curvularia* (Bezerra et al., 2012, 2013). The genera *Curvularia* was also isolated by Oliveira et al. (2013) in a study on filamentous fungi diversity obtained from the soil of the semiarid area (Caatinga), Pernambuco, Brazil.

Although, the species *C. australiensis* and *C. raphigera* of endophytic fungi have been reported in isolates from other plant species, our study is the first to report on these fungi isolated from a plant of the semiarid region of Brazil. However, *C. australiensis* and *C. raphigera* were isolated as endophytic fungi in seed of *Withania somnifera* (L.) Dunal collected in a semiarid region of Pakistan (Khan et al., 2010). These species have also been isolated from leaves of *Ziziphus* sp. collected in the mountains of arid regions located at the South of the Arabian Gulf (El-Nagerabi et al., 2013). Nevertheless, in the semiarid region of Pernambuco, Brazil, the species *C. australiensis* was found in soil (Oliveira et al., 2013), and the genus *Drechslera* (nowadays known as Curvularia) was also isolated as endophyte in seeds of Cowpea (*Vigna unguiculata* (L.) Walp.), a plant of the Fabaceae family (Rodrigues and Menezes, 2002).

In our work, there was an occurrence of fungi that was not possible to sporulate in culture after a certain incubation period and they were classified as sterile mycelia. This was not a surprise since sterile mycelia were prevalent in several studies with endophytes (Xing et al., 2010; Siqueira et al., 2011; Sun et al., 2012; Bezerra et al., 2013). We hypothesized that the difficulty that some fungi have to develop reproductive structures is probably related to the fact that artificial culture media do not offer the same set of conditions that these fungi encounter in their host plants. These fungi have been identified in some studies with the aid of molecular biology (Guo et al., 2003; Giordano et al., 2009; Lacap et al., 2003), and the species classified as sterile mycelia in this study will also be identified through these tools in future studies.

Table 2 shows the diversity indices of endophytic fungal species isolated at different study sites and season, which compose three groups in accordance with the component of diversity that express richness (S), evenness (*J'*) and dominance (D). The results obtained indicate that the richness of fungal species at the different study sites was S=5 during the rainy season, but showed variations in the dry season with S=4 in the Atlantic Coastal Forest and S = 3 in the Caatinga. The diversity was analyzed by Shannon-Wiener (*H'*) and Simpson (*D'*)

**Table 2.** Diversity, evenness and species richness of endophytic fungi isolated from *I. suffruticosa* at Atlantic Coastal Forest and Caatinga (tropical dry forest) in rainy and dry season (Brazil).

| Site of collection | *H'* | *D'* | *J'* | S |
|---|---|---|---|---|
| **Atlantic coastal forest** | | | | |
| Rainy season | 1.334 | 3.215 | 0.388 | 5 |
| Dry season | 2.328 | 8.000 | 0.660 | 4 |
| **Caatinga** | | | | |
| Rainy season | 1.490 | 4.048 | 0.537 | 5 |
| Dry season | 0.926 | 2.272 | 0.284 | 3 |

Shannon-Wiener (*H'*) and Simpson (*D'*) diversity indices, evenness (*J'*) and richness (S).

indices, and showed distinct variation of fungi, without repetition of the results at any of the study sites or period of the year. The highest diversity indices and evenness (*J'*) was found in Atlantic Coastal Forest in the dry season. Based on the Shannon-Wiener (*H'*) and Simpson (*D'*) indices, it is possible to see that there is a dominance of species during the rainy season as compared to the dry season in Caatinga was significant, while during the dry season in relation to the rainy season in Atlantic Coastal Forest, it was not significant. The differences in fungal endophytic assemblages observed in this study, indicate that variations in the study site and period of the year influence the species colonization and distribution in *I. suffruticosa* leaves, since the differences observed in the rainy and dry seasons in the different study sites were considered statistically significant. However, other factors may also influence endophytic colonization, such as the age of the plant collected, the differences in nutritional supply of host tissue and the differences in climatic conditions for example, relative humidity and intensity of light exposure (Talley et al., 2002; Vieira et al., 2011).

Moreover, some studies have indicated that the endophytic diversity in dry areas is low due to enviromental factors, such as reduced rainfall and low vegetation density (Arnold et al., 2003; Suryanarayanan et al., 2002, 2003, 2005). For instance, Tejesvi et al. (2005) found only five species in a study with endophytic fungal assemblages from inner bark and twig of *Terminalia arjuna* (Roxb.) Wight & Arn. In a study on leaves and stems of plants from desert areas in China, Sun et al. (2012) found values for Shannon's index varying from 0.29 to 4.78, and for Simpson's index from 1.00 to 6.60. However, in contrast to our results, some studies have reported high endophytic diversity in plants of Caatinga, Pernambuco. A total of 71 fungi species involving 23 genera was found within four hundred seeds of Cowpea (*Vigna unguiculata* (L.) Walp) collected in Caruaru and Serra Talhada counties (Rodrigues and Menezes, 2002). Bezerra et al. (2012) studing the cactus *Opuntia ficus-indica* (L.) Mill. obtained 44 endophytic fungi, belonging to 12 genera and 13 species. In another survey with cactus *C. jamacaru*, Bezerra et al. (2013) reported values for Shannon's index varying from 2.273 to 2.597, and for Simpson's index from 0.8127 to 0.9008.

According to the dendrogram shown in Figure 1, it is possible to perceive that there is a greater similarity between fungi isolated in Atlantic Coastal Forest and Caatinga in the dry season as compared to the rainy season, suggesting that it may be showing the predominance of seasonality rather than geographical factor.

The results presented in this work are the first study of endophytic fungi from leaves of *I. suffruticosa* growing in Atlantic Coastal Forest and Caatinga in Brazil. This study documents that the properties of the Atlantic Coastal Forest biome associated with increased rainfall seem to favor greater endophytic colonization of *I. suffruticosa*, in comparison with the Caatinga. In this study, it is reported for the first time, the isolation of endophytic fungi *C. australiensis* and *C. raphigera* from a plant of the Brazilian Caatinga. Finally, the results indicate that there is a diversity of the endophytic fungi from *I. suffruticosa,* which are of ecological importance to plant growing in different areas studied, and also these fungi may be important source for future study in searching for new natural compounds with potential antimicrobial properties.

## Conflict of interests

The authors did not declare any conflict of interest.

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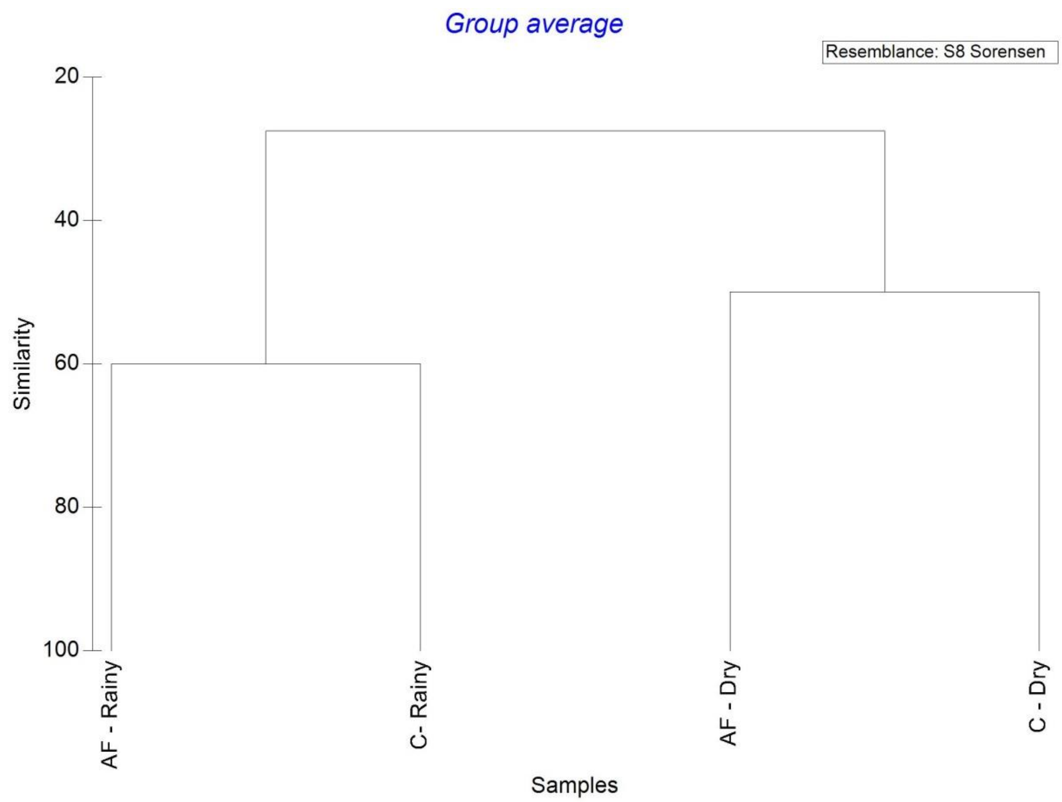


**Figure 1.** Dendogram showing the relationship of communities of endophytic fungi isolated from *I. suffruticosa* at Atlantic Coastal Forest and tropical dry forest (Caatinga) in rainy and dry season (Brazil) from Sørensen similarity index. AF, Atlantic forest; C, Caatinga.

## REFERENCES

Ab'Saber AN (1974). O domíınio morfoclimatico semi-árido das caatingas brasileiras. Instituto de Geografia, USP, São Paulo Geomorfologia 43:1-3.

Agra MF, Freitas PF , José Maria Barbosa-Filho JM (2007). Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. Rev. Bras. Farmacogn. Braz. J. Pharmacogn. 17(1):114-140.

Albuquerque UP, Araújo EL, El-Deir ACA, Lima ALA, Souto A, Bezerra BM, Ferraz EMN, Freire EMX, Sampaio EVSB, Las-Casas FMG, Moura GJB, Pereira GA, Melo JG, Ramos MA, Rodal MJN, Schiel N, Lyra-Neves RM, Alves RRN, Azevedo-Júnior SM, Júnior WRT, Severi W (2012). Caatinga revisited: ecology and conservation of an important seasonal dry forest. Sci. World J. 2012:1-18.

Almeida ER (1993). Plantas Medicinais Brasileiras: Conhecimentos populares e científicos. São Paulo: Hemus Editora Ltda.

Almeida ER, Chaves TM, Luna RLA, Silva AR, Aragão-Neto AC, Silva LLS, Couto GBL (2013). Anticonvulsant effect of *Indigofera suffruticosa* Mill: Indication of involvement of the GABAergic system. Afr. J. Pharm. Pharmacol. 7:622-628.

Aly AH, Debbab A, Proksch P (2011). Fifty years of drug discovery from fungi. Fungal Divers. 50:3-19.

Alzugaray D, Alzugaray C (1988). Enciclopédia de plantas brasileiras. Editora Três, São Paulo, Brazil.

APAC – Agência Pernambucana de Águas e Clima (2013). Monitoramento Pluviométrico do Estado de Pernambuco. Available in http://www.apac.pe.gov.br/meteorologia/monitoramento-pluvio.php Accessed 16 July 2013.

Araújo WL, Marcon J, Maccheroni Jr W, Van Elsas JD, Van Vuurde JWL, Azevedo JL (2002). Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. Appl. Environ. Microbiol. 68:4906-4914.

Arnold AE, Mejía LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA (2003). Fungal endophytes limit pathogen damage in a tropical tree. Proc. Natl. Acad. Sci. USA. 100:15649–15654.

Azevedo JL (2014). Endophytic fungi from Brazilian tropical hosts and their biotechnological applications. In: Kharwar RN, Upadhyay R, Dubey N, Raghuwansh R (eds) Microbial diversity and biotechnology in food security, Springer, New Delhi, pp. 17-22.

Barnett HL, Hunter BB (1987). Illustrated genera of imperfect fungi, 4th edn. American Phytopathological Society, St. Paul: APS Press, Minnesota, USA.

Basso LA, Silva LHP, Fett-Neto AG, Azevedo Jr WF, Moreira IS, Palma MS, Calixto JB, Astolfi Filho S, Santos RR, Soares MBP, Santos DS (2005). The use of biodiversity as source of new chemical entities against defined molecular targets for treatment of malaria, tuberculosis, and T-cell mediated diseases - a review. Mem. Inst. Oswaldo Cruz 100:475-506.

Bezerra dos Santos AT, Araújo TFDS, Da Silva LCN, Silva CBD, Oliveira AFMD, Araújo JM, Lima VLM (2015). Organic extracts from *Indigofera suffruticosa* leaves have antimicrobial and synergic actions with Erythromycin against *Staphylococcus aureus*. Front. Microbiol. 6:13.

Bezerra JDP, Santos MGS, Barbosa RN, Svedese VM, Lima DMM, Fernandes MJS, Gomes BS, Paiva LM, Almeida-Cortez JS, Souza-Motta CM (2013). Fungal endophytes from cactus *Cereus jamacaru* in Brazilian tropical dry forest: a first study. Symbiosis 60:53-63.

Bezerra JDP, Santos MGS, Svedese VM, Lima DMM, Fernandes MJS, Paiva LM, Souza-Motta CM (2012). Richness of endophytic fungi isolated from *Opuntia ficus-indica* Mill. (Cactaceae) and preliminary screening for enzyme production. World. J. Microbiol. Biotechnol. 28:1989-1995.

Bhardwaj A, Agrawal P (2014). A review fungal endophytes: As a store house of bioactive compound. World J. Pharm. Pharm. Sci. 3:228-

237.

Cardoso DBOS, Queiroz LP (2011). Caatinga no contexto de uma metacomunidade: evidencias da biogeografia, padrões filogenéticos e abundância das espécies em Leguminosas. In: de Carvalho CJB, Almeida EAB (eds) Biogeografia América do Sul - Padrões e Processos, Roca, São Paulo, pp. 241-260.

Clarke BB, White JFJ, Hurley RH, Torres MS, Sun S, Huff DR (2006). Endophyte-mediated suppression of dollar spot disease in fine fescues. Plant Dis. 90:994-998.

Collado J, Platas G, González I, Peláez F (1999). Geographical and seasonal influences on the distribution of fungal endophytes in *Quercus ilex*. New Phytol. 144:525-532.

Ellis MB (1971). Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, UK.

Ellis MB (1976). More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, UK.

El-Nagerabi SAF, Elshafie AE, Alkhanjari SS (2013). Endophytic fungi associated with *Ziziphus* species and new records from mountainous area of Oman. Biodiversitas 14:10-16.

Giordano L, Gonthier P, Varese GC, Miserere L, Nicolotti G (2009). Mycobiota inhabiting sapwood of healthy and declining Scots pine (*Pinus sylvestris* L.) trees in the Alps. Fungal Divers. 38: 69-83.

Göre ME, Bucak C (2007). Geographical and seasonal influences on the distribution of fungal endophytes in *Laurus nobilis*. For. Pathol. 37:281-288.

Guo LD, Huang GR, Wang Y (2008). Seasonal and Tissue Age Influences on Endophytic Fungi of *Pinus tabulaeformis* (Pinaceae) in the Dongling Mountains, Beijing. J. Integr. Plant Biol. 50:997-1003.

Guo LD, Huang GR, Wang Y, He WH, Zheng WH, Hyde KD (2003). Molecular identification of white morphotype strains of endophytic fungi from *Pinus tabulaeformis*. Mycol. Res. 107:680-688.

Hanlin RT (2000). Illustrated genera of Ascomycetes, 2nd edn. American Phytopathological Society, St. Paul: APS Press, Minnesota, USA.

Hashizume Y, Fukuda K, Sahashi N (2010). Effects of summer temperature on fungal endophyte assemblages in Japanese beech (*Fagus crenata*) leaves in pure beech stands. Botany 88:266-274.

Hashizume Y, Sahashi N, Fukuda K (2008). The influence of altitude on endophytic mycobiota in *Quercus acuta* leaves collected in two areas 1000 km apart. For. Pathol. 38:218-226.

Hastings RB (1990). Medicinal legumes of Mexico, Fabaceae, Papilionoideae, Part One. Econ. Bot. 44:336-348.

Helander M, Ahlholm J, Sieber TN, Hinneri S, Saikkonen K (2007). Fragmented environment affects birch leaf endophytes. New Phytol. 175:547-553.

Khan R, Shahzad S, Choudhary MI, Khan SA, Ahmad A (2010). Communities of endophytic fungi in medicinal plant *Withania somnifera*. Pak. J. Bot. 42:1281-1287.

Krishnamurthy YL, Naik SB, Jayaram S (2008). Fungal communities in herbaceous medicinal plants from the Malnad region, southern India. Microbes Environ. 23:24-28.

Kusari P, Kusari S, Spiteller M, Kayser O (2013). Endophytic fungi harbored in *Cannabis sativa* L.: diversity and potential as biocontrol agents against host plant-specific phytopathogens. Fungal Divers. 60:137-151.

Kusari S, Spiteller M (2011). Are we ready for industrial production of bioactive plant secondary metabolites utilizing endophytes? Nat. Prod. Rep. 28:1203-1207.

Lacap DC, Hyde KD, Liew ECY (2003). An evaluation of the fungal “morphotype” concept based on ribosomial DNA sequences. Fungal Divers. 12: 53-66.

Larran S, Perelló A, Simón MR, Moreno V (2002). Isolation and analysis of endophytic microorganisms in wheat (*Triticum aestivum* L.) leaves. World J. Microb. Biotechnol. 18:683–686.

Leal IR, Tabarelli M, Silva JMC, Larcher TE (2005). Changing the course of biodiversity conservation in the Caatinga of Northeastern Brazil. Conserv. Biol. 19:701-706.

Leite SP, Medeiros PL, Silva EC, Maia MBS, Lima VLM, Saul DE (2004). Embryotoxicity *in vitro* with extract of *Indigofera suffruticosa* leaves. Reprod. Toxicol. 18:701-705.

Leite SP, Vieira JRC, Madeiros PL, Leite RMP, Lima VLM, Xavier HS, Lima E (2006). Antimicrobial activity of *Indigofera suffruticosa*. Evid. Based Complement. Alternat. Med. 2:261-265.

Li H, Wei D, Shen M, Zhou Z (2012). Endophytes and their role in phytoremediation. Fungal Divers. 54:11-18.

Lôbo D, Leão T, Melo FPL, Santos AMM, Tabarelli M (2011). Forest fragmentation drives Atlantic forest of northeastern Brazil to biotic homogenization. Divers. Distrib. 17:287-296.

Lou J, Fu L, Luo R, Wang X, Luo H, Zhou L (2013). Endophytic fungi from medicinal herb *Salvia miltiorrhiza* Bunge and their antimicrobial activity. Afr. J. Microbiol. Res. 7:5343-5349.

Luiz-Ferreira A, Cola M, Barbastefano V, Farias-Silva E, Calvo TR, Almeida ABA, Pellizon CH, Hiruma-Lima CA, Vilegas W, Souza-Brito ARM (2011). *Indigofera suffruticosa* Mill as new source of healing agent: Involvement of prostaglandin and mucus and heat shock proteins. J. Ethnopharmacol. 137:192-198.

Magurran AE (1988). Ecological diversity and its measurement. Princeton University Press, Princeton, New Jersey, USA.

Malinowski DP, Belesky DP (2000). Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. Crop Sci. 40:923-94.

Mandyam K, Jumpponen A (2005). Seeking the elusive function of the root-colonising dark septate endophytic fungi. Stud. Mycol. 53:173-189.

Martín P, Pajares JA, Nanos N, Diez JJ (2004). Site and seasonal influences on the fungal community on leaves and stems of *Pinus* and *Quercus* seedlings in forest nurseries. Sydowia 56:23-37.

Martín Pinto P, Pajares Alonso JA, Pando Fernández V, Díez Casero JJ (2006). Fungi isolated from diseased nursery seedlings in Spain. New Forests 31:41-56.

Matos FJA (1999). Plantas da medicina popular do Nordeste: propriedades atribuídas e confirmadas. Editora UFC, Fortaleza, p.78.

Morton FJ, Smith G (1963). The genera Scopulariopsis Bainier, Microascus Zukal and Doratomyces Corda. Commonwealth Mycological Institute, Kew, UK.

Oliveira LG, Cavalcanti MAQ, Fernandes MJS, Lima DMM (2013). Diversity of filamentous fungi isolated from the soil in the semiarid area, Pernambuco, Brazil. J. Arid Environ. 95:49-54.

Oliveira-Filho AT, Fontes MAL (2000). Patterns of floristic differentiation among Atlantic forests in south-eastern Brazil, and the influence of climate. Biotropica 32:793-810

Orlandelli RC, Alberto RN, Rubin Filho CJ, Pamphile JA (2012). Diversity of endophytic fungal community associated with *Piper hispidum* (Piperaceae) leaves. Genet. Mol. Res. 11:1575-1585.

Pharamat T, Palaga T, Piapukiew J, Whalley AJS, Sihanonth P (2013). Antimicrobial and anticancer activities of endophytic fungi from *Mitrajyna javanica* Koord and Val. Afr. J. Microbiol. Res. 7:5565-5572.

Porras-Alfaro A, Bayman P (2011). Hidden fungi, emergent properties: endophytes and microbiomes. Annu. Rev. Phytopathol. 49:291-315.

Prado DE (2003). As Caatingas da America do Sul. In Leal IR, Tabarelli M, Silva JMC (eds) Ecologia e conservação da Caatinga. Universidade Federal de Pernambuco, Recife, Brazil, pp. 3-74.

Rajulu MBG, Thirunavukkarasu N, Suryanarayanan TS, Ravishankar JP, El Gueddari NEE, Moerschbacher BM (2011). Chitinolytic enzymes from endophytic fungi. Fungal Divers. 47:43-53.

Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM (2002). Thermotolerance generated by plant/fungal symbiosis. Science 298:1581.

Ribeiro MC, Metzger JP, Martensen PFJ, Ponzoni FJ, Hirota MM (2009). The Brazilian Atlantic forest: how much is left, and how is the remaining forest distributed? Implications for conservation. Biol. Conserv. 142:1141-1153.

Rivera-Orduña FN, Suarez-Sanchez RA, Flores-Bustamante ZR, Gracida-Rodriguez JN, Flores-Cotera LB (2011). Diversity of endophytic fungi of *Taxus globosa* (Maxican yew). Fungal Divers. 47:65-74.

Rodal MJN, Barbosa MRV, Thomas WW (2008). Do the seasonal forests in northeastern Brazil represent a single floristic unit? Braz. J. Biol. 68:467-475.

Rodrigues AAC, Menezes M (2002). Detecção de fungos endofíticos em sementes de caupi provenientes de Serra Talhada e de Caruaru, Estado de Pernambuco. Fitopatol. Bras. 27:532-537.

Rodriguez R, Redman R (2008). More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. J. Exp. Bot. 59:1109-1114.

Roig JT (1988). Plantas medicinales, aromaticas o venenosas de Cuba. Editorial Cientifica-Tecnica, La Havana.

Simões-Ambrosio LM, Gregório LE, Sousa JP, Figueiredo-Rinhel AS, Azzolini AE, Bastos JK, Lucisano-Valim YM (2010). The role of seasonality on the inhibitory effect of Brazilian green propolis on the oxidative metabolism of neutrophils. Fitoterapia 81:1102-1108.

Siqueira VM, Conti R, Araújo JM, Souza-Motta CM (2011). Endophytic fungi from the medicinal plant *Lippia sidoides* Cham. and their antimicrobial activity. Symbiosis 53:89-95.

Stierle A, Strobel GA, Stierle D (1993). Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science 260:214-216.

Sun Y, Wang Q, Lu X, Okane I, Kakishima M (2012). Endophytic fungal community in stems and leaves of plants from desert areas in China. Mycol. Prog. 11:781-790.

Suryanarayanan TS, Murali TS, Venkatesan G (2002). Occurrence and distribution of fungal endophytes in tropical forest across a rainfall gradient. Can. J. Bot. 80:818-826.

Suryanarayanan TS, Venkatesan G, Murali TS (2003). Endophytic fungal communities in leaves of tropical forest trees: diversity and distribution patterns. Curr. Sci. 85:489-493.

Suryanarayanan TS, Wittlinger SK, Faeth SH (2005). Endophytic fungi associated with cacti in Arizona. Mycol. Res. 109:635-639.

Sutton BC (1980). The Coelomycetes: Fungi imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycological Institute, Kew, UK.

Talley SM, Coley PD, Kursar TA (2002). The effects of weather on fungal abundance and richness among 25 communities in the Intermountain West. BMC Ecol. 2:1-7.

Tan RX, Zou WX (2001). Endophytes: a rich source of functional metabolites. Nat. Prod. Rep. 18:448-459.

Teiten MH, Mack F, Debbab A, Aly AH, Dicato M, Proksch P, Diederich M (2013). Anticancer effect of altersolanol A, a metabolite produced by the endophytic fungus *Stemphylium globuliferum*, mediated by its pro-apoptotic and anti-invasive potential via the inhibition of NF-κB activity. Bioorg. Med. Chem. 21:3850-3858.

Tejesvi MV, Mahesh B, Nalini MS, Prakash HS, Kini KR, Subbiah V, Hunthrike SS (2005). Endophytic fungal assemblages from inner bark and twig of *Terminalia arjuna* W. & A. (Combretaceae). World J. Microbiol. Biotechnol. 21:1535-1540.

Vaz ABM, Fontenla S, Rocha FS, Brandão LR, Vieira MLA, Garcia V, Góes-Neto A, Rosa CA (2014). Fungal endophyte β-diversity associated with Myrtaceae species in an Andean Patagonian forest (Argentina) and an Atlantic forest (Brazil). Fungal Ecol. 8:28-36.

Vieira MLA, Hughes AFS, Gil VB, Vaz ABM, Alves TMA, Zani CL, Rosa CA, Rosa LH (2011). Diversity and antimicrobial activities of the fungal endophyte community associated with the traditional Brazilian medicinal plant *Solanum cernuum* Vell. (Solanaceae). Can. J. Microbiol. 58:54-66.

Xing X, Guo S, Fu J (2010). Biodiversity and distribution of endophytic fungi associated with *Panax quinquefolium* L. cultivated in a forest reserve. Symbiosis 51:161-166.

Zalamea PC, Sarmiento C, Stevenson PR, Rodríguez M, Nicolini E, Heuret P (2013). Effect of rainfall seasonality on the growth of *Cecropia sciadophylla*: intra-annual variation in leaf production and node length. J. Trop. Ecol. 29:361-365.