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# Pollen types and levels of total phenolic compounds in propolis produced by *Apis mellifera* L. (Apidae) in an area of the Semiarid Region of Bahia, Brazil

VANESSA R. MATOS[1], SEVERINO M. ALENCAR[2] and FRANCISCO A.R. SANTOS[1]

[1]Programa de Pós-Graduação em Botânica, Universidade Estadual de Feira de Santana, Avenida Transnordestina, s/n, Novo Horizonte, 44036-900 Feira de Santana, BA, Brasil
[2]Departamento de Agroindústria, Alimentos e Nutrição, Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz, Av. Pádua Dias, 11, São Dimas, 13418-900 Piracicaba, SP, Brasil

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## ABSTRACT

Twenty-two propolis samples produced by *Apis mellifera* L. in an area of the Semiarid region the the State of Bahia (Agreste of Alagoinhas), Brazil, were palynologically analyzed and quantified regarding their levels of total phenolic compounds. These samples were processed using the acetolysis technique with the changes suggested for use with propolis. We found 59 pollen types belonging to 19 families and 36 genera. The family Fabaceae was the most representative in this study with nine pollen types, followed by the family Asteraceae with seven types. The types *Mikania* and *Mimosa pudica* occurred in all samples analyzed. The types *Mimosa pudica* and *Eucalyptus* had frequency of occurrence above 50% in at least one sample. The highest similarity index (c. 72%) occurred between the samples ER1 and ER2, belonging to the municipality of Entre Rios. Samples from the municipality of Inhambupe displayed the highest (36.78±1.52 mg/g EqAG) and lowest (7.68 ± 2.58 mg/g EqAG) levels of total phenolic compounds. Through the Spearman Correlation Coefficient we noticed that there was a negative linear correlation between the types *Mimosa pudica* (rs = -0.0419) and *Eucalyptus* (rs = -0.7090) with the profile of the levels of total phenolic compounds of the samples.

**Key words:** apicultural products, floristic similarity, melissopalynology, phenolic compounds.

## INTRODUCTION

Propolis is an apicultural product resulting from the mixing of resinous substances collected from plant structures with the wax and salivary enzymes of bees. It is composed of resin (50-60%), wax (30-40%), essential oils (5-10%), pollen grains (5%), trace elements (aluminum, calcium, strontium, iron, copper, manganese) and vitamins (B1, B2, B6, C and E) (Ghisalberti 1979, Alencar 2002). The best known propolis is that produced by the bee *Apis mellifera* L (Apidae). The function of this product is protective, acting as a thermal insulator and preventing intruders from entering the hive (Barth and Luz 2003, Park et al. 2005, Freitas et al. 2010).

Propolis is widely used in folk medicine and also heavily used by cosmetic, pharmaceutical and food industries due to the numerous biological activities attributed to it, such as antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, cicatrizing, anesthetic, and anticariogenic (Ghisalberti 1979, Marcucci and Bankova 1999, Park et al. 2005). Such a huge variety of therapeutic properties

Correspondence to: Vanessa Ribeiro Matos
E-mail: vanessamatos18@yahoo.com.br

described by researchers for propolis is attributed to the presence of several phenolic compounds in this product (Adelmann 2005).

The chemical composition of propolis is highly variable and complex, being related to the phytogeography of the region. The ecology of the local flora where the bees are foraging is directly related to the composition (Castro et al. 2007, Sousa et al. 2007, Buriol et al. 2009, Cabral et al. 2009). The chemical composition of Brazilian propolis is mentioned in several studies as being one the best in the world (Buriol et al. 2009).

Brazilian legislation, through the normative instruction No. 3 of 19 January 2001, regulates the identity and quality of propolis produced in the country. According to this law, the minimum amount of phenolic compounds should be 0.5% (m/m) (Adelmann 2005).

The phenolic profile of bee products, such as propolis, has a great range of therapeutic properties, but it is dependent on their floral source. Among these properties, it is the antioxidant capacity very searched by society (Silva et al. 2013).

In Bahia, despite the diversity of the flora and its high apicultural potential, there is little information about the plants of importance in the elaboration of propolis (Moreti et al. 2000). The pollen spectrum present in propolis contains pollen grains brought by bees and also pollen grains which are brought by wind (anemophilous) and adhered to the resin. Thus, pollen analysis is a valuable tool for the verification and labeling of samples of this apicultural product, since it allows for the determination of their geographical origin, indicating the different regions of production and the season in which they were made (Barth 1998, Barth et al. 1999). Knowledge about the apicultural flora is necessary for the conservation of these plants in order to promote a sustainable apiculture (Sodre et al. 2008).

Given the economic growth and scientific research on propolis in the country and the scarceness of information about the product in Bahia, this paper aims to outline the palynological profile (through identification of pollen types) and quantify the levels of total phenolic compounds (by comparing them to what has been described in literature for other Brazilian propolis) in propolis samples produced in the territory of the Agreste of Alagoinhas, State of Bahia.

## MATERIALS AND METHODS

### Investigated Propolis

We analyzed 22 samples of raw propolis originating from nine municipalities from the territory of the Agreste of Alagoinhas, Bahia (Figure 1). The climate is semiarid, where pluviometric indexes where quite low and long periods of drought where common (SEI 2002). The samples were donated by the Apicultural Association of Bahia, through the Empresa Baiana de Desenvolvimento Agrícola (EBDA), being collected in 2010 and 2011.

### Palynological Analysis

Samples of propolis were dissolved in 95% ethylic alcohol and the sediment obtained was subjected to a hot potassium hydroxide solution (10%), with the subsequent residue being dehydrated with glacial acetic acid and finally subjected to the acetolysis method of Erdtman (1960). After successive centrifugations, residual pollen grains were mounted between slide and coverslip with glycerin gelatin. The preparations were analyzed in an optical microscope, with the pollen grains counted to establish a pollen census. We counted a minimum of 500 pollen grains per propolis sample. The determination and identification of the botanical affinity of the pollen types were made according to the instructions of Santos (2011).

To establish the frequency distribution of pollen types (taxa) between samples, we observed the number of samples in which a given pollen type occurred. Samples were categorized into the following frequency classes of Jones and Bryant Jr (1996): very frequent – >50%; frequent – 20-50%; infrequent – 10-20%, and rare – <10%.

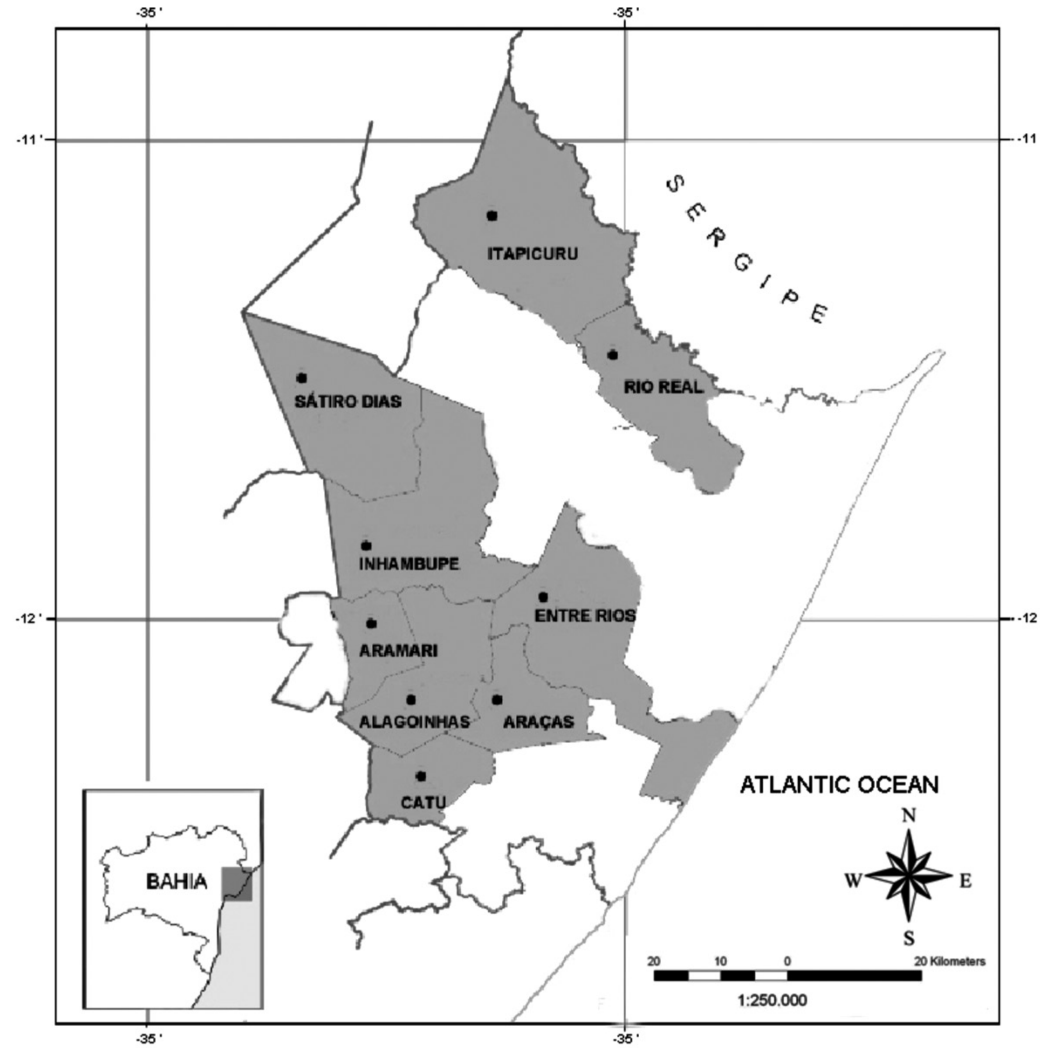


**Figure 1 -** Territory of the Agreste of Alagoinhas, highlighting the municipalities (in gray) where the propolis samples were collected (Adapted from SEI 2002).

Pollen grains were identified through comparison with the samples deposited in the collection of the Laboratory of Plant Micromorphology (UEFS), where we deposited all slides prepared in this study. We also used pollen catalogues and other works to aid in the identification of the botanical origin of the pollen types. All pollen types were photographed using an optical microscope.

QUANTIFICATION OF THE LEVELS OF TOTAL PHENOLIC COMPOUNDS

To quantify the levels of total phenolic compounds in propolis we used the Folin-Ciocalteau method (Singleton and Rossi 1965). Measurements were based on readings from the spectrophotometer at wavelength 740 nm.

STATISTICAL ANALYSIS

For the analysis of pollen similarity between samples we made a dendrogram using the software PAST - Palaeontological Statistics, ver. 1.89 (Hammer et al. 2001). The similarity coefficient used was the Jaccard coefficient because it does not consider shared absences as evidence of similarity. Thus, only the identified pollen types entered the analysis.

To correlate the main pollen types identified in the study with the levels of total phenolic compounds of the samples, we used the Spearman coefficient, which calculates the correlation via non-parametric methods that describe the relationships between two variables without making any assumptions about their frequency distribution (Menezes et al. 2010). Using this methodology we made the correlation between the levels of total phenolic compounds and the pollen types occurring in over 50% of the analyzed samples.

## RESULTS

A total of 59 pollen types were found in the analyzed propolis samples from the nine municipalities of the territory of the Agreste of Alagoinhas. Of these, 49 were properly identified botanically and belong to 19 families and 36 genera (Table I). The family Fabaceae had prominence with nine pollen types, followed by the family Asteraceae with seven. The families Rubiaceae and Myrtaceae also had large representativeness in the study, with respectively six and five pollen types being identified. The number of pollen types per sample ranged from 11 in the sample CA1 to 30 in the sample ER2 (Table I).

Only two pollen types were present with 100% frequency in the pollen spectrum of all the 22 samples studied (Figure 2): *Mikania*, Asteraceae (Figure 3D), and *Mimosa pudica*, Fabaceae (Figure 3H). Other 10 pollen types displayed frequency above 50% in the samples investigated (Figure 2). The family Fabaceae embraces a third of this group of more frequent pollen types, with the types *Mimosa tenuiflora* (Figure 3I), *Senna* I and *Chamaecrista*, besides the already mentioned *M. pudica* type.

The highest frequency of occurrence of a pollen type in a sample was 78.8%, namely the type *M. pudica* in the sample IT4 (Table I). Only two pollen types had a frequency of occurrence above 50% in at least one sample: *M. pudica* (AL3, IN2, IN3, IT1, 1T4, SD1 and SD2) and *Eucalyptus* (AL1, AL2, AR1, AM1, ER2 and IN4). The remainder pollen types occurred in the samples with frequency lower than 50% of counted pollen grains.

The analysis of pollen similarity between the samples, revealed that they were grouped into five basic clades (Figure 4, AE). In the same clade the samples were not grouped by municipality, indicating its pollen heterogeneity and also reflecting the floristic heterogeneity of the municipalities. However, samples from the municipality of Entre Rios were all positioned in clade E and grouped with the sample IT3 of Itapicuru. Two of these samples displayed the highest similarity (72%) among them, ER1 and ER2, both from the municipality of Entre Rios. Samples of this city possessed the highest amount of pollen types (Table I). The sample ER3 showed a 65% similarity index with the sample IT3 (from the municipality of Itapicuru), with these samples having 21 pollen types in common.

The lowest pollen similarity between groups of samples was 35%, observed between the sample from the municipality of Catu and the sample AL2 from Alagoinhas (Figure 4, clade A), which had in common the pollen types Asteraceae I, *Banisteriopsis*, *Chamaecrista*, *Eucalyptus*, *Mikania, Mimosa pudica* and *Myrcia* I.

Regarding the levels of total phenolic compounds, the analyzed samples displayed levels ranging from 7.68±2.58 to 36.78±1.52 mg GAE/g (Table II).

The highest level of total phenolic compounds found in the study (36.78±1.52 mg GAE/g) corresponds to the municipality of Inhambupe, and 52.8% of the pollen grains from this sample belonged to *Mimosa pudica*. The lowest level of total phenolic compounds found in the study (7.68±2.58 mg GAE/g), which also belonged to a sample from the municipality of Inhambupe, displayed 71% of pollen grains from the type *Eucalyptus*.

The correlation (Spearman's coefficient) between levels of phenolic compounds and concentration of the main pollen types proved to be negative in some cases and positive in others (Table III).

**TABLE I**

**Frequency (%) of pollen types found in propolis samples analyzed from municipalities of the territory of the Agreste of Alagoinhas. Municipalities: AL. Alagoinhas; AR. Araçás; AM. Aramari; CA. Catu; ER. Entre Rios; IN. Inhambupe; IT. Itapicuru; RR. Rio Real; SD. Sátiro Dias.**

| Pollen types | Samples | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AL1 | AL2 | AL3 | AL4 | AL5 | AR1 | AM1 | CA1 | ER1 | ER2 | ER3 | IN1 | IN2 | IN3 | IN4 | IT1 | IT2 | IT3 | IT4 | RR1 | SD1 | SD2 |
| **Amaranthaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Alternathera ramosissima* | | | 0.2 | | | | | | | | 1.2 | 10.0 | 0.2 | 0.4 | 1.8 | 4.6 | 1.4 | 6.6 | | 9.8 | 0.2 | 0.2 |
| *Gomphrena* | | 0.2 | | | | | | | 1.2 | 0.8 | | | | | | | | | | | | |
| **Anacardiaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Schinus* | | | 0.6 | 0.2 | | | 0.4 | | 4.6 | 3.0 | 4.6 | 0.4 | | | | | | 1.4 | | | | |
| **Arecaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Syagrus* | 0.4 | | | 0.6 | | 0.4 | 3.2 | 0.4 | 3.6 | 2.2 | 3.2 | 3.6 | 0.4 | 0.6 | | 0.8 | 0.6 | 4.0 | 0.2 | 7.6 | 0.8 | 4.2 |
| **Asteraceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Asteraceae* I | 1.2 | 2.2 | 0.8 | 1.2 | 1.4 | 1.6 | 0.4 | 2.2 | 6.4 | 1.2 | 1.4 | 1.0 | 0.4 | 0.2 | 1.4 | | 4.0 | 1.0 | 0.2 | 0.4 | 0.6 | 0.6 |
| *Eupatorium* I | 0.4 | 0.4 | | | | | | | | 0.2 | | 0.8 | | | | | 2.2 | 3.8 | | | 0.2 | |
| *Eupatorium* II | | 0.2 | 0.2 | | | | | | | | | | 0.8 | | | | | | | | | |
| *Mikania* | 1.8 | 6.2 | 0.6 | 4.2 | 0.6 | 6.0 | 1.0 | 1.2 | 5.2 | 4.2 | 0.1 | 1.2 | 1.6 | 1.8 | 1.0 | 1.6 | 6.4 | 2.4 | 0.8 | 2.2 | 1.2 | 1.8 |
| *Venonia* I | | | | | | | 0.6 | | 0.6 | 0.6 | 1.8 | 0.8 | 0.2 | | 0.2 | | | 8.4 | | | 0.2 | |
| *Venonia* II | | | | | | | | | 0.2 | 0.2 | | | | | | | | 0.4 | | | | |
| *Venonia* III | | | | 0.4 | 0.8 | | | 0.4 | | | | 0.4 | | 0.2 | | | 1.0 | | | 3.2 | | |
| **Convolvulaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Evolvulus* | | 0.2 | | | | | 0.2 | | | | | 1.0 | | | | | | 0.2 | | | | |
| *Jacquemontia* | | | | | | | | | 0.2 | 0.8 | | | | | | | 1.0 | | | | | |
| *Merremia* | | | | | | | | | | | | | | | | | | | | 0.4 | | |
| **Euphorbiaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Croton* | | | | | | | | | 0.6 | | 0.2 | 0.2 | | | | | 0.2 | 0.6 | | | | |
| *Phyllanthus* | | | | | | | | | | | 4.2 | | | | | | 4.8 | 3.8 | | 8.0 | | |
| **Fabaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Acacia* | | | | | | | | | | 0.2 | 20 | | | | 0.4 | | | | | | | 0.2 |
| *Bauhinia* | | | | 0.2 | | | | | | | | | | | | | | | | | | |
| *Chaemecrista* | 0.2 | 0.2 | 1.0 | 0.2 | 0.2 | | 0.2 | 0.4 | | | | | | | | 0.2 | 3.4 | | | 0.6 | | 0.2 |
| *Desmanthus* | | | | | | | | | 0.6 | 0.6 | | | | | | | | | | | | |
| *Mimosa pudica* | 20.0 | 25.6 | 70.2 | 43.0 | 38.7 | 18.2 | 18.8 | 71.6 | 21.8 | 8.0 | 40.0 | 8.4 | 52.2 | 52.8 | 12.2 | 72.6 | 18.0 | 17.0 | 78.8 | 24.6 | 60.2 | 63.0 |
| *Mimosa tenuiflora* | 1.0 | 1.2 | 2.0 | 0.4 | 1.5 | 0.6 | 3.8 | | 4.0 | 0.8 | 5.8 | 1.2 | 2.6 | 5.6 | 8.0 | 9.4 | 8.2 | 3.0 | 0.6 | 2.6 | 16.2 | 14.4 |
| *Senna* I | 0.6 | | | 0.6 | 2.2 | 2.8 | 1.4 | | | 0.2 | 0.4 | 1.4 | | | 0.8 | | 0.2 | 0.4 | 0.2 | 0.6 | | |
| *Senna* II | | | | | | 0.4 | | | | | | | | | | | | | | | | |
| *Zornia* | | | | | 0.2 | | | | | | | | | | | | | | | | | |

**TABLE I(continuation)**

| Pollen types | Samples | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **AL1** | **AL2** | **AL3** | **AL4** | **AL5** | **AR1** | **AM1** | **CA1** | **ER1** | **ER2** | **ER3** | **IN1** | **IN2** | **IN3** | **IN4** | **IT1** | **IT2** | **IT3** | **IT4** | **RR1** | **SD1** | **SD2** |
| **Lamiaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Hyptis* | | | | 0.6 | 0.4 | | 0.4 | | 0.6 | 0.4 | 0.6 | | | | | | | 1.8 | | 0.6 | 0.6 | 0.4 |
| *Salvia* | | | | | | | | | | | 3.2 | | | | | | 6.8 | 0.8 | | | | |
| **Malpighiaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Banisteriopsis* | | 0.6 | | 0.6 | 0.2 | | | 0.2 | 1.0 | 4.2 | | | | | | | 0.2 | | | | 1.2 | |
| **Malvaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Sida* | 0.2 | 0.4 | | | | | | | 0.8 | 1.0 | 0.2 | | | | | | | 0.4 | | | | |
| **Myrtaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Eucalyptus* | 69.6 | 57.4 | 20.0 | 43.4 | 49.6 | 53.2 | 59.2 | 16.4 | 22.4 | 50.8 | 1.4 | 51.2 | 38.6 | 33.0 | 71.0 | | | | 10.4 | 10.6 | 5.2 | 7.4 |
| *Eugenia* I | | | | | 0.2 | 1.8 | | | | | 1.6 | | | 0.8 | | | | 1.0 | 2.0 | | 3.2 | 0.8 |
| *Eugenia* II | | | | | | | | | | | 2.4 | | | | | | | | 0.8 | 0.6 | | |
| *Myrcia* I | 2.2 | 4.0 | | 3.6 | 5.4 | | 3.0 | 4.8 | 5.0 | 3.0 | 1.2 | | | 0.8 | 1.4 | 8.0 | 19.8 | 1.4 | 0.2 | 3.0 | 7.4 | 3.4 |
| *Myrcia* II | | | | | | 4.8 | | | 1.0 | 0.6 | 0.6 | 2.2 | 1.2 | 1.0 | | | | | | | | |
| **Plantaginaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Angelonia* | | 0.4 | | | | | 0.2 | | | 6.6 | 2.0 | | | | | | | | 2.0 | | | |
| **Poaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Poaceae* | 0.2 | | | | 0.8 | 0.8 | 0.2 | | 0.4 | 0.8 | 0.1 | 0.8 | | 1.8 | | | 5.0 | 1.8 | 0.4 | 0.8 | 0.6 | 0.2 |
| **Rubiaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Borreria verticillata* | | | 0.4 | 0.2 | 0.6 | 1.6 | 5.2 | | 10.0 | 4.6 | 13.0 | 9.8 | 0.5 | 0.8 | 0.8 | 0.8 | 6.6 | 0.6 | | 18.2 | 1.2 | 2.4 |
| *Borreria* I | 1.2 | | | | | | | | 6.6 | 2.0 | | | | | | | 2.2 | 30.6 | 0.4 | | | |
| *Borreria* II | | | | | | | | 0.8 | | | | | | | | | | | | | | |
| *Diodia radula* | | | 0.4 | | | | 0.4 | | | | | | | | | | 0.8 | | | | 0.4 | 0.6 |
| *Mitracarpus longicalyx* | 0.6 | 0.4 | 3.6 | 0.4 | 0.6 | 1.2 | | | 2.2 | 0.6 | 3.0 | 4.0 | 0.4 | | 0.8 | 0.8 | 4.0 | 1.6 | 1.4 | 6.0 | | |
| *Richardia* | | | | | 0.2 | | 0.2 | | 0.6 | 1.0 | | | 0.5 | | | | 2.4 | 4.2 | | | 0.2 | |
| **Sapindaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Serjania* I | | | | | | 0.4 | | | | 0.6 | 2.2 | | | | | 0.2 | | 2.0 | 0.2 | | | |
| *Serjania* II | | | | | | | | | | 0.2 | | | | | | | | | | | | |
| *Serjania* III | | | | 0.2 | 0.6 | | | | | | | | | | | | | | | | | |
| **Tuneraceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Turnera* | | | | | | | 1.0 | | | | | 1.6 | | | | | | | | | | |
| **Urticaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Cecropia* | | | | | 1.0 | | | 1.6 | 0.4 | 0.4 | 3.6 | | | | | 0.8 | | 0.6 | 1.4 | | | |
| **Verbenaceae** | | | | | | | | | | | | | | | | | | | | | | |
| *Lippia* | | | | | | | | | | | | | | | | | | | | | | 0.2 |
| **Pollen types not identified (%/no.)** | 0.4/1 | 0.4/2 | | | 0.2/1 | 6.2/1 | 0.2/1 | | | 0.2/1 | | | 0.4/1 | 0.2/1 | 0.2/1 | 0.2/1 | 0.8/2 | 0.2/1 | | | 0.4/1 | |
| **Total of pollen types (no.)** | 15 | 17 | 12 | 17 | 20 | 15 | 20 | 11 | 24 | 30 | 26 | 19 | 14 | 14 | 13 | 12 | 24 | 27 | 16 | 18 | 18 | 16 |

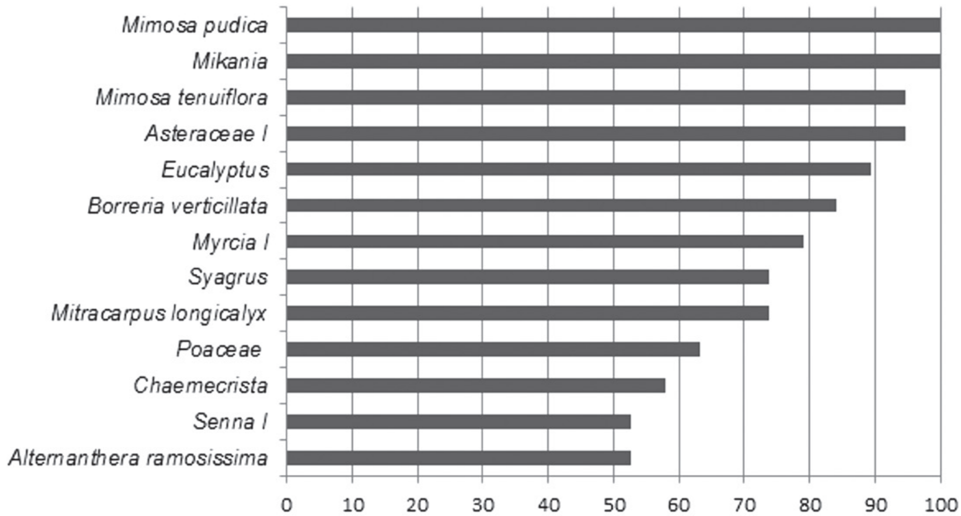


**Figure 2 -** Pollen types occurring in over 50% of the propolis samples analyzed, produced in the territory of the Agreste of Alagoinhas.

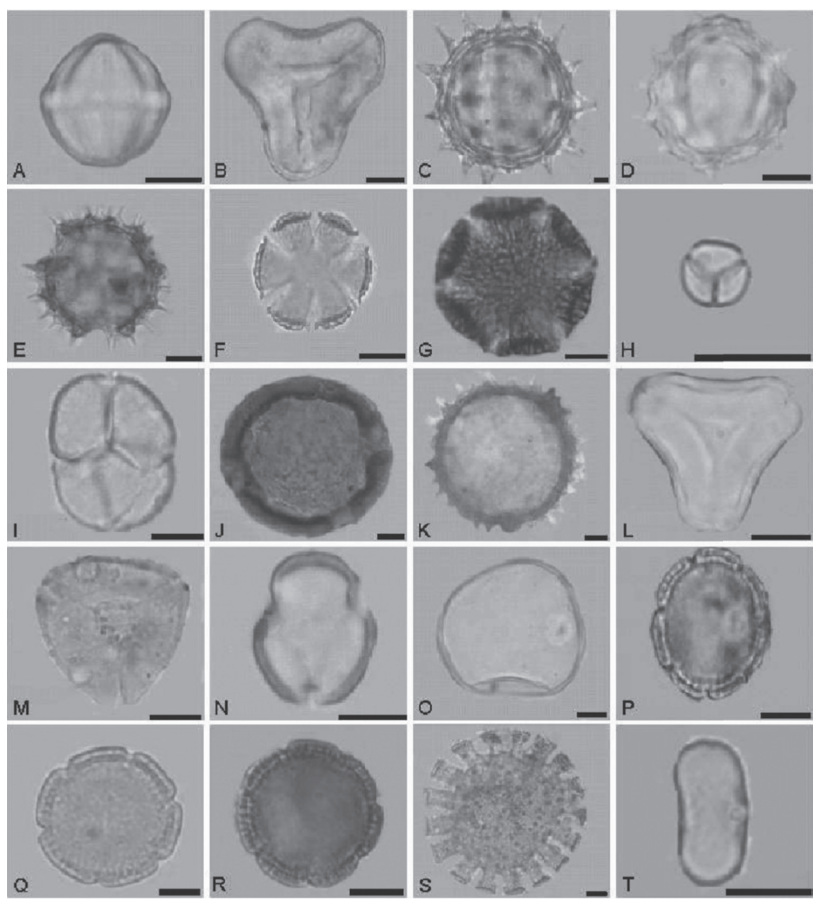


**Figure 3 -** Pollen types found in the propolis samples investigated from the territory of the Agreste of Alagoinhas. **A.** Anarcadiaceae, Schinus. **B.** Arecaceae, *Syagrus*. **C-E.** Asteraceae: **C.** *Eupatorium* I. **D.** *Mikania*, **E.** *Venonia* I. **F-G.** Lamiaceae: **F.** *Hyptis*, **G.** *Salvia* **H-I.** Fabaceae: **H.** *Mimosa pudica*, I. *Mimosa tenuiflora* **J.** Malpighiaceae, *Banisteriopsis* **K**. Malvaceae, *Sida* **L-M.** Myrtaceae: **L**. *Eucalyptus* **M.** *Myrcia* I. **N.** Plantaginaceae, *Angelonia* **O.** Poaceae. **P-S.** Rubiaceae: **P.** *Borreria verticillata*, **Q.** *Borreria* I. **R.** *Mitracarpus longicalyx*, **S**. *Richardia*. **T.** Urticaceae, *Cecropia*. (Bar = 10 µm).

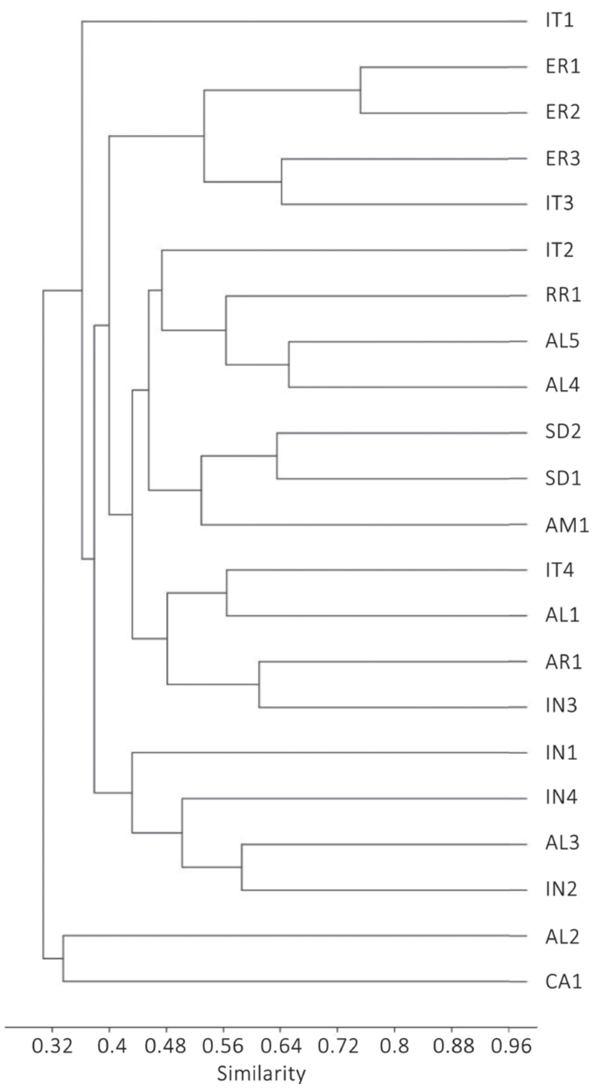


**Figure 4 -** Similarity (Jaccard coefficient) between propolis samples produced in municipalities from the territory of the Agreste of Alagoinhas. Formation of six clades (A-F) according to pollen similarity.

**TABLE II**
**Levels of total phenolic compounds found in the analyzed propolis samples from the municipalities of the territory of the Agreste of Alagoinhas.**

| Samples | Municipalities | Levels of total phenolic compounds (mg/g EqAG ) |
|---|---|---|
| AL1 | Alagoinhas | 17.24±0.58 |
| AL2 | Alagoinhas | 19.02±0.77 |
| AL3 | Alagoinhas | 19.08±0.83 |
| AL4 | Alagoinhas | 18.66±0.60 |
| AL5 | Alagoinhas | 9.43±1.18 |
| AR1 | Araçás | 19.99±1.32 |
| AM1 | Aramari | 14.90±0.76 |
| CA1 | Catu | 14.14±1.08 |
| ER1 | Entre Rios | 32.80±2.41 |
| ER2 | Entre Rios | 31.61±2.18 |
| IN1 | Inhambupe | 23.37±0.69 |
| IN2 | Inhambupe | 12.18 ±0.30 |
| IN3 | Inhambupe | 36.78±1.52 |
| IN4 | Inhambupe | 7.68± 2.58. |
| IT1 | Itapicuru | 12.22±0.44 |
| IT2 | Itapicuru | 18.43±0.78 |
| RR1 | Rio Real | 13.19±0.85 |
| SD1 | Sátiro Dias | 17.38±0.50 |
| SD2 | Sátiro Dias | 21.64±1.04 |

**TABLE III**
**The correlation (Spearman coefficient) between the levels of phenolic compounds and the concentration of the main pollen types of the territory of the Agreste of Alagoinhas.**

| Pollen types | Correlation coefficient (Rs) |
|---|---|
| Asteraceae | 0.178 |
| *Borreria verticillata* | 0.378 |
| *Chamaecrista* | -0.297 |
| *Eucalyptus* | -0.709 |
| *Mikania* | 0.297 |
| *Mimosa pudica* | -0.041 |
| *Mimosa tenuiflora* | -0.004 |
| *Mitracarpus longicalyx* | 0.245 |
| *Myrcia* I | -0.005 |
| *Senna* I | -0.158 |
| *Poaceae* | -0.080 |
| *Syagrus* | -0.234 |

## DISCUSSION

The twenty-two analyzed propolis samples possessed a total of 59 pollen types, and with the occurrance in over 50% of these samples, of the Eucalyptus and *Mimosa pudica*. Barth (1998) and Barth et al. (1999) analyzed propolis samples from Southern and Southeastern Brazil and identified respectively 31 and 34 pollen types, with the types *Eupatorium* and *Eucalyptus* being the most representative in these studies (>50%). Freitas et al. (2010) analyzed 24 propolis samples produced in the State of Bahia and identified 45 pollen types, with the type *Eucalyptus* occurring in over 50% of the samples.

Pollen grains of the genus *Mimosa* occur in high frequency in samples of Brazilian propolis. *Mimosa verrucosa* was the most representative type found in the analysis of Freitas et al. (2011) in a propolis sample from the State of Bahia. However, the palynological analyses conducted by Barth and Luz (2009) in red propolis produced in Bahia did not find any pollen type belonging to the genus *Mimosa*.

The pollen type *M. pudica* was highly representative in this study, being identified in all samples analyzed. Thus it can be regarded as indicative of a possible propolis source from the territory of the Agreste of Alagoinhas. The species *Mimosa pudica* L. is considered by apiarists as a highly polliniferous plant. According to Queiroz (2009), it is a very common invasive species, occurring frequently in degraded areas and roadsides. This extensive availability of plants with high pollen productivity makes this a species of high apicultural potential.

In recent studies in Brazil on pollen identification from propolis, the pollen type *Eucalyptus* was frequently found. This fact led some authors to consider the species of this genus from the family Myrtaceae as one of the possible sources of Brazilian propolis (Bastos et al. 2000, Santos et al. 2003, Freitas et al. 2010). *Eucalyptus* L'Hér. is a genus that produces a lot of pollen. It has its origin in Australia but it is widely used in reforestation programs in Brazil. In 1992 it started to be planted in commercial scale (for cellulose production) and in tests of introduction of species in the territory of the Agreste de Alagoinhas, and nowadays there are extensive plantations of these trees for paper production (Araújo et al. 2004).

The identification of apicultural plants is extremely important for apiarists because it indicates the food sources used for the collection of nectar and pollen aiming at maximizing the use of trophic resources, both in the implantation and maintenance of natural vegetation (Menezes et al. 2010). The recognition of which genera supply these floral resources in the territory of the Agreste of Alagoinhas is an important step in promoting a more sustainable apiculture in the state, especially with regards to the species supplying resin, a substance essential for the production of propolis.

The pollen types that occur at low frequency in propolis samples can be regarded as reference of the botanicals species supplying resin; thus the propolis analyses should be very detailed and include a reasonable amount of samples per study area, with the care of the perception that the pollen types found in low frequency in the samples are important indicators of the flora of the study site (Bastos 2001, Freitas et al. 2010). In the pollen spectrum of the analyzed samples, some pollen types belonging to the family Asteraceae and the pollen type *Schinus* can be especially considered as indicative of resin sources (Sawaya et al. 2006).

The observed differences in the similarity index could be related to the pollen types with low frequency in each municipality, since some types appeared in only a few samples, forming isolated clades (Moreti et al. 2000, Freitas et al. 2010).

Samples with high quantity of pollen types such as those from clade E (ER1, ER2, ER3 and IT3) displayed the highest similarity among them, forming an axis with c. 50% of similarity. The samples with the lowest amount of pollen types such as those from clade A (CA1 and IT1) formed an axis with less than 40% of similarity. This is partly due to the statistical test used, which only considers the shared presences and not the absences.

The positioning of samples from the same municipality in different clades, especially the samples from Alagoinhas, Inhambupe and Itapicuru, highlights the apicultural floristic potential of this region which provides bees with a varied range of plants for foraging.

Castro et al. (2007) conducted a study with propolis samples collected in the municipality of Entre Rios, and obtained the levels of total phenolic compounds for six consecutive months, with the largest level obtained in July (39.38±0.01 mg GAE/g) and the lowest in May (22.03±0.01 mg GAE/g). The value obtained here for the propolis samples from the same municipality fits the values found in the months September (32.16±0.01 mg GAE/g) and October (32.13±0.00 mg GAE/g). Furthermore, the values found for the propolis samples from other municipalities of the territory of the Agreste of Alagoinhas are very close to those found by Castro et al. (2007).

Menezes et al. (2010) conducted a study of the correlation between the pollen types *Mimosa pudica* and *Eucalyptus*, which are the most frequent types in the apicultural pollen from the municipality of Alagoinhas (Bahia), and the levels of phenolic compounds of the same samples. In their analyses of the samples with the highest occurrence of *Mimosa pudica*, the average levels of phenolic compounds was 33.34±0.46 mg GAE/g, and in the samples with the highest occurrence of *Eucalyptus* the average levels of phenolic compounds was 67.83±0.93 mg GAE/g. Through the Spearman correlation coefficient they identified a negative correlation between levels of total phenolic compounds with the pollen type *Mimosa pudica* (Rs=-0.5066) and a positive correlation with the type *Eucalyptus* (Rs=0.6666), whereas in our study of samples from the territory of the Agreste of Alagoinhas the correlation was negative for both types. There was however a positive correlation with the types identified as Asteraceae I, *Borreria verticillata*, *Mikania* and *Mitracarpus longicalyx*. Further studies including a larger number of samples are needed to establish whether the association of the correlation of the levels of total phenolic compounds with these pollen types is indeed valid for propolis produced in this region.

## CONCLUSIONS

The unanimous presence in the samples of the pollen types *Mimosa pudica* and *Eucalyptus* characterized the propolis produced in the territory, however, the plants associated to these pollen types are not good geographic markers since they represent widely distributed plants, specifically an invasive species and a cellulose-producing species planted in large monocultures.

The levels of total phenolic compounds found in the propolis samples from the territory of the Agreste of Alagoinhas fit the standards of the Brazilian legislation. It is, therefore, important that studies such as ours corroborate the quality of propolis produced in the state, since the quality of propolis is directly related to the levels of phenolic compounds.

The correlations between the occurrence of the two main pollen types identified and the levels of total phenolic compounds in the propolis samples from the territory of the Agreste of Alagoinhas were both negative, unlike what was found in another study. However, the other study investigated apicultural pollen; in propolis the largest source of total phenolic compounds comes from the resin, unlike what happens in pollen. Because no other study was found in the specialized literature addressing this subject matter in propolis, we recommend that more analyses be conducted so that we can get more data that could corroborate the information contained herein.

The study detailed here has its importance in the primacy of addressing propolis samples exclusively from the territory of the Agreste of Alagoinhas. The results presented are the basis for future studies, in order to provide means for the certification of this apicultural product.

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## RESUMO

Vinte e duas amostras da própolis produzida por *Apis mellifera* L. em uma região do Semiárido no Estado da Bahia (Agreste de Alagoinhas), Brasil, foram analisadas palinologicamente e quantificadas em relação aos níveis de compostos fenólicos totais. Essas amostras foram processadas utilizando a técnica da acetólise com as modificações sugeridas para uso de própolis. Foram encontrados 59 tipos pertencentes a 19 famílias e 36 gêneros. A família Fabaceae foi a mais representativa neste estudo com nove tipos de polens, seguida da família Asteraceae com sete tipos. Os tipos *Mikania* e *Mimosa pudica* estiveram presentes em todas as amostras analisadas. Os tipos *Mimosa pudica* e *Eucalyptus* apresentaram frequência de ocorrência superior a 50% em pelo menos uma amostra. O maior índice de similaridade (c. de 72%) ocorreu entre as amostras ER1 e ER2 pertencentes ao município de Entre Rios. As amostras da cidade de Inhambupe apresentaram os maiores (36,78±1,52 mg/g EqAG) e menores (7,68±2,58 mg/g EqAG) níveis de compostos fenólicos totais. Através do Coeficiente de Correlação de Spearman notou-se que existia uma correlação linear negativa entre os tipos *Mimosa pudica* (rs= -0,0419) e *Eucalyptus* (rs = -0,7090) com o perfil dos níveis totais de compostos fenólicos das amostras.

**Palavras-chave:** produtos apícolas, similaridade florística, melissopalinologia, compostos fenólicos.

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