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Original Article

Pollen analysis of honey and pollen stored by *Melipona (Melikerria) fasciculata* Smith, 1854 (Apidae Meliponini), in an Amazon and Cerrado transition area, Maranhão, Brazil

Análise polínica do mel e pólen estocado por *Melipona* (*Melikerria*) *fasciculata* Smith, 1854 (Apidae, Meliponini), em área de transição Amazônia e Cerrado, Maranhão, Brasil

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Abstract

The identification of meliponicultural flora is fundamental for the preservation of bees, as well as enabling the development of pollinator management and reforestation programs. In this context, this study aimed to investigate the types of pollen found in the honey and pollen pots of the *Melipona fasciculata* bee in Arari, State of Maranhão, between August 2022 and July 2023. 40 types of pollen were identified, distributed among 18 botanical families, with Fabaceae standing out (46.15% in pollen pots and 37.86% in honey pots), Myrtaceae (11.53% in pollen and 13.51% in honey) and Rubiaceae (7.69% in pollen and 5.40% in honey). Sapindaceae, in the honey pots, accounted for 5.40%. The other families showed lower percentages, 3.84% in the pollen pots and 2.70% in the honey pots. In terms of similarity, two distinct groups were observed in both the pollen and honey pots. In the pollen pots, group A (May-June ~ 97%) and group B (Feb-Mar ~ 99%) stood out, while in the honey pots, group A (Mar-Apr ~ 98%) and group B (Jun-Jul-Sept ~ 98%) showed the highest percentages. These findings highlight the wide range of resources used by the *M. fasciculata* species, as well asits preference for Fabaceae and Myrtaceae, due to the diversity and availability of trophic resources. An understanding of the meliponicultural flora is essential to support effective conservation strategies, which aim not only to guarantee the survival of the bees, but also to ensure the continued production of honey, a resource of great importance to local communities.

Keywords: meliponiculture flora, melissopalynology, Tiúba, Baixada Maranhense.

Resumo

A identificação da flora com potencial meliponícola é fundamental para a preservação das abelhas, além de possibilitar o desenvolvimento de programas de manejo de polinizadores e reflorestamento. Nesse contexto, este estudo teve como objetivo investigar os tipos de pólen encontrados nos potes de mel e pólen da abelha *Melipona fasciculata* em Arari-Ma, durante o período de agosto de 2022 a julho de 2023. Foram identificados 40 tipos de pólen distribuídos em 18 famílias botânicas, com destaque para Fabaceae (46,15% nos potes de pólen e 37,86% nos potes de mel), Myrtaceae (11,53% em pólen e 13,51% em mel) e Rubiaceae com 7,69% em pólen e 5,40% em mel. Sapindaceae, nos potes de mel, representou 5,40%. As demais famílias apresentaram percentuais menores, sendo 3,84% nos potes de pólen e 2,70% nos potes de mel. Quanto à similaridade, tanto nos potes de pólen quanto nos de mel, foram observados dois grupos distintos. Nos potes de pólen, o grupo A (mai-jun ~ 97%) e o grupo B (fev-mar ~ 99%) se destacaram, enquanto nos potes de mel, o grupo A (mar-abr ~ 98%) e o grupo B (jun-jul-set ~ 98%) apresentaram maiores percentuais. Essas descobertas ressaltam a ampla gama de recursos utilizados pela espécie *M. fasciculata,* bem como sua preferência por Fabaceae e Myrtaceae, devido à diversidade e disponibilidade dos recursos tróficos. A compreensão da flora meliponícola é fundamental para embasar estratégias eficazes de conservação, que visam não apenas garantir a sobrevivência das abelhas, mas também assegurar a continuidade da produção de mel, um recurso de grande importância para as comunidades locais.

Palavras-chave: flora meliponícola, melissopalinologia, Tiúba. Baixada Maranhense.

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1. Introduction

The meliponiculture flora can be defined as the set of plants visited by bees to collect floral resources such as pollen, nectar and resin (Nordi and Barreto, 2016). Several plant species are visited by bees, including economically valuable cultivated, ornamental, medicinal and forest plants (Nascimento et al., 2021).

Some plant species used by bees include polliniferous plants, which exclusively provide pollen (Barth, 1989; Santos et al., 2018). Nectar plants provide a greater volume of nectar in relation to the volume of pollen (Almeida et al., 2003, Santos et al., 2018). There are also resin plants, which are exploited to produce propolis (Santos et al., 2018). They all provide essential resources for the survival and development of bee colonies.

Stingless bees are of great importance in the pollination of various crops, accounting for up to 90% of the pollination of wild plant species in tropical environments (Holzschuh et al., 2012). *Melipona* (*Melikerria*) *fasciculata* Smith, 1854, a native stingless bee, plays a crucial role as a pollinator in the natural ecosystems of Maranhão. Its presence in regional meliponiculture has been a significant source of income for the Baixada Maranhão region (Carvalho et al., 2016; Gostinski et al., 2017).

In the research by Souza et al. (2023) on the interconnection between floristic composition and the practice of meliponiculture in the Eastern Amazon, a diversity of tree, shrub and herbaceous species with the potential to be used in this activity in the region was evidenced. Understanding which plants are used by bees to feed and build their nests is crucial to preserving and maintaining bee species with the potential to produce honey in the Amazon region (Absy et al., 2018).

Identifying flora with meliponiculture potential in a specific region is crucial, especially for preserving bees in their natural habitats. This is essential for understanding the interaction between bees and plants, enabling the development of pollinator management, reforestation and environmental recovery programs (Luz et al., 2007). In addition, surveying the flora also helps with bee farming activities, contributing significantly with information about plant species used by the bees and the types of honeys to be produced.

The aim of this research was to obtain information on the flora used by the species *M. fasciculata* through pollen analysis of pollen and honey samples.

The aim of this study was to obtain data on the flora exploited by the species M. fasciculata through pollen analysis of pollen and honey samples, as well as highlighting the relevant botanical groups that offer resources in a transition region between the Cerrado and the Amazon.

2. Methods

2.1. Study area

The municipality of Arari, is located in the geographical mesoregion Norte Maranhense in the microregion of Baixada Maranhense (Maranhão State, Northeast region of Brazil). It covers different biomes such as the Amazon and Cerrado, with varied vegetation such as pioneer areas, river and/or lake influence, agricultural activities and secondary vegetation (IBGE, 2022).

There is a diversity of predominant vegetation, such as grasslands, coconut forests and mangroves, each with its own specific characteristics. The grasslands have tall grasses, the mangroves are made of medium-sized trees adapted to the environment and the cocais forest is a transition between Cerrado, Caatinga and Amazon forest, with spaced trees such as babassu, buriti and embaúba, as well as other species such as araticum, black sucupira and ipê (CPRM, 2011). Arari has patches of mangrove due to the influence of salt water and areas of natural grassland due to the flooding of the Mearim River and its tributaries.

Three communities, Bubasa (S 03° 34'01.9" H 044° 40' 48.6"), Trizidela (S 03° 23' 15.3" H 044° 47'19.9") and Bonfim (S 03° 23'48.1" H 044° 48' 08.5") (Figure 1), were selected based on different phytophysiognomies that cover the various biomes of the Arari region (Figure 2). The distance from Bubasa to Trizidela is 20 km, from Bubasa to Bonfim is 30 km, and from Trizidela to Bonfim is 11 km. In addition, the presence of at least one meliponary in each community was taken into account.

2.2. Sampling

To collect honey and pollen, a meliponary was chosen from each community. Three colonies were chosen at random from each meliponary.

Each colony was identified by a number, C1 for hive 1, C2 for hive 2 and C3 for hive 3. From each colony, 2-3g of pollen was collected from each colony using spatulas and 10mL of honey using disposable syringes to avoid contamination. Pollen and honey were collected from open and closed food jars. After being collected, the honey was stored in falcon tubes and the pollen in eppendorfs. The samples were transported to the laboratory and stored in a refrigerator. The samples collected monthly from August 2022 to July 2023 were labeled according to their origin and analyzed individually.

The pollen samples were diluted in acetic acid, while the honey samples were diluted in warm distilled water. They were then subjected to acetolysis, following the method described by Erdtman (1960), prior to mounting the permanent slides. The pollen types were identified based on their morphological characteristics by comparing them with specialized literature (Roubik and Moreno, 1991; Carreira et al., 1996). In addition, the identifications were also made using the reference palynoteca, which was made using plant samples from the region. Pollen grains were characterized according to their morphology, as "pollen type", including one or more species as taxonomic categories (de Klerk and Joosten, 2007)

2.3. Plant collection

To help identify the meliponiculture flora, the monthly flowering plants around the meliponary were collected in order to build a reference palynotheca. To do this, trails were used to collect branches and flower buds of the plant species found in a radius of approximately 1,000 meters

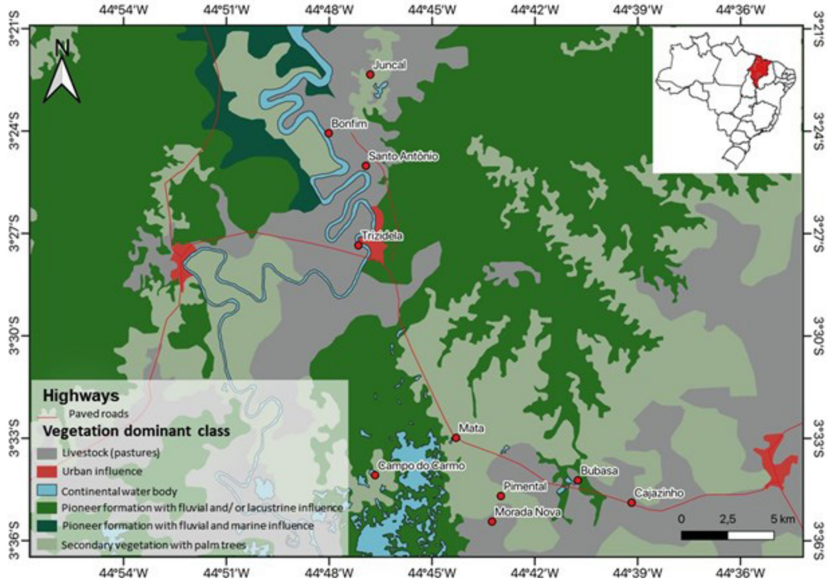

Figure 1. Communities in the municipality of Arari-Maranhão.

around the meliponary of the three communities chosen in the municipality of Arari.

The branches were dried, exsiccated and later identified by the Rosa Mochel Herbarium (SLUI) at the State University of Maranhão, São Luís. The flower buds of the plant species collected underwent the acetolysis process (Erdtman, 1960), after which permanent slides were made to compose the region's palynotheca.

2.4. Data analysis

Pollen grain analysis was conducted by counting 500 grains for each sample grouped into monthly percentages (Freitas et al., 2015). The pollen types were classified as follows. Louveaux et al. (1978) according to the number of pollen grains per sample, considering predominant pollen (≥ 45%), secondary pollen (16-45%), importante minor pollen (3-15%) and minor pollen (<3%). To assess the similarity of the pollen spectra from the three communities, a similarity dendrogram was drawn up. For this analysis, the data from the months of collection was used, applyning Past software version 4.04 (Hammer et al., 2001).

3. Results

The pollen analysis of the pollen pots identified 27 types pollen pollen used by *M. fasciculata*, among them representatives of the families: Arecaceae (1 pollen type); Asteraceae (1); Combretaceae (1); Convolvulaceae (1); Fabaceae (12); Lamiaceae (1); Melastomastaceae (2); Mytaceae (3); Ochnaceae (1); Pontederiaceae (1); Rubiaceae (2) and the Solanaceae family (1) (Figure 3), (Table 1).

In the pollen analysis of the honey pots, 37 types pollen were identified, the same as the pollen pots, but with the exceptions of *Attalea speciosa, Pontederia* and *Crotalaria*

Figure 2. Communities in the municipality of Arari-Maranhão. Bubasa (A), Trizidela (B) and Bonfim (C).

Table 1. Types of pollen collected by *Melipona fasciculata* from August 2022 to July 2023.

Family	Pollen types	Pollen pot	Honey pot
Acanthaceae	*Avicennia germinans*	-	X
Arecaceae	*Astrocaryum vulgare*	-	X
	Attalea speciosa	X	-
Amaranthaceae	*Alternanthera brasialiana*	-	X
Asteraceae	Asteraceae	X	X
Bignoniaceae	*Adenocalymma inundatum*	-	X
Combretaceae	*Terminalia lucida*	X	X
Convolvulaceae	Convolvulaceae	X	X
Euphorbiaceae	*Croton*	-	X
Fabaceae	*Bauhinia forficata*	-	X
	Crotalaria retusa	X	-
	Crotalaria pallida	-	X
	Chamaecrista diphylla	X	X
	Chamaecrista	X	X
	Centrosema	X	X
	Copaifera	-	X
	Neptunia plena	X	X
	Mimosa pudica	X	X
	Mimosa candollei	X	X
	Mimosa caesalpiniifolia	X	X
	Mimosa sensitiva	X	X
	Mimosa	X	X
	Senna	X	X
	Fabaceae	X	X
Lamiaceae	*Hyptis atrorubens*	X	X
Melastomataceae	*Mouriri acutiflora*	X	X
Myrtaceae	*Syzygium cumini*	-	X
	Myrcia	X	X
	Eugenia	-	X
	Eucalyptus	X	X
	Myrtaceae	X	X
Ochnaceae	*Ouratea*	X	X
Pontederiaceae	*Pontederia*	X	-
	Eichhornia	-	X
Rubiaceae	*Borreria verticilata*	X	X
	Borreria scabiosoides	X	X
Sapindaceae	*Pauhinia*	-	X
	Sapindaceae	-	X
Solanaceae	*Solanum*	X	X
Passifloraceae	*Turnera subulata*	-	X
	Indeterminate 1	-	X
	Indeterminate 2	-	X
	Indeterminate 3	-	X
	Indeterminate 4	-	X
	Indeterminate 5	-	X

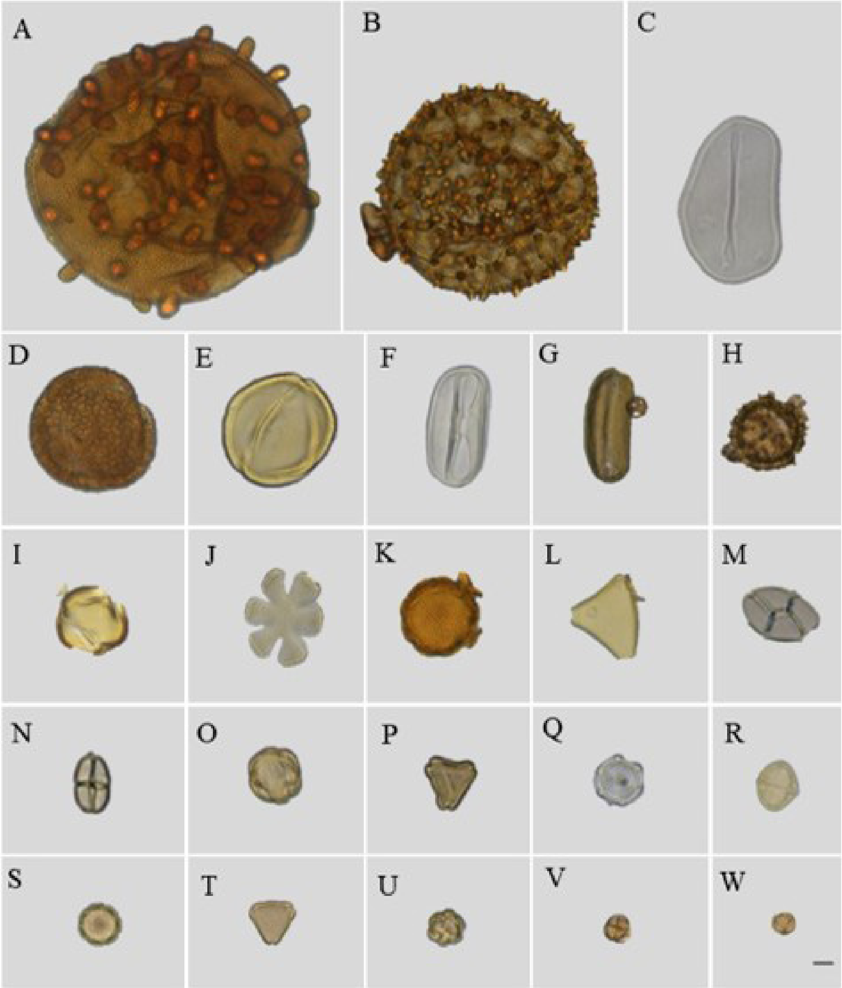

Figure 3. Types of pollen identified in the pollen and honey pots of *Melipona fasciculata*. (A) *Bauhinia forficata* **, (B) Convolvulaceae ***, (C) *Attalea speciosa* *, (D) *Turnera subulata* **, (E) *Neptunia plena* ***, (F) *Chamaecrista* *** (G) *Pontederia* * (H) Astaraceae ***, (I) *Senna* ***, (J) *Hyptis atrorubens* ***, (K) *Borreria scabiosoides* ***, (L) Sapindaceae **, (M) *Mimosa candolei* ***, (N) *Mimosa* , (O) *Mouriri acutiflora* ***, (P) *Eucalyptus* ***, (Q) *Alternanthera brasiliana* **, (R) *Solanum* *** (S) *Borreria verticilata* *** (T) *Myrcia* *** (U) *Terminalia lucida* ***, (V) *Mimosa caesalpiniifolia* *** and (W) *Mimosa pudica* ***. Scale: 10μm. (*pollen pots, ** honey pots and *** pollen pots e honey pots).

retusa. And 14 types of pollen unique to honey pots were identified, such as: *Avicennia germinans* (Acanthaceae); *Astrocaryum vulgare* (Arecaceae); *Alternanthera brasiliana* (Amaranthaceae); *Adenocalymma inudatum* (Bignoniaceae); *Croton* (Euphorbiaceae); *Bauhinia forficata*, *Crotalaria pallida* and *Copaifera* (Fabaceae); *Syzygium cumini* and *Eugenia* (Myrtaceae); *Eichhornia* (Pontederiaceae); *Paullinia* and Sapindaceae (Sapindaceae) and *Turnera subulata* (Passifloraceae) (Figure 3), (Table 1). Only five pollen types were not identified.

The most representative botanical families with the highest number of species in both pollen and honey pots were: Fabaceae (Pollen= 46.15% and Honey=37.86%) and Myrtaceae (Pollen = 11.53% and Honey = 3.51%), followed by Rubiaceae (Pollen = 7.69% and Honey = 5.40%), and only in the honey pots Sapindaceae with 5.40% each. The other 14 families individually showed 3.84% in the pollen pots and 2.70% in the honey pots.

In the analysis of the pollen pots, the predominant types of pollen were were *Senna* in all months, *Mimosa candolei* in February, March and April, and *Mimosa pudica* in May and June.

In the analysis of the honey pots, the types pollen that were predominant were *Senna* in the following months: August, September, October, November, January, May, June and July. *Terminalia lucida* which occurred in November and *Mimosa caesalpiniifolia* in March and April.

A total of 4 types pollen occured as secondary they were: *M. pudica* in October, December, January, February, April, May and July; *Senna* in October, November, March, May and June; *M. caesalpiniifolia* in October and November; and *Chamaecrista* only in September.

And in the honey pots there were 11 secondary types pollen: *Senna* in October, November, December, May and July; *M. caesalpiniifolia* in September, October, November, February and June; *Eucalyptus* in September, October and December; *T. lucida* in September, October, December, January and May; *M. pudica* in May and June; type Myrtaceae in October and November; and the Other 5 pollen types occured only in one month, *A. inundatum*. (February), *Solanum* (December), *Chamaecrista* (May), *Eichhornia* and *Borreria scabiosoides* (May).

The four types pollen classified as important minor in the pollen pots were: *M. pudica* in August, September, October, November, February, March and July; *M. candolei* in March and April; *Neptunia plena*, in September and November; Myrtaceae in November, January and July; *M. caesalpiniifolia* in March and July; and the others eight occurred in only one month, *Eucalyptus* (September), *Borreria verticillata* (November), *Mouriri acutiflora* (February), *Solanum* (March), *Chamaecrista* (May), *Myrcia* (May), *Pontederia* (April) and *B. scabiosoides* (May).

As for the honey pots, the types pollen pollen that were classified as important minor were: *M. pudica* in August, September, October, November, December, March, April, May, June and July; *M. candolei* in January, February, March, April and May; *M. caesalpiniifolia* in September, January, March and July; *T. lucida* in August, September, October and July; Myrtaceae in August, September, October and July; *Senna* in August, January and May. *T. lucida* in August, September, October and November; *Eucalyptus* in September, October, November, December, January and May; Myrtaceae in August, September, January and July; *Senna* in January, February, March, April, June and July; *Solanum* in January, February and June; *Chamaecrista diphylla* in May and June; Fabaceae in June and July; *A. inundatum* in January and April, Convolvulaceae in March and April; *M. acutiflora* in May and June; *Solanum* in November and June; and the others only occurred in one month, *Hyptis atrorubens* (November) and *B. scabiosoides* (March).

The other types pollen both in the pollen and honey pots were minor pollen.

The similarity dendrogram of the pollen pots resulted in two main groups, with the April sample being the most different from the others. Group A (May and June) and B (February, March, October, December, January, July, September, August and November). The percentage of similarities between the months of May and June was approximately 97%. In group B, the percentage of similarities was very high, between the samples from February and March (~ 99%), which were grouped together with the samples from October, December, January, July (~ 97%), September and August (~ 98%). And November, together with the other sample from group B (~ 87%) (Figure 3).

The combination of the honey pots resulted in two large groups. The samples from February, March and April (Group A) and the samples from January, May, June, July, August, September, November, October and December (Group B). The percentage of similarity in Group A was (~ 39%), but the months of March and April showed similarity of approximately (~ 98%). In Group B, the percentage of similarity was high between the June, July, August and September samples (~ 98%), which were grouped together with November and October (~ 97%). January and the other months in group B showed a similarity of approximately (~ 82%) and December showed a similarity of (~70%) (Figure 4).

4. Discussion

In the analysis of the pollen and honey pots, 40 different types of pollen were identified, as well as five unidentified pollen types, which is less than the number found by Kerr et al. (1986) who identified 79 plant species that provide nectar and/or pollen for *M. fasciculata* bees in the state of Maranhão.

However, our results show a greater variety of pollen types compared to the work of of Carneiro and Albuquerque (2005), who identified 14 pollen types used by *M. fasciculata* in the Baixada Maranhense region. Another survey of the food resources used by *M. fasciculata* in the Baixada Maranhense region was carried out, in which the author identified 11 pollen types in the pollen pots and 13 types in the honey pots (Silva, 2006). Ribeiro et al. (2016) identified a total of 121 pollen types in of flooded fields areas in the state of Maranhão.

The most representative families are similar to the findings of Carvalho et al. (2016) when examining the

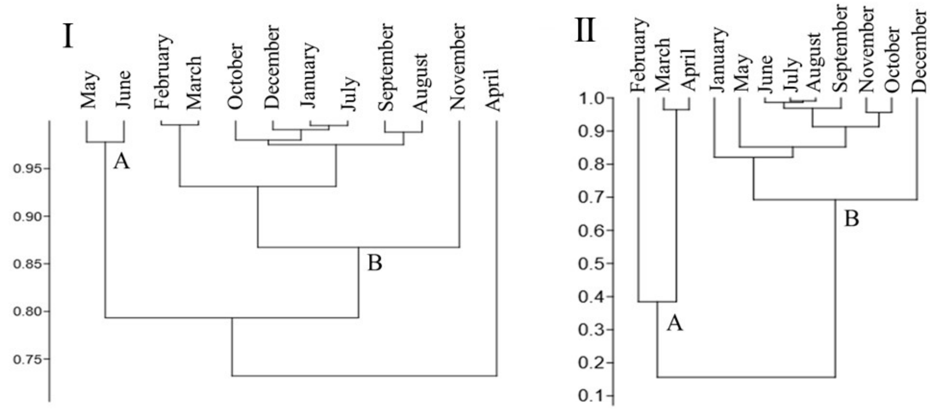

Figure 4. Similarity dendrogram based on monthly data from *Melipona fasciculata* pollen pots (I) and honey pots (II) collections.

importance of pollen in the flora used by *M. fasciculata* in the Baixada Maranhense region. In that study, it was found that the Fabaceae, Melastomataceae and Myrtaceae were among the most representative families.

Furthermore, the results of this study regarding the most representative families are also similar to those found in the study carried out by Rezende et al. (2020) in the municipality of Maués, in the state of Amazonas, where the Fabaceae and Myrtaceae families also stand out as the most frequently collected. The results of Ferreira et al. (2021) also revealed a similarity, as the most representative botanical families were Melastomataceae, Fabaceae, Anacardiaceae and Arecaceae in a study involving stingless bees of the genus *Melipona.*

The pollen types that were found to be predominant make an important contribution to characterizing the meliponiculture flora of the region and probably flower all year round. In the *Senna* genus, the flowers do not néctar produce and pollination usually occurs by bees capable of vibrating the stamens to release pollen, which characterizes "*buzz pollination*" (Silva et al., 2018). *Buzz pollination* (Proença, 1992) is a strategy used by the tiúba bee (M. fasciculata) to collect food (Carvalho et al., 2016)

The genus *Mimosa* has some species that provide a small amount of nectar, but are great suppliers of pollen grains (Barth, 1989). Therefore, they are considered important for the meliponiculture pasture in the Baixada Maranhense region, as the bees need a good amount of pollen to maintain their colonies. In the study by Correia et al. (2020), the genus *Mimosa* stood out as one of the most significant pollen taxa for bees of the genus *Melipona* in the region of Acre, which belongs to the Amazon biome. The authors attribute this to *Mimosa* ability to grow abundantly in different areas, to have mass flowering and consecutive flowering periods, and to adapt well to disturbed environments.

Out of the pollen types that stand out as secudary class, the genus *Eichhornia* has several aquatic species that provide nectar for *M. fasciculata* (Silva, 1996). *M. caesalpiniifolia* is a plant that produces large quantities of pollen and nectar (Carvalho, 2007), and is important for the production of tiúba honey in Maranhão (Kerr et al., 1986). Both are considered important sources of food for bees.

The pollen types *Eucalyptus* and Myrtaceae also stand out as accessories. They are representatives of the Myrtaceae family, which is known to supply large amounts of pollen, the main resource offered by this family, and also provides a small amount of nectar (Wilms and Wiechers, 1997). According to Simeão et al. (2015) Eucalyptus is a source of both pollen and nectar for bees. The presence of this family in the Baixada Maranhense region is important, especially in meliponiculture areas, as the bees would have it as a source of resources.

The other pollen types that only occurred in one month do not reflect the real importance of these plants for the tiúbas, but we must take into account the possibility that at a certain time of year they contributed to the production of honeys and the maintenance of the colonies. The representativeness of a pollen type will depend on the time of year the sample was collected and how long the pollen has been stored in the cerumen pots (Santos, 2006).

The herbaceous plants *Neptunia* and *Pontederia*, which stood out as important minor pollens, are typical of humid zones. In the analysis of tiúba honey from Maranhão carried out by Martins et al. (2011), the pollen types *Neptunia* and *Pontederia* were significant and indicate that these pollen types do not depend on rain to flower and can be considered a constant resource for *M. fasciculata*, as it is available almost all year round.

The other pollen types in both the pollen and honey pots occurred occasionally. According to Ribeiro et al. (2016) these pollen types should be considered in the evaluation because they produce pollen (entomophilous) and are good indicators of geographical origin. In addition, pollen classified as occasional is important as bee pasture for the sustainability of bee species (Santana et al., 2011).

In relation to the dendrogram of similarities, group A (~97%) and group B (in February and March ~99%) of the pollen pots, occurred because there was a high frequency of the pollen types *M. pudica*, *M. candolei*, type *Mimosa*, type *Senna*, and type Myrtaceae. The same occurred in the honey pots with similarity in group A (in March and April ~98%) and group B (in June, July, August and September ~98%). In the other months, representatives of the Fabaceae and Myrtaceae families were more frequent. In a survey of the flora of pollen importance used by *M. fasciculata*, carried out by Carvalho et al. (2016), the authors also found the same result regarding the Fabaceae and Myrtaceae families, as both were the most prominent.

This result shows that *M. fasciculata* has a preference for species from these families. In a study carried out by Santos et al. (2021), the Fabaceae family is among the most representative in terms of number of species, and is considered an important supplier of floral resources for meliponines, and is therefore very representative in the flora sampled. In the survey by Ribeiro et al. (2016), the Fabaceae family was also observed with the highest number of pollen types.

The Myrtaceae family is extremely attractive to the tiúba bee (*M. fasciculata*), due to the sweet smell of its white flowers and its numerous stamens with abundant, powdery pollen (Carvalho et al., 2016).

Analysis of the pollen spectrum used by the *M. fasciculata* species reveals that it uses a wide range of plant sources to collect floral resources. In addition, similarity analyses indicate a feeding preference for species from the Fabaceae and Myrtaceae families. Understanding the meliponiculture flora used by *M. fasciculata* is crucial, as it can guide breeders in identifying the main plant species that provide floral resources for these insects. This not only guarantees the survival of the colonies, but also promotes an increase in bee productivity.

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