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# Fungi Presence In Africanized Honeybees Hives Affected By Brazilian Sac Brood

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# Introduction

- The Brazilian Sac Brood (BSB) is a serious disease established in the southeastern of Brazil that attacks honey bees when they are fed with pollen of *Stryphnodendron* flowers (Mimosoideae).
- These plants are rich in toxic substances, tannins, leading a high mortality at the pre-pulps, and the symptoms are similar to the European Sac Brood that is caused by a virus.



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# Introduction

- The crescent losses of hives at different periods of time and regions of Rio de Janeiro State associated to the impossibility of finding the suspected tree near the infected apiaries permits suggesting a hypothesis that the disease is not caused by the toxic pollen.
- The fungi contamination from bee hives samples may be a clue to clarify the etiology of BSB.



# Introduction

- Fungi have worldwide distribution and can adapt themselves to different environmental conditions, including the environment of the hive.
- They were one of the main pathogens responsible for the destruction of millions of hives in the United States, Europe and Asia with alarming losses to agriculture (2007).



# Introduction

**Mycotoxins**

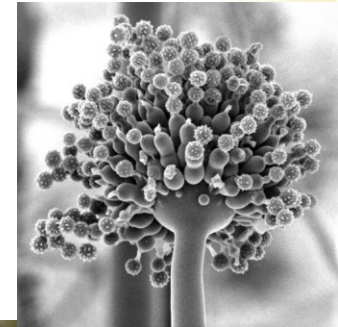


Secondary toxic metabolites

*Aspergillus*

*Penicillium*

*Fusarium*



Aflatoxins

Fumonisins

Ochratoxin A

Zearalenone

Tricothecens



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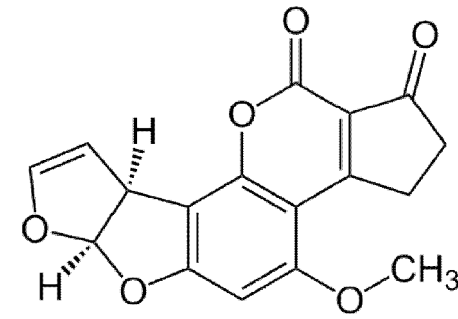
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# Introduction

## *Aspergillus*

- ✓ *A. flavus*
- ✓ *A. parasiticus*

- Mutagenic
- Carcinogenic
- Teratogenic
- Hepatotoxic
- Immunosuppressive



**Aflatoxin B<sub>1</sub>**

- Tropical and subtropical regions
- Different types of substrates
- Classified in the Class 1 carcinogen according to the International Agency for Research on Cancer (IARC, 1993).
- Most potent known biological hepatocarcinogen



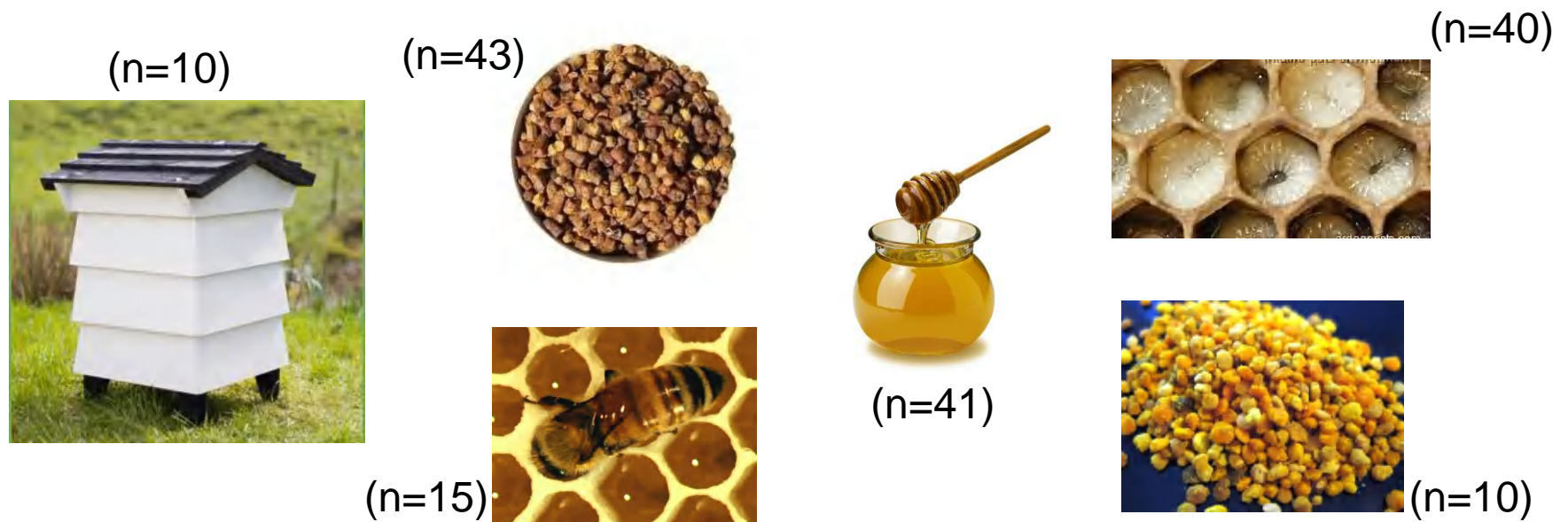
# Aims

- The aims of this study was to verify the occurrence of fungi infection in the africanized beehives in a region affected by the BSB, and to detect the presence of mycotoxins.



# Material and Methods

- The study took place in two apiaries from regions of Rio de Janeiro State where BSB occurs.

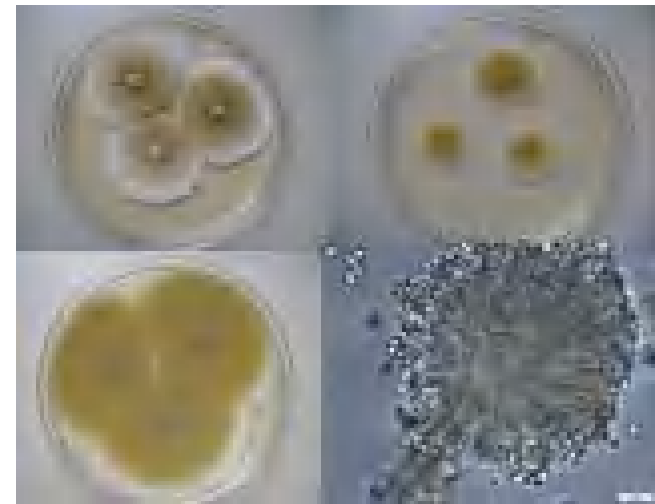
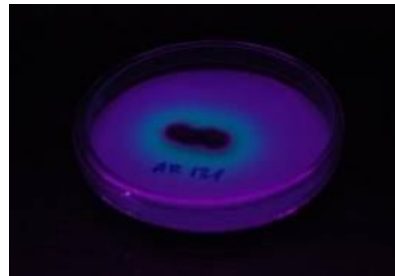
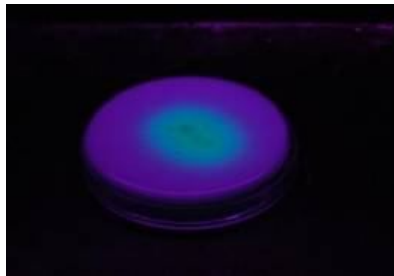


- The samples were collected in sterile flasks and kept under refrigeration during transport to processing.



# Material and Methods

- Water activity level ( $A_w$ )
- Fungi enumeration (CFU  $g^{-1}$ )
- Fungi identification
- Toxigenic profile of strains



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# Results

- The  $A_w$  is high in bee foods ( $0.70 \pm 0.10$ ), which promotes the fungi growth.
- Bee pollen, mainly bee bread, had remarkable fungi contamination in the hives ( $> 6 \cdot 10^4$  CFU  $g^{-1}$ ).
- The presence of toxigenic strains enhances the fungi action on bee bread ( $> 5 \cdot 10^5$  CFU  $g^{-1}$ ) (p-value  $< 0.001$ ), where was also detected aflatoxin  $B_1$ .

# Results

- *Aspergillus* sp., *Cladosporium* sp. and *Penicillium* sp. were the predominant genus in the samples.
- In bee food, the most frequently isolated fungi were *A. flavus*, *A. niger* aggregates, *A. fumigatus*, *A. ochraceus* and *P. citrinum*, which are potentially capable to produce different mycotoxins.

# Results

- 55% of the isolated strains of *A. flavus* in bee bread and 33% in bee pollen were able to produce aflatoxins on *in vitro* assays.
- We highlight the presence of entomopathogenic fungi to honeybees: *A. niger* aggregates, *A. flavus* and *A. fumigatus*.



# Conclusion

- The bee hives environment proved to be a good substrate for the growth of a high diversity of fungal genera and species.
- The presence of entomopathogenic fungi and mycotoxins can decrease the immune response, leaving the honeybees susceptible to various diseases, including the BSB itself.



# Conclusion

- The message we want to make with this work is that microbial contamination can be a factor of great impact on bee health and therefore the regulations around the world should include monitoring of these agents as a means of prevention.





# Thank you for your attention!

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