

# Microbiota, fungal resistance and mycotoxigenic potential in herbal teas

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FINAL CONSIDERATIONS

A top-down view of various tea leaves and processing tools on a light grey surface. There are several wooden bowls and scoops containing different types of tea leaves, including dark green rolled leaves, light green loose leaves, and white tea buds. Some tools have wooden handles with silver rivets. The background is scattered with loose tea leaves and small pieces of dried fruit or flowers.

# INTRODUCTION

# TEA

- ❖ Most common beverages worldwide, being prepared by infusing it with boiling water over the leaves or tea bags of the *Camellia sinensis*
- ❖ Several categories of tea, based on the degree of fermentation
- ❖ Current growth in tea consumption is explained by the recent trends in a healthy lifestyle



Black  
tea



White  
tea



# HERBAL

- ❖ Herbal and fruit infusions are amongst the world's most popular and widely enjoyed beverages, thanks to their almost unlimited variety and their convenience .
- ❖ Full range of flavour to suit every taste and any occasion
- ❖ **Do not** originate from the tea plant *Camellia sinensis*

Boldo

• *Peumus boldus*

Cavalinha

• *Equisetum hyemal*

Equinácia

• *Echinacea*

Cidreira

• *Melissa officinalis*



# KEY CONCEPTS

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## FUNGAL CONTAMINATION



In all stages of the tea  
and plants circuit

Poor agricultural  
practices, processing,  
drying, packaging,  
improper storage, and  
transportation  
conditions

## MYCOTOXINS



Risk for the production  
of mycotoxins

Influenced by the  
genotype of the fungi, by  
the available nutrients  
and by environmental  
factors

Potential risks to human  
health

## AZOLE RESISTANCE



Medically authorized  
antifungal medicines  
used for agricultural  
purposes

Acquired azolic  
resistance for  
continuous exposure

# OBJECTIVES

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1

quantify the microbiota present in the extraction and infusion of tea and herbal samples.



2

identify the mycobiota present in tea and herbal samples obtained through macroscopic characteristics and microscopy



3

determine the resistance profile of fungi to antifungal drugs of the azole group.

A photograph of a traditional tea set on a light-colored woven placemat. The set includes a dark brown, textured ceramic teapot with a curved handle and lid, and two smaller matching cups. The background is dark with some green foliage visible. A yellow rectangular overlay is centered over the image, containing the word 'METHODOLOGY' in a serif font. A faint leaf outline is visible behind the text.

# METHODOLOGY

# SAMPLES

Samples	Type	Agriculture	Origin	Packaging
1	Black tea	biological	Portugal	bulk
2	White tea	biological	EU	bulk
3	<i>Equisetum hyemale</i>	conventional	EU	tea bags
4	<i>Melissa officinalis</i>	conventional	EU	tea bags
5	<i>Peumus boldus</i>	conventional	Outside EU	bulk
6	<i>Echinacea</i>	conventional	EU	bulk
7	<i>Equisetum hyemale</i>	conventional	Portugal	bulk
8	<i>Melissa officinalis</i>	conventional	Portugal	bulk
9	<i>Echinacea</i>	conventional	EU	tea bags
10	Black tea	biological	EU	bulk
11	Black tea with aroma	biological	EU	tea bags
12	Black tea with aroma	conventional	Outside EU	tea bags
13	Black tea with aroma	biological	Outside EU	tea bags
14	Black tea	biological	Outside EU	tea bags
15	Black tea	conventional	Outside EU	tea bags
16	Black tea	conventional	Outside EU	tea bags
17	Black tea with aroma	conventional	Outside EU	tea bags
18	Black tea with aroma	conventional	Outside EU	tea bags
19	Black tea	conventional	n.d.	tea bags
20	Black tea with aroma	conventional	n.d.	tea bags
21	Black tea	conventional	n.d.	tea bags
22	White tea with aroma	conventional	n.d.	tea bags
23	White tea	biological	Portugal	bulk



The tea and herbal samples were purchased from two different markets, one of them being an established hypermarket and an herbalist shop, both located in the Lisbon Metropolitan Area

# SAMPLE TREATMENT

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## EXTRACTION

- 4.4g of the sample + 40ml of a NaCl 0,9%+Tween 80 0,05% solution
- agitated for 30min at 250rpm in an orbital shaker (OHAUS®)
- filtered with the help of a strainer
- glycerol was added
- kept at -80°C until use



## INFUSION

- 1g of the sample + 50 mL of boiling water for 5 minutes
- filtered with the help of a strainer
- glycerol was added
- kept at -80°C until use.

# MICROBIOLOGY

## Preparation of Culture Media



According to fabricant's recommendations  
Autoclave 15min  
121°C  
Plated on 90mm  
Petri dishes

## Inoculation



150ul were placed  
and spread  
throughout the  
plate

## Incubation



### TSA & RB

- 30°C for 7 days – lid facing down
- Counted on the 3rd, 5th and 7th day

### MEA & DG18

- 25°C for 6 days – lid facing up
- Counted on the 6th day

### SDA + azoles

- 25°C for 4 days – lid facing up
- Counted on the 4th day

# COUNTING

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BACTERIOTA

bacteria colonies are counted  
on the third, fifth and seventh  
day



MYCOBIOTA

fungi colonies are grouped  
based on their organoleptic  
characteristics and later  
counted on the sixth day

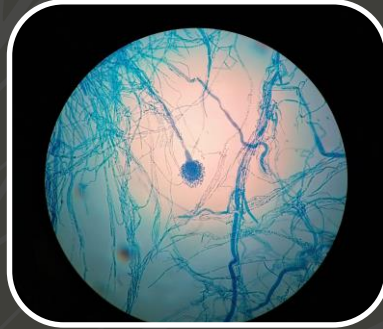
# IDENTIFICATION

## Slides Preparation

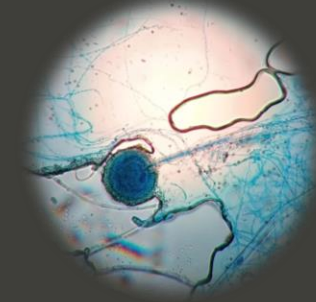
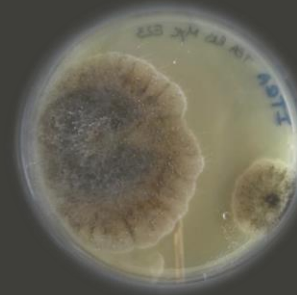


- drop of lactophenol cotton blue solution was placed on the slide
- piece of the fungi colony was cut and placed on it.
- lamella was placed on top
- excess lactophenol cotton blue solution was removed

## Mycobiota Identification



The identification is conducted by an expert. Morphological identification was achieved through macro and microscopic characteristics as noted by De Hoog et al. (2016).



# RESULTS & DISCUSSION



# TOTAL MICROBIOTA

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## BACTERIOTA

### Extraction

- TSA: 0 to 2121,2 CFU/g
- RB: 0 to 1000 CFU/g

### Infusion

- TSA: 0 to 67333,3 CFU/g
- RB: 0 to 33,3 CFU/g



## MYCOBIOTA

### Extraction

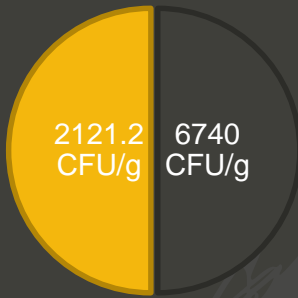
- TSA: 0 to 1212,1 CFU/g
- RB: 0 to 6606,1 CFU/g

### Infusion

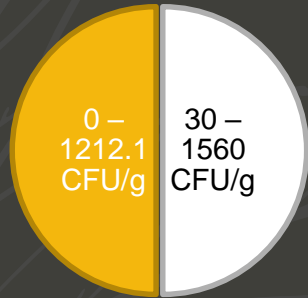
- TSA: 0 to 1000 CFU/g
- RB: 0 to 666,7 CFU/g

# RESULTS COMPARISON

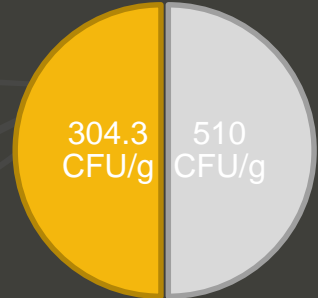
Max. bacteria in extracted samples



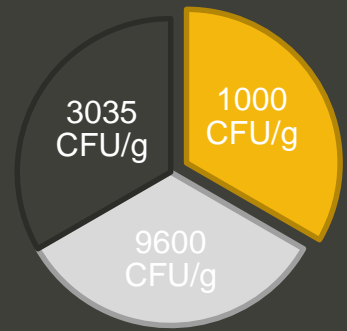
Mycobiota range in extracted samples



Mycobiota mean in infused samples



Max. Mycobiota in infused samples



● This study

● Pakshir *et al.*

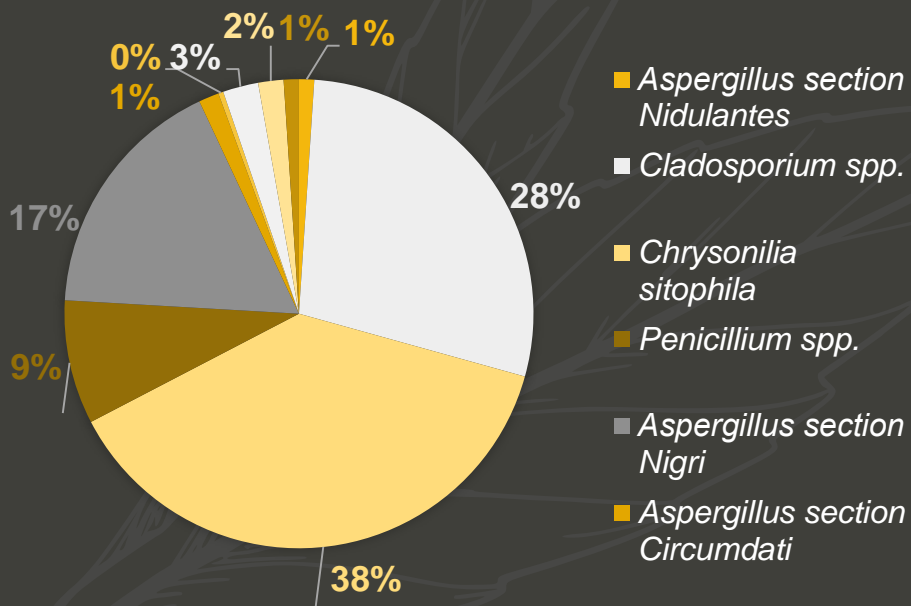
● Carraturo *et al.*

● Reinholds *et al.*

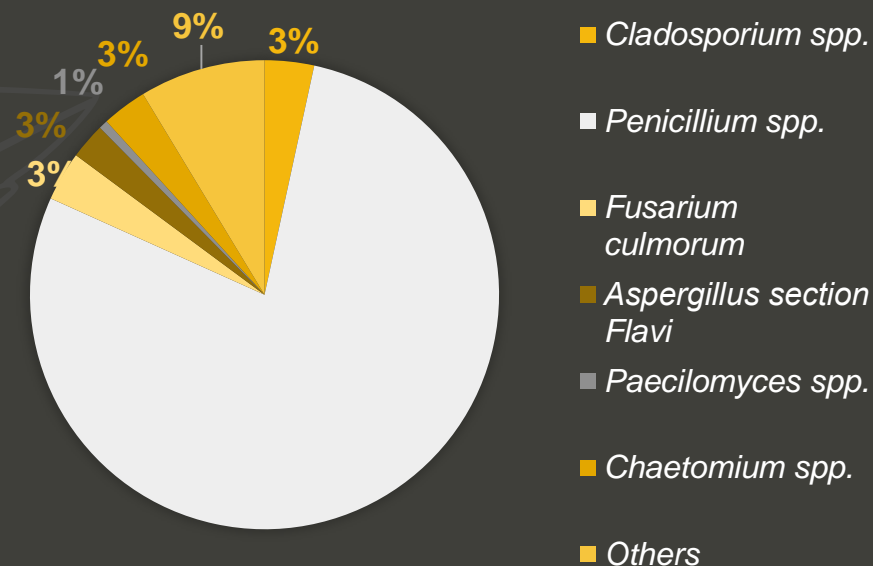
Pakshir K, Mirshekari Z, Nouraei H, Zareshahrabadi Z, Zomorodian K, Khodadadi H, et al. Mycotoxins Detection and Fungal Contamination in Black and Green Tea by HPLC-Based Method. J Toxicol. 2020;2020.  
Carraturo F, De Castro O, Troisi J, De Luca A, Masucci A, Cennamo P, et al. Comparative assessment of the quality of commercial black and green tea using microbiology analyses. BMC Microbiol. 2018;18(1):1–12.  
Reinholds I, Bogdanova E, Pugajeva I, Alksne L, Stalberga D, Valcina O, et al. Determination of Fungi and Multi-Class Mycotoxins in Camelia sinensis and Herbal Teas and Dietary Exposure Assessment. Toxins (Basel). 2020;12(9):1–30.

# MYCOBIOTA IDENTIFICATION

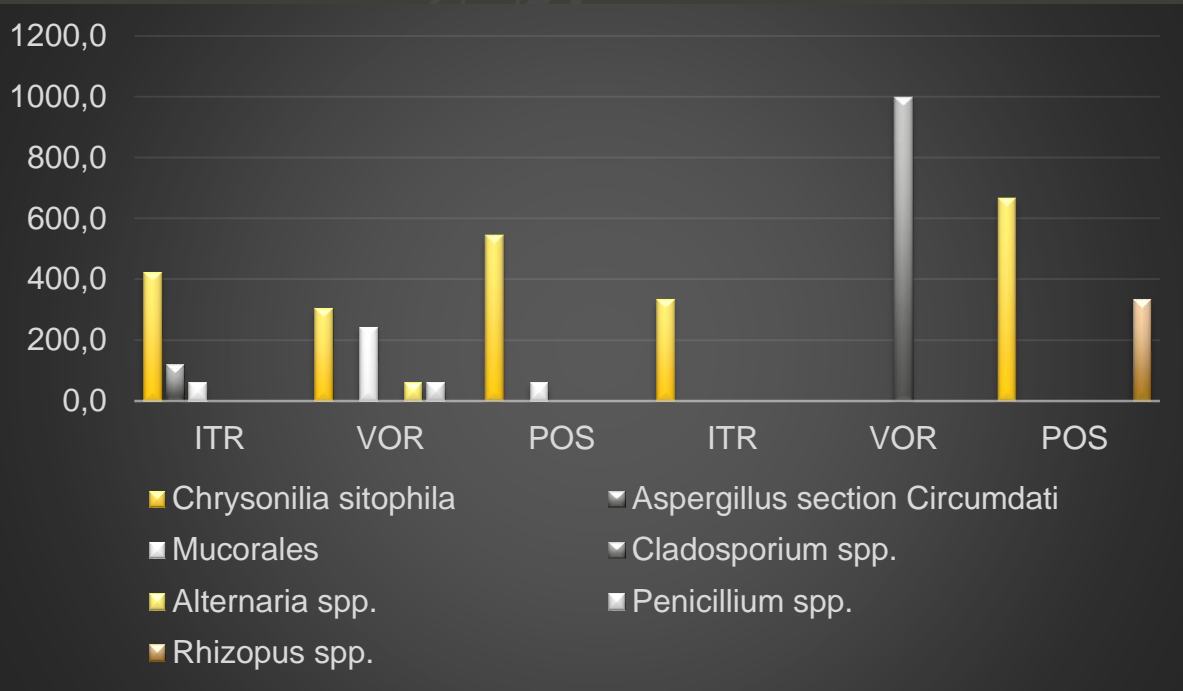
## MYCOBIOTA IDENTIFICATION IN TEA SAMPLES



## MYCOBIOTA IDENTIFICATION IN HERBAL SAMPLES



# AZOLE RESISTANCE PROFILE



Viegas *et al.* for green tea

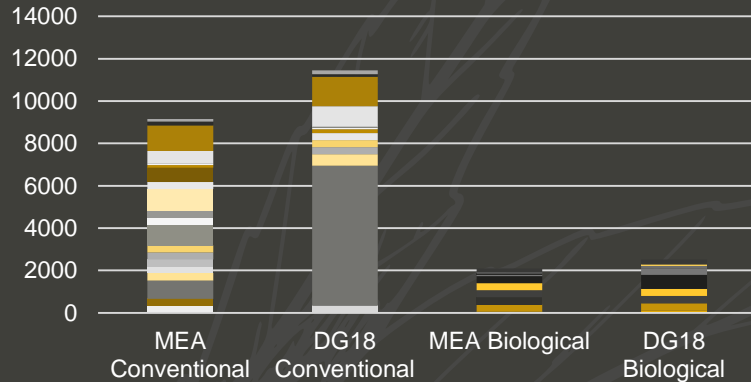
*Penicillium spp.*

*Rhizopus spp.*

*Chrysonilia sitophila*

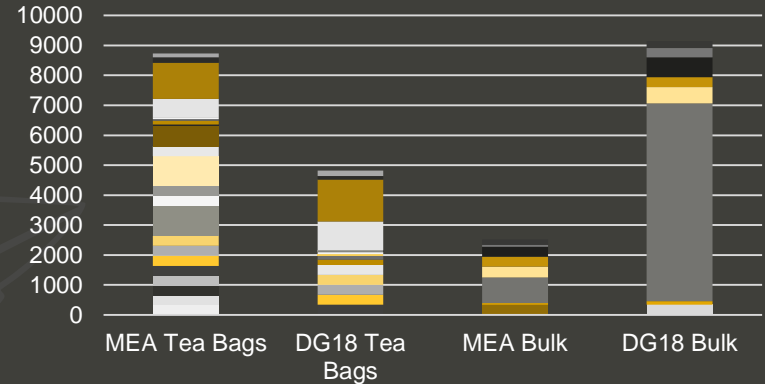
*Aspergillus section nigri*

## Conventional VS Biological Tea and Herbal Samples



Regarding conventional and biological agriculture samples, the biological samples analysed, compared to non-biological tea matrices, present lower microbial loads, which is what Carraturo and colleagues' stated in their study as well.

## Tea Bags VS Bulk Tea and Herbal Samples



Reinholds *et al.* study also presented a link between tea packaged in tea bags and in bulk. The mean mould counts in the bagged tea samples ( $6.47 \times 10^2$  CFU.g<sup>-1</sup>) were higher than those in the loose-leaf tea samples ( $3.69 \times 10^2$  CFU.g<sup>-1</sup>)

# CURRENTLY UNDER ANALYSES

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MYCOTOXINS

STATISTICAL ANALYSIS



# OBRIGADA

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Dúvidas? Questões?

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