

Mycobiota and Mycotoxins in Arabic Coffee and their biological control by using bacterial VOC's.

Abstract

All tested coffee samples were found contaminated with OTA and AF. Traditional roasting and brewing demonstrated reduction in mycotoxins levels. *In vitro* coinubation of *Bacillus simplex* showed promising results in inhibition of fungal growth and mycotoxin synthesis. *B. simplex* VOCs are important contributors in inhibition. *B. simplex* shows promising results against a large spectrum of fungi.

Background

- Coffee is one among the commonly consumed drink around the world.
- Mycotoxins such as OTA (Ochratoxin) & AF (aflatoxin) are secondary metabolites that are produced by mycotoxigenic fungi.
- OTA & AF have adverse health effect on humans, they are nephrotoxic and hepatotoxic and possibly carcinogens.
- Scientists attention turning towards investigating biological strategies due to the persistence and stability of mycotoxins.
- Bacillus simplex* VOCs (volatile organic Compounds) are showing promising results in the biological control of both fungal growth and mycotoxins synthesis.

Objectives

- Investigate the level of OTA & AF in green, roasted coffee beans and ground coffee marketed in Qatar.
- Study the effect of Qatari traditional coffee roasting and brewing methods on the reduction of mycotoxins content.
- Explore *Bacillus simplex* as a biological control against fungal growth and mycotoxins synthesis in green coffee beans.

Methodology

Isolation and morphological identification of mycotoxigenic fungi from Arabic coffee

Coffee powders were diluted while beans (green & roasted) after surface disinfection were directly plated on DRCB. Isolates were then transferred to CYA and MEA for morphological identification.

Analysis of mycotoxins in Arabic coffee samples

The extraction of aflatoxins and ochratoxin A from coffee samples will be carried out by following standard extraction procedures according to the recommendation of ELISA kits.

Effect of traditional Qatari roasting and brewing methods on the reduction of mycotoxins in coffee

Green coffee beans were artificially contaminated with known levels of OTA and AFs and are then roasted and brewed in the traditional Qatari way and later analyzed by ELISA.

Application of bacterial volatile organic compounds (VOCs) against mycotoxigenic fungi

Surface disinfected green coffee beans were be contaminated with *A. carbonarius* spores and will be exposed to *B. Simplex* volatiles in a closed glass chamber.

GC-MS based analysis of bacterial VOCs to identify the active antifungal compounds(s)

Gas chromatography mass spectrometry (GC-MS) based analysis will be performed to identify active VOC responsible for fungal growth inhibition on the coffee bean samples. Activated charcoal (AC) will be used for the capturing of VOCs produced *B. simplex*.

Exploration of the spectrum of antifungal activities of *B. simplex* on mycotoxigenic fungi

In vitro coinubation of *Bacillus simplex* with different fungi was done to demonstrate the antifungal activity and the ability to reduce fungal growth and inhibit sporulation.

Results and Discussion

Isolation and morphological identification of mycotoxigenic fungi in Arabic coffee

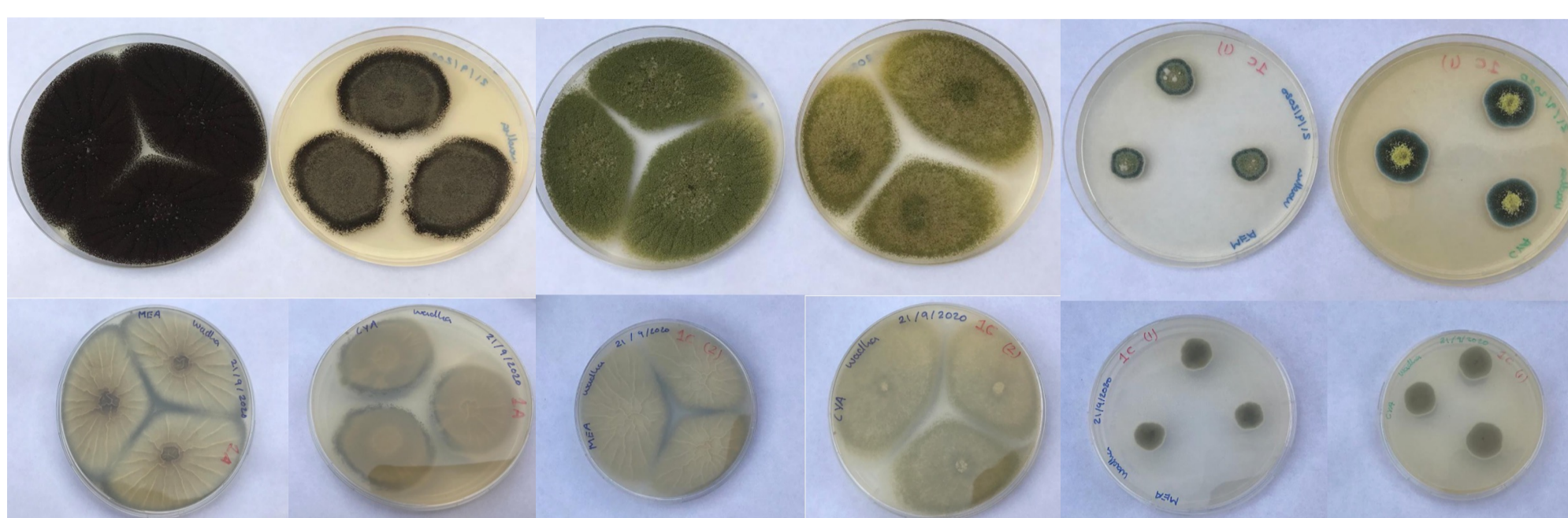


Figure 1: Isolation and morphological identification of fungi in green coffee on MEA and CYA

- Aspergillus* and *Penicillium* were found more abundant in green coffee.
- A. flavus* and *A. carbonarius* were more common.

Effect of traditional roasting and brewing on reducing mycotoxins in coffee

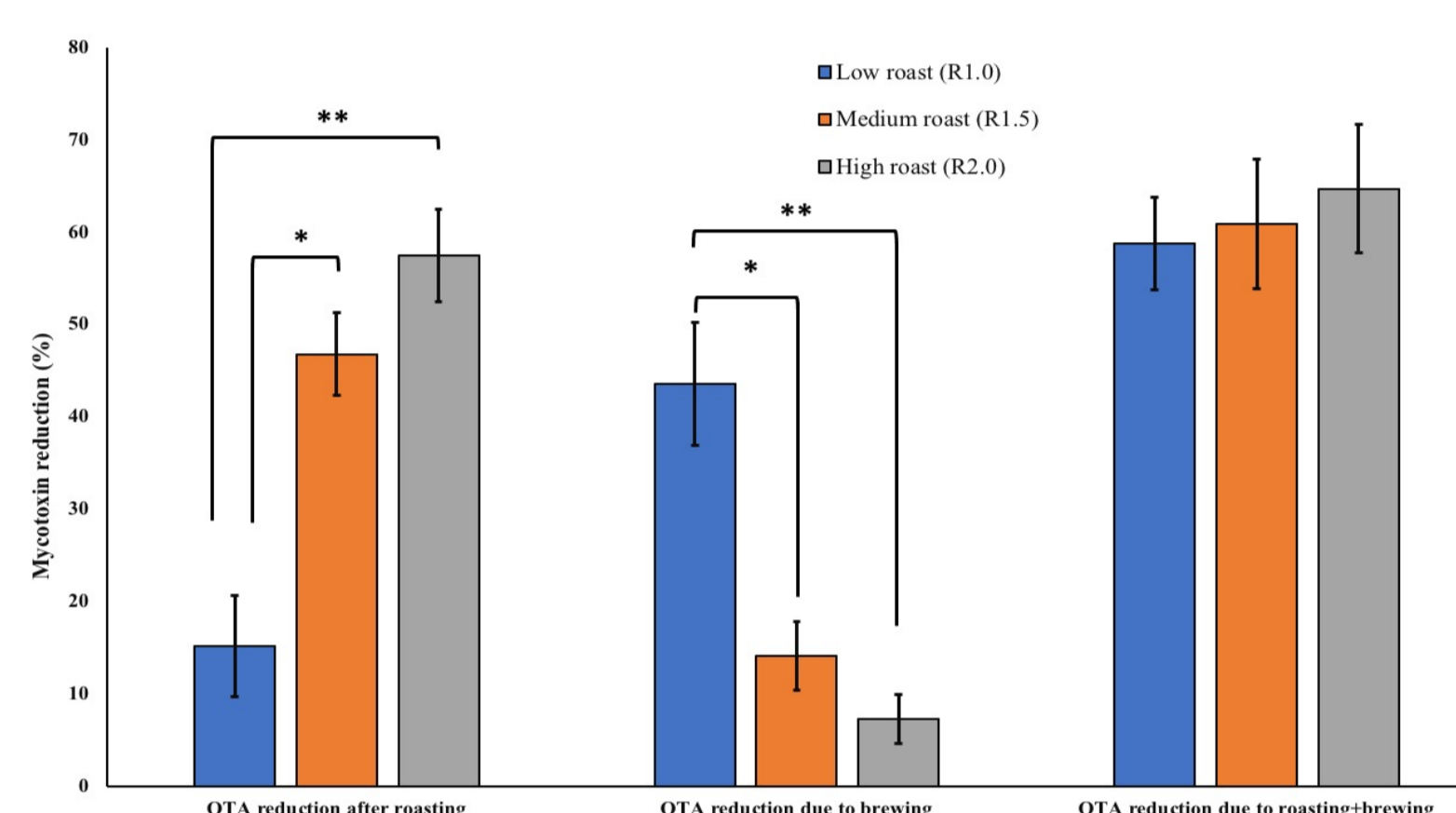


Figure 2: Effect of traditional roasting and brewing on OTA reduction (%) in naturally contaminated coffee beans.

- Roasting and brewing reduced OTA and AF levels in coffee samples.
- Higher roasting condition showed higher % reduction of mycotoxins (OTA & AF)

Bacterial VOCs against mycotoxigenic fungi

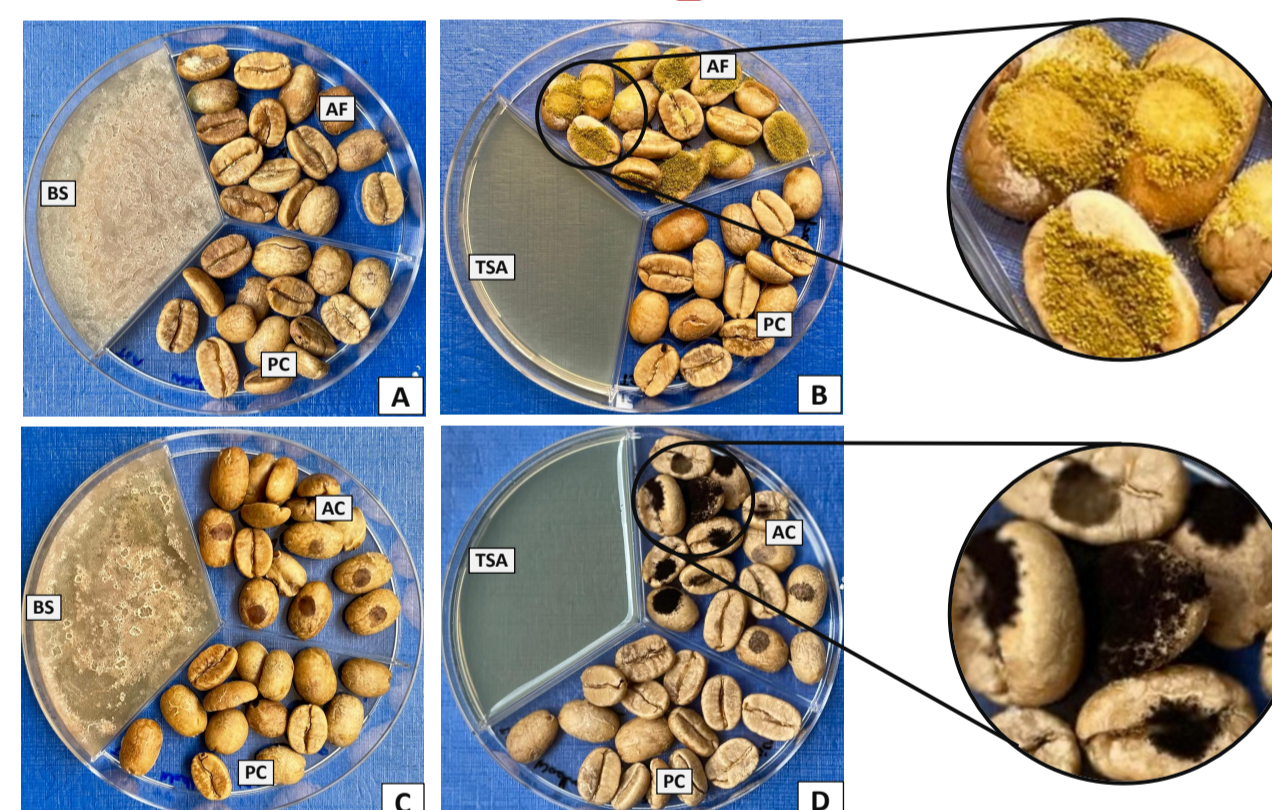


Figure 4 : In vivo antagonistic activity of *B. simplex* 350-3 volatiles against the growth of toxigenic fungi on infected coffee beans.

- Control plates showed fungal growth (*A. carbonarius* & *A. flavus*).
- Spreading bacteria on TSA caused almost complete inhibition of fungal growth.

Table 2: Effect of bacterial volatiles on the inhibition of mycotoxins synthesis

| Treatment | Aflatoxin (µg/kg) | OTA (µg/kg) |
|---|-------------------|-------------|
| Control (plan coffee beans) | 30.05 ± 3.27 | 1.39 ± 0.12 |
| *Fungal infected coffee beans | 63.60 ± 8.52 | 4.13 ± 0.81 |
| Fungal infected coffee incubated in the presence of <i>B. licheniformis</i> | 23.60 ± 5.07 | 1.53 ± 0.42 |
| Fungal infected coffee incubated in the presence of <i>B. simplex</i> | 26.65 ± 3.33 | 1.88 ± 0.33 |

* Coffee beans were either infected with toxigenic *A. flavus* or *A. carbonarius* for AFs and OTA synthesis, respectively.

- All samples showed OTA and AF contamination.
- Uninfected coffee that was not exposed to bacterial VOC, we observed some OTA and AF showing that coffee beans naturally have some levels of mycotoxins.

Identification of VOCs introduced by *B. simplex*

Table 3: Analysis of *B. simplex* 350-3 volatiles through GCMSMS

| S. No. | Molecule | Retention time (min) | Peak area (%) |
|--------|--------------------|----------------------|---------------|
| 1 | Quinoline | 18.42 | 2.44 |
| 2 | Benzenemethanamine | 24.98 | 5.58 |
| 3 | 1-Octadecene | 34.38 | 4.48 |

- Antifungal activity of *B. simplex* was shown alone or combined with Quinoline, Benzene-methanamine and 1-Octadecene

Investigation of the mycotoxigenic content

Table 1: Mycotoxins level (µg/kg) in marketed coffee samples

| Sample types | Parameter | Mycotoxins (µg/kg) | |
|----------------------------|-----------|--------------------|--------------|
| | | Aflatoxins | Ochratoxin A |
| Green coffee beans (n=8) | Mean ± SD | 9.07 ± 5.26 | 2.15 ± 0.51 |
| | Range | 0 - 14.50 | 1.75 - 3.34 |
| Roasted coffee beans (n=8) | Mean ± SD | 33.61 ± 18.83 | 2.76 ± 1.46 |
| | Range | 7.55 - 67.42 | 1.68 - 6.14 |
| Powdered coffee (n=9) | Mean ± SD | 90.58 ± 39.08 | 8.95 ± 3.80 |
| | Range | 29.01 - 136.08 | 2.52 - 12.74 |

- 25% roasted coffee exceeded permissible levels.
- 44% powder coffee exceeded permissible levels.
- All green coffee samples were within the acceptable limit.

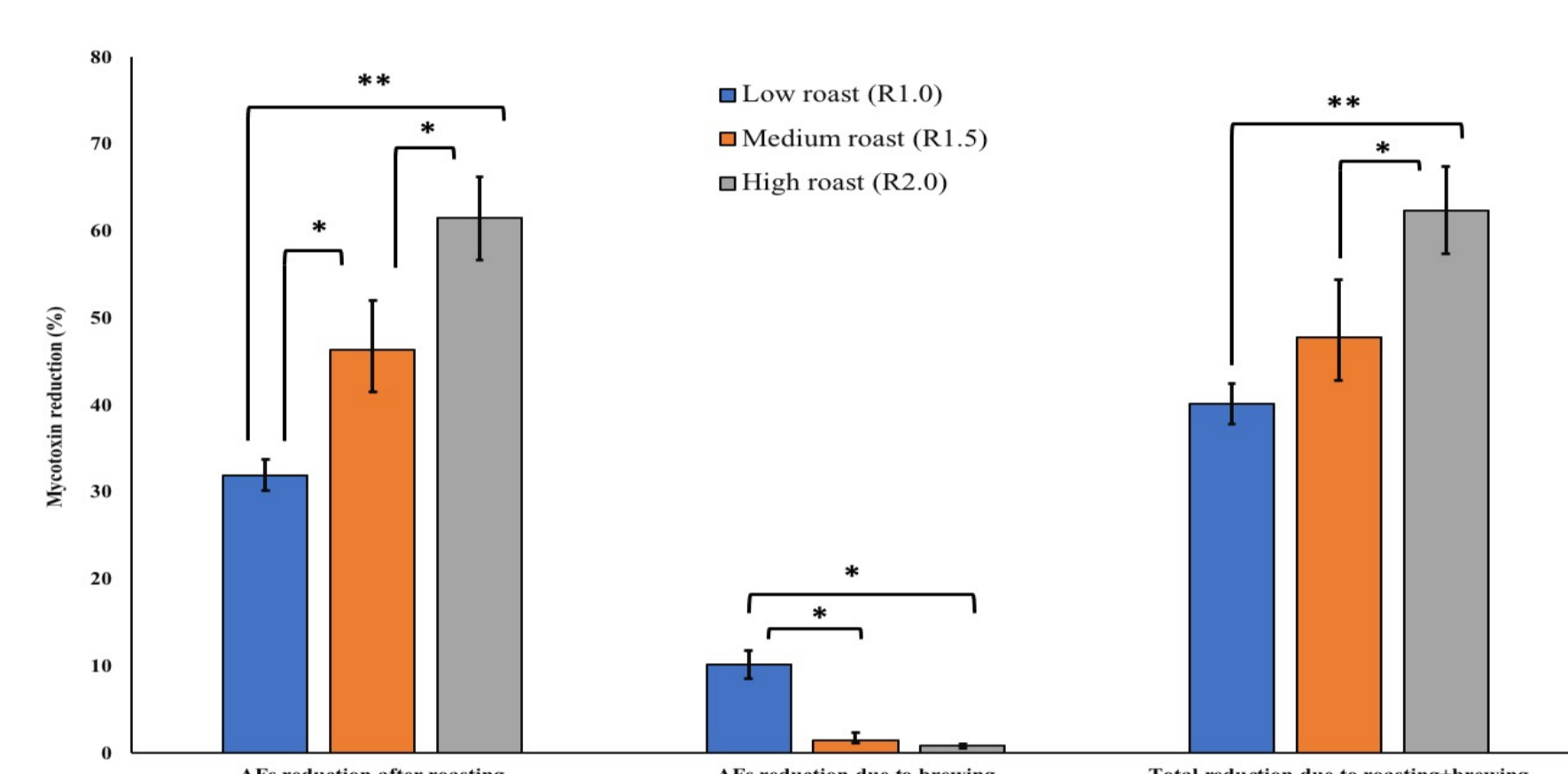


Figure 3: Effect of traditional roasting and brewing on AFs reduction (%) on naturally contaminated coffee beans.

Bacterial VOCs against mycotoxigenic fungi

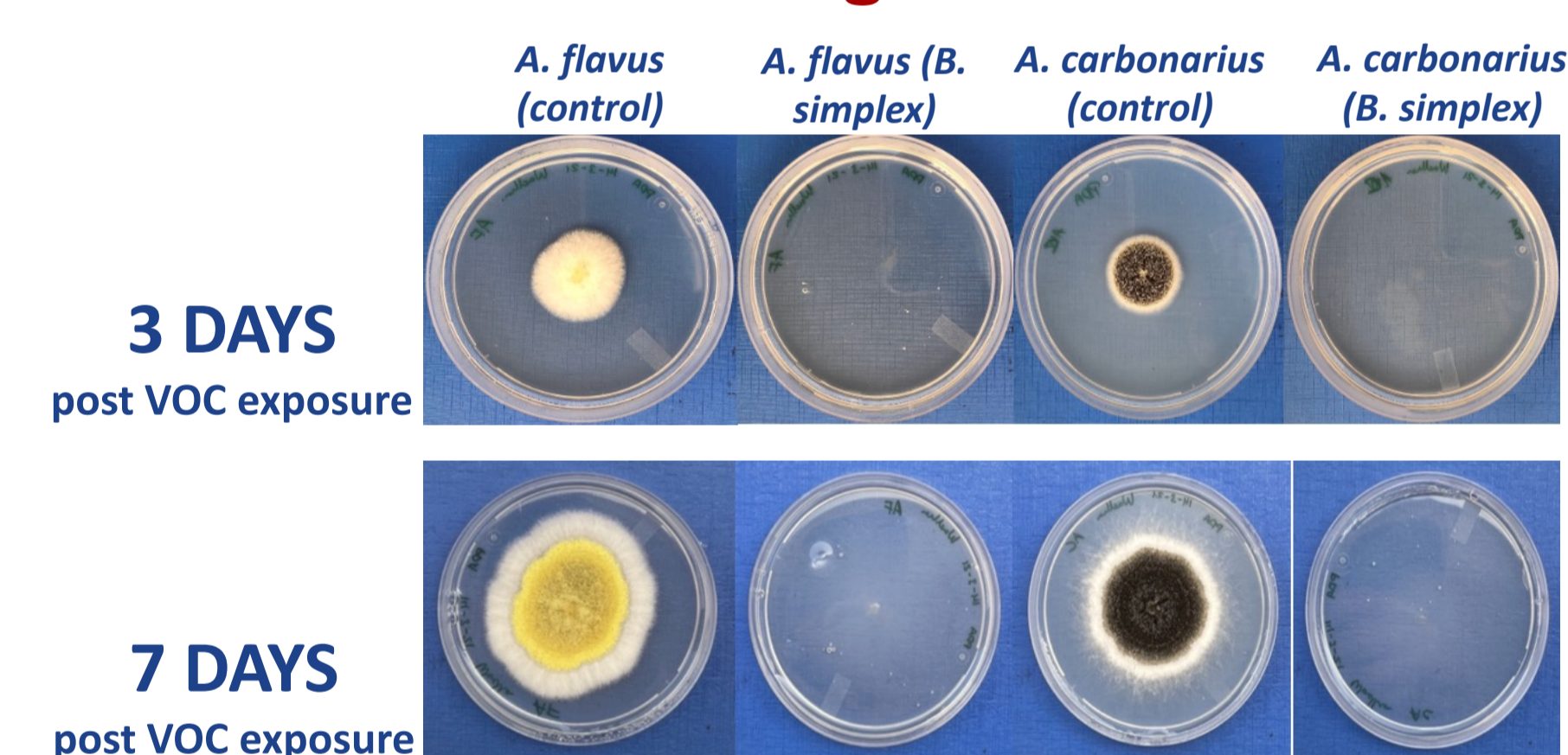


Figure 5: In vitro coinhibition of *B. simplex* with spectrum of fungi

- At day 3, many fungal strains growth were completely inhibited.
- At day 7, the diameter of fungi is smaller compared to the control.

Conclusion

- Arabic coffee is considered as one of the main beverages in Qatar.
- All tested coffee samples were found contaminated with mycotoxigenic fungi synthesizing OTA and AF.
- B. simplex* shows promising results in the biological control of both fungal growth and mycotoxins synthesis.
- Bacterial VOCs were found to help in fungal growth inhibition.

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